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Vasoactive Molecules and the Kidney

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CHAPTER OUTLINE

KEY POINTS

- Beyond the classical endocrine renin–angiotensin system (RAS) that pivots around the actions of systemically circulating angiotensin II, an additional, quasi-independent local RAS operates within the kidney in paracrine, autocrine, and possibly even intracrine modes.
- Although much of our understanding of the RAS relates to the actions of angiotensin II, a range of other angiotensin-related proteins, enzymes, and receptors also have important biological actions in both kidney physiology and disease.
- Therapies that block the RAS have been employed to slow renal decline in chronic kidney disease for decades and agents that either prevent (e.g., endothelin receptor antagonists) or augment (e.g., neprilysin inhibitors in combination with angiotensin receptor blockade) the actions of other vasoactive molecules are under investigation.
- Endothelin type A receptor blockade is undergoing phase III evaluation for the treatment of diabetic nephropathy, although earlier clinical trials were hampered by dose-dependent fluid retention.
- Inhibition of neprilysin prevents the degradation of the natriuretic peptides atrial natriuretic peptide and brain natriuretic peptide, but its effects are limited by an unopposed RAS. Concurrent angiotensin receptor blockade and neprilysin inhibition improves outcomes in patients with heart failure, although its effects on hard renal outcomes are currently unknown.
- Concurrent angiotensin-converting enzyme inhibition and neprilysin inhibition are associated with an increased risk of angioedema.
- The development of therapies that affect other vasoactive molecules (e.g., the kallikrein–kinin system, urotensin II, guanylin, uroguanylin, and adrenomedullin) has been limited.

Vasoactive peptides, arising from both the systemic circulation and from local tissue-based generation, play important roles in kidney physiology, not only in the regulation of renal blood flow (RBF) but also in electrolyte exchange, acid–base balance, and diuresis. More recent interest has focused on the role of these peptide systems in kidney development and in the pathogenesis of organ injury.

RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM

In their now seminal 1898 report, *Niere und Kreislauf,* Robert Tigerstedt and Per Bergman, while working at the Karolinska Institute in Sweden, described the prolonged vasopressor effects of crude kidney extracts.[1] Although recognizing the impurity of the extract, Tigerstedt named the unidentified active substance, "renin," based on its organ of origin. More than 110 years later, our understanding of the renin–angiotensin system (RAS) continues to evolve with recent insights into its pivotal role in pathophysiological as well as physiological processes. Underlying this effort to fully understand the RAS is not only a desire for knowledge but also a profound appreciation of the therapeutic importance of its blockade that emanates from the renoprotective effects of angiotensin-converting enzyme inhibition first described by Anderson, Meyer, Rennke, and Brenner in 1985 in a rodent model of progressive kidney disease.[2]

Clinical Relevance

The renin–angiotensin system (RAS) plays a fundamental role in blood pressure, plasma volume, electrolyte, and acid–base homeostasis. Beyond its function in kidney physiology, however, angiotensin II, the primary effector molecule of the RAS, raises intraglomerular pressure, induces proteinuria, and stimulates the production of extracellular matrix that leads to glomerulosclerosis and interstitial fibrosis. Accordingly, blockade of angiotensin II synthesis by angiotensin-converting enzyme inhibition or antagonizing its action at the angiotensin I receptor with an angiotensin receptor blocker is at the cornerstone of strategies that attenuate progressive kidney function decline in most forms of chronic kidney disease.

CLASSICAL RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM

The classical view of the RAS focuses on the endocrine aspects of this peptidergic system. Angiotensinogen synthesized by the liver enters the circulation where it is cleaved to form angiotensin I by renin, a peptidase that is secreted from the juxtaglomerular apparatus (JGA) of the kidney. The terminal two amino acids of angiotensin I are then removed to form angiotensin II, as it traverses through the circulation, exposed to angiotensin-converting enzyme (ACE), a peptidase robustly expressed on endothelial cells of the pulmonary vasculature. Angiotensin II, the principal effector molecule of the RAS, then binds to its type 1 receptor (AT_1R), resulting in vasoconstriction, sodium retention, thirst, and aldosterone secretion. This traditional view of the RAS is still valid but has been considerably augmented in recent years, not only by the discovery of new enzymes, peptides, and receptors, but also by an appreciation that the RAS has an independently functioning local tissue–based component that acts through paracrine, autocrine, and possibly intracrine mechanisms (Fig. 11.1).

ANGIOTENSINOGEN

Angiotensinogen is primarily, although by no means exclusively, synthesized in the liver, particularly the pericentral zone of the hepatic lobules.[3] In humans, it is coded by a single gene, composed of five exons and four introns, that spans about 13 kb of genomic sequence on chromosome 1 (1q42-q43). It is translated to a 453 amino acid globular glycoprotein with a molecular weight between 45 and 65 kDa, depending on the extent of its glycosylation, that then undergoes posttranslational cleavage of a 24– or 33–amino acid signal peptide,[4] giving rise to the mature circulating form of angiotensinogen.[5]

Structurally, angiotensinogen bears substantial homology to the serpin superfamily of protease inhibitors and like many members of its family behaves as an acute phase reactant in the inflammatory setting,[6] reflecting the presence of an acute-phase response element that binds the transcription factor, NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells).[7]

RENIN

Like angiotensinogen, the gene encoding renin is also located on the long arm of chromosome 1 (1q32) and contains 10 exons and 9 introns, similar to other aspartyl proteases.[8] Unlike humans and rats that have only a single renin gene, the mouse has two genes, *Ren1* and *Ren2*, expressed primarily in the submandibular gland and kidney, respectively.

Following its synthesis as a 406–amino acid preprohormone, the 23–amino acid leader sequence of preprorenin is cleaved in the rough endoplasmic reticulum, giving rise to prorenin (also called inactive renin and "big" renin), which may be then rapidly secreted directly from the Golgi apparatus or from protogranules.[4] Alternatively, and virtually exclusively in the JGA, prorenin may be packaged into mature, dense granules that instead of being immediately secreted, undergo further processing to the active enzyme, renin (active renin). Contrasting with the more constitutive secretion of prorenin, the release of renin-containing granules is tightly regulated.[8]

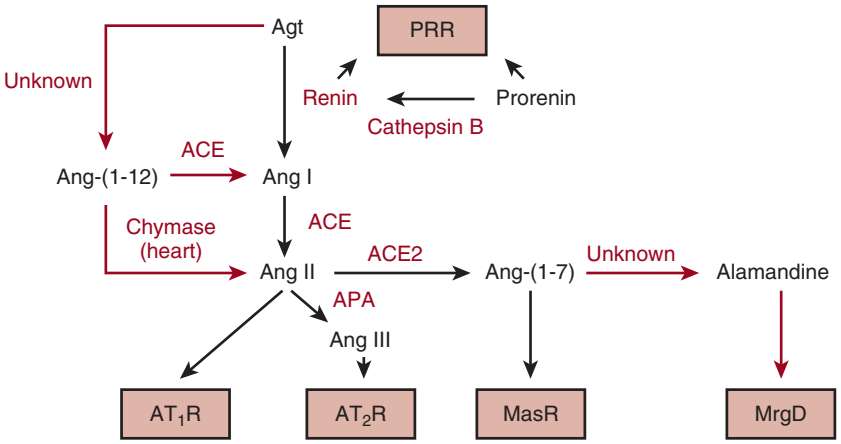

Fig. 11.1 Schematic depiction of the renin–angiotensin system components and selected actions. The enzymes of the system are shown in *red.* Newly described enzymatic pathways are shown as *red arrows.* Receptors are shown in the *boxes*. *ACE,* Angiotensin-converting enzyme; *Agt,* angiotensinogen; *Ang,* angiotensin; *APA,* aminopeptidase A; *AT_1R,* angiotensin type-1 receptor; *AT_2R,* angiotensin type-2 receptor; *MasR, Mas* receptor; *MrgD, Mas*-related G-protein–coupled receptor; *PRR,* (pro)renin receptor. (Modified from Carey RM. Newly discovered components and actions of the renin-angiotensin system. *Hypertension.* 2013;62: 818–822.)

Mature, active renin is a variably glycosylated 340–amino acid 37–40-kDa aspartyl protease that is active at neutral pH, and in contrast to the more promiscuous activities of most other proteases in this class, has only a single known substrate, cleaving the decapeptide angiotensin I from the amino terminal of angiotensinogen. Although the kidney produces both renin and prorenin, a range of extrarenal tissues including the adrenals, gonads, and placenta produce prorenin and contribute to its presence in plasma. However, as evidenced by the near total absence of active renin in anephric patients, the kidney, and the JGA in particular, appears to be the only source of circulating renin in humans.

Factors that chronically stimulate renin secretion, such as a low sodium diet and ACE inhibition, lead to an increase in the number of renin secreting cells rather than an increase in cell size or the number of granules that each JGA cell contains. This expansion of the renin secreting mass occurs proximally by metaplastic transformation of smooth muscle cells within the walls of the afferent arteriole. Although sometimes mentioned, ectopic renin expression within the extraglomerular mesangium appears to be an uncommon event.[9]

PRORENIN ACTIVATION

Prorenin is maintained as an inactive zymogen through the occupation of its catalytic cleft by its prosegment. Removing this prosegment by either proteolytic or nonproteolytic means yields active renin, a term that denotes its enzymatic activity rather than its amino acid sequence (Fig. 11.2).

Within the dense core secretory granules of the JGA, acidification by vacuolar adenosine triphosphatases (ATPases) provides the optimal pH for the prosegment cleaving enzymes (proconvertase 1 and cathepsin B), and may also assist the pH-dependent, nonenzymatic activation of prorenin as well.[9–11] Although various peptidases such as trypsin, plasmin, and kallikrein can also cleave the prosegment of prorenin in vitro, these do not appear to contribute to the generation of renin in the in vivo setting. Although traditionally viewed as occurring only in the JGA, recent cell culture–based studies suggest that proteolytic activation of renin can also occur in cardiac and vascular smooth muscle cells by as yet unidentified serine proteases.[12–14] The significance of these findings in the intact organism, however, remains to be established.

In addition to proteolytic cleavage of its prosegment, prorenin can also be reversibly activated nonenzymatically by a conformational change such that the prosegment no longer occupies the enzymatic cleft. Under usual circumstances, less than 2% of prorenin is in this open active conformation. This process can, however, be induced by acid (pH 4.0)[15,16] and to a lesser extent by cold.[17] More recently, the putative (pro)renin receptor (PRR; discussed later) has also been shown to nonproteolytically activate prorenin.[18]

REGULATION OF RENIN SECRETION

Mechanical, neurological, and chemical factors regulate the activity of the RAS by modulating renin secretion.

Renal Baroreceptor

The existence of a renal baroreceptor mechanism was first conceptualized by Skinner and colleagues to explain how renin secretion increases when afferent arteriolar perfusion pressure falls.[19] Studies in conscious dogs show that changes in renal perfusion pressure have only a small effect on renin secretion until a threshold of about 90 mm Hg is reached, below which renin secretion abruptly increases, doubling with every 2 to 3 mm Hg fall in pressure.[20] Accordingly, reduction in pressure below this level profoundly stimulates renin secretion, thereby acutely activating the RAS and resulting in a range of angiotensin II–dependent phenomena that collectively serve to restore systemic pressure. Despite the importance of the baroreceptor function, several decades of research have not identified precisely how the pressure signal is transduced into renin release, though postulated mediators include stretch-activated calcium channels, endothelins (ETs), and prostaglandins.

Neural Control

The JGA is endowed with a rich network of noradrenergic nerve endings and their β1 receptors. Stimulation of the

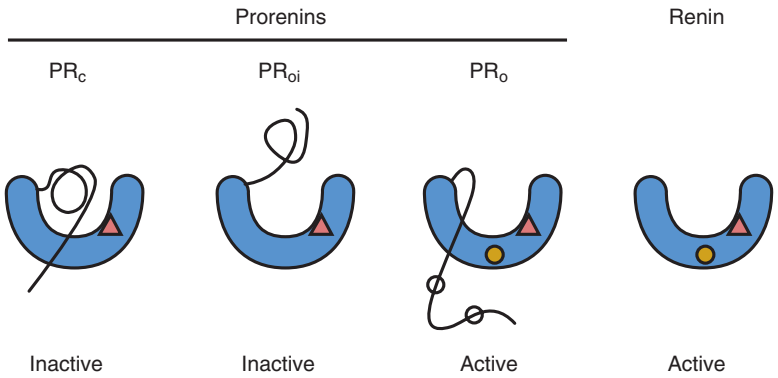

Fig. 11.2 The conformational changes and the expression of immunoreactive epitopes associated with the activation of prorenin are depicted. The main body of the molecule *(blue)*, the substrate-binding cleft, and the prosegment *(black line)* are shown. The closed triangle represents the epitope of the main body expressed by PR_c (prorenin in the inactive closed conformation), PR_{oi} (prorenin in the inactive intermediary open conformation), PR_o (prorenin in the active open conformation), and renin. The closed circle *(yellow)* represents the epitope of the main body, expressed by PR_o and renin, but not by PR_c and PR_{oi}. The *open circles* represent epitopes of the prosegment expressed by PR_o but not by PR_c and PR_{oi}. (Modified from Schalekamp MA, Derkx FH, Deinum J, et al. Newly developed renin and prorenin assays and the clinical evaluation of renin inhibitors. *J Hypertension.* 2008;26:928–937.)

renal sympathetic nerve activity leads to renin secretion that is independent of changes in RBF, glomerular filtration rate (GFR), or Na^+ reabsorption. Moreover, this effect can be blocked surgically (denervation) and pharmacologically, by the administration of β-adrenoreceptor blockers.[20] The role of cholinergic, dopaminergic, and adrenergic activation is controversial, though these agents have also been shown to modulate renin release under certain circumstances.

Tubule Control

Chronic diminution in luminal NaCl delivery to the macula densa is a potent stimulus for renin secretion, reflecting a coordinate interaction between a range of mediators including adenosine, nitric oxide (NO), and prostaglandins that not only affect renin release but also its transcription.[21] This mechanism is thought to account for the chronically high plasma renin activity (PRA) in subjects ingesting a low-salt diet.[22]

Metabolic Control

The tricarboxylic acid (TCA) cycle provides a final common pathway by which carbohydrates, fatty acids, and amino acids converge in the process of adenosine triphosphate (ATP) generation by aerobic electron transfer. Although the TCA cycle operates within mitochondria, its intermediates can be detected within the extracellular space, increasing in abundance when local energy supply and demand are mismatched or when cells are exposed to hypoxia, toxins, or injury.[23] Succinate, for instance, has been shown to stimulate renin release and its intravenous administration leads to hypertension, though the mechanisms underlying this effect have only recently been unraveled. In 2004, He and colleagues reported that alpha keto-glutarate and succinate are ligands for the previously orphaned G protein–coupled receptors (GPCRs), GPR99 and GPR91, respectively, and that succinate-induced hypertension is abolished in GPR91-deficient mice.[24] Indeed, in follow-up studies from this group, GPR91 was localized to the apical plasma membrane of macula densa cells where succinate stimulation was shown to activate p38 and Erk 1/2 mitogen-activated protein (MAP) kinases (MAPKs), inducing cyclooxygenase-2 (COX-2)-dependent synthesis of prostaglandin E2, a well-established paracrine mediator of renin release.[25] Moreover, the ability of tubule succinate to induce JG renin secretion suggests that this phenomenon is likely an important determinant of JGA function in both physiological and pathophysiological settings. In diabetic rats, for instance, elevated succinate has been detected in both plasma and urine.[25]

Vitamin D Receptor

The vitamin D receptor (VDR) is a negative regulator of the RAS such that VDR-null mice display a marked increase in renin expression and angiotensin II production in conjunction with hypertension and cardiac hypertrophy.[26] Importantly, these effects occurred independently of calcium and parathyroid hormone, both of which have been reported to also modulate renin expression. The molecular basis for the interaction between vitamin D and renin expression has also been, at least partly, unraveled in a series of studies by Yuan et al.[27] Under usual circumstances, activation of the cyclic adenosine monophosphate (cAMP)–protein kinase A pathway by the sympathetic nervous system or macula densa leads to phosphorylation of a cAMP response element (CRE) binding protein (CREB) and recruitment of CREB-binding protein/p300 to the CRE in the promoter region of the renin gene. VDR-bound 1,25 $(OH)_2D_3$, however, blocks binding of CREB to the CRE DNA *cis*-element, leading to a reduction in renin gene transcription (Fig. 11.3).

From a clinical perspective, this interaction between vitamin D and renin may explain the well-documented inverse relationship between plasma vitamin D_3, blood pressure, and PRA. While several studies examining the effects of vitamin D supplementation as a renoprotective or antihypertensive measure have been undertaken, their findings have been mixed and long-term, randomized controlled trials with clinically meaningful endpoints are awaited.

Other Local Factors

In addition to the factors discussed earlier, a large range of locally produced biologically active molecules have also been shown to alter renin secretion. These include peptides [ANP, kinins, vasoactive intestinal polypeptide, ET, calcitonin gene–related peptide (CGRP)], amines (dopamine and histamine), and arachidonic acid derivatives.[20]

PLASMA PRORENIN AND RENIN

Under usual circumstances, the plasma concentration of prorenin is approximately 10 times greater than renin. In some patients with diabetes, however, plasma prorenin is disproportionately increased, where it predicts the development of diabetic nephropathy (including microalbuminuria) and retinopathy.[28,29]

In addition to its role in the research setting, measurement of plasma renin is an important clinical assay, providing important information, for example, when evaluating patients with possible hyperaldosteronism, assessing volume status, and in predicting the response to, or monitoring drug

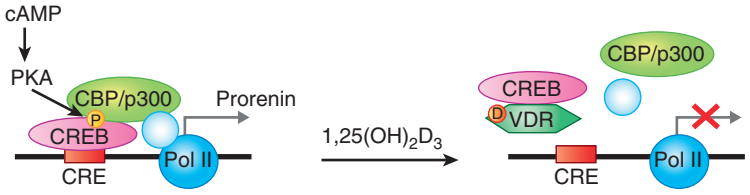

Fig. 11.3 Model of $1,25(OH)_2D_3$-induced transrepression of renin gene expression. The cAMP–PKA pathway activates CREB by phosphorylation, leading to recruitment of CBP/p300. In the presence of $1,25(OH)_2D_3$, liganded VDR interacts with CREB and blocks its binding to CRE, leading to reduction of renin gene transcription. *cAMP,* Cyclic AMP; *CBP,* CREB-binding protein; *CRE,* cAMP response element; *CREB,* CRE-binding protein; *D,* $1,25(OH)_2D_3$; *P,* phosphorylation; *PKA,* protein kinase A; *Pol II,* RNA polymerase II; *VDR,* vitamin D receptor. (Modified from Yuan W, Pan W, Kong J, et al. 1,25-Dihydroxyvitamin D_3 suppresses renin gene transcription by blocking the activity of the cyclic AMP response element in the renin gene promoter. *J Biol Chem.* 2007;282:29821–29830.)

adherence to, an ACE inhibitor or angiotensin receptor blocker (ARB). In broad terms, plasma renin is determined by either activity or immunological assay methods.[30] The most commonly used method involves the measurement of PRA. With this method, the rate at which angiotensin I is produced from plasma angiotensinogen is assayed. To prevent angiotensin I's degradation or its conversion to angiotensin II, inhibitors of angiotensinase and ACE are added to the assay. Accordingly, PRA is not only dependent on renin and endogenous angiotensinogen concentrations but will also overestimate the extent of inhibition by renin inhibitors due to the displacement of protein-bound drug by the peptidase inhibitors. The latter scenario may be diminished by using an antibody capture method in which antiangiotensin I antibody, instead of peptidase inhibitors, is used to protect angiotensin I from further catabolism.[31]

The nomenclature of renin assays can be quite confusing in that plasma renin concentration (PRC) may be measured by both activity and immunological assays. With the activity method (PRCa), exogenous angiotensinogen is added to the assay, thereby avoiding the influence that endogenous levels of the substrate might have. However, PRCa may also be affected by the presence of renin inhibitors, though like PRA may take advantage of antibody capture methodology.[30] In the immunological assay for renin (PRCi), the concentrations of renin and prorenin in its active, open conformation are assessed, so that like PRA and PRCa, the PRCi assay is also time and temperature dependent because lower temperatures will increase the proportion of prorenin in its active conformation. Moreover, renin inhibitors, by binding to the active site of prorenin in its open conformation, prevent the refolding of the prosegment and may therefore lead to an overestimation of PRCi.[30,31]

ANGIOTENSIN-CONVERTING ENZYME

ACE is a zinc-containing dipeptidyl carboxypeptidase that cleaves the terminal histidyl-leucine from angiotensin I to form the octapeptide angiotensin II. In contrast to the single-substrate specificity of renin, ACE is not specific, cleaving the two terminal acids from peptides with the C′-terminal sequence R_1-R_2-R_3-OH, where R_1 is the protected (noncleaved) amino acid, R_2 is any nonproline L-amino acid, and R_3 is any nondicarboxylic (cysteine, ornithine, lysine, arginine) L-amino acid with a free carboxyl terminal.[4] Importantly, therefore, ACE also catalyzes the inactivation of bradykinin. Although encoded by a single ACE gene, two distinct tissue-specific messenger RNAs (mRNAs) are transcribed, each with different initiation and alternative splice sites.[32] The somatic form, present in almost all tissues, is a 1306–amino acid, 140–160-kDa glycoprotein with two active sites, whereas the 90- to 100- kDa testicular or germinal form is found exclusively in postmeiotic male germ cells, and contains a single active site and appears to be involved in spermatogenesis.[4,33,34] The somatic form of ACE is widely distributed with activity present not only in tissues but also in most biological fluids. In the human kidney, ACE is present to the greatest extent within proximal and distal tubules, however, both its magnitude of expression and its site-specific distribution may be altered by disease.[35]

ANGIOTENSIN TYPE 1 RECEPTOR

The AT_1R mediates most of the known physiological effects of angiotensin II. The gene for this widely distributed 359–amino acid, 40-kDa, seven-transmembrane GPCR is located on chromosome 3 in humans.[36]

Within the kidney, AT_1Rs are widely expressed. In the glomerulus, they are found in both afferent and efferent arterioles as well as in the mesangium, endothelium, and on podocytes.[37] Consistent with angiotensin II's role in Na^+ reabsorption, AT_1Rs are highly abundant on the brush borders of proximal tubule epithelial cells.[38] Prominent expression has also been found in renal medullary interstitial cells, located between the renal tubules and vasa recta, where angiotensin II is purported to have a potential role in the regulation of medullary blood flow.[39]

Angiotensin II binding to AT_1R initiates cell signaling by several different pathways that have been mostly studied in vascular smooth muscle cells.[40] These include G protein–mediated pathways, and the activation of tyrosine kinases, NADH/NADPH oxidases, and serine/threonine kinases.[36]

G Protein–Mediated Signaling

In the classical G protein–mediated pathway, AT_1R ligand binding leads to activation of phospholipases C, D, and A_2. Phospholipase C rapidly hydrolyses phosphatidylinositol bisphosphate to inositol trisphosphate and diacylglycerol (DAG), initiating calcium release from intracellular stores and protein kinase C (PKC) activation, respectively. Phospholipase D similarly generates DAG and activates PKC, whereas phospholipase A2 (PLA_2) leads to the formation of various vasoactive and proinflammatory arachidonic acid derivatives.

Reactive Oxygen Species

Although previously regarded as toxic waste products, emerging evidence indicates that reactive oxygen species may also act as second messengers, not only activating other cell signaling cascades such as p38 MAPK but also a number of transcription pathways implicated in the pathogeneses of inflammatory and degenerative disease.[36] Although the mechanisms by which the AT_1R stimulates NADH/NADPH are not well understood, angiotensin II binding to this receptor results in the generation of both superoxide and hydrogen peroxide.

Tyrosine Kinases

Angiotensin II binding to the AT_1R "transactivates" a number of nonreceptor tyrosine kinases [Src, Pyk2, Focal adhesion kinase (FAK), and Janus kinase (JAK)] as well as the growth factor receptor tyrosine kinases for epidermal growth factor (EGF)[41,42] and platelet-derived growth factor (PDGF).[43,44] By binding to the AT_1R, angiotensin II initiates the translocation of tumor necrosis factor-alpha (TNF-α)–converting enzyme (TACE, ADAM17) to the cell surface. TACE then cleaves TNF-α from its membrane-associated precursor (pro-TNF-α), allowing it to bind to the EGF receptor (EGFR) on the cell surface. This ligand–receptor interaction then induces EGFR autophosphorylation and activates its downstream signaling pathways that include Akt, Erk-1/2, and mammalian target of rapamycin (Fig. 11.4). The in vivo relevance of this transactivation pathway has been recently confirmed. Using mice that express a dominant negative form of EGFR, Lautrette and colleagues showed that despite similar blood pressures, mutant mice infused with angiotensin II had less proteinuria and renal fibrosis than did their wild-type

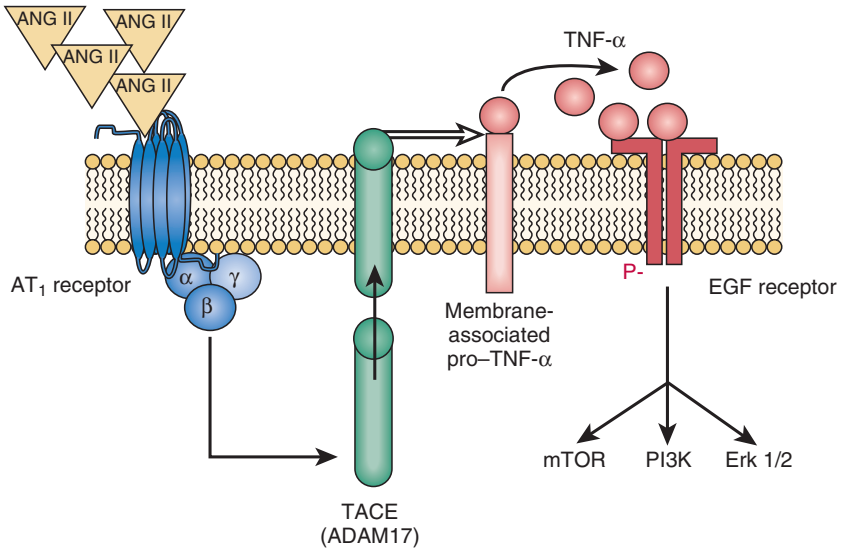

Fig. 11.4 Angiotensin II *(ANG II)* binds to its angiotensin II type 1 *(AT_1)* receptor, a G protein–coupled receptor lacking intrinsic tyrosine kinase activity. Through as-yet-undescribed mechanisms, this interaction leads to the translocation of the metalloprotease tumor necrosis factor-α *(TNF-α)*–converting enzyme *(TACE)* from the cytosol to the cell surface, where it cleaves TNF-α from its membrane-associated promolecule, allowing it to bind and activate the epidermal growth factor (EGF) receptor. *Erk 1/2,* Extracellular signal–regulated kinases 1 and 2; *mTOR,* mammalian target of rapamycin; *P13K,* phosphatidylinositol-3-kinase. (Modified from Wolf G. "As time goes by": angiotensin II–mediated transactivation of the EGF receptor comes of age. *Nephrol Dial Transplant.* 2005;20:2050–2053.)

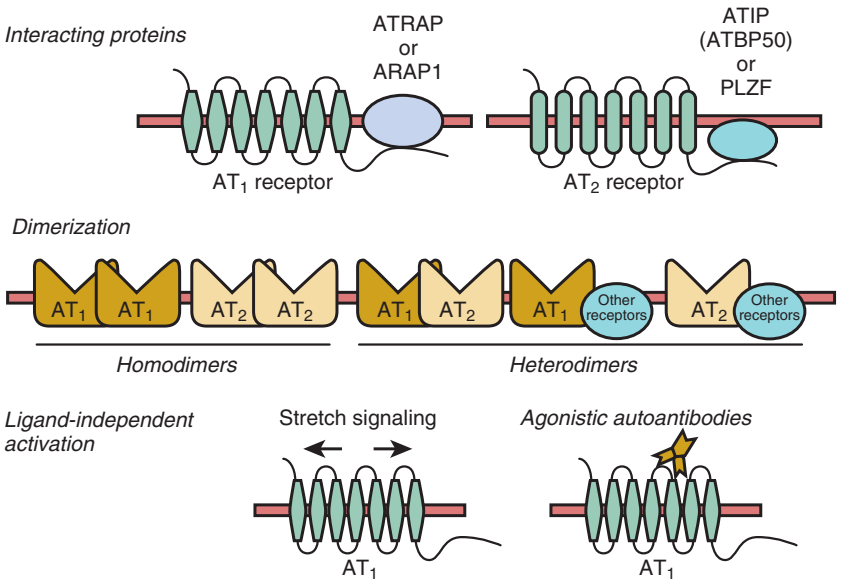

Fig. 11.5 Regulation of angiotensin receptors. *ARAP1,* AT_1 receptor–associated protein 1; *ATBP50,* AT_2 receptor–binding protein of 50 kDa; *ATIP,* angiotensin type 2 receptor–interacting protein; *AT_1 receptor,* angiotensin type 1 receptor; *AT_2 receptor,* angiotensin type 2 receptor; *ATRAP,* AT_1 receptor–associated protein; *PLZF,* promyelocytic leukemia zinc finger. (Modified from Mogi M, Iwai M, Horiuchi M. New insights into the regulation of angiotensin receptors. *Curr Opin Nephrol Hypertens.* 2009;18:138–143.)

counterparts.[45] Consistent with these findings and the pivotal role of the RAS in diabetic nephropathy, studies using a specific EGFR tyrosine kinase inhibitor (i.e., PKI 166) have also shown a reduction in early structural injury in a rat model of diabetic nephropathy.[46]

Like EGFR, the transactivation of the PDGF receptor (PDGFR) by AT_1R is also complex, involving the adaptor protein Shc.[43,44] In addition to studies that have explored the angiotensin–PDGFR interaction in cell culture or organ baths,[47] a more recent report has shown that despite continued hypertension, inhibition of the PDGFR kinase in vivo can also dramatically attenuate angiotensin II–induced vascular remodeling.[48]

Angiotensin Type 1 Receptor Internalization

In addition to the conventional ligand–receptor mediated pathways, a range of other signaling mechanisms that involve the AT_1R have also been described. These include the discovery of receptor interacting proteins, heterologous receptor dimerization, and ligand-independent activation (Fig. 11.5).[49]

These new insights, although adding greater complexity to our understanding of the RAS, also provide the potential for new therapeutic targets in disease prevention and management.

Following ligand binding and the initiation of signal transduction, AT_1Rs are rapidly internalized, followed by either lysosomal degradation or recycling back to the plasma membrane. Several mechanisms account for AT_1R internalization, including interaction with caveolae, phosphorylation of its carboxyl terminal by G-protein receptor kinases,[36] and association with the newly described AT_1R interacting proteins.[49] To date, two such interacting proteins, *AT*$_1$ *r*eceptor–*a*ssociated *p*rotein (ATRAP)[50] and *AT*$_1$ *r*eceptor–*a*ssociated *p*rotein-1 (ARAP1),[51] have been described. ATRAP interacts with the C terminal of AT_1R, downregulating cell surface AT_1R expression and attenuating angiotensin II–mediated effects.[49] ARAP1, though somewhat similar to ATRAP, promotes AT_1R recycling to the plasma membrane such that its kidney-specific overexpression induces hypertension and renal hypertrophy.[51]

Angiotensin Type 1 Receptor Dimerization

In addition to their ability to induce cell signaling in their monomeric state, GPCRs like AT_1R may also associate to form both homodimers and heterodimers.[52] Beyond its constitutive homodimerization,[53] AT_1R may dimerize with angiotensin type 2 receptor (AT_2R) and also form heterooligomers with receptors for bradykinin (B2), epinephrine (β2), dopamine (D1,3,5), ET (B), Mas, and EGF that modulate their function.[54–57]

Ligand-Independent Angiotensin Type 1 Receptor Activation

Without involvement of angiotensin II, cell stretch induces a conformational switch that initiates AT_1R's intracellular signaling pathways.[58,59] As might be expected from an understanding of this mechanism, an AT_1R blocker, acting as an inverse agonist, will abrogate these effects, as described in both cardiac[59] and mesangial cells.[60] A similar means of ligand-independent activation has also been shown to result from the binding of agonist antibodies to AT_1R in some women with preeclampsia[61] and in certain cases of renal allograft rejection.[62]

PHYSIOLOGIC EFFECTS OF ANGIOTENSIN II IN THE KIDNEY

The traditional actions of angiotensin II relate primarily to its effects on vascular tone and fluid balance that are mediated by its actions on the vasculature, heart, kidney, brain, and adrenal glands by the AT_1R. In vascular smooth muscle, stimulation of AT_1Rs by angiotensin II induces cell contraction and consequent vasoconstriction. In the adrenal cortex, this ligand– receptor interaction stimulates aldosterone release, thereby promoting sodium reabsorption in the distal nephron. Moreover, angiotensin II will directly enhance sodium retention by the proximal tubule and in the brain it will stimulate thirst and salt craving. Additional effects include sympathoadrenal stimulation and the augmentation of cardiac contractility. Together, these effects serve to maintain extracellular fluid volume and systemic blood pressure. Given the central role that the kidney has in the regulation of these key aspects of mammalian homeostasis, it is not surprising that angiotensin II should have profound effects on renal physiology.

HEMODYNAMIC ACTIONS

The effects of exogenously administered angiotensin II are dose dependent. At low doses, angiotensin II infusion increases renal vascular resistance (RVR) and lowers RBF without affecting GFR so that the filtration fraction is increased. At higher doses of angiotensin II, RVR is further increased, leading to an augmented reduction in RBF and fall in GFR.[63] However, because GFR is reduced to a lesser extent than renal plasma flow, the filtration fraction remains elevated. Such studies are consistent with the view that limited stimulation of the RAS would mostly serve to enhance tubule sodium, as is seen, for instance, in societies unaccustomed to contemporary diets.[22] Greater activation of the RAS, by contrast, as might be found in the setting of severe volume depletion, would result in angiotensin II–dependent reduction in RBF that would aid in sustaining systemic blood pressure while further stimulating sodium reabsorption.

Kidney micropuncture has been used extensively to explore the intrarenal sites of angiotensin II's effects on vascular resistance. These studies demonstrate that although angiotensin II increases both afferent and efferent arteriolar resistance, intraglomerular capillary pressure (P_{GC}) is consistently elevated[64] and the ultrafiltration coefficient (K_f) is reduced.[63] Moreover, as predicted by mathematical modeling, the glomerular hypertension induced by angiotensin II does not lead to acute proteinuria, because the structural barriers to macromolecular passage remain intact.[63] Chronic angiotensin II infusion with sustained intraglomerular hypertension, by contrast, leads to glomerular capillary damage and substantial proteinuria.

TUBULE TRANSPORT

Sodium

Consistent with its importance in the regulation of volume status, angiotensin II has profound effects on renal Na^+ handling. The proximal tubule is responsible for the reabsorption of approximately two-thirds of the sodium from the glomerular filtrate and binding sites for angiotensin II are particularly abundant in the proximal tubule with immunohistochemical localization of the AT_1R to both apical and basolateral surfaces.[65] At picomolar concentrations, angiotensin II stimulates the luminal Na^+/H^+ exchanger, the basolateral $Na^+/HCO3^-$ cotransporter, and the Na^+–K^+ ATPase. However, at concentrations greater than 10^{-9} M, angiotensin II inhibits the very same transporters. The mechanisms underlying this dose-dependent effect of angiotensin II on Na^+ transport, that seem to also occur in the loop of Henle,[65] are incompletely understood. In the distal tubule, the effects of angiotensin II on Na^+ transport are site dependent. In the early distal tubule, for instance, angiotensin II stimulates apical Na^+/H^+ exchange while in the late distal tubule it stimulates the amiloride-sensitive sodium channel.[65]

Acid–Base Regulation

The kidney has a key role in the maintenance of physiological pH by regulating the secretion/reabsorption of acids and bases. As for Na^+, angiotensin II also has substantial effects on acid–base transport in the proximal tubule, distal tubule, and collecting duct. Recent interest has focused, in particular, on its actions in the collecting duct. In the collecting duct,

angiotensin II not only stimulates Na^+/H^+ exchangers and $Na^+/HCO3^-$ cotransporters but has also been shown to stimulate the vacuolar H^+-ATPase in intercalated A-cells via its AT_1R receptor.[66] Moreover, elegant and detailed electron microscopic studies have helped to unravel the mechanisms by which angiotensin II exerts its effects at this site, revealing translocation of the H^+-ATPase from the cytoplasm to the apical surface in response to ligand stimulation.[67]

EXPANDED RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM: ENZYMES, ANGIOTENSIN PEPTIDES, AND RECEPTORS

ANGIOTENSIN TYPE 2 RECEPTOR

In humans, the AT_2R is a 363–amino acid protein that maps to the X chromosome and is highly homologous to its rat and mouse counterparts.[68] Like AT_1R, AT_2R is also a seven-transmembrane GPCR, though it shares only 34% homology.

Despite substantial research, the actions of AT_2R are still not well understood and remain somewhat controversial.[69] In general, however, the actions of AT_2R stimulation oppose those of AT_1R. For instance, whereas AT_1R vasoconstricts and promotes Na^+ retention, AT_2R stimulation leads to vasodilation[70] and natriuresis,[71] consistent with its abundance on the epithelium of the proximal tubule.[72] The vasodilatory effects of AT_2R stimulation are mediated by increasing NO synthesis and cyclic guanosine monophosphate (cGMP) by bradykinin-dependent and -independent mechanisms.[73] Its natriuretic effects, however, seem to be dependent on angiotensin II's conversion to angiotensin III by aminopeptidase N.[74]

Like the AT_1R, the activity of AT_2R may also be modulated by oligomerization, in association with various interacting proteins and ligand-independent effects.[73]

(PRO)RENIN RECEPTOR

In 2002 an apparently novel, 350-amino acid, single-transmembrane protein that binds both renin and prorenin with high affinity was identified.[18] Ligand binding to this protein was shown to induce a fourfold increase in the catalytic cleavage of angiotensinogen as well as stimulating intracellular signaling with activation of MAPKs extracellular signal–regulated kinases 1 and 2 (ERK1/2),[18] leading to it being named the (pro)renin receptor [(P)RR]. The designation (pro)renin refers to its ability to interact with both renin and prorenin.

Given its localization to the mesangium in initial studies, its actions in augmenting local angiotensin II production, and its ability to increase mesangial transforming growth factor-β (TGF-β) production,[75] the (P)RR was understandably implicated in the pathogenesis of kidney disease.[76] However, despite the appeal, it has been difficult to reconcile this view of the (P)RR with a number of other experimental findings, regarding not only its potentially pathogenetic role, but also its pattern of distribution within the kidney and its homology to other proteins. For instance, given the purported pathogenetic role of the (P)RR, the increased abundance of renin that follows the use of ACE inhibitors and ARBs would be expected to be adverse, yet these classes of drugs have been repeatedly shown to be renoprotective. Second, although the (P)RR was initially localized to the glomerular mesangium, more recent detailed studies have shown that (P)RR is primarily expressed in the collecting duct.[77] Third, although initially reported as having no homology with any known membrane protein,[18] database interrogation shows that the (P)RR is identical to two other proteins: CAPER (endoplasmic reticulum–localized type 1 transmembrane adaptor precursor) and ATP6ap2 (ATPase, H^+ transporting, lysosomal accessory protein 2),[78–82] a protein that associates with the vacuolar H^+-ATPase.[83] Indeed, the predominant expression of the (P)RR at the apex of acid secreting cells in the collecting duct, in conjunction with its colocalization and homology with an accessory subunit of the vacuolar H^+-ATPase, suggests that the (P)RR may function primarily in urinary acidification.[77] However, the vacuolar H^+-ATPase is not restricted to the kidney but is widely distributed in the plasma membrane and the membranes of organelles in several tissues where it functions, not only in urinary acidification but also in endocytosis, conversion of proinsulin to insulin, and osteoclast bone resorption.[84] Whereas the prevailing data indicate that (P)RR is an accessory subunit of the vacuolar H^+-ATPase that also binds renin and prorenin, the precise functions of the prorenin- and renin-binding subunit remain to be unraveled in the kidney and elsewhere.

ANGIOTENSIN-CONVERTING ENZYME 2

In 2000, two groups independently reported the existence of the first ACE homolog, ACE2, an apparently novel zinc metalloprotease but with considerable homology (40% identity and 61% similarity) to ACE.[85,86] The gene-encoding ACE2, located on the X chromosome (Xp22), contains 18 exons, several of which bear considerable similarity to the first 17 exons of human *ACE*. Its transcript is 3.4 kb, generating an 805–amino acid peptide that is most highly expressed in kidney, heart, and testis but is also present in plasma and urine.[87,88] In contrast to ACE, ACE2 functions as a carboxypeptidase, removing the terminal phenylalanine from angiotensin II to yield the vasodilatory heptapeptide, angiotensin 1-7 [Ang (1-7)]. ACE2 may also indirectly lead to the formation of Ang (1-7) by cleaving the C-terminal leucine from angiotensin I, thereby generating angiotensin 1-9 [Ang (1-9)] which may then give rise to Ang (1-7) under the influence of ACE or neutral endopeptidase (NEP).[88] Thus ACE2 contributes to both angiotensin II degradation and Ang (1-7) synthesis. Accordingly, ACE and ACE2 were initially viewed as having opposing actions with regard to vascular tone and tissue injury. However, emerging data suggest that the situation is far from clear. For instance, while lentivirus-induced overexpression of ACE2 in the heart exerted a protective influence following experimental myocardial infarction,[89] ACE2 overexpression led to cardiac dysfunction and fibrosis, despite lowering systemic blood pressure.[90]

In the kidney, ACE2 colocalizes with ACE and angiotensin receptors in the proximal tubule while in the glomerulus it is predominantly expressed within podocytes and to a lesser extent in mesangial cells, contrasting the endothelial predilection of ACE at that site.[91] Numerous studies have explored changes in ACE2 expression in human kidney disease as well as in a range of animal models, reporting both increased and decreased levels.[92] As such, it is uncertain whether increased ACE2 might be detrimental or a beneficial response to injury. With this in mind, the findings of intervention studies are of particular importance.

In experimental diabetic nephropathy, for instance, pharmacological ACE2 inhibition with MLN-4760 led to

worsening albuminuria and glomerular injury[91]; similar findings were reported in ACE2 knockout mice that were crossed with the Akita model of type 1 diabetes.[93] As might be expected from these findings, augmenting ACE2 activity by the infusion of human recombinant protein (hrACE2) was shown to attenuate diabetic kidney injury in the Akita mouse. In this study, hrACE2 not only improved kidney structure and function but also showed that the protective effects were likely due to reduction in angiotensin II and an increase in Ang (1-7) signaling.[94] In addition to its role in the RAS, ACE2 has been shown to be the receptor for the severe adult respiratory syndrome (SARS) coronavirus.[95] The relevance of some of these findings to the human setting will hopefully be clarified when results of the recently initiated clinical trials of a recombinant human ACE2 peptide (GSK2586881) become available.

ANGIOTENSIN PEPTIDES

Angiotensin III, or Angiotensin-(2-8)

Formed by the actions of aminopeptidase A, the heptapeptide angiotensin III (angiotensin 2-8), like angiotensin II (angiotensin 1-8), exerts its effects by binding to the AT_1R and AT_2R.[96] Initially, angiotensin III was thought to have a predominant role in regulating vasopressin release.[97] However, more recent studies indicate that while angiotensin III is equipotent to angiotensin II with regard to its effects on blood pressure, aldosterone secretion, and renal function, its metabolic clearance rate is approximately five times as rapid.[98]

Angiotensin IV, or Angiotensin-(3–8)

Angiotensin IV is generated from angiotensin III by the actions of aminopeptidase M. Although some of its actions are mediated by the AT_1R, the majority of angiotensin IV's biological effects are thought to result from its binding to insulin-regulated aminopeptidase.[99] Previously viewed as inactive, there has been considerable recent interest in angiotensin IV with regard to its actions in the central nervous system (CNS), where it not only enhances learning and memory but also possesses anticonvulsant properties and protects the brain from ischemic injury.[99]

In addition to its CNS effects, angiotensin IV has also been implicated in atherogenesis, principally related to its ability to activate NF-κB and upregulate several proinflammatory factors that include monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1, interleukin-6 (IL-6), and TNF-α, as well as enhancing the synthesis of the prothrombotic factor plasminogen activator inhibitor-1.[100,101] In the kidney, angiotensin IV is reported to have variable effects on blood flow and natriuresis.[99]

Angiotensin-(1-7)

The ostensibly vasodilatory and antitrophic angiotensin 1-7 may be formed by the actions of several endopeptidases that include removal of the terminal tripeptide of angiotensin I by NEP, cleavage of the C-terminal phenylalanine of angiotensin II by ACE2, and the excision of the dipeptidyl group from the C terminal of angiotensin 1-9 by ACE. Evolving evidence indicates that the actions of this heptapeptide are mediated by its binding to the orphan GPCR *masR*.[102] Angiotensin 1-7 induces vasodilation by a number of mechanisms that include the amplification of bradykinin's effects, stimulating cGMP synthesis, and inhibiting the release of norepinephrine.[103] Additionally, angiotensin 1-7 inhibits vascular smooth muscle proliferation and prevents neo-intima formation following balloon injury of the carotid arteries.[104] Contrasting these findings, however, is a recent report that exogenous angiotensin-(1-7), rather than ameliorating diabetic nephropathy, as might have been predicted based on the prevailing paradigm, actually accelerated the progression of the disease.[93]

Angiotensin-(2-10)

In addition to angiotensin II and the other C-terminal cleavage products discussed earlier, angiotensin I (1-10) may also give rise to a number of other potentially biologically active peptides that result from removal of amino acids from its N terminus. Of these, angiotensin 2-10, produced by the actions of aminopeptidase A has been found to modulate the pressor activity of angiotensin II in rodents.[105]

Angiotensin-(1-12)

Angiotensin-(1-12) is a dodecapeptide, first isolated in rat intestine but also found to be present in the kidney and heart, that is cleaved from angiotensinogen by a heretofore unidentified nonrenin enzyme.[106] Notably, in the kidney, angiotensin-(1-12), akin to other components of the intrarenal RAS, is primarily localized to the proximal tubule epithelium.[107] Although its biological activity is incompletely understood, its main mode of action is thought to be mediated by its ability to serve as a precursor to angiotensin II by the site- and possibly species-specific actions of ACE and chymase.[108] Other pathways may, however, also contribute to the overall effects of angiotensin-(1-12), which in the rat kidney may also include the formation of angiotensin-(1-7) and angiotensin-(1-4) by neprilysin.[107]

Angiotensin A and Alamandine

Identified by mass spectroscopy, angiotensin A and alamandine are characterized by the decarboxylation of N-terminal aspartic acid to alanine in angiotensin II and angiotensin-(1-7), respectively.[109] While the extremely low concentrations of angiotensin A suggest that it is unlikely to play a physiological role, this may not be the case for alamandine, which circulates in human plasma and at increased concentrations in patients with end-stage renal disease (ESRD).[109] With its ability to lower blood pressure and reduce fibrosis, the actions of alamandine resemble those of angiotensin-(1-7). However, rather than exerting its effects via the *Mas* receptor, alamandine's actions occur through a related receptor, the *Mas*-related GPCR (*Mrg*D).[110]

INTRARENAL RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM

In the traditional view of the RAS, angiotensin II functions as a hormone that, in classical endocrine fashion, circulates systemically to act at sites distant from those where it was formed. However, since the cloning of its components, it has become increasingly clear that there is an additional local, tissue-based RAS that functions quasi-independently from its systemic counterpart, acting in paracrine, autocrine, and possibly even intracrine modes.[1] This is most clearly seen in the kidney where pioneering work of Navar and others[111–113] has shown that the kidney possesses all the necessary molecular machinery to synthesize angiotensin II and other bioactive

angiotensin peptides. Moreover, their concentrations in glomerular filtrate, tubule fluid, and interstitium are between 10- and 1000-fold higher than in plasma.[38,114]

Within the kidney, renin-expressing cells have traditionally been considered to be terminally differentiated and confined to the JGA. However, in a series of elegant studies using a fate-mapping cre-loxP system, Sequeira López and coworkers showed that renin-expressing cells are precursors to a range of other cell types in the kidney, including those of the arteriolar media, mesangium, Bowman's capsule, and proximal tubule.[115] While normally quiescent, these cells may undergo metaplastic transformation to synthesize renin when homeostasis is challenged.[115] Such threats include not only those related to volume depletion but also tissue injury. For instance, in the setting of single nephron hyperfiltration and consequent progressive dysfunction that follows renal mass reduction, Gilbert and colleagues noted the de novo expression of renin mRNA and angiotensin II peptide in tubule epithelial cells.[116]

In addition to resident kidney cells, infiltrating mast cells may also contribute to activation of the local RAS in disease. Traditionally associated with allergic reactions and host responses to parasite infestation, mast cells have been increasingly recognized for their role in inflammation, immunomodulation, and chronic disease. In the kidney, interstitial mast cell infiltration accompanies most forms of CKD where their abundance correlates with the extent of tubulointerstitial fibrosis and declining GFR, though not proteinuria.[117] Notably, mast cells have been shown to synthesize renin,[118] such that their degranulation will release large quantities of both renin and chymase, accelerating angiotensin II formation in the local environment.

Intracrine Renin–Angiotensin–Aldosterone System

Peptide hormones traditionally bind to their cognate receptors on the plasma membrane and produce their effects through the generation of secondary intermediates. However, emerging evidence suggests that certain peptides may also act directly within the cell's interior, having arrived there by either internalization or intracellular synthesis. For instance, angiotensin II has not only been localized within the cytoplasm and nucleus but its introduction into the cytoplasm was shown more than a decade ago to have major effects on intracellular calcium currents.[119] Uptake of angiotensin II from the extracellular space likely contributes to its intracellular activity; however, recent studies have focused predominantly on its endogenous synthesis. Consistent with the potential role for intracellular angiotensin II, transgenic mice that express an enhanced cyan fluorescent protein–angiotensin II fusion protein that lacks a secretory signal so that it is retained intracellularly develop hypertension with microthrombi in glomerular capillaries and small vessels.[120] To date, numerous canonical and noncanonical pathways in the cytoplasm, nucleus, and mitochondria have been implicated in the intracrine RAS,[121,122] providing a new forefront for the role of the RAS in physiology, pathophysiology, and therapeutics.

RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM IN KIDNEY PATHOPHYSIOLOGY

In a critical series of experiments in the 1980s, Brenner, Hostetter, and colleagues studied the hemodynamic effects of renal mass ablation in 5/6 nephrectomized rats, a now well-established model of progressive kidney disease.[123] In the setting of nephron loss, those glomeruli that remain undergo compensatory enlargement with increased single nephron GFR (SNGFR) and elevations in intraglomerular pressure (P_{GC}), argued to initiate glomerulosclerosis and loss of function. That this phenomenon might be related to angiotensin II was suggested by previous work in which angiotensin II infusion was demonstrated to also result in elevated P_{GC}.[124] Together, these studies suggested that intraglomerular hypertension, as a consequence of angiotensin II's action, was a pivotal factor underlying the inexorable progression of kidney disease and that strategies to reduce P_{GC} would lead to its amelioration. Indeed, in proof-of-concept studies, blockade of angiotensin II formation with the ACE inhibitor, enalapril, was shown to dilate the glomerular efferent arteriole, reduce P_{GC}, and disease progression in 5/6 nephrectomized rats.[125] By contrast, combination therapy with hydralazine, reserpine, and hydrochlorothiazide, though equally effective in lowering systemic blood pressure, failed to ameliorate intraglomerular hypertension and disease progression.[125] These studies were soon followed by similar ones in other disease models, particularly diabetes, which like the 5/6 nephrectomized rat is also characterized by increased SNGFR and elevated P_{GC}.[126]

FIBROSIS

During the past 20 years considerable research has focused on many of the nonhemodynamic effects of angiotensin II. For instance, in addition to their effects on P_{GC}, ACE inhibitors and ARBs are also highly effective in reducing interstitial fibrosis and tubule atrophy, each close correlates of progressive kidney dysfunction. Underlying these effects is the ability of angiotensin II to potently induce expression of the profibrotic and proapoptotic growth factor and TGF-β in a range of kidney cell types.[127,128] Consistent with these in vitro studies, TGF-β overexpression is seen in both the glomerular and tubulointerstitial compartments in 5/6 nephrectomized rats and diabetic rats, where studies also showed that both ACE inhibitors and ARBs were effective at reducing TGF-β and disease progression.[129,130] Similarly, in human diabetic nephropathy, the ACE inhibitor perindopril was found to reduce TGF-β mRNA in a sequential renal biopsy study[131] and losartan was shown to lower urinary TGF-β excretion.[132]

PROTEINURIA

The development of proteinuria is both a cardinal manifestation of glomerular injury and a pathogenetic factor in the progression of renal dysfunction. While P_{GC} remains an important factor in determining the transglomerular passage of albumin, more recent work has focused on the potential contribution of the podocyte. Indeed, podocyte injury is a cardinal manifestation of proteinuric renal disease where foot process effacement has been shown to be prevented by both ACE inhibition and angiotensin receptor blockade.[133] In consideration of its crucial role in the development and function of the glomerular filtration barrier, other studies have focused on the podocyte slit pore membrane protein nephrin. Of note, podocytes express the AT_1R and respond to the addition of angiotensin II to the cell culture medium by dramatically decreasing their expression of nephrin.[134] Consistent with these findings, the reduction in nephrin

expression in patients with diabetic nephropathy was shown to be ameliorated by ACE inhibitor treatment for 2 years.[135]

INFLAMMATION, IMMUNITY, AND THE RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM

Inflammatory cell infiltration is a long-recognized feature of CKD that is attenuated in rodent models by agents that block the RAS.[136] In the in vitro setting, angiotensin II activates NF-κB by both AT_1R- and AT_2R-dependent pathways, stimulating the expression of a number of potent chemokines such as MCP-1 and regulated on activation, normal T cell expressed and secreted (RANTES) as well as cytokines, like IL-6.[137] In addition to angiotensin II, angiotensin-(1-7), acting via the Mas receptor, activates NF-κB, inducing proinflammatory effects in the kidney under both basal and disease settings.[138]

In addition to macrophages, mast cells, and other components of the innate immune system, the adaptive immune system also appears to be involved in the pathogenesis of angiotensin II–mediated organ injury. Of note, suppression of the adaptive immune system prevents the development of angiotensin II–dependent hypertension in experimental models[139] and adoptive transfer of $CD4^+CD25^+$ regulatory T cells is able to ameliorate angiotensin II–dependent injury.[140]

DIABETES PARADOX

Despite the fact that patients with long-standing diabetes characteristically have low plasma renin,[141–143] suggesting that the RAS is not activated by the disease, agents that block the RAS are the mainstay of therapy in diabetic nephropathy. Compounding this apparent paradox, although PRA is normal or low in diabetes, plasma prorenin is characteristically elevated. This dichotomy suggests differences in cell-specific responses to diabetes since the JGA is the primary source of renin secretion while prorenin is secreted by a much wider range of cell types. In a recent commentary, Peti-Peterdi et al. have ventured to explain the (pro)renin paradox of diabetes.[144] Although early diabetes would lead to augmented succinate and enhanced JGA renin release, elevated angiotensin II levels would thereafter suppress JGA renin secretion. Contrasting this negative feedback at the JGA, angiotensin II has been shown to have the opposite effect in the tubule, with diabetes causing a 3.5-fold increase in collecting duct renin that could be reduced by AT_1R blockade.[145]

ENDOTHELIN

Clinical Relevance

The actions of the endothelins (ETs) are mediated by two receptors: ET types A (ET-A) and B (ET-B). ET receptor antagonists (particularly ET-A receptor antagonists) are being investigated for their efficacy in slowing renal decline in chronic kidney diseases, especially diabetic kidney disease. However, whereas ET receptor antagonists lower proteinuria, their development has been hampered by fluid retention and a narrow therapeutic window.

ETs are potent vasoconstrictors that, although expressed primarily in the vascular endothelium, are also notably present within the renal medulla. The biologic effects of the ET system are mediated by two receptors: ET types A (ET-A) and B (ET-B). In the kidneys, these receptors contribute to the regulation of RBF, salt and water balance, and acid–base homeostasis, as well as potentially mediating tissue inflammation and fibrosis. An important therapeutic role has emerged for ET receptor antagonism in the treatment of pulmonary hypertension;[146–149] ET receptor antagonists have been granted regulatory authority approval for this indication in the United States and in Europe.[150] ET receptor blockade as a therapeutic strategy has been investigated in a range of renal diseases. Recent clinical trials have demonstrated the antiproteinuric and antihypertensive properties of ET receptor antagonists, which have a relatively narrow therapeutic window for the treatment of CKD.

STRUCTURE, SYNTHESIS, AND SECRETION OF THE ENDOTHELINS

ETs consist of three 21–amino acid isoforms that are structurally and pharmacologically distinct: ET-1, ET-2, and ET-3. The dominant isoform in the cardiovascular system is ET-1. Differences in the amino acid sequence among the isopeptides are minor. All three isoforms share a common structure with a typical hairpin-loop configuration that results from two disulfide bonds at the amino terminus and a hydrophobic carboxy terminus that contains an aromatic indol side chain at Trp_{21} (Fig. 11.6). Both the carboxy terminus and the two disulfide bonds are responsible for the biologic activity of the peptide. ETs are synthesized from preprohormones by posttranslational proteolytic cleavage mediated by furin and other enzymes. Dibasic pair–specific processing endopeptidases, which recognize Arg-Arg or Lys-Arg paired amino acids, cleave preproETs, reducing their size from approximately 203 to 39 amino acids. Subsequent proteolytic cleavage of the largely biologically inactive big ETs is mediated by endothelin-converting enzymes (ECEs), the key enzymes in the ET biosynthetic pathway. ECE1 and ECE2 are type II membrane-bound metalloproteases whose amino acid sequence is significantly homologous to that of neprilysin (NEP 24.11).

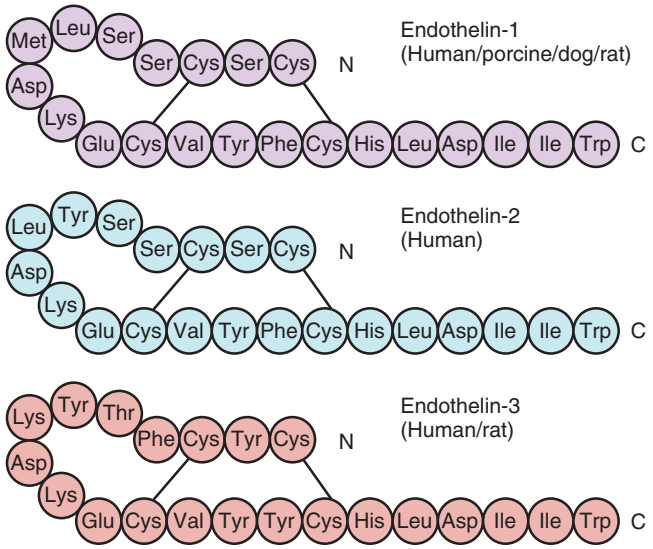

Fig. 11.6 Molecular structure of the three endothelin isoforms. (Modified from Schiffrin EL. Vascular endothelin in hypertension. *Vascul Pharmacol.* 2005;43:19–29.)

Table 11.1 Endothelin Gene and Protein Expression

Stimulation	
Vasoactive Peptides	**Growth Factors**
Angiotensin II	Epidermal growth factor
Bradykinin	Insulin-like growth factor
Vasopressin	Transforming growth factor-β
Endothelin-1	**Coagulation**
Epinephrine	Thromboxane A_2
Insulin	Tissue plasminogen activator
Glucocorticoids	**Other**
Prolactin	Calcium
Inflammatory Mediators	Hypoxia
Endotoxin	Shear stress
Interleukin-1	Phorbol esters
Tumor necrosis factor-α	Oxidized low-density lipoproteins
Interferon-β	
Inhibition	
Atrial natriuretic peptide	Prostacyclin
Brain natriuretic peptide	Protein kinase A activators
Bradykinin	Nitric oxide
Heparin	Angiotensin-converting enzyme inhibitors

Secretion of ET-1 is dependent on de novo protein synthesis, which is constitutive. However, a range of stimuli may also increase ET synthesis through both transcriptional and posttranscriptional regulation (Table 11.1). Once it is synthesized, ET-1 is secreted by endothelial cells into the basolateral compartment, toward the adjacent smooth muscle cells. Because of its abluminal secretion, plasma levels of ET-1 do not necessarily reflect its production.[151]

Within the kidneys, ET-1 expression is most abundant in the inner medulla. In fact, this region possesses the highest concentration of ET-1 of any tissue bed.[152] In addition to their presence in the inner medullary collecting ducts (IMCDs), ETs have also been described in glomerular endothelial cells,[153] glomerular epithelial cells,[154] mesangial cells,[155] vasa recta,[156] and tubule epithelial cells.[157] The kidneys also synthesize ET-2 and ET-3, although at much lower levels than they do ET-1.[158] As with ET-1, ECE1 mRNA is also more abundant in the renal medulla than in the cortex under normal conditions. However, in disease states such as chronic heart failure, ECE1 mRNA is upregulated primarily within the cortex.[159] In human kidneys, ECE1 has been localized to endothelial cells and tubule epithelial cells in the cortex and medulla.[160]

ENDOTHELIN RECEPTORS

ETs bind to two seven-transmembrane domain GPCRs, ET-A and ET-B. Within the vasculature, ET-A receptors are found on smooth muscle cells, where they mediate vasoconstriction. Although ET-B receptors localized on vascular smooth muscle cells can also mediate vasoconstriction, they are also expressed on endothelial cells, where their activation results in vasodilation through the production of NO and prostacyclin.[161] In addition to their role in mediating vascular tone, ET-B receptors also act as clearance receptors for ET-1,[162] particularly in the lung, where ET-B receptor binding accounts for approximately 80% of clearance.[163] Because of its natriuretic and vasodilatory actions, the ET-B receptor is generally considered to confer predominantly renoprotective effects.

In the kidneys, expression of both ET-A and ET-B receptors is most prominent within the IMCDs, although binding of ET-1 also occurs in smooth muscle cells, endothelial cells, renomedullary interstitial cells, thin descending limbs, and medullary thick ascending limbs.[156] ET-A receptors are localized to several renovascular structures, including vascular smooth muscle cells, arcuate arteries, and pericytes of descending vasa recta, as well as glomeruli. ET-B receptors, although prominently represented within the medullary collecting system, have also been demonstrated in proximal convoluted tubules, collecting ducts of the inner cortex, medullary thick ascending limbs, and podocytes.[164]

PHYSIOLOGIC ACTIONS OF ENDOTHELIN IN THE KIDNEY

The ETs have several effects on normal renal function, including regulation of RBF, sodium and water balance, and acid–base homeostasis. Although ET-1 has hemodynamic effects in almost all vessels, the sensitivity of different vascular beds varies. The renal vasculature, along with the mesenteric vessels, is the most sensitive: vasoconstriction occurs at picomolar concentrations of ET-1,[165,166] increasing RVR and decreasing RBF. However, long-lasting vasoconstriction that is mediated by the ET-A receptor may be preceded by a transient ET-B receptor–mediated vasodilation.[167] Because of the site-specific distribution of ET receptors, ET-1 may exert different vasoconstrictive and vasodilatory effects in different regions of the kidneys. For example, by inducing NO release from adjacent tubule epithelial cells, ET-1 may actually increase blood flow in the renal medulla, where ET-B receptors predominate.[168]

In addition to effects on RBF, the ET system also plays a direct role in renal sodium and water handling.[169] In the renal medulla, ET is regulated by sodium intake and exerts its natriuretic and diuretic effects through the ET-B receptor.[170–172] In addition to natriuretic and diuretic effects, the ET-B receptor may also contribute to acid–base homeostasis by stimulating proximal tubule sodium/proton exchanger isoform 3 (NHE3).[173] Although the role of ET-B receptor activation in urinary sodium excretion has been appreciated for some time, more recent evidence suggests that renal medullary ET-A receptors may also mediate natriuresis.[174] This may partly explain the edema that can occur as a side effect of ET-A or dual ET receptor antagonism.

ROLE OF ENDOTHELIN IN ESSENTIAL HYPERTENSION

In view of its potent vasoconstrictive properties, it is not surprising that ET-1 has been implicated in the pathogenesis of hypertension. In preclinical models of hypertension, ET antagonism may ameliorate heart failure, vascular injury, and renal failure, as well as reduce the incidence of stroke.[175,176] ET-A receptor antagonism has also been shown to normalize blood pressure in rats exposed to eucapnic intermittent hypoxia, which is analogous to sleep apnea in humans.[177]

PreproET-1 mRNA is increased in the endothelium of subcutaneous resistance arteries in patients with moderate to severe hypertension[178] and according to a recent meta-analysis, plasma ET-1 concentrations are increased in individuals with hypertension.[179] However, plasma ET-1 levels are not universally elevated[175]; an increase is found more commonly in the presence of end-organ damage or in salt-depleted, salt-sensitive patients with a blunted renin response.[180] A major component of this increase in disease is often decreased clearance by the kidneys. These findings suggest that certain patient subgroups may be more responsive than others to ET receptor blockade. Females appear to be relatively protected from the pressor effects of ET-1 by virtue of both increased ET-B expression and a blunted hemodynamic response to ET-A receptor activation.[181]

Clinical trials of the antihypertensive effects of ET receptor antagonism have been hampered by difficulties with selectivity for the ET-A receptor, study design, dosing regimens, and adverse events.[182] Because the ET-B receptor exerts diuretic and natriuretic effects, induces vasodilation, and clears ET-1, selective ET-A receptor antagonists may be expected to demonstrate a more favorable antihypertensive profile.[182] Mixed ET-A/B and specific ET-A receptor antagonists are distinguished by their in vitro binding affinities with mixed ET-A/B receptor antagonists demonstrating a selectivity for ET-A of <100-fold, and ET-A selective antagonists having an affinity for the ET-A receptor of 100-fold or higher. However, it has been suggested that a 1000-fold or higher affinity may be required in order to induce ET-A receptor-specific effects in vivo.[183,184] In an early study, treatment of patients with essential hypertension with the nonselective ET receptor antagonist bosentan decreased blood pressure as effectively as enalapril, without reflex neurohumoral activation, over a 4-week period.[185] Similarly, in 115 patients with resistant hypertension who were taking three or more agents, the selective ET-A receptor antagonist darusentan significantly reduced blood pressure at 10 weeks.[186] In a subsequent study of 379 individuals with resistant hypertension, darusentan treatment for 14 weeks reduced blood pressure by approximately 18/10 mm Hg with no evidence of dose dependence across a range of 50 to 100 mg/day.[187] However, in a second study of similar design a large placebo effect meant that darusentan treatment failed to achieve its primary endpoint of change in office blood pressure and the development of the drug for this indication was halted.[188] Interestingly, in both of these studies ambulatory blood pressure monitoring revealed a reduction in systolic blood pressure with active treatment.[183,189] However, also in both studies, peripheral edema or fluid retention was more common in patients treated with darusentan than those receiving placebo.[187,188]

ROLE OF ENDOTHELIN IN RENAL INJURY

Beyond its effects on the regulation of vascular tone, the ET system also likely plays a direct role in the pathogenesis of fibrotic injury in CKD.[190] In patients with CKD, plasma ET-1 concentrations are elevated, as a result of both increased production and decreased renal clearance.[191] Urinary levels of ET-1 are also increased, which is indicative of increased renal ET-1 expression.[192] One mechanism for increased renal ET-1 in CKD is a direct effect of urinary protein on ET-1 expression in tubule epithelial cells.[193,194] Beyond direct effects of urine protein, a number of proinflammatory factors induce ET-1 expression in the kidneys, including hypoxia, angiotensin II, thrombin, thromboxane A_2, TGF-β, and shear stress (Table 11.1).

Several distinct mechanisms may account for the injurious effects of ET-1 on the kidneys. Locally derived ET-1 has direct hemodynamic effects, increasing P_{GC} at high doses and causing vasoconstriction of the vasa recta and peritubular capillaries, with a resultant reduction in tissue oxygen tension. ET-1 acts as a chemoattractant for inflammatory cells, which may express the peptide themselves, stimulating interstitial fibroblast and mesangial cell proliferation and mediating the production of a number of factors associated with collagenous matrix deposition, including TGF-β, matrix metalloproteinase-1, and tissue inhibitors of metalloproteinases-1 and -2. In mesangial cells, ET-1 can induce cytoskeletal remodeling and cell contraction[195] and, in these cells, ET-A receptor activation appears to be important to the development of Alport glomerular disease.[196]

A particularly important role of ET-1 in mediating injury of glomerular podocytes is also beginning to emerge. For instance, increased passage of protein across the filtration barrier causes podocyte cytoskeletal rearrangements and coincident upregulation of ET-1, which may act in an autocrine manner to further propagate ultrastructural injury in the same cells.[197–199] ET-1 promotes podocyte dedifferentiation and migration by activating ET-A and increasing β-arrestin-1 expression which ultimately results in EGFR transactivation, phosphorylation of β-catenin, and increased expression of the transcription factor Snail, an inducer of epithelial–mesenchymal transition.[200] ET-1 induces calcium signaling in podocytes through both the ET-A and ET-B receptors, and mice with deletion of both the ET-A receptor and ET-B receptor from podocytes are protected from the glomerular injury associated with streptozotocin-induced diabetes.[201] Using these mice, investigators have subsequently gone on to demonstrate that ET-1 causes podocytes to release heparanase that damages the endothelial glycocalyx facilitating albumin passage across the filtration barrier.[202]

THE ENDOTHELIN SYSTEM IN CHRONIC KIDNEY DISEASE AND DIABETIC NEPHROPATHY

PRECLINICAL STUDIES OF ET RECEPTOR ANTAGONISTS IN DIABETIC KIDNEY DISEASE

ET receptor antagonists have been employed to study the role of ETs in renal pathophysiology in a range of experimental models, including the rat remnant kidney, lupus nephritis, and diabetes. In the remnant kidney model of progressive renal disease, although beneficial effects have been reported with nonselective ET receptor antagonists,[203] selective ET-A receptor inhibition appears to yield superior outcomes with concomitant inhibition of ET-B receptors, potentially abrogating any beneficial effects.[204]

Data with regard to an effect of high glucose concentrations on ET synthesis and secretion are conflicting. Mesangial cell p38 MAPK activation in response to ET-1, angiotensin II, and PDGF is enhanced in the presence of high glucose levels.[205] By contrast, mesangial contraction in response to ET-1 is diminished under high-glucose conditions.[206,207] Circulating ET-1 concentrations are elevated in animal models of both type 1 and type 2 diabetes. Increased expression of

ET-1 and its receptors has been found in glomeruli and in tubule epithelial cells,[194,208] although increased expression of ET receptors has not been a universal finding.[209] Diabetes also causes an increase in renal ECE1 expression, the effect being synergistic with that of radiocontrast media.[210]

A number of researchers have investigated the effect of both nonselective and selective ET-A receptor antagonists in experimental diabetic nephropathy. In streptozotocin-diabetic rats, the nonselective ET receptor antagonist bosentan has yielded conflicting results,[211,212] whereas another nonselective ET receptor antagonist, PD142893, improved renal function when administered to streptozotocin-diabetic rats that were already proteinuric.[194] More recently, acute ET-A receptor antagonism was shown to improve oxygen availability in the kidneys of streptozotocin-diabetic rats.[213] In Otsuka Long Evans Tokushima Fatty (OLETF) rats with type 2 diabetes, selective ET-A receptor blockade attenuated albuminuria, without affecting blood pressure, whereas ET-B receptor blockade had no effect.[214] In a study of streptozotocin-diabetic apolipoprotein E knockout mice, the renoprotective effects of the predominant ET-A receptor antagonist avosentan were comparable or superior to the ACE inhibitor quinapril.[215] Supporting a protective role for the ET-B receptor, diabetic ET-B receptor–deficient rats developed severe hypertension and progressive renal failure.[216]

Accumulation of reactive oxygen species plays a major role in the pathogenesis of diabetic complications, particularly diabetic nephropathy,[217,218] and several observations suggest that the ET system may contribute to oxidative stress. In low-renin hypertension, ET-1 increases superoxide in carotid arteries,[219] and ET-A receptor blockade decreases vascular superoxide generation.[220,221] Similarly, ET-1 infusion increased urinary excretion of 8-isoprostane prostaglandin $F_2\alpha$ in rats, which is indicative of increased generation of reactive oxygen species.[222] By contrast, however, other preclinical studies have suggested a predominantly proinflammatory role for ET-1 in diabetic nephropathy. For instance, the selective ET-A receptor antagonist ABT-627 prevented the development of albuminuria in streptozotocin-diabetic rats without an improvement in markers of oxidative stress but with a reduction in macrophage infiltration and urinary excretion of TGF-β and prostaglandin E_2 metabolites.[223]

CLINICAL STUDIES OF ET RECEPTOR ANTAGONISTS IN CKD AND DIABETIC NEPHROPATHY

Both plasma and urinary ET-1 levels are increased in patients with CKD[191,224,225] with plasma ET-1 levels inversely correlating with estimated GFR. In a study of hypertensive patients with CKD, both selective ET-A receptor blockade and nonselective ET receptor blockade lowered blood pressure.[226] However, ET-A receptor blockade increased both RBF and effective filtration fraction and decreased RVR, whereas dual blockade had no effect.[226]

In a study of 22 nondiabetic individuals with CKD, intravenous infusion of the ET-A receptor antagonist BQ-123 reduced pulse wave velocity and proteinuria to a greater extent than the calcium channel antagonist nifedipine which comparably lowered blood pressure.[227] These findings suggest a potentially blood pressure–independent mechanism of action for the antiproteinuric effect observed. In a subsequent study by the same investigators, 27 subjects with proteinuric CKD were treated with the ET-A receptor antagonist sitaxsentan for 6 weeks in a three-way crossover study design. Sitaxsentan treatment was associated with a reduction in blood pressure, proteinuria, and pulse-wave velocity, whereas nifedipine reduced pulse-wave velocity and blood pressure but had no effect on urine protein excretion.[228] Subsequently, sitaxsentan was also shown to restore the nocturnal dip in blood pressure in people with CKD.[229] A fall in GFR with sitaxsentan therapy observed in this study is analogous to that seen with RAS blockade.[228] Although no clinically significant adverse effects were seen, sitaxsentan development has subsequently been halted due to hepatotoxicity.

The effect of the ET-A receptor antagonist avosentan was examined, in addition to standard treatment with an ACE inhibitor or ARB, in a placebo-controlled trial of 286 patients with diabetic nephropathy and macroalbuminuria.[230] At 12 weeks, avosentan was found to decrease urine albumin excretion rate without affecting blood pressure. These results led to the initiation of the ASCEND trial (a randomised, double blind, placebo controlled, parallel group study to assess the effect of the endothelin receptor antagonist avosentan on time to doubling of serum creatinine, end-stage renal disease or death in patients with type 2 diabetes mellitus and diabetic nephropathy).[231] ASCEND set out to examine the effects of avosentan, on top of RAS blockade, in 1392 individuals with type 2 diabetes and nephropathy but was terminated after a median duration of 4 months due to adverse events.[231] In that study, despite a more than 40% reduction in urine albumin-to-creatinine ratio with avosentan, adverse events, predominantly fluid overload and congestive heart failure, occurred more frequently in those receiving active therapy than placebo.[231]

Since the publication of ASCEND, it has become increasingly apparent that the outcome of that study was hampered by the relatively high dose of avosentan that was selected and that ET-A receptor antagonists likely have a relatively narrow therapeutic window. However, if this therapeutic window is appropriately targeted and patients carefully selected, then ET-A receptor antagonists may still find a clinical niche for the treatment of diabetic nephropathy. The Reducing Residual Albuminuria in Subjects with Diabetes and Nephropathy with Atrasentan trial and an identical study conducted in Japan (RADAR/JAPAN) explored the effects of two different doses of the ET-A receptor antagonist atrasentan (0.75 and 1.25 mg/day, respectively) in 211 patients with type 2 diabetes and albuminuria (estimated GFR [eGFR] 30–75 mL/min/1.73 m^2).[232] In comparison with placebo, 0.75 and 1.25 mg/day atrasentan, on top of maximum tolerated doses of ACE inhibitor or ARB, reduced urine albumin-to-creatinine ratio by an average of 35% and 38%, respectively, without major side effects.[232] Atrasentan treatment was also associated with decreases in 24-hour blood pressure, low-density lipoprotein cholesterol, and triglycerides.[232] Importantly, despite a comparable lowering of albuminuria to the 0.75 mg/day dose of atrasentan, the 1.25 mg/day atrasentan dose was accompanied by more fluid retention.[232] In a post hoc analysis, fluid retention was more likely in participants with lower eGFR and a higher dose of atrasentan, whereas the degree of urinary albumin lowering was not linked to the degree of fluid retention.[233] Thus it is likely that the antiproteinuric and fluid retaining effects of ET receptor antagonists are mediated by different mechanisms; plausibly the former by vascular or glomerular actions, and the latter by direct effects of ET receptor blockade on sodium transport in the renal tubule.[233] Regardless, based on the promising results of RADAR/JAPAN, the 0.75 mg/day dose of atrasentan

is currently being investigated in the phase 3 Study of Diabetic Nephropathy with Atrasentan (SONAR) trial, which has a primary outcome of hard renal endpoints (time to doubling of serum creatinine or ESRD, including renal death; NCT01858532). This study aims to recruit over 4000 participants and is estimated to complete at the end of 2018.

COMBINED ECE AND NEPRILYSIN INHIBITION

Distinct from ET receptor antagonism, blockade of ET-1-induced signaling has been explored in the clinical setting with the use of the combined ECE and neprilysin inhibitor daglutril.[234] In an 8-week, crossover design study of participants with type 2 diabetes, blood pressure <140/90 mm Hg, and urinary albumin excretion 20–999 μg/min, daglutril (300 mg/day) did not significantly reduce albuminuria compared with placebo, although blood pressure was reduced.[234] The failure to reach the primary endpoint of albuminuria reduction may relate to concurrent neprilysin inhibition, which may diminish ET-1 degradation.[235] Alternatively, it may reflect an overall diminution of ET-1 with consequent decreased activation of ET-B as well as ET-A.[236]

THE ENDOTHELIN SYSTEM AND OTHER KIDNEY DISEASES

In addition to diabetic and nondiabetic CKD, the role of the ET system has also been investigated in a number of other kidney diseases. Overall, these studies have suggested some degree of renoprotection with either selective ET-A or nonselective ET receptor inhibitors.

SICKLE CELL DISEASE-ASSOCIATED NEPHROPATHY

Administration of the selective ET-A receptor antagonist ambrisentan preserved GFR and prevented the development of albuminuria in a humanized mouse model of sickle cell disease (SCD).[237] However, the renoprotective effects of ambrisentan were only partially recapitulated by treatment with the combined ET-A/ET-B receptor antagonist A-182086, highlighting the importance of selectively targeting the ET-A receptor in SCD.[237]

RENOVASCULAR DISEASE

A series of recent studies in pigs provide support for ET-A receptor blockade in the treatment of renovascular disease. In an initial study, investigators treated pigs with unilateral renal artery stenosis with an ET-A receptor antagonist beginning at the onset of renovascular disease and continuing for 6 weeks.[238] In these experiments, researchers observed that ET-A receptor blockade preserved renal hemodynamics, renal function, and microvascular architecture in the stenotic kidney.[238] Similarly, ET-A receptor blockade (but not ET-B receptor blockade) reversed microvascular rarefaction and diminished renal inflammation and fibrosis when it was initiated 6 weeks after the induction of renal artery stenosis.[239] Finally, ET-A receptor blockade also led to an improvement in microvascular density and renal function recovery compared with placebo when it was administered following percutaneous transluminal renal angioplasty/stenting.[240]

ACUTE KIDNEY INJURY

ET-1 may play a role in sepsis-mediated acute renal failure,[241] although experimental findings have been conflicting, dependent to some extent on the ET receptor antagonist employed. For example, in a rat model of early normotensive endotoxemia, neither an ET-A receptor antagonist nor a combined ET-A/ET-B receptor blockade improved GFR,[242] whereas ET-B receptor blockade alone resulted in a marked reduction in RBF.[242] By contrast, in a porcine model of endotoxemic shock, the dual ET receptor antagonist tezosentan attenuated the decrease in RBF and increase in plasma creatinine.[243] Pointing to a role of ET-1/ET-A signaling in the progression from acute kidney injury to CKD, transient unilateral renal ischemia induced upregulation of ET-1 and ET-A receptor in mice and ET-A receptor antagonism (but not ET-B receptor antagonism) prevented progressive kidney injury.[244]

SYSTEMIC LUPUS ERYTHEMATOSUS

Urinary ET-1 excretion is correlated with disease activity in patients with systemic lupus erythematosus (SLE),[245] and serum from such patients has been shown to stimulate ET-1 release from endothelial cells in culture.[246] In accordance with a pathogenetic role for the ET system in SLE, the ET-A receptor antagonist FR139317 attenuated renal injury in a murine model of lupus nephritis.[247]

PRIMARY FOCAL AND SEGMENTAL GLOMERULOSCLEROSIS

Sparsentan is a dual ET-A receptor/ARB antagonist being developed for the treatment of primary focal and segmental glomerulosclerosis (FSGS) by Retrophin Inc. In late 2016, the company reported results from the phase 2 DUET study that examined the effect of three different doses of sparsentan (200, 400, and 800 mg/day) when compared with the ARB irbesartan (300 mg/day) in 96 participants over an 8-week period. The mean reduction in proteinuria in sparsentan-treated patients was 45% in comparison to a 19% reduction in those receiving irbesartan.[248]

SCLERODERMA

The Zibotentan Better Renal Scleroderma Outcome Study (ZEBRA) is a 3-part phase 2 study (ZEBRA 1, ZEBRA 2A, and ZEBRA 2B) exploring the safety and therapeutic potential of the ET-A receptor antagonist zibotentan in acute and chronic renal complications of scleroderma (NCT02047708). The primary outcome measure is the plasma level of soluble vascular cell adhesion molecule-1 as a biomarker of scleroderma renal involvement.

HEPATORENAL SYNDROME

Plasma ET-1 concentrations are increased in individuals with cirrhosis and ascites and in patients with type 2 hepatorenal syndrome (diuretic-resistant or refractory ascites with slowly progressive renal decline) in whom systemic vasodilation accompanies paradoxical renal vasoconstriction.[249] To investigate the therapeutic potential of ET receptor antagonism in this setting, the combined ET-A/ET-B receptor blocker tezosentan was administered to six patients in an early phase clinical trial.[250] In this study, treatment was discontinued early in five patients, in one case because of systemic hypotension and in four because of concerns about worsening renal function.[250] These adverse effects are consistent with a dose-dependent decline in renal function in patients with acute heart failure treated with tezosentan, and they highlight the need for caution with the use of ET receptor antagonists in certain patient populations.

PREECLAMPSIA

A role for ET-1 in the development of preeclampsia is suggested by the observations that infusion of fms-like tyrosine kinase-1 and TNF-α into pregnant rats induced ET-A-dependent hypertension,[251–253] whereas ET-A receptor antagonism attenuated placental ischemia-induced hypertension in a rat model.[254] Despite the mechanistic role of ET-1 in the pathogenesis of preeclampsia, however, ET receptor antagonists are very unlikely to be used in this condition given their known teratogenicity.[251]

SAFETY PROFILE OF ENDOTHELIN RECEPTOR ANTAGONISTS

The therapeutic development of ET receptor antagonists has been slowed by the adverse side effect profile of available agents, particularly the dose dependency of certain adverse effects. Most notable has been the development of fluid retention, peripheral edema, and congestive heart failure despite the use of predominant ET-A receptor antagonists. The mechanisms that underlie the fluid retention associated with ET-A receptor antagonism have not been fully resolved. It has been suggested that the use of comparatively high doses of ET-A receptor antagonists may have resulted in concurrent ET-B receptor blockade. However, inhibition of nephron ET-A receptors may also be implicated.[167,255] For instance, mice with nephron or collecting duct ET-A receptor deletion were protected from the fluid retention associated with ET-A receptor blockade.[256] Hepatotoxicity may be a class effect or may be restricted to particular subclasses of ET receptor antagonist. A rise in hepatic transaminases has been observed with both bosentan and sitaxsentan, which are both sulfonamide-based agents, but not with ambrisentan or darusentan, which are propionic acid based.[183,186,187,257,258] As discussed earlier, teratogenicity would preclude the use of this class of agents in pregnancy, whereas the potential for testicular toxicity has also been described, although testicular damage has not been reported in patients taking ET receptor antagonists for the treatment of pulmonary hypertension.[236]

NATRIURETIC PEPTIDES

Clinical Relevance

Neprilysin inhibitors prevent the enzymatic degradation of the natriuretic peptides. When used alone they do not produce sustained antihypertensive effects, likely a consequence of compensatory upregulation of the renin–angiotensin system. The combination of neprilysin inhibition and angiotensin-converting enzyme inhibition is associated with an increased risk of angioedema. The combination of angiotensin receptor blockade and an inhibitor of neprilysin has a more favorable side-effect profile and has demonstrated efficacy in the treatment of heart failure. The effect of combination angiotensin receptor blockade/neprilysin inhibition on hard renal outcomes is currently unknown.

The NPs are a family of vasoactive hormones that play a role in salt and water homeostasis. The family consists of at least five structurally related peptides: ANP, BNP, CNP, *Dendroaspis* natriuretic peptide (DNP), and urodilatin. ANP was originally isolated from human and rat atrial tissues in 1984.[259] Since then, the NP family has been found to include several other members, all of which share a common 17–amino acid ring structure that is stabilized by a cysteine bridge and that contains several invariant amino acids.[260] Both BNP[261] and CNP[262] were originally identified in porcine brain tissue, and DNP was first isolated from the venom of the green mamba snake *Dendroaspis angusticeps*.[263] Urodilatin is an NH2-terminally extended form of ANP that was initially described in human urine.[264] NP inactivation occurs through at least two distinct pathways: binding to a clearance receptor (natriuretic peptide receptor [NPR]-C) and enzymatic degradation. Other peptides that may be involved in salt and water balance include guanylin, uroguanylin, and adrenomedullin.

ANP and BNP act as endogenous antagonists of the RAS-mediating natriuresis, diuresis, vasodilation, and suppression of sympathetic activity, as well as inhibiting cell growth and decreasing secretion of aldosterone and renin.[265] The role of NPs in cardiovascular and renal disease, particularly BNP, has led to their adoption into clinical practice as indicators of disease states and, to some extent, as therapeutic agents.

STRUCTURE AND SYNTHESIS OF THE NATRIURETIC PEPTIDES

ATRIAL NATRIURETIC PEPTIDE

ANP is a 28–amino acid peptide comprising a 17–amino acid ring linked by a disulfide bond between two cysteine residues and a COOH-terminal extension that confers its biologic activity (Fig. 11.7). The gene for ANP, *NPPA,* is found on chromosome 1p36 and encodes the precursor preproANP, which is between 149 and 153 amino acids in length according to the species of origin. Human preproANP consists of 151 amino acids and is rapidly processed to the 126–amino acid proANP. ANP is identical in mammalian species except for a single amino acid substitution at residue 110, which is isoleucine in rat, rabbit, and mouse and methionine in human, pig, dog, sheep, and cow.

ANP synthesis occurs primarily within atrial cardiomyocytes, in which it is stored as proANP, the main constituent of the atrial secretory granules. The major stimulus to ANP release is mechanical stretch of the atria that is secondary to increased wall tension. In addition to atrial stretch, ANP synthesis and release may be stimulated by neurohumoral factors such as glucocorticoids, ET, vasopressin, and angiotensin II, partly through changes in atrial pressure and partly through direct cellular effects. Although ANP mRNA levels are approximately 30- to 50-fold higher in the cardiac atria than in the ventricles, ventricular expression is dramatically increased in the developing heart and in conditions of hemodynamic overload such as heart failure and hypertension. Beyond the heart, ANP has also been demonstrated in the kidneys, brain, lungs, adrenal glands, and liver. In the kidneys, alternate processing of proANP adds four amino acids to the NH_2 terminus of the ANP peptide to generate a 32–amino acid peptide: proANP 95-126, or urodilatin.

ANP is stored, primarily as proANP, in the secretory granules of the atrial cardiomyocytes and is released by fusion of the granules with the cell surface. During this process, proANP is cleaved to an NH_2-terminal 98–amino acid peptide (ANP

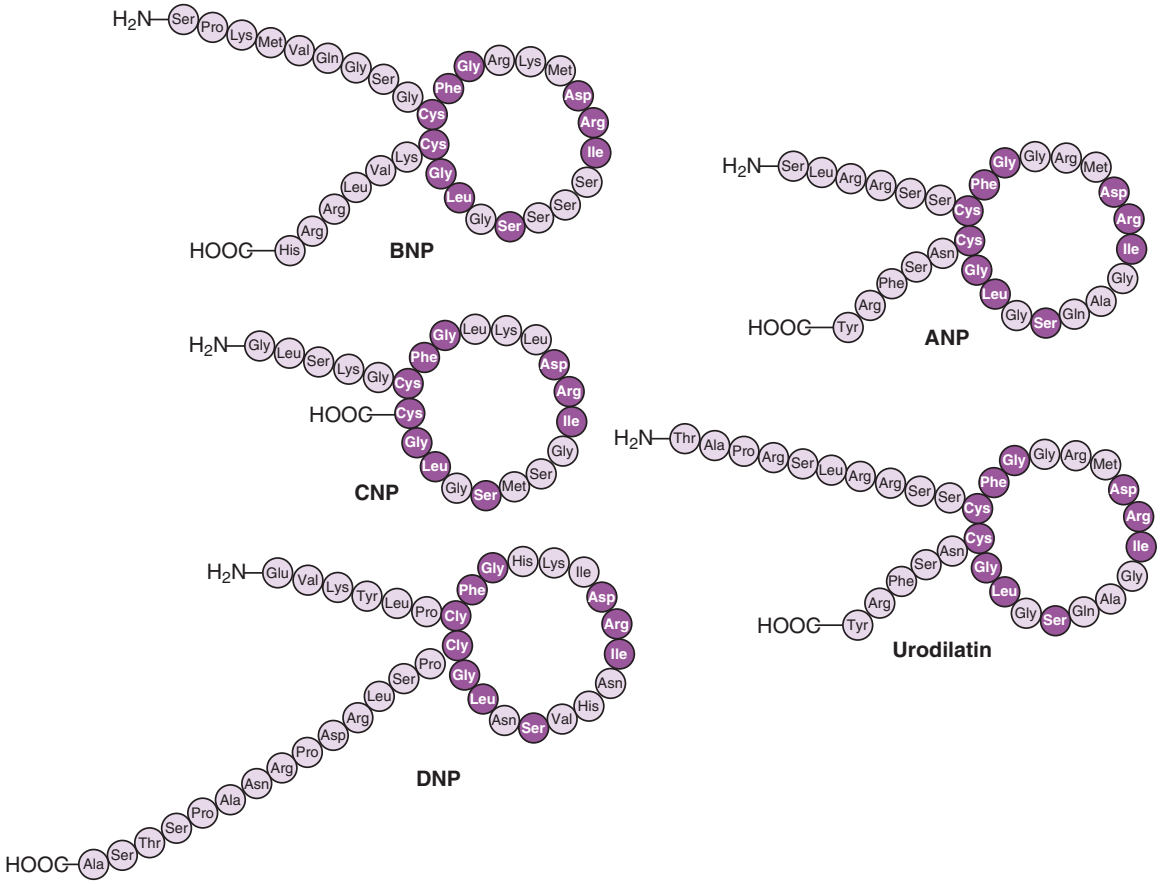

Fig. 11.7 Molecular structure of the natriuretic peptides. *ANP,* Atrial natriuretic peptide; *BNP,* brain natriuretic peptide; *CNP,* C-type natriuretic peptide; *DNP, Dendroaspis* natriuretic peptide. (Modified from Cea LB. Natriuretic peptide family: new aspects. *Curr Med Chem Cardiovasc Hematol Agents.* 2005;3:87–98.)

1-98) and the COOH-terminal 28–amino acid biologically active fragment (ANP 99-126). Both fragments circulate in the plasma; further processing of the NH_2-terminal fragment leads to the generation of peptides ANP 1-30 (long-acting NP), ANP 31-67 (vessel dilator), and ANP 79-98 (kaliuretic peptide), all of whose biologic actions may be similar to those of ANP.[266]

BRAIN NATRIURETIC PEPTIDE

The BNP gene, NPPB, is located only about 8 kb upstream of the ANP gene on the short arm of chromosome 1 in humans, which suggests that the two genes may share both evolutionary origin and transcriptional regulation. By contrast, NPPC, the gene encoding CNP, is found separately, on chromosome 2. CNP is highly conserved across species; thus it may represent the evolutionary ancestor of ANP and BNP. BNP, like ANP, is synthesized as a preprohormone, between 121 and 134 amino acids in length, according to species of origin. Human preproBNP (134 amino acids) is cleaved to produce the 108–amino acid precursor proBNP. Further processing leads to the production of the 32–amino acid, biologically active BNP (which corresponds to the C terminal of the precursor), as well as a 76–amino acid N-terminal fragment (NT-proBNP).[267] Active BNP, NT-proBNP, and pro-BNP all circulate in the plasma. Circulating BNP contains the characteristic 17–amino acid ring structure closed by a disulfide bond between two cysteine residues, along with a nine–amino acid N-terminal tail and a six–amino acid C-terminal tail (Fig. 11.7).[268]

The term *brain natriuretic peptide* is somewhat misleading, given that the primary sites of synthesis of BNP are the cardiac ventricles, and expression also occurs, to a lesser extent, in atrial cardiomyocytes. Like ANP, expression of BNP is regulated by changes in intracardiac pressure and stretch. However, unlike ANP, which is stored and released from secretory granules, BNP is regulated at the gene expression level and is synthesized and secreted in bursts. BNP expression is increased in heart failure, hypertension, and renal failure. Its plasma half-life is approximately 22 minutes; by contrast, the half-life of circulating ANP is 3 to 5 minutes, and the half-life of the biologically inactive NT-proBNP is 120 minutes. This difference is relevant to the utility of NP measurement as a biologic marker of cardiorenal disease. Changes in pulmonary capillary wedge pressure may be reflected by plasma BNP concentrations every 2 hours and by NT-proBNP levels every 12 hours.[269,270] The physiologic actions of BNP are similar to those of ANP, including effects on the kidneys (natriuresis and diuresis), vasculature (hypotension), endocrine system (inhibition of plasma renin and aldosterone secretion), and the brain (central vasodepressor activity).

C-TYPE NATRIURETIC PEPTIDE

As is the case for ANP and BNP, CNP is derived from a prepropeptide that undergoes posttranslational proteolytic

cleavage. The initial translation product preproCNP is 126 amino acids in length and is cleaved to produce the 103–amino acid prohormone. Cleavage of proCNP yields two mature peptides made up of 22 and 53 amino acids: CNP and NH_2-terminally extended form of CNP, respectively. Of the 17 amino acids within the CNP ring structure, 11 are identical to those in the other NPs, although, uniquely, CNP lacks an amino tail at the carboxy terminus (Fig. 11.7). Whereas ANP and BNP are ligands for a guanylyl cyclase–coupled receptor, the NPR-A receptor, CNP is a specific ligand for the NPR-B receptor. CNP primarily functions in an autocrine/paracrine manner with effects on vascular tone and muscle cell growth.[271] Expression of the CNP gene by the endothelial cells, the presence of CNP receptors on vascular smooth muscle cell, and the antiproliferative effect of CNP on vascular smooth muscle cells suggest that CNP is produced by the endothelium and acts on adjacent cells, serving as an autocrine/paracrine endothelium-derived locally active vasoregulatory system. Accordingly, plasma concentrations of CNP are very low, although they are increased in the conditions of heart failure and renal failure. CNP is present in the heart, kidneys, and endothelium, and its receptor is also expressed in abundance in the hypothalamus and pituitary gland, which suggests that the peptide may also play a role as a neuromodulator or neurotransmitter. Regulation of CNP expression is distinct from that of ANP and BNP and is controlled by a number of vasoactive mediators, including insulin, vascular endothelial growth factor, TGF-β, TNF-α, and IL-1β.[271]

The principle enzymes responsible for the conversion of proANP, proBNP, and proCNP to their active forms are the serine proteases, corin and furin.[272] Corin converts proANP to ANP,[273] furin converts proCNP to CNP[274] and both corin and furin cleave proBNP.[275] Corin is highly expressed in the heart and to a lesser extent in the kidney[272] and it is the rate-limiting enzyme in ANP activation.[276] In response to pressure overload, corin-deficient mice develop hypertension together with cardiac hypertrophy and dysfunction.[277,278] In the kidneys, corin colocalizes with ANP[279] and decreased urinary corin excretion has been observed in patients with CKD.[280] Interestingly, studies combining observations made in organ-specific corin-deficient mice together with human correlative experiments have identified a role for impaired uterine corin/ANP function in the pathogenesis of preeclampsia.[281]

DENDROASPIS NATRIURETIC PEPTIDE

The physiologic role of DNP has been controversial since its original identification in the venom of the Green Mamba snake, *D. angusticeps,* in 1992.[263,282] DNP is a 38–amino acid peptide that shares the 17–amino acid ring structure common to all NPs, except that it has unique N- and C-terminal regions (Fig. 11.7).[270] Immunoreactivity for DNP has been reported in human plasma and atrial myocardium, and DNP has also been described in rat[283] and rabbit[284] kidneys, rat colon,[285] rat aortic vascular smooth muscle cells,[286] and pig ovarian granulosa cells.[287] DNP binds to NPR-A[288] and the clearance receptor NPR-C,[289] which may be of particular relevance in view of the peptide's apparent resistance to enzymatic degradation.[290] In dogs, either under normal conditions or in a pacing-induced heart failure model, administration of synthetic DNP decreased cardiac filling pressures; increased GFR, natriuresis, and diuresis; and lowered blood pressure, suppressing renin release and increasing plasma and urine cGMP levels.[291,292] Despite these propitious findings, several aspects of the biologic role of DNP remain contentious. In particular, the gene for the peptide has not been identified in mammals and the fractionation of DNP from human samples has not been reported.[282] These uncertainties have led some authors to question whether DNP is, in fact, expressed at all in humans.[282]

URODILATIN

Urodilatin is a structural homolog of ANP that shares the same 17–amino acid ring structure and COOH-terminal tail. It is synthesized in renal distal tubule cells and differentially processed to a 32–amino acid NH_2-terminally extended form of ANP.[293] Urodilatin is not found in plasma; instead, it acts in a paracrine manner within the kidneys on receptors in the glomeruli and IMCDs to promote natriuresis and diuresis. Urodilatin is upregulated in diabetic animals[294] and in the remnant kidney[295] and is relatively resistant to enzymatic degradation, which may explain its more potent renal effects.

NATRIURETIC PEPTIDE RECEPTORS

NPs mediate their biologic effects by binding to three distinct guanylyl cyclase NPRs. The terminology can be somewhat confusing: NPR-A binds ANP and BNP, and NPR-B binds CNP, whereas NPR-C acts as a clearance receptor for all three peptides.

NPR-A and NPR-B are structurally similar but share only 44% homology in the extracellular ligand-binding segment; this difference is probably responsible for the differences in ligand specificity. Both NPR-A and NPR-B have a molecular weight of approximately 120 kDa and consist of a ligand-binding extracellular domain, a single transmembrane segment, an intracellular kinase domain, and an enzymatically active guanylyl cyclase domain.[260] The kinase homology domain of NPR-A and NPR-B shares 30% homology with protein kinases but has no kinase activity. Ligand binding of NPR-A and NPR-B prevents the normal inhibitory action exerted by the kinase homology domain on the guanylyl cyclase domain, allowing the generation of cGMP, which acts as a second messenger responsible for most of the biologic effects of the NPs. NPR-C, in contrast to NPR-A and -B, lacks both the kinase homology domain and the catalytic guanylyl cyclase domain and therefore does not signal through a second-messenger system. Instead, the receptor contains the extracellular ligand-binding segment, a transmembrane domain, and a 37–amino acid cytoplasmic domain containing a G protein–activating sequence.[296] In NPR-C–knockout mice, blood pressure is reduced and the plasma half-life of ANP is increased; this finding supports the role of NPR-C as a clearance receptor.[297]

NPR-C binds all members of the NP family with high affinity. It is the most abundantly expressed of the NPRs—present in the kidneys, vascular endothelium, smooth muscle cells, and heart—and represents approximately 95% of the total receptor population. Preferential binding of NPR-C to ANP over BNP may explain the relatively increased plasma half-life of BNP.[270] NPR-C clears NPs from the circulation through a process of receptor-mediated endocytosis and lysosomal

degradation before rapid recycling of the internalized receptor to the cell surface. Although the primary function of NPR-C is as a clearance receptor, ligand binding may exert biologic effects on the cell through G protein–mediated inhibition of cAMP.[298] The biologic effects of NPs are largely dependent on the distribution of their receptors. NPR-A mRNA is present mainly in the kidneys, especially in the IMCD cells, although the receptor is also notably present within the glomeruli, renal vasculature, and proximal tubules. The distribution of NPR-B overlaps with that of NPR-A; the receptor is found in the kidneys, vasculature, and brain. However, in accordance with the paracrine effects of CNP on vascular tone, mitogenesis, and cell migration, NPR-B is expressed in greater abundance than is NPR-A within the vascular endothelium and smooth muscle, whereas expression levels are relatively lower within the kidneys.

NEPRILYSIN

Receptor-mediated endocytosis probably accounts for about 50% of clearance of the NPs from the circulation; catalytic degradation by the enzyme neprilysin (NEP 24.11) is responsible for the majority of the rest, and direct renal excretion accounts for only a minor contribution.[270] Receptor clearance probably plays an even smaller role in conditions associated with chronically elevated NP levels, because of increased receptor occupancy and downregulation of NPR-C expression.

Neprilysin is a membrane-bound zinc metalloproteinase, originally termed *enkephalinase* because of its ability to degrade opioid receptors in the brain. The enzyme has structural and catalytic similarity to other metallopeptidases, including aminopeptidase; ACE; ECE; and carboxypeptidases A, B, and E and, in addition to the NPs, numerous other substrates have been described for neprilysin (Table 11.2). The primary mechanism of action of neprilysin is to hydrolyze peptide bonds on the NH_2 side of hydrophobic amino acid residues. In the case of ANP, neprilysin cleaves the Cys^{105}-Phe^{106} bond to disrupt the ring structure and inactivate the peptide. The Cys-Phe bond of BNP is relatively insensitive to enzymatic cleavage. Neprilysin has a nearly ubiquitous tissue distribution; expression has been demonstrated in the kidneys, liver, heart, brain, lungs, gut, and adrenal glands. The metallopeptidase is present not only on the surface of endothelial cells but also on smooth muscle cells, fibroblasts, and cardiac myocytes[299]; it is most abundant in the brush border of the proximal tubules of the kidneys, where it rapidly degrades filtered ANP, preventing the peptide from reaching more distal luminal receptors.

ACTIONS OF THE NATRIURETIC PEPTIDES

RENAL EFFECTS OF THE NATRIURETIC PEPTIDES

The natriuretic and diuretic actions of the NPs are consequences of both vasomotor effects and direct effects on the renal tubule. Both ANP and BNP cause an increase in glomerular capillary hydrostatic pressure and a rise in GFR by inducing afferent arteriolar vasodilation and efferent arteriolar vasoconstriction. These contrasting effects of the NPs on the afferent and efferent arterioles differ from the actions of classical vasodilators such as bradykinin. In addition to direct effects on vascular tone, ANP can increase GFR through cGMP-mediated mesangial cell relaxation and consequent changes in the ultrafiltration coefficient. Plasma levels of ANP that do not increase GFR can induce natriuresis, indicating the potential for direct tubule effects, which may involve either locally produced NPs acting in a paracrine manner, such as urodilatin, or circulating NPs. A number of mechanisms may be responsible for the natriuresis, including direct effects on sodium transport in tubule epithelial cells and indirect effects through inhibition of renin secretion after increased sodium delivery to the macula densa.

NPs also antagonize vasopressin in the cortical collecting ducts. Similar mechanisms probably underlie the response to ANP, BNP, and urodilatin. By contrast, CNP has little natriuretic or diuretic effect, which may indicate a requirement for the presence of the C-terminal extension of the peptide for renal effects. The NPs may have antifibrotic effects within the kidneys, as evidenced by an increase in renal fibrosis in NPR-A–knockout mice after unilateral ureteric obstruction.[300] In cultured proximal tubule cells, ANP attenuates high glucose–induced activation of TGF-β_1, Smad, and collagen synthesis, which illustrates the potentially antifibrotic properties of the peptide in the context of diabetic nephropathy.[301]

Table 11.2 Peptides That Have Been Described as Substrates for Neprilysin[393,547]

Atrial natriuretic peptide	Cholecystokinin	Interleukin-1β
Brain natriuretic peptide	Corticotropin-releasing hormone	β-Lipotropin
C-type natriuretic peptide	Dynorphins	Luliberin
Endothelin-1	Endorphins	Luteinizing hormone-releasing hormone
Bradykinin and kallidin	Endothelin-2	α-Melanocyte-stimulating hormone
Substance P	Endothelin-3	Neurokinin A
Angiotensin I	Enkephalins	Neuropeptide Y
Angiotensin II	N-Formylmethionine-leucyl-phenylalanine	Neurotensin
Angiotensin 1-7	Fibroblast growth factor-2	Oxytocin
Adrenocorticotrophic hormone	Gastric-inhibitory peptide	Peptide YY
Adrenomedullin	Gastrin-releasing peptide	Secretin
Amyloid-β peptide	Glucagon	Somatostatin
Big endothelin-1	Gonadotropin-releasing hormone	Thymopentin
Bombesin-like peptides	Incretins	Vasoactive intestinal peptide
Calcitonin gene–related peptide	Insulin B chain	Vasopressin

CARDIOVASCULAR EFFECTS OF THE NATRIURETIC PEPTIDES

All NPs have vasodilatory and hypotensive properties. Heterozygous mutant mice with a disrupted proANP gene display evidence of salt-sensitive hypertension,[302] whereas hypotension is a feature of transgenic mice overexpressing ANP.[303] In a human patient population, a variant in the ANP promoter was associated with both lower levels of plasma ANP and increased susceptibility to early development of hypertension.[304] However, infusion of high concentrations of ANP can actually induce a rise in blood pressure, which suggests that counterregulatory baroreceptors may be activated.[305]

ANP lowers blood pressure through two major direct mechanisms. First, it increases vascular permeability with a shift of fluid from the intravascular to extravascular compartments by capillary hydraulic pressure. Second, ANP increases venous capacitance and lowers preload.[306] In addition, ANP and BNP antagonize the vasoconstrictive effects of the RAS, ET, and the sympathetic nervous system[265] by decreasing sympathetic peripheral vascular tone, thereby suppressing the release of catecholamines and reducing central sympathetic outflow.[267] By lowering the activation threshold of vagal afferents, ANP prevents the vasoconstriction and tachycardia that normally follow a reduction in preload and thereby produces a sustained drop in blood pressure. CNP is a more potent vasodilator than either ANP or BNP. In fact, CNP relaxes human subcutaneous resistance arteries, whereas ANP and BNP have no effect.[307]

NPs have a number of other effects on the cardiovascular system distinct from their action on vasomotor tone. For example, NPs play a major role in cardiac remodeling. Mice with genetic deficiencies of ANP exhibit an increase in cardiac mass,[302] whereas heart size is diminished in mice transgenically overexpressing ANP.[303] The antimitogenic and antitrophic effects of NPs, which appear to be mediated by cGMP, have also been demonstrated in a range of cultured cell types, including cultured vascular cells, fibroblasts, and myocytes, and in vivo in response to balloon angioplasty. Further evidence for the role of ANP in mediating cardiac hypertrophy was obtained from population studies, in which variants in either the *NPPA* promoter (associated with reduced circulating ANP) or the NPR-A gene, NPR1, have been associated with left ventricular hypertrophy.[308,309] BNP has been shown to have antifibrotic properties within the heart. In vitro, BNP antagonizes TGF-β–induced fibrosis in cardiac fibroblasts,[310] and in vivo, targeted genetic disruption of BNP in mice is associated with an increase in cardiac fibrosis, in the absence of either hypertension or ventricular hypertrophy.[311]

Cardiac CNP is increased in heart failure where it may play a role in ventricular remodeling.[312] Comparison of plasma CNP levels in samples taken from the aorta and renal vein, at the time of diagnostic heart catheterization, has demonstrated that CNP is indeed synthesized and secreted by the kidney.[313] Moreover, this effect was found to be blunted in patients with heart failure, potentially contributing to renal sodium retention.[313] In rats subjected to unilateral ureteric obstruction, recombinant CNP decreased blood urea nitrogen and creatinine levels and attenuated renal fibrosis.[314]

OTHER EFFECTS OF THE NATRIURETIC PEPTIDES

Even though they do not cross the blood–brain barrier, NPs exert important CNS effects that may augment their peripheral actions. ANP, BNP, and particularly CNP are all expressed within the brain. Circulating NPs may also exert central effects through actions at sites that are outside the blood–brain barrier. The NPR-B receptor is expressed throughout the CNS, which reflects the wide distribution of CNP, whereas the NPR-A receptor is expressed in areas adjacent to the third ventricle, which is indicative of a role of peripherally circulating ANP and BNP, as well as centrally expressed peptides. Complementing their natriuretic and diuretic effects, NPs inhibit both salt appetite and water drinking. ANP also prevents release of vasopressin and possibly adrenocorticotropic hormone from the pituitary gland, whereas sympathetic tone is increased by the actions of the NPs on the brain stem.

Clinical and experimental evidence suggests that NPs play a role in mediating metabolism. Circulating levels of NPs are decreased in obese individuals[315] and among patients with the metabolic syndrome,[316,317] correlating inversely with both plasma glucose and fasting insulin levels.[318] In accordance with these epidemiologic observations, infusion of ANP activates hormone-sensitive lipase from fat cells, which is indicative of lipolysis.[319] In vitro, ANP inhibits preadipocyte proliferation,[320] the lipolytic properties of the peptide being mediated by cGMP phosphorylation.[321,322]

Knockout mouse studies have revealed that CNP plays a predominant role in the regulation of skeletal growth, specifically cartilage homeostasis and endochondral bone formation.[323] Mice with genetic deficiencies of either CNP or its receptor NPR-B lack growth of longitudinal bones and vertebrae and have a shortened life span as a consequence of respiratory insufficiency secondary to abnormal ossification of the skull and vertebrae.[324,325] Transgenic mice that overexpress CNP are relatively protected from glucocorticoid-induced growth retardation.[326] Mutations in the NPR-B gene have also been reported in patients with the autosomal recessive skeletal dysplasia and acromesomelic dysplasia–type Maroteaux, and obligate carriers of the mutations have heights that are below predicted levels.[325] Accordingly, CNP analog therapy is being investigated as a possible treatment for achondroplasia.[327]

NATRIURETIC PEPTIDES AS BIOMARKERS OF DISEASE

Both ANP and BNP have been studied as clinical biomarkers of heart failure and renal failure. The short half-life of ANP (2–5 minutes) restricts its applicability.[328] However, the biologically inactive NH_2-terminal 98–amino acid peptide ANP 1-98 does not bind to NPR-A or NPR-C and so remains in the circulation longer than ANP does. In heart failure, ANP 1-98 levels closely reflect the degree of renal function.[329] Plasma concentrations of the midregional epitopes of the stable prohormones of both ANP and adrenomedullin are predictive of the progression of renal decline in patients with nondiabetic CKD.[330] The prognostic performance of midregional proANP is not superior to that of NT-proBNP or BNP in hemodialysis patients[331] and measurement of ANP or one of its prohormone derivatives is currently not part of routine clinical care. Signal peptides from both ANP and BNP are present in venous blood and rise rapidly following myocardial infarction,

suggesting that their detection may aid in the diagnosis of cardiac ischemia.[332,333] Commercial assays are widely available for measurement of either BNP or the biologically inactive peptide fragment NT-proBNP. Correspondingly, since 2000, measurement of circulating BNP and NT-proBNP levels has been incorporated into several clinical practice guidelines for the management of heart failure. Important differences distinguish BNP and NT-proBNP from each other as clinical biomarkers. NT-proBNP is not removed from the circulation by binding to the clearance receptor NPR-C, and hence its circulating half-life of approximately 2 hours is significantly longer than that of BNP (approximately 20 minutes). In addition, both BNP and NT-proBNP are affected by renal impairment,[334] but the magnitude of the effect is greater for NT-proBNP.[335]

BRAIN NATRIURETIC PEPTIDE AND N-TERMINAL PROBRAIN NATRIURETIC PEPTIDE AS BIOMARKERS OF HEART FAILURE

Measurement of circulating levels of either BNP or NT-proBNP has effectively helped guide clinical practice in several aspects of the management of heart failure, including diagnosis, screening, prognosis, and monitoring of therapy.[260] The primary role of BNP measurement in the assessment of dyspnea is as a "ruling out" test: A plasma BNP level lower than 100 pg/mL has a negative predictive value for heart failure of 90%.[336] In the ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) study, an NT-proBNP level lower than 300 pg/mL was optimal in ruling out heart failure, with a negative predictive value of 99%.[337] Screening for BNP[338] and NT-BNP[339] levels has also proven useful in identifying individuals at risk for heart failure for whom aggressive medical therapy should be targeted. The utility of serial BNP or NT-BNP measurements in guiding the treatment of patients with heart failure was the subject of a 2016 Cochrane Review.[340] This review concluded that low-quality evidence shows that NP-guided treatment is associated with a reduction in hospital admissions for heart failure and that low-quality evidence shows uncertainty with respect to the effect of NP-guided treatment on mortality or all-cause hospital admissions.[340] In the interpretation of plasma levels of BNP and NT-proBNP, a number of other biologic variables should be taken into account. NP levels rise with age and are higher in women, the latter effect possibly secondary to estrogen regulation, inasmuch as hormone replacement therapy increases BNP levels.[341] Conversely, NP levels fall with increasing obesity. Although BNP levels of heart failure patients are higher in Asian or African American patients than in Caucasian or Hispanic patients, they provide prognostic value regardless of race or ethnicity.[342]

ROLE OF BRAIN NATRIURETIC PEPTIDE AND N-TERMINAL PROBRAIN NATRIURETIC PEPTIDE AS BIOMARKERS IN RENAL DISEASE

The interpretation of NP concentrations in patients with renal disease merits special consideration. NP levels are increased in individuals with impaired renal function. This increase is probably multifactorial in origin and not solely the consequence of increased intravascular volume. Other factors that contribute to increased NP levels include decreased NP responsiveness, subclinical ventricular dysfunction, hypertension, left ventricular hypertrophy, subclinical ischemia, myocardial fibrosis, and RAS activation,[343] as well as decreased filtration and reduced clearance by NPR-C and NEP.[344] Although, on the basis of observational studies, it has been widely considered that renal clearance plays a greater role in the removal of NT-proBNP from the circulation than removal of BNP, one study has challenged this view. By measuring both NT-proBNP and BNP in the renal arteries and veins of 165 subjects undergoing renal arteriography, investigators found that both NT-proBNP and BNP are equally dependent on renal clearance.[345] However, the NT-proBNP-to-BNP ratio did increase with declining GFR, which suggests that the two peptides may be differentially cleared at GFRs lower than 30 mL/min/1.73 m^2.[345]

Even though both BNP and NT-proBNP are affected by renal impairment, their clinical utility for the prediction of heart failure persists in CKD patients in the context of appropriately adjusted reference ranges. For example in the Breathing Not Properly study, BNP cut point values were approximately threefold higher to diagnose heart failure in patients with an estimated GFR lower than 60 mL/min relative to the conventional cut point value of 100 pg/mL.[346] In a cohort of 831 patients with dyspnea and a GFR less than 60 mL/min, both BNP and NT-proBNP were effective predictors of heart failure, although NT-proBNP was superior in predicting mortality.[347] In asymptomatic patients with CKD, both BNP and NT-proBNP were equivalent and effective in indicating the presence of left ventricular hypertrophy or coronary artery disease.[348] In patients with CKD, BNP and NT-proBNP may be predictive of the progression of renal decline[349,350] and cardiovascular disease and mortality. In a nondialysis CKD population, NT-proBNP, but not BNP, was an independent predictor of death[351]; in 994 black patients with hypertensive renal disease (GFR = 20 to 65 mL/min/1.73 m^2), NT-proBNP was predictive of cardiovascular disease and mortality, particularly among individuals with proteinuria.[352] In pediatric CKD patients, both BNP and pro-BNP (but not troponins I and T) were indicative of left ventricular hypertrophy or dysfunction.[353]

BNP and NT-proBNP have been studied extensively in dialysis recipients both as prognostic indicators and as markers of volume status. The molecular weights of BNP (3.5 kDa) and NT-proBNP (8.35 kDa) are low enough that both peptides may be cleared by high-flux dialysis.[354,355] Nevertheless, in contrast to ANP, which falls sharply after either hemodialysis or peritoneal dialysis, levels of BNP and NT-proBNP are less affected.[354,356] The role of NP levels as indicators of volume status in either hemodialysis or peritoneal dialysis recipients is confounded by the common coexistence of left ventricular abnormalities.[334,346,357–360] Both BNP and NT-proBNP levels are predictive of mortality, heart failure, and coronary artery disease in the population undergoing dialysis.[361–366] However, no definite cut point values for diagnosing heart failure in dialysis patients have been defined.[346]

CIRCULATING C-TYPE NATRIURETIC PEPTIDE LEVELS AS A BIOMARKER FOR RISK OF MYOCARDIAL INFARCTION

Although CNP usually functions in a paracrine manner, its presence in the plasma may provide utility as a biomarker of cardiovascular risk. In a study of 1841 individuals from the general population, individuals with plasma CNP levels in the highest quartile were at increased risk of myocardial

infarction and, unlike BNP levels, plasma CNP levels were unaffected by sex and only weakly associated with age.[367]

THERAPEUTIC USES OF NATRIURETIC PEPTIDES

Even though NP levels are increased in heart failure, their biologic effects are blunted. Intravenous administration of recombinant NPs increases their circulating levels several-fold, overcoming this resistance. As such, two recombinant NPs are currently available as therapeutic agents for the treatment of heart failure: recombinant ANP (carperitide), which is available in Japan for the treatment of pulmonary edema, and recombinant BNP (nesiritide), which is licensed in several countries, including the United States, for the treatment of acute decompensated heart failure.

RECOMBINANT ATRIAL NATRIURETIC PEPTIDE

ANP has a short half-life and a high total body clearance. Its intravenous administration causes a reduction in blood pressure, diuresis, and natriuresis in healthy individuals; this response is reduced in the setting of acute heart failure. In a 6-year open-label study of 3777 patients with acute heart failure treated with carperitide, clinical improvement was reported in 82%.[368] Whereas early experimental studies were suggestive of a potential benefit of exogenous ANP in acute renal failure, results in patients have generally been disappointing. Nevertheless, the peptide may have a limited role in selected patient populations. For example, low-dose carperitide preserved renal function in patients undergoing repair of abdominal aortic aneurysm[369] and reduced the incidence of contrast-induced nephropathy in patients after coronary angiography.[370] However, a meta-analysis suggested that recombinant ANP has no effect on mortality in patients with acute renal injury, although a trend toward a reduction in the need for renal replacement therapy was shown.[371] In a separate meta-analysis of studies conducted in cardiovascular surgery patients, ANP infusion decreased peak serum creatinine, incidence of arrhythmia, and need for renal replacement therapy, whereas both ANP and BNP decreased the length of intensive care unit and hospital stay.[372] Among 367 high-risk individuals undergoing coronary artery bypass grafting (CABG), recombinant ANP decreased the incidence of major adverse cardiovascular and cerebrovascular events and the need for dialysis, immediately and up to 2 years postoperatively, although survival was unaffected.[373] Similarly, among CKD patients undergoing CABG, those receiving recombinant ANP experienced a smaller rise in serum creatinine, fewer cardiac events, and lower requirement for dialysis, although mortality did not differ from those that did not receive ANP.[374] However, when employed in an effort to treat rather than prevent acute kidney injury following cardiac surgery, recombinant ANP had no significant effect on renal function, the need for renal replacement, length of stay, or medical costs.[375]

RECOMBINANT BRAIN NATRIURETIC PEPTIDE

Nesiritide is recombinant human BNP, manufactured from *Escherichia coli* and identical in structure to native human BNP, with a mean terminal half-life of 18 minutes in patients with heart failure.[376] Intravenous administration of nesiritide lowers pulmonary and systemic vascular resistance, decreases right atrial pressure, and increases cardiac output (presumably through effects on ventricular afterload) in a concentration-dependent fashion.[377] In the kidneys, nesiritide increases RBF and GFR through both direct vasodilatory effects and indirect effects on cardiac output and norepinephrine inhibition.[378] Diuresis and natriuresis may also occur, although these effects are modest and may not be seen at the approved doses. Additional effects of nesiritide may also include inhibition of renin secretion in the kidneys and aldosterone production in the heart and adrenal glands.

In response to meta-analysis data suggesting that nesiritide treatment may be associated with a worsening of renal function and an increase in the rate of early death,[379,380] the Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure (ASCEND-HF) trial was initiated.[381] In this study of 7141 patients hospitalized with acute heart failure, nesiritide neither increased nor decreased the rate of death or rehospitalization, rates of worsening renal function were unaffected, and there was a small but nonsignificant improvement in self-reported rates of dyspnea.[381] Based on these results, the investigators concluded that nesiritide cannot be recommended for routine use in the broad population of patients with acute heart failure.[381]

THERAPEUTIC USES OF OTHER NATRIURETIC PEPTIDES

The effects of urodilatin (ularitide) have been assessed in both heart failure and acute renal failure. However, the diuretic effect of urodilatin appears to be attenuated in heart failure patients, which reflects a blunted response, as observed for ANP and BNP.[382,383] Similarly, as with ANP and BNP, hypotension appears to be a dose-limiting side effect of ularitide therapy.[383,384] In the Safety and Efficacy of an Intravenous Placebo-Controlled Randomized Infusion of Ularitide in a Prospective Double-blind Study in Patients with Symptomatic, Decompensated Chronic Heart Failure (SIRIUS II) study, a phase II trial of 221 patients hospitalized for decompensated heart failure, a single 24-hour infusion of ularitide preserved short-term renal function.[385] The NP vessel dilator may offer theoretical advantages for the treatment of acute decompensated heart failure in comparison with current NP-based therapies.[386,387] In particular, vessel dilator may produce a greater and more sustained natriuresis than does ANP or BNP, without a blunted response in patients with heart failure, and may also improve renal function in the setting of experimental acute renal injury.[388] An alternative therapeutic approach is the development of novel chimeric peptides. For example, researchers have synthesized a peptide (cenderitide) that represents fusion of the 22–amino acid peptide CNP together with the 15–amino acid linear C terminus of DNP.[389] In vitro, this peptide activates cGMP and attenuates cardiac fibroblast proliferation. In vivo, cenderitide is both natriuretic and diuretic and increases GFR with less hypotension than does BNP.[389,390] Cenderitide is more resistant to degradation by neprilysin than the naturally occurring NPs and is eightfold more potent in inducing glomerular cGMP production than CNP.[391]

COMBINATION ANGIOTENSIN RECEPTOR BLOCKADE AND NEPRILYSIN INHIBITION

Notwithstanding concerns regarding the efficacy and cost effectiveness of recombinant NP therapy, a major limitation is the requirement for systemic administration, which is unsuitable for chronic treatment. Alternative methods to

increase the biologic activity of NPs may offer a more feasible approach for chronic therapy. In particular, inhibition of the enzymatic degradation of NPs by neprilysin has been the focus of drug discovery efforts for a number of years. Neprilysin is a zinc metallopeptidase with catalytic similarity to ACE and with a wide tissue distribution, although abundant at the proximal tubule brush border. Several pharmacologic neprilysin inhibitors have been investigated (e.g., candoxatril, thiorphan, and phosphoramidon). Although these agents, in general, lead to an increase in plasma levels of the NPs and, under some experimental conditions, induce natriuresis and diuresis with peripheral vasodilation, results of clinical trials in hypertension and heart failure have generally been disappointing. Specifically, sustained antihypertensive effects have not been demonstrated, and some researchers have reported a paradoxical rise in blood pressure. This may be a consequence of the induction of both neprilysin and ACE expression with neprilysin inhibition[392] and a consequent increase in angiotensin II levels.[393] The biologic actions of the NPs are, however, restored in the presence of an inhibited RAS and this has led to the development of two classes of agent: (1) vasopeptidase inhibitors that inhibit both neprilysin and ACE and (2) combined angiotensin receptor blockade/neprilysin inhibition, the latter having gained regulatory authority approval for the treatment of heart failure.

The rational design of vasopeptidase inhibitors—such as mixanpril (S21402), CGS30440, aladotril, MDL 100173, sampatrilat, and omapatrilat—was made possible because of the similar structural characteristics of the catalytic sites of both neprilysin and ACE.[299] Despite the theoretical advantages of vasopeptidase inhibitors, phase III clinical studies have not been able to demonstrate superiority of vasopeptidase inhibition over ACE inhibition, and an increase in the incidence of angioedema has raised safety concerns. For instance, in the Omapatrilat Cardiovascular Treatment vs. Enalapril (OCTAVE) trial of 25,302 hypertensive patients, angioedema occurred in 2.17% of omapatrilat-treated patients, in comparison with 0.68% of patients treated with the ACE inhibitor enalapril.[394] In the Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events (OVERTURE) study, the incidence of angioedema was, again, increased among subjects receiving omapatrilat in comparison with those receiving enalapril (0.8% vs. 0.5%).[395] The increased angioedema with vasopeptidase inhibition is likely a consequence of decreased degradation of bradykinin and substance P with combined inhibition of the two metallopeptidases.[396]

The rationale that concurrent RAS blockade may potentiate the therapeutic effects of neprilysin inhibition yet concurrent ACE inhibition increases the risk of angioedema encouraged the development of a new class of drug that combines an ARB and neprilysin inhibitor. This class has been termed ARNi (i.e., angiotensin receptor-neprilysin inhibitor), although ARBs do not inhibit enzymatic activity. In July 2015, the first in the class, valsartan/sacubitril gained approval from the U.S. Food and Drug Administration for the treatment of heart failure with reduced ejection fraction. Valsartan/sacubitril is a single molecule composed of molecular moieties of the ARB, valsartan, and the neprilysin inhibitor prodrug sacubitril (formerly AHU-377) in a 1:1 ratio.[397] During development, the combination drug of valsartan/sacubitril was referred to as LCZ696. After ingestion, valsartan/sacubitril dissociates into valsartan and sacubitril, and sacubitril is subsequently converted to its active form sacubitrilat (LBQ657) by esterases.[396] In a study of 1328 patients, valsartan/sacubitril conferred greater blood pressure lowering than valsartan alone with no cases of angioedema reported.[398] In the Prospective comparison of ARNi with ARB on Management Of heart failUre with preserved ejectioN fracTion (PARAMOUNT) study of 301 individuals with heart failure with preserved ejection fraction, valsartan/sacubitril lowered NT-proBNP levels to a greater extent than valsartan after 12 weeks of treatment and was well tolerated.[399] Although the reduction in NT-proBNP was sustained at 36 weeks, the difference in NT-proBNP levels between participants randomized to valsartan/sacubitril and valsartan was no longer significant.[399] However, left atrial remodeling and heart failure symptoms were improved.[399]

The case for regulatory authority approval for valsartan/sacubitril was based on the findings of the phase III prospective comparison of ARNi with ACEi (Determine Impact on Global Mortality and Morbidity in Heart Failure [PARADIGM-HF] trial).[400] PARADIGM-HF compared the effects of valsartan/sacubitril (200 mg twice daily) and enalapril (10 mg twice daily) in 8442 patients with heart failure (New York Heart Association class II–IV) and a reduced ejection fraction (≤40%).[400] The primary outcome, a composite of cardiovascular death and hospitalization for heart failure, occurred in 21.8% of participants treated with valsartan/sacubitril and 26.5% of participants treated with enalapril (hazard ratio 0.80, confidence interval 0.73–0.87, $P < .001$). Hypotension was more common in participants receiving valsartan/sacubitril, whereas cough, hyperkalemia, and renal impairment were more common in those receiving enalapril.[400] Importantly, there was no significant difference in the number of cases of angioedema between participants receiving valsartan/sacubitril than those receiving enalapril, although the number of cases of angioedema was numerically higher in the valsartan/sacubitril group (19 vs. 10, $P = .13$). The effect of valsartan/sacubitril in patients with heart failure and preserved ejection fraction is currently being evaluated in the Prospective Comparison of ARNI with ARB Global Outcomes in Heart Failure with preserved Ejection Fraction (PARAGON-HF) trial (NCT01920711), and other molecules combining ARB and NEP inhibition are also currently under development.[401,402]

Despite the promising findings of PARADIGM-HF, there are some difficulties with the design of the study and there are some theoretical considerations around the use of neprilysin inhibitors in a broad population. In terms of the active comparator in PARADIGM-HF, it is noteworthy that valsartan/sacubitril was not compared with valsartan alone and that the dose of enalapril (10 mg twice daily) may have been insufficient. For instance, in the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) of patients with heart failure, the target dose of enalapril was up to 20 mg twice daily.[403] Separately, whether the apparently low risk of angioedema observed in PARADIGM-HF translates to the real-world setting remains to be determined. Only 5% of participants in PARADIGM-HF were black, a group at increased risk of angioedema associated with ACE inhibition or neprilysin inhibition.[396] Furthermore, 78% of participants in PARADIGM-HF had been previously treated with an ACE inhibitor and inclusion of a run-in period where all participants were exposed to enalapril may have resulted in an underrepresentation in the number of cases of angioedema.[400]

Other theoretical risk concerns surrounding chronic neprilysin inhibition largely relate to the other substrates that are normally degraded by neprilysin (Table 11.2). Based on the breadth of activity of these substrates, the possibility has been raised that long-term neprilysin inhibition could have deleterious effects on bronchial reactivity, pain, inflammation, tumorigenesis, and neuronal function.[393] Of particular note has been the recognition that neprilysin is important in the metabolism of amyloid-β peptides and that its inhibition could predispose to the development of Alzheimer's disease, age-related macular degeneration, and cerebral amyloid angiopathy which may take many years to manifest.[393] A trial comparing the efficacy and safety of valsartan/sacubitril with valsartan on cognitive function in patients with heart failure and preserved ejection fraction is ongoing (NCT02884206).

RENAL EFFECTS OF VALSARTAN/SACUBITRIL IN PATIENTS WITH HEART FAILURE AND PRESERVED EJECTION FRACTION

The renal effects of valsartan/sacubitril were assessed in a post hoc analysis of participants in the PARAMOUNT trial of individuals with heart failure and preserved ejection fraction.[404] Participants received treatment with valsartan/sacubitril titrated to 200 mg twice daily or valsartan titrated to 160 mg twice daily, which each give similar levels of systemic exposure to valsartan.[397,398] In the PARAMOUNT trial, eGFR declined less with valsartan/sacubitril treatment than it did with valsartan treatment over a 36-week period (–1.5 vs. –5.2 mL/min/1.73 m^2, $P = .002$), whereas the geometric mean of the urinary albumin-to-creatinine ratio increased from baseline in the valsartan/sacubitril group (2.4–2.9 mg/mmol) and was unchanged in the valsartan group (2.1–2.0 mg/mmol; *P* value for difference between groups = .016).[404] The finding of a relative preservation in eGFR with valsartan/sacubitril is consistent with the observation from the PARADIGM-HF trial that valsartan/sacubitril-treated patients experienced less renal impairment that necessitated cessation of therapy than enalapril-treated patients.[400] Both the effect on eGFR and albuminuria are reminiscent of the effect of systemic ANP administration, raising the possibility that they are a consequence of increased levels of biologically active ANP with neprilysin inhibition.[404] Participants in the PARAMOUNT trial were required to have an eGFR of at least 30 mL/min/1.73 m^2 at enrollment[399] and thus the effects of combination ARB and neprilysin inhibition in more advanced renal disease are currently unknown, as are the effects on hard renal endpoints in at-risk populations.

OTHER NATRIURETIC PEPTIDES

GUANYLIN AND UROGUANYLIN

The existence of intestinal NPs has been suggested by initial observations that sodium excretion is greater after an oral salt load than after an intravenous salt load.[405,406] These intestinal peptides include guanylin and uroguanylin. However, a study of 15 healthy volunteers found that sodium excretion was similar in response to either oral or intravenous sodium load during either a low- or high-sodium–containing diet.[407] Moreover, serum concentrations of either prouroguanylin or proguanylin were unchanged following either oral or intravenous sodium load and showed no correlation with sodium excretion.[407] Collectively, these observations challenge the notion of a gastrointestinal–renal natriuretic axis mediated by the guanylin peptide family.[407,408] It thus appears likely that the natriuretic, kaliuretic, and diuretic effects of guanylin and uroguanylin, which occur without change in GFR or RBF, are mediated by local production of the peptides within the kidney.[408]

ADRENOMEDULLIN

Adrenomedullin is a 52–amino acid peptide originally isolated from human pheochromocytoma cells,[409] although it is synthesized mainly by vascular smooth muscle cells, endothelial cells, and macrophages[410] and is present in the plasma, vasculature, lungs, heart, and adipose tissue. The peptide is upregulated in patients with cardiovascular disease and has positive inotropic and vasodilatory properties. Systemic administration of adrenomedullin induces an NO-dependent natriuresis and an increase in GFR both under normal conditions and in patients with congestive heart failure; it also decreases plasma aldosterone levels without affecting renin activity. Individuals with type 2 diabetes and plasma levels of midregional proadrenomedullin (MR-proADM) peptide in the highest tertile are at an increased risk of severe nephropathy (doubling of plasma creatinine and/or ESRD), which may reflect a reactive rise in MR-proADM.[411]

KALLIKREIN–KININ SYSTEM

The KKS is a complex network of peptide hormones, receptors, and peptidases that is evolutionarily conserved with homologs in nonmammalian species.[412] Discovery of the KKS is attributed to Abelous and Bardier, who reported in 1909 that experimental injection of urine resulted in an acute fall in systemic blood pressure.[412a] Since that time, investigators have recognized that the physiologic actions of the KKS also include regulation of tissue blood flow, transepithelial water and electrolyte transport, cellular growth, capillary permeability, and inflammatory responses. The main components of the KKS are the enzyme kallikrein, its substrate kininogen, effector hormones known as *kinins* (especially bradykinin and kallidin [also termed *lys-bradykinin*]) and their inactivating enzymes, which include kininases I and II (ACE) and neprilysin.

Kinins exert their biologic effects through binding to two receptors: the bradykinin B1 receptor (B1R) and bradykinin B2 receptor (B2R). The B2R is widely expressed and mediates all the physiologic actions of the kinins under normal conditions. The B1R is activated predominantly by des-Arg-bradykinin, a natural degradation product of bradykinin, generated by cleavage of the peptide by kininase I. The KKS may be subdivided into a circulatory (plasma) KKS and a tissue (including renal) KKS, which may be distinguished by their principal effector molecules, bradykinin and kallidin, respectively. In the kidneys, the kinins play a significant role in the modulation of renal hemodynamics and salt and water homeostasis.

COMPONENTS OF THE KALLIKREIN–KININ SYSTEM

KININOGEN

Humans possess a single kininogen gene, *KNG1,* which is localized to chromosome 3q26 and encodes both high–molecular

weight (HMW) kininogens (626 amino acids, 88 to 120 kDa) and low–molecular weight (LMW) kininogens (409 amino acids, 50–68 kDa) through alternate splicing from 11 exons spread over a 27-kb genomic region. A second kininogen gene has been identified in mice.[413] In humans, kininogen deficiency may be relatively asymptomatic[414]; the kininogen-deficient Brown Norway Katholiek rat strain, however, shows increased sensitivity to the pressor effects of salt, angiotensin II, and mineralocorticoid.[415,416]

KALLIKREIN

HMW and LMW kininogen are cleaved by the serine protease kallikrein. The name "kallikrein" is derived from the Greek term *kallikreas,* meaning "pancreas," after the work of Frey and others, in the 1930s, who extracted a kinin-producing enzyme from the pancreas of dogs.[416a] Since then, 15 tissue kallikreins have been identified, although, in humans, only one (KLK1) is involved in local kinin production. The human kallikrein genes are clustered on chromosome 19 at loci q13.3-13.4. Plasma kallikrein is found in the circulation and is involved largely with the coagulation cascade and activation of neutrophils. The tissue kallikreins are acid glycoproteins that are variably and extensively glycosylated. Human renal kallikrein is synthesized as a zymogen (prekallikrein) with a 17–amino acid signal peptide and a 7–amino acid activation sequence, which must be cleaved in order to activate the enzyme. In most mammals, including humans, tissue kallikrein cleaves kallidin (lys-bradykinin) from kininogens, whereas plasma kallikrein releases bradykinin.

Although the physiologic effects of kallikrein have been attributed to increased kinin generation, the enzyme may also have direct effects on the B2R, as well as actions independent of the kinin receptors.[417,418] For example, in kininogen-deficient Brown Norway Katholiek rats, local injection of kallikrein into the myocardium after coronary artery ligation had a cardioprotective effect that was abolished by the NO synthase inhibitor Nω-nitro-L-arginine methyl ester and the selective B2R inhibitor icatibant (Hoe 140).[417] As a serine protease, kallikrein may also elicit kinin receptor–independent effects on endothelial cell migration and survival through cleavage of growth factors and matrix metalloproteinases.[419] Transgenic mice overexpressing human kallikrein exhibit a sustained reduction in systemic blood pressure throughout their life span, which is indicative of the lack of sufficient compensatory mechanisms to reverse the hypotensive effect of kallikrein.[420] In humans, polymorphisms of the kallikrein gene *KLK1* or its promoter can impair enzymatic activity, potentially influencing both kinin-dependent and kinin-independent effects. Among normotensive men with a common loss-of-function *KLK1* polymorphism (R53H), an increase in wall shear stress and a paradoxical reduction in artery diameter and lumen were noted, although flow-mediated and endothelium-independent vasodilation were unaffected.[421]

KININS

The kinins are bradykinin and kallidin in humans and bradykinin and kallidin-like peptide in rodents.[422] Plasma aminopeptidase can convert kallidin (10 amino acids: Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) to bradykinin (9 amino acids: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) by cleavage of the first N-terminal lysine residue. Cleavage of the carboxy-terminal arginine residue by kininase I (carboxypeptidase-N) and carboxypeptidase-M generates their des-Arg derivatives, which are agonists of the B1R.[422] Removal of two C-terminal amino acids (Phe and Arg) by ACE (kininase II), neprilysin, or ECE is responsible for inactivation of the peptides.[422]

BRADYKININ RECEPTORS

B1R and B2R share 36% homology, and both are GPCRs with seven transmembrane domains. The genes for the two receptors are in tandem on a compact locus (14q23) separated by only 12 kb.[423] The B2R is the principal receptor mediating the actions of both kinins, is expressed in abundance by vascular endothelial cells, and is present in most tissues, including those of the kidneys, heart, skeletal muscle, CNS, vas deferens, trachea, intestines, uterus, and bladder. In general, the distribution and action of B1Rs are similar to those of the B2Rs. The B1R, by contrast, is expressed at low levels under normal conditions but is upregulated in response to inflammatory stimuli (e.g., lipopolysaccharide, endotoxins, and cytokines such as IL-1β and TNF-α)[424] and in the setting of diabetes[425] and ischemia-reperfusion injury.[426] B2R binds both bradykinin and kallidin, whereas bradykinin has almost no effect at the B1R. The carboxypeptidase required to generate the des-Arg B1R-active kinin fragments is closely associated with the B1R on the cell surface.[427] This association would enable B2R agonists to rapidly activate B1Rs, particularly in response to inflammation.[427]

Ligand binding of both receptor subtypes induces activation of phospholipase C, which results in intracellular calcium mobilization through production of inositol 1,4,5-triphosphate and DAG via activation of G proteins, including $G\alpha_q$ and $G\alpha_i$. The physiologic effects of bradykinin receptor activation are mediated through generation of both endothelial NO synthase–derived NO and prostaglandins. B2R activation leads to a rise in intracellular calcium concentrations in vascular endothelial cells.[422] However, bradykinin-induced vasodilation is not abolished by coadministration of NO synthase and COX inhibitors, which indicates that additional effectors are also likely to be involved, possibly an endothelium-derived hyperpolarizing factor. In addition, through binding to both B1R[428] and B2R,[429] bradykinin also increases the expression of inducible NO synthase (iNOS), at least in rodents. It is very difficult to induce the iNOS gene in human tissues, especially the vascular endothelium. Mice that have genetic deficiencies of B2R,[430] B1R,[431] or both receptors[432] have been generated; the reported phenotypes of the different knockout strains have been varied, which may be a result of different genetic backgrounds, or, in the case of the single knockouts, differing compensatory effects of the remaining receptor. For example, some studies of B2R-deficient mice revealed an increase in resting systemic blood pressure, an exaggerated pressor response to angiotensin II[433] and salt sensitivity,[434] whereas others revealed no difference in resting blood pressure between B2R- or B1R-deficient mice and wild-type animals.[431,435] Double B2R-/B1R-knockout mice were also reported to have resting blood pressure identical to that in wild-type mice and were resistant to lipopolysaccharide-induced hypotension.[432,436] By contrast, transgenic mice expressing the human B2R had a lower resting blood pressure than did wild-type controls.[437] Transgenic mice expressing the rat B1R (as well as their native murine B2R) were normotensive but

showed an exaggerated hypotensive response to lipopolysaccharide and, unexpectedly, a hypertensive response to des-Arg bradykinin.[438]

KALLISTATIN

Kallistatin is an endogenous serpin inhibitor of kallikrein that acts by forming a heat-stable complex with the enzyme. Surprisingly, administration of human kallistatin to rodents induced vasodilation and a decline in systemic blood pressure, which was unaltered by either an NO synthase inhibitor or the B2R antagonist icatibant; this suggests that the vasodilatory properties of kallistatin may be mediated through a smooth muscle mechanism independent of bradykinin receptor activation.[439]

KININASES

With the exception of the metabolites des-Arg-bradykinin and des-Arg-kallidin, kinin-cleavage products are biologically inactive. Kinins are cleaved by a number of enzymes, including carboxypeptidases, ACE, and neprilysin. ACE also truncates its own reaction product, bradykinin-(1–7), further to form bradykinin-(1–5). Neprilysin, like ACE, cleaves bradykinin at the 7 to 8 position and has a broad substrate specificity (Table 11.2). The amino-terminal of bradykinin possesses two proline residues and is susceptible to cleavage by the proline-specific exopeptidase aminopeptidase P. The resultant peptide, bradykinin-(2–9), may be further cleaved by proteases that include the endothelial enzyme dipeptidyl peptidase-4, which reduces this metabolite to bradykinin-(4–9).

PLASMA AND TISSUE KALLIKREIN–KININ SYSTEM

The two independent KKSs in humans (plasma and tissue) can be distinguished by the specific subtypes of kallikreins, kininogens, and kinins involved. The circulating plasma KKS includes HMW kininogen and plasma prekallikrein, both of which are synthesized in the liver and secreted in the plasma, in which kallikrein is generated by the cell matrix–associated prekallikrein activator prolylcarboxypeptidase.[440] Of importance is that bradykinin is the main effector molecule of the plasma KKS. The tissue-specific KKS consists of locally synthesized or liver-derived kininogen (HMW and LMW), tissue kallikrein, and the effector molecules kallidin in humans and kallidin-like peptide in rodents. The half-life of kinins is 10 to 30 seconds, but in tissues with high kallikrein content, including the kidneys, local and plasma-derived LMW kininogen can be continuously cleaved to produce kallidin. Fig. 11.8 illustrates the enzymatic cascades of the plasma and tissue KKSs.

RENAL KALLIKREIN–KININ SYSTEM

The tissue KKS contributes to the physiologic functions of the kidneys with effects on RVR, natriuresis, diuresis, and other vasoactive mediators, such as renin and angiotensin, eicosanoids, catecholamines, NO, vasopressin, and ET. In the kidneys, large quantities of kininogen and kallikrein are synthesized by the tubule epithelium and are excreted in the urine. Locally formed kinin is also detectable in the urine,

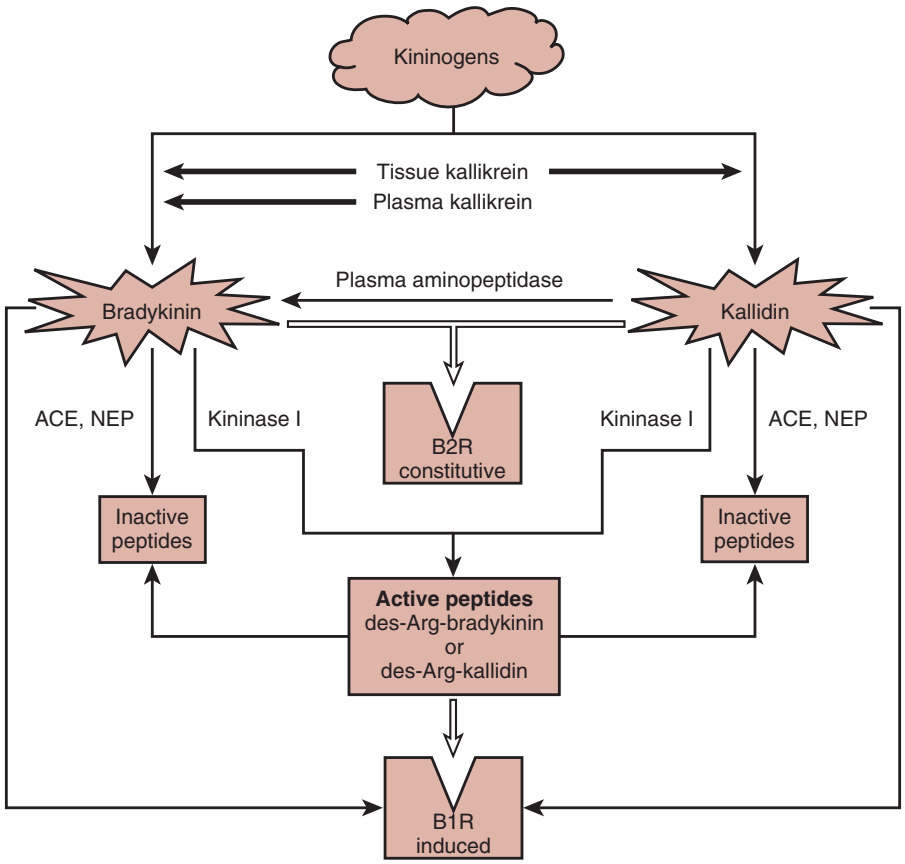

Fig. 11.8 Enzymatic cascade of the kallikrein–kinin system. *ACE,* Angiotensin-converting enzyme; *B1R,* bradykinin B1 receptor; *B2R,* bradykinin B2 receptor; *NEP,* neutral endopeptidase.

renal interstitial fluid, and renal venous blood. In the human kidneys, kallikrein is localized to the connecting tubules with close anatomic association between the kallikrein-expressing tubules and the afferent arterioles of the JGA. Results of some studies suggest that renal kallikrein mRNA is also detectable by in situ hybridization at the glomerular vascular pole. This anatomic association highlights the physiologic relationship between the KKS and the RAS and is consistent with a paracrine function for the KKS in the regulation of RBF, GFR, and renin release. In this regard, it has been suggested that, through effects on prostaglandin production, kinins may lower tubuloglomerular feedback sensitivity.

Expression of kallikrein within the kidneys is altered during development and is regulated by estrogen and progesterone, salt intake, thyroid hormone, and glucocorticoid.[441–444] The enzyme is not normally filtered at the glomerulus in the absence of glomerular injury. Kininogens are localized mostly to connecting tubule principal cells near kallikrein, which can be found in the connecting tubules of the same nephron. Once activated, renal kallikrein cleaves both HMW and LMW kininogens to release kallidin. The majority of the physiologic effects of kinins are mediated through activation of constitutively expressed B2Rs, with little or no B1R mRNA detectable in normal kidneys. In rats, administration of lipopolysaccharide, however, induces expression of B1R throughout the nephron (except the outer medullary collecting ducts), with strong expression in the efferent arteriole, medullary limb, and distal tubule.[445]

The KKS is involved in the regulation of both renal hemodynamics and tubule function. Diuretic and natriuretic effects play a pivotal role in the contribution of the renal KKS to fluid and electrolyte balance. Kinins have been reported to increase RBF and papillary blood flow and to mediate the hyperfiltration induced by a high-protein diet. Kinins also inhibit conductive sodium entry in the IMCDs,[446] and B2R-deficient mice demonstrate increased urinary concentration in response to vasopressin, which indicates that, through the B2R, endogenous kinins oppose the antidiuretic effect of vasopressin.[447] Kinins may therefore affect sodium reabsorption through direct effects on sodium transport along the nephron, through vasodilatory effects, and through changes in the osmotic gradient of the renal medulla. In addition to the effects on renal vascular tone, salt homeostasis, and water homeostasis, experiments with the B2R antagonist icatibant have yielded evidence that kinins may also have antihypertrophic and antiproliferative properties in mesangial cells, fibroblasts, and renomedullary interstitial cells. The antiproliferative effect of bradykinin in mesangial cells may be mediated through interaction of the B2R with the protein-tyrosine phosphatase SH2 domain–containing phosphatase-2.[448]

REGULATION OF TUBULE TRANSPORT BY TISSUE KALLIKREIN

Independent of its ability to generate kinin, tissue kallikrein also exerts separate effects on tubule solute transport by regulating the activity of the epithelial Na^+ channel (ENaC), the colonic H^+, K^+ ATPase, and the epithelial calcium channel TRPV5 (transient receptor potential channel vanilloid subtype 5).[449] The connecting tubules secrete a large amount of tissue kallikrein which, through its enzymatic activity, can alter the function of ion transporters expressed on the luminal surface of cells downstream of its site of secretion.[449] For instance, tissue kallikrein may participate in the proteolytic processing of ENaC increasing its activity, whereas tissue kallikrein–deficient mice have decreased ENaC activity.[450] Despite decreased ENaC activity, however, coincident upregulation of ENaC-independent electroneutral NaCl absorption ensures that tissue kallikrein is not essential for sodium homeostasis.[449,451] The cortical collecting ducts from tissue kallikrein–deficient mice also demonstrate enhanced activity of the colonic H^+, K^+ ATPase in intercalated cells, resulting in net K^+ absorption.[449,452] Finally, tissue kallikrein functions to stabilize the TRPV5 channel at the plasma membrane, promoting Ca^{2+} reabsorption, whereas tissue kallikrein knockout mice exhibit robust hypercalciuria.[453] Distal tubule defects in potassium[454] and calcium[455] handling have also been reported in humans with the loss-of-function R53H polymorphism in the tissue kallikrein gene.

THE KALLIKREIN–KININ SYSTEM IN RENAL DISEASE

HYPERTENSION

Although it has been known for many years that kinin infusion results in an acute drop in systemic blood pressure by reducing peripheral resistance, the role of the KKS in mediating primary or secondary hypertension has yet to be fully established. Decreased activity of kallikrein has been reported in the urine of hypertensive patients and hypertensive rats. An inverse relationship between urinary kallikrein excretion and blood pressure in humans may be suggestive of a role for the renal KKS in protecting against hypertension.[456] However, an alternative interpretation may be that preexisting or hypertension-induced renal disease may itself lead to a reduction in renal kallikrein excretion. In the Dahl salt-sensitive rat model of hypertension, ACE inhibitors attenuate the progression of proteinuria and hypertensive nephrosclerosis better than do ARBs.[457] That this difference may be mediated by enhanced kinin activity with ACE inhibition is supported by the observations that infusion of either kallikrein[458] or bradykinin[459] in this model attenuated glomerulosclerosis without affecting blood pressure. Studies in two-kidney, one-clip hypertension have yielded conflicting results. The incidence of two-kidney, one-clip hypertension was increased in B2R-deficient mice, in comparison with wild-type animals.[460] By contrast, with regard to the response between tissue kallikrein–deficient mice and wild-type animals, there was no difference with respect to kidney size, renin release, systemic blood pressure increase, and cardiac remodeling.[461]

Despite the uncertainty about the role of the KKS in mediating the pathogenesis of hypertension, a variety of genetic mutations of the KKS have been associated with hypertension in animal models and in humans.[462] Inactivating mutations in the kallikrein gene have been identified in spontaneously hypertensive rats,[463] and an association between mutations in the regulatory region of the kallikrein gene *KLK1* and hypertension has also been described in African Americans[464] and Chinese Han people.[465] The loss-of-function *KLK1* R53H mutation is found in 5% to 7% of the Caucasian population,[466] but this one single-nucleotide polymorphism (SNP) has not in itself been found to markedly alter blood pressure.[467] ACE polymorphisms, responsible for different plasma levels of the enzyme and, accordingly, altered kinin

levels, have been identified as independent risk factors for progression of various diseases, including diabetic nephropathy, but they do not affect blood pressure. Finally, a number of SNPs in both the B2R and B1R genes have been associated with hypertension[468,469] and coronary risk in hypertensive individuals.[470]

DIABETIC NEPHROPATHY

Observations in both experimental animal models and in humans indicate a role for altered KKS activity in the pathogenesis of diabetic nephropathy, although results in experimental studies have been conflicting. The KKS is markedly altered in rats with streptozotocin-induced diabetes and changes are correlated with those in renal plasma flow and GFR.[471] Renal and urinary levels of active kallikrein are increased in rats with moderate hyperglycemia in association with reduced RVR, increased GFR, and increased renal plasma flow and treatment of diabetic rats with the kallikrein inhibitor aprotinin or with a B2R antagonist reduced RBF and GFR.[471] By contrast, in non–insulin-treated streptozotocin-treated rats with severe hyperglycemia and hypofiltration, kallikrein excretion and expression were reduced.[471,472] In addition to its hemodynamic effects, the KKS may also play a renoprotective role in diabetic nephropathy through its antiinflammatory and antiproliferative properties.[424]

Results of receptor antagonist studies initially suggested that the KKS had a limited role in preserving renal structure and function in diabetic nephropathy: Treatment of diabetic rats with icatibant had no effect on glomerular structure or on albuminuria, nor did it alter the attenuating effect of ACE inhibition on either of these parameters.[473] In contrast to this finding, however, the results of more contemporary work suggest that the beneficial effects of ACE inhibitors in experimental diabetic nephropathy may be attenuated by coadministration of a B2R antagonist.[474–476] In Akita diabetic mice lacking the B2R, there was a marked increase in mesangial sclerosis and a worsening of albuminuria,[477] in association with an increase in oxidative stress and mitochondrial damage[478]; however, another study reported contrary results in that B2R-knockout mice were relatively protected from the renal injury caused by streptozotocin-induced diabetes.[479] Upregulation of B1R occurs in response to B2R knockout and could plausibly contribute to renal pathology or alternatively confer a renoprotective benefit. In support of the latter thesis, Akita-diabetic mice deficient in both B2R and B1R exhibited augmented renal injury in comparison to those lacking B2R alone.[480]

In further support of a renoprotective effect of the KKS in diabetic nephropathy, one study showed that induction of diabetes by streptozotocin in mice caused a twofold increase in mRNA for kininogen, tissue kallikrein, kinins, and kinin receptors, with a doubling in albumin excretion in kallikrein-knockout mice in comparison with wild-type animals.[481] In another study, gene delivery of human tissue kallikrein with an adeno-associated virus vector attenuated renal injury in diabetes and decreased urinary albumin excretion.[482] When exogenous pancreatic kallikrein was administered to diabetic mice, it caused a reduction in albuminuria, renal fibrosis, inflammation, and oxidative stress.[483] Conversely, however, kallistatin, which decreases kallikrein activity, also attenuated renal injury in diabetic mice when it was overexpressed using ultrasound microbubble-mediated gene transfer.[484]

Urinary kallikrein excretion in patients with type 1 diabetes demonstrates a similar association with GFR as observed in rats with streptozotocin-induced diabetes.[485] Active kallikrein excretion is increased in hyperfiltering individuals in comparison with both patients with type 1 diabetes who have a normal GFR and normal controls, and it is correlated with both GFR and distal tubule sodium reabsorption.[485] Results of genetic association studies in patients with diabetes have, however, been conflicting: One study demonstrated an association between B2R polymorphisms and albuminuria in 49 patients with type 1 diabetes and 112 patients with type 2 diabetes,[486] whereas another revealed no association between either B1R or B2R polymorphisms and incipient or overt nephropathy in 285 patients with type 2 diabetes.[487] Plasma levels of HMW kininogen fragments were observed to be elevated among individuals with type 1 diabetes and progressive renal decline.[488]

ISCHEMIC RENAL INJURY

In models of ischemia-reperfusion injury, ACE inhibitors appear to be superior to ARBs in protecting against tubule necrosis, loss of endothelial function, and excretory dysfunction.[489] This superiority may be attributed to enhanced kinin activity with ACE inhibition, inasmuch as the effect is negated by B2R antagonists and inhibitors of NO synthase.[490,491] Bradykinin suppresses the opening of mitochondrial pores,[492] and NO suppresses oxidative metabolism; both observations indicate that the KKS may exert its protective effects in ischemia-reperfusion injury through attenuation of oxidative damage. In mice with genetic deficiencies in either the B2R alone or both B1R and B2R, ischemic damage was enhanced in comparison with wild-type mice; injury was most severe in mice that lacked both receptors.[436] By contrast, tissue kallikrein infusion aggravated renal ischemia-reperfusion injury in rats,[493] whereas expression of the human kallistatin gene with an adenoviral vector protected mice from renal ischemia-reperfusion injury.[494] Thus although physiologic kinin levels may be protective in this setting, higher levels may be detrimental, possibly through pathologic reperfusion.[422]

CHRONIC KIDNEY DISEASE

In the remnant kidney model of progressive renal disease, adenovirus-mediated or adeno-associated virus–mediated gene delivery of kallikrein attenuated the decline in renal function.[495] In the model of unilateral ureteric obstruction, both genetic ablation of the B2R and pharmacologic blockade of the B2R increased tubulointerstitial fibrosis.[496] By contrast, expression of the B1R is increased after unilateral ureteric obstruction,[497] and treatment with a nonpeptide B1R antagonist reduced macrophage infiltration and fibrosis.[497] In the same model, B1R-deficient mice similarly showed less upregulation of inflammatory cytokines, reduced albumin excretion, and diminished fibrosis in comparison with wild-type mice.[498] In an Adriamycin-induced mouse model of FSGS, B1R antagonist therapy attenuated and B1R agonist therapy aggravated renal dysfunction.[499] Together, these observations suggest that although the B2R is renoprotective, under some circumstances (and in contrast to the observations made in Akita-diabetic B1R/B2R knockout mice) compensatory B1R upregulation may contribute to the pathogenesis of renal fibrosis. In humans, polymorphisms in both the B1R gene[500,501]

and the B2R gene[500,502] have been associated with the development of ESRD.

LUPUS NEPHRITIS/ANTI–GLOMERULAR BASEMENT MEMBRANE DISEASE

Evidence has linked the KKS to the pathogenesis of the immune-mediated nephritides, SLE, Goodpasture syndrome (antiglomerular basement membrane [GBM] disease), and spontaneous lupus nephritis. Mice strains differ in their susceptibility to anti-GBM antibody–induced nephritis. Comparison of disease-sensitive and control strains, by microarray analysis of renal cortical tissue, revealed that 360 gene transcripts were differentially expressed.[503] Of the underexpressed genes, one-fifth belonged to the kallikrein gene family.[503] Furthermore, in disease-sensitive mice, B2R antagonism augmented proteinuria after anti-GBM challenge, whereas bradykinin administration attenuated disease.[503] In the same study, SNPs in the *KLK1* and *KLK3* promoters were also described in patients with SLE and lupus nephritis.[503] Extending their work further, the same investigators showed that adenoviral delivery of the *KLK1* gene attenuated renal injury in congenic mice possessing a lupus-susceptibility interval on chromosome 7.[504]

ANTINEUTROPHIL CYTOPLASMIC ANTIBODY–ASSOCIATED VASCULITIS

Granulomatosis with polyangiitis (GPA) may be associated with a necrotizing glomerulonephritis. The major antigenic target in GPA is neutrophil-derived proteinase 3 (PR3). Incubation of PR3 with HMW kininogen resulted in the generation of a novel tridecapeptide kinin, termed "PR3-kinin."[505] PR3-kinin binds to B1R directly and can also activate B2R after further processing to form bradykinin.[505] These observations suggest that, in GPA, PR3 may activate the kinin pathway in a kallikrein-independent manner. B1R upregulation has been observed in biopsies from patients with Henoch–Schönlein purpura nephropathy or with antineutrophil cytoplasmic antibody–associated vasculitis.[506] Similarly, B1R upregulation was also observed in a murine serum–induced glomerulonephritis model, whereas treatment with a B1R antagonist attenuated renal decline.[506]

UROTENSIN II

Urotensin II (U-II) is a potent vasoactive cyclic undecapeptide originally isolated from the caudal neurosecretory organ of teleost fish. The system is now known to be present in humans. The two principal regulatory peptides derived from this organ are urotensin I (U-I), which is homologous to mammalian corticotropin-releasing factor, and U-II, which bears sequence similarity to somatostatin[507] and has notable hemodynamic, gastrointestinal, reproductive, osmoregulatory, and metabolic functions in fish. Homologs of U-II have been identified in many species, including humans.

SYNTHESIS, STRUCTURE, AND SECRETION OF UROTENSIN II

Human U-II is derived from two prepropeptide alternate splice variants of 124 and 139 amino acids, differing only in the N-terminal sequence.[508,509] The C terminus is cleaved by prohormone convertases to yield the mature 11–amino acid U-II peptide. U-II contains a cyclic Cys-Phe-Trp-Lys-Tyr-Cys hexapeptide sequence that is conserved across species and is essential for its biologic activity[510] (Fig. 11.9). The N-terminal region of the precursor is highly variable across species. Prepro–U-II mRNA has been described in a range of cell types, including vascular smooth muscle cells, endothelial cells, neuronal cells, and cardiac fibroblasts. Multiple monobasic and polybasic amino acid sequences have been identified as posttranslational cleavage sites of the prohormone. However, a specific U-II–converting enzyme has not yet been described. With respect to its tissue distribution, immunohistochemical staining has identified U-II protein in the blood vessels of various organs and also within the tubule epithelial

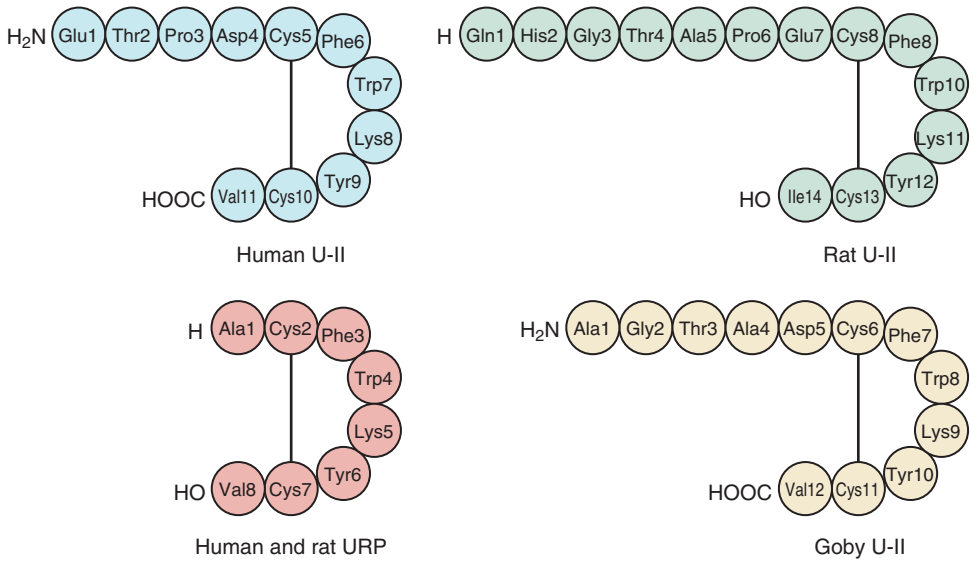

Fig. 11.9 Molecular structure of human, rat, and goby urotensin II (U-II). *URP,* Urotensin-related peptide. (Modified from Ashton N. Renal and vascular actions of urotensin II. *Kidney Int.* 2006;70:624–629.)

cells of the kidneys.[507,511,512] A significant arteriovenous gradient exists across the heart, liver, and kidneys, which indicates that these organs are important sites of U-II production.[513]

In 1999, Ames and colleagues[509] identified U-II as the ligand for the previously orphan rat receptor GPR14/SENR. The U-II receptor (commonly referred to as the UT receptor) is a seven-transmembrane, GPCR, encoded on chromosome 17q25.3 in humans,[514] that bears structural similarity to both somatostatin receptor subtype 4 and the opioid receptors. Ligand binding of the receptor results in G protein–mediated activation of PKC, calmodulin, and phospholipase C; evidence also links MAPKs ERK1/2, the Rho kinase pathway, and peroxisome proliferator-activated receptor α in the intracellular signaling cascade.[515–518]

The relationship between U-II and the UT receptor is not exclusive; the receptor also binds alternative U-II fragments such as U-II(4-11) and U-II(5-11), as well as urotensin-related peptide (URP).[519,520] URP was originally isolated from rat brain and binds with high affinity to the UT receptor.[520] Although this 8–amino acid peptide retains the cyclic hexapeptide sequence, it is derived from a different precursor to U-II and may have different physiologic properties.[521]

PHYSIOLOGIC ROLE OF UROTENSIN II

U-II is the most potent vasoconstrictor known, being 16 times more potent than ET-1 in the isolated rat thoracic aorta.[509] However, its vasoconstrictive properties are not universal, varying between species and between vascular beds. For example, U-II has little or no effect on venous tone, and it does not cause constriction of rat abdominal aorta, femoral arteries, or renal arteries.[522] It also lacks systemic pressor activity when administered intravenously to anesthetized rats.[509,523] In cynomolgus monkeys, bolus intravenous injection of U-II induced myocardial depression, circulatory collapse, and death.[509] In contrast to the vasoconstrictive properties of vascular smooth muscle UT receptor, endothelial UT receptor may mediate vasodilation in pulmonary and mesenteric vessels.[524] The response to U-II may be dependent on the caliber of the artery; a small-vessel response is more endothelium mediated, and a large-vessel response is more dependent on vascular smooth muscle.[507] These disparities are among many examples of how the role of U-II may be influenced by a number of factors, including animal model, vascular bed, method of exogenous U-II administration, and the presence of comorbid conditions.

UROTENSIN II IN THE KIDNEY

The kidneys are major sites of U-II production; this is indicated by both the arteriovenous gradient of plasma U-II across the kidneys and the observation that urinary U-II clearance exceeds urinary creatinine clearance.[507,513] In fact, in humans, urinary concentrations of U-II are approximately three orders of magnitude higher than plasma concentrations.[525] U-II is present in a number of kidney cell types, including the smooth muscle cells and endothelium of arteries, proximal convoluted tubules, and particularly the distal tubules and collecting ducts.[512] UT receptor mRNA is also present in the kidneys, especially within the renal medulla,[525–527] which suggests that the peptide may have autocrine or paracrine functions at this site. In addition, URP mRNA has been described in both rat and human kidneys.[520,526,527] Studies of the role of U-II in normal renal physiology have yielded conflicting findings. In one report, continuous infusion of U-II into the renal artery of anesthetized rats caused NO-dependent increases in GFR, urinary water excretion, and urinary sodium excretion.[528] By contrast, another study showed that bolus injection of picomolar concentrations of U-II produced a dose-dependent decrease in GFR and a reduction in urine flow and urinary sodium excretion.[527] Furthermore, a third group of researchers reported that intravenous bolus injection of U-II in nanomolar amounts induced only a minor reduction in GFR and had no effect on sodium excretion.[529] These researchers also investigated the effect of U-II administration in the context of experimental congestive heart failure, in which the peptide induced an almost 30% increase in GFR.[529]

OBSERVATIONAL STUDIES OF UROTENSIN II IN RENAL DISEASE

Variations in the concentration of U-II in the plasma and urine have been found in a number of diseases with, again, sometimes conflicting results. U-II levels in plasma may be increased in hypertensive individuals in comparison with normotensive controls and are correlated with systolic blood pressure.[530] In one study, U-II concentrations in plasma were increased twofold in patients with renal disease not on hemodialysis and threefold in patients on hemodialysis.[531] In a separate study, the same investigators observed U-II levels in both plasma and urine to be higher in patients with type 2 diabetes and renal disease than in such patients with normal renal function.[532] Higher U-II levels in urine have been described in patients with essential hypertension, in patients with glomerular disease and hypertension, and in patients with renal tubule disorders, but not in normotensive patients with glomerular disease.[525] Increased expression of both U-II and the UT receptor have also been demonstrated in biopsy samples of patients with diabetic nephropathy[533]; increased U-II levels have also been described in glomerulonephritis[534] and in minimal change disease.[535] By contrast, a more recent study described a reduction in U-II levels with CKD. Here, investigators reported that plasma U-II concentrations were highest in healthy individuals, lower in individuals with ESRD, and lowest in subjects with non-ESRD CKD, while hypothesizing that the discordance with earlier work may reflect the different populations studied or the assays used.[536]

INTERVENTIONAL STUDIES OF UROTENSIN II IN THE KIDNEY

Both peptide and nonpeptide UT receptor antagonists have been studied. Urantide is a derivative of human U-II.[537] Continuous infusion of urantide into rats induces an increase in GFR and natriuresis,[527] although it is not clear whether the natriuresis is a consequence of altered renal vascular tone or a direct effect of U-II on the tubule epithelium. Whereas urantide is a potent antagonist of the rat UT receptor,[537] it has been found to have agonist properties in cells expressing the human UT receptor.[538] An alternative U-II peptide antagonist, UFP-803, also has partial agonist properties in human UT receptor–expressing cells,[539] which complicates the interpretation of a peptide-based approach to U-II inhibition. Two compounds in the nonpeptide group of U-II antagonists

have been studied: palosuran (ACT-058362) and SB-611812. Intravenous administration of palosuran protected against renal ischemia in a rat model.[540] The same compound was also studied in rats with streptozotocin-induced diabetes, in which it was found to significantly reduce the severity of albuminuria.[541] In a study of 19 individuals with type 2 diabetes and macroalbuminuria, palosuran attenuated urine albumin excretion after 2 weeks.[542] However, in a subsequent 4-week study of 54 individuals with type 2 diabetes, hypertension, and nephropathy, palosuran had no effect on albuminuria, blood pressure, GFR, or renal plasma flow,[543] effectively halting the development of this drug for this indication. SB-611812 decreased the carotid intima-to-media ratio in a rat model of balloon angioplasty–induced stenosis.[544] The same compound attenuated myocardial remodeling and was associated with a reduced rate of mortality in a rat model of ischemic cardiomyopathy.[545,546] At present, there are no reports of the effect of SB-611812 in renal disease.

Complete reference list available at ExpertConsult.com.

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BOARD REVIEW QUESTIONS

1. The predominant site for angiotensinogen synthesis is?
   a. The juxtaglomerular apparatus
   b. Vascular endothelium
   c. Pericentral zone hepatocytes
   d. Peritubular interstitial fibroblasts

   Answer: c

   Rationale: The RAS is a multi-organ endocrine system wherein specific cell types have key roles. Understanding which cell in which organ does what is an integral part of knowing how the system works.

2. Acute angiotensin type 1 receptor stimulation causes all but one of the following?
   a. Elevated intraglomerular pressure (P_{GC})
   b. Reduction in the ultrafiltration coefficient (K_f)
   c. Proteinuria
   d. Increased afferent and efferent arteriolar tone

   Answer: c

   Rationale: The effects of angiotensin II and the activation of its type 1 receptor on glomerular hemodynamics is pivotal in understanding the role of the RAS in physiology, pathophysiology and therapeutics.

3. Concerning angiotensin-converting enzyme 2, which of the following are true?
   a. Shares >85% homology with angiotensin-converting enzyme in terms of its amino acid sequence
   b. Cleaves angiotensin II to angiotensin III
   c. Is the receptor for the severe adult respiratory syndrome (SARS) coronavirus
   d. Within the glomerulus is expressed predominantly in mesangial cells

   Answer: c

   Rationale: Angiotensin converting enzyme 2 is a relatively new but important addition to the RAS. Knowledge of its origin and actions are necessary to fully appreciate the complexites of the system.

4. B-type natriuretic peptide binds to which of the following receptors?
   a. Natriuretic peptide receptor-A
   b. Natriuretic peptide receptor-B
   c. Natriuretic peptide receptor-B and natriuretic peptide receptor-C
   d. Natriuretic peptide receptor-A and natriuretic peptide receptor-C

   Answer: d

   Rationale: The nomenclature for the natriuretic peptide receptors can be confusing. NPR-A binds ANP and BNP. NPR-B binds CNP and NPR-C is a clearance receptor for all three peptides.

5. Which of the following is not a known substrate of neprilysin?
   a. Vascular endothelial growth factor-A
   b. Amyloid β-peptide
   c. C-type natriuretic peptide
   d. Vasopressin

   Answer: a

   Rationale: Neprilysin is a zinc metalloproteinase that catalyzes the degradation of the natriuretic peptides and that has activity against over 40 other substrates (Table 11.2). Vascular endothelial growth factor-A is not amongst the reported substrates of neprilysin.

12

Aldosterone and Mineralocorticoid Receptors: Renal and Extrarenal Roles

David Pearce | Vivek Bhalla | John W. Funder

CHAPTER OUTLINE

KEY POINTS

- Inappropriate aldosterone hypersecretion relative to sodium status is much more common in hypertensives (5%–15%) than is generally appreciated and is even found in a significant percentage of normotensives.
- Most unilateral aldosterone-producing adenomas harbor disease-causing gene mutations, the most common of which is in the K^+ channel gene, *KCNJ5*. Germline mutations in *KCNJ5* cause familial bilateral adrenal hyperplasia. Most recently, the chloride channel gene, *CLCN2,* has been implicated in early-onset primary aldosteronism, with clinical findings most consistent with bilateral hyperplasia.
- Recent evidence supports the idea that the actions of aldosterone on the distal convoluted tubule are indirect and mediated by changes in K^+. Aldosterone stimulates ENaC in connecting tubule and cortical collecting ducts, and the ensuing hypokalemia acts directly in DCT cells to stimulate the Na-Cl cotransporter, NCC.
- SGK1 gene transcription is regulated by aldosterone, and it in turn stimulates ENaC, thereby enhancing Na^+ reabsorption and K^+ secretion. SGK1 also responds to and integrates a variety of other hormonal and nonhormonal signals, including insulin and IL-17, which stimulate SGK1 activity and contribute to salt-sensitive hypertension.
- Studies in mice demonstrate a role for T cells in the pathogenesis of hypertension through a mechanism involving IL-17A and SGK1. Mice lacking T cell SGK1 are protected from angiotensin II induced hypertension and renal inflammation.
- The classic thinking that Na^+ is dissolved, fully exchangeable, and osmotically active in both intracellular and extracellular compartments has been proving inadequate. Recent studies have identified a nonexchangeable pool of Na^+ in extracellular compartments, most notably in subcutaneous tissues in an osmotically inactive form, perhaps sequestered within negatively charged glycosaminoglycans.

In mammals, the control of extracellular fluid volume and blood pressure is intimately intertwined with the regulation of epithelial ion transport. Aldosterone, which is essential for survival, is the central hormone regulating the relevant epithelial transport processes, particularly of ions such as Na^+, K^+, and Cl^-. All circulating aldosterone is generated in the adrenal glomerulosa, where its synthesis and secretion are under the control of angiotensin II and potassium, and its major epithelial actions occur in the distal nephron and colon. The former extends from the late distal convoluted tubule (DCT) through the connecting segment and the entire cortical and medullary collecting ducts. These segments, rich in mineralocorticoid receptor (MR), are often referred to as the "aldosterone-sensitive distal nephron" (ASDN).[1] Most, if not all, effects of aldosterone are mediated by MR, a hormone-regulated transcription factor related closely to the glucocorticoid receptor and more distantly to other members of the nuclear receptor superfamily. The physiologic effects of aldosterone on epithelia entail direct gene-regulatory actions of MR. Thus, a sound foundation for understanding aldosterone's physiologic effects on the extracellular fluid, blood pressure, and electrolyte concentrations can be understood through familiarity with the MR-dependent effects on the transcription of various genes, which, in turn, alter epithelial ion transport. Aldosterone actions in certain disease states involve both genomic and nongenomic effects in epithelial and nonepithelial tissues. Furthermore, physiologic and pathophysiologic effects of cortisol are also mediated in part by MR, which binds cortisol with high affinity. This chapter addresses the cellular and molecular mechanisms underlying aldosterone—and, to some extent, cortisol—action, focusing primarily on effects on ion transport in epithelia but also highlighting key aspects of nonepithelial actions, which are of substantial importance to its pathophysiologic effects.

GENERAL INTRODUCTION TO ALDOSTERONE AND MINERALOCORTICOID RECEPTORS

Steroid hormones are derived from cholesterol and produced in systemically relevant amounts in a relatively narrow range of tissues (e.g., adrenal glands, gonads, placenta, skin). In mammalian physiology, six classes of steroid hormones are commonly recognized— mineralocorticoid, glucocorticoid, androgen, estrogen, progestin, and the secosteroid vitamin D_3. This classification was based on observed effects of these hormones and has proven robust, despite current appreciation of a much more diverse physiology of steroid hormones over and above their classic roles. In further support of this original classification is the characterization of six intracellular receptors—MR; glucocorticoid, GR; androgen, AR; estrogen, ER; progestin, PR; and vitamin D_3, VDR. As further addressed later, it is now appreciated that a one-to-one relationship between receptor and hormone does not hold, and this is particularly the case for MR.

Aldosterone was isolated and characterized in 1953. Crucial for its isolation was the application of radioisotopic techniques to measure $[Na^+]$ and $[K^+]$ flux across epithelia in the laboratory of Sylvia Simpson, a biologist, and Jim Tait, a physicist.[2,3] Because of this, the active principle was initially called "electrocortin"; the name was soon changed to aldosterone when its unique aldehyde (rather than methyl) group at carbon 18 was discovered in collaborative studies between investigators in London and Basel.[4] Aldosterone is commonly depicted so as to highlight this aldehyde group (Fig. 12.1, *right*). In vivo, the very reactive aldehyde group cyclizes with the β-hydroxyl group at carbon 11 to form the 11,18- hemiacetal and, in addition, may exist in an 11,18-hemiketal form. This cyclization of the 11β-hydroxyl group protects aldosterone from dehydrogenation by the enzyme 11β-hydroxysteroid dehydrogenase in epithelial tissue and by some neuronal and smooth muscle cells, which enables it to activate epithelial MR and thus regulate ion transport at very low (subnanomolar) circulating levels.[5,6]

There is broad evidence that aldosterone is not the only cognate ligand for MR, its essential effects via MR on epithelial ion transport notwithstanding. MR is found in high abundance in the hippocampus and cardiomyocytes and, in these nonepithelial tissues—which lack 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2; see later, "11β-Hydroxysteroid Dehydrogenase Type 2")—they are essentially constitutively occupied by glucocorticoids (cortisol in humans, corticosterone in rodents). This is due to the comparable affinity and markedly higher plasma free levels (≥100-fold) of endogenous

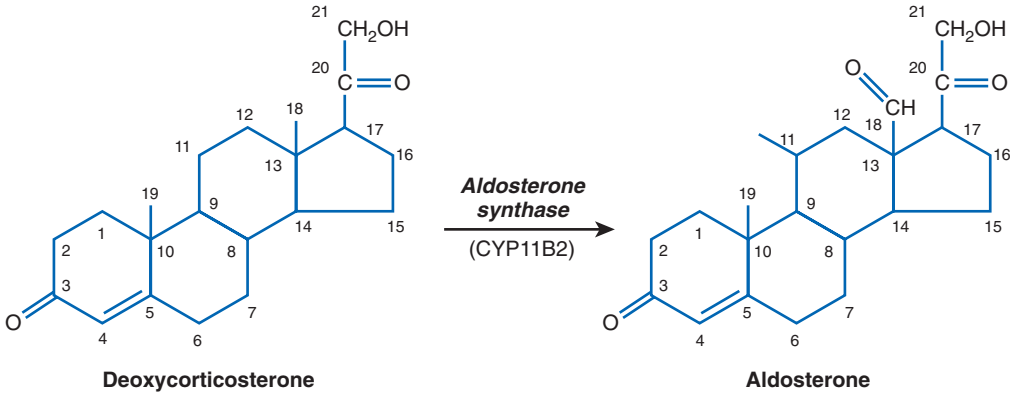


Fig. 12.1 Final step in aldosterone synthesis. Note that the aldehyde form of aldosterone is shown. Most aldosterone (>99%) exists as the hemiacetal form, which is cyclized and does not allow access of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) to the 11-hydroxyl. See text for details.

glucocorticoids compared with those of aldosterone. In terms of evolution, MR appeared well before aldosterone synthase (e.g., in fish).[7] It was commonly assumed that MR and GR share a common immediate evolutionary precursor,[8] although this has been challenged on sequence grounds,[9] which implicate MR as the first of the MR-GR-AR-PR subfamily to branch off an ancestral receptor. A final reason not to equate MR and aldosterone action derives from a comparison of the MR knockout and aldosterone synthase knockout ($AS^{-/-}$) phenotypes. MR knockout mice (which lack all functional MR) cannot survive sodium restriction; $AS^{-/-}$ mice (which have no detectable aldosterone) survive even stringent sodium restriction but die when their fluid intake is restricted to that of wild-type animals.[10] The survival of $AS^{-/-}$ mice on a low-Na^+ intake may reflect, in part, Na^+ retention via renal tubular intercalated cells, in which MRs (not 11β-HSD2–protected) are activated by glucocorticoids in the context of high ambient angiotensin concentrations.[11,12] Their inability to survive fluid restriction suggests an as yet poorly defined dependence on aldosterone for vasopressin action.[13] Potassium homeostasis is also surprisingly intact in $AS^{-/-}$ mice, although they do not tolerate extremes of K^+ loading.[13a]

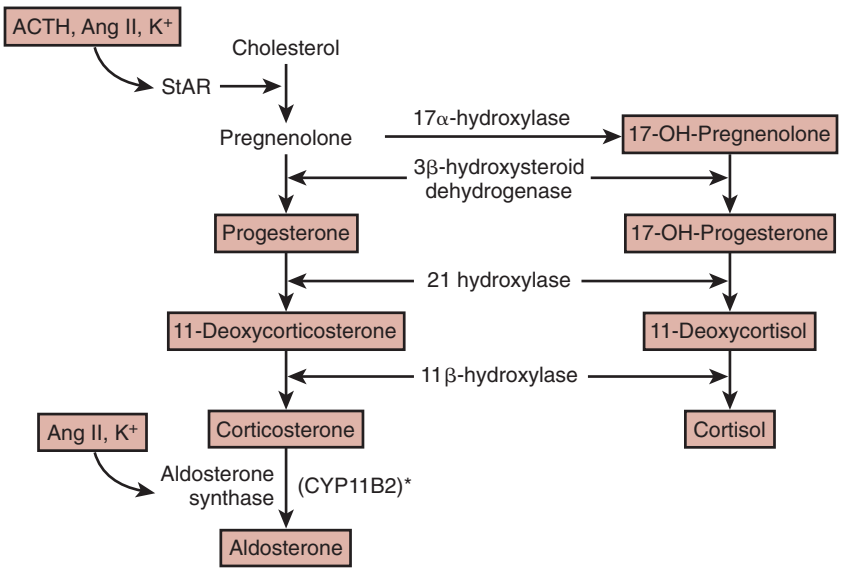
ALDOSTERONE SYNTHESIS

Aldosterone is synthesized in the adrenal cortex, which has three functional zones. The outermost layer of cells represents the zona glomerulosa, which is the unique site of aldosterone biosynthesis in normal physiology (see later; aldosterone is produced in excessive amounts in patients with glucocorticoid-remediable aldosteronism). Cortisol is synthesized in the middle zone, the zona fasciculata, and the innermost zona reticularis secretes adrenal androgens in many species, including humans, but not in rats or mice. Normally, the glomerulosa secretes aldosterone at the rate of 50 to 200 μg/day to give plasma levels of 4 to 21 μg/dL; in contrast, secretion of cortisol is at levels 200- to 500-fold higher. Underlying the separate synthesis of cortisol and aldosterone is expression of the enzyme 17α-hydroxylase uniquely in the zona fasciculata and that of aldosterone synthase uniquely in the glomerulosa.

In most species, aldosterone synthase, or cytochrome P450 (CYP) enzyme 11B2, is responsible for the conversion of deoxycorticosterone to aldosterone in a three-step process of sequential 11β-hydroxylation, 18-hydroxylation, and 18-methyl oxidation, to produce the characteristic C18-aldehyde from which aldosterone derives its name (see Fig. 12.1, *left*). Although CYP11B2 is distinct from CYP11B1 (11β-hydroxylase) in most species,[14,15] in some species (e.g., bovine), only a single CYP11B is expressed. How this enzyme is responsible for the three-step process of aldosterone synthesis in the glomerulosa but not the fasciculata has yet to be determined.

Fig. 12.2 also illustrates key steps in the biosynthesis of cortisol to illustrate the overlap and similarities with those of aldosterone. The genes encoding CYP11B1 and CYP11B2 lie close to one another on human chromosome band 8q24.3, so that an unequal crossing over at meiosis has been shown to be responsible for the syndrome of glucocorticoid-remediable aldosteronism (now known as "familial hyperaldosteronism type I"), in which the 5′ end of the CYP11B1 is fused to the 3′ end of CYP11B2. The chimeric gene product[16] is expressed in the fasciculata and responds to adrenocorticotropic hormone (ACTH) with aldosterone synthesis, producing a syndrome of juvenile-onset hyperaldosteronism and hypertension.

Normal glomerulosa secretion of aldosterone is primarily regulated by angiotensin II in response to posture and acute



*Aldosterone synthase = CYP11B2

Fig. 12.2 Overview of aldosterone synthetic pathway showing key regulatory nodes. Note that adrenocorticotropic hormone *(ACTH)*, angiotensin II *(Ang II)*, and K^+ regulate steroidogenic acute regulatory protein *(StAR)*, which stimulates cholesterol uptake by mitochondria and thus substrate availability for synthesis of all of the steroid hormones. Aldosterone synthase (gene name, *CYP11B2*), which is selectively expressed in the adrenal glomerulosa, mediates the final step in aldosterone synthesis. It is also regulated by Ang II and K^+. Aldosterone synthesis is shown on the left. Cortisol synthesis is also shown *(right)* to emphasize the interconnections and similarities between these pathways.

lowering of circulating volume, to plasma [K^+] in response to elevated potassium levels, particularly in settings of Na^+ deficiency,[17] and to ACTH to the extent of entrainment of the circadian fluctuation in plasma aldosterone levels with those of cortisol. Aldosterone secretion is lowered by high levels of atrial natriuretic peptide and by the administration of heparin, somatostatin, and dopamine. As yet, incompletely characterized molecules of adipocyte origin have been shown to stimulate aldosterone secretion in vitro, and roles in the metabolic syndrome have been proposed on this basis.[18]

Angiotensin and plasma [K^+] stimulate aldosterone secretion primarily by increasing the expression and activity of key steroidogenic enzymes, as well as the steroidogenic acute regulatory protein (StAR).[19] StAR is required for cholesterol transport into mitochondria and hence for its availability for steroid synthesis.[20] Regulated steroidogenic enzymes include side chain cleavage enzyme 3β-hydroxysteroid dehydrogenase and, most notably, aldosterone synthase. Common to the mechanism of stimulation by angiotensin II and [K^+] is elevation of intracellular [Ca^{2+}]. Angiotensin II activates the G-protein–coupled angiotensin type I receptor (AT1R) in the glomerulosa cell membrane, which in turn activates phospholipase C, which catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol trisphosphate (IP3) and diacylglycerol (DAG). DAG stimulates protein kinase C, whereas IP3 stimulates Ca^{2+} release from intracellular stores, both of which affect the aldosterone biosynthetic pathway. AT1R also separately stimulates Ca^{2+} influx, which is important for sustained stimulation of aldosterone secretion.[21] Elevated [K^+] increases intracellular [Ca^{2+}] by depolarizing the cell membrane and activating voltage-sensitive Ca^{2+} channels.[22,23] Patients taking angiotensin-converting enzyme inhibitors or angiotensin receptor blockers usually show a degree of suppression of aldosterone secretion, reflected in a modest (0.2–0.3 mEq/L) elevation in plasma [K^+] levels. This is often sufficient to establish a new steady state, with plasma aldosterone levels rising into the normal range, a process best termed "breakthrough" rather than "escape," given the time-honored usage of the latter to refer to the escape from progression of the salt and water effect of mineralocorticoid excess in the medium and long term.[24]

Studies have shed new light on the regulation of aldosterone production by the adrenal glomerulosa in health and disease. Choi and associates have found recurrent somatic mutations in the K^+ channel Kir3.4 (encoded by the gene *KCNJ5*), which were present in more than one-third of spontaneous human aldosterone-producing adenomas studied.[25] These mutations increased Na^+ conductance through Kir3.4 and resulted in increased Ca^{2+} entry and enhanced aldosterone production and glomerulosa cell proliferation. Interestingly, an inherited mutation in *KCNJ5* is associated with hypertension associated with marked bilateral adrenal hyperplasia (now known as "familial hyperaldosteronism type III" [FH-III]).[26] These findings suggest that *KCNJ5* may provide tonic inhibition of aldosterone production and glomerulosa cell proliferation. In glomerulosa cells harboring the mutant channel, both proliferation rate and aldosterone synthesis are increased. These initial studies were continued and extended by a much wider survey by Boulkroun and colleagues[27]; subsequently, less common but similarly somatic mutations in the adrenal cortex *(ATP1A1, ATP2A3, CACNA1D,* and *CTNNB1)* have been associated with hyperaldosteronism caused by adrenal adenomas.[28,29]

MECHANISMS OF MINERALOCORTICOID RECEPTOR FUNCTION AND GENE REGULATION

Mammals cannot survive without MR, except with substantial NaCl supplementation. This member of the nuclear receptor superfamily appears to have both genomic and nongenomic actions; however, the latter do not appear to play a significant role in the control of epithelial ion transport. This section thus focuses exclusively on the function of MR as a hormone-regulated transcription factor.

MINERALOCORTICOID RECEPTOR FUNCTION AS A HORMONE-REGULATED TRANSCRIPTION FACTOR: GENERAL FEATURES AND SUBCELLULAR LOCALIZATION

In the presence of agonists, MR binds to specific genomic sites and alters the transcription rate of a subset of genes. Fig. 12.3 shows the fundamental paradigm of MR function. All nuclear receptors shuttle in and out of the nucleus; however, in the absence of hormone, some, such as the estrogen and vitamin D receptors, are predominantly nuclear, whereas others, like GR, are almost exclusively cytoplasmic. In the absence of hormone, MR is distributed relatively evenly between nuclear and cytoplasmic compartments but, in the presence of hormone, it is highly concentrated in the nucleus (Fig. 12.4).[30,31] It is also notable that in addition to this marked

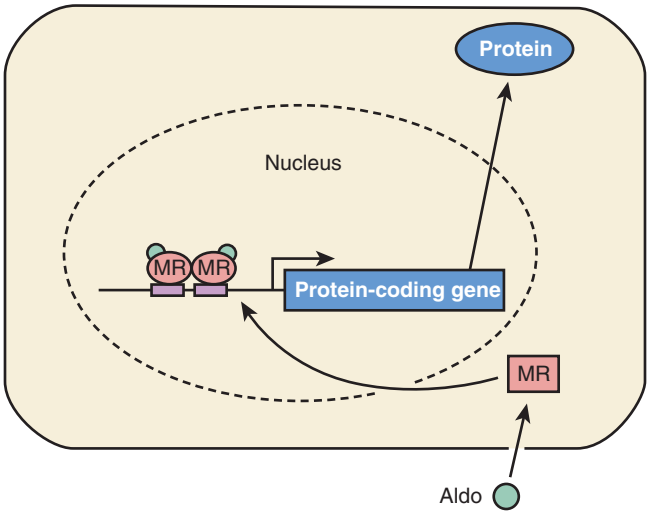


Fig. 12.3 General mechanism of aldosterone action through the mineralocorticoid receptor *(MR).* This simple schematic shows the general features of MR regulation of a "simple" hormone response element (HRE), common to a large subset (but not all) of aldosterone-stimulated genes. Note that in the absence of hormone, MR is found in both nucleus and cytoplasm (see Fig. 12.4). Aldosterone *(Aldo)* triggers nuclear translocation of cytoplasmic MR, binding as a dimer to HREs, and stimulation of transcription initiation complex formation (*arrow,* upstream of the so-called protein-coding gene, defines the site of transcription initiation). See text for further details.

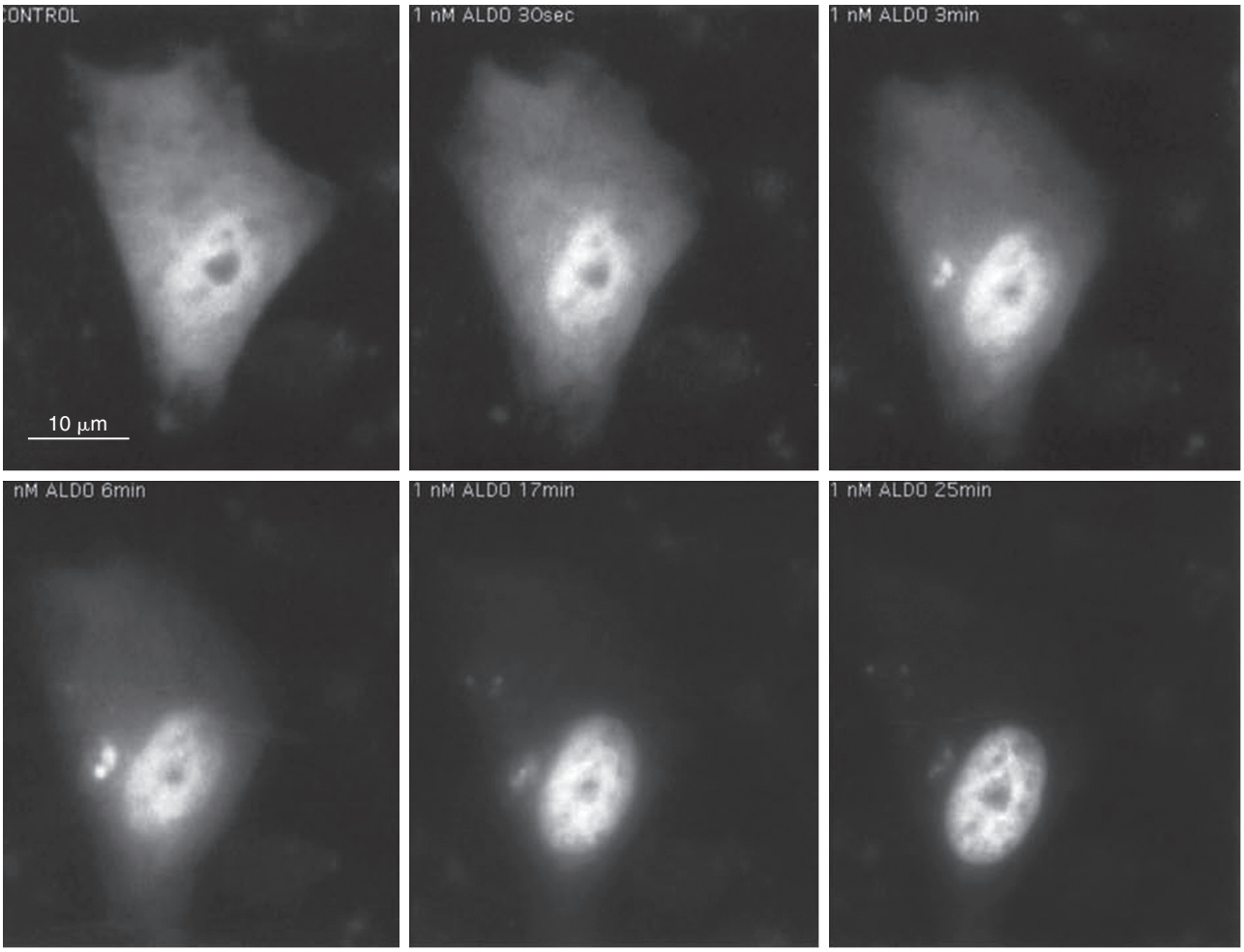


Fig. 12.4 Time-dependent nuclear translocation of the mineralocorticoid receptor (MR) in the presence of aldosterone. Cultured cells expressing green fluorescent protein–MR fusion protein (GFP-MR) were grown in a steroid-free medium and treated with 1 nmol/L aldosterone. Translocation of GFP-MR was followed in real time, and images were captured at indicated times. It is notable that the nuclear accumulation of GFP-MR started within 30 seconds, was half-maximal at 7.5 minutes, and was complete by 10 minutes. Control: MR distribution before addition of aldosterone. (From Fejes-Toth G, Pearce D, Naray-Fejes-Toth A. Subcellular localization of mineralocorticoid receptors in living cells: effects of receptor agonists and antagonists. *Proc Natl Acad Sci U. S. A.* 1998; 95[6]:2973–2978.)

change in MR cellular distribution, its subnuclear organization and protein-protein interactions are also changed.[31] Like its close cousin the GR, the unliganded MR (in the absence of hormone) is complexed with a set of chaperone proteins, which include the heat shock proteins hsp90, hsp70, and hsp56 and immunophilins.[32,33] This chaperone complex is essential for several aspects of MR function, notably high-affinity hormone binding and trafficking to the nucleus.[33] It was thought for many years that after binding hormone, the hsp90-containing chaperone complex is jettisoned. However, it has become clear that this complex remains associated with the receptor and plays an important role in nuclear trafficking.[32] Several members of the immunophilin family, including FKBP52, FKBP51, and CyP40, are present in the chaperone complex and provide a bridge between hsp90 and the cytoplasmic motor protein dynein, which moves the receptor-hsp90 complex retrogradely along microtubules to the nuclear envelope.[33] Here, the receptor is handed off to the nuclear pore protein, importin-α, and translocated into the nucleus, where it functions as a transcription factor, stimulating the transcription of certain genes and repressing the transcriptional activity of others. In the regulation of ion transport, stimulation of key target genes is paramount. Transcriptional repression may be essential for effects in nonepithelial cells, including neurons, cardiomyocytes, smooth muscle cells, and macrophages.[34]

DOMAIN STRUCTURE OF MINERALOCORTICOID RECEPTORS

The MRs of all vertebrates are highly conserved. There are only minor differences between MRs in rodents and MRs in humans.[34] In general, the steroid and nuclear receptors have been divided into three major domains (Fig. 12.5):

1. An N-terminal transcriptional regulatory domain
2. A central DNA-binding domain (DBD)
3. A C-terminal ligand/hormone-binding domain (LBD)

Each of these broadly defined domains has more than one function, and not all the functions can be neatly assigned to separate distinct domains; however, much of the action

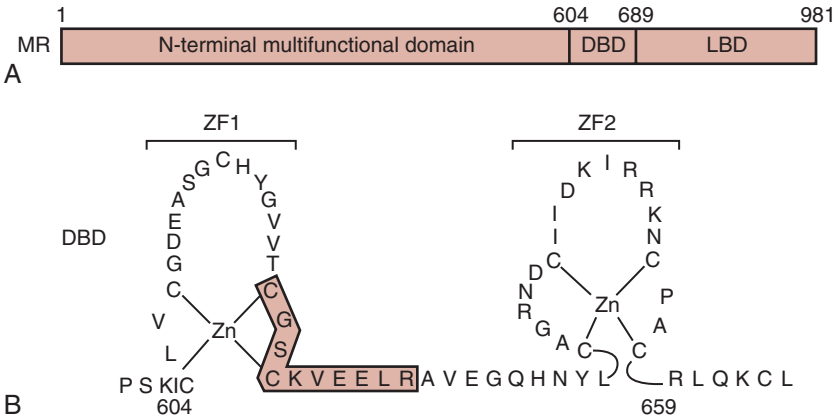


Fig. 12.5 Domain structure of the mineralocorticoid receptor *(MR).* Three major domains have been defined, which are common to all steroid and nuclear receptors. Further refinements have led some to use a six-letter system, which is shown; however, this adds little to the understanding of receptor structure or function, and we prefer the three–global domain system. These large receptor sections should not be confused with the many small functional domains that have been identified, as discussed in the text. The size and amino acid designations used here are for rat MR (981 amino acids total); they apply with minor variations to human MR (984 amino acids total). (A) Strip diagram of MR. The N-terminus is to the *left,* C-terminus to the *right.* (B) Schematic of MR DNA-binding domain *(DBD)*, showing the two zinc fingers and the positions of the coordinating Zn ions. *Boxed region* is the alpha helix, which intercalates into the major groove of the DNA and provides the major protein–DNA interaction contacts. The dimerization interface comprises amino acids within the second zinc finger, which form van der Waals and salt bridge interactions. *LBD,* Ligand/hormone–binding domain.

of MRs can be understood from this point of view. In the following sections, the domains are described roughly in the historical order in which they were characterized, which also parallels the clarity of functional and structural knowledge about them.

DNA-BINDING DOMAIN

The essential quality of MR function as a transcription factor is its ability to bind specifically to DNA. This protein-DNA interaction is mediated by the receptor's compact modular DBD (amino acids 603–688 of human MR; Fig. 12.5 shows a strip diagram and two-dimensional structure), which forms a variety of contacts with a specific 15-nucleotide DNA sequence termed a "hormone response element" (HRE). Receptor binding to the HRE in the vicinity of regulated genes promotes the recruitment of coactivators and components of the general transcription machinery, such as the TATA-binding protein, which binds to the thymidine- and adenosine-rich DNA sequence found upstream of many genes and is required for correct transcription initiation. These types of HREs have been identified near or in many of the key MR-regulated genes, such as serum- and glucocorticoid-regulated kinase 1 *(SGK1),* glucocorticoid-induced leucine zipper *(GILZ),* and amiloride-sensitive sodium channel subunit α *(α-ENaC).* Although, in many cases, differential binding to HREs is a key determinant of the specificity of many transcription factors, it should be noted that some steroid receptors (notably MR and GR) have only minor (<10%) differences within this domain and have identical DNA-binding properties.[35] Specificity in these cases is determined through other mechanisms.[34,36]

The canonical MR HRE is a 15-nucleotide sequence that forms a partial palindrome (inverted repeat), which binds a receptor homodimer. A dimer interface embedded within the DBD is essential for MR to form these requisite homodimers, as well as to form heterodimers with GR.[37,38] Mutations that disrupt this interface have complex effects on receptor activity in animals[39] and cultured cells,[40] and similar mutations in other receptors (AR in particular) result in disease states.[41] Also, in at least one kindred of the autosomal dominant form of pseudohypoaldosteronism type I, an MR DBD mutation appears to be causative, although the mechanistic basis has not been elucidated.[42]

In addition to supporting DNA binding and dimerization, the DBD also harbors a nuclear localization signal,[43,44] as well as surfaces that contact distant parts of the receptor and that mediate interactions with other proteins, as has been shown for GR and, in some cases, MR.[45–47]

LIGAND/HORMONE-BINDING DOMAIN

The MR LBD comprises amino acids 689 to 981 (see Fig. 12.5A). Like the DBD, the LBD has multiple functions; in addition to binding with high affinity to various MR agonists and antagonists, it also harbors interaction surfaces for coactivators, dimerization, and N-C interactions.[48–50] MR is distinct from GR in that it binds with equally high affinity to cortisol, corticosterone (the physiologic glucocorticoid in rats and mice), and aldosterone. Indeed, as discussed later, MR appears to function as a high-affinity glucocorticoid receptor in some tissues, including the brain and heart.

High-resolution representations of the crystal structures of wild-type and mutant MR have identified the structural features of the LBD and specific amino acid contacts involved in binding to the mineralocorticoid desoxycorticosterone (Fig. 12.6).[51,52] Key features include the following:

1. The LBD of MR, like that of other nuclear receptors, is arranged into 11 α-helices and four small β-strands.
2. The C-terminal alpha helix (α-helix), H12, contains the activation function AF2.
3. Ligand is deeply embedded into a pocket comprising α-helices H3, H4, H5, H7, and H10 and two beta strands (β-strands); numerous contacts are made between amino acids of the pocket and hormone.

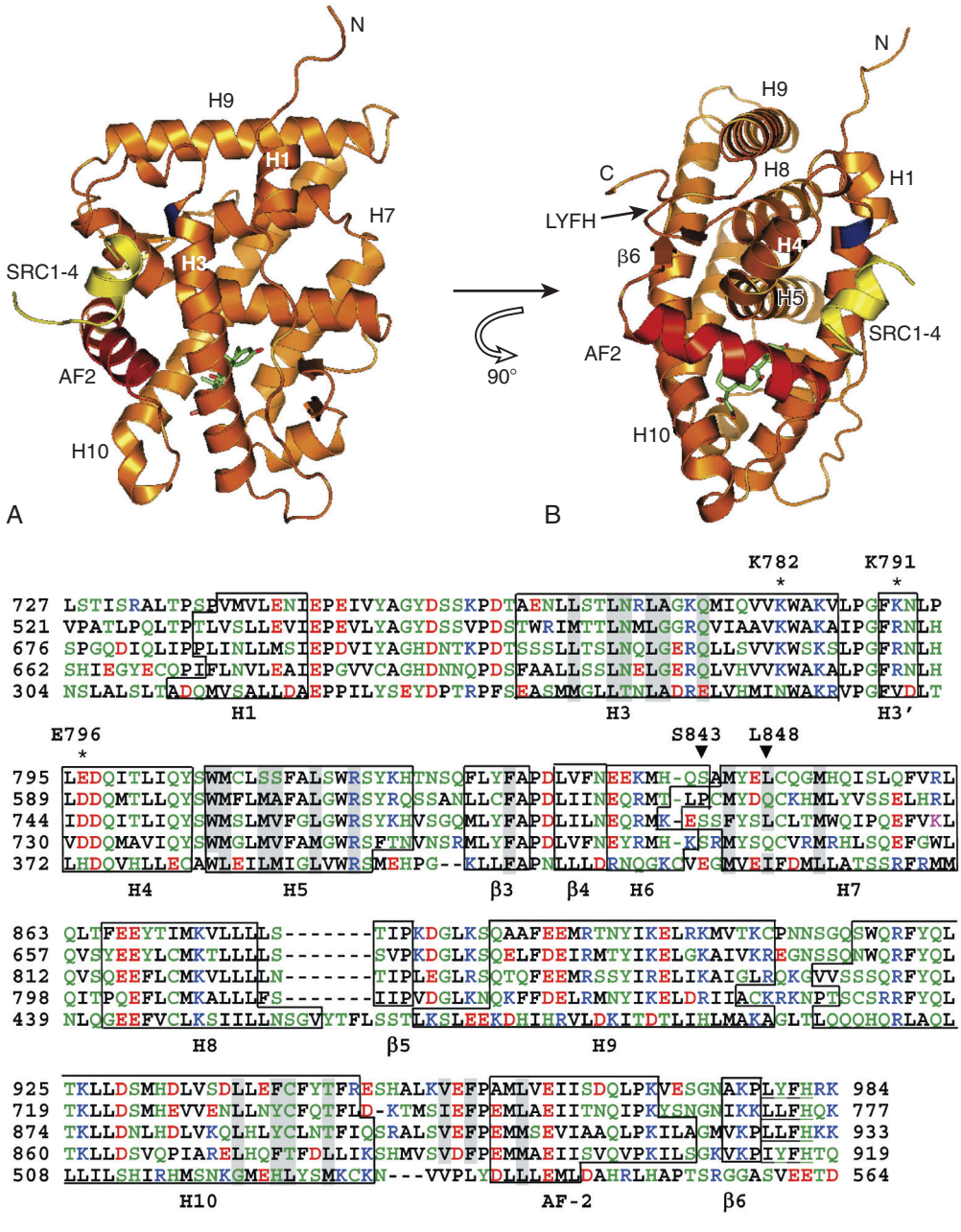


Fig. 12.6 Mineralocorticoid receptor *(MR)* ligand/hormone–binding domain (LBD) crystal structure. Structure of the MR LBD bound to corticosterone and coactivator peptides (SRC1-4). (A and B) Two views of the complex (rotated by 90 degrees about the vertical axis) are shown in this ribbon representation. MR is colored in *gold* and SRC1-4 peptide in *yellow.* Corticosterone, which binds MR with an affinity comparable with that of aldosterone, is shown in a *ball-and-stick* representation. Note that hormone is located in a deep pocket formed by helices 3, 5, and 7 (*H3, H5,* and *H7*), which explains the slow off-rate and high affinity. (C) Sequence alignment of the human MR LBD with other steroid hormone receptors (*GR,* glucocorticoid; *AR,* androgen; *PR,* progesterone; and *ER,* estrogen). Residues that form the steroid-binding pockets are shaded in *gray.* Key structural features for the binding of SRC peptides are noted with *stars,* and the residues that determine MR-GR hormone specificity are labeled by *arrowheads.* See Li and others[394] for further details. (From Li Y, Suino K, Daugherty J, et al. Structural and biochemical mechanisms for the specificity of hormone binding and coactivator assembly by mineralocorticoid receptor. *Mol Cell.* 2005;19:367–380.)

This accounts for the slow off-rate and high affinity of aldosterone, corticosterone, and cortisol for MR.[51] The crystal structure of the mutant (S810L) MR, in which progesterone acts as an agonist rather than an antagonist,[53] reveals that H12 is stabilized with AF2 in the active conformation.[52] The crystal structure of wild-type MR LBD also provides insight into the mechanisms underlying some forms of pseudohypoaldosteronism type I. Notably, MR/S810L has an LBD mutation in helix 5, which is predicted to disrupt interaction with the steroid ring structure,[54] whereas Q776R and L979P have been demonstrated to have markedly reduced aldosterone binding.[42] Structural analysis reveals that Q776 is located in helix H3 at the extremity of the hydrophobic ligand-binding pocket and anchors the steroid C3–ketone group.

MR binds cortisol and corticosterone with an affinity similar to that of aldosterone. 11β-HSD2 is an essential determinant of aldosterone specificity, through its effect in metabolizing glucocorticoids to their receptor-inactive keto-congeners in collecting duct principal cells, as discussed later. In tissues that do not coexpress 11β-HSD2, the physiologic ligand for MR is cortisol (corticosterone in rats and mice).[55–57] The extent to which such "unprotected" MR can be pathophysiologically activated in aldosterone excess states is discussed later (see "Disease States: Primary Aldosteronism" and "Nonepithelial Actions of Aldosterone").

N-TERMINAL DOMAIN

As its noncommittal name implies, the N-terminal region of MR has diverse functions, which appear to revolve primarily around protein–protein interactions and the recruitment of coactivators and corepressors. It has two potent transcriptional regulatory motifs, usually termed *AF1a* and *AF1b*.[48,58] This domain bears some functional and sequence similarity to the homologous region of GR and is capable of stimulating gene transcription when fused to an unrelated DBD.[48] Overall, however, MR and GR differ markedly in the N-terminal domain, and this region of the receptor is a central determinant of specificity.[59] Other studies have supported the idea that this domain has functional sequences that limit receptor activity through the recruitment of corepressors, in addition to transcriptional activation functions.[47,60] Its role in coactivator and corepressor recruitment is addressed further in the following section.

MINERALOCORTICOID RECEPTOR REGULATION OF TRANSCRIPTION INITIATION: COACTIVATORS AND COREPRESSORS

The major mechanism of MR action is its effects on transcription initiation; however, there may also be effects on transcript elongation.[61,62] Much has been learned about the generation of an initiation complex and the particular roles that steroid receptors play in this process. Several review articles and book chapters have provided in-depth examinations of the biochemistry of the general transcription machinery, transcription initiation, promoter escape, and processive elongation.[63–65] Most of the coactivators identified so far interact with the C-terminal AF2 domain and include the prototypical GRIP1/TIF2 and SRC,[66,67] which sequentially recruit a series of different components of the transcriptional machinery and result in the formation of a preinitiation complex (PIC). This PIC includes all the key components of the transcription machinery, including RNA polymerase II. A detailed picture of MR-dependent PIC formation has not been determined. However, the general features are likely similar to those for ER[68] and involve the sequential recruitment by the receptor of the following: (1) chromatin-remodeling SWI/SNF and CARM1/PRTM1 proteins, which promote chromatin remodeling and initiation of complex formation; (2) histone acetylase CBP/P300 (cyclic adenosine monophosphate [cAMP]–responsive element–binding protein), which promotes an active chromatin conformation[64]; and (3) direct or indirect recruitment of the TATA-binding protein and other components of the general transcription machinery.[68]

The aforementioned mechanisms are generic and are used by many transcription factors, including all steroid receptors, through interactions with the C-terminal AF2 domain. The N-terminal region of MR, which harbors the AF1 domain, diverges from the other steroid receptors, and other studies have identified coregulators that interact selectively with this receptor domain. ELL (11-19 lysine-rich leukemia factor) is a coactivator for MR that specifically interacts with AF1b and assists in PIC formation.[61] It was originally identified as an elongation factor, and it may also affect transcript elongation. Other specific coregulators include the synergy inhibitory protein PIAS1,[60] Ubc9,[69] and p68 RNA helicase.[70] In many cases, interactions of these regulators with MR require receptor posttranslational modifications—for example, by phosphorylation, acetylation, or sumoylation.[71]

REGULATION OF SODIUM ABSORPTION AND POTASSIUM SECRETION

GENERAL MODEL OF ALDOSTERONE ACTION

Aldosterone enters the cell passively, binds to MR, triggers changes in gene transcription (as addressed later; see "Mechanisms of Mineralocorticoid Receptor Function and Gene Regulation"), and potentially has nongenomic effects. Aldosterone effects in the ASDN have been divided into three major phases: latent, early, and late.[1] This designation goes back to the early observations by Ganong and Mulrow that after aldosterone infusion into experimental animals, no effect was observed for at least 15 to 20 minutes.[72] A similar delay was observed in isolated epithelia.[73] The early phase, which is now known to involve primarily MR-dependent regulation of signaling mediators such as SGK1, culminates in increased apical localization—and, possibly, increased probability of the open state—of EnAc. In the late phase, aldosterone stimulates transcription of a variety of effector genes, including those that encode components of the ion transport machinery, notably the epithelial sodium channel (ENaC) and Na^+-K^+–adenosine triphosphatase (Na^+-K^+-ATPase) subunits. The major direct effect is to increase Na^+ reabsorption, which is accompanied variably by Cl^- reabsorption and/or K^+ secretion and, ultimately, water reabsorption. Aldosterone's actions in the principal cells of the connecting segment and collecting duct (Fig. 12.7) are of primary significance; however, this also has been shown to influence fluid and electrolyte transport in other tubule segments, as well as in other organs. These actions of aldosterone can be surmised from the clinical features of individuals with aldosterone-secreting tumors; they have volume expansion with high blood pressure and are commonly (>50% of patients) hypokalemic.[74,75] In general, the effects of aldosterone on Na^+ absorption and K^+ secretion work together. However, there are ways whereby these actions can be separated, as discussed later.

The two basic cell processes that aldosterone regulates—Na^+ absorption and K^+ secretion—are depicted in Fig. 12.7. Most aspects of this mechanism are relevant to the various aldosterone target tissues.

Na^+-K^+-ATPase, located on the basolateral membrane (blood side), establishes the essential electrochemical gradients that drive ion transport (see Chapters 5 and 6). Importantly, it operates well below its V_{max}, and is seldom, if ever, the rate-limiting step in transepithelial Na^+ transport.[76] Rather, apical

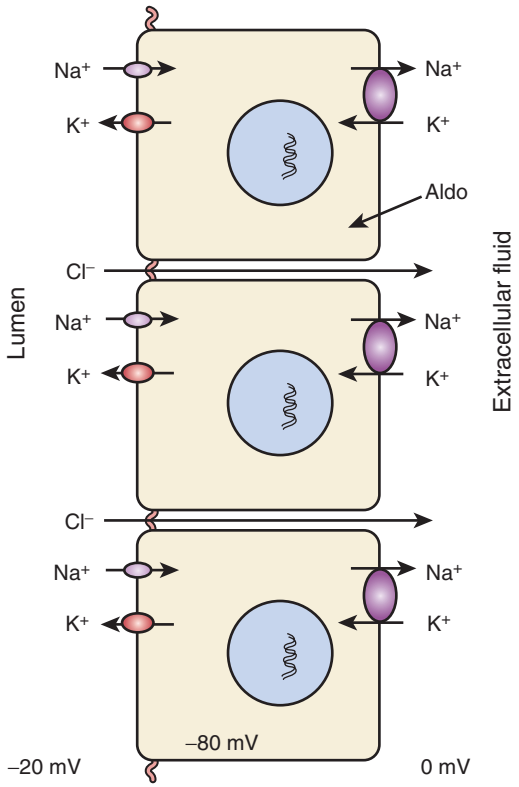


Fig. 12.7 Schematic of principal cells in the aldosterone-sensitive distal nephron (ASDN). The ASDN includes the distal third of the distal convoluted tubule (DCT), connecting tubule, and collecting duct. The Na^+-K^+–adenosine triphosphatase (Na^+-K^+-ATPase) establishes the gradients for passive apical entry of sodium through the epithelial sodium channel (ENaC). Transport of sodium through the ENaC creates a negative lumen potential that drives potassium secretion into the lumen. Potassium is also recycled at the basolateral surface, which facilitates potassium exchange across the Na^+-K^+-ATPase. Chloride (Cl^-) moves via paracellular and transcellular pathways. There is evidence to support aldosterone *(Aldo)* actions in other segments, particularly the sodium-chloride cotransporter–expressing portions of the DCT (DCT1 and DCT2).

Na^+ entry into the cell via the epithelial Na^+ channel, ENaC, is the rate-limiting step for Na^+ reabsorption by the ASDN and the key locus of regulation.

The discovery of the molecular composition of ENaC in 1993[77,78] opened the door to understanding how aldosterone functions to regulate this critically important ion channel. Most Na^+ transporters are encoded by a single gene product. In contrast, ENaC is composed of three similar but distinct subunits, each encoded by a unique gene. All three subunits come together (probably as a heterotrimer) to form an ion channel with unique biophysical characteristics, the most striking of which is the relatively long time it stays open or closed.[79] The complete loss of any one of these subunits in mice is incompatible with life,[80–82] and mutations in channel subunits cause profound disease manifestations in humans.[83] The apical entry of Na^+ into the cell via ENaC is the rate-limiting step in both Na^+ absorption and K^+ secretion.[84] Na^+ enters the cell down a steep electrochemical gradient; intracellular [Na^+] is approximately 10 mmol/L, and the membrane voltage is high (inside negative). Intracellular Na^+ is pumped out across the basolateral membrane by Na^+-K^+-ATPase, as addressed in detail in Chapter 6. Most epithelial cells have a greater density of K^+ channels on the basolateral membrane and thus recycle K^+ back into the blood. The distal nephron is unique in that it has an unusually high density of K channels on the apical membrane (primarily Kir 1.1 [renal outer medullary potassium (ROMK)] and BK channels) relative to the apical membrane of other epithelia.[85,86] This distribution of K^+ channels permits a large amount of K^+ that enters the cell via Na^+-K^+-ATPase to exit the cell into the lumen and be excreted into the urine. The vast majority of K^+ that appears in the urine is secreted by the distal nephron.

Much attention has been focused on the early phase of aldosterone action because it appears to be more tractable to dissection, and most changes in Na^+ current occur during this phase. This separation is probably somewhat artificial, however, and there is considerable overlap in events that define the early and late phases. Moreover, many efforts to manipulate mediators of the early phase (through overexpression and knockdown experiments) have been evaluated after prolonged alteration. Nevertheless, there is some heuristic value in considering the early and late phases of aldosterone action separately.

In cultured collecting duct cells deprived of corticosteroids and then exposed to high concentrations of aldosterone, an increase in ENaC-mediated Na^+ transport can be observed in well under 1 hour, which is consistent with animal studies.[72,87] Na^+ transport continues to increase for 2 to 3 hours, then plateaus for a few hours, and then gradually increases over the next several hours. After 12 hours of exposure to saturating aldosterone concentrations, the increase in ENaC activity is near maximal. The molecular basis for this increase in ENaC activity has been intensively investigated, and several key events are now apparent.

For aldosterone to increase ENaC activity, a change in gene transcription must occur. One of the earliest response genes is *SGK1*.[88–90] This serine–threonine kinase, which mediates a substantial portion of the early effects of aldosterone,[91,92] is addressed in greater detail later in this chapter, together with its major target, Nedd4-2. The genetic disease Liddle syndrome provided key first clues to ENaC, as addressed further later[93,94] and in Chapter 44.

ALDOSTERONE AND EPITHELIAL SODIUM CHANNEL TRAFFICKING

The major action of aldosterone is to increase the number of functional ENaC units on the apical membrane. This process can involve an increase in the number of channel complexes on the surface, activation of existing complexes, or both. There is evidence to support both, although the bulk of evidence favors the idea that a change in the number of ENaC units predominates.[95–97] The redistribution of ENaC to the apical membrane can be detected in less than 2 hours after aldosterone exposure.[87]

It is less well established whether the number of channels is increased through increased insertion, decreased removal, or both. Aldosterone probably contributes to both processes. Rapid insertion of ENaC is best understood with regard to the actions of cAMP.[98] The extent to which the molecules

involved in cAMP-mediated insertion are also involved in aldosterone action is uncertain, but some common mechanisms are probably used. Trafficking to the apical membrane appears to involve hsp70,[99] SNARE (soluble NEM-sensitive factor attachment protein receptor) proteins,[100] and the aldosterone-induced protein melanophilin.[101] The mitogen-activated protein kinase pathway may also be involved, because interruption of ERK phosphorylation by GILZ[102] increases ENaC surface expression.

Considerably more is known about how ENaC complexes are retrieved from the apical membrane. This understanding is the direct result of dissecting the molecular consequences of Liddle syndrome, in which mutations in the C terminus of ENaC lead to increased residence time in the apical membrane.[93,94] The missing or mutated domains in the β- or γ-subunit of ENaC in this syndrome normally bind to Nedd4-2, a ubiquitin ligase, which ultimately is responsible for initiating endocytosis and degradation.[103,104] The interaction of Sgk1 and Nedd4-2 in the actions of aldosterone is discussed later. ENaC is internalized via clathrin-coated vesicles, processed into early endosomes, and then further processed into recycling endosomes and late endosomes.[105,106] Degradation is via lysosomes or proteasomes.[107,108] The processing of ENaC by vesicular trafficking and its regulation by aldosterone has been reviewed by Butterworth et al.[109]

Phosphatidylinositol-3-kinase (PI3K)–dependent signaling is essential for epithelial Na^+ transport. It controls SGK1 activity (see later) and also appears to have independent effects on ENaC open probability through direct actions of 3-phosphorylated phosphoinositides, particularly phosphatidylinositol (3,4,5)-trisphosphate.[110,111] Ras-dependent signaling may also regulate ENaC and the pump in complex ways that depend on downstream signaling through Raf, MEK, and ERK, as well as through PI3K.[112–117]

The late phase of ENaC activation by aldosterone is less well understood than the early phase. A simple evaluation of the late phase is that aldosterone increases the transcription and protein abundance of the ENaC α-subunit. This idea comes from the fact that aldosterone increases the mRNA and protein abundance of α-ENaC in the kidney[96,118] after a lag of several hours.[119] Although less well studied, aldosterone appears to produce an increase in β- and γ-subunit expression in the colon.[119,120] Dietary Na^+ restriction, a physiologically relevant maneuver that increases aldosterone secretion, clearly increases ENaC surface expression in the renal distal nephron.[97] However, there appear to be some important differences between chronic aldosterone administration to a Na^+-replete animal and chronic dietary Na^+ restriction.[118,121] Furthermore, it should be noted that increased α-ENaC expression, in and of itself, does not increase ENaC activity in models of collecting duct and lung epithelia, although limiting its expression does restrict aldosterone stimulation.[122] It appears that increased expression of α-ENaC may be important for the consolidation of the increase, but it is not sufficient to reproduce the steroid-mediated increase in ENaC activity.

BASOLATERAL MEMBRANE EFFECTS OF ALDOSTERONE

Over the years, research on aldosterone action has focused with varying degrees of intensity on apical effects,[123,124] basolateral effects,[125–127] and effects on metabolism.[73] There is general agreement now that the early effects of aldosterone are on apical events, primarily on ENaC, and that basolateral and metabolic effects occur later. In addition, although it is somewhat less settled whether the basolateral effects are direct or result indirectly from the enhanced entry of Na^+ into cells, the bulk of evidence favors the latter view. Notably, increased Na^+ entry has been found to control more than 80% of increased Na^+-K^+-ATPase activity and basolateral membrane density in the rat[128,129] and rabbit[129] cortical collecting tubules. Furthermore, striking increases in basolateral membrane folding and surface area occur in aldosterone-treated animals,[130] an effect that is markedly attenuated in animals fed a low-Na^+ diet. This result strongly suggests that apical Na^+ entry is required for basolateral changes to occur. However, there is good evidence for direct transcriptional stimulation of Na^+-K^+-ATPase subunit expression,[131,132] as well as reports supporting some direct effects of aldosterone in increasing basolateral pump activity[125,133] or at least in constituting the pool of latent pumps, which are then recruited to the basolateral membrane in response to a rise in intracellular $[Na^+]$.[134]

ACTIVATION OF THE EPITHELIAL SODIUM CHANNEL BY PROTEOLYTIC CLEAVAGE

There is now clear evidence that when ENaC is delivered to the apical membrane, it can be activated by proteolytic cleavage. The first hint of this process was the demonstration that rats fed a low-Na diet or given aldosterone over the long term showed the appearance of a proteolytic fragment of the γ-ENaC subunit.[96] Subsequently, investigators have shown that both the α- and the γ- but not the β-ENaC subunits can be cleaved. Furthermore, cleavage at each site apparently initiates a degree of activation of the channel complex. ENaC complexes are activated by cleavage because specific regions of the large extracellular domain (26 residues in the α-ENaC and 46 residues in the γ-ENaC) are excised. These regions contain inhibitory sequences that when introduced exogenously, can inhibit ENaC function. Removing these regions by proteolytic cleavage releases this inhibition.[135]

Several proteases can cleave either the α- or γ-ENaC subunits. Among them are furin, prostasin, CAP2, kallikrein, elastase, matriptase, plasmin, and trypsin. It is not clear whether activation of ENaC by proteolytic cleavage can be regulated by aldosterone, but the idea certainly has attractive features. If aldosterone could regulate expression of one or more rate-limiting proteases, it would be able to regulate both the number of complexes in the apical membrane and the ability of the channel complex to be active. It appears that aldosterone may regulate the expression of prostasin.[136] Aldosterone may also regulate the expression of the protease nexin-1 (an inhibitor of prostasin) and other proteases.[137]

The discovery of ENaC activation by cleavage helps explain how aldosterone might increase ENaC activity by increasing both surface expression and the activity of a single ENaC complex. By phosphorylating Nedd4-2 via SGK1 and reducing its ability to bind to the PY domains of the ENaC subunits, aldosterone increases ENaC residence time on the apical membrane. This additional time permits proteolytic activation by one or more endogenous proteases.[138]

POTASSIUM SECRETION AND ALDOSTERONE

One of the major effects of aldosterone is to increase K^+ secretion (and thus excretion). This phenomenon has been demonstrated in countless patients with aldosterone-secreting tumors and in hundreds of studies in animals given excess amounts of aldosterone. The general mechanism whereby aldosterone increases K^+ secretion is depicted in Fig. 12.7. The key feature of this process—with respect to the direct effects of aldosterone per se—involves the stimulation of Na^+ absorption via ENaC. The dependence of K^+ secretion on Na^+ absorption is the basis of the action of the so-called K-sparing diuretics, amiloride and triamterene, both of which inhibit ENaC. These drugs have no direct effect on apical K^+ channels.

Increasing ENaC activity produces two major secondary effects that in turn enhance K^+ secretion. First, the enhanced Na^+ conductance of the apical membrane produces depolarization and hence a more favorable electrical driving force for K^+ efflux into the lumen. The second effect relates to the activity of the Na^+-K^+ pump on the basolateral membrane. The more Na^+ that enters across the apical membrane, the more that must be extruded by the pump. Because the pump operates well below its V_{max} under baseline conditions, a slight increase in intracellular Na^+ concentration markedly stimulates pump activity and more K^+ enters the cell. In isolated, perfused cortical collecting ducts, the amount of secreted K^+ is highly related to the amount of absorbed Na^+ when the stimulus for Na^+ absorption is mineralocorticoid hormone.[139]

Two types of K^+ channels are found in the apical membrane of the ASDN: small conductance (SK, 30–40 picosiemens [pS]) channels encoded by the *ROMK* gene, and large conductance (BK, 100–200 pS) channels found in many other cell types, including the apical membrane of the colon. Most of the K^+ channels on the apical membrane of the principal cells appear to be SK, at least as far as can be assessed by patch-clamp analysis. The activity of either channel is not directly increased by aldosterone.[140,141]

A feature of K^+ secretion is that although apical K^+ channels are abundant in the proximal portion of the ASDN (connecting tubule and cortical collecting duct), they are strikingly less abundant in the medullary collecting duct.[142–144] Because apical K channels are not regulated by aldosterone, their absence in the medullary collecting duct might uncouple aldosterone-regulated Na^+ reabsorption from K^+ secretion in this segment.

SEPARATION OF SODIUM ABSORPTION AND POTASSIUM SECRETION BY THE ALDOSTERONE-SENSITIVE DISTAL NEPHRON

The preceding sections establish a picture that parsimoniously accounts for the effect of aldosterone to stimulate Na^+ reabsorption and K^+ secretion at the same rate. The simple stimulation of electrogenic Na^+ reabsorption (via ENaC) is sufficient to stimulate K^+ secretion, which fits well for organisms faced with a combined low-Na^+, high-K^+ diet, which was maintained most of the time through millions of years of vertebrate evolution. However, organisms do not ingest a fixed amount of Na^+ and K^+, so an inexorable linkage between Na^+ absorption and K^+ secretion by the ASDN cannot possibly occur all the time. Investigators have proposed several possibilities to explain how these processes can be separated.

ROLE OF DISTAL TUBULE FLUID DELIVERY

A traditional view for differential Na^+ and K^+ handling stems from the differing role that aldosterone plays in potassium secretion depending on the tubular flow (and thus sodium flow) rate. Studies from adrenalectomized dogs have demonstrated that the primary regulators of potassium excretion are the serum potassium concentration and tubular flow rate. A higher serum potassium concentration yields a higher filtered load of potassium. Hyperkalemia also stimulates natriuresis from upstream segments of the nephron.[145,146] This latter effect can increase tubular flow rate, which, in turn, diminishes potassium concentration in the lumen and activates flow-stimulated BK channel–mediated potassium secretion in the collecting duct. In the setting of sufficient distal delivery of sodium, potassium loading will not yield a higher steady-state concentration of aldosterone because the two mechanisms previously mentioned are sufficient to normalize the serum potassium level.[147] However, under conditions of sodium depletion, proximal Na^+ reabsorption is increased, which further diminishes distal delivery of sodium and hence tubular flow rate.[148] This diminishes flow-mediated potassium secretion, so that aldosterone secretion is necessary to normalize potassium balance.

INDEPENDENT REGULATION OF SODIUM AND POTASSIUM TRANSPORTERS

Other possible mechanisms have been suggested, which involve separate regulation of sodium and potassium transport (e.g., ENaC and ROMK) by specific stimuli, depending on the state of Na^+ and K^+ intake. With a constant Na^+ intake, one could envision that a high-potassium diet could enhance the activity of ROMK, whereas a low-potassium diet would reduce its activity. Such an effect would cause more or less of the K^+ entering the cell via the Na^+-K^+ pump to be recycled across the basolateral membrane. This mechanism, although probably very complex in its execution, is appealing in its simplicity.

ROLE OF WNKS

Advances in understanding genetic forms of hypertension have uncovered a key functional role for a family of kinases that have potent effects on pathways regulating Na^+ and K^+ transport in the distal nephron. These kinases, called "WNKs" (*w*ith *n*o lysine; *K* is the one-letter code for lysine), were initially thought to lack a conserved lysine residue in the catalytic domain, which binds adenosine triphosphate (ATP), and is essential for catalysis. Interestingly, a lysine performing the same function is indeed present, but is shifted from its canonical location in subdomain II to subdomain I to permit entry of chloride into the catalytic domain.[149] This modification contributes to the chloride regulation of WNK activity,

The spectrum of regulatory roles of WNKs is complex and remains controversial. It is clear that the Na^+,Cl^- cotransporter (NCC) is regulated by WNK4, and it is likely that ROMK and ENaC are regulated by WNK1.[150,151] Recent evidence has also supported the idea that the chloride-binding properties of WNKs provide the basis for their functional regulation by extracellular K^+ concentration.[152] In the DCT, in particular,

low plasma (and hence extracellular) K^+ concentration depolarizes the basolateral membrane via Kir4.1, which leads to a reduction in intracellular chloride, thereby activating WNK4 and SPAK (STE20/SPS1-related proline-alanine–rich kinase) and hence NCC phosphorylation.[152,153] A high plasma K^+ concentration has the opposite effect and inhibits NCC phosphorylation; however, the mechanism appears to be chloride-independent. Thus, through its effects to raise the plasma K^+ concentration, a high-K^+ diet has effects both locally in the kidneys and through aldosterone to stimulate K^+ excretion. Aldosterone acts directly in the ASDN to stimulate ENaC, which increases the driving force for K^+ secretion through ROMK and BK channels..[154] In conjunction, an elevated K^+ concentration acts directly in DCT cells to inhibit NCC phosphorylation and enhance delivery of Na^+ to the ENaC-expressing segments. According to this view, aldosterone does not have a direct effect on NCC-mediated electroneutral Na^+ transport. Further details of the mechanisms of K transport and the role of WNKs in the distal nephron are presented in Chapter 6.

ROLE OF CHLORIDE TRANSPORT REGULATION

There is also evidence supporting the independent regulation of Cl^- transport in the collecting duct. Cl^- can be absorbed by the paracellular pathway (i.e., between cells) driven by the lumen-negative voltage across the epithelium. This pathway can be influenced by aldosterone.[155] Cl^- can also be absorbed through the cells by specific transporters. One example of a Cl^- transporter in the collecting duct is pendrin, an anion exchanger present on the apical membrane of intercalated cells. Mice that lack this transporter do not tolerate NaCl restriction as well as normal mice.[156] Its activity is dependent on Cl^- delivery to the distal nephron, and it is coregulated by angiotensin II and aldosterone.[157,158,159]

As discussed in the next section, the ability of the MR in intercalated cells (but not in principal cells) to respond to aldosterone is regulated by the phosphorylation of its ligand-binding domain. It should also be noted that modulation of Na^+ absorption in the medullary collecting duct may also play a role in the balance of Na^+ reabsorption and K^+ secretion; this segment has little capacity to secrete K^+, and endogenous paracrine factors such as prostaglandins E_2 and transforming growth factor-β, which have potent inhibitory effects on Na^+ transport, are increased in response to a high-NaCl diet.[160,161]

DIFFERENTIAL REGULATION OF INTERCALATED CELL MINERALOCORTICOID RECEPTOR

It has become increasingly clear that intercalated cell Cl^- transport contributes to collecting duct NaCl absorption, and this plays an important role in allowing distinct responses to aldosterone in states of volume depletion versus hyperkalemia.[162,163] A central feature of this proposed regulation is differential phosphorylation of the MR LBD in intercalated cells, as shown schematically in Fig. 12.8. When phosphorylated at S843 in the LBD, MR cannot bind aldosterone (or cortisol) and thus cannot be activated. This phosphorylation, which is stimulated by hyperkalemia, occurs selectively in intercalated cells but not in principal cells. Angiotensin II, on the other hand, induces S843 dephosphorylation in intercalated cells, markedly increasing ligand binding and, therefore, activation. Intercalated cells are known predominantly to mediate H^+ transport; however, other studies have implicated them in electroneutral NaCl transport via the combined actions of the Na^+-dependent Cl^--HCO_3^- exchanger (NDCBE)[164] and the apical Cl^--HCO_3^- exchanger, pendrin.[165,166] Thus, when MR is active in these cells (S843 dephosphorylated), electroneutral NaCl transport occurs, without enhancing the driving force for K^+ secretion. Because intercalated cells lack 11β-HSD2 under these conditions, it is cortisol that binds to and activates MR. When intercalated cell MR is inactive (S843 phosphorylated), aldosterone acts in principal cells to stimulate ENaC-dependent electrogenic Na^+ transport, which enhances K^+ secretion.

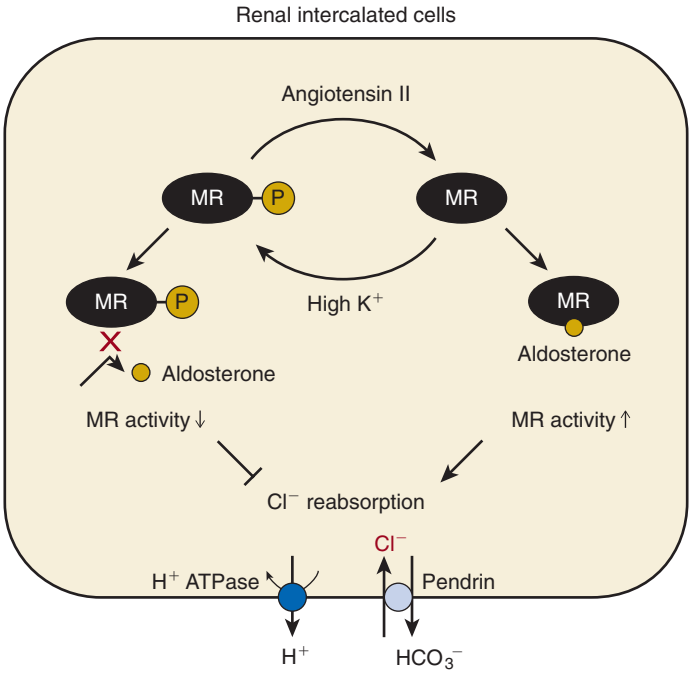


Fig. 12.8 The role of mineralocorticoid receptor *(MR)* ligand-hormone–binding domain (LBD) phosphorylation in controlling chloride reabsorption by intercalated cells. When phosphorylated at Ser-843 in the LBD, MR cannot bind ligand and hence cannot be activated. This phosphorylated state of MR is found only in intercalated cells, not in neighboring principal cells. In states of volume depletion, an elevated angiotensin II level decreases MR phosphorylation at Ser-843, allowing activation. In intercalated cells, MR mediates stimulation of both the proton pump and Cl^--HCO_3^- exchangers, thereby increasing Cl^- reabsorption and promoting increased plasma volume while inhibiting K^+ secretion. In contrast, in states of hyperkalemia, phosphorylation of Ser-843 is increased, and hence Cl^- reabsorption by intercalated cells is decreased and the principal cell–dependent K^+ secretion is increased. (From Shibata S, Rinehart J, Zhang J, et al. Regulated mineralocorticoid receptor phosphorylation controls ligand binding and renal response to volume depletion and hyperkalemia. *Cell Metab.* 2013;18:660–671.)

ALDOSTERONE-INDEPENDENT ENAC-MEDIATED SODIUM REABSORPTION IN THE DISTAL NEPHRON

The term "aldosterone-sensitive distal nephron" emphasizes the primacy of this key steroid in the control of ion transport in this region of the nephron. However, ENaC activity and aldosterone sensitivity exhibit axial heterogeneity from the late distal convoluted tubule (DCT2) through the connecting

tubule to the cortical collecting duct and, finally, to the medullary collecting duct. In mice on a standard sodium diet, total ENaC expression increases with progression from the DCT2 to the connecting tubule,[167] although ENaC apical localization and activity are higher in the DCT2. Only under conditions of a low-sodium diet or aldosterone administration does the primacy of the connecting tubule in particular, and to a lesser extent the cortical collecting duct, emerge. The total luminal surface area in the DCT2 and connecting tubule is several-fold higher than in the cortical collecting duct,[168] and together these two segments appear to be sufficient to maintain sodium balance, even in the absence of detectable ENaC along the collecting duct. Mice lacking ENaC selectively in the collecting duct come into balance, even on a low-sodium diet.[169] Congruent with these findings, deletion of α-ENaC from the DCT2, connecting tubule, and collecting duct results in severe sodium wasting.[170] Notably, it is the connecting tubule that appears to be most important in the response to aldosterone, whereas DCT2 has the highest baseline transport in the absence of MR activation.[171] The cortical collecting duct is not as critical as was originally thought for either baseline or aldosterone-stimulated sodium reabsorption, probably due to its smaller surface area compared with the DCT2 and connecting tubule.

As we continue to traverse the nephron, further sodium reabsorption is minimal in the medullary collecting duct, on a normal sodium diet, and is not significantly stimulated by aldosterone.[139]

SITES OF MINERALOCORTICOID RECEPTOR EXPRESSION AND LOCUS OF ACTION ALONG THE NEPHRON

ALDOSTERONE-SENSITIVE DISTAL NEPHRON

In the kidney, MR is expressed at the highest levels in distal nephron cells extending from the last third of the DCT through the medullary collecting duct,[172] which is frequently referred to as the ASDN (Fig. 12.9).[1] This pattern of expression was first demonstrated using labeled hormone–binding studies performed before the cloning of MR[173] and has been confirmed since by several methods, including the polymerase chain reaction assay,[174] in situ hybridization,[175] and immunohistochemical analysis.[176] Effects of aldosterone on electrogenic Na^+ and K^+ transport in principal cells have been found consistently in these nephron segments,[172] which also express ENaC, and 11β-HSD2, as addressed in detail earlier.

Collecting duct intercalated cells also express MR and respond specifically to aldosterone and alter proton secretion. Aldosterone directly increases the activity of the H^+-ATPase in the collecting duct, and its absence results in decreased proton secretion.[177–179] Interestingly, nongenomic stimulation of H^+-ATPase activity in type A intercalated cells has been demonstrated in isolated murine collecting ducts.[180] Consistent with these effects, aldosterone deficiency results in distal renal tubular acidosis type 4, and excess aldosterone results in metabolic alkalosis.[181] It should be noted that aldosterone also stimulates H^+ secretion due to effects on principal cell Na^+ transport, which alter the electrical gradient. These older studies must also now be interpreted in the context of more recent data,[162] which, as noted earlier, demonstrate that the effect of aldosterone on intercalated cells depends on the genesis of the signal.

OTHER SITES OF EXPRESSION

MR has been identified at some level in all parts of the nephron examined, including the glomerulus.[174,182–187] Its effects, at least at some of these sites, are likely to be physiologically relevant in states of volume depletion and acid–base disturbances; however, the data are not as robust and consistent as those for the ASDN.

Glomerulus

MR (but not 11β-HSD2) is expressed in glomerular mesangial cells, where it is thought to affect proliferation and production of reactive oxygen species[188,189] and to have profibrotic effects through SGK1.[190] These effects have been suggested to be important in the progression of renal damage, particularly in diabetic nephropathy,[191] in which glucocorticoids mimic the activity of aldosteronism in the context of tissue damage. However, the physiologic role of mesangial cell MR is uncertain.

Proximal Convoluted Tubule

Hierholzer and Stolte have shown, through elegant microperfusion studies, that the sodium reabsorptive capacity of the proximal convolution is decreased in adrenalectomized animals and restored by administration of aldosterone.[192] Chronic volume depletion increases sodium reabsorption in the proximal convoluted tubule, which is in part mediated by MR. The mechanisms of action in this nephron segment are controversial. Some studies have indicated an MR-dependent increase in the activity of Na^+-H^+-exchanger isoform 3, possibly through an increase in trafficking of the transporter to the membrane.[193–196] This transporter contributes to sodium and bicarbonate reabsorption. MR activation, in turn, may activate the Na^+-K^+-ATPase in the basolateral membrane of the proximal convoluted tubule to maintain a gradient for sodium reabsorption.[197–200]

Medullary Thick Ascending Limb

In the medullary thick ascending limb, mineralocorticoids but not glucocorticoids increase sodium and chloride reabsorption. In rodents, adrenalectomy impairs the reabsorption of NaCl in the medullary thick ascending limb, and aldosterone restores this process.[201,202] This reabsorptive defect may contribute to the urinary concentrating and diluting abnormality measured in patients with Addison disease and in mice lacking aldosterone synthase.[178,192,203] The medullary thick ascending limb also participates in the regulation of acid–base balance by reabsorbing most of the filtered HCO_3 that is not reabsorbed by the proximal tubule. In this context, aldosterone has been shown to stimulate the Na^+-H^+ exchanger in the amphibian thick ascending limb, possibly through a rapid nongenomic effect.[204] Other studies have also implicated regulation of the Na-K-2Cl cotransporter type 2 in the thick ascending limb—as well as the NCC in the DCT (see later)—by oxidative stress response kinase 1 (OSR1) and STE20/SPS1-related proline-alanine–rich kinase (SPAK) (OSR1/SPAK).[205,206]

Distal Convoluted Tubule

The studies of potassium regulation described earlier have also provided insight into the role of direct or indirect aldosterone-induced NCC transport. Aldosterone increases

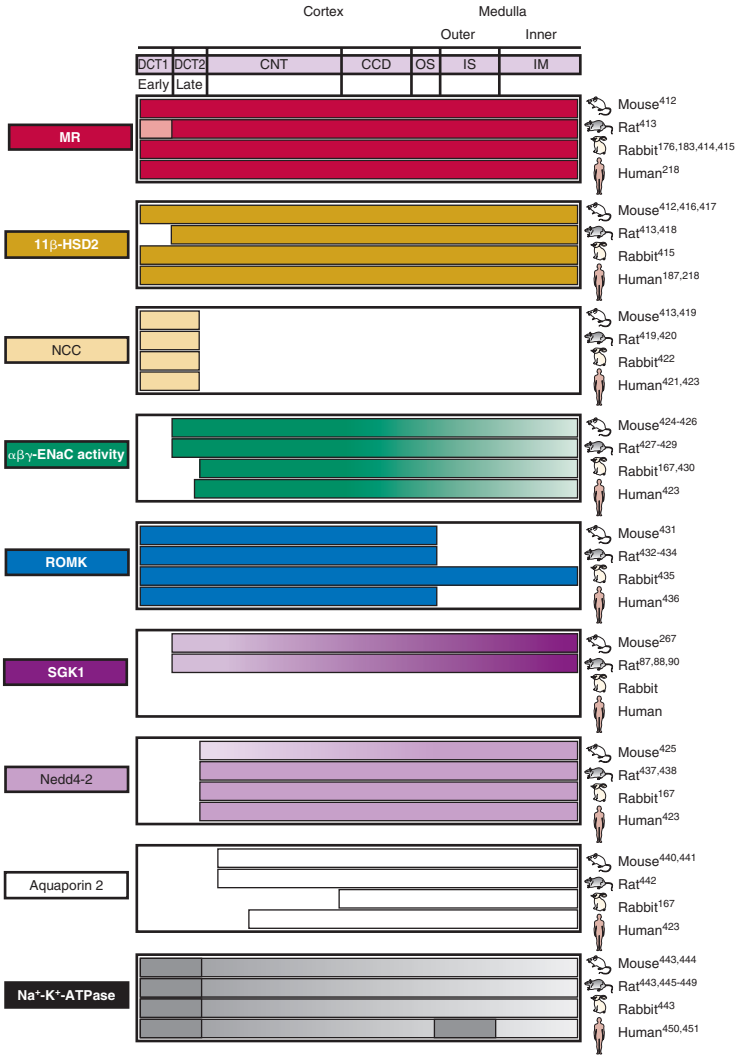


Fig. 12.9 Expression and/or activity of the mineralocorticoid-dependent transport machinery in principal cells along the mature aldosterone-sensitive distal nephron (ASDN). Mineralocorticoid specificity is conferred by the presence of the mineralocorticoid receptor *(MR)* and 11β-hydroxysteroid dehydrogenase type 2 *(11β-HSD2)*, beginning primarily from the latter part of the distal convoluted tubule *(DCT)*. The thiazide-sensitive sodium chloride cotransporter *(NCC)* is expressed exclusively in the DCT, but after the transition from the DCT to the connecting tubule (CNT), sodium reabsorption is distinctly determined by amiloride-sensitive sodium channel *(ENaC)* activity. ENaC activity is strongest in the CNT and decreases down to the inner medulla collecting duct. Variation in gene expression or activity along the nephron is indicated by the intensity of shading. Note that there is some variation in gene expression from mouse to human. However, the machinery for sodium reabsorption in the ASDN is predominantly conserved across species. Each nephron segment is drawn to scale, but expression of channels and transporters in intercalated cells is omitted. Expression and/or activity is based on messenger RNA, protein, and biochemical studies. *G,* Glomerulus; *PCT,* proximal convoluted tubule; *ROMK channel,* renal outer medullary potassium; *SGK1,* serum- and glucocorticoid-regulated kinase 1. (Modified from Loffing J, Korbmacher C: Regulated sodium transport in the renal connecting tubule [CNT] via the epithelial sodium channel [ENaC]. *Pflugers Archiv.* 2009;458[1]:111–135.)

NCC phosphorylation and total protein[207] abundance but, until recently, the mechanism of this upregulation was unclear. Elegant work by several groups have shown that aldosterone indirectly stimulates NCC via the activation of ENaC-mediated sodium transport and the resultant potassium secretion[208,209] and hypokalemia. In turn, a lower plasma potassium level activates NCC.[152,153] Another potential mechanism is through direct regulation by aldosterone. In rodent studies, aldosterone stimulates serum and glucocorticoid kinase 1, which inhibits the ubiquitin ligase Nedd4-2, which, in turn, can regulate WNK1 and NCC phosphorylation.[210] As discussed later, this pathway mirrors a well-known mechanism of aldosterone-mediated disinhibition of Nedd4-2 and subsequent ENaC degradation in principal cells. The specific physiologic contexts for these different direct and indirect modes of aldosterone-dependent NCC are still unknown.

NONRENAL ALDOSTERONE-RESPONSIVE TIGHT EPITHELIA

The mineralocorticoid effects of aldosterone have predominantly been studied in the distal nephron, but do influence other—mostly ENaC-expressing—tight epithelia. ENaC is present in visceral epithelial cells of the distal colon, distal lung, salivary glands, sweat glands, and taste buds.

COLON

Under physiologic conditions, approximately 1.3 to 1.8 L of electrolyte-rich fluid is reabsorbed per day from the colonic epithelium, which accounts for about 90% of the salt and water that enter the proximal colon from the terminal ileum. In nonmammalian vertebrates, sodium conservation by the colon plays an even more significant role.[211] This transport is regulated by several transporters and channels, including ENaC. Like the nephron, the proximal colon reabsorbs sodium via an electroneutral, ENaC-independent process. In the distal colon, electrogenic Na^+ absorption via ENaC channels is the predominant mode of sodium transport.[212–215] In disease states such as inflammatory bowel disease, ENaC-mediated sodium reabsorption can be reduced,[216] although in diarrheal states, elevated aldosterone levels may attenuate sodium and water loss from the colon.[217] It should be noted that in the colon, as in the distal nephron, MR signaling is aldosterone selective, reflecting the activity of 11β-HSD2.[218] Aldosterone increases electrogenic sodium absorption and potassium secretion and inhibits electroneutral absorption.[219] This is in contrast to glucocorticoids, which, at higher concentrations, activate GR to stimulate electroneutral absorption in the proximal and distal colon.[84,220] As in the distal nephron, the aldosterone response can be characterized by an early and late response. The early response gene, *SGK1,* is upregulated by aldosterone via MR.[221] However, in contrast with the kidney, aldosterone and a low-salt diet have been shown to stimulate transcription of β-ENaC but not α-ENaC in rat models.[222,223]

Aldosterone stimulates electrogenic potassium secretion from colonic epithelia. The significance of this secretion is evident in anuric patients. Potassium secretion from the colon is much higher in patients undergoing long-term hemodialysis than in patients not undergoing dialysis.[224–226] Indeed, administration of fludrocortisone, a mineralocorticoid agonist, to dialysis patients has been shown to reduce hyperkalemia in small clinical trials.[227] Low doses of the common MR antagonist spironolactone do not result in significant hyperkalemia.[228–230]

LUNG

Vectorial transport of salt and water across the distal airway epithelium (ciliated Clara cells, nonciliated cuboidal cells) and alveoli (types I and II alveoli) primarily determines fluid clearance from the lung. ENaC is the rate-limiting step in sodium transport in the lung and plays a primary role in several physiologic and pathophysiologic conditions determined by fluid clearance.[231] At birth, the lung assumes a resorptive phenotype, and lack of functional ENaC channels leads to neonatal respiratory distress syndrome in mouse knockout models.[232] In children, lack of functional ENaC (e.g., autosomal recessive pseudohypoaldosteronism type I)[233–235] results in increased rates of recurrent infection due to increased airway liquid.[234] In the mature lung, defective ENaC channels can lead to pulmonary edema and pathologic conditions (e.g., acute respiratory distress syndrome,[236] high-altitude pulmonary edema[237]). Conversely, hyperabsorption through ENaC is emerging as an important mechanism of decreased mucus clearance in cystic fibrosis.[238]

The molecular apparatus for mineralocorticoid-stimulated liquid reabsorption via ENaC (concomitant MR and 11β-HSD2) is present in late gestational and mature adult lung in humans[218,239,240] and rats,[241] and there is some evidence for a significant physiologic role of aldosterone in ENaC-mediated sodium transport,[241] although glucocorticoids acting via GR are likely to play the predominant role in lung.[118,221,242–245] Importantly, glucocorticoids, but not mineralocorticoids, play a critical role in lung maturation in humans, and GR knockout mice, like α-ENaC knockout mice, die of respiratory insufficiency within hours of birth. In contrast, MR knockout mice demonstrate a severe salt-wasting phenotype but no significant lung phenotype.[177,246]

EXOCRINE GLANDS AND SENSORS

ENaC-mediated sodium reabsorption is also measurable in the salivary and sweat glands.[247] The importance of these tissues for sodium and water homeostasis is underscored by rare genetic mutations that result in elevated plasma aldosterone levels and pseudohypoaldosteronism, with normal renal tubular function but significant sodium loss from salivary or sweat glands.[248,249] ENaC channels also play an important role in transduction of sodium salt taste in the anterior papillae of the tongue.[250,251] The appropriate molecular machinery for mineralocorticoid-responsive sodium reabsorption is expressed in these organs,[218,252,253] and these epithelia are model systems for the study of ENaC regulation.[247,254] As in colonic epithelia, aldosterone stimulates the expression of β- and γ-ENaC and sodium transport in glands and taste buds in animal models.[251,255] Moreover, in humans, changes in dietary sodium are inversely proportional to sodium transport across salivary epithelia.[256] Similar to the aldosterone-responsive distal nephron and distal colon, sodium uptake is coupled with potassium secretion in salivary epithelia. This effect is evident in humans with hyperaldosteronism. Such patients have a salivary $[Na^+]/[K^+]$ ratio significantly lower than that of subjects without the disorder,[257,258] although this has not been accepted as a valid means to screen for hyperaldosteronism.

ROLE OF SERUM- AND GLUCOCORTICOID-REGULATED KINASE IN MEDIATING ALDOSTERONE EFFECTS

INDUCTION OF SGK1 BY ALDOSTERONE

In the early to mid-1960s, primarily from the work of Edelman and colleagues, it became clear that aldosterone, like cortisol, exerted most, if not all, of its key physiologic effects by altering transcription rates of a specific subset of mRNA-encoding genes.[259] In particular, hormone-induced changes in gene transcription were shown to be essential for its effects on epithelial Na^+ transport.[73] Transporters involved in Na^+ reabsorption (Na^+-K^+-ATPase and ENaC), are regulated by aldosterone at the transcriptional level. However, these effects are manifest several hours after most of the change in Na^+ transport has already occurred and hence could not explain the early and greatest proportion of effects of aldosterone.[1] Considerable effort by many groups went into unbiased screening for aldosterone-regulated proteins[260] and later aldosterone-regulated mRNAs (reviewed in Verrey[1]). In 1999, SGK1 was identified as the first early-onset, aldosterone-induced gene

product, which clearly stimulates ENaC-mediated sodium reabsorption in the distal nephron[221,261] without pleiotropic effects on other cellular processes. The physiologic relevance of SGK1 has now been firmly established, and investigations by numerous laboratories into its mechanism of action have revealed critical general features of the mechanism underlying hormone-regulated ion transport. It is therefore addressed in some detail here.

SGK1 mRNA levels are increased within 15 minutes, and protein levels within 30 minutes, in cultured cells on stimulation by aldosterone[262,263] and in the collecting duct by aldosterone or a low-salt diet (a physiologic stimulus for aldosterone secretion).[87,221,264] Notably, SGK1 is increased more abundantly in the kidney cortex (the connecting tubule and cortical collecting duct) than in the medulla, which is congruent with the potency of aldosterone-induced ENaC activation in these nephron segments, as noted earlier.[265,266] SGK1 is expressed in other nephron segments, including glomeruli, proximal tubule, and papillae[87,221,267]; however, its rapid induction in the ASDN appears to provide most of the basis for its role in aldosterone-regulated sodium and potassium transport. SGK1, induced by high sodium in infiltrating T cells, is also important for inflammation in the kidney and hypertension, although the specific nephron segments are unknown.[268,269]

MOLECULAR MECHANISMS OF SGK1 ACTION IN THE ALDOSTERONE-SENSITIVE DISTAL NEPHRON

SGK1 is a serine-threonine kinase of the AGC protein kinase superfamily,[270] and its kinase activity appears to be essential for Na^+ transport regulation. Although it has effects on proliferation and apoptosis in kidney cells, these effects appear to be minor, and the control of ENaC and other transporters[271] predominates. SGK1 is interesting as a signaling kinase in that both its expression level and activity are highly regulated. SGK1 transcription is induced by a variety of stimuli in addition to aldosterone. As its name implies, these include serum and glucocorticoids, but also follicle-stimulating hormone, transforming growth factor-β, and osmotic stress.[272–275]

SGK1 activation is primarily regulated through phosphorylation, which is required for its stimulation of ENaC.[276–278,279,280] Like that of its close relative, Akt, SGK1 phosphorylation is stimulated by a variety of growth factors, including insulin and insulin-like growth factor-1[279–282]; these act through PI3K to trigger phosphorylation at two key residues, an activation loop (residue T256) and a hydrophobic motif (S422). Specifically, the α-isoform of the p110 subunit of PI3K stimulates PI3K-dependent kinase 1 (PDK1) to phosphorylate T256, and also stimulates mammalian target of rapamycin [mTOR] in its complex 2 variant (mTORC2, formerly called PDK2) to phosphorylate S422.[276,280,282–286] mTORC2 also uses a cofactor, SIN1, to specify activation of SGK1 rather than related family members, such as Akt.[287] In turn, the upstream kinases, PDK1 and mTORC2, phosphorylate and thereby activate SGK1 kinase, Thus, SGK1 serves as a convergence point for different classes of stimuli, which act on the one hand to control its expression (aldosterone) and on the other to control its activity (insulin and other activators), which results in the coordinate regulation of ENaC.

In the study of the physiologic and pathophysiologic roles of SGK1 in the ASDN, mice lacking SGK1 under different physiologic stimuli have provided considerable insight. Unlike MR knockout mice,[177] mice lacking SGK1 survive the neonatal period and appear normal when consuming a normal sodium diet, although circulating aldosterone is markedly elevated. When subjected to a low-sodium diet, these mice have a profound sodium-wasting phenotype, akin to pseudohypoaldosteronism type I.[288,289] Additional mouse models of SGK1 deletion have demonstrated diminished processing of ENaC subunits I.[290,291] Notably, this is a significantly milder phenotype than with deletion of MR or α-ENaC.[292] These comparisons suggest that disruption of SGK1 signaling may be insufficient to eliminate aldosterone-mediated sodium transport due to additional aldosterone-induced and aldosterone-repressed proteins, which could compensate for the lack of SGK1.

SGK1 may also play a significant role in states of aldosterone excess or upregulation of hormonal activators of SGK1 (e.g., insulin). SGK1 knockout mice are protected from the development of salt-sensitive hypertension, which accompanies the hyperinsulinemia of the metabolic syndrome.[293,294] Taken together, SGK1 is an important component of ENaC regulation to maintain both sodium and potassium homeostasis.

Despite its accepted role as a mediator of aldosterone-stimulated sodium reabsorption, the mechanisms whereby SGK1 stimulates ENaC are not fully characterized. Several mechanistic studies have demonstrated that SGK1 is rapidly induced but also rapidly degraded.[221,295,296] The N-terminus of the kinase, which distinguishes SGK1 from other kinase family members (e.g., Akt), is important for stimulation of sodium transport but is also the target for rapid degradation of the kinase via the ubiquitin-proteasome system.[297–303] The pathophysiologic implications of the N-terminus for sodium transport are unclear, but they may involve a negative feedback loop to limit sodium reabsorption in states of hypertension. The molecular mechanisms of ENaC stimulation by SGK1 can be divided into three known categories (Fig. 12.10): (1) posttranslational effects on the E3 ubiquitin ligase Nedd4-2; (2) posttranslational Nedd4-2–independent effects; and (3) transcription of gene products such as α-ENaC.

SGK1 INHIBITS THE UBIQUITIN LIGASE NEDD4-2

Before the discovery of SGK1 as an aldosterone-induced early gene product, the E3 ubiquitin ligase known as "neural developmentally downregulated isoform 4-2" (Nedd4-2) was shown to interact with the C-terminal tails of β-ENaC and γ-ENaC[304] and decrease surface expression of the channel via channel ubiquitination, hence inhibiting the sodium current.[108,305] The genetic defect in Liddle syndrome (ENaC-mediated hypertension, hypokalemia, and metabolic alkalosis) consists of a gain-of-function mutation in the C-terminal tail of these subunits, which results in decreased inhibition by Nedd4-2 and hence increased ENaC activity.[306] Lack of Nedd4-2 in vivo results in increased ENaC activity and salt-sensitive hypertension,[307,308] recapitulating a Liddle syndrome–like phenotype. SGK1 interacts with and phosphorylates Nedd4-2[263,277] in an ENaC signaling complex[113] and enhances cell surface expression of ENaC,[262,309] a determinant of ENaC activity (see Fig. 12.10A). This interaction coordinates the phosphorylation-dependent binding of 14-3-3 proteins to inhibit Nedd4-2 and prevent the ubiquitination of ENaC.[310–313] This disinhibition of ENaC parallels a recurring theme in the regulation of ion transport in the kidney seen with the WNK kinases and NCC, other aldosterone-regulated

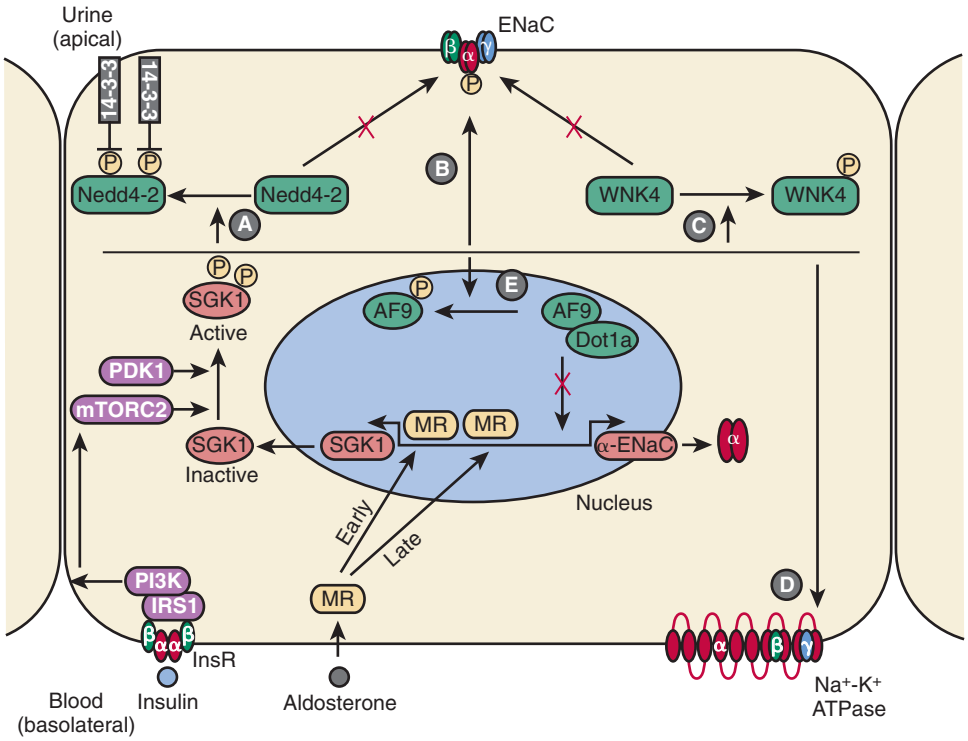


Fig. 12.10 Mechanisms of serum- and glucocorticoid-regulated kinase 1 *(SGK1)*–mediated stimulation of the amiloride-sensitive sodium channel *(ENaC)*. Within principal cells of the mammalian kidney, SGK1 is transcriptionally upregulated as an early aldosterone-induced gene product. SGK1 is then phosphorylated twice via a phosphatidylinositol-3-kinase *(PI3K)*–dependent cascade of upstream kinases leading to active SGK1. Active SGK1 has multiple effects: it increases apical plasma membrane ENaC by inhibiting Nedd4-2 and Raf-1, and it induces transcription of the α-ENaC (thereby influencing late effects of aldosterone). (A–E, *clockwise*) Shown are the individual mechanisms that have been elucidated in principal cells. See text for details. *InsR,* Insulin receptor; *IRS1,* insulin receptor substrate 1; *MR,* mineralocorticoid receptor; *mTORC2,* mammalian target of rapamycin complex 2; *PDK1,* 3-phosphoinositide-dependent protein kinase type 1.

gene products (e.g., GILZ) and ENaC, and NHERF2 and ROMK.[270,314] Similarly, SGK1 has also been implicated in the stimulation of NCC via inhibition of Nedd4-2 and increased abundance of PY motif-containing WNK1.[210,315,316]

SGK1 ENHANCES EPITHELIAL SODIUM CHANNEL ACTIVITY INDEPENDENTLY OF NEDD4-2

In cell culture systems, mutation of SGK1 phosphorylation sites on Nedd4-2 does not completely abolish the ability of SGK1 to stimulate ENaC.[277] Furthermore, SGK1 has been shown to stimulate ENaC channels with Liddle syndrome mutations, which are unable to bind Nedd4-2.[221,262] Consequently, other Nedd4-2–independent mechanisms of SGK1 stimulation have been proposed. SGK1 directly phosphorylates a serine residue in the intracellular C-terminal tail of α-ENaC, which directly activates channels at the cell surface (see Fig. 12.10B).[317,318] SGK1 has been implicated in the stimulation of ENaC via the phosphorylation of WNK4, a kinase mutated in pseudohypoaldosteronism type II (see Fig. 12.10C).[319] Cell surface–expressed SGK1 may also increase open probability of the channel.[318,320] In addition to showing effects on ENaC, SGK1 has been found to stimulate the activity of basolateral Na^+-K^+-ATPase, which separately increases ENaC-mediated sodium transport (see Fig. 12.10D).[321,322] The time course of these effects and their relative importance compared with Nedd4-2–dependent inhibition have not been explored.[317] The next generation of molecular studies of SGK1 will elucidate the relative importance of each of these pathways.

SGK1 STIMULATES THE COMPONENTS OF SODIUM TRANSPORT MACHINERY

SGK1 also regulates the expression of late aldosterone-responsive genes, primarily α-ENaC.[323,324] Active SGK1 is an important mediator of aldosterone-sensitive α-ENaC transcription in vivo via inhibition of a transcriptional repression element, the disruptor of telomeric silencing alternative splice variant a (Dot1a)–ALL1–fused gene from the chromosome 9 (Af9) complex.[324] SGK1 phosphorylates Af9 and reduces the interaction between Dot1a and Af9. This releases suppression of ENaC transcription by this complex (see Fig. 12.10E). Thus, SGK1 not only acts on ENaC channels to enhance sodium channel activity rapidly through the increase of active channels at the apical surface and the increase of Na^+-K^+-ATPase at the basolateral surface, but also stimulates the transcription of elements of the machinery for sodium transport to promote a sustained response to aldosterone. SGK1 is an early-onset gene, but its effects influence both immediate- and long-term aldosterone-stimulated sodium reabsorption.

SGK1 STIMULATES POTASSIUM SECRETION IN THE ALDOSTERONE-SENSITIVE DISTAL NEPHRON

Further evidence of a role for SGK1 in the regulation of sodium transport in the ASDN has been revealed by the study of potassium secretion. If SGK1 enhances ENaC-mediated sodium transport, the potential difference across

the apical to basolateral surface of principal cells should be higher (more negative) and thus should indirectly stimulate potassium secretion. SGK1 knockout mice are unable to secrete potassium adequately in the short and long terms when challenged with a high-potassium diet, and mice with constitutive or inducible deletion of SGK1 are prone to hyperkalemia.[325–327] Moreover, the potential difference across collecting duct epithelia from these knockout mice indicates that the effect of SGK1 on potassium secretion occurs via ENaC, not through direct regulation of ROMK.[328] SGK1 also directly inhibits Nedd4-2, and deletion of Nedd4-2 predisposes low-potassium–fed mice to hypokalemia via the constitutive stimulation of ENaC-mediated sodium transport.[329] Thus, SGK1 and its effectors have physiologically relevant roles in sodium and potassium transport in the ASDN.

ALTERNATE MODES OF REGULATION OF ENAC-MEDIATED SODIUM TRANSPORT BY ALDOSTERONE

Although SGK1 remains the best characterized aldosterone-regulated gene, other early aldosterone-induced mRNAs, including K-ras, GILZ, kidney-specific WNK1, Usp45, melanophilin, and promyelocytic leukemia zinc finger,[330–335] have also been implicated in the stimulation of ENaC. Their distinct mechanisms of action are beyond the scope of this chapter, but more recent data have suggested that micro-RNAs stimulated by aldosterone may play a prominent role in regulating both SGK1 and ENaC.

Aldosterone can upregulate or downregulate several microRNAs in cultured cells and in vivo. These micro-RNAs can then indirectly increase or decrease protein levels of intermediate regulators of ENaC-mediated transport. The first micro-RNA cluster (mmu-miR-335-3p, mmu-miR-290-5p, and mmu-miR-1983) to be described by Butterworth was downregulated by aldosterone within 24 hours and thereby released the 3′ untranslated region of ankyrin 3 to increase apical trafficking of α-ENaC.[336,337] Aldosterone also increases micro-RNAs that inhibit a negative regulator of ENaC, intersectin 2.[338] Aldosterone has also been shown to promote the rapid induction of SGK1 mRNA by decreasing micro-RNA 466g in cultured cells.[339] Taken together, SGK1 plays a prominent role in transducing the effect of aldosterone to stimulate ENaC for both regulation of blood pressure and potassium homeostasis. Additionally, there are alternate effectors of aldosterone, but the physiologic contexts of these other pathways have not been as well established.

11β-HYDROXYSTEROID DEHYDROGENASE TYPE 2

ESSENTIAL DETERMINANT OF MINERALOCORTICOID SPECIFICITY

The physiologic glucocorticoid cortisol (corticosterone in rats and mice) has a high affinity for MR, equivalent to that of aldosterone and, as noted earlier, circulates at plasma free concentrations that are 100-fold or more higher than those of aldosterone. Central to the ability of MR to respond to aldosterone selectively in the ASDN is the coexpression of the enzyme 11β-HSD2.[5,6] 11β-HSD2 converts cortisol (corticosterone) to receptor-inactive 11-keto steroids (cortisone in humans, 11-dehydrocorticosterone in rats and mice), using nicotinamide adenine dinucleotide (NAD) as a cosubstrate and generating sufficient amounts of the reduced form of NAD (NADH) to alter the redox potential of the cell. This dependence sets it in contrast to 11β-HSD1, which uses the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), preferentially catalyzes the conversion of the oxidized to the reduced form, and has received substantial attention as a target for the treatment of metabolic syndrome.[340] Aldosterone has a very reactive aldehyde group at carbon 18 (see Fig. 12.1), which forms an 11,18-hemiacetal and is protected from dehydrogenation by 11β-HSD2.[5,6]

SITES OF EXPRESSION

In the kidney, 11β-HSD2 is expressed at high levels throughout the ASDN,[175,186,341] where it is coexpressed with MR and ENaC (see Fig. 12.9).[342] It is also coexpressed in DCT with NCC.[343,344] Interestingly, expression has also been found in the thick ascending limb,[186] although expression levels appear to be substantially lower, and increase progressively in DCT. Expression is the highest in the connecting tubule and cortical collecting duct.[341,344] It is also expressed in the aldosterone-sensitive segments of the colon, particularly the distal colon, as is the case for MR, although there is species variability.[345] 11β-HSD2 expression has also been described in several nonepithelial tissues, including placenta,[346] the nucleus tractus solitarius in the brain,[347] and the vessel wall,[56] which makes all these tissues potential aldosterone target tissues.

IMPACT ON MINERALOCORTICOID RECEPTOR ACTIVITY

The initial[5,6] and still widely held interpretation of the role of 11β-HSD2 was that of excluding active glucocorticoids from epithelial MR, which allowed aldosterone unfettered access. This is only part of the picture, however; to reduce the signal to noise ratio from 100-fold to 10% would require that 999 of every 1000 cortisol molecules entering the cell be metabolized to cortisone, a very tall order in an organ such as the kidney, which commands 20% to 25% of cardiac output. 11β-HSD2 in epithelia (and in other tissues in which it is expressed) clearly reduces glucocorticoid levels by an order of magnitude[348] but still leaves them with intracellular levels well above those of aldosterone. At the same time, although it is clear that when 11β-HSD2 is operative, glucocorticoid-occupied MR is not transcriptionally active, it is also clear that when enzyme activity is insufficient (as in apparent mineralocorticoid excess) or deficient (as in licorice abuse or by genetic mutation), cortisol can activate MR and ion transport. Although the subcellular mechanisms involved have yet to be established, it appears that glucocorticoid-MR complexes are conformationally distinct from aldosterone-MR complexes. One intriguing possibility is that these hormone-receptor complexes, in contrast to aldosterone–MR complexes, are held inactive by the obligate generation of NADH from the cosubstrate NAD, required for the operation of 11β-HSD2.[349] There is direct evidence to support the idea that redox potential affects the activity of the glucocorticoid receptor through effects on thioredoxin.[350]

APPARENT MINERALOCORTICOID EXCESS: A DISEASE OF DEFECTIVE 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 2

Apparent mineralocorticoid excess was first described by New, and the molecular mechanisms responsible were established after an intense but fruitless search for a novel mineralocorticoid.[351] The condition reflects a partial or complete deficiency of 11β-HSD2 activity, is more common in consanguinity, and manifests as severe juvenile hypertension (see also Chapters 17 and 44).[352] Confectionery licorice (or that added to chewing tobacco) contains glycyrrhizic and glycyrrhetinic acids, suicide substrates for 11β-HSD2, which thus acts as a potent inhibitor of the enzyme. Lack of functional 11β-HSD2 results in MR activation by cortisol and inappropriate mineralocorticoid-like stimulation of ENaC-mediated Na^+ reabsorption. This causes severe hypertension, often accompanied by hypokalemia. Plasma renin, angiotensin II, and aldosterone are suppressed. Treatment of apparent mineralocorticoid excess is the use of MR antagonists and additional antihypertensives, as required. Treatment of licorice abuse is moderation.

ROLE IN BLOOD VESSELS

Studies of 11β-HSD2 in the human vascular wall[353] have defined the activity of aldosterone and cortisol in this physiologic aldosterone target tissue. Aldosterone at nanomolar concentrations causes a rapid rise in the intracellular pH, reflecting nongenomic activation of the Na^+-H^+ exchanger. Cortisol alone over a range of doses produced no effect, but when carbenoxolone was added to inhibit 11β-HSD2, cortisol mimicked aldosterone. Inhibitor studies have revealed that the effects of both aldosterone and cortisol are mediated by classic MR. In other studies involving tissue damage, mineralocorticoid antagonists were protective, whereas aldosterone or cortisol worsened injury. The inference from these results was that cortisol becomes an MR agonist in the context of tissue damage (or when 11β-HSD2 is pharmacologically inhibited), with alteration of reactive oxygen species generation and redox potential.[354] It is further notable that aldosterone has been shown to have both vasodilatory and vasoconstricting effects in animals and humans.[355] These contradictory results have not been fully reconciled,[356] but may well reflect a combination of direct effects on vascular smooth muscle to stimulate myosin light-chain phosphorylation through ERK activation[357,358] on the one hand, and stimulatory effects on endothelial cell nitric oxide synthase[355] on the other. Finally, it is of considerable interest that vascular smooth muscle cells express ENaC, in addition to MR, and that the channel might play a role in vascular tone.[359]

SUMMARY OF 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 2 ROLES

In summary, the enzyme 11β-HSD2 is crucial for the aldosterone-selective activation of epithelial MR and possibly of MR in other tissues, including blood vessels, nucleus of the solitary tract, and placenta. It does this in part by debulking intracellular glucocorticoids by an order of magnitude, which is not sufficient to account for its blockade of cortisol agonist activity. Current evidence supports the possibility that 11β-HSD2–mediated generation of NADH renders glucocorticoid-occupied MR inactive. Partial or complete deficiency of 11β-HSD2 results in the syndrome of apparent mineralocorticoid excess, which is mimicked by licorice abuse.

NONGENOMIC EFFECTS OF ALDOSTERONE

The classic effects of aldosterone on ion transport are genomic, with MR acting at the nuclear level to regulate DNA-directed, RNA-mediated protein synthesis and thereby sodium transport. Such genomic effects are characterized by a lag period of 45 to 60 minutes before changes in ion transport can be measured, commensurate with a homeostatic role for aldosterone action in regulating sodium and potassium status in response to dietary intake. In other circumstances (e.g., orthostasis, acute blood volume depletion), aldosterone secretion rises rapidly, and acute nongenomic effects are an understandable response. Such rapid effects were first demonstrated over 30 years ago in the laboratory[360]; in human vascular tissues, they have been amply demonstrated both in vitro[353] and in vivo.[361] Although most of these rapid nongenomic effects appear to be mediated via activation of classic MR,[353,360] there is evidence from atomic force microscopy studies for non-MR membrane sites binding aldosterone with high affinity on cultured endothelial cells.[362] Such nongenomic effects are not unique to aldosterone, having been shown for the other recognized classes of steroid hormones[363] and reported for dehydroepiandrosterone (DHEA).[355] Genomic effects commonly have a lag period of 20 minutes or longer and are abrogated by inhibitors of transcription, such as actinomycin D. Most nongenomic effects of steroids have time courses from onset to plateau of 5 to 10 minutes and are mediated by a variety of pathways.

MR does not have a myristoylation site (e.g., unlike estrogen receptors[364]), and there is little evidence for membrane-associated classic MR. Most rapid nongenomic effects of aldosterone appear to be mediated by classic MR in that they are inhibited by the MR antagonist RU 28318. In some cases,[353] spironolactone is ineffective as an inhibitor; exclusive reliance on blockade by spironolactone has led to the assumption of a widely distributed aldosterone receptor distinct from classic MR and a long and unsuccessful search for such a membrane-bound species.[365] The physiology of nongenomic aldosterone actions has been slow to be accepted, which in part reflects the major emphasis on the clearly genomic actions of aldosterone in the kidney. The most obvious example is the conjunction of rapid secretion of aldosterone in response to orthostasis and its demonstrated rapid vascular effects.[356,366] With more interest in the pathophysiologic effects of MR activation, particularly in nonclassic aldosterone target tissues, there has been renewed interest in the rapid nongenomic effects of aldosterone (and the physiologic glucocorticoids) via classic MR. Further details of the nongenomic actions of aldosterone can be found in reports by Funder[366] and other sources.[367,368]

DISEASE STATES

PRIMARY ALDOSTERONISM

Clinically, the most prevalent disorder directly involving aldosterone is Conn syndrome, or primary aldosteronism.[369]

In this syndrome, aldosterone secretion is elevated and (relatively) autonomous as a result of an adrenal adenoma or, more frequently, bilateral adrenal hyperplasia and, very rarely, adrenal carcinoma or the inherited disorder glucocorticoid remediable aldosteronism (FH-1). Once considered rare (<1% of all cases of hypertension), necessarily characterized by hypokalemia and relatively benign, primary aldosteronism is now thought to account for approximately 8% to 13% of all hypertension, which reflects improved case detection and diagnosis. In contrast with previous teachings, frank hypokalemia is found in only 25% to 30% of cases, and the incidence of cardiovascular pathology (e.g., fibrosis, fibrillation, infarct, stroke) is substantially higher than in age-, gender-, and blood pressure–matched individuals with essential hypertension.[370,371]

Guidelines for the case detection, diagnosis, and management of primary aldosteronism have been published[372] as a first step in addressing what has been increasingly recognized as a major public health issue. It has long been thought and taught that the role of aldosterone in blood pressure regulation reflects its epithelial effects leading to retention of sodium, and with it water, which thus increases circulating volume. This increase, in turn, is reflected in an increased cardiac output, which is reflexively normalized by vasoconstriction and thus elevation of blood pressure (in keeping with the Guyton hypothesis[373]). Although the epithelial effects of aldosterone on vascular volume are indisputably homeostatically important, there have been compelling experimental and clinical studies to suggest a role for nonepithelial effects in mineralocorticoid-induced hypertension.[374,375] In addition to MR-mediated central nervous system and vascular effects in hypertension, roles for macrophages have been demonstrated by two groups using distinct and complementary experimental approaches.[376,377]

Other studies have suggested a role for a mutated K^+ channel (KCNJ5) in the pathogenesis of aldosterone-producing adrenal adenomas and in the rare condition of FH type III.[25,26] See earlier, "Aldosterone Synthesis," for additional details.

CONGESTIVE HEART FAILURE

Aldosterone has been implicated in the pathophysiology of congestive heart failure since soon after its discovery in the mid-1950s.[378,379] Until fairly recently, most of the focus has been on the counterproductive effects of aldosterone in epithelia. More recently, the beneficial effects of MR antagonists in congestive heart failure have suggested an additional effect in myocardium itself.[380] In the Randomized Aldactone Evaluation Study (RALES),[380] addition of low-dose (mean, 26 mg/day) spironolactone to standard of care treatment in patients with progressive heart failure produced a 30% reduction in mortality and 35% fewer hospitalizations. This result is often attributed to spironolactone antagonizing the effect of aldosterone on cardiomyocyte MR, but actually reflects its antagonizing of cortisol acting as an MR agonist under ischemic conditions. Subsequently, the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) examined the effect of eplerenone, an MR antagonist with improved specificity relative to spironolactone, on heart failure due to systolic dysfunction complicating acute myocardial infarction. The study showed that adding eplerenone (25 mg/day) to conventional therapy significantly decreased mortality due to all causes (31%) and cardiovascular mortality (13%).[381] Potassium concentration was only slightly higher in the eplerenone-treated group than in the placebo-treated group (4.47 mmol/L and 4.54 mmol/L, respectively). Coupled with studies on the direct vascular effects of aldosterone addressed earlier, these data suggest that MR antagonists have a beneficial effect that cannot be accounted for by diuretic actions in the kidney alone.[382]

It is also notable that a trial (Eplerenone in Mild Patients Hospitalization And SurvIval Study in Heart Failure [EMPHASIS-HF]) examining the effect of eplerenone in New York Heart Association (NYHA) class II heart failure (milder than previously examined), was stopped early because a significant benefit was found in the treated group.[383] In summary, the pathophysiologic effects of aldosterone excess on the cardiovascular system in primary aldosteronism have been well documented, and MR also plays an important role in essential hypertension and heart failure. Importantly, MR expressed in cardiac and vascular cells may commonly be activated by cortisol rather than aldosterone, which is present in serum at levels that are higher by 100-fold or more and mimics aldosterone in the context of tissue damage.

CHRONIC KIDNEY DISEASE

The role of MR blockade in slowing the progression of chronic kidney disease has been considered in a recent study and commentary[384,385] and in Chapter 59. The study, a double-blind, randomized, placebo-controlled trial, examined the antialbuminuric effect of the MR antagonist eplerenone in nondiabetic hypertensive patients with albuminuria.

NONEPITHELIAL ACTIONS OF ALDOSTERONE

In addition to the classic epithelial tissues involved in ion transport—kidney, colon, sweat gland, salivary gland—there are documented effects of aldosterone in the brain, vascular wall, and possibly the placenta, as previously noted. Many other tissues and organs have been postulated as physiologic aldosterone target tissues, largely on the inadequate evidence that they express MR and can be shown in vitro to respond by some measure to aldosterone. What underpins these hypotheses is the misconception that aldosterone is the cognate ligand for MR, which is true for epithelia but not for cells not expressing 11β-HSD2, coupled with disregard for the role of cortisol. Cortisol was not only the ligand for MR in cartilaginous and bony fish, millions of years before the appearance of aldosterone synthase, but is the overwhelming occupant of MR that is not protected by 11β-HSD2 (primarily nonepithelial MR) throughout the body. It is notable that some nonepithelial MR is also protected by 11β-HSD2—for example, in the nucleus tractus solitarius.

The fact that aldosterone can activate MR under experimental conditions without 11β-HSD2 was illustrated by the work of Gómez-Sánchez and colleagues more than 2 decades ago.[386] Very low doses of aldosterone that did not affect blood pressure when infused systemically elevated blood pressure when infused into the lateral ventricle of conscious, free-living rats. That this did not reflect a physiologic role for aldosterone,

however, was shown by the co-infusion of one, two, and five times the dose of corticosterone, which progressively blocked the blood pressure effect of the infused aldosterone, evidence for the absence of 11β-HSD2 in the hypothalamic nuclei involved and the overwhelming occupancy of their MR by the physiologic glucocorticoid.

The two established nonepithelial aldosterone target tissues are the vascular wall and nucleus tractus solitarius in the brain. Both these tissues express 11β-HSD2, as noted earlier, allowing aldosterone-selective MR activation; both can be reasonably envisaged as having important ancillary roles supporting the primary epithelial role of aldosterone on fluid and electrolyte homeostasis. Aldosterone vasoconstricts blood vessels, acutely and in the longer term, in response to volume depletion; similarly, it acts on the nucleus tractus solitarius to stimulate salt appetite. Both actions are thus harnessed into the physiologic role of aldosterone in maintaining fluid and electrolyte balance.

It is commonly assumed that in pathophysiologic states of high aldosterone levels, such as primary aldosteronism, the deleterious effects are mediated by aldosterone occupying and inappropriately activating nonprotected MR in cardiomyocytes, for example. It is plausible that instead of the approximately 1% physiologic occupancy (given the ≈100-fold higher levels of plasma free cortisol), aldosterone occupancy of cardiomyocyte MR might rise to 3% to 5%. Relatively minor degrees of MR occupancy have been shown to be effective for spironolactone, acting as a protective inverse agonist[387]; similarly, therefore, minor degrees of cardiomyocyte MR occupancy by aldosterone could potentially produce the deleterious effects seen.

This explanation, however, is almost certainly incorrect. Plasma aldosterone levels are as high or higher in chronic sodium deficiency (or in the effectively volume-depleted condition of secondary hyperaldosteronism), with no deleterious cardiovascular effects. In primary and secondary aldosteronism, and in chronic sodium deficiency, physiologic target tissues, both renal tubular and coronary vascular, are exposed to (and respond to) maintained high levels of aldosterone. It is thus unlikely that the cardiovascular damage in primary aldosteronism reflects increased MR activation in blood vessels, coronary and peripheral. The key difference between these circumstances is that primary aldosteronism is a state of aldosterone and sodium excess and the others of sodium and volume depletion.

A plausible but untested mechanism of aldosterone-induced damage is that it is secondary to increased renal sodium reabsorption and the action of endogenous ouabain on blood vessels. Endogenous ouabain is incompletely explored, but its levels are elevated in primary aldosteronism.[388] Like aldosterone, its secretion is elevated by ACTH and angiotensin (the latter via AT_2R); in stark contrast with aldosterone, it is raised (not lowered) in states of sodium excess.[389–391] It acts via Na^+-K^+-ATPase in vessel walls as a vasoconstrictor, presumably physiologically to produce a pressure natriuresis as a homeostatic response. Thus, it may be that the cardiovascular damage in primary aldosteronism reflects a combination of the effects of aldosterone plus endogenous ouabain on the vasculature; if this is the case, the source and origin of the nonepithelial effects of aldosterone remain squarely in the renal tubule and the exaggerated sodium retention therein.

RECENT ADVANCES IN NONRENAL MINERALOCORTICOID RECEPTOR–MEDIATED DISEASE PATHOLOGY

Recent developments in the use of MR antagonists for nonrenal disease are not described in detail here. See the report by Jaisser and Farman[392] for a review. It is particularly worth noting the surprising recent use of spironolactone for the treatment of retinal diseases. MR is expressed in several retinal cell types, including glial cells, which are essential for retinal water and ion homeostasis, epithelial cells, and choroidal endothelial cells. The activation of MR has been implicated in multiple retinal pathologies, and MR antagonists, particularly spironolactone, have been shown to have therapeutic benefit.[392,393]

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Our coauthor, colleague, and friend John Stokes died in 2012, just before work on the 10th edition of this textbook began. John was senior author on the chapter "Aldosterone Regulation of Ion Transport," written as a new chapter for the 9th edition of Brenner and Rector, and inspired our writing and focus for subsequent editions, including the present chapter for the 11th edition. John had a profound knowledge of aldosterone action in the renal tubules, a sharp wit, and unsurpassed work ethic. For all these reasons, he was the model coauthor and colleague. John also made enormous primary contributions to aldosterone and renal tubule research, which enriched all of us, as did his humor and enthusiasm for life. For those interested in reading more about John's inspiring life and contributions to nephrology and renal research, see the eloquent eulogy at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3715930/.

Complete reference list available at ExpertConsult.com.

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BOARD REVIEW QUESTIONS

1. The most appropriate initial treatment for a patient with primary aldosteronism due to bilateral adrenal hyperplasia is:
   a. Triamterene
   b. Bilateral adrenalectomy
   c. Spironolactone
   d. Beta blockers
   e. Thiazide diuretics
   Answer: c

2. The enzyme conferring aldosterone-specificity on mineralocorticoid receptors in epithelial tissues is:
   a. CYPIIB1
   b. CYPIIB2
   c. 11βOHSD1
   d. 11βOHSD2
   e. Aldosterone synthase
   Answer: d

3. Mineralocorticoid receptors are aldosterone regulated:
   a. Transcription factors.
   b. Receptor tyrosine kinases.
   c. G-protein coupled receptors.
   d. Mitochondrial enzymes.
   e. Epithelial sodium channels.
   Answer: a

4. Serum- and glucocorticoid-regulated kinase 1 (SGK1) acts in principal cells to:
   a. Inhibit K+ channels
   b. Stimulate the epithelial sodium channel (ENaC)
   c. Stimulate aldosterone uptake
   d. Stimulate cortisol break down
   e. Inhibit the Krebs cycle
   Answer: b

5. Which of the following is true?
   a. Acute aldosterone regulated potassium secretion is primarily via direct stimulation of potassium channels
   b. Mineralocorticoid receptors are exclusively expressed in the distal nephron
   c. Hyperkalemia-induced aldosterone secretion leads to high blood pressure
   d. Sodium reabsorption increases the driving force for potassium and proton efflux in the distal nephron.
   Answer: d

13

Arachidonic Acid Metabolites and the Kidney

Raymond C. Harris | Ming-Zhi Zhang | Richard M. Breyer

CHAPTER OUTLINE

KEY POINTS

- The kidney is source of eicosanoids (prostaglandins, lipoxygenase metabolies, cytochrome P450 metabolites.
- Eicosanoids derived from arachidonic acid play important roles in kidney physiology and pathophysiology.
- Prostaglandins can regulate renal hemodynamics, the renin-angiotensin system and salt and water excretion.
- Eicosanoids are important mediators of systemic blood pressure.

CELLULAR ORIGIN OF EICOSANOIDS

Eicosanoids comprise a family of biologically active, oxygenated arachidonic acid (AA) metabolites. AA is a polyunsaturated fatty acid possessing 20 carbon atoms and four double bonds (C20:4) and is formed from linoleic acid (C18:2) by the addition of two carbons to the chain and further desaturation. In mammals, linoleic acid is derived strictly from dietary sources. Essential fatty acid deficiency occurs when dietary fatty acid precursors, including linoleic acid, are omitted, thereby depleting the hormone-responsive pool of AAs. Essential fatty acid deficiency thereby reduces the intracellular availability of AA in response to hormonal stimulation and abrogates many biologic actions of hormone-induced eicosanoid release.[1]

Of an approximate 10 g of linoleic acid ingested/day, only about 1 mg/day is eliminated as end products of AA metabolism. Following its formation, AA is esterified into cell membrane phospholipids, principally at position 2 of the phosphatidylinositol fraction (i.e., sn-2–esterified AA), the major hormone-sensitive pool of AA that is susceptible to release by phospholipases.

Multiple stimuli lead to the release of membrane-bound AA via the activation of cellular phospholipases, principally the phospholipase A2 family (PLA2).[2] This cleavage step is rate limiting in the production of biologically relevant arachidonate metabolites. In the case of PLA2 activation, membrane receptors activate guanine nucleotide-binding (G) proteins, leading to the release of AA directly from membrane phospholipids. Activation of PLC or PLD, on the other hand, releases AA via the sequential action of the phospholipase-mediated production of diacylglycerol (DAG), with the subsequent release of AA from DAG by DAG lipase.[3] This pathway may also lead to the formation of the esterified AA metabolites arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG), the endocannabinoids. These endocannabinoids can subsequently be converted to free AA by the action of monoacylglycerol lipases.[4] When considering eicosanoid formation, the physiologic significance of AA release by these other phospholipases remains uncertain because, at least in the setting of inflammation, PLA2 action appears to be essential for the generation of biologically active AA metabolites.[5]

More than 15 proteins with PLA2 activity are known to exist, including secreted (sPLA2) and cytoplasmic PLA2 (cPLA2) isoforms.[6,7] A mitogen-activated cytoplasmic PLA2 has been found to mediate AA release in a calcium-/calmodulin-dependent manner. Other hormones and growth factors,

including epidermal growth factor (EGF) and platelet-derived growth factors, activate PLA2 directly through tyrosine residue kinase activity, allowing the recruitment of co-activators to the enzyme without an absolute requirement for the intermediate action of Ca^{2+}-calmodulin or other cellular kinases.

Following de-esterification, AA is rapidly re-esterified into membrane lipids or avidly bound by intracellular proteins, hence making AA unavailable for further metabolism. Should it escape re-esterification and protein binding, free AA becomes available as a substrate for one of three major enzymatic transformations, the common result of which is the incorporation of oxygen atoms at various sites of the fatty acid backbone, with accompanying changes in its molecular structure (e.g., ring formation).[8,9] This results in the formation of biologically active molecules, referred to as *eicosanoids*. The specific nature of the products generated is a function of the initial stimuli for AA release, as well as the metabolic enzyme available, which is determined, in part, by the cell type involved.[9,10]

These products, in turn, either mediate or modulate the biologic actions of the agonist in question. AA release may also result from nonspecific stimuli, such as cellular trauma, including ischemia and hypoxia,[11] oxygen free radicals,[12] and osmotic stress.[13] The identity of the specific AA metabolite generated in a particular cell system depends on the proximate stimulus and availability of the downstream AA metabolizing enzymes present in that cell.

Three major enzymatic pathways of free AA metabolism are present in the kidney—cyclooxygenases, lipoxygenases, and cytochrome P-450 (Fig. 13.1). The cyclooxygenase pathway mediates the formation of prostaglandins (PGs) and thromboxanes. The lipoxygenase pathway mediates the formation of mono-, di-, and trihydroxyeicosatetraenoic acids (HETEs), leukotrienes (LTs), and lipoxins (LXs). Whereas the cytochrome P-450– dependent oxygenation of AA mediates the formation of epoxyeicosatrienoic acids (EETs), their corresponding diols, HETEs, and monooxygenated AA derivatives. Fish oil diets, rich in ω-3 polyunsaturated fatty acids,[14] interfere with metabolism via all three pathways by competing with AA oxygenation, resulting in the formation of biologically inactive end products.[15] Interference with the production of proinflammatory lipids has been hypothesized to underlie the beneficial effects of fish oil in immunoglobulin A (IgA) nephropathy, and other cardiovascular diseases.[16] The following sections deal with the current understanding of the chemistry, biosynthesis, renal metabolism, mechanisms of release, receptor biology, signal transduction pathways, biologic activities, and functional significance of each of the metabolites generated by the three major routes of AA metabolism in the kidney.

THE CYCLOOXYGENASE PATHWAY

See Fig. 13.2.

MOLECULAR BIOLOGY

The cyclooxygenase (COX) enzyme system is the major pathway for AA metabolism in the kidney. COX (prostaglandin synthase G2/H2) is the enzyme responsible for the initial conversion of AA to prostaglandin G2 and subsequently to prostaglandin H2. The COX protein was first purified from ram seminal vesicles, and cDNA encoding COX was cloned in 1988. The protein is widely expressed, and the level of activity is not dynamically regulated. Other studies have supported the presence of a COX isoform that was dynamically regulated and responsible for increased prostanoid production in inflammation. This second inducible COX isoform was identified shortly after the cloning of the initial enzyme and designated as COX-2, whereas the initially isolated isoform is now designated as COX-1.[8,17,18] COX-1 and COX-2 are encoded by distinct genes located on different chromosomes. The human COX-1 gene (PTGS1 [prostaglandin synthase 1]) is distributed over 40 kB on 11 exons on chromosome 9, whereas COX-2 is localized on chromosome 1 and spans approximately 9 kB. The genes are also subject to dramatically different regulatory signals.

REGULATION OF COX GENE EXPRESSION

At the cellular level, COX-2 expression is highly regulated by several processes that alter its transcription rate, message

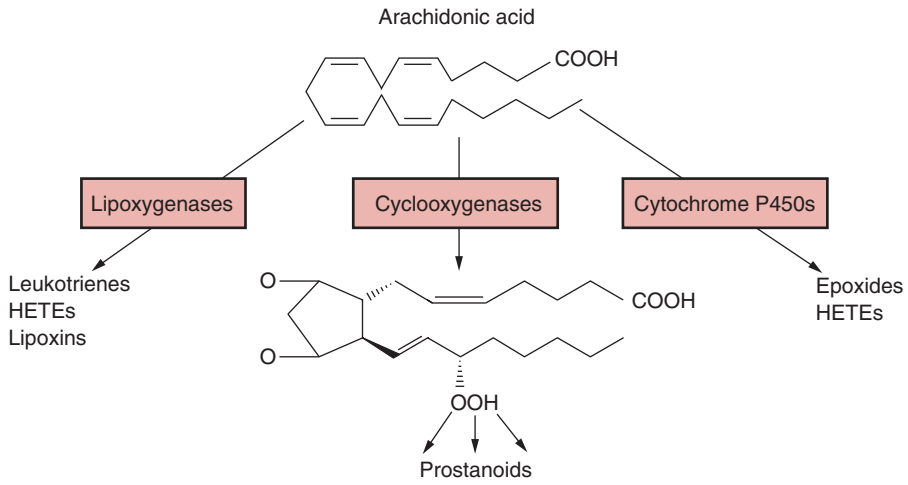


Fig. 13.1 Pathways of enzymatically mediated arachidonic acid metabolism. Arachidonic acid can be converted into biologically active compounds by cyclooxygenase (COX), lipoxygenase (LOX), or cytochrome P450 (CYP450)–mediated metabolism. *HETE,* Hydroxyeicosatetraenoic acid.

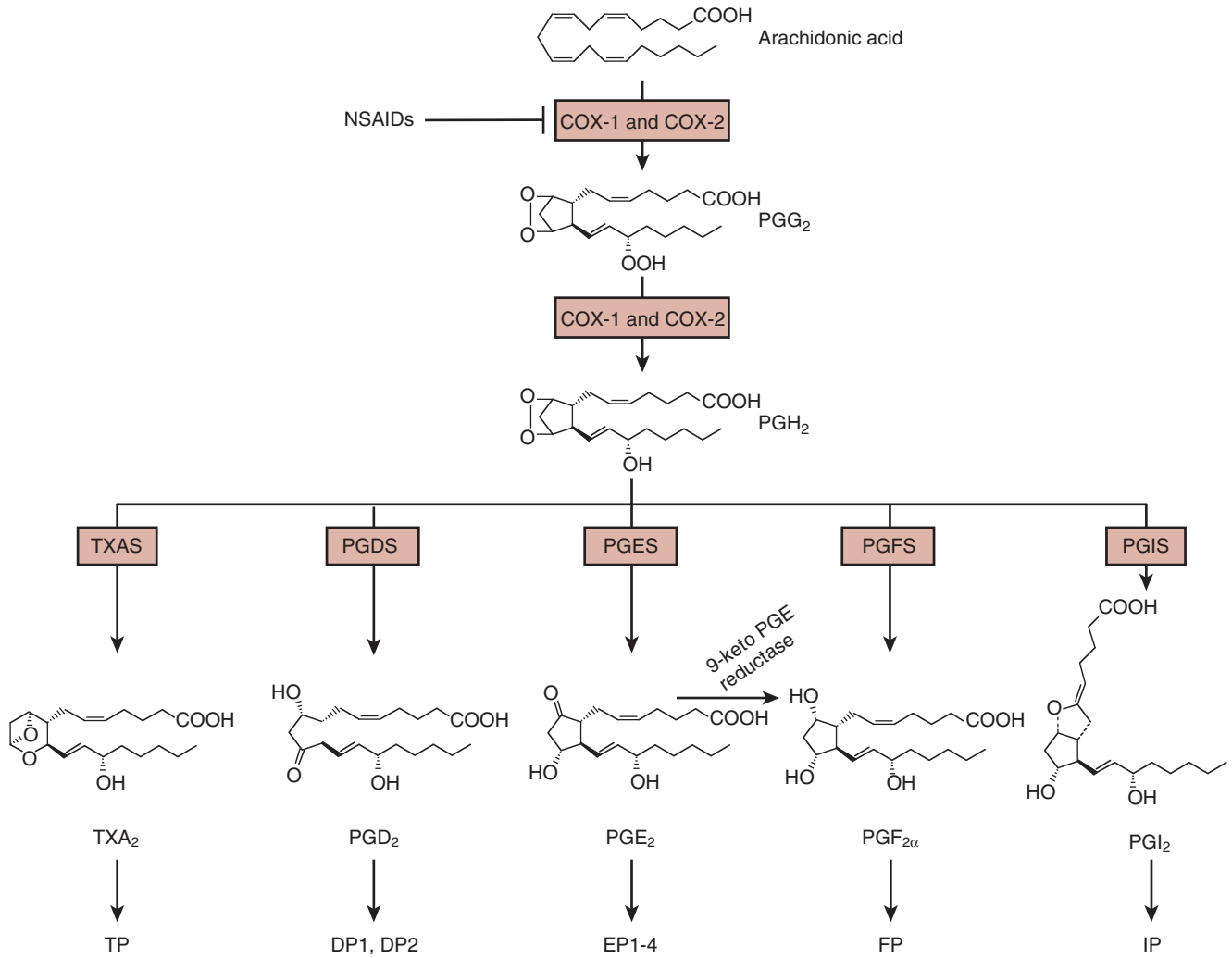


Fig. 13.2 Cyclooxygenase (COX) metabolism of arachidonic acid (AA). Both COX-1 and COX-2 convert AA to prostaglandin (PG) H2 (PGH2), which is then acted on by specific synthases to produce prostanoids that act at G protein–coupled receptors that increase or decrease cyclic adenosine monophosphate (cAMP) or increase intracellular calcium. *NSAID,* Nonsteroidal antiinflammatory drug; *TXAS,* thromboxane synthase; *PGDS,* prostaglandin D synthase; *PGES,* prostaglandin E synthase; *PGFS,* prostaglandin F synthase; *PGIS,* prostacyclin synthase.

export from the nucleus, messenger RNA (mRNA) stability, and efficiency of mRNA translation.[19,20] These processes tightly control the expression of COX-2 in response to many of the same cellular stresses that activate arachidonate release (e.g., cell volume changes, shear stress, hypoxia),[11,21] as well as a variety of cytokines and growth factors, including tumor necrosis factor (TNF), interleukin-1ß, epidermal growth factor, and platelet-derived growth factor (PDGF). Activation of COX-2 gene transcription is mediated via the coordinated activation of several transcription factors that bind to and activate consensus sequences in the 5′ flanking region of the COX-2 gene for nuclear factor-kappa B (NF-κB), NF–IL-6/C–enhancer-binding protein (CEBP), and a cyclic adenosine monophosphate (cAMP) response element (CRE).[22] The induction of COX-2 mRNA transcription by endotoxin (lipopolysaccharide) may also involve CRE[23] and NF-κB sites.[24]

REGULATION OF COX EXPRESSION BY ANTIINFLAMMATORY STEROIDS

A molecular basis linking the antiinflammatory effects of COX-inhibiting nonsteroidal antiinflammatory drugs (NSAIDs) and antiinflammatory glucocorticoids has long been sought. A novel mechanism for the suppression of arachidonate metabolism by corticosteroids involving the translational inhibition of COX formation had been suggested before the molecular recognition of COX-2. With the cloning of COX-2, it became well established that glucocorticoids suppress COX-2 expression and prostaglandin synthesis, an effect now viewed as central to the antiinflammatory effects of glucocorticoids. Posttranscriptional control of COX-2 expression represents another robust mechanism whereby adrenal steroids regulate COX-2 expression.[25] Accumulating evidence has suggested that COX-2 is modulated at multiple steps in addition to transcription rate, including stabilization of the mRNA and enhanced translation.[19,26] Glucocorticoids, including dexamethasone, downregulate COX-2 mRNA, in part by destabilizing the mRNA.[26] The 3′-untranslated region of the COX-2 mRNA contains 22 copies of an AUUUA motif, which are known cis-RNA sequences important in destabilizing the COX-2 message in response to dexamethasone; other 3′ sequences appear to be important for COX-2 mRNA stabilization in response to IL-1ß.[26] Effects of the 3′-untranslated region

(3′-UTR), as well as other factors regulating the efficiency of COX-2 translation, have also been suggested.[19] The factors determining the expression of COX-1 are more obscure.

ENZYMATIC CHEMISTRY

Despite these differences, both prostaglandin (PG) synthases catalyze a similar reaction, resulting in the cyclization of C8 to C12 of the AA backbone, forming cyclic endoperoxide, accompanied by the concomitant insertion of two oxygen atoms at C15 to form PGG2 (a 15-hydroperoxide). In the presence of a reduced glutathione-dependent peroxidase, PGG2 is converted to the 15-hydroxy derivative, PGH2. The endoperoxides (PGG2 and PGH2) have very short half-lives, about 5 minutes, and are biologically active in inducing aortic contraction and platelet aggregation.[27] However, under some circumstances, the formation of these endoperoxides may be strictly limited via the self-deactivating properties of the enzyme.

The expression of recombinant enzymes and determination of the crystal structure of COX-2 have provided further insight into the observed physiologic and pharmacologic similarities to, and differences from, COX-1. It is now clear that NSAIDs work by inhibiting cyclooxygenases by sterically blocking access of AA to the heme-containing active enzymatic site.[28] Aspirin, also known as acetylsalicylic acid (ASA), predominantly targets COX-1. Particularly well conserved are COX-1 sequences surrounding the aspirin-sensitive serine residue at position 529 of COX-1, at which acetylation by aspirin irreversibly inhibits activity.[29] More recent evidence has shown that COX-1 and COX-2 are capable of forming heterodimers and sterically modulating each other's function.[30] The substrate-binding pocket of COX-2 is larger and therefore accepting of bulkier inhibitors and substrates. This difference has allowed the development and marketing of relatively and highly selective COX-2 inhibitors for clinical use as analgesics,[31] antipyretics,[32] and antiinflammatory agents.[31] In addition to its central role in inflammation, aberrantly upregulated COX-2 expression has been implicated in the pathogenesis of a number of epithelial cell carcinomas[33] and in Alzheimer disease and other degenerative neurologic conditions.[34]

RENAL COX-1 AND COX-2 EXPRESSION

See Fig. 13.3.

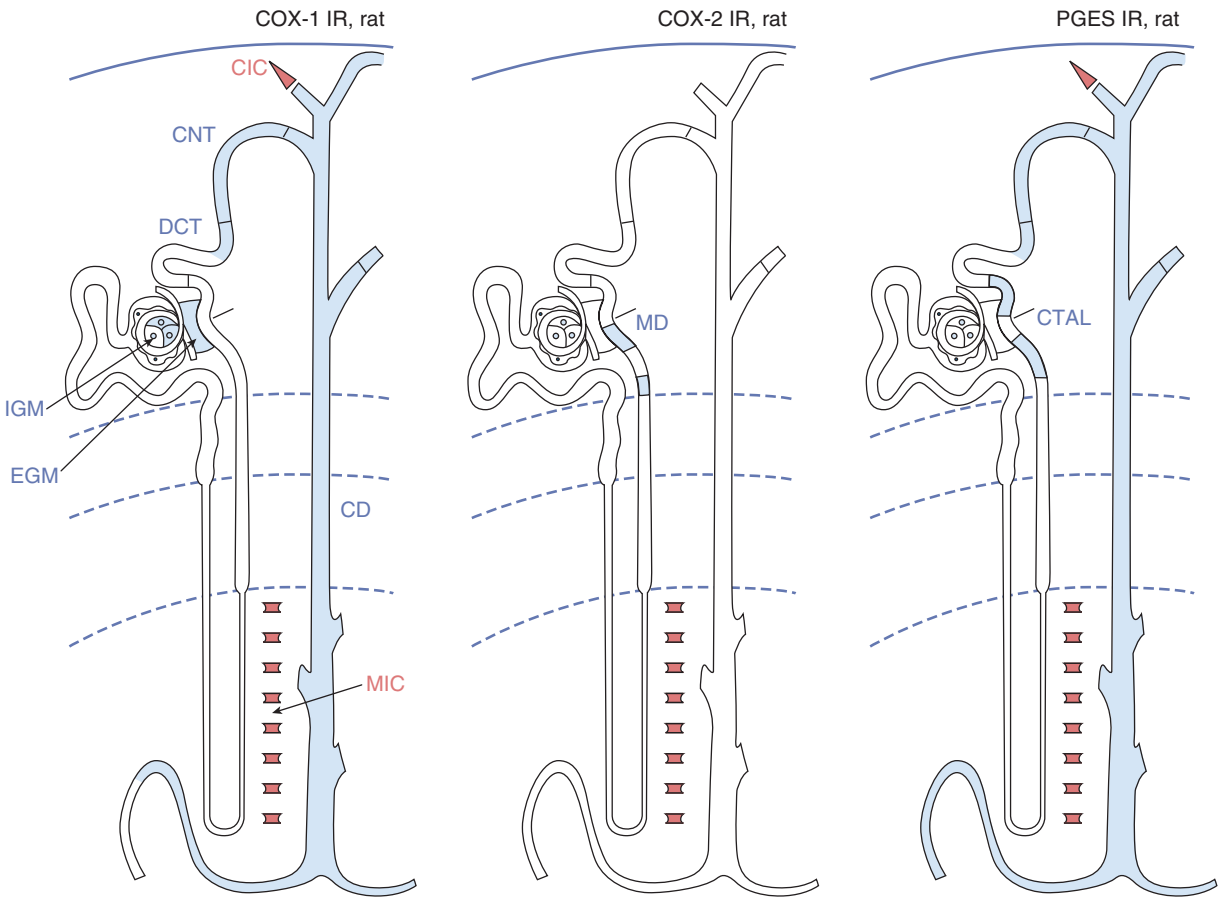


Fig. 13.3 Localization (indicated in *blue shaded areas*) of immunoreactive (IR) cyclooxygenase 1 and 2 (COX-1, COX-2) and microsomal prostaglandin E synthase *(PGES)* along the rat nephron. Eicosanoid expression in cortical (CIC) and medullary (MIC) interstitial cells is shaded in *red*. *CD,* Collecting duct; *CNT,* connecting tubule; *CTAL,* cortical thick ascending limb; *DCT,* distal convoluted tubule; *EGM,* extraglomerular mesangium; *IGM,* intraglomerular mesangium; *MD,* macula densa. (Modified from Campean V, Theilig F, Paliege A, et al. Key enzymes for renal prostaglandin synthesis: site-specific expression in rodent kidney [rat, mouse]. *Am J Physiol Renal Physiol.* 2003;285:F19–F32.)

COX-2 EXPRESSION

COX-2 EXPRESSION IN THE KIDNEY

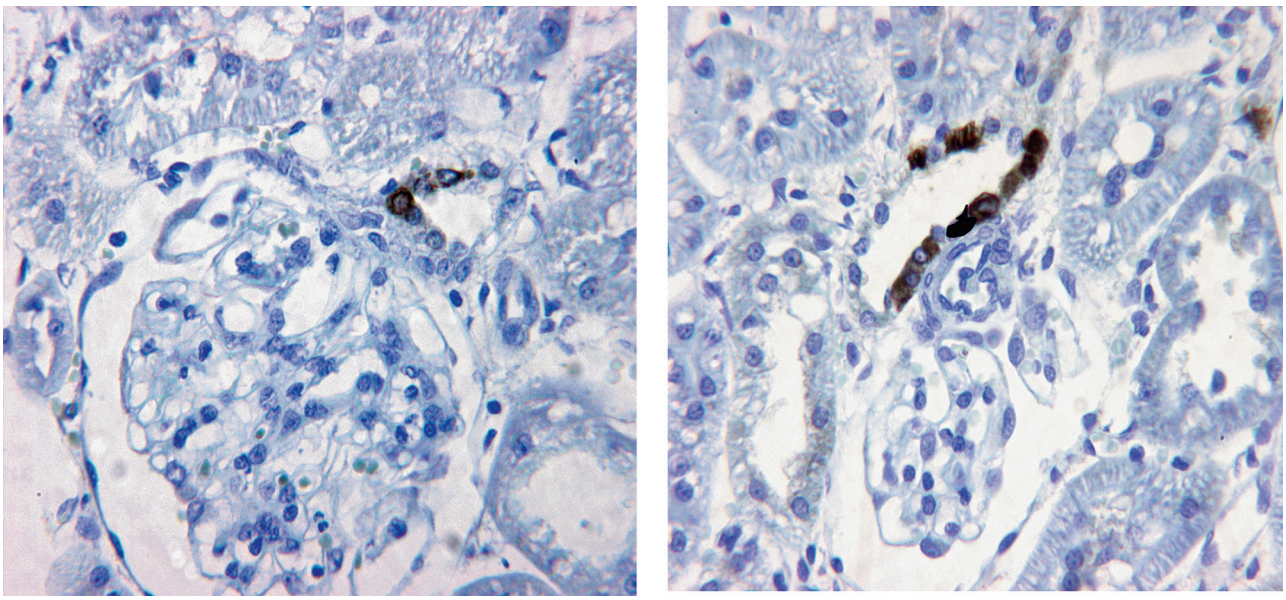
There is now definitive evidence for significant COX-2 expression in the mammalian kidney. This contrasts with the usual view that COX-2 is induced by cellular injury in other tissues, such as blood vessels. COX-2 mRNA and immunoreactive COX-2 are present at low but detectable levels in the normal adult mammalian kidney, where in situ mRNA hybridization and protein immunolocalization have demonstrated constitutive localized expression of COX-2 mRNA and immunoreactive protein in the cells of the macula densa and in a few cells in the cortical thick ascending limb (TAL) cells immediately adjacent to the macula densa.[35,36] COX-2 expression is also abundant in the lipid-laden medullary interstitial cells in the tip of the papilla.[35,37] Some investigators have reported that COX-2 may be expressed in inner medullary collecting duct cells or intercalated cells in the renal cortex in the collecting duct.[38] Nevertheless, COX-1 expression is also constitutive and clearly the most abundant isoform in the collecting duct, so the potential existence and physiologic significance of COX-2 co-expression in this segment remains uncertain.

COX-2 EXPRESSION IN THE RENAL CORTEX

It is now well documented that COX-2 is expressed in macula densa–cortical thick ascending limb of Henle (cTALH) and in the mammalian kidney,[1,36] including the human kidney, especially in kidneys of older adults,[39,40] patients with diabetes mellitus, congestive heart failure,[41] and Bartter-like syndrome.[42]

The presence of COX-2 in the unique group of cells comprising the macula densa, points to a potential role for COX-2–derived prostanoids in regulating glomerular function.[43] Studies of the prostanoid-dependent control of the glomerular filtration rate (GFR) by the macula densa suggest effects via both dilator and constrictor effects of prostanoids contributing to tubuloglomerular feedback (TGF).[44,45] Some studies have suggested that COX-2–derived prostanoids are predominantly vasodilators.[46,47] By inhibiting the production of dilator prostanoids contributing to the patency of adjacent afferent arteriole, COX-2 inhibition may contribute to the decline in the GFR observed in patients taking NSAIDs or selective COX-2 inhibitors[48] (see later).

The volume-depleted state is typified by low NaCl delivery to the macula densa, and COX-2 expression in the macula densa is increased in states associated with volume depletion (Fig. 13.4).[35] Of note, COX-2 expression in cultured macula densa cells and cTAL cells is also increased in vitro by reducing the extracellular Cl^- concentration. Studies in which cortical thick limbs and associated glomeruli were removed and perfused from rabbits pretreated with a low-salt diet to upregulate macula densa COX-2 demonstrated COX-2–dependent release of PGE2 from the macula densa in response to decreased chloride perfusate.[49] Furthermore the induction of COX-2 by low Cl^- levels can be blocked by a specific p38 MAP kinase inhibitor.[50,51] Finally, in vivo, renal cortical immunoreactive pp38 expression (the active form of p38) predominantly localizes to the macula densa and cTALH and increases in response to a low-salt diet..[50] These findings point to a molecular pathway whereby enhanced COX-2 expression occurring in circumstances associated with intracellular volume depletion could result from decreased luminal chloride delivery. The carbonic anhydrase inhibitor, acetazolamide, and dopamine may both indirectly regulate macula densa COX-2 expression by inhibiting proximal reabsorption and thereby increasing luminal macula densa chloride delivery.[52,53] In mice deficient in the Na^+-H^+ exchanger subtype 2 (NHE2), the macula densa is shrunken, accompanied by increased COX-2 expression



Control Low salt

Fig. 13.4 Cyclooxygenase 2 (COX-2) expression is regulated in renal cortex in rats (original magnification: ×400). Under basal conditions, sparse immunoreactive COX-2 is localized to the macula densa and surrounding cortical thick ascending limb. Following chronic administration of a sodium-deficient diet, macula densa–cortical thick ascending limb COX-2 expression increases markedly.

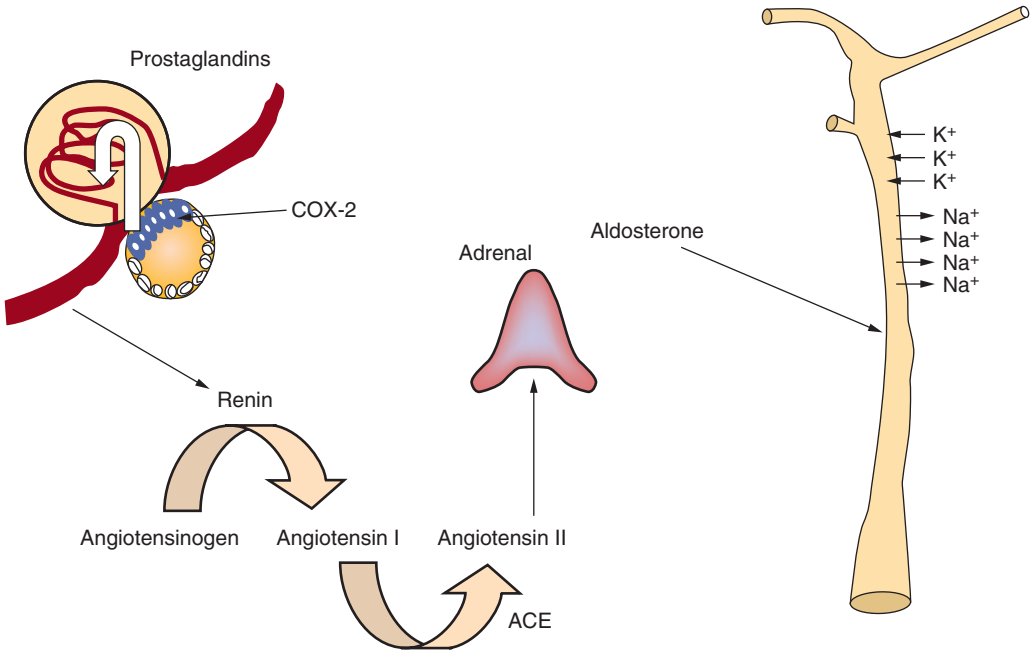


Fig. 13.5 Proposed intrarenal roles for vasodilatory prostaglandins to regulate renal function and blood pressure control. Prostaglandins released from the macula densa and/or the afferent arteriole can vasodilate the afferent arteriole and modulate renin release from juxtaglomerular cells. *ACE,* Angiotensin-converting enzyme; *COX-2,* cyclooxygenase 2.

and juxtaglomerular renin expression, suggesting that NHE2 appears to be the major isoform associated with macula densa cell volume regulation.[54]

In the mammalian kidney, the macula densa is involved in regulating renin release by sensing alterations in luminal chloride via changes in the rate of Na^+-K^+-$2Cl^-$ cotransport (Fig. 13.5).[55] In vivo measurements in isolated perfused kidney and an isolated perfused juxtaglomerular preparation have all indicated that the administration of nonspecific COX inhibitors prevents the increases in renin release mediated by macula densa sensing of decreases in luminal NaCl.[44] Induction of a high-renin state by the imposition of a salt-deficient diet, angiotensin-converting enzyme (ACE) inhibition, diuretic administration, or experimental renovascular hypertension all significantly increase macula densa/cTALH COX-2 mRNA and immunoreactive protein levels.[43] COX-2–selective inhibitors blocked elevations in plasma renin activity, renal renin activity, and renal cortical renin mRNA in response to loop diuretics, ACE inhibitors, or a low-salt diet[43,56–58] and, in an isolated perfused juxtaglomerular preparation, increased renin release in response to lowering the perfusate NaCl concentration that was blocked by COX-2 inhibition.[59] In COX-2 knockout mice, increases in renin in response to low salt or ACE inhibitors were significantly blunted[60,61] but were unaffected in COX-1 knockout mice.[62,63] COX-2–derived PGE2, activating the type 4 (EP4) receptors on juxtaglomerular cells, has been shown to be important for the macula densa regulation of renin release.[49,64] Macula densa COX-2–derived prostanoids appear to be predominantly involved in setting tonic levels of juxtaglomerular renin expression rather than necessarily mediating acute renin release,[65,66] but PGE2 from macula densa does stimulate CD44+ mesenchymal stromal-like cells to migrate to the juxtaglomerular apparatus (JGA) and increase renin production, an effect mediated by EP4 receptors.[67] There is evidence that the effect of ACE inhibitors and angiotensin receptor blockers (ARBs) to increase macula densa COX-2 expression is mediated by feedback of angiotensin II on the macula densa, with AT1 and AT2 receptors inhibiting COX-2 expression.[56] In addition, prorenin and/or renin may stimulate macula densa COX-2 expression through activation of the prorenin receptor.[68]

COX-2 inhibitors have also been shown to decrease renin production in models of renovascular hypertension,[69,70] and studies in mice with targeted deletion of the prostacyclin receptor have suggested a predominant role for prostacyclin in mediating renin production and release in these models.[70] In a model of sepsis, COX-2 expression increased in macula densa and both cortical and medullary TAL. This increased COX-2 expression was mediated by Toll-like receptor 4 (TLR4) and, in $TLR4^{-/-}$ mice, JGA renin expression was absent.[71]

In addition to mediating juxtaglomerular renin expression, COX-2 metabolites may also modulate TGF. However, using different methodologies, investigators have reported that COX-2 metabolites predominantly modulate TGF by the production of vasodilatory prostanoids[47,72] or mediate afferent arteriolar vasoconstriction by activating thromboxane receptors through the generation of thromboxane A2 and/or PGH2.[73] Further studies will be required to reconcile these divergent results.

There is evidence that macula densa COX-2 expression is sensitive not only to alterations in intravascular volume but also to alterations in renal metabolism. Specifically, the G protein–coupled receptor, GPR91, has been shown to be a receptor for succinate, an intermediate of the citric acid cycle (Krebs cycle).[74] GPR91 is expressed in macula densa, and both GPR91 and the intrarenal production of succinate are increased in diabetes. Studies have suggested that succinate

activation of GPR91 leads to increased macula densa COX-2 expression.[75,76]

COX-2 EXPRESSION IN THE RENAL MEDULLA

The renal medulla is a major site of prostaglandin synthesis and abundant COX-1 and COX-2 expression (Fig. 13.6).[77] COX-1 and COX-2 exhibit differential compartmentalization within the medulla, with COX-1 predominating in the medullary collecting ducts and COX-2 predominating in medullary interstitial cells.[43] In the collecting duct, COX-2 has also been localized to intercalated cells but is absent in principal cells.[78] Intercalated cell COX-2 expression increases in response to angiotensin II (Ang II), and deletion exacerbates Ang II-mediated hypertension.[79] COX-2 may also be expressed in endothelial cells of the vasa recta supplying the inner medulla.

In medullary interstitial cells, dynamic regulation of COX-2 expression appears to be an important adaptive response to physiologic stresses, including water deprivation, increased dietary sodium, and exposure to endotoxins.[38,77,80,81] In contrast, COX-1 expression is unaffected by water deprivation. Although hormonal factors could also contribute to COX-2 induction, shifting cultured renal medullary interstitial cells to hypertonic media (using NaCl or mannitol) is sufficient to induce COX-2 expression directly. Because prostaglandins play an important role in maintaining renal function during volume depletion or water deprivation, induction of COX-2 by hypertonicity provides an important adaptive response.

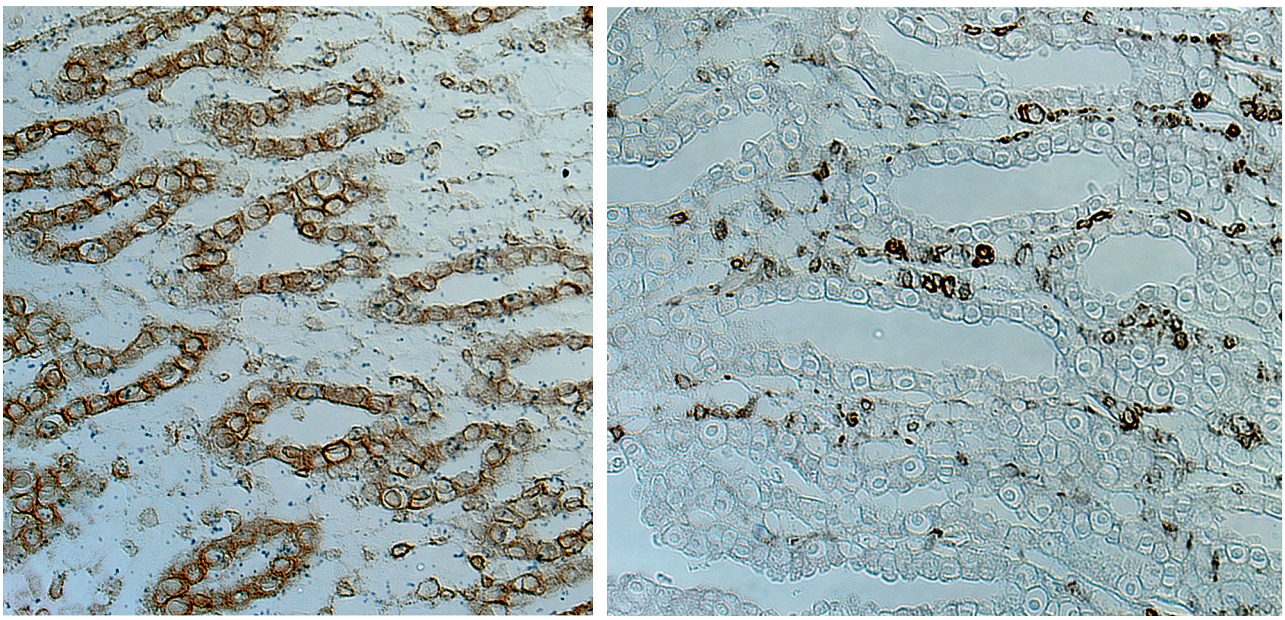
As is the case for the macula densa, medullary interstitial cell COX-2 expression is transcriptionally regulated in response to renal extracellular salt and tonicity. Water deprivation and a high-sodium diet both induce COX-2 expression in medullary interstitial cells by activating the nuclear factor-kappa B (NF-κB) pathway.[77,81] There is also evidence that nitric oxide may modulate medullary COX-2 expression through mitogen-activated protein (MAP) kinase–dependent pathways.[82]

The mechanisms underlying the upregulation of medullary COX-2 expression in response to volume expansion are probably multifactorial. There is clear evidence that increased medullary tonicity increases medullary COX-2 expression. Different studies have indicated a role for NF-kB,[77] EGF receptor (EGFR) transactivation,[83] and mitochondria-generated reactive oxygen species (ROS).[84] Whether these represent parallel pathways or are all interrelated is not yet clear; however, it should be noted that the described EGFR transactivation is mediated by cleavage of the EGFR ligand, TGF-α, by ADAM17 (TACE), which is known to be activated by src, which can be activated by ROS. In addition to medullary COX-2, cortical COX-2 expression increases in salt-sensitive hypertension, especially in the glomerulus, and is inhibited by the superoxide dismutase mimetic, Tempol, or an ARB.[85] There is also recent evidence that COX-2 expression increases in renal macrophages in response to a high-salt diet, and selective inhibition of macrophage COX-2 expression exacerbates salt-sensitive hypertension.[86]

COX-1 EXPRESSION

COX-1 EXPRESSION IN THE KIDNEY

Although well-defined factors regulating COX-2 and determining the role of COX-2 expression in the kidney are coming to light, the role of renal COX-1 remains more obscure. COX-1 is constitutively expressed in platelets,[87] in the renal microvasculature, and glomerular parietal epithelial cells (Fig. 13.7). In addition, COX-1 is abundantly expressed in the collecting duct, but there is little COX-1 expressed in the



COX-1 COX-2

Fig. 13.6 Differential immunolocalization of cyclooxygenase 1 (COX-1) and COX-2 in the renal medulla of rodents (original magnification ×250). COX-1 is predominantly localized to the collecting duct and is also found in a subset of medullary interstitial cells; COX-2 is predominantly localized to a subset of interstitial cells.

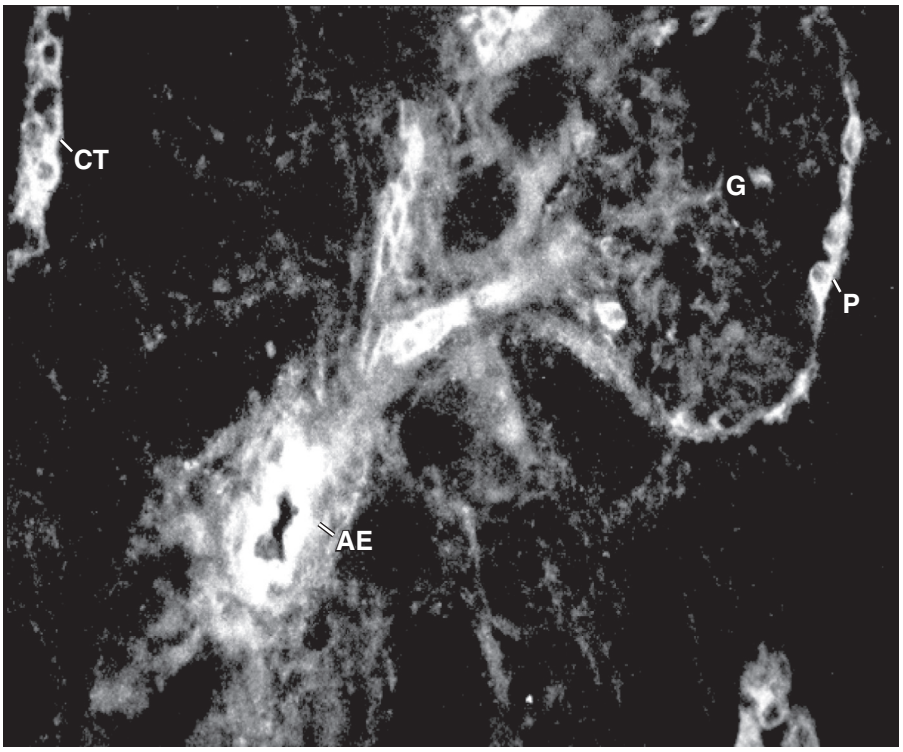


Fig. 13.7 Renal cortical cyclooxygenase 1 (COX-1) expression. Immunoreactive COX-1 is predominantly localized to the afferent arteriole *(AE),* glomerular mesangial cells *(G),* parietal glomerular epithelial cells *(P),* and the cortical collecting tubule (CT). (Modified from Yokoyama C, Yabuki T, Shimonishi M, et al. Prostacyclin-deficient mice develop ischemic renal disorders, including nephrosclerosis and renal infarction. *Circulation.* 2002;106(18):2397–403.)

proximal tubule or TAL.[46,88] Although COX-1 expression levels do not appear to be dynamically regulated and, consistent with this observation, the COX-1 promoter does not possess a TATA box, vasopressin does increase COX-1 expression in collecting duct epithelial cells and in interstitial cells in the inner medulla.[88] The factors accounting for the tissue-specific expression of COX-1 are uncertain but may involve histone acetylation and the presence of two tandem Sp1 sites in the upstream promoter region of the gene.[89]

RENAL COMPLICATIONS OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS

SODIUM RETENTION, EDEMA, AND HYPERTENSION

The use of nonselective NSAIDs may be complicated by the development of significant Na^+ retention, edema, congestive heart failure, and hypertension.[90] These complications are also apparent in patients using COX-2 selective NSAIDs. Studies with celecoxib and rofecoxib have demonstrated that like nonselective NSAIDs, these COX-2 selective NSAIDs reduce urinary sodium (Na^+) excretion and are associated with modest Na^+ retention in otherwise healthy subjects.[91,92] The evidence supporting a role for NSAID exacerbating hypertension, especially COX-2 inhibitors, continues to grow. COX-2 inhibition likely promotes salt retention via multiple mechanisms (Fig. 13.8). A reduced GFR may limit the filtered Na^+ load and salt excretion.[93,94] In addition, PGE2 directly inhibits Na^+ absorption in the TAL and collecting duct.[95] The relative abundance of COX-2 in medullary interstitial cells places this enzyme adjacent to both these nephron segments, allowing for COX-2–derived PGE2 to modulate salt absorption. COX-2 inhibitors decrease renal PGE2 production[91,96] and thereby may enhance renal sodium retention. Finally, a reduction in renal medullary blood flow by inhibition of vasodilator prostanoids may significantly reduce renal salt excretion and promote the development of edema and hypertension. COX-2–selective NSAIDs have been demonstrated to exacerbate salt-dependent hypertension.[97,98] Similarly, patients with preexisting treated hypertension commonly experience hypertensive exacerbations with COX-2–selective NSAIDs.[92] Taken together these data suggest that COX-2 selective NSAIDs have similar effects as nonselective NSAIDs with respect to salt excretion.

HYPERKALEMIA

Nonselective NSAIDs cause hyperkalemia due to suppression of the renin-aldosterone axis. Both a decreased GFR and inhibition of renal renin release may compromise renal K^+ excretion. Patients on a salt-restricted diet also have decreased urinary potassium excretion when treated with a COX-2–selective inhibitor,[93,94] and COX-2–selective inhibitors may pose an equal or greater risk as nonselective NSAIDs for the development of hyperkalemia.[99]

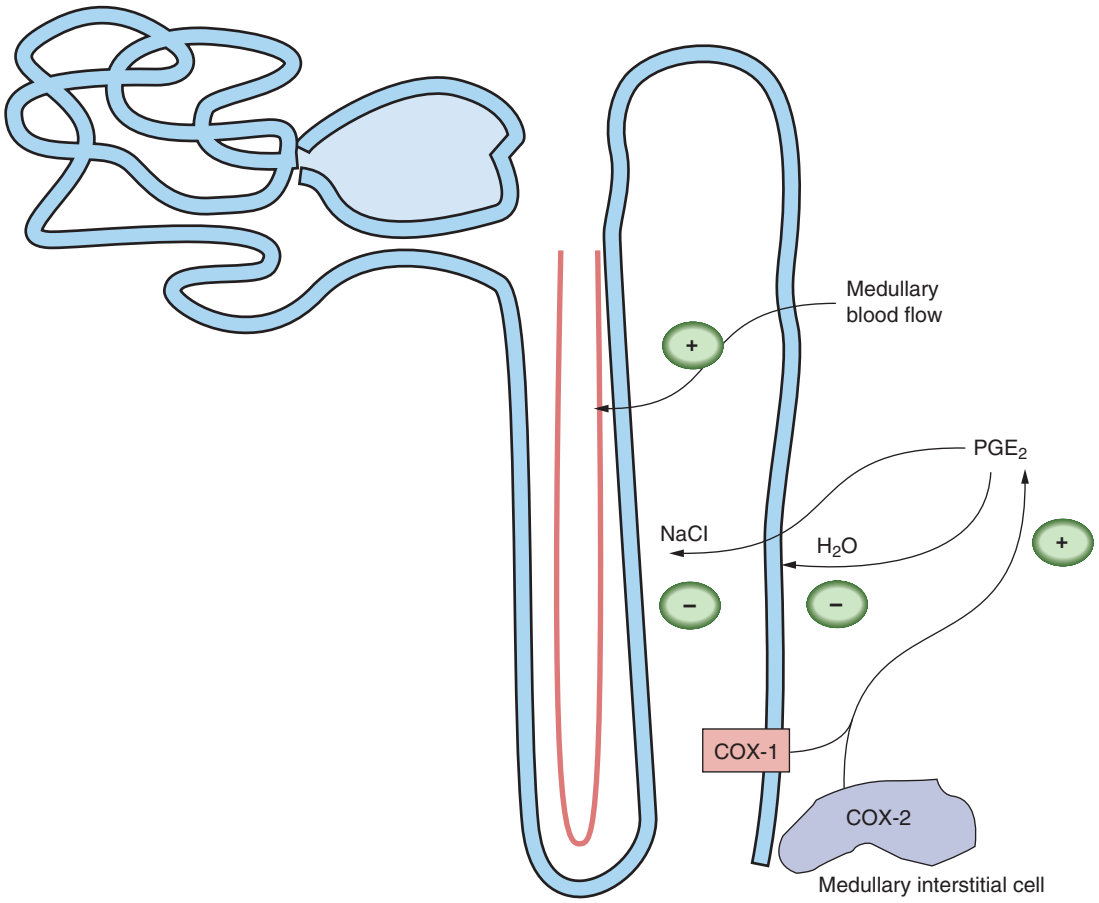


Fig. 13.8 Integrated role of prostaglandin E2 *(PGE2)* on the regulation of salt and water excretion. PGE2 can both increase medullary blood flow and directly inhibit NaCl reabsorption in the medullary thick ascending limb (mTAL) and water reabsorption in the collecting duct. *COX-1,* Cyclooxygenase 1; *COX-2,* cyclooxygenase 2.

PAPILLARY NECROSIS

Both acute and subacute forms of papillary necrosis have been observed with NSAID use.[100–102] Acute NSAID-associated renal papillary injury is more likely to occur in the setting of dehydration, suggesting a critical dependence of renal function on COX metabolism in this setting.[77] Long-term use of NSAIDs has been associated with papillary necrosis and progressive renal structural and functional deterioration, much like the syndrome of analgesic nephropathy observed with acetaminophen, aspirin, and caffeine combinations.[101] Experimental studies have suggested that renal medullary interstitial cells are an early target of injury in analgesic nephropathy.[103] COX-2 has been shown to be an important survival factor for cells exposed to a hypertonic medium.[37,77,104,105] The coincident localized expression of COX-2 in these interstitial cells[37,77] raises the possibility that like nonselective NSAIDs, long-term use of COX-2–selective NSAIDs may contribute to development of papillary necrosis.[106]

ACUTE RENAL INSUFFICIENCY

Acute kidney injury (AKI) is a well-described complication of NSAID use.[90] This is generally considered to be a result of altered intrarenal microcirculation and glomerular filtration secondary to the inability to produce beneficial endogenous prostanoids when the kidney is dependent on them for normal function. Like the traditional nonselective NSAIDs, COX-2–selective NSAIDs will also reduce glomerular filtration in susceptible patients.[90] Although rare overall, NSAID-associated renal insufficiency occurs in a significant proportion of patients with underlying volume depletion, renal insufficiency, congestive heart failure, diabetes, and old age.[90] These risk factors are additive and rarely are present in patients included in study cohorts used for a safety assessment of these drugs. It is therefore relevant that the COX-2–selective inhibitors celecoxib and rofecoxib cause a slight but significant fall in the GFR rate in salt-depleted but otherwise healthy subjects.[93,94] Similar to nonselective NSAIDs, AKI can occur also secondary to COX-2–selective NSAIDs.[48,107] Preclinical studies have supported the concept that the inhibition of COX-2–derived prostanoids generated in the macula densa contributes to a fall in the GFR by reducing the diameter of the afferent arteriole. In vivo video microscopy studies have documented a reduced afferent arteriolar diameter following the administration of a COX-2 inhibitor.[47] These animal data not only support the concept that COX-2 plays an important role in regulating the GFR but also the clinical observations that COX-2–selective inhibitors can cause renal insufficiency similar to that reported with nonselective NSAIDs.

INTERSTITIAL NEPHRITIS

The gradual development of renal insufficiency characterized by a subacute inflammatory interstitial infiltrate may occur after several months of continuous NSAID ingestion. Less commonly, the interstitial nephritis and renal failure may be fulminant. The infiltrate is typically accompanied by eosinophils; however, the clinical picture is typically much less dramatic than classic drug-induced allergic interstitial nephritis, lacking fever or rash.[108] This syndrome has also been reported with the COX-2–selective drug celecoxib.[109,110] Dysregulation of the immune system is thought to play an important role in the syndrome, which typically abates rapidly following discontinuation of the NSAID or COX-2 inhibitor.

NEPHROTIC SYNDROME

Like interstitial nephritis, nephrotic syndrome typically occurs in patients chronically ingesting any one of a myriad of NSAIDs over the course of months.[108,111] The renal pathology is usually consistent with minimal change disease, with foot process fusion of glomerular podocytes observed on electron microscopy (EM), but membranous nephropathy has also been reported.[112] Typically, the nephrotic syndrome occurs together with the interstitial nephritis.[108] Nephrotic syndrome without interstitial nephritis may occur, as well as immune-complex glomerulopathy, in a small subset of patients receiving NSAIDs. It remains uncertain whether this syndrome results from mechanism-based COX inhibition by these drugs, an idiosyncratic immune drug reaction, or a combination of both.

RENAL DYSGENESIS

Reports of renal dysgenesis and oligohydramnios in the offspring of women administered nonselective NSAIDs during the third trimester of pregnancy have implicated prostaglandins in the process of normal renal development.[113,114] A similar syndrome of renal dysgenesis has been reported in mice, with targeted disruption of the COX-2 gene, as well as mice treated with the specific COX-2 inhibitor SC58236.[115] Because neither COX-1$^{-/-}$ mice or mice treated with the COX-1 selective inhibitor SC58560 exhibited altered renal development, a specific role for COX-2 in nephrogenesis has been suggested.[116–118] A report of renal dysgenesis in the infant of a woman exposed to the COX-2–selective inhibitor nimesulide has suggested that COX-2 also plays a role in renal development in humans.[113]

The intrarenal expression of COX-2 in the developing kidney peaks in mice at postnatal day 4 and in the rat in the second postnatal week.[115,119] It has not yet been determined if a similar pattern of COX-2 is seen in human kidneys. Although the most intense staining is observed in a small subset of cells in the nascent macula densa and cortical TAL, expression in the papilla has also been observed.[115,119] In vitro studies have shown that exogenous PGE2 promotes renal metanephric development[120] and is a critical growth factor for renal epithelia cells; studies in zebrafish have indicated that PGE2, acting through EP2 and EP4, is a regulator of nephron formation in the zebrafish embryonic kidney.[121] Studies have indicated that angiotensin II–AT1 receptor signaling mediates COX-2–dependent postnatal development in mice.[122]

CARDIOVASCULAR EFFECTS OF COX-2 INHIBITORS

EFFECTS OF COX-2 INHIBITION ON VASCULAR TONE

In addition to their propensity to reduce renal salt excretion and decrease medullary blood flow, NSAIDs and selective COX-2 inhibitors have been shown to exert direct effects on systemic resistance vessels. The acute pressor effect of angiotensin infusion in human subjects was significantly increased by pretreatment with the nonselective NSAID indomethacin at all Ang II doses. The administration of selective COX-2 inhibitors or COX-2 gene knockout has been shown to accentuate the pressor effects of Ang II in mice.[46] These studies have also demonstrated that Ang II-mediated blood pressure increases are markedly reduced by the administration of a selective COX-1 inhibitor or in COX-1 gene knockout mice.[46] These findings support the conclusion that COX-1–derived prostaglandins participate in, and are integral to, the pressor activity of Ang II, whereas COX-2–derived prostaglandins are vasodilators that oppose and mitigate the pressor activity of Ang II. Other animal studies have shown more directly that that both NSAIDs and COX-2 inhibitors blunt arteriolar dilation and decrease flow through resistance vessels.[123]

INCREASED CARDIOVASCULAR THROMBOTIC EVENTS

COX-2 is known to be induced in vascular endothelial cells in response to shear stress,[124] and selective COX-2 inhibition reduces circulating prostacyclin levels in normal human subjects.[125] Therefore, increasing evidence has indicated that COX-2–selective antagonism may carry increased thrombogenic risks due to selective inhibition of the endothelial-derived antithrombogenic prostacyclin without any inhibition of the prothrombotic platelet-derived thromboxane generated by COX-1.[126] Although animal studies have provided conflicting results about the role of COX-2 inhibition on development of atherosclerosis,[127–131] there have been recent indications that COX-2 inhibition may destabilize atherosclerotic plaques.[132] This has been suggested by studies indicating increased COX-2 expression and colocalization with microsomal PGE synthase-1 and metalloproteinases-2 and -9 in carotid plaques from individuals with symptomatic disease before endarterectomy.[133] Because of the concerns about increased cardiovascular risk, two selective COX-2 inhibitors, rofecoxib and valdecoxib, have been withdrawn from the market, and remaining coxibs and other NSAIDs have been relabeled to highlight the increased risk for cardiovascular events.

PROSTANOIDS

PROSTANOID SYNTHASES

Once PGH2 is formed in the cell, it can undergo a number of possible transformations, yielding biologically active prostaglandins and thromboxane A2. As seen in Fig. 13.9,

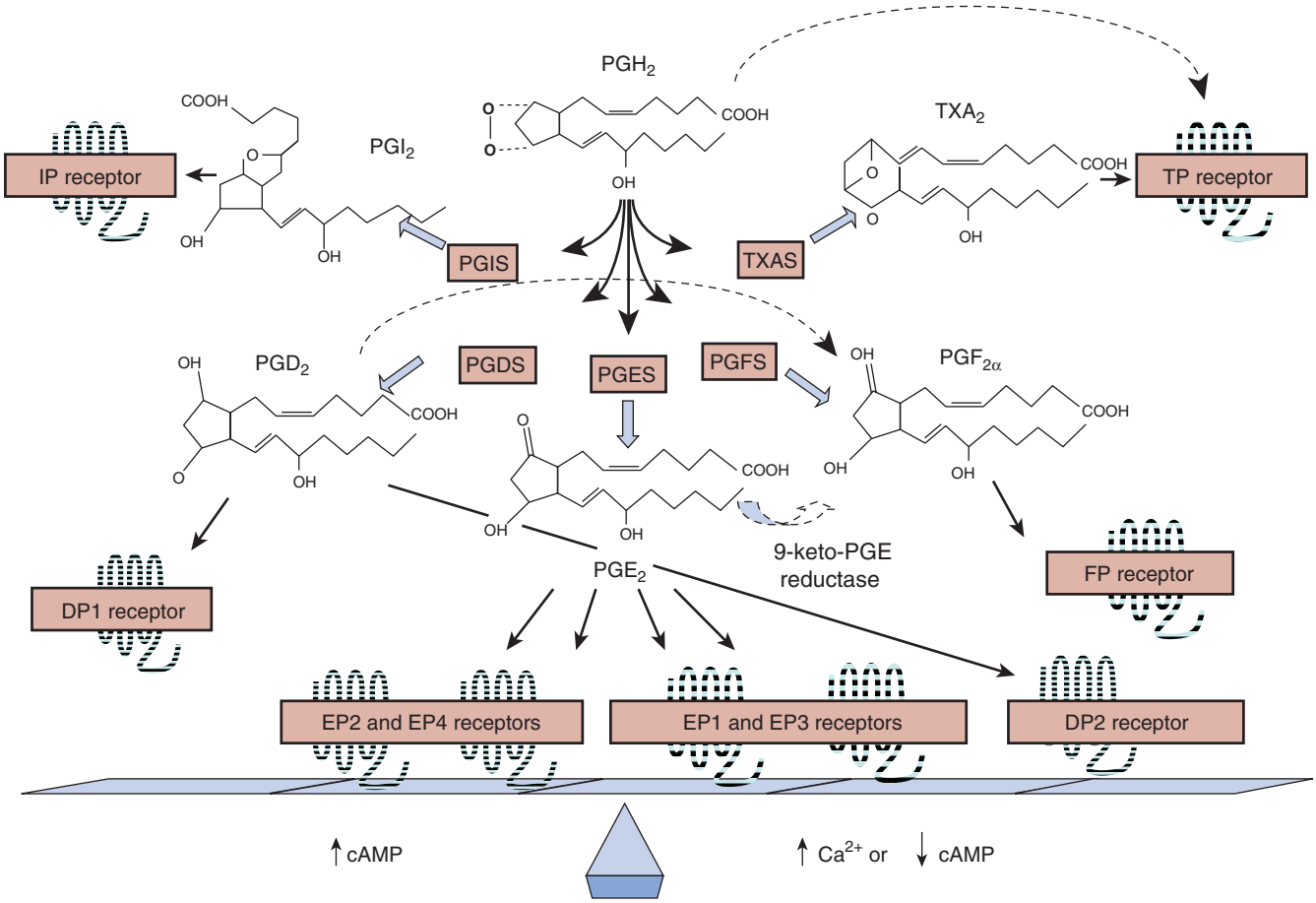


Fig. 13.9 Prostaglandin synthases. *cAMP,* Cyclic adenosine monophosphate; *PGDS,* prostaglandin D synthase; *PGES,* prostaglandin E synthase; *PGFS,* prostaglandin F synthase; *PGIS,* prostacyclin synthase; *TXAS,* thromboxane synthase.

in the presence of isomerase and reductase enzymes, PGH2 is converted to PGE2 and PGF2α, respectively. Thromboxane synthase converts PGH2 into a bicyclic oxetane-oxane ring metabolite, thromboxane A2 (TXA2), a prominent reaction product in platelets and an established synthetic pathway in the glomerulus. Prostacyclin synthase, a 50-kDa protein located in plasma and nuclear membranes and found mostly in vascular endothelial cells, catalyzes the biosynthesis of prostacyclin (PGI2). PGD2, the major prostaglandin product in mast cells, is also derived directly from PGH2, but its role in the kidney is uncertain. The enzymatic machinery and their localization in the kidney are discussed in detail later.

SOURCES AND NEPHRONAL DISTRIBUTION OF COX PRODUCTS

COX activity is present in arterial and arteriolar endothelial cells, including glomerular afferent and efferent arterioles.[43] The predominant metabolite from these vascular endothelial cells is PGI2.[134,135] Whole glomeruli generate PGE2, PGI2, PGF2α, and TXA2.[1] The predominant products in rat and rabbit glomeruli are PGE2, followed by PGI2 and $PGF_{2\alpha}$ and finally TXA2.

Analyses of individual cultured glomerular cell subpopulations have also provided insight into the localization of prostanoid synthesis. Cultured mesangial cells are capable of generating PGE2 and, in some cases, PGF2α and PGI2 have also been detected.[136] Other studies have suggested that mesangial cells may produce the endoperoxide PGH2 as a major COX product.[137] Glomerular epithelial cells also appear to participate in prostaglandin synthesis, but the profile of COX products generated in these cells remains controversial. Immunocytochemical studies of rabbit kidney have demonstrated intense staining for COX-1, predominantly in the parietal epithelial cells. Glomerular capillary endothelial cell PG generation profiles remain undefined but may well include prostacyclin.

The predominant synthetic site of PG synthesis along the nephron is the collecting duct (CD), particularly its medullary portion (MCT).[138] In the presence of exogenous arachidonic acid, PGE2 is the predominant PG formed in the collecting duct, the variations among the other products being insignificant.[1] PGE2 is also the major COX metabolite generated in medullary interstitial cells.[139] The role that specific prostanoid synthases may play in the generation of these products is outlined subsequently.

Thromboxane Synthase

TXA2 is produced from PGH2 by thromboxane synthase (TXAS), a microsomal protein of 533 amino acids with a predicted molecular weight of around 60 kDa. The amino acid sequence of the enzyme exhibits homology to the cytochrome P-450s and is now classified as CYP5A1.[140] The

human gene is localized on chromosome 7q and spans 180 kB. TXAS mRNA is highly expressed in hematopoietic cells, including platelets, macrophages, and leukocytes. TXAS mRNA is expressed in the thymus, kidney, lung, spleen, prostate, and placenta. Immunolocalization of TXAS demonstrates high expression in the dendritic cells of the interstitium, with lower expression in glomerular podocytes of human kidney.[141] TXA2 synthase expression is regulated by dietary salt intake.[142] Furthermore, the experimental use of ridogrel, a specific TXAS inhibitor, reduced blood pressure in spontaneously hypertensive rats.[143] The clinical use of TXA2 synthase inhibitors is complicated by the fact that its endoperoxide precursors (PGG2 and PGH2) are also capable of activating its downstream target, the TP receptor.[27]

Prostacyclin Synthase

The biologic effects of prostacyclin are numerous. They include nociception, antithrombosis, and vasodilator actions, which have been targeted therapeutically to treat pulmonary hypertension.

PGI2 is produced by the enzymatic conversion of PGH2 via prostacyclin synthase (PGIS). The cloned cDNA contains a 1500–base pair open reading frame that encodes a 500–amino acid protein of approximately 56 kDa. The human prostacyclin synthase gene is present as a single-copy haploid genome and is localized on chromosome 20q. Northern blot analysis shows that prostacyclin synthase mRNA is widely expressed in human tissues and is particularly abundant in the ovary, heart, skeletal muscle, lung, and prostate. PGI synthase expression exhibits segmental expression in the kidney, especially in kidney inner medulla tubules and interstitial cells.

PGI2 synthase null mice[144] have reduced PGI2 levels in the plasma, kidneys, and lungs, documenting the role of this enzyme as an in vivo source of PGI2. Blood pressure and blood urea nitrogen and creatinine levels in the PGIS knockout mice were significantly increased, and renal pathologic findings included surface irregularity, fibrosis, cysts, arterial sclerosis, and hypertrophy of vessel walls. Thickening of the thoracic aortic media and adventitia were observed in aged PGI null mice.[144] Interestingly, this is a distinct phenotype from that reported for the IP receptor knockout mouse.[145] These differences point to the presence of additional IP-independent PGI2-activated signaling pathways. Regardless, these findings demonstrate the importance of PGI2 to the maintenance of blood vessels and to the kidney.

Prostaglandin D Synthase

Prostaglandin D2 is derived from PGH2 via the action of specific enzymes designated as PGD synthases. Two major enzymes are capable of transforming PGH2 to PGD_2, including a lipocalin-type PGD synthase and a hematopoietic-type PGD synthase.[146,147] Mice lacking the lipocalin D synthase gene exhibit altered sleep and pain sensation.[148] PGD2 is the major prostanoid released from mast cells following challenge with immunoglobulin E (IgE). The kidney also appears capable of synthesizing PGD2. RNA for the lipocalin type PGD synthase has been reported to be widely expressed along the rat nephron, whereas the hematopoietic type of PGD synthase is restricted to the collecting duct.[149] Urinary excretion of lipocalin D synthase has been proposed as a biomarker predictive of renal injury,[150] and lipocalin D synthase knockout mice appear to be more prone to diabetic nephropathy.[151] However, the physiologic roles of these enzymes in the kidney remain less certain. Once synthesized, PGD2 is available to interact with the DP1 or DP2 receptors (see later) or undergo further metabolism to a PGF2α-like compound.

Prostaglandin F Synthesis

PGF2a is a major urinary COX product. It may be synthesized either directly from PGH2 via a PGF synthase[152] or indirectly by metabolism of PGE2 via a 9-ketoreductase.[152] Another more obscure pathway for PGF formation is by the action of a PGD2 ketoreductase, yielding a stereoisomer of PGF2α9a, 11ß-PGF2 (11epi-PGF2α).[152] This reaction, and the conversion of PGD2 into an apparently biologically active metabolite (9a,11b-PGF2α) has been documented in vivo.[153] Interestingly, this isomer can also ligate and activate the FP receptor.[154] The physiologically relevant enzymes responsible for renal PGF2α formation remain incompletely characterized.

Prostaglandin 9 Ketoreductase

Physiologically relevant transformations of COX products occur in the kidney via a nicotinamide adenine dinucleotide phosphate (NADPH)–dependent 9-ketoreductase, which converts PGE2 into PGF2α. This enzymatic activity is typically cytosolic[152] and may be detected in homogenates from the renal cortex, medulla, or papilla. The activity appears to be particularly robust in suspensions from the TALH. Renal PGE2 9-ketoreductase also exhibits 20α-hydroxysteroid reductase activity that could affect steroid metabolism.[152] This enzyme appears to be a member of the aldoketoreductase family 1C.[155]

Interestingly, some studies have suggested that activity of a 9-ketoreductase may be modulated by salt intake and AT_2 receptor activation and may play an important role in hypertension.[156] Mice deficient in the AT_2 receptor exhibit salt-sensitive hypertension, increased PGE2 production, and reduced production of PGF2α,[157] consistent with reduced 9-ketoreductase activity. Other studies have suggested that dietary potassium intake may also enhance the activity of conversion from PGE2 to PGF2α.[158] The intrarenal sites of expression of this enzymatic activity remain to be characterized.

Prostaglandin E Synthases

PGE2 is the other major product of COX-initiated AA metabolism in the kidney and is synthesized at high rates along the nephron, particularly in the collecting duct. Two membrane-associated PGE2 synthases have been identified, a 33-kDa and a 16-kDa membrane-associated enzyme.[159,160] The initial report describing the cloning of a glutathione-dependent microsomal enzyme (the 16-kDa form) that specifically converts PGH2 to PGE2[160] noted that mRNA for this enzyme is highly expressed in reproductive tissues, as well as in kidney. Genetic disruption confirms that $mPGES1^{-/-}$ mice exhibit a marked reduction in inflammatory responses compared with $mPGES1^{+/+}$[161] and indicate that mPGES1 is also critical for the induction of inflammatory fever.[162]

Intrarenal expression of mPGES1 has been demonstrated and mapped to the collecting duct, with lower expression in medullary interstitial cells and the macula densa[138,163] (see Fig. 13.3). Thus, in the kidney, this isoform colocalizes with both COX-1 and COX-2. In contrast, in inflammatory cells, this PGE synthase is co-induced with COX-2 and appears to

be functionally coupled to it.[164] Notably, the kidneys of mPGES1$^{-/-}$ mice are normal and do not exhibit the renal dysgenesis observed in COX-2$^{-/-}$ mice,[117,165] nor do these mice exhibit perinatal death from a patent ductus arteriosus, which is observed with the prostaglandin EP4 receptor knockout mouse.[166]

More recently, another membrane-associated PGE synthase, with a relative mass of around 33 kDa, was purified from the heart. The recombinant enzyme was activated by several thiol (SH)-reducing reagents, including dithiothreitol, glutathione (GSH), and betamercaptoethanol. Moreover, the mRNA distribution was high in the heart and brain and was also expressed in the kidney, but the mRNA was not expressed in the seminal vesicles. The intrarenal distribution of this enzyme is, at present, uncharacterized.[159]

Other cytosolic proteins exhibit lower PGE synthase activity, including a 23-kDa glutathione S-transferase (GST) requiring cytoplasmic, PGES,[167] that is expressed in the kidney and lower genitourinary tract.[168] Some evidence has suggested that this isozyme may constitutively couple to COX-1 in inflammatory cells. In addition, several cytosolic glutathione-S-transferases have the capability to convert PGH2 to PGE2; however, their physiologic role in this process remains uncertain.[169]

PROSTANOID RECEPTORS

See Figs. 13.10 and 13.11.

TP RECEPTORS

The TP receptor was originally purified by chromatography using a high-affinity ligand to capture the receptor.[170] This was the first eicosanoid receptor cloned; it is a G protein–coupled transmembrane receptor capable of activating a calcium-coupled signaling mechanism. Other prostanoid receptors were cloned by finding cDNAs homologous to this TP receptor cDNA. Two alternatively spliced variants of the human thromboxane receptor have been described[171] that differ in their carboxyl-terminal tail distal to arginine (Arg). Similar patterns of alternative splicing have been described for both the EP3 and FP receptors.[172] Heterologous cAMP-mediated signaling of the thromboxane receptor may occur via its heterodimerization with the prostacyclin (IP) receptor.[173]

Either the endoperoxide, PGH2, or its metabolite, TXA2, can activate the TP receptor.[27] Competition radioligand binding studies have demonstrated a rank order of potency on the human platelet TP receptor of the ligands I-BOP—S145 > SQ29548 > STA_2 > U-46619.[174,175] Whereas I-BOP, STA_2, and U-46619 are agonists, SQ29548 and S145 are high-affinity TP receptor antagonists.[176] Studies have suggested that the TP receptor may mediate some of the biologic effects of the nonenzymatically-derived isoprostanes,[177] including modulation of tubuloglomerular feedback.[178] This latter finding may have significance in pathophysiologic conditions associated with increased oxidative stress.[179] Signal transduction studies have shown that the TP receptor activates phosphatidylinositol hydrolysis (PIP_2)–dependent Ca^{2+} influx.[170,180] Quantitative polymerase chain reaction (PCR) analysis of mouse tissues has revealed that the highest level of TP mRNA expression is in the thymus, followed by the aorta, adrenal gland, vena cava, and spleen, with lower levels of expression in the pituitary gland, kidney, uterus, and brain.[181]

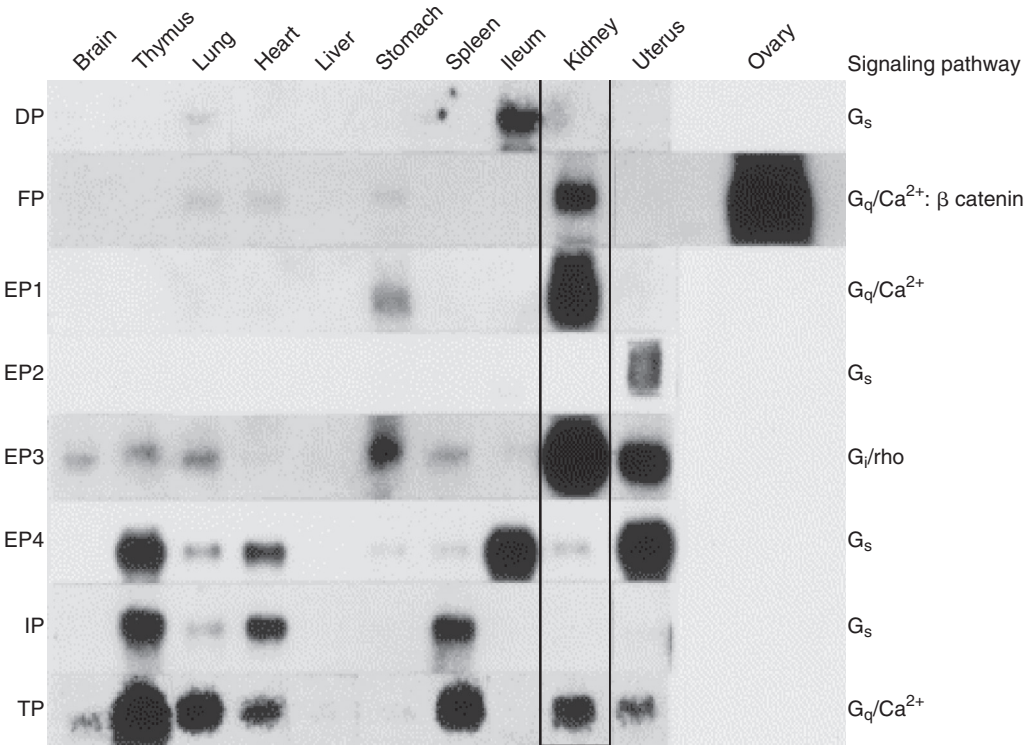


Fig. 13.10 Tissue distribution of prostanoid receptor mRNA. (Modified from Chaudhari A, Gupta S, Kirschenbaum M. Biochemical evidence for PGI2 and PGE2 receptors in the rabbit renal preglomerular microvasculature. *Biochim Biophys Acta.* 1990;1053(2-3):156–161.)

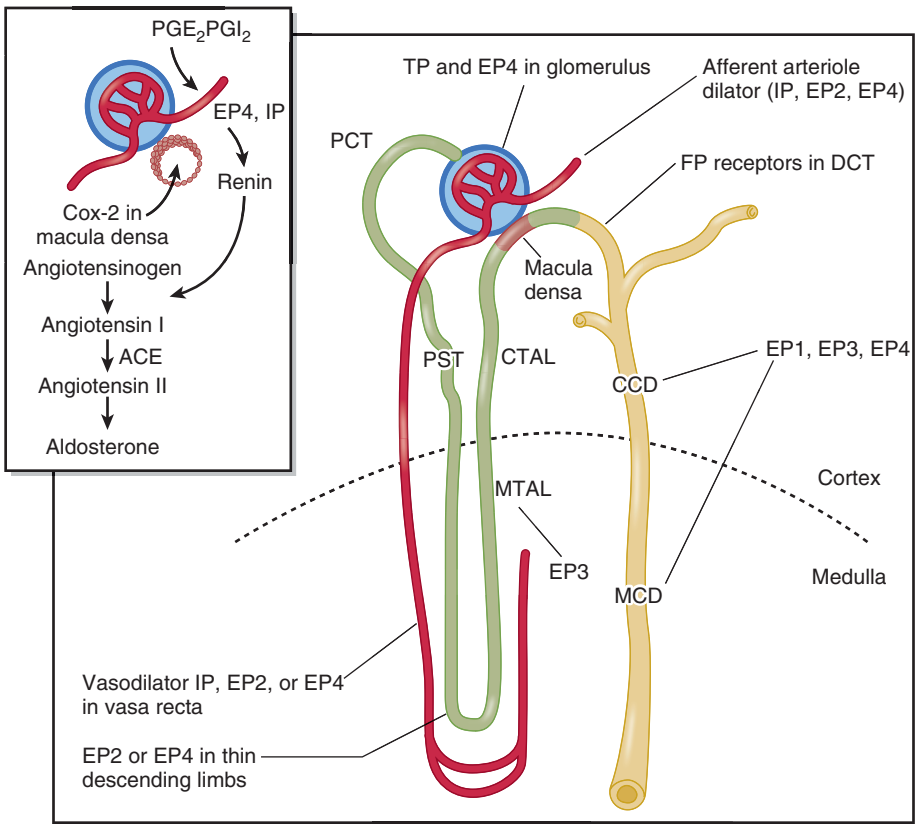


Fig. 13.11 Intrarenal localization of prostanoid receptors. *Inset,* Prostanoid signaling in the juxtaglomerular apparatus.

Table 13.1 Published Phenotypes of Prostanoid Receptor Knockout Mice

Receptor	Renal Expression	Renal Phenotype	Other Knockout Phenotypes
DP1	Minimal?	No	Reduced allergic asthma, reduced niacin flushing
DP2	Minimal?	++ Reduced fibrosis in UUO	Decreased cutaneous inflammatory responses
IP	++ Afferent arterioles	±	Reduced inflammation, increased thrombosis
TP	+ Glomerulus, tubules?	No	Prolonged bleeding time, platelet defect
FP	+++ Distal tubules	No	Failure of parturition
EP1	++++ MCD	No	Decreased Ang II hypertension
EP2	++ Interstitial stromal	++ Salt-sensitive hypertension	Impaired female fertility
EP3	++++ TAL, MCD	+	Impaired febrile response
EP4	+++ Glomerulus, + distal tubules	++ Reduced fibrosis in UUO	Perinatal death from persistent patent ductus arteriosus

Ang II, Angiotensin II; *MCD,* medullary collecting duct; *TAL,* thick ascending limb; *UUO,* unilateral ureter obstruction.

TX is a potent modulator of platelet shape change and aggregation, as well as smooth muscle contraction and proliferation. Moreover, a point mutation (Arg^{60} to leucine [Leu]) in the first cytoplasmic loop of the TXA2 receptor was identified in a dominantly inherited bleeding disorder in humans, characterized by a defective platelet response to TXA2.[182] Targeted gene disruption of the murine TP receptor also resulted in prolonged bleeding times and a reduction in collagen-stimulated platelet aggregation (Table 13.1). Conversely, overexpression of the TP receptor in vascular tissue increases the severity of vascular pathology following injury.[1] Increased thromboxane synthesis has been linked to cardiovascular diseases, including acute myocardial ischemia, heart failure, and inflammatory renal diseases.[1]

In the kidney, TP receptor mRNA has been reported in the glomeruli and vasculature. Radioligand autoradiography using ^{125}I-BOP has suggested a similar distribution of binding sites in mouse renal cortex, but additional renal medullary binding sites were observed.[183] These medullary TXA2 binding sites are absent following disruption of the TP receptor gene,

suggesting that they also represent authentic TP receptors.[184] Glomerular TP receptors may participate in potent vasoconstrictor effects of TXA2 analogues on the glomerular microcirculation associated with a reduced GFR.[1] Mesangial TP receptors coupled to phosphatidylinositol hydrolysis, protein kinase C activation, and glomerular mesangial cell contraction may also contribute to these effects.[185]

An important role for TP receptors in regulating renal hemodynamics and systemic blood pressure has also been suggested. Administration of a TP receptor antagonist reduces blood pressure in spontaneously hypertensive rats (SHRs)[143] and in angiotensin-dependent hypertension.[186] The TP receptor also appears to modulate renal blood flow in Ang II–dependent hypertension[187] and in endotoxemia-induced renal failure.[188] Modulation of renal TP receptor mRNA expression and function by dietary salt intake has also been reported.[189] These studies have also suggested an important role for luminal TP receptors in the distal tubule to enhance glomerular vasoconstriction indirectly via effects on the macula densa and TGF.[190] However, other studies have revealed no significant difference in tubuloglomerular feedback between wild-type and TP receptor knockout mice.[45]

A major phenotype of TP receptor disruption in mice and humans appears to be reduced platelet aggregation and prolonged bleeding time.[184] TX may also modulate the glomerular fibrinolytic system by increasing the production of an inhibitor of plasminogen activator-1 (PAI-1) in mesangial cells, which would promote fibrin accumulation.[191] Although a specific renal phenotype in the TP receptor knockout mouse has not yet been reported, important pathogenic roles for TXA2 and glomerular TP receptors in mediating renal dysfunction in glomerulonephritis, diabetes mellitus, and sepsis seem likely.

In an Ang II-dependent mouse model of hypertension, deletion of the TP receptor gene ameliorated hypertension and reduced cardiac hypertrophy, but had no effect on proteinuria.[192] In NG-nitro-L-arginine methyl ester (L-NAME) hypertension, TP receptors again contributed to elevated blood pressure and cardiac hypertrophy. However, in this same model, TP receptors also provided unexpected protection against kidney injury—TP deletion led to an increase in worsening of histopathology and significant renal hypertrophy. This suggested that the TP receptor may play a renal protective role in some settings.[193]

PROSTACYCLIN RECEPTORS

The cDNA for the IP receptor encodes a transmembrane protein of approximately 41 kDa. The IP receptor is selectively activated by the analogue cicaprost.[194] Iloprost and carbaprostacyclin potently activate the IP receptor but also activate the EP1 receptor. Selexipag is first in a class of long-acting IP agonists with good selectivity. Most evidence has suggested that the PGI2 receptor signals via stimulation of cAMP generation; however, at 1000-fold higher concentrations, the cloned mouse PGI2 receptor also signaled via PIP_2.[195] It remains unclear whether PIP_2 hydrolysis plays any significant role in the physiologic action of PGI2.

The IP receptor mRNA is widely expressed and is especially abundant in mouse bone marrow, vasculature, spleen, and heart[181] and in human kidney, liver, and lung.[196] In situ hybridization shows IP receptor mRNA predominantly in neurons of the dorsal root ganglia and vascular tissue, including the aorta, pulmonary artery, and renal interlobular and glomerular afferent arterioles.[197] The expression of IP receptor mRNA in the dorsal root ganglia is consistent with a role for prostacyclin in pain sensation. Mice with IP receptor gene disruption exhibit a predisposition to arterial thrombosis, diminished pain perception, and inflammatory responses.[145]

PGI2 has been demonstrated to play an important vasodilator role in the kidney,[198] including in the glomerular microvasculature,[199] as well as regulating renin release.[200,201] The capacity of PGI2 and PGE2 to stimulate cAMP generation in the glomerular microvasculature is distinct and additive,[202] demonstrating that the effects of these two prostanoids are mediated via separate receptors. IP receptor knockout mice also exhibit salt-sensitive hypertension.[203] Prostacyclin is a potent stimulus of renal renin release, and studies using $IP^{-/-}$ mice have confirmed an important role for the IP receptor in the development of renin-dependent hypertension of renal artery stenosis.[70] Selexipag has been shown to attenuate albuminuria in mouse models of diabetes in a nephrin-dependent manner.[203a]

Renal epithelial effects of PGI2 in the TAL have also been suggested,[204] and IP receptors have been reported in the collecting duct,[205] but the potential expression and role of prostacyclin in these segments are less well established. Of interest, in situ hybridization has also demonstrated significant expression of prostacyclin synthase in medullary collecting ducts,[206] consistent with a role for this metabolite in this region of the kidney. In summary, although IP receptors appear to play an important role in regulating renin release and as a vasodilator in the kidney, their role in regulating renal epithelial function seems likely.

DP RECEPTORS

The DP1 receptor has been cloned and, like the IP and EP2/4 receptors, the DP receptor predominantly signals by increasing cAMP generation. The human DP receptor binds PGD2 with a high-affinity binding of 300 pM and a lower affinity site of 13.4 nM.[207] DP-selective PGD2 analogues include the agonist BW 245C.[208] DP receptor mRNA is highly expressed in leptomeninges, retina, and ileum but is not detected in the kidney.[209] Northern blot analysis of the human DP receptor has demonstrated mRNA expression in the small intestine and retina,[210] whereas in the mouse, the DP receptor mRNA had highest expression in the olfactory epithelium, testes, and trachea.[181] PGD2 has also been shown to affect the sleep-wake cycle,[211] pain sensation,[148] and body temperature.[212] Peripherally, PGD2 has been shown to mediate vasodilation as well as possibly inhibiting platelet aggregation. PGD2 production is especially robust in mast cells. Inconsistent with this latter finding, the DP receptor knockout mice displayed reduced inflammation in the ovalbumin model of allergic asthma.[213] PGD2 is a major mediator of niacin-induced flushing. Development of the antagonist laropiprant was undertaken to inhibit this niacin-induced vasodilation flushing response.[214] Although the kidney appears capable of synthesizing PGD2, its role in the kidney remains poorly defined. Intrarenal infusion of PGD2 resulted in a dependent increase in renal artery flow, urine output, creatinine clearance, and sodium and potassium excretion.[215]

A second DP receptor was originally cloned as an orphan chemoattractant receptor from eosinophils and T cells (TH2 subset) and designated the CRTH2 receptor.[216] This receptor,

now designated as the DP2 receptor, bears no significant sequence homology to the family of prostanoid receptors discussed previously, and couples to increased cell calcium rather than increased cAMP. It binds agonists with an order of potency as follows: PGD2 = PGJ2 = 15 d PGJ2 ≫ PGF2α, PGE2 > PGI_2, TAX2. DP2 receptor action is blocked by the antagonist ramatroban, a drug used to treat allergic rhinitis and originally described as a TP receptor antagonist.[217] DP2 is widely expressed in a number of mouse tissues, including the kidney, and is most highly expressed in the uterus and testes.[181] Deletion of the DP2 receptor gene was protective in a mouse unilateral ureteral obstruction (UUO) model of fibrosis.[218] The recognition of this molecularly unrelated receptor allows for the possibility of the existence of a distinct and new family of prostanoid-activated membrane receptors.

FP RECEPTORS

The cDNA encoding the PGF2α receptor (FP receptor) was cloned from a human kidney cDNA library and encodes a protein of 359 amino acid residues. The bovine and murine FP receptors, similarly cloned from the corpora lutea, encode proteins of 362 and 366 amino acid residues, respectively. Transfection of HEK293 cells with the human FP receptor cDNA conferred preferential ^{3}H-P PGF2α binding with a K_D of 4.3 ± 1.0 nM.[176,219] Selective activation of the FP receptor may be achieved using fluprostenol or latanoprost.[176] ^{3}H-PGF2α binding was displaced by a panel of ligands with a rank order potency as follows: PGF2α = fluprostenol > PGD2 > PGE2 > U46619 > iloprost.[194] When expressed in oocytes, PGF2α or fluprostenol induced a Ca^{2+}-dependent Cl^- current. Increased cell calcium has also been observed in fibroblasts expressing an endogenous FP receptor.[220] FP receptors may also activate protein kinase C–dependent and Rho-mediated–PKC-independent signaling pathways.[221] An alternatively spliced isoform with a shorter carboxy-terminal tail has been identified that appears to signal via a similar manner as the originally described FP receptor.[222] Studies have also suggested that these two isoforms may exhibit differential desensitization and may also activate a glycogen synthase kinase/ß-catenin–coupled signaling pathway.[223]

Tissue distribution of FP receptor mRNA shows highest expression in the ovary, followed by the heart, trachea, and kidney.[181] Expression of the FP receptor in the corpora lutea is critical for normal birth, and homozygous disruption of the murine FP receptor gene results in failure of parturition in females, apparently due to failure of the normal preterm decline in progesterone levels.[224] PGF2α is a potent constrictor of smooth muscle in the uterus, bronchi, and blood vessels; however, an endothelial FP receptor may also play a dilator role.[225] The FP receptor is also highly expressed in skin, where it may play an important role in carcinogenesis.[226] A clinically important role for the FP receptor in the eye has been demonstrated to increase uveoscleral outflow and reduce ocular pressure. The FP-selective agonist latanoprost, an ester prodrug that is activated in the cornea, has been used clinically as an effective treatment for glaucoma.[227] Bimatoprost is a structural analogue of prostaglandin F2α (PGF2α). Like other PGF2α analogues, such as latanoprost, it increases the outflow of aqueous fluid from the eye and lowers intraocular pressure. It has a curious side effect of increasing eye growth. However, in contrast to latanoprost, it does not act on the FP receptor nor on any other known prostaglandin receptor.

The role of FP receptors in regulating renal function is only partially defined. FP receptor expression has been mapped to the cortical collecting duct in mouse and rabbit kidneys.[228] FP receptor activation in the collecting duct inhibits vasopressin-stimulated water absorption via a pertussis toxin–sensitive (presumably Gi) dependent mechanism. Although PGF2α increased cell Ca^{2+} levels in the cortical collecting duct, the FP-selective agonists latanoprost and fluprostenol did not increase calcium.[229] Because PGF2α can also bind to EP1 and EP3 receptors,[194,230,231] these data suggest that the calcium increase activated by PGF2α in the collecting duct may be mediated via an EP receptor. PGF2α also increases Ca^{2+} in cultured glomerular mesangial cells and podocytes,[232,233] suggesting that an FP receptor may modulate glomerular contraction. In contrast to these findings, the demonstration of glomerular FP receptors at the molecular level has not been forthcoming. Other vascular effects of PGF2α have been described, including selective modulation of renal production of PGF2α by sodium or potassium loading and AT2 receptor activation.[156]

Some reports have uncovered a role of the FP receptor in regulating renin expression. Interestingly, FP agonists increased renin mRNA expression in the JGA in a dose-dependent manner but, unlike IP receptor agonists, it did not increase intracellular cAMP. Deletion of the FP receptor resulted in decreased renin levels and decreased systemic blood pressure. These data suggest that FP receptor blockade may be a novel target for the treatment of hypertension.[234]

MULTIPLE EP RECEPTORS

Four EP receptor subtypes have been identified.[235] Although these four receptors uniformly bind PGE2 with a higher affinity than other endogenous prostanoids, the amino acid homology of each is more closely related to other prostanoid receptors that signal through similar mechanisms.[175] Thus, the relaxant cAMP-coupled EP2 receptor is more closely related to other relaxant prostanoid receptors, such as the IP and DP receptors, whereas the constrictor/Ca^{2+}-coupled EP1 receptor is more closely related to the other Ca^{2+}-coupled prostanoid receptors, such as the TP and FP receptors.[236] These receptors may also be selectively activated or antagonized by different analogues. EP receptor subtypes also exhibit differential expression along the nephron, suggesting distinct functional consequences of activating each EP receptor subtype in the kidney.[237]

EP1 Receptors

The human EP1 receptor cDNA encodes a 402–amino acid polypeptide that signals via IP3 generation and increased cell Ca^{2+} with IP3 generation. Studies of EP1 receptors may use one of several relatively selective antagonists, including ONO-871, SC19220, and SC53122. EP1 receptor mRNA has widespread expression, presumably from its vascular expression,[181] and is expressed in the kidney ≫ gastric muscularis mucosae > adrenal.[238] Renal EP1 mRNA expression determined by in situ hybridization is expressed primarily in the collecting duct and increases from the cortex to the papillae.[238] Activation of the EP1 receptor increases intracellular calcium levels and inhibits Na^+ and water reabsorption absorption in the collecting duct,[238] suggesting that renal EP1 receptor activation might contribute to the natriuretic and diuretic effects of PGE2.

Hemodynamic microvascular effects of EP1 receptors have also been reported. The EP1 receptor was originally described as a smooth muscle constrictor.[239] The EP1 receptor may also be present in cultured glomerular mesangial cells,[240] where it could play a role as a vasoconstrictor and a stimulus for mesangial cell proliferation. Although a constrictor PGE2 effect has been reported in the afferent arteriole of rat,[241] apparently produced by EP1 receptor activation,[242] there does not appear to be very high expression of the EP1 receptor mRNA in preglomerular vasculature or other arterial resistance vessels in mice or rabbits.[243] Other reports have suggested that EP1 receptor knockout mice exhibit hypotension and hyperreninemia, supporting a role for this receptor in maintaining blood pressure.[244]

EP2 Receptors

Two cAMP-stimulating EP receptors, designated EP2 and EP4, have been identified. The EP2 receptor can be pharmacologically distinguished from the EP4 receptor by its sensitivity to butaprost.[245] Before 1995, the cloned EP4 receptor was designated as the EP2 receptor, but then a butaprost-sensitive EP receptor was cloned,[246] the original receptor was reclassified as the EP4 receptor and the newer butaprost sensitive protein as the EP2 receptor.[247] A pharmacologically defined EP2 receptor has now also been cloned for the mouse, rat, rabbit, dog, and cow.[248] The human EP2 receptor cDNA encodes a 358–amino acid polypeptide, which signals through increased cAMP. The EP2 receptor may also be distinguished from the EP4 receptor, the other major relaxant EP receptor, by its relative insensitivity to the EP4 agonist PGE1-OH and to the weak EP4 antagonist AH23848[245] and the high-affinity EP4 antagonists ONO-AE3-208 and L-161982.[249] The EP2 antagonist PF-04418948 has been described,[250] as has the EP2/DP_1 antagonist TG4-155,[251] which should greatly facilitate the characterization of EP2 versus EP4 effects in vivo.

The precise distribution of the EP2 receptor mRNA has been partially characterized. This reveals a major mRNA species of around 3.1 kb, which is most abundant in the uterus, lung, and spleen, exhibiting only low levels of expression in the kidney.[248] Studies using PCR across a range of tissue have demonstrated highest expression in the bone marrow > ovary > lung, consistent with these earlier findings.[181] EP2 mRNA is expressed at much lower levels than EP4 mRNA in most tissues.[252] There is scant evidence to suggest segmental distribution of the EP2 receptor along the nephron.[248] Interestingly, it is expressed in cultured renal interstitial cells, supporting the possibility that the EP2 receptor is predominantly expressed in this portion of the nephron.[248] Studies in knockout mice have demonstrated a critical role for the EP2 receptor role in ovulation and fertilization.[253] In addition, these studies have suggested a potential role for the EP2 receptor in salt-sensitive hypertension.[253] This latter finding supports an important role for the EP2 receptor in protecting systemic blood pressure, perhaps via its vasodilator effect or its effects on renal salt excretion. Evidence for the latter role has been revealed in studies demonstrating that a high-salt diet increases PGE2 production, and infusion of EP-selective agonists identified the EP2 receptor as mediating PGE2-evoked naturesis. Moreover, deletion of the EP2 receptor ablated the naturetic effect of PGE2.[254]

EP3 Receptors

The EP3 receptor generally acts as a constrictor of smooth muscle.[255] Ribonuclease protection and Northern blot analysis of mRNA levels have demonstrated relatively high levels of EP3 receptor expression in several tissues, including the kidney, uterus, adrenal, and stomach, with riboprobes hybridizing to major mRNA species at around 2.4 and around 7.0 kb.[256] A metabolic pattern of expression was found by PCR with high levels of expression in the pancreas, as well as and brown fat tissue in addition to expression in the kidney.[181] This receptor is unique in that there are multiple (more than eight) alternatively spliced variants, differing only in their C-terminal cytoplasmic tails.[257–259] The EP3 splice variants bind PGE2 and the EP3 agonists MB28767 and sulprostone with similar affinity and, although they exhibit common inhibition of cAMP generation via a pertussis toxin–sensitive G_i-coupled mechanism, the tails may recruit different signaling pathways, including Ca^{2+}-dependent signaling[175,245] and the small G protein, rho.[260] A Ptx-insensitive pathway for the inhibition of cAMP generation via Gz has also been described.[261] Differences in agonist-independent activity have been observed for several of the splice variants, suggesting that they may play a role in constitutive regulation of cellular events.[262] The physiologic roles of these different C-terminal splice variants and sites of expression within the kidney remain uncertain.

In situ hybridization has demonstrated that EP3 receptor mRNA is abundant in the TAL and collecting duct.[263] This distribution has been confirmed by reverse transcriptase (RT)–PCR on microdissected rat and mouse collecting ducts and corresponds to the major binding sites for radioactive PGE2 in the kidney.[264] An important role for a G_i-coupled PGE receptor in regulating water and salt transport along the nephron has been recognized for many years. PGE2 directly inhibits salt and water absorption in both microperfused TALs and collecting ducts (CDs). PGE2 directly inhibits Cl^- absorption in the mouse or rabbit medullary TAL from the luminal or basolateral surfaces.[265] PGE2 also inhibits hormone-stimulated cAMP generation in TAL. Good and George have demonstrated that PGE2 modulates ion transport in the rat TAL by a pertussis toxin–sensitive mechanism.[265] Interestingly, these effects also appear to involve protein kinase C activation,[266] possibly reflecting activation of a novel EP3 receptor signaling pathway and possibly corresponding to alternative signaling pathways, as described earlier.[260] Taken together, these data support a role for the EP3 receptor in regulating transport in the collecting duct and TAL.

Blockade of endogenous PGE2 synthesis by NSAIDs enhances urinary concentration. It is likely PGE2-mediated antagonism of vasopressin-stimulated salt absorption in the TAL, and water absorption in the collecting duct contributes to its diuretic effect. In the in vitro microperfused collecting duct, PGE2 inhibits both vasopressin-stimulated osmotic water absorption and vasopressin-stimulated cAMP generation.[229] Furthermore, PGE2 inhibition of water absorption and cAMP generation are both blocked by pertussis toxin, suggesting effects mediated by the inhibitory G protein, G_i.[229] When administered in the absence of vasopressin, PGE2 actually stimulates water absorption in the collecting duct from the luminal or basolateral side.[267] These stimulatory effects of PGE2 on transport in the collecting duct appear to be related to activation of the EP4 receptor.[267] Despite the presence of

this absorption-enhancing EP receptor, in vivo studies have suggested that in the presence of vasopressin, the predominant effects of endogenous PGE2 on water transport are diuretic. Based on the preceding functional considerations, one would expect EP3$^{-/-}$ mice to exhibit an inappropriately enhanced urinary concentration. Surprisingly, EP3$^{-/-}$ mice exhibited a comparable urinary concentration following desmopressin (DDAVP), similar 24-hour water intake, and similar maximal and minimal urinary osmolality The only clear difference was that in mice allowed free access to water, indomethacin increased urinary osmolality in normal mice but not in the knockout animals. These findings raise the possibility that some of the renal actions of PGE2 normally mediated by the EP3 receptor have been co-opted by other receptors (e.g., the EP1 or FP receptor) in the EP3 knockout mouse. This remains to be formally tested.

The significance of EP3 receptor activation to animal physiology has been significantly advanced by the availability of mice with targeted disruption of this gene. Mice with targeted deletion of the EP3 receptor exhibit an impaired febrile response, suggesting that EP3 receptor antagonists could be effective antipyretic agents.[268] Other studies have suggested that the EP3 receptor plays an important vasopressor role in the peripheral circulation of mice.[243] Studies in knockout mice have also supported a potential role for the EP3 receptor as an important systemic vasopressor.[243,269] In the intrarenal circulation, the PGE2 has variable effects, acting as a vasoconstrictor in the larger proximal portion of the intralobular arteries and changing to a vasodilator effect in the smaller distal intralobular arteries and afferent arterioles.[270]

EP4 Receptor

Although, like the EP2 receptor, the EP4 receptor signals through increased cAMP,[271] it has been found to signal through a number of other pathways as well.[272] These other pathways include arrestin-mediated signaling, PI3 kinase, signaling β-catenin, and G_i coupling. The human EP4 receptor cDNA encodes a 488–amino acid polypeptide with a predicted molecular mass of ~53 kDa.[273] Note that care must be taken in reviewing the literature prior to 1995, when this receptor was generally referred to as the EP2 receptor.[247] In addition to the human receptor, EP4 receptors for the mouse, rat, rabbit, and dog have been cloned. EP4 receptors can be pharmacologically distinguished from the EP1 and EP3 receptors by insensitivity to sulprostone and from EP2 receptors by its insensitivity to butaprost and relatively selective activation by PGE1-OH.[176] EP4-selective agonists (ONO-AE1-329, ONO-4819) and antagonists (ONOAC-3208, L-161982) have been generated[245] and have been used to investigate the role of EP4 in vivo. Activation of the EP4 receptor was able to ameliorate the phenotype of a mouse model of nephrogenic diabetes insipidus.[274]

EP4 receptor mRNA is highly expressed relative to the EP2 receptor and widely distributed, with a major species of around 3.8 kb detected by Northern blot analysis in the thymus, ileum, lung, spleen, adrenal, and kidney.[252,275,275a] Dominant vasodilator effects of EP4 receptor activation have been described in venous and arterial beds.[208,255] A critical role for the EP4 receptor in regulating the perinatal closure of the pulmonary ductus arteriosus has also been suggested by studies of mice with targeted disruption of the EP4 receptor gene.[166,276] On a 129-strain background, EP4$^{-/-}$ mice had nearly 100% perinatal mortality due to persistent patent ductus arteriosus.[276] Interestingly, when bred on a mixed genetic background, only 80% of EP4$^{-/-}$ mice died, whereas around 21% underwent closure of the ductus and survived.[166] Preliminary studies in these survivors have supported an important role for the EP4 receptor as a systemic vasodepressor;[277] however, their heterogeneous genetic background complicates the interpretation of these results because survival may select for modifier genes that not only allow ductus closure but also alter other hemodynamic responses.

Other roles for the EP4 receptor in controlling blood pressure have been suggested, including the ability to stimulate aldosterone release from zona glomerulosa cells.[278] In the kidney, EP4 receptor mRNA expression is primarily in the glomerulus, where its precise function is uncharacterized,[275,279] but might contribute to the regulation of the renal microcirculation as well as renin release.[280] Studies in mice with genetic deletion of selective prostanoid receptors have indicated that EP4$^{-/-}$ mice, as well as IP$^{-/-}$ mice to a lesser extent, fail to increase renin production in response to loop diuretic administration, indicating that macula densa–derived PGE2 increases renin primarily through EP4 activation.[281] This corresponds to other studies suggesting that EP4 receptors are expressed in cultured podocytes and juxtaglomerular apparatus cells.[232,280] PGE2 may mediate increased podocyte COX-2 expression through EP4-mediated increased cAMP, which activates P38 through an independent process.[282] Finally, the EP4 receptor in the renal pelvis may participate in regulation of salt excretion by altering afferent renal nerve output.[283]

REGULATION OF RENAL FUNCTION BY EP RECEPTORS

PGE2 exerts a number of effects in the kidney, presumably mediated by EP receptors. PGE2 not only dilates the glomerular microcirculation and vasa rectae, supplying the renal medulla,[284] but also modulates salt and water transport in the distal tubule (Fig. 13.5).[285] The maintenance of normal renal function during physiologic stress is particularly dependent on endogenous prostaglandin synthesis. In this setting, the vasoconstrictor effects of Ang II, catecholamines, and vasopressin are more effectively buffered by prostaglandins in the kidney than in other vascular beds, preserving normal renal blood flow, the GFR, and salt excretion. Administration of COX-inhibiting NSAIDs in the setting of volume depletion interferes with these dilator effects and may result in a catastrophic decline in the GFR, resulting in overt renal failure.[286]

Other evidence points to vasoconstrictor and prohypertensive effects of endogenous PGE2. PGE2 stimulates renin release from the juxtaglomerular apparatus,[287] leading to a subsequent increase in the vasoconstrictor Ang II. In conscious dogs, chronic intrarenal PGE2 infusion increases renal renin secretion, resulting in hypertension.[288] Treatment of salt-depleted rats with indomethacin not only decreases plasma renin activity, but also reduces blood pressure, suggesting that PGs support blood pressure during salt depletion via their capacity to increase renin.[289] Direct vasoconstrictor effects of PGE2 on vasculature have also been observed.[243] It is conceivable that these latter effects might predominate in circumstances where the kidney is exposed to excessively high perfusion pressures. Thus, depending on the setting, the primary effect of PGE2 may be to increase or decrease

vascular tone, effects that appear to be mediated by distinct EP receptors.

RENAL CORTICAL HEMODYNAMICS

The expression of the EP4 receptor in the glomerulus suggests that it may play an important role in regulating renal hemodynamics. PGs regulate the renal cortical microcirculation and, as suggested previously, both glomerular constrictor and dilator effects of PGs have been observed.[243,290] In the setting of volume depletion, endogenous PGE2 helps maintain the GFR by dilating the afferent arteriole.[290] Some studies have suggested roles for EP and IP receptors coupled to increased cAMP generation in mediating vasodilator effects in the preglomerular circulation.[44,280,291] PGE2 exerts a dilator effect on the afferent arteriole but not the efferent arteriole, consistent with the presence of an EP2 or EP4 receptor in the preglomerular microcirculation.

RENIN RELEASE

Other studies have suggested that the EP4 receptor may also stimulate renin release. Soon after the introduction of NSAIDs, it was recognized that endogenous PGs play an important role in stimulating renin release.[44] Treatment of salt-depleted rats with indomethacin not only decreases plasma renin activity, but also causes blood pressure to fall, suggesting that PGs support blood pressure during salt depletion via their capacity to increase renin. Prostanoids also play a central role in the pathogenesis of renovascular hypertension, and administration of NSAIDs lowers blood pressure in animals and humans with renal artery stenosis.[292] PGE2 induces renin release in isolated preglomerular juxtaglomerular apparatus cells.[287] Like the effect of ß-adrenergic agents, this effect appears to be through a cAMP-coupled response, supporting a role for an EP4 or EP2 receptor.[287] EP4 receptor mRNA has been detected in microdissected JGAs,[293] supporting the possibility that renal EP4 receptor activation contributes to enhanced renin release. Finally, regulation of plasma renin activity and intrarenal renin mRNA does not appear to be different in wild-type and EP2 knockout mice,[294] arguing against a major role for the EP2 receptor in regulating renin release. Conversely, one report has suggested that EP3 receptor mRNA is localized to the macula densa, suggesting that this cAMP-inhibiting receptor may also contribute to the control of renin release.[279]

RENAL MICROCIRCULATION

The EP2 receptor also appears to play an important role in regulating afferent arteriolar tone.[290] In the setting of systemic hypertension, the normal response of the kidney is to increase salt excretion, thereby mitigating the increase in blood pressure. This so-called "pressure natriuresis" plays a key role in the ability of the kidney to protect against hypertension.[295] Increased blood pressure is accompanied by increased renal perfusion pressure and enhanced urinary PGE2 excretion.[296] Inhibition of PG synthesis markedly blunts (although it does not eliminate) pressure natriuresis.[297] The mechanism whereby PGE2 contributes to pressure natriuresis may involve changes in resistance of the renal medullary microcirculation.[298] PGE2 directly dilates descending vasa recta, and increased medullary blood flow may contribute to the increased interstitial pressure observed as renal perfusion pressure increases, leading to enhanced salt excretion.[284] The identity of the dilator PGE2 receptor controlling the contractile properties of the descending vasa recta remains uncertain, but EP2 or EP4 receptors seem likely candidates.[208] Studies demonstrating salt-sensitive hypertension in mice with targeted disruption of the EP2 receptor[253] have suggested that the EP2 receptor facilitates the ability of the kidney to increase sodium excretion, thereby protecting systemic blood pressure from a high-salt diet. Given its defined role in vascular smooth muscle,[253] these effects of the EP2 receptor disruption seem more likely to relate to its effects on renal vascular tone. In particular, loss of a vasodilator effect in the renal medulla might modify pressure natriuresis and could contribute to hypertension in EP2 knockout mice. Nonetheless, a role for the EP2 or EP4 receptor in regulating renal medullary blood flow remains to be established. In conclusion, direct vasomotor effects of EP4 receptors, as well as effects on renin release, may play critical roles in regulating systemic blood pressure and renal hemodynamics.

EFFECTS ON SALT AND WATER TRANSPORT

COX-1 and COX-2 metabolites of arachidonate have important direct epithelial effects on salt and water transport along the nephron.[299] Thus, functional effects can be observed that are thought to be independent of any hemodynamic changes produced by these compounds. Because biologically active AA metabolites are rapidly metabolized, they act predominantly in an autocrine or paracrine fashion, and thus, their locus of action will be close to their point of generation. One can expect, therefore, that direct epithelial effects of these compounds will result when they are produced by the tubule cells themselves or the neighboring interstitial cells, and the tubules possess an appropriate receptor for the ligand.

PROXIMAL TUBULE

Neither the proximal convoluted tubule nor the proximal straight tubule appear to produce amounts of biologically active COX metabolites of arachidonic acid. As will be discussed in a subsequent section, the dominant arachidonate metabolites produced by proximal convoluted and straight tubules are metabolites of the cytochrome P-450 pathway.[300]

Early whole-animal studies have suggested that PGE2 might have an action in the proximal tubule because of its effects on urinary phosphate excretion. PGE2 blocks the phosphaturic action of calcitonin infusion in thyroparathyroidectomized rats. Nevertheless, studies using in vitro perfused proximal tubules have failed to show an effect of PGE2 on sodium chloride or phosphate transport in the proximal convoluted tubule. More recent studies have suggested that PGE2 may play a key role in the phosphaturic action of FGF-23[301] because phosphaturia in hyp mice with X-linked hyperphosphaturia is associated with markedly increased urine PGE2 excretion, and phosphaturia was normalized by indomethacin.[302] Nevertheless, there are very little data on the actions of other COX metabolites in proximal tubules and scant molecular evidence for the expression of classic G protein–coupled prostaglandin receptors in this segment of the nephron.

LOOP OF HENLE

The nephron segments making up the loop of Henle also display limited metabolism of exogenous AA through the

COX pathway although, given the realization that COX-2 is expressed in this segment, it is of note that PGE2 was uniformly greater in the cortical segment than in the medullary TAL. The TAL has been shown to exhibit PGE2 receptors in high density.[303] Studies have also demonstrated high expression levels of mRNA for the EP3 receptor in medullary TAL of both rabbits and rats[231] (see earlier section on the EP3 receptor). Subsequent to the demonstration that PGE2 inhibits sodium chloride absorption in the medullary TAL of the rabbit TAL perfused in vitro, it was shown that PGE2 blocks antidiuretic hormone (ADH) but not cAMP-stimulated sodium chloride absorption in the medullary TAL of the mouse. It is likely that the mechanism involves activation of G_i and inhibition of adenyl cyclase by PGE2, possibly via the EP3 receptors expressed in this segment.

COLLECTING DUCT SYSTEM

In vitro perfusion studies of rabbit cortical collecting tubule have demonstrated that PGE2 directly inhibits sodium transport in the collecting duct when applied to the basolateral surface of this nephron segment. It is now apparent that PGE2 uses multiple signal transduction pathways in the cortical collecting duct, including those that modulate intracellular cAMP levels and Ca^{2+}. PGE2 can either stimulate or suppress cAMP accumulation. The latter may also involve stimulation of phosphodiesterase. Although modulation of cAMP levels appears to play an important role in PGE2 effects on water transport in the cortical collecting duct (see following section), it is less clear that PGE2 affects sodium transport via the modulation of cAMP levels.[229] PGE2 has been shown to increase cell calcium possibly coupled with PKC activation in in vitro perfused cortical collecting ducts.[304] This effect may be mediated by the EP1 receptor subtype coupled to phosphatidylinositol hydrolysis.[238]

WATER TRANSPORT

Vasopressin-regulated water transport in the collecting duct is markedly influenced by COX products, especially PGs. When COX inhibitors are administered to humans, rats, or dogs, the antidiuretic action of arginine vasopressin is markedly augmented. Because vasopressin also stimulates endogenous PGE2 production by the collecting duct, these results have suggested that PGE2 participates in a negative feedback loop, whereby endogenous PGE2 production dampens the action of arginine vasopressin (AVP).[305] In agreement with this model, the early classical studies of Grantham and Orloff directly demonstrated that PGE1 blunted the water permeability response of the cortical collecting duct to vasopressin.[305a] In these early studies, the action of PGE1 appeared to be at a pre-cAMP step. Interestingly, when administered by itself, PGE1 modestly augmented basal water permeability. These earlier studies have been confirmed with respect to PGE2. PGE2 also stimulates basal hydraulic conductivity and suppresses the hydraulic conductivity response to AVP in the rabbit cortical collecting duct.[306,307] Inhibition of both AVP-stimulated cAMP generation and water permeability appears to be mediated by the EP1 and EP3 receptors, whereas the increase in basal water permeability may be mediated by the EP4 receptor.[267] In contrast, EP4 receptors may mediate vasopressin-independent water reabsorption because selective collecting duct deletion of EP4 decreases aquaporin 2 expression and leads to a urine-concentrating defect.[308]

PROSTAGLANDINS

METABOLISM OF PROSTAGLANDINS

15-KETODEHYDROGENASE

The half-life of PGs is 3 to 5 minutes and that of TXA2 is approximately 30 seconds. The elimination of PGE2, PGF2α, and PGI_2 proceeds through enzymatic and nonenzymatic pathways, whereas that of TXA2 is nonenzymatic. The end products of all these degradative reactions generally possess minimal biologic activity, although this is not uniformly the case (see later). The principal enzyme involved in the transformation of PGE2, PGI_2, and PGF2α is 15-hydroxyprostaglandin dehydrogenase (PGDH), which converts the 15 alcohol group to a ketone.[309]

15-PGDH is an nicotinamide adenine dinucleotide (NAD^+)/nicotinamide adenine dinucleotide phosphate ($NADP^+$)–dependent enzyme that is 30 to 49 times more active in the kidney of the young rat (3 weeks of age) than in the adult. The K_m for PGE2 is 8.4 μM and 22.6 μM for PGF2α.[309] It is mainly localized in the cortical and juxtamedullary zones,[310] with little activity detected in papillary slices. At baseline, it is found in the proximal tubule, TAL, and collecting duct. However, it was present in macula densa in COX-2 knockout mice and in the presence of a high-salt diet and in cultured macula densa cells, COX inhibition increases expression.[311] Disruption of the 15-PGDH gene in mice results in persistent patent ductus arteriosus (PDA), thought to be a result of failure of circulating PGE2 levels to fall in the immediate peripartum period.[312] Thus administration of COX-inhibiting NSAIDs rescues the knockout mice by decreasing PGs and allowing the animals to survive.

Subsequent catalysis of 15-hydroxy products by a delta-13 reductase leads to the formation of 13,14-dihydro compounds. PGI2 and TXA2 undergo rapid degradation to 6-keto-PGF1a and TXB2, respectively.[309] These stable metabolites are usually measured, and their rates of formation taken as representative of those of the parent molecules.

ω/ω-1-HYDROXYLATION OF PROSTAGLANDINS

Both PGA2 and PGE2 have been shown to undergo hydroxylation of the terminal or subterminal carbons by a cytochrome P450–dependent mechanism.[313] This reaction may be mediated by a CYP4A family member or CYP4F enzymes. Both CYP4A[314] and CYP4F members have been mapped along the nephron.[315] Some of these derivatives have been shown to exhibit biologic activity.

CYCLOPENTENONE PROSTAGLANDINS

The cyclopentenone PGs include PGA2, a PGE2 derivative, and PGJ2, a derivative of PGD2. Although it remains uncertain whether these compounds are actually produced in vivo, this possibility has received increasing attention because some cyclopentenone prostanoids have been shown to be activating ligands for nuclear transcription factors, including proliferator-activated receptor (PPAR)-δ and PPARγ.[316–318] The realization that the antidiabetic thiazolidinedione drugs act through PPARγ to exert their antihyperglycemic and insulin-sensitizing effects[319] has generated intense interest in the possibility that the cyclopentenone PGs might serve as the

endogenous ligands for these receptors. Interestingly, DP2, unlike DP1 or indeed other members of the PGG protein-coupled receptor (GPCR) family, binds and is activated by PGD2 metabolites such as 15-deoxy-Δ12,14-PGJ2, which acts at nanomolar concentrations.[320] An alternative biologic activity of these compounds has been recognized in their capacity to covalently modify thiol groups, forming adducts with cysteine of several intracellular proteins, including thioredoxin 1, vimentin, actin, and tubulin.[321] Studies regarding the biologic activity of cyclopentenone prostanoids abound, and the reader is referred to several excellent sources in the literature.[322–324] Although there is evidence supporting the presence of these compounds in vivo,[325] it remains uncertain whether they can be formed enzymatically or are an unstable spontaneous dehydration product of the E and D ring PGs.[326]

NONENZYMATIC METABOLISM OF ARACHIDONIC ACID

It has long been recognized that oxidant injury can result in the peroxidation of lipids. In 1990, Morrow and colleagues reported that a series of PG-like compounds can be produced by free radical–catalyzed peroxidation of arachidonic acid that is independent of COX activity.[327] These compounds, which are termed *isoprostanes*, have been increasingly used as a sensitive marker of oxidant injury in vitro and in vivo.[328] In addition, at least two of these compounds, 8-iso-PGF2α (15-F2-isoprostane) and 8-iso-PGE2 (15-E2-isoprostane) are potent vasoconstrictors when administered exogenously. 8-Iso-PGF2α has been shown to constrict the renal microvasculature and decrease the GFR, an effect that is prevented by thromboxane receptor antagonism.[329] However, the role of endogenous isoprostanes as mediators of biologic responses remains unclear.

PROSTAGLANDIN TRANSPORT AND URINARY EXCRETION

It is notable that most of the PG synthetic enzymes have been localized to the intracellular compartment, yet extracellular prostaglandins are potent autacoids and paracrine factors. Thus prostanoids must be transported extracellularly to achieve efficient metabolism and termination of their signaling. Similarly, enzymes that metabolize PGE2 to inactive compounds are also intracellular, requiring uptake of the PG for its metabolic inactivation. The molecular basis of these extrusion and uptake processes are slowly being defined.

As a fatty acid, PGs may be classified as an organic anion at a physiologic pH. Early microperfusion studies have documented that basolateral PGE2 could be taken up into proximal tubules cells and actively secreted into the lumen. Furthermore, this process could be inhibited by a variety of inhibitors of organic anion transport, including Para-aminohippurate (PAH), probenecid, and indomethacin. Studies of basolateral renal membrane vesicles have also supported the notion that this transport process occurs via an electroneutral anion exchanger. These studies are of note because renal PGs enter the urine in the loop of Henle, and late proximal tubule secretion could provide an important entry mechanism.[1]

A molecule that mediates PGE2 uptake in exchange for lactate has been cloned and referred to as PGT, prostaglandin transporter.[330] PGT is a member of the SLC21/SLCO: organic anion transport family, and its cDNA encodes a transmembrane protein of 100 amino acids that exhibits broad tissue distribution (heart, placenta, brain, lung, liver, skeletal muscle, pancreas, kidney, spleen, prostate, ovary, small intestine, and colon).[331–333] Immunocytochemical studies of PGT expression in rat kidneys have suggested expression primarily in glomerular endothelial and mesangial cells, arteriolar endothelial and muscularis cells, principal cells of the collecting duct, medullary interstitial cells, medullary vasa rectae endothelia, and papillary surface epithelium.[334] PGT appears to mediate PGE2 uptake rather than release,[335] allowing target cells to metabolize this molecule and terminate signaling.[336] PGT expression is decreased with low salt and increased with high salt in the collecting duct, which may allow regulation of PG excretion by taking up more PGs excreted from the luminal surface, the site of PG transporter, thereby allowing more accumulation at the basolateral surface.[337]

Other members of the organic cation-anion-zwitterion transporter family SLC22 have also been shown to transport PGs[330] and have been suggested to mediate PG excretion into the urine. Specifically, OAT1 and OAT3 are localized on the basolateral proximal tubule membrane, where they likely participate in the urinary excretion of PGE2.[338,339] Conversely, members of the multidrug resistance protein (MRP) have been shown to transport PGs in an adenosine triphosphate (ATP)–dependent fashion.[340,341] MRP2 (also designated as ABBC2) is expressed in kidney proximal tubule brush borders and may contribute to the transport (and urinary excretion) of glutathione-conjugated PGs.[342,343] This transporter has more limited tissue expression, restricted to the kidney, liver, and small intestine, and could contribute not only to renal para-aminohippurate (PAH) excretion but also to PG excretion as well.[344]

INVOLVEMENT OF CYCLOOXYGENASE METABOLITES IN RENAL PATHOPHYSIOLOGY

EXPERIMENTAL AND HUMAN GLOMERULAR INJURY

GLOMERULAR INFLAMMATORY INJURY

COX metabolites have been implicated in functional and structural alterations in glomerular and tubulointerstitial inflammatory diseases.[345] Essential fatty acid deficiency totally prevents the structural and functional consequences of the administration of nephrotoxic serum (NTS) to rats, an experimental model of antiglomerular basement membrane glomerulonephritis.[329] Changes in arteriolar tone during the course of this inflammatory lesion are mediated principally by locally released COX and lipoxygenase (LO) metabolites of AA.[329]

TXA2 release appears to play an essential role in mediating the increased renovascular resistance observed during the early phase of this disease.[1] Subsequently, increasing rates of PGE2 generation may account for the progressive dilation of renal arterioles and increases in renal blood flow (RBF) at later stages of the disease. Consistent with this hypothesis, TXA2 antagonism ameliorated the falls in RBF and GFR 2 hours post-NTS administration, but not after 24 hours. During the latter heterologous phase of NTS, COX metabolites mediate the renal vasodilation and reduction in the glomerular ultrafiltration coefficient (K_f) that characterize this

phase.[329] The net functional result of COX inhibition during this phase of experimental glomerulonephritis, therefore would depend on the relative importance of renal perfusion versus the preservation of K_f to the maintenance of the GFR. Evidence also indicates that COX metabolites are mediators of pathologic lesions and the accompanying proteinuria in this model.[1] COX-2 expression in the kidney increases in experimental anti–glomerular basement membrane (GBM) glomerulonephritis[346,347] and after the systemic administration of lipopolysaccharide.[348]

A beneficial effect of fish oil diets (enriched in eicosapentaneoic acid), with an accompanying reduction in the generation of COX products, has been demonstrated on the course of genetic murine lupus (MRL-lpr mice). In subsequent studies, enhanced renal TXA2 and PGE2 generation was demonstrated in this model, as well as in NZB mice, another genetic model of lupus.[1] In addition, studies in humans have demonstrated an inverse relation between TXA2 biosynthesis and GFR and improvement of renal function following short-term therapy with a thromboxane receptor antagonist in patients with lupus nephritis.[1] More recently, studies have indicated that in humans, as well as NZB mice, COX-2 expression is upregulated in patients with active lupus nephritis, with colocalization to infiltrating monocytes, suggesting that monocytes infiltrating the glomeruli contribute to the exaggerated local synthesis of TXA2.[349,350] COX-2 inhibition selectively decreased thromboxane production, and chronic treatment of NZB mice with a COX-2 inhibitor and mycophenolate mofetil significantly prolonged survival.[350] Taken together, these data, as well as others from animal and human studies, support a major role for the intrarenal generation of TXA2 in mediating renal vasoconstriction during inflammatory and lupus-associated glomerular injury. In contrast, an EP4-selective agonist was shown to reduce glomerular injury in a mouse model of anti-GBM disease.[351]

The demonstration of a functionally significant role for COX metabolites in experimental and human inflammatory glomerular injury has raised the question of the cellular sources of these eicosanoids in the glomerulus. In addition to infiltrating inflammatory cells, resident glomerular macrophages, glomerular mesangial cells, and glomerular epithelial cells represent likely sources for eicosanoid generation. In the anti-Thy1.1 model of mesangioproliferative glomerulonephritis, COX-1 staining was transiently increased in diseased glomeruli at day 6 and was localized mainly to proliferating mesangial cells. COX-2 expression in the macula densa region also transiently increased at day 6.[352,353] Glomerular COX-2 expression in this model has been controversial, with one group reporting increased podocyte COX-2 expression,[347] and two other groups reporting minimal, if any, glomerular COX-2 expression.[352,353] However, it is of interest that selective COX-2 inhibitors have been reported to inhibit glomerular repair in the anti-Thy1.1 model.[353] In both anti-Thy1.1 and anti-GBM models of glomerulonephritis, the nonselective COX inhibitor, indomethacin, increased monocyte chemoattractant protein-1 (MCP-1), suggesting that prostaglandins may repress the recruitment of monocytes and macrophages in experimental glomerulonephritis.[354]

A variety of cytokines has been reported to stimulate PGE2 synthesis and COX-2 expression in cultured mesangial cells. Furthermore, complement components, in particular C5b-9, which are known to be involved in the inflammatory models described previously, have been implicated in the stimulation of PGE2 synthesis in glomerular epithelial cells (GECs). Cultured GECs express predominantly COX-1, but exposure to C5b-9 significantly increases COX-2 expression[1].

GLOMERULAR NONINFLAMMATORY INJURY

Studies have suggested that prostanoids may also mediate altered renal function and glomerular damage following subtotal renal ablation, and glomerular PG production may be altered in such conditions. Glomeruli from remnant kidneys, as well as animals fed a high-protein diet, have increased prostanoid production.[1] These studies have suggested an increase in COX enzyme activity per se rather than, or in addition to, increased substrate availability, because increases in prostanoid production were noted when excess exogenous AA was added.

Following subtotal renal ablation, there are selective increases in renal cortical and glomerular COX-2 mRNA and immunoreactive protein expression, without significant alterations in COX-1 expression.[355] This increased COX-2 expression was most prominent in the macula densa and surrounding cTALH. In addition, COX-2 immunoreactivity was also present in podocytes of remnant glomeruli, and increased PG production in isolated glomeruli from remnant kidneys was inhibited by a COX-2–selective inhibitor but was not decreased by a COX-1–selective inhibitor.[355] Of interest, in the fawn-hooded rat, which develops spontaneous glomerulosclerosis, there is increased cTALH/macula densa COX-2 and neuronal nitric oxide synthase (nNOS) and juxtaglomerular cell renin expression preceding the development of sclerotic lesions.[356] Studies have indicated that selective overexpression of COX-2 in podocytes in mice increases sensitivity to development of glomerulosclerosis, an effect that is mediated by TX receptor activation.[357–359]

When given 24 hours after subtotal renal ablation, a nonselective NSAID, indomethacin, normalized increases in renal blood flow and single-nephron GFR; similar decreases in hyperfiltration were noted when indomethacin was given acutely to rats 14 days after subtotal nephrectomy although in this latter study, the increased glomerular capillary pressure (P_{GC}) was not altered because both afferent and efferent arteriolar resistances increased.[1] Previous studies have also suggested that nonselective COX inhibitors may acutely decrease hyperfiltration in diabetes and inhibit proteinuria and/or structural injury[1]; more recent studies have indicated that selective COX-2 inhibitors will decrease the hyperfiltration seen in experimental diabetes or increased dietary protein.[360,361] Of note, NSAIDs have also been reported to be effective in reducing proteinuria in patients with refractory nephrotic syndrome.[1] Similarly, selective COX-2 inhibition decreased proteinuria in patients with both diabetic and nondiabetic renal disease, without alterations in blood pressure.[362]

The prostanoids involved have not yet been completely characterized, although it is presumed that vasodilatory prostanoids are involved in the mediation of the altered renal hemodynamics. Defective autoregulation of renal blood flow due to decreased myogenic tone of the afferent arteriole is seen after subtotal ablation or excessive dietary protein and is corrected by the inhibition of COX activity. In these hyperfiltering states, TGF is reset at a higher distal tubular flow rate.[1] Such a resetting dictates that afferent arteriolar vasodilation will be maintained in the face of increased distal

solute delivery. It was previously shown that the alterations in TGF sensitivity after reduction in renal mass are prevented with the nonselective COX inhibitor, indomethacin.[1] An important role has been suggested for neuronal nitric oxide synthase, which is localized to the macula densa, in the vasodilatory component of TGF.[363–365] Of interest, studies by Ichihara and colleagues have determined that this nNOS-mediated vasodilation is inhibited by the selective COX-2 inhibitor, NS398, suggesting that COX-2–mediated prostanoids may be essential for arteriolar vasodilation.[47,72]

Administration of COX-2–selective inhibitors decreased proteinuria and inhibited development of glomerular sclerosis in rats with reduced functioning renal mass.[366,367] In addition, COX-2 inhibition decreased mRNA expression of TGF-ß$_1$ and types III and IV collagen in the remnant kidney.[366] Similar protection was observed with the administration of nitroflurbiprofen (NOF), an NO-releasing NSAID without gastrointestinal toxicity.[368] Prior studies have also demonstrated that TXAS inhibitors retard the progression of glomerulosclerosis, with decreased proteinuria and glomerulosclerosis in rats with remnant kidneys and in diabetic nephropathy, in association with increased renal prostacyclin production and lower systolic blood pressure.[369] Studies in models of types 1 and 2 diabetes have indicated that COX-2–selective inhibitors retard progression of diabetic nephropathy.[370,371] Schmitz and colleagues have confirmed increases in TXB2 excretion in the remnant kidney and correlated decreased arachidonic and linoleic acid levels with increased thromboxane production because the TXAS inhibitor U63557A restores fatty acid levels and retards progressive glomerular destruction.[372]

Enhanced glomerular synthesis and/or urinary excretion of both PGE2 and TXA2 have been demonstrated in passive Heymann nephritis (PHN), and adriamycin-induced glomerulopathies in rats. Both COX-1 and COX-2 expression are increased in glomeruli with PHN.[373] Both TXAS inhibitors and selective COX-2 inhibitors also decreased proteinuria in PHN.[1]

In contrast to the putative deleterious effects of TX, the prostacyclin analogue, cicaprost, retarded renal damage in uninephrectomized dogs fed a high-sodium and high-protein diet, an effect that was not mediated by the amelioration of systemic hypertension.[374] Similarly both EP2 and EP4 agonists decreased glomerular and tubulointerstitial fibrosis in a model of subtotal renal ablation.[362] Other studies have also indicated that in models of polycystic kidney disease, there is increased COX-2 expression and increased PGE2 and TX in cyst fluid. Either COX-2 inhibition or EP2 receptor inhibition decreased cyst growth and interstitial fibrosis.[375,376]

Prostanoids have also been shown to alter extracellular matrix production by mesangial cells in culture. TXA2 stimulates matrix production by both TGF-ß–dependent and TGF-ß–independent pathways.[377] PGE2 has been reported to decrease steady-state mRNA levels of alpha 1(I) and alpha 1(III) procollagens, but not alpha 1(IV) procollagen and fibronectin mRNA, and to reduce the secretion of all studied collagen types into the cell culture supernatants. Of interest, this effect did not appear to be mediated by cAMP.[378] PGE2 has also been reported to increase production of matrix metalloproteinase-2 and mediate Ang II-induced increases in MMP-2.[379] Whether vasodilatory prostaglandins mediate decreased fibrillar collagen production and increased matrix degrading activity in glomeruli in vivo has not yet been studied; however, there is compelling evidence in nonrenal cells that prostanoids may mediate or modulate matrix production.[380] Cultured lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis exhibit decreased ability to express COX-2 and synthesize PGE2.[381]

ACUTE KIDNEY INJURY

When cardiac output is compromised, as in extracellular fluid volume depletion or congestive heart failure, systemic blood pressure is preserved by the action of high circulating levels of systemic vasoconstrictors (e.g., norepinephrine, Ang II, AVP). Amelioration of their effects within the renal vasculature serves to blunt the development of otherwise concomitant marked depression of renal blood flow. The intrarenal generation of vasodilator products of AA, including PGE2 and PGI2, is a central part of this protective adaptation. Increased renal vascular resistance induced by exogenously administered Ang II or renal nerve stimulation (increased adrenergic tone) is exaggerated during the concomitant inhibition of prostaglandin synthesis. Experiments in animals with volume depletion have demonstrated the existence of intrarenal AVP-prostaglandin interactions, similar to those described previously for Ang II.[1] Studies in patients with congestive heart failure have confirmed that enhanced prostaglandin synthesis is crucial in protecting kidneys from various vasoconstrictor influences in this condition.

Renal dysfunction accompanying the acute administration of endotoxin in rats is characterized by progressive reductions in RBF and GFR in the absence of hypotension. Renal histology in these animals is normal, but cortical generation of COX metabolites is markedly elevated. A number of reports have provided evidence for a role for TXA2-induced renal vasoconstriction in this model of renal dysfunction.[382] In addition, roles for PGs and TXA2 in modulating or mediating renal injury have been suggested in ischemia and reperfusion,[383] and models of toxin-mediated acute tubular injury, including those induced by uranyl nitrate,[384] amphotericin B,[385] aminoglycosides,[386] and glycerol.[387] In experimental acute renal failure, administration of vasodilator PGs has been shown to ameliorate injury.[388] Similarly, the administration of nonselective or COX-2–selective NSAIDs exacerbates experimental ischemia-reperfusion injury.[389]

COX-2 expression decreases in the kidney in response to acute ischemic injury.[390] The role of COX products in ischemia-reperfusion injury is controversial. Roles for PGs and TXA2 in modulating or mediating renal injury have been suggested in ischemia-reperfusion[383] and in models of toxin-mediated acute tubular injury, including those induced by uranyl nitrate,[384] amphotericin B,[385] aminoglycosides,[386] and glycerol.[387] Furthermore, fibrosis resulting from prolonged ischemic injury has been shown to be ameliorated by nonspecific COX inhibition.[391] In contrast, renal injury in response to ischemia-reperfusion is worsened by COX-2–selective inhibitors or in COX-2$^{-/-}$ mice,[389] and administration of vasodilator PGs has been shown to ameliorate injury,[388] possibly through a PPARα-dependent mechanism.[392]

URINARY TRACT OBSTRUCTION

Following the induction of chronic (>24 hours) ureteral obstruction, renal PG and TXA2 synthesis are markedly

enhanced, particularly in response to stimuli such as endotoxins or bradykinins. Enhanced prostanoid synthesis, especially TX, likely arises from infiltrating mononuclear cells, proliferating fibroblast-like cells, interstitial macrophages, and interstitial medullary cells.[345] Selective COX-2 inhibitors may prevent renal damage in response to unilateral ureteral obstruction.[393,394] However, PGE2 acting through the EP4 receptor can limit tubulointerstitial fibrosis resulting from UUO.[395] Prostaglandins derived from medullary COX-2 are mediators of the early phase of diuresis seen after the relief of ureteral obstruction because COX-2 inhibition prevents the acute (24-hour) phase of postobstructive diuresis. However, more persistent, chronic postobstructive diuresis is not PG-dependent but results from the downregulation of NKCC2 and decreases aquaporin-2 phosphorylation and translocation to the collecting duct membrane.[396]

ALLOGRAFT REJECTION AND CYCLOSPORINE NEPHROTOXICITY

ALLOGRAFT REJECTION

Acute administration of a TXA2 synthesis inhibitor is associated with significant improvement in rat renal allograft function.[397] A number of other experimental and clinical studies have also demonstrated increased TXA2 synthesis during allograft rejection,[398,399] leading some to suggest that increased urinary TXA2 excretion may be an early indicator in renal and cardiac allograft rejection.

CALCINEURIN INHIBITOR NEPHROTOXICITY

Numerous investigators have demonstrated effects for cyclosporine A (CY-A) on renal PG and TXA2 synthesis and provided evidence for a major role for renal and leukocyte TXA2 synthesis in mediating acute and chronic CY-A nephrotoxicity in rats.[400] Fish oil–rich diets, TXA2 antagonists, or administration of CY-A in fish oil as vehicle have all been shown to reduce renal TXA2 synthesis and may therefore afford protection against nephrotoxicity. Moreover, CY-A has been reported to decrease renal COX-2 expression.[401]

HEPATIC CIRRHOSIS AND HEPATORENAL SYNDROME

Patients with cirrhosis of the liver show an increased renal synthesis of vasodilating PGs, as indicated by the high urinary excretion of PGs and/or their metabolites. Urinary excretion of 2-3-dinor-6-keto PGF1α, an index of systemic PGI2 synthesis, is increased in patients with cirrhosis and hyperdynamic circulation, thus raising the possibility that systemic synthesis of PGI2 may contribute to the arterial vasodilation of these patients. Inhibition of COX activity in these patients may cause a profound reduction in renal blood flow and GFR, a reduction in sodium excretion, and an impairment of free water clearance.[402] The sodium-retaining properties of NSAIDs are particularly exaggerated in patients with cirrhosis of the liver, attesting to the dependence of renal salt excretion on vasodilatory PGs. In the kidneys of rats with cirrhosis, COX-2 expression increases, while COX-1 expression is unchanged; however, in these animals, selective inhibition of COX-1 leads to impaired renal hemodynamics and natriuresis, whereas COX-2 inhibition has no effect.[403,404]

Diminished renal PG synthesis has been implicated in the pathogenesis of the severe sodium retention seen in hepatorenal syndrome, as well as in the resistance to diuretic therapy.[405,406] There is reduced renal synthesis of vasodilating PGE2 in the presence of activation of endogenous vasoconstrictors and a maintained or increased renal production of TXA2.[402,407] Therefore, an imbalance between vasoconstricting systems and the renal vasodilator PGE2 has been proposed as a contributing factor to the renal failure observed in this condition. However, administration of exogenous prostanoids to patients with cirrhosis is not effective in ameliorating renal function or preventing the deleterious effect of NSAIDs.[402]

DIABETES MELLITUS

In the streptozotocin-induced model of diabetes in rats, COX-2 expression is increased in the cTALH–macula densa region,[360,370] as well as in podocytes.[408] possibly mediated by epigenetic processes.[409] COX-2 immunoreactivity has also been detected in the macula densa region in human diabetic nephropathy.[41] Studies have suggested that COX-2 dependent vasodilator prostanoids play an important role in the hyperfiltration seen early in diabetes mellitus,[410,360,411–413] as well as in response to a high-protein diet.[414] The increased COX-2 expression appears to be mediated at least in part by increased ROS production in diabetes, because the superoxide dismutase analogue, Tempol, blocks the increased expression.[415]

It has been found that the chronic administration of a selective COX-2 inhibitor significantly decreases proteinuria and reduces extracellular matrix deposition, as indicated by decreases in immunoreactive fibronectin expression and mesangial matrix expansion.[370,416] In addition, COX-2 inhibition reduced expression of TGF-ß, PAI-1, and vascular endothelial growth factor (VEGF) in the kidneys of the diabetic hypertensive animals. Increasing intrarenal dopamine production also ameliorates diabetic nephropathy progression, at least in part by inhibiting renal cortical COX-2 expression.[417] The vasoconstrictor TXA2 may play a role in the development of albuminuria and basement membrane changes with diabetic nephropathy (DN). In addition, the administration of a selective PGE2 EP1 receptor antagonist prevented the development of experimental DN,[418] whereas EP4 receptor activation may exacerbate DN.[419] In contrast to the beneficial effect of global inhibition of COX-2, selective inhibition of renal macrophage COX-2 actually exacerbates diabetic renal injury.[420]

PREGNANCY

Most, but not all, investigators have not reported increases in vasodilator PG synthesis or suggested an essential role for prostanoids in the mediation of the increased GFR and renal plasma flow (RPF) of normal pregnancy;[421] however, diminished synthesis of PGI2 has been demonstrated in humans and in animal models of pregnancy-induced hypertension,[422] which is associated with decreased expression of COX-2 and PGI2 synthase in placental villi.[423] In animal models, the inhibition of TXA2 synthetase has been associated with resolution of the hypertension, suggesting a possible pathophysiologic role.[424] A moderate beneficial effect of reducing TXA2 generation, while preserving PGI2 synthesis, by low-dose aspirin therapy (60–100 mg/day) has been demonstrated

in patients at high risk for pregnancy-induced hypertension and preeclampsia.[425,426]

LITHIUM NEPHROTOXICITY

Lithium chloride is a mainstay of treatment in psychiatry for bipolar illness. However, it is routinely complicated by polyuria and even frank nephrogenic diabetes insipidus. In vitro and in vivo studies have demonstrated lithium-induced renal medullary interstitial cell COX-2 protein expression via inhibition of glycogen synthase kinase-3ß (GSK-3ß). COX-2 inhibition prevented lithium-induced polyuria. COX-2 inhibition also resulted in the upregulation of aquaporin 2 (AQP2) and Na-K-2Cl co-transporter (NKCC2).[427,428]

ROLE OF REACTIVE OXYGEN SPECIES AS MEDIATORS OF COX-2 ACTIONS

In addition to NADPH oxidase, nitric oxide synthase, and xanthine oxidase, COX-2 can also be a source of oxygen radicals.[429] COX-2 enzymatic activity is commonly accompanied by associated oxidative mechanisms (co-oxidation) and free radical production.[430] The catalytic activity of COX consists of a series of radical reactions that use molecular oxygen and generate intermediate ROS.[431] Elevated levels of COX-2 protein are associated with increased ROS production and apoptosis in cultured renal cortical cells[432] and human mesangial cells.[433] It has been suggested that COX-2–mediated lipid peroxidation, rather than PGs, can induce DNA damage via adduct formation.[434] A COX-2 specific inhibitor, NS-398, was able to reduce the oxidative activity with prevention of oxidant stress.[435]

In addition to ROS generated by COX per se, prostanoids may also activate intracellular pathways that generate ROS. Locally generated ROS may damage cell membranes, leading to lipid peroxidation and release of AA. Prostanoids released during inflammatory reactions cause rapid degenerative changes in some cultured cells, and their potential cytotoxic effect has been suggested to occur by accelerating intracellular oxidative stress. TX[436] and PGE2 acting through the EP1 receptor[437] have been reported to induce NADPH oxidase and ROS production. Of interest, PGE2 acting through the EP4 receptor inhibits macrophage oxidase activity. [438,439] As mentioned previously, there is also evidence for crosstalk between COX-2 and ROS, such that ROS may induce COX-2 expression.[434] Interestingly, during aging, there is ROS-mediated NF-κB expression, which increases COX-2 expression in the kidney.[440] Furthermore, this appears to induce a vicious cycle, because COX-2 then serves as a source of ROS. This interaction of COX-derived PG and ROS production has been posited to play a role in the development of hypertension.[441] The amount of renal ROS resulting from COX activity increases with age, so that up to 25% of total kidney ROS production in aged rat kidneys is inhibited by NSAID administration.

THE LIPOXYGENASE PATHWAY

The lipoxygenase enzymes metabolize AA to form LTs, HETEs, and LXs (Fig. 13.12). These lipoxygenase metabolites are primarily produced by leukocytes, mast cells, and macrophages in response to inflammation and injury. There are three lipoxygenase enzymes—5-, 12-, and 15-lipoxygenase—so named for the carbon of AA where they insert an oxygen. The lipoxygenases are products of separate genes and have distinct distributions and patterns of regulation. Glomeruli, mesangial cells, cortical tubules, and vessels also produce

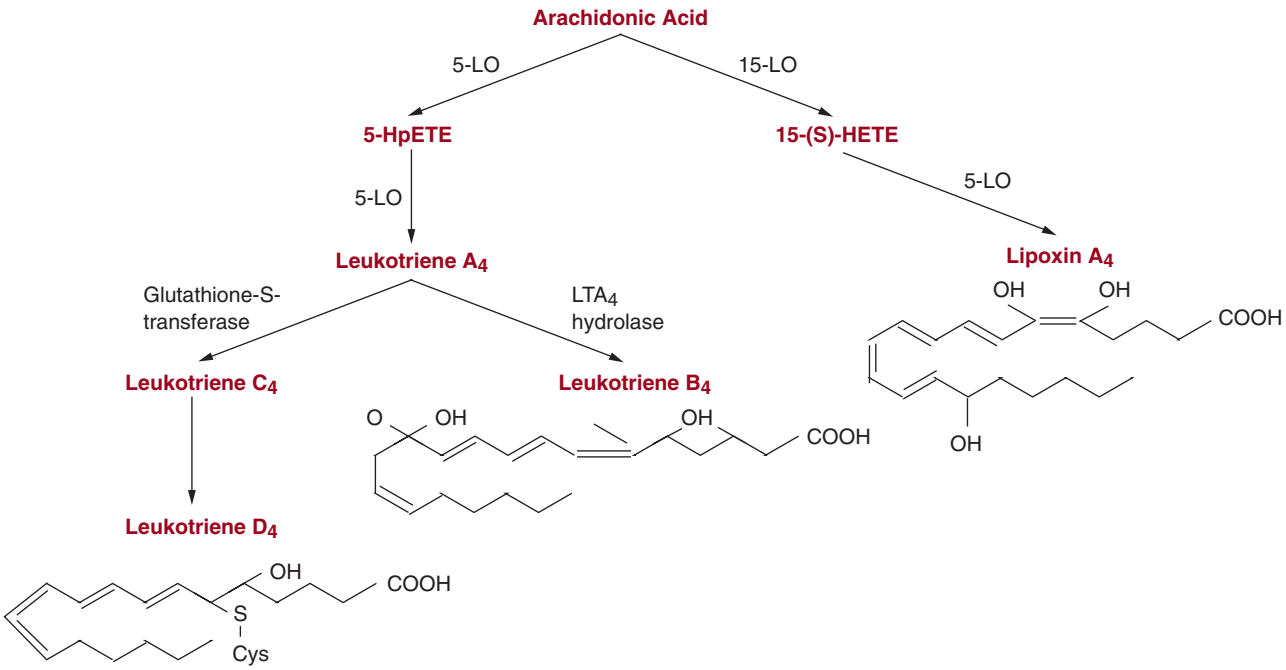


Fig. 13.12 Pathways of lipoxygenase (LOX) metabolism of arachidonic acid. *5-LO,* 5-Lipoxygenase; *15-(S)-HETE,* 15(S)-hydroxyeicosatetraenoic acid; *5-HpETE,* 5-hydroperoxyeicosatetraenoic acid.

the 12-lipoxygenase (12-LOX) product, 12(*S*)-HETE, and the 15-LOX product, 15-HETE. Studies have localized 15-LOX mRNA primarily to the distal nephron and 12-LOX mRNA to the glomerulus. 5-LOX mRNA and 5-LOX-activating protein (FLAP) mRNA were expressed in the glomerulus and the vasa recta.[442] In polymorphonuclear leukocytes (PMNs), macrophages, and mast cells, 5-LOX mediates the formation of leukotrienes.[443] 5-LOX, which is regulated by FLAP, catalyzes the conversion of AA to 5-HpETE and then to leukotriene A4 (LTA4).[444] LTA4 is then further metabolized to the peptidyl leukotrienes (LTC4 and LTD4) by glutathione-S-transferase or to LTB4 by LTA4 hydrolase. Although glutathione-S-transferase expression is limited to inflammatory cells, LTA4 hydrolase is also expressed in glomerular mesangial cells and endothelial cells;[445] PCR analysis has actually demonstrated ubiquitous LTA4 hydrolase mRNA expression throughout the rat nephron.[442] LTC4 synthase mRNA could not be found in any nephron segment.[442]

Two cysteinyl leukotriene receptors (CysLTR) have been cloned and identified as members of the G protein–coupled superfamily of receptors. They have been localized to vascular smooth muscle and endothelium of the pulmonary vasculature.[446–448] In the kidney, the CysLTR type 1 is expressed in the glomerulus, whereas CysLTR type 2 mRNA has not been detected in any nephron segment to date.[442]

The peptidyl leukotrienes are potent mediators of inflammation and vasoconstrictors of vascular, pulmonary, and gastrointestinal smooth muscle. In addition, they increase vascular permeability and promote mucous secretion.[449] Because of the central role that peptidyl leukotrienes play in the inflammatory trigger of asthma exacerbation, effective receptor antagonists have been developed and are now an important component of asthma treatment.[450]

In the kidney, LTD4 administration has been shown to decrease RBF and GFR, and peptidyl leukotrienes are thought to be mediators of decreased RBF and GFR associated with acute glomerular inflammation. Micropuncture studies have revealed that the decreases in GFR are the result of afferent and arteriolar vasoconstriction, with more pronounced efferent vasoconstriction and a decrease in K_f.[1] In addition both LTC4 and LTD4 increase the proliferation of cultured mesangial cells.

The LTB4 receptor is also a seven-transmembrane G protein–coupled receptor. On PMNs, receptor activation promotes chemotaxis, aggregation, and attachment to endothelium. In the kidney LTB4, mRNA is localized to the glomerulus[442]. A second, low-affinity LTB4 receptor is also expressed,[451] which may mediate calcium influx into PMNs, thereby leading to activation. LTB4 receptor blockers lessen acute renal ischemic-reperfusion injury[452] and nephrotoxic nephritis in rats,[453] and PMN infiltration and structural and functional evidence of organ injury by ischemia and reperfusion are magnified in transgenic mice overexpressing the LTB4 receptor.[454] In addition to the activation of cell surface receptors, L TB4 has also been shown to be a ligand for the nuclear receptor PPARα.[455]

15-LOX leads to the formation of 15-S-HETE. In addition, dual oxygenation in activated PMNs and macrophages by 5- and 15-LOX leads to formation of the lipoxins. LX synthesis also can occur via transcellular metabolism of the leukocyte-generated intermediate, LTA4, by 12-LOX in platelets or adjoining cells, including glomerular endothelial cells.[456,457]

15-S-HETE is a potent vasoconstrictor in the renal microcirculation;[458] however, 15-LOX–derived metabolites antagonize proinflammatory actions of leukotrienes, both by inhibiting PMN chemotaxis, aggregation, and adherence and by counteracting the vasoconstrictive effects of the peptidyl leukotrienes.[459,460] Administration of 15-S-HETE reduced LTB4 production by glomeruli isolated from rats with acute nephrotoxic, serum-induced glomerulonephritis, and it has been proposed that 15-LOX may regulate 5-LOX activity in chronic glomerular inflammation because it is known that in experimental glomerulonephritis, lipoxin A4 (LXA4) administration increases renal blood flow and GFR, mainly by inducing afferent arteriolar vasodilation, an effect mediated in part by release of vasodilator PGs.[1] LXA4 also antagonized the effects of LTD4 to decrease the GFR, although not RBF, even though administration of LXA4 and LXB4 directly into the renal artery induced vasoconstriction. Glomerular micropuncture studies have revealed that LXA4 leads to moderate decreases in K_f.[459] Lipoxins signal through a specific G protein–coupled receptor denoted ALXR. This receptor is related at the nucleotide sequence level to chemokine and chemotactic peptide receptors, such as N-formyl peptide receptor.[461] It is also noteworthy that in isolated perfused canine renal arteries and veins, LTC4 and LTD4 were found to be vasodilators, which were partially dependent on an intact endothelium; this was mediated by nitric oxide production.[462]

A potential interaction between COX- and LOX-mediated pathways has been reported. Although aspirin inhibits PG formation by COX-1 and COX-2, aspirin-induced acetylation converts COX-2 to a selective generator of 15-(*S*)-HETE. This product can then be released, taken up in a transcellular route by PMNs and converted to 15-epilipoxins, which have similar biologic actions as the lipoxins.[463]

Similar to 15-HETE, 12(*S*)-HETE also potently vasoconstricts glomerular and renal vasculature.[456] 12(*S*)-HETE increases protein kinase C and depolarizes cultured vascular smooth muscle cells. Afferent arteriolar vasoconstriction and increases in smooth muscle calcium in response to 12(*S*)-HETE, were partially inhibited by voltage-gated, L-type calcium channel inhibitors.[464] 12(*S*)-HETE has also been proposed to be an angiogenic factor, because in cultured endothelial cells, 12-LOX inhibition reduces cell proliferation, and 12-LOX overexpression stimulates cell migration and endothelial tube formation.[465] 12- and 15-LOX inhibitors and elective elimination of the leukocyte 12-LOX enzyme also ameliorate the development of diabetic nephropathy in mice.[466] There is also interaction between 12- and 15-LOX pathways and TGF-ß–mediated pathways in the diabetic kidney.[467] 12(*S*)-HETE has also been proposed to be a mediator of renal vasoconstriction by Ang II, with inhibition of the 12-LOX pathway attenuating Ang II-mediated afferent arteriolar vasoconstriction and decreased renal blood flow.[468] LOX inhibition also blunted renal arcuate artery vasoconstriction by norepinephrine and KCl.[469] However, 12-LOX products have also been implicated as inhibitors of renal renin release.[470,471]

Although the major significance of LOX products in the kidney derives from their release from infiltrating leukocytes or resident cells of macrophage or monocyte origin, there is evidence to suggest that intrinsic renal cells are capable of generating LTs and LXs, either directly or through transcellular metabolism of intermediates.[472] Human and rat glomeruli

can generate 12- and 15-HETE, although the cells of origin are unclear. LTB4 can be detected in supernatants of normal rat glomeruli, and its synthesis could be markedly diminished by mechanisms that deplete glomeruli of resident macrophages, such as irradiation or fatty acid deficiency. In addition, 5-,12-, and 15-HETEs were detected from pig glomeruli, and their structural identity was confirmed by mass spectrometry[1]. 12-LOX products are increased in mesangial cells exposed to hyperglycemia and in diabetic nephropathy.[473] There also appears to be crosstalk between 12- and 15-LOX and COX-2. Both are increased with diabetes or high glucose levels and, in cultured cells, 12(*S*)-HETE increases COX-2, whereas PGE2 increases 12- and 15-LOX. Knockdown of 12- and 15-LOX expression with short hairpin RNA (shRNA) decreases COX-2 expression, whereas 12- and 15-LOX overexpression increases COX-2 expression.[430]

Glomeruli subjected to immune injury release LTB4,[474] and LTB4 generation was suppressed by resident macrophage depletion. Synthesis of peptido-LTs by inflamed glomeruli has also been demonstrated,[475] but leukocytes could not be excluded because its primary source LXA4 is generated by immune-injured glomeruli.[476] Rat mesangial cells generate LXA4 when provided with LTA4 as substrate, thereby providing a potential intraglomerular source of LXs during inflammatory reactions. In nonglomerular tissue, 12-HETE production has been reported from rat cortical tubules and epithelial cells and 12- and 15-HETE from rabbit medulla[1].

BIOLOGIC ACTIVITIES OF LIPOXYGENASE PRODUCTS IN THE KIDNEY

In early experiments, the systemic administration of LTC4 in the rat and administration of LTC4 and LTD4 in the isolated perfused kidney revealed potent renal vasoconstrictor actions of these eicosanoids. Subsequently, micropuncture measurements revealed that LTD4 exerts preferential constrictor effects on postglomerular arteriolar resistance and depresses K_f and GFR. The latter is likely due to receptor-mediated contraction of glomerular mesangial cells, which has been demonstrated for LTC4 and LTD4 in vitro (see earlier). These actions of LTD4 in the kidney are consistent with its known smooth muscle contractile properties. LTB4, a potent chemotactic and leukocyte-activating agent, is devoid of constrictor action in the normal rat kidney. Lipoxin A4 dilates afferent arterioles when infused into the renal artery, without affecting efferent arteriolar tone. This results in elevations in intraglomerular pressure and plasma flow rate, thereby augmenting the GFR.[1]

INVOLVEMENT OF LIPOXYGENASE PRODUCTS IN RENAL PATHOPHYSIOLOGY

Increased generation rates of LTC_4 and LTD4 have been documented in glomeruli from rats with immune complex nephritis and mice with spontaneously developing lupus nephritis.[443,476] Moreover, results from numerous physiologic studies using specific LTD4 receptor antagonists have provided strong evidence for the release of these eicosanoids during glomerular inflammation. In four animal models of glomerular immune injury (anti-GBM nephritis, anti-Thy1.1 antibody-mediated mesangiolysis, passive Heymann nephritis, and murine lupus nephritis), acute antagonism of LTD4 by receptor binding competition or inhibition of LTD4 synthesis led to highly significant increases in GFR in nephritic animals.[477] The principal mechanism underlying the improvement in GFR was reversal of the depressed values of K_f, which is characteristically compromised in immune-injured glomeruli. In other studies in PHN, Katoh and colleagues provided evidence that endogenous LTD4 not only mediates reductions in K_f and GFR, but that LTD4-evoked increases in intraglomerular pressure underlie, to a large extent, the accompanying proteinuria.[477] Cysteinyl leukotrienes have been implicated in cyclosporine nephrotoxicity.[478] Of interest, 5- LOX deficiency accelerates renal allograft rejection.[479]

LTB4 synthesis, measured in the supernates of isolated glomeruli, is markedly enhanced early in the course of several forms of glomerular immune injury.[480] Cellular sources of LTB4 in injured glomeruli include PMNs and macrophages. All studies concur as to the transient nature of LTB4 release. LTB4 production decreases 24 hours after onset of the inflammation, which coincides with macrophage infiltration, a major source of 15-LOX activity.[481] 15-HPETE incubation decreased lipopolysaccharide-induced TNF expression in a human monocytic cell line,[482] and HVJ-liposome–mediated glomerular transfection of 15-LOX in rats decreased markers of injury (e.g., blood urea nitrogen [BUN], proteinuria) and accelerated functional (GFR, RBF) recovery in experimental glomerulonephritis (GN).[483] In addition, MK501, a FLAP antagonist, restored size selectivity and decreased glomerular permeability in acute GN.[484]

The suppression of LTB4 synthesis beyond the first 24 hours of injury is rather surprising, because both PMNs and macrophages are capable of effecting the total synthesis of LTB4; they contain the two necessary enzymes that convert AA to LTB4—namely, 5-LOX and LTA4 hydrolase. It has therefore been suggested, based on in vitro evidence, that the major route for LTB4 synthesis in inflamed glomeruli is through transcellular metabolism of leukocyte-generated LTA4 to LTB4 by LTA4 hydrolase present in glomerular mesangial, endothelial, and epithelial cells. Because the transformation of LTA4 to LTB4 is rate-limiting, regulation of LTB4 synthetic rate might relate to the regulation of LTA4 hydrolase gene expression or catalytic activity in these parenchymal cells, rather than to the number of infiltrating leukocytes. In any case, leukocytes represent an indispensable source for LTA4, the initial 5-LOX product and the precursor for LTB4, because endogenous glomerular cells do not express the 5-LOX gene.[485] Thus, it was demonstrated that the PMN cell-specific activator, N-formyl-Met-Leu-Phe, stimulated LTB4 production from isolated perfused kidneys harvested from NTS-treated rats to a significantly greater degree than from control animals treated with nonimmune rabbit serum.[486] The renal production of LTB4 correlated directly with renal myeloperoxidase activity, suggesting interdependence of LTB4 generation and PMN infiltration.

The acute and long-term significance of LTB4 generation in conditioning the extent of glomerular structural and functional deterioration has been highlighted in studies in which LTB4 was exogenously administered or in which its endogenous synthesis was inhibited. Intrarenal administration of LTB4 to rats with mild NTS-induced injury was associated with an increase in PMN infiltration, reduction in renal plasma flow rate, and marked exacerbation of the fall in the GFR, with the latter correlating strongly with the number of

infiltrating PMNs and glomeruli, whereas inhibition of 5-LOX led to preservation of the GFR and abrogation of proteinuria.[486] Similarly, both 5-LOX knockout mice and wild-type mice treated with the 5-LOX inhibitor zileuton had reduced renal injury in response to ischemia and reperfusion.[487] Thus although devoid of vasoconstrictor actions in the normal kidney, increased intrarenal generation of LTB4 during early glomerular injury amplifies leukocyte-dependent reductions in glomerular perfusion and filtration rates and inflammatory injury, likely due to enhancement of PMN recruitment and activation.

12(*S*)-HETE has been reported to increase AT1 receptor (AT1R) mRNA and protein expression in cultured rat mesangial cells by stabilizing AT1R mRNA and enhancing the profibrotic effects of Ang II.[488] Ang II AT1R blockade has been shown to inhibit the development of diabetic nephropathy via the upregulation of glomerular nephrin and P-cadherin expression through inhibition of 12- and 15-LOX activation in rats.[489] Genetic or pharmacologic inhibition of 12- and 15-LOX led to decreases in 12(*S*)-HETE production, proteinuria, renal oxidative stress, and collagen deposition in type I diabetes.[490] Recently, 12(*S*)-HETE was found to increase profibrotic gene expression and enhance the permissive histone lysine modification at their promoters by upregulation of protein levels of SET7, a histone H3 lysine 4 methyltransferase, its nuclear translocation, and enhancement at profibrotic gene promoters in mesangial cells.[491]

Both LOX and leukotriene signaling pathways have been reported to be involved in the development of cisplatin-mediated acute kidney injury in Wistar albino rats.[492] Urinary 12(S)- and 15(S)-HETE levels have been shown to correlate positively with elevated serum creatinine levels after kidney transplantation.[493] In the JCR:LA-corpulent rat, a model of the metabolic syndrome, fish oil (ω-3 polyunsaturated fatty acid) supplements markedly reduced albuminuria and glomerulosclerosis in association with decreases in 5(S)-, 12(S)-, and 15(S)-HETE.[494] The nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 (NLRP3) inflammasome promotes renal inflammation and contributes to chronic kidney disease through the generation of proinflammatory cytokines IL-β and IL-18.[495] Docosahexaenoic acid (DHA, in the form of fish oil), a well-known ω-3 polyunsaturated fatty acid, inhibits inflammation and exerts a beneficial action in numerous inflammatory human diseases. Recent studies have indicated that the LOX-mediated DHA metabolite, resolving D1, attenuates podocyte injury during hyperhomocysteinemia by inhibiting NLRP3 inflammasome activation.[496]

THE CYTOCHROME P450 PATHWAY

Following their elucidation and characterization as endogenous metabolites of AA, numerous studies have investigated the possibility that cytochrome P450 (CYP450) AA metabolites subserve physiologic and/or pathophysiologic roles in the kidney. In whole-animal physiology, these compounds have been implicated in the mediation of release of peptide hormones, regulation of vascular tone, and regulation of volume homeostasis. On the cellular level, CYP AA metabolites have been proposed to regulate ion channels and transporters and to act as mitogens. See Fig. 13.13.

CYP450 monooxygenases are mixed-function oxidases that use molecular oxygen and NADPH as cofactors[497,498] and will

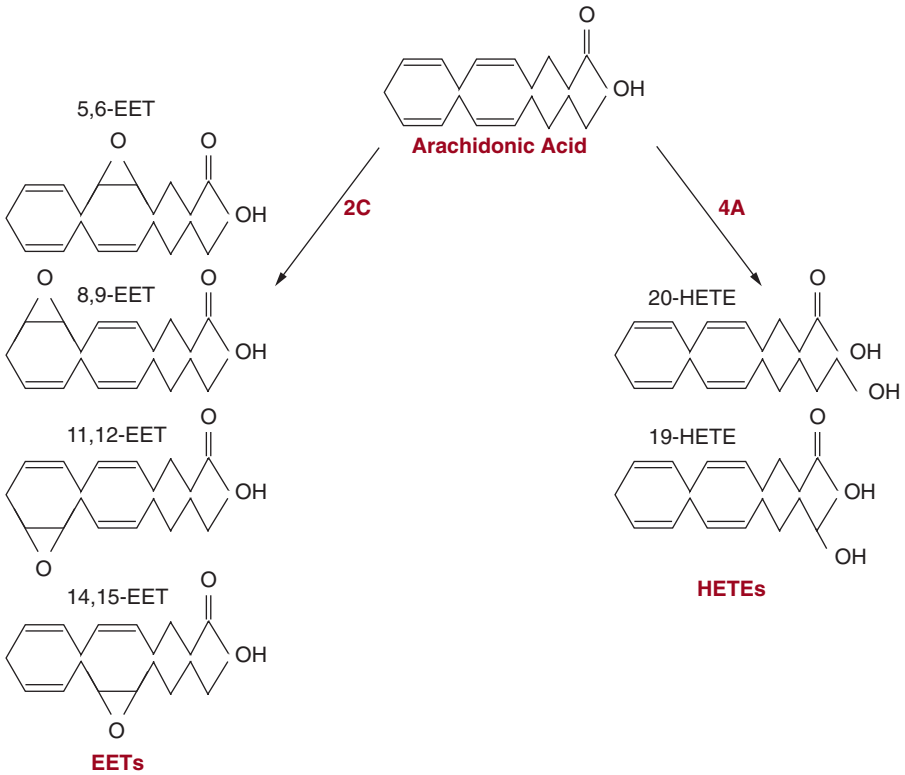


Fig. 13.13 Pathways of CYP450 metabolism of arachidonic acid. *EET,* Epoxyeicosatrienoic acid; *HETE,* hydroxyeicosatetraenoic acid.

add an oxygen molecule to AA in a region- and stereo-specific geometry. CYP450 monooxygenase pathways metabolize AA to generate HETEs and epoxyeicosatrienoic acids (EETs); EETs can be hydrolyzed to dihydroxyeicosatrienoic acids (DHETs).[497,499,500] The kidney displays one of the highest CYP450 activities of any organ and produces CYP450 AA metabolites in significant amounts.[497,500,501] HETEs are formed primarily via CYP450 hydroxylase enzymes and EETs, and DHETs are formed primarily via CYP450 epoxygenase enzymes.[501] The CYP450 4A gene family is the major pathway for synthesis of hydroxylase metabolites, especially 20-HETE and 19-HETE,[300,501] whereas the production of epoxygenase metabolites is primarily via the 2C gene family. [497,502] A member of the 2J family that is an active epoxygenase is also expressed in the kidney.[503]

CYP450 enzymes have been localized to vasculature and tubules.[300] The 4A family of hydroxylases is expressed in preglomerular renal arterioles, glomeruli, proximal tubules, the TALH, and macula densa.[504]

The 2C and 2J families of epoxygenases are expressed at the highest levels in the proximal tubule and collecting duct.[503,505] When isolated nephron segments expressing CYP450 protein have been incubated with AA, the production of CYP450 AA metabolites can be detected. 20-HETE and EETs are both produced in the afferent arterioles,[506] glomerulus,[507] and proximal tubule.[508] 20-HETE is the predominant CYP450 AA metabolite produced by the TALH and in the pericytes surrounding vasa recta capillaries,[507] whereas EETs are the predominant CYP450 AA metabolites produced by the collecting duct.[509]

Renal production of both epoxygenase and hydroxylase metabolites has been shown to be regulated by hormones and growth factors, including Ang II, endothelin, bradykinin, parathyroid hormone, and epidermal growth factor.[300,498,502] Alterations in dietary salt intake also modulate CYP450 expression and activity.[510] Alterations in the production of CYP450 metabolites have also been reported with uninephrectomy, diabetes mellitus, and hypertension.[300,499] Glycerol-containing epoxygenase metabolites are produced endogenously and serve as high-affinity ligands for cannabinoid receptors, implicating these compounds as endocannabinoids.[511]

VASCULATURE

20-TRIHYDROXYEICOSATETRAENOIC ACID

In rat and dog renal arteries and afferent arterioles, 20-HETE is a potent vasoconstrictor,[506] whereas it is a vasodilator in rabbit renal arterioles. The vasoconstriction is associated with membrane depolarization and a sustained rise in intracellular calcium. 20-HETE is produced in the smooth muscle cells, and its afferent arteriolar vasoconstrictive effects are mediated by the closure of K_{Ca} channels through a tyrosine kinase- and ERK-dependent mechanism (Fig. 13.14). Recent studies have indicated that 20-HETE–induced hypertension is a mediator of kidney disease in diabetes.[512]

An interaction between CYP450 AA metabolites and nitric oxide has also been demonstrated. NO can inhibit the formation of 20-HETE in renal vascular smooth muscle (VSM) cells. A significant portion of NO's vasodilator effects in the preglomerular vasculature appear to be mediated by the inhibition of tonic 20-HETE vasoconstriction, and inhibition of 20-HETE formation attenuates the pressor response and fall in RBF seen with NO synthase inhibition.[513,514]

EPOXIDES

Unlike CYP450 hydroxylase metabolites, epoxygenase metabolites of AA increase RBF and GFR.[300,498,502] 11,12-EET and 14,15-EET vasodilate the preglomerular arterioles

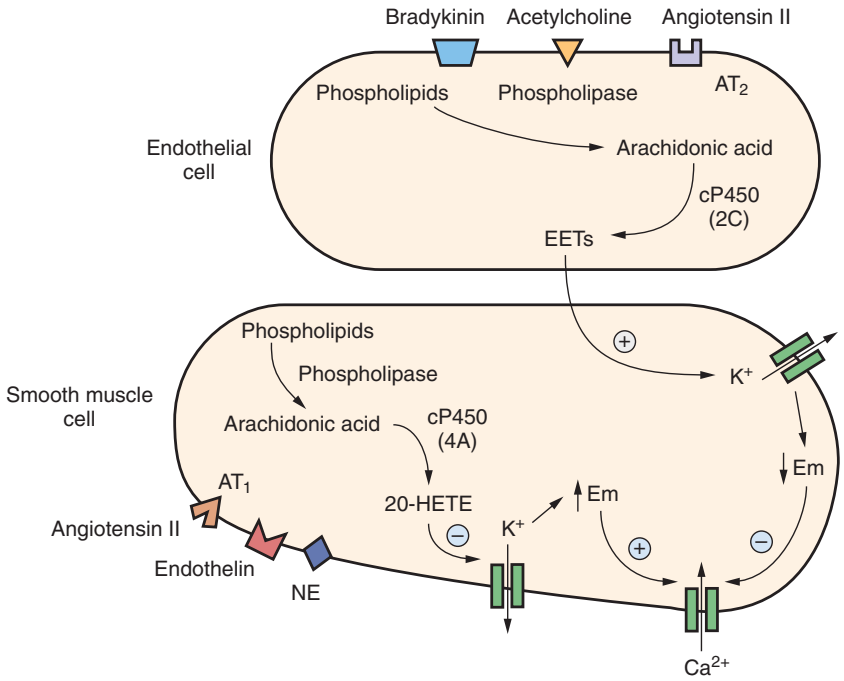


Fig. 13.14 Proposed interactions of CYP450 arachidonic acid metabolites derived from vascular endothelial cells and smooth muscle cells to regulate vascular tone. *EET,* Epoxyeicosatrienoic acid; *HETE,* hydroxyeicosatetraenoic acid; *NE,* norepinephrine.

independently of COX activity, whereas 5,6-EET and 8,9-EET cause COX-dependent vasodilation or vasoconstriction.[515] It is possible that these COX-dependent effects are mediated by COX conversion of 5,6-EET and 8,9-EET to PG- or TX-like compounds.[516] EETs are produced primarily in the endothelial cells and exert their vasoactive effects on the adjacent smooth muscle cells. In this regard, it has been suggested that EETs, and specifically 11,12-EET, may serve as an endothelium-derived hyperpolarizing factor (EDHF) in the renal microcirculation.[502,517] EET-induced vasodilation is mediated by activation of K_{Ca} channels through cAMP-dependent stimulation of protein kinase C.

CYP450 metabolites may serve as second messengers or as modulators of the actions of hormonal and paracrine agents. Vasopressin increases renal production of CYP450 metabolites,[518] and increases in intracellular calcium and proliferation in cultured renal mesangial cells are augmented by EET administration.[518] CYP450 metabolites also may serve to modulate the renal hemodynamic responses of endothelin-1, with 20-HETE as a possible mediator of the vasoconstrictive effects and EETs counteracting the vasoconstriction.[502,519] The formation of 20-HETE does not affect the ability of ET-1 to increase free intracellular calcium transient in renal vascular smooth muscle but appears to enhance the sustained elevations that represent calcium influx through voltage-sensitive channels.

CYP450 metabolites have also been implicated in mediation of renal vascular responses to Ang II. In the presence of AT1 receptor blockers, Ang II produces an endothelial-dependent vasodilation in rabbit afferent arterioles that is dependent on CYP450 epoxygenase metabolite production by AT2 receptor activation.[520] With intact AT1 receptors, Ang II increases 20-HETE release from isolated preglomerular microvessels through an endothelium-independent mechanism.[521] Ang II's vasoconstrictive effects are in part the result of 20-HETE–mediated inhibition of K_{Ca}, which enhances sustained increases in intracellular calcium concentration by calcium influx through voltage-sensitive channels. Inhibition of 20-HETE production reduces the vasoconstrictor response to Ang II by more than 50% in rat renal interlobular arteries in which the endothelium has been removed.[521]

AUTOREGULATION

CYP450 metabolites of AA have been shown to be mediators of RBF autoregulatory mechanisms. When PG production was blocked in canine arcuate arteries, AA administration enhanced myogenic responsiveness, and renal blood flow autoregulation was blocked by CYP450 inhibitors.[300,500] Similarly, in the rat juxtamedullary preparation, selective blockade of 20-HETE formation significantly decreased afferent arteriolar vasoconstrictor responses to elevations in perfusion pressure, and inhibition of epoxygenase activity enhanced vasoconstriction.[522] This suggests that 20-HETE is involved in afferent arteriolar autoregulatory adjustment, whereas release of vasodilatory epoxygenase metabolites in response to increases in renal perfusion pressure acts to attenuate the vasoconstriction. In vivo studies have also implicated 20-HETE as a mediator of the autoregulatory response to increased perfusion pressure.[523] Bradykinin-induced efferent arteriolar vasodilation has been shown to be mediated in part by the direct release of EETs from this vascular segment. In addition, bradykinin-induced release of 20-HETE from the glomerulus can modulate the EET-mediated vasodilation.[524] Deficient 20-HETE production worsens ischemic kidney injury by impairing medullary blood flow.[525]

TUBULOGLOMERULAR FEEDBACK

CYP450 metabolites may also be involved in the tubuloglomerular feedback response.[300] As noted, 20-HETE is produced by both the afferent arteriole and macula densa, and studies have suggested the possibility that HETEs may serve as a vasoconstrictive mediator of TGF released by the macula densa or as a second messenger in the afferent arteriole in response to mediators released by the macula densa, such as adenosine or ATP.[526] 20-HETE may also be a mediator of regulation of intrarenal distribution of blood flow.[527,528] In addition, there is evidence for "connecting tubule–glomerular feedback," in which increased sodium reabsorption in the connecting segment, which abuts the afferent arteriole, leads to increased AA release, leading to increased production of EETs and vasodilatory PGs. These then diffuse to the adjacent afferent arteriole and dilate it.[529]

TUBULES

Both 20-HETE and EETs inhibit tubular sodium reabsorption.[300,498] Renal cortical interstitial infusion of the nonselective CYP450 inhibitor 17-ODYA increases papillary blood flow, renal interstitial hydrostatic pressure, and sodium excretion without affecting total RBF or GFR. High dietary salt intake in rats increases expression of the renal epoxygenase 2C23 and production and urinary excretion of EETs while decreasing 20-HETE production in the renal cortex.[497,510] 14,15-EET has also been shown to inhibit renin secretion;[530] however, clotrimazole, which is a relatively selective epoxygenase inhibitor, induced hypertension in rats fed a high-salt diet, suggesting a role in the regulation of blood pressure.[510]

Proximal Tubule

The proximal tubule contains the highest concentration of CYP450 in the mammalian kidney and expresses minimal COX and LOX activity.[497] The 4A CYP450 family of hydroxylases that produce 19- and 20-HETE is highly expressed in the mammalian proximal tubule.[314] CYP450 enzymes of both the 2C and 2J family, which catalyze the formation of EETs, are also expressed in the proximal tubule.[497] Both EETs and 20-HETE have been shown to be produced in the proximal tubule and have been proposed to be modulators of sodium reabsorption in the proximal tubule.

Studies in isolated perfused proximal tubule have indicated that 20-HETE inhibits sodium transport, whereas 19-HETE stimulates sodium transport, suggesting that 19-HETE may serve as a competitive antagonist of 20-HETE.[508,531] Administration of EETs inhibits amiloride-sensitive sodium transport in primary cultures of proximal tubule cells[532] and in LLC-PK1 cells, a nontransformed, immortalized cell line from pig kidney with proximal tubule characteristics.[533,534]

It has been proposed that 20-HETE can be a mediator of the hormonal inhibition of proximal tubule reabsorption by parathyroid hormone (PTH), dopamine, Ang II, and EGF. Although the mechanisms of 20-HETE's inhibition have not yet been completely elucidated, there is evidence that it can inhibit Na^+-K^+-ATPase activity by phosphorylation of the Na^+-K^+-ATPase alpha subunit through a protein kinase C–dependent pathway.[535,536]

EETs may also serve as second messengers in the proximal tubule for EGF.[537] The mechanisms whereby CYPP450 AA metabolites modulate proximal tubule reabsorption have not been completely elucidated; they may involve both luminal (NHE3) and basolateral (Na^+-K^+-ATPase) transporters.[532,535] CYP450 AA metabolites may modulate the proximal tubule component of the pressure-natriuresis response.[538] Of note, intrarenal dopamine, which originates from the proximal tubule, induces the production of EETs and, if EET production is inhibited genetically, dopamine-mediated diuresis and natriuresis are inhibited.[539]

THICK ASCENDING LIMB OF HENLE

In addition, 20-HETE serves as a second messenger to regulation transport in the TAL. It is produced in this nephron segment[504] and can inhibit net Na-K-Cl cotransport by direct inhibition of the transporter and by blocking the 70-pS apical K^+ channel.[540] In addition, 20-HETE has been implicated as a mediator of the inhibitory effects of Ang II[541] and bradykinin[542] on TALH transport.

COLLECTING DUCT

In the collecting duct, EETs and/or their diol metabolites serve as inhibitors of the hydroosmotic effects of vasopressin, as well as inhibitors of sodium transport in this segment.[509,543] Patch clamp studies have indicated that the eNaC sodium channel activity in the cortical collecting duct is inhibited by 11,12-EET.[544,545] Studies using mice with selective deletion of CYP2C44, the major kidney epoxygenase, have confirmed that EETs modulate eNaC activity and that EET production is mediated in part by activation of collecting duct epidermal growth factor receptors and ERK1/2 activation.[546–549] There is also an intriguing association of 20-HETE with circadian clock sodium regulation in the collecting duct.[550]

ROLE IN ACUTE AND CHRONIC KIDNEY DISEASE

EET-mediated increases in rat mesangial cell proliferation was the first direct evidence that CYP450 AA metabolites are cellular mitogens.[551] In cultured rabbit proximal tubule cells, CYP450 inhibitors blunted EGF-stimulated proliferation in proximal tubule cells.[537] In $LLCPKcl_4$, EETs were found to be potent mitogens, cytoprotective agents, and second messengers for EGF signaling. 14,15-EET–mediated signaling and mitogenesis are dependent on EGF receptor transactivation, which is mediated by the metalloproteinase-dependent release of heparin-binding (HB)-EGF.[552] In addition to the EETs, 20-HETE has been shown to increase thymidine incorporation in primary cultures of rat proximal tubule and LLC-PK1 cells[553] and vascular smooth muscle cells.[554] EETs are also pro-angiogenic factors.[555]

There is increasing evidence that the activation of EETs or the administration of EET analogues can protect against acute kidney injury.[556–558] Conversely, inhibition of 20-HETE is beneficial in AKI.[559]

EETs may also be protective in chronic models of renal injury, such as diabetic nephropathy, and in a 5/6 nephrectomy model.[560,561] Increasing EET levels by the inhibition of soluble epoxide hydrolase decreases the inflammation and fibrosis in a model of unilateral ureteral obstruction.[562] Similarly, an EET analogue was found to inhibit the development of radiation-induced renal fibrosis.[563]

ROLE IN HYPERTENSION

There is increasing evidence that the renal production of CYP450 AA metabolites is altered in a variety of models of hypertension and that blockade of the formation of compounds can alter blood pressure in several of these models. CYP450 AA metabolites may have both pro- and antihypertensive properties. At the level of the renal tubule, both 20-HETE and EETs inhibit sodium transport. However, in the vasculature, 20-HETE promotes vasoconstriction and hypertension, whereas EETs are endothelial-derived vasodilators that have antihypertensive properties. Rats fed a high-salt diet increase expression of the CYP450 epoxygenase 2C23[564] and develop hypertension if treated with a relatively selective epoxygenase inhibitor. Because EETs have antihypertensive properties, efforts are underway to develop selective inhibitors of soluble epoxide hydrolase (sEH), which converts active EETs to their inactive metabolites, DHETs, and thereby increase EET levels. Studies in rats have indicated that one such sEH inhibitor, 1-cyclohexyl-3-dodecylurea, lowers blood pressure and reduces glomerular and tubulointerstitial injury in an Ang II-mediated model of hypertension in rats.[565] Furthermore, genetic deletion of CYP2C44, the major kidney epoxygenase, leads to the development of salt-sensitive hypertension.[547]

In deoxycorticosterone acetate (DOCA)-salt hypertension, the administration of a CYP450 inhibitor prevented the development of hypertension.[519,566] Ang II stimulates the formation of 20-HETE in the renal circulation,[567] and 20-HETE synthesis inhibition attenuates Ang II-mediated renal vasoconstriction[521,568] and reduces Ang II-mediated hypertension.[566]

The CYP450 4A2 gene is regulated by salt and is overexpressed in spontaneously hypertensive rats (SHRs);[569] the production of both 20-HETEs and diHETEs is increased and the production of EETs is reduced.[314,570] CYP450 inhibitors or antisense oligonucleotides directed against CYP4A1 and 4A2 lowered blood pressure in SHRs.[514,571] Conversely, studies in humans have indicated that a variant of the human CYP4A11, with reduced 20-HETE synthase activity, is associated with hypertension.[572]

In Dahl salt-sensitive rats (Dahl S), pressure natriuresis in response to salt loading is shifted so that the kidney requires a higher perfusion pressure to excrete the same amount of sodium as normotensive salt-resistant (Dahl R) rats;[300,497,498] this is because, at least in part, of increased TALH reabsorption. The production of 20-HETE and expression of CYP4A protein are reduced in the outer medulla and TALH of Dahl S rats relative to Dahl R rats, which is consistent with the observed effect of 20-HETE to inhibit TALH transport. In addition, Dahl S rats do not increase EET production in response to salt loading.

Studies have indicated that Ang II acts on AT2 receptors on renal vascular endothelial cells to release EETs that may then counteract AT1-induced renal vasoconstriction and influence pressure natriuresis.[515,573,574] AT2 receptor knockout mice develop hypertension,[575] which is associated with blunted pressure natriuresis, reduced RBF and GFR, and defects in kidney 20-HETE production.[575] There is also evidence that the natriuretic effects of dopamine are mediated by EETs and 20-HETE.[539,576]

There has been recent interest in the role of soluble epoxide hydralase (sEH), which is the major enzyme

mediating the metabolism of EETs to the inactive dHETEs in the regulation of blood pressure. Ang II induces sEH in the vasculature, which may contribute to the hypertensive effects by increasing EET metabolism.[577] Progressively more selective sEH inhibitors are being developed and have been shown to be effective in reducing blood pressure in a number of experimental models of hypertension.[578]

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BOARD REVIEW QUESTIONS

1. Prostaglandins are produced from arachidonic acid by:
   a. Lipoxygenases
   b. Cytochrome P450
   c. Cyclooxygenases
   d. Soluble epoxide hydrolases
   e. All of the above
   Answer: c

2. Prostaglandins in the kidney can modulate which of the following functions (more than one answer possible)?
   a. Regulation of blood flow
   b. Regulation of renin secretion
   c. Regulation of vitamin D synthesis
   d. Regulation of collecting duct function
   e. Regulation of erythropoietin production
   Answer: a, b, e

3. Choose the Prostanoid Receptor(s) that are vasodilatory (more than one answer possible).
   a. EP1
   b. IP
   c. EP4
   d. TP
   e. EP2
   Answer: b, c, e

4. The lipoxygenase enzymes metabolize arachidonic acid to make all of the below except:
   a. HETEs
   b. Lipoxins
   c. EETs
   d. Leukotrienes
   Answer: c

14 Disorders of Sodium Balance

Itzchak N. Slotki | Karl Skorecki

CHAPTER OUTLINE

Sodium (Na^+) and water balance and their distribution among the various body compartments are essential for the maintenance of fluid homeostasis, particularly intravascular volume. Disturbances of either or both of these components have serious medical consequences, are relatively frequent and are among the most common conditions encountered in clinical practice. In fact, abnormalities of Na^+ and water balance are responsible for, or associated with, a wide spectrum of medical and surgical admissions or complications. The principal disorders of Na^+ balance are manifested clinically as hypovolemia or hypervolemia, whereas disruption in water balance can be diagnosed only in the laboratory as hyponatremia or hypernatremia. Although disorders of Na^+ and water balance are often interrelated, the latter are considered separately in Chapter 15. In this chapter, the physiologic and pathophysiologic features of Na^+ balance are discussed. Because Na^+ is restricted predominantly to the extracellular compartment, this chapter also addresses perturbations of extracellular fluid (ECF) volume homeostasis.

PHYSIOLOGY

Approximately 60% of adult body mass is composed of solute-containing fluids divided into extracellular and intracellular compartments. Because water flows freely across cell membranes in accordance with the prevailing osmotic forces on either side of the membrane, the solute/water ratios in the intracellular fluid (ICF) and ECF are almost equal. However, the solute compositions of the ICF and ECF are quite different, as shown in Fig. 14.1. The principal ECF cation is sodium; other cations are potassium (K^+), calcium, and magnesium. In contrast, potassium is the major ICF cation. The accompanying anions in the ECF are chloride, bicarbonate and plasma proteins (mainly albumin), whereas electroneutrality of the ICF is maintained by phosphate and the negative charges on organic molecules. The difference in cationic composition of the two compartments is maintained by a pump-leak mechanism consisting of sodium–potassium adenosine triphosphatase (Na^+-K^+-ATPase), which operates in concert with sodium and potassium conductance pathways in the cell membrane.

The free movement of water across the membrane ensures that the ECF and ICF osmolalities are the same. However, the intracellular volume is greater because the amount of potassium salts inside the cell is larger than that of sodium salts outside the cell. The movement of water is determined by the "effective osmolality," or tonicity, of each compartment, so that if tonicity of the ECF rises—for example, as a result of excess Na^+—water will move from the ICF to ECF to restore tonicity. On the other hand, addition of solute-free water leads to a proportionate decrease in both osmolality and tonicity of all body fluid compartments (see Chapters 10 and 15 for a detailed discussion). The restriction of Na^+ to the ECF compartment by the pump-leak mechanism, in combination with maintenance of the osmotic equilibrium between ECF and ICF, ensures that ECF volume is determined mainly by total body Na^+ content. Departures from this paradigm relate to the identification in recent years of subcutaneous Na^+ pools, which do not participate in movement of water in response to osmotic equilibrium, and therefore do not respond equivalently to ECF volume regulation as described below. Further departures from the classical paradigm that maintenance of osmotic balance in response to salt surfeit is mediated by increased water consumption to balance the salt load, were recently suggested by studies demonstrating that under the influence of glucocorticoid and mineralocorticoid hormone fluctuations, long-term increased sodium intake increased water retention rather than consumption in human subjects. Parallel studies in murine models attributed the water retention to increased medullary urea and urea transport.[1,2]

To maintain constancy of the ECF and ICF and thereby safeguard hemodynamic stability, even minute changes in

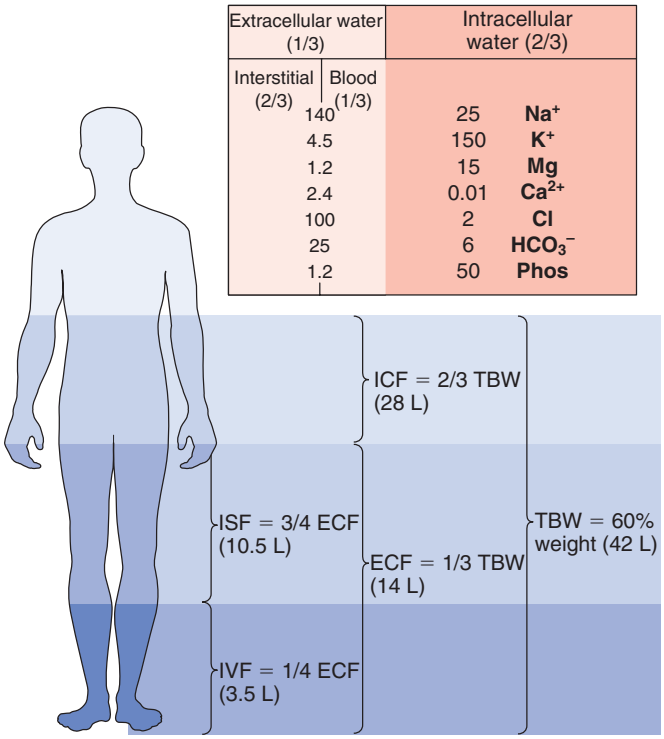

Fig. 14.1 Composition of body fluid compartments. This is a schematic representation of electrolyte composition *(upper panel)* and volumes *(lower panel)* of the body fluid compartments in humans. In the *upper panel*, electrolyte concentrations are in millimoles per liter; intracellular concentrations are typical values obtained from muscle. In the *lower panel*, *shaded areas* depict the approximate size of each compartment as a function of body weight. In a normally built individual, the total body water content is roughly 60% of body weight. Because adipose tissue has a low concentration of water, the relative water/total body weight ratio is lower in obese individuals. Relative volumes of each compartment are shown as fractions; approximate absolute volumes of the compartments (in liters) in a 70-kg adult are shown in parentheses. *ECF*, Extracellular fluid; *ICF*, intracellular fluid; *ISF*, interstitial fluid; *IVF*, intravascular fluid; *TBW*, total body water. (From Verbalis JG. Body water osmolality. In Wilkinson B, Jamison R, editors: *Textbook of nephrology,* London: Chapman & Hall; 1997: pp 89–94. Reproduced with permission of Hodder Arnold.)

these parameters can be detected by a number of sensing mechanisms. These sensory signals lead to activation of neural and hormonal factors, which, in turn, cause appropriate adjustments in urinary Na^+ and water excretion (Fig. 14.2). Constancy of ECF volume ensures a high degree of circulatory stability, whereas constancy of ICF volume protects against significant brain cell swelling or shrinkage.

SODIUM BALANCE

Na^+ balance is the difference between intake (diet or supplementary fluids) and output (renal, gastrointestinal, perspiratory, and respiratory). In healthy humans in steady state, dietary intake is closely matched by urinary output of Na^+. Thus, a person consuming a chronically low-Na^+ diet (20 mmol/day, or ≈1.2 g of salt/day) excretes, in the steady state, a similar quantity of Na^+ in the urine (minus extrarenal losses). Conversely, on a high-Na^+ diet (200 mmol/day, or 12 g of salt/day), approximately 200 mmol of Na^+ is excreted in the urine. Any perturbation of this balance leads to activation of the sensory and effector mechanisms outlined in the following discussions. In practice, any deviation in ECF volume in relation to its capacitance is sensed and translated, under the influence of neural and hormonal factors, into the appropriate change in Na^+ excretion, principally through the kidneys but also, to a much lesser degree, through stool and sweat.

According to the traditional two-compartmental model, body sodium balance and partitioning of extracellular fluid volume (ECFV) is based solely on exchangeable and osmotically active Na^+. For normal functioning of the afferent sensing and efferent effector mechanisms that regulate ECF volume, the integrity of the intravascular and extravascular subcompartments of the ECF is crucial[3] (see Fig. 14.2). Although the composition and concentration of small, noncolloid electrolyte solutes in these two subcompartments are approximately equal (slight differences are due to the Gibbs-Donnan effect), the concentration of colloid osmotic particles (mainly albumin and globulin) is higher in the intravascular compartment. The balance between transcapillary hydraulic and colloid osmotic (oncotic) gradients (Starling forces) favors the net transudation of fluid from the intravascular to interstitial compartment. However, this is countered by movement of lymphatic fluid from the interstitial to intravascular compartment via the thoracic duct. The net effect is to restore and maintain the intravascular subcompartment at 25% of the total ECF volume (corresponding to 3.5 L of plasma); the remaining 75% is contained in the interstitial space (equivalent to 10.5 L in a 70-kg man; see Fig. 14.1). The constancy of ECF volume and the appropriate partitioning of the fluid between intravascular and interstitial subcompartments are crucial for maintaining hemodynamic stability. In particular, intravascular volume in relation to overall vascular capacitance is a major determinant of left ventricular filling volume and, hence, cardiac output and mean arterial pressure (MAP).

Recently, the traditional two-compartment model of volume regulation (according to which the intravascular and interstitial spaces are in equilibrium) has been challenged. It now appears that Na^+ can be bound to and stored on proteoglycans in interstitial sites, where it becomes osmotically inactive; accordingly, a novel mechanism of volume regulation has been elucidated.[4–11] In rats fed a high-salt diet, this uniquely bound Na^+ induced a state of subcutaneous interstitial hypertonicity and systemic hypertension.[8] This hypertonicity is sensed by macrophages,[5] which then produce vascular endothelial growth factor C (VEGF-C), an angiogenic protein. In turn, VEGF-C stimulates increased numbers and density of lymphatic capillaries. In parallel, macrophages subjected to osmotic stress display activation of a transcription factor, tonicity-responsive enhancer–binding protein (TonEBP). This factor is known to activate osmoprotective genes in other hypertonic environments, such as the renal medulla.[12] Moreover, the VEGF-C promoter contains two TonEBP binding sites, which are upregulated in parallel with VEGF-C. The effect of TonEBP on VEGF-C was shown to be specific, inasmuch as small interfering RNA for TonEBP or deletion of the murine TonEBP gene, but not nonspecific small interfering RNA, inhibited the VEGF-C upregulation and increased blood pressure (BP). Furthermore, macrophage depletion or inhibition of VEGF-C signaling led to

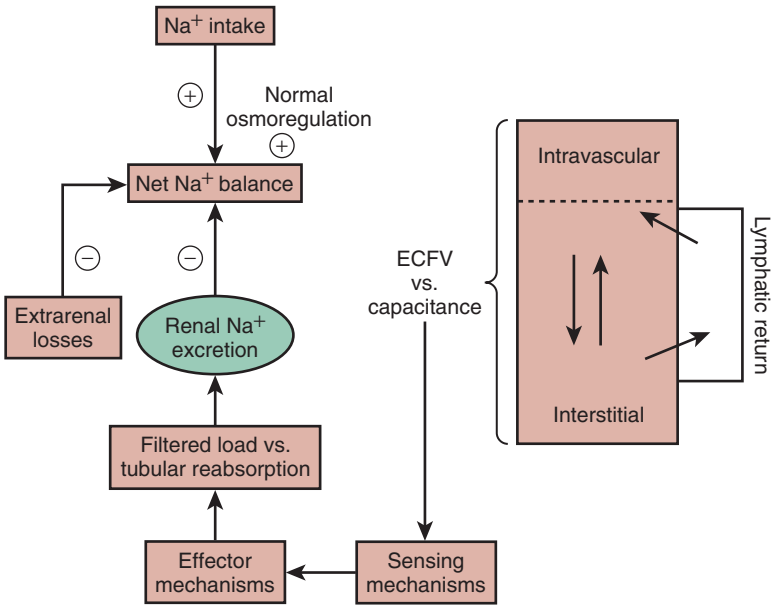

Fig. 14.2 Traditional two-compartment scheme for body sodium balance and partitioning of extracellular fluid volume (ECFV), based on exchangeable and osmotically active Na^+. In the setting of normal osmoregulation, extracellular Na^+ content is the primary determinant of ECFV. Overall Na^+ homeostasis depends on the balance between losses (extrarenal and renal) and intake. Renal Na^+ excretion is determined by the balance between filtered load and tubule reabsorption. This latter balance is modulated under the influence of effector mechanisms, which, in turn, are responsive to sensing mechanisms that monitor the relationship between ECFV and capacitance. In rats, a high-salt diet leads to interstitial hypertonic Na^+ accumulation in skin, resulting in increased density and hyperplasia of the lymphatic capillary network.

exacerbation of high-salt diet–induced hypertension.[5] Also, VEGFR-3, an antibody that blocks the lymph-endothelial VEGF-C receptor, selectively inhibited macrophage-driven increases in cutaneous lymphatic capillary density, led to skin chloride (Cl^-) accumulation and induced salt-sensitive hypertension. Mice overexpressing soluble VEGFR-3 in epidermal keratinocytes exhibited hypoplastic cutaneous lymph capillaries and increased Na^+, Cl^- and water retention in skin and salt-sensitive hypertension.[13] A high-salt diet also led to elevated skin osmolality above plasma levels. In addition, in humans with relatively resistant hypertension, elevated levels of VEGF-C were found,[5] and skin Na^+ concentration correlated with the presence of left ventricular hypertrophy in chronic kidney disease.[14] These data are consistent with a role for VEGF-C in the redistribution of excess volume to the intravascular space and exacerbation of hypertension.[15]

Interestingly, mice skin, but not muscle, arterioles isolated from animals fed a high-salt diet compared with those on a normal salt diet exhibited increased contractile sensitivity to concentrations of angiotensin II (Ang II) $\geq 10^{-10}$ M and to norepinephrine (NE) at 10^{-5} to 10^{-4} M. Finally, a unique study involving astronauts on a simulated Mars expedition, who received diets with fixed salt intake that varied between 6 and 12 g daily, each for 35 days, was recently reported (reviewed in Titze et al. [16]). At each level of salt in the diet, the astronauts reached overall equilibrium between intake and output, as measured in 24-hour urine collections, within the expected 6 days. In parallel, there were the expected early changes in body weight, ECF water and inverse relationship with the urine aldosterone level. However, changes in total body Na^+ only occurred after 7 days and BP reached a new steady state after 3 weeks. Moreover, on the 12-g salt diet, BP continued to rise over a further 4 weeks, with an initial rise and then subsequent fall in body weight and ECF water. During this period, urine aldosterone levels did not change, whereas total body Na^+ decreased back to original levels, despite the maintained high salt intake. From these data, it appears that intrinsic rhythms with a periodicity of 30 days or more exist for aldosterone and Na^+ retention, independent of salt intake. Taken together, all these results clearly demonstrate that the skin contains a hypertonic interstitial fluid compartment in which macrophages exert homeostatic and BP regulatory control by local organization of interstitial electrolyte clearance via TonEBP and VEGF-C/VEGFR-3–mediated modification of cutaneous lymphatic capillary function.[13] This compartment may be associated with increased vasoreactivity in precapillary arterioles, the major resistance vessel of rat skin, which could increase peripheral resistance and contribute, independently of the kidney, to higher BP in salt-sensitive individuals.[17,18]

Fig. 14.3 summarizes the novel three-compartment model of Na^+ balance. The reader is also referred to an excellent recent review of this fascinating subject.[16]

EFFECTIVE ARTERIAL BLOOD VOLUME

To understand the mechanisms regulating ECF volume, it is important to appreciate that what is sensed is the effective arterial blood volume (EABV), defined as that part of the ECF in the arterial blood system that effectively perfuses the tissues. In physiologic terms, what is sensed is the threat to arterial pressure induced by the EABV[19] that perfuses the arterial baroceptors in the carotid sinus and glomerular afferent arterioles. Any change in perfusion pressure (or stretch) at these sites evokes appropriate compensatory responses. EABV is usually correlated with actual ECF volume and is proportional to total body Na^+. This means that the regulation of Na^+ balance and the maintenance of EABV are

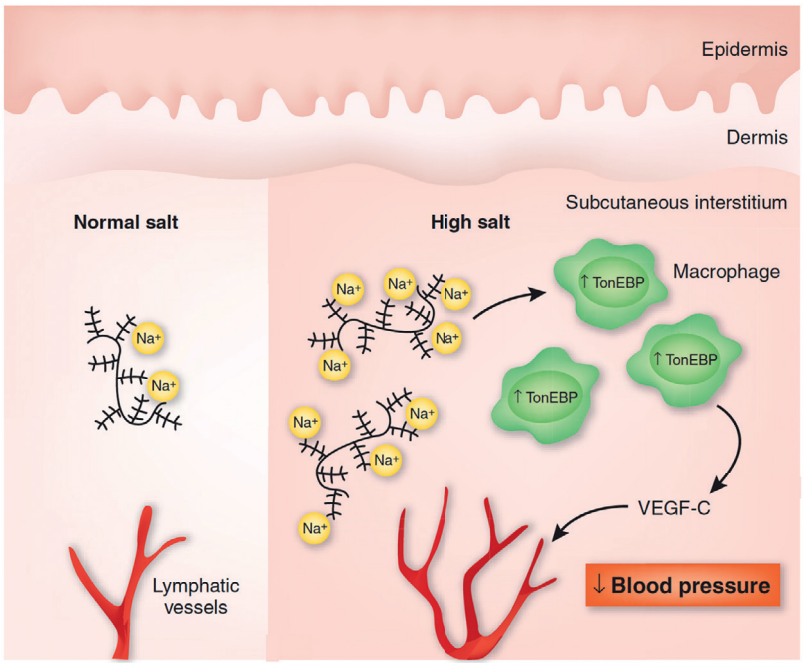

Fig. 14.3 New model of body electrolyte balance and blood pressure homeostasis. Increased subcutaneous Na^+ modulates lymphangiogenesis and blood pressure. After high salt intake, osmotically inactive Na^+ accumulates in the skin interstitium, binding proteoglycans. Macrophages accumulate in the subcutaneous compartment, and increased interstitial tonicity activates tonicity-responsive enhancer–binding protein (TonEBP). TonEBP transactivates the *VEGFC* gene and increases vascular endothelial growth factor C secretion by macrophages. This increases lymph capillary density and attenuates the blood pressure response to high salt. (Modified from Marvar PJ, Gordon FJ, Harrison DG. Blood pressure control: salt gets under your skin. *Nature Medicine*. 2009;15:487–488.)

closely related functions. Na^+ loading generally leads to EABV expansion, whereas loss leads to depletion. However, in some situations, EABV and actual blood volume are not well correlated (see Table 14.5). For example, in heart failure (HF), a primary decrease in cardiac output leads to lowered perfusion pressure of the baroceptors and reduced EABV is sensed. This leads to renal Na^+ retention and ECF volume expansion. The net result is increased plasma and total ECF volume, in association with increased EABV. The increase in plasma volume is partially appropriate in that intraventricular filling pressure rises leading to increasing myocardial stretching and improved ventricular contractility, thereby raising cardiac output and restoring systemic BP and baroceptor perfusion. However, this response is also maladaptive in that the elevated intraarterial pressure promotes fluid movement out of the intravascular space into the tissues, which leads to peripheral and pulmonary edema.

In HF, EABV is dependent on cardiac output; in other disease settings, however, these two parameters may be dissociated. For example, in the presence of an arteriovenous fistula cardiac output rises in proportion to the blood flow through the fistula. However, the flow through the fistula shunts blood away from the capillaries perfusing the tissues and, therefore, the EABV does not rise in conjunction with the rise in cardiac output. Similarly, a fall in systemic vascular resistance (SVR)—which, together with cardiac output, is a determinant of BP—leads to reductions in BP and EABV.

Another situation in which cardiac output and EABV change in opposite directions is advanced cirrhosis with ascites. ECF volume expands because of the ascites and plasma volume is increased because of fluid accumulation in the splanchnic circulation, in which the vessels are dilated but flow is sluggish. Although cardiac output may increase modestly because of arteriovenous shunting, marked peripheral vasodilation leads to a fall in SVR, with reductions in EABV and BP. In the presence of reduced EABV, renal perfusion is impaired; under the influence of hormones, such as renin, NE, and antidiuretic hormone (or arginine vasopressin [AVP])—released in response to the perceived hypovolemia—further Na^+ and water retention ensue (see later section, "Efferent Limb: Effector Mechanisms for Maintaining Effective Arterial Blood Volume").

To summarize, EABV is an unmeasured index of tissue perfusion that usually, but not always, reflects actual arterial blood volume. Therefore, EABV can be viewed as a functional parameter of organ perfusion.

The diagnostic hallmark of reduced EABV is evidence of renal sodium retention, manifested as a urinary sodium (U_{Na}) less than 15 to 20 mmol/L. This relationship holds true with the following exceptions. If renal Na^+ wasting occurs because of diuretic therapy or intrinsic tubular disease or injury, then U_{Na} is relatively high, despite low EABV. Conversely, the presence of selective renal or glomerular ischemia (e.g., because of bilateral renal artery stenosis or acute glomerular injury) will be misinterpreted as poor renal perfusion and is associated with renal Na^+ retention (low U_{Na}).

REGULATION OF EFFECTIVE ARTERIAL BLOOD VOLUME

Regulation of EABV can be divided into two stages, afferent sensing and efferent effector mechanisms. A number of mechanisms for sensing low EABV exist, all of them primed to stimulate renal Na^+ retention.

AFFERENT LIMB: SENSING OF EFFECTIVE ARTERIAL BLOOD VOLUME

Volume sensors are strategically situated at critical points in the circulation (Table 14.1). Each sensor reflects a specific characteristic of overall circulatory function so that atrial and ventricular sensors sense cardiac filling, arterial sensors respond to cardiac output, and renal, central nervous system (CNS) and gastrointestinal (GI) tract sensors monitor perfusion of the kidneys, brain and gut, respectively. The common mechanism whereby volume is monitored is by physical alterations in the vessel wall, such as stretch or tension. The process of mechanosensing probably is dependent on afferent sensory nerve endings in the vessel wall and activation of endothelial cells. Signal transduction mechanisms in endothelial cells include stretch-activated ion channels, cytoskeleton-associated protein kinases, integrin-cytoskeletal interactions, cytoskeletal-nuclear interactions and generation of reactive oxygen species.[20,21] In addition, mechanical stretch and tension of blood vessel walls, as well as the frictional forces of the circulation or shear stress, can lead to alterations in gene expression, mediated by specific recognition sites in the upstream promoter elements of responsive genes.[22,23] These signals induce efferent effector mechanisms that lead to modifications in renal Na^+ excretion, appropriate to the volume status.

Sensors of Cardiac Filling

Atrial Sensors. The role of the atria in volume regulation in humans was elucidated in experiments involving head-out water immersion (HWI) and exposure to head-down tilt or nonhypotensive lower body negative pressure (LBNP). During HWI, the increased hydrostatic pressure of the water on the lower limbs leads to redistribution of the intravascular fluid from the peripheral to central circulation. The resulting increase in central blood volume causes a rise in cardiac output, which in turn produces a brisk increase in Na^+ and water excretion in an attempt to restore euvolemia.[24] In contrast, LBNP results in a redistribution of blood to the lower limbs, thereby reducing central venous and cardiac filling pressures without affecting arterial pressure, heart rate, or atrial diameter. The resulting retention of Na^+ and water occurs without any change in renal plasma flow (RPF).[25]

A second and, possibly, more dominant, mechanism of HWI-induced Na^+ and water dieresis involves the external hydrostatic pressure of the water reducing the hydrostatic pressure gradient across the capillary wall in the legs, leading to a net transfer of fluid from the interstitial to intravascular compartment. The resulting hemodilution causes a fall in the colloid osmotic pressure (COP).[26,27] The combination of hemodilution and central hypervolemia, through atrial stretch, induces neurohumoral changes that bring about the subsequent diuresis and natriuresis.

Neural Pathways. Two types of neural receptors in the atrium have been described, type A and type B. They are thought to be branching ends of small medullated fibers running in the vagus nerve. Only type B receptor activity is increased by atrial filling and stretch.[28] The signal is then thought to travel along cranial nerves IX and X to the hypothalamic and medullary centers, where a series of responses is initiated—inhibition of AVP release (left atrial signal),[29] a selective decrease in renal but not lumbar sympathetic nerve discharge,[30,31] and decreased tone in peripheral precapillary and postcapillary resistance vessels. Conversely, reduction in central venous pressure and atrial volume stimulates renal sympathetic nerve activity (RSNA).[32,33]

The effects just described occur in response to acute atrial stretch, whereas chronic atrial stretch leads to adaptation and downregulation of the neural responses.[33] Cardiac nerves appear to be essential only for the restoration of Na^+ balance in states of repletion, but not for the renal response to acute volume depletion.[34] For example, after human cardiac transplantation, a natural model of cardiac denervation, the expected suppression of the renin-angiotensin-aldosterone (RAAS) system in response to chronic volume expansion is not observed.[35]

Humoral Pathways. Even after cardiac denervation the natriuresis and diuresis induced by atrial distension is maintained, due to the presence of natriuretic peptides (NP) of cardiac origin.[36,37] The NP family is comprised of atrial NP (ANP), brain NP (BNP), C-type NP (CNP), *Dendroaspis* NP (DNP), and urodilatin. Although their structures are quite similar, each is encoded by different genes and has distinct, albeit overlapping, functions.[38–41] The actions of NPs and their interaction with other hormone systems are discussed in detail later (see section, "Efferent Limb: Effector Mechanisms for Maintaining Effective Arterial Blood Volume" and Chapter 11). This section is confined to a discussion of the afferent mechanisms of NP stimulation.

From studies in animals and humans, it is clear that acute increments in atrial stretch or pressure cause a brisk release of ANP. The process involves cleavage of the prohormone, located in preformed stores in atrial granules, to the mature 28-amino acid C-terminus peptide, in a sequence-specific manner, by corin, a transmembrane serine protease.[42] Release of the hormone appears to occur in two steps, the first a Ca^{2+}-sensitive K^+ channel-dependent release of ANP from myocytes into the intercellular space and then

Table 14.1 Mechanisms for Sensing Regional Changes in Effective Arterial Blood Volume

Sensors of Cardiac Filling
Atrial
Neural pathways
Humoral pathways
Ventricular
Pulmonary
Sensors of Cardiac Output
Carotid and aortic baroceptors
Sensors of Organ Perfusion
Renal sensors
CNS sensors
GI tract sensors
Hepatic receptors
Guanylin peptides

CNS, Central nervous system; *GI*, gastrointestinal.

a Ca^{2+}-independent translocation of the hormone into the atrial lumen.[43] The afferent mechanism for ANP release is activated by intravascular volume expansion, supine posture, HWI, saline administration, exercise, Ang II, tachycardia and ventricular dysfunction.[44,45] Conversely, volume depletion induced by Na^+ restriction, furosemide administration or LNBP-mediated reduction in central venous pressure causes a fall in plasma ANP concentration.

In contrast to the effects of acute changes in atrial pressure on ANP release, the role of this peptide in the long-term regulation of plasma volume appears to be minimal. For example, although incremental oral salt loading was associated with correspondingly higher baseline plasma ANP levels, only intravenous (not oral) salt loading led to increased ANP levels.[46] Moreover, in humans given intravenous or oral salt loads, no correlation could be found between changes in ANP levels and the degree of natriuresis.[47,48] The contrasting relationships among acute and chronic Na^+ loading, plasma ANP levels and natriuresis have been elegantly demonstrated in ANP gene knockout mice. These mice display a reduced natriuretic response to acute ECFV expansion in comparison with their wild type counterparts. However, no differences in cumulative Na^+ and water excretion were observed between the knockout and wild type mice after a high- or low-Na^+ diet for 1 week. The only difference between the two types of mice was a significant increase in MAP. Further experiments using disruptions of the genes for ANP or its receptor, guanylate cyclase A (GC-A), have shown the importance of this system in the maintenance of normal BP and in modulating cardiac hypertrophy.[49]

In contrast to ANP, the other members of the NP family appear not to be involved in the physiologic regulation of Na^+ excretion.[38,50]

Ventricular and Pulmonary Sensors. Volume sensors have been found in the ventricles, coronary arteries, main pulmonary artery and bifurcation,[51] and juxtapulmonary capillaries in the interstitium of the lungs,[52] but not in the intrapulmonary circulation.[53] These sensors apparently mediate reflex changes in heart rate and SVR through modulation of the sympathetic nervous system (SNS) and ANP. This also appears to be true for the coronary baroceptor reflex in anesthetized dogs, by which changes in coronary artery pressure lead to alterations in lumbar and renal sympathetic discharge and a coronary artery response much slower than that of the carotid and aortic baroceptors.[54] However, ventricular and pulmonary sensors, at least in dogs, may also detect changes in blood volume through increased left ventricular pressure, which causes a reflex inhibition of plasma renin activity (PRA).[55,56]

Sensors of Cardiac Output

The sensors described so far are situated in low-pressure sites, where they sense the fullness of the circulation and are probably more important for defending against excessive volume expansion and the consequent cardiac failure. The arterial high-pressure sensors, on the other hand, are geared more toward detecting low cardiac output or SVR, manifested as underfilling of the vascular tree (i.e., EABV depletion) threatening arterial pressure[19], and as signaling the kidneys to retain Na^+. These high-pressure sensors are found in the aortic arch, carotid sinus, and renal vessels.

Carotid and Aortic Baroceptors. The carotid baroceptor has a large content of elastic tissue in the tunica media, which makes the vessel wall highly distensible in response to changes in intraluminal pressure, thereby facilitating transmission of the stimulus intensity to sensory nerve terminals. A rise in MAP induces depolarization of these sensory endings, resulting in action potentials. Transient receptor potential vanilloid receptors may mediate this process.[57] Afferent signals from the baroceptors are integrated in the nucleus tractus solitarius (NTS) of the medulla oblongata,[58] which leads to reflex changes in systemic and renal sympathetic nerve activity and, to a lesser degree, release of AVP. Baroceptor reflex modulation in the NTS may be mediated by endocannabinoid CB(1) receptor activation, via the 5-hydroxytryptamine type 1A (5-HT1A) receptor, leading to endogenous anandamide release.[59,60,61] Conversely, hypovolemia-induced activation of NTS (A1) adenosine receptors may serve as a negative feedback regulator of sympathoinhibitory reflexes integrated in the NTS.[62] An important additional function of the carotid baroceptors is maintenance of adequate cerebral perfusion. The aortic baroceptor appears to behave in a way similar to the carotid baroceptor. Finally, there is evidence in dogs for an interaction between pulmonary arterial and carotid baroceptor reflexes.[63]

Sensors of Organ Perfusion

Renal Sensors. Not only is the kidney the major effector target responding to signals that indicate the need for adjustments in Na^+ excretion, but also has a central role in the afferent sensing of volume homeostasis by virtue of the local sympathetic innervation. However, despite considerable knowledge concerning the mechanisms of renal sensing of EABV, the molecular identity and exact cellular location of the renal sensor(s) remains elusive.[61] The integral relationship between afferent and efferent RSNA and the central arterial baroceptors was highlighted by Kopp and colleagues.[64] They showed that a high-Na^+ diet increases afferent RSNA, which then decreases efferent RSNA and leads to natriuresis. Using dorsal rhizotomy to induce afferent renal denervation in rats maintained on a high-Na^+ diet, they demonstrated increased MAP that was dependent on impaired arterial baroreflex suppression of efferent RSNA. Animals fed a normal-Na^+ diet displayed no changes in arterial baroceptor function. Kopp and coworkers concluded that arterial baroreflex function contributes to increased efferent RSNA, which, in the absence of intact afferent RSNA, would eventually lead to Na^+ retention and hypertension. The role of RSNA in Na^+ regulation is discussed further later in this chapter (see section, "Neural Mechanisms: Renal Nerves and Sympathetic Nervous System").

An additional level of renal sensing depends on the close anatomic proximity of the sensor and effector limbs to one another: Volume changes may be sensed through alterations in glomerular hemodynamics and renal interstitial pressure. These alterations result simultaneously in adjustments in the physical forces governing tubular Na^+ handling (see section, "Efferent Limb: Effector Mechanisms for Maintaining Effective Arterial Blood Volume").

The kidneys have the ability to maintain a constant blood flow and glomerular filtration rate (GFR) at varying arterial pressures. This phenomenon, termed "autoregulation," operates over a wide range of renal perfusion pressures (RPPs).

Autoregulation of renal blood flow (RBF) occurs through three mechanisms—the myogenic response, tubuloglomerular feedback (TGF) and a third mechanism. In the myogenic response, changes in RPP are sensed by smooth muscle elements that serve as baroreceptors in the afferent glomerular arteriole and dynamically respond by adjusting transmural pressure and tension across the arteriolar wall.[65] An example of this can be seen in Ang II–infused rats on a high salt intake. These animals show reduced dynamic autoregulation of RBF, an effect mediated, at least in part, by superoxide[66] and attenuated by endothelial nitric oxide synthase (eNOS)–dependent production of nitric oxide (NO).[67]

The second mechanism, TGF, is operated by the juxtaglomerular apparatus (JGA), comprised of the afferent arteriole and, to a lesser extent, the cells of the macula densa in the early distal tubule.[65,68] The JGA is also important because of its involvement in the synthesis and release of renin,[68] which is controlled by three pathways, all driven by EABV status. First, renin release is inversely related to RPP and directly related to intrarenal tissue pressure. When RPP falls below the autoregulatory range, renin release is further enhanced. Second, renin secretion is influenced by solute delivery to the macula densa. Increased NaCl delivery past the macula densa leads to inhibition of renin release, whereas a decrease has the opposite effect. Sensing at the macula densa is mediated by NaCl entry through the Na^+-K^+-$2Cl^-$ cotransporter (NKCC2),[69,70] which leads to increased intracellular Ca^{2+}, production of prostaglandin E_2 (PGE_2),[71] adenosine,[72] and, subsequently, renin release. Third, changes in RSNA influence renin release. Renal nerve stimulation increases renin release through direct activation of β-adrenergic receptors on juxtaglomerular cells. This effect is independent of major changes in renal hemodynamics.[73] Sympathetic stimulation also affects intrarenal baroreceptor input, composition of the fluid delivered to the macula densa and renal actions of Ang II so that renal nerves may serve primarily to potentiate other regulatory signals.[73]

The nature of the third mechanism of RBF autoregulation is still unclear, but Seeliger and associates,[74] using a normotensive Ang II clamp in anesthetized rats, were able to abolish the resetting of autoregulation during incremental shaped RPP changes. Under control conditions, the initial TGF response was dilatory after total occlusions but constrictive after partial occlusions. The initial third mechanism response was a mirror image of TGF; it was constrictive after total occlusions but dilatory after partial occlusions. The angiotensin clamp suppressed TGF and converted the initial third mechanism response after total occlusions into dilation. Seeliger and coworkers concluded: 1. pressure-dependent renin angiotensin system (RAS) stimulation was a major factor behind hypotensive resetting of autoregulation; 2. TGF sensitivity depended strongly on pressure-dependent changes in RAS activity; 3. the third mechanism was modulated, but not mediated, by the RAS; and 4. the third mechanism acted as a counterbalance to TGF.[74] These findings may be related to feedback between the connecting tubule and glomerulus.[75] TGF is discussed later (see section, "Integration of Changes in Glomerular Filtration Rate and Tubular Reabsorption") and in detail in Chapter 3.

Central Nervous System Sensors. Certain areas in the CNS appear to act as sensors to detect alterations in body salt balance, at least in rats. Thus, intracerebral injection of hypertonic saline led to reduced RSNA and natriuresis,[76,77] and administration of Ang II into the cerebral ventricles and changes in dietary Na^+ modulated baroreflex regulation of RSNA. Stimulation of neurons in the paraventricular nucleus and in a region extending to the anteroventral third ventricle led to ANP release, inducing Ang II blockade and inhibition of salt and water intake. Conversely, disruption of these neurons, as well as of the median eminence or neural lobe, led to decreased ANP release and impaired response to volume expansion.[78] However, the exact nature, mode of operation, and relative importance of this aspect of sensing remains unclear.

Gastrointestinal Tract Sensors. Under normal physiologic conditions, Na^+ and water reach the ECF by absorption in the GI tract. Therefore, it is not surprising that sensing and regulatory mechanisms of ECF volume have been found in the GI tract itself. The evidence for this phenomenon comes from experiments that showed more rapid natriuresis after an oral salt load than after a similar intravenous load. Moreover, infusions of hypertonic saline into the portal vein led to greater natriuresis than similar infusions into the femoral vein. These findings were consistent with the presence of Na^+-sensing mechanisms in the splanchnic or portal circulation, or both[79] and are probably important in the pathogenesis of the hepatorenal syndrome (HRS; see later).

Hepatoportal Receptors. The two main neural reflexes, termed the "hepatorenal" and "hepatointestinal reflexes," originate from receptors in the hepatoportal region. They transduce portal plasma Na^+ concentration into hepatic afferent nerve activity; before a measurable increase in systemic Na^+ concentration occurs, the hepatointestinal reflex attenuates intestinal Na^+ absorption via the vagus nerve and the hepatorenal reflex augments Na^+ excretion both in humans and experimental animals.[80–82] These reflexes are impaired in the chronic bile duct ligation model of cirrhosis and portal hypertension,[83] leading to Na^+ retention, mediated in part by the A_1 adenosine receptor (A1AR)[84,85] and, possibly, also the NKCC2 cotransporter.[86] In addition, the hepatic artery shows significant autoregulatory capacity, dilating when perfusion pressure falls and constricting when pressure rises, thereby maintaining hepatic arterial blood flow over a wide range of perfusion pressures. This indicates the presence of a sensor in the hepatic artery, which responds to changes in the contribution of the portal vein to total hepatic blood flow.[87]

In addition to hepatoportal Na^+-sensing chemoreceptors, the liver also contains mechanoreceptors. Increased intrahepatic hydrostatic pressure, as seen in the Budd-Chiari syndrome,[88] is associated with enhanced RSNA and renal Na^+ retention in various experimental models,[89,90] so that hepatic volume-sensing mechanisms probably play a role in renal Na^+ retention (see section, "Specific Treatments Based on the Pathophysiology of Sodium Retention in Cirrhosis").

Intestinal Natriuretic Hormones. As described previously, the natriuretic response to a Na^+ load in experimental animals is more rapid when the load is delivered orally than intravenously.[73] The different responses are observed without changes in plasma aldosterone,[91] suggesting that the gut produces one or more substances that signal the kidneys to

excrete excess Na^+. The two main candidate substances are guanylins (guanylin and uroguanylin)[92,93] and gastrin.[94]

Guanylins are small (15 to 16 amino acids), heat-stable peptides with intramolecular disulfide bridges and are found in mammals, birds, and fish.[93] Both guanylin and uroguanylin are synthesized as prepropeptides, primarily in the intestine. The former, produced mainly by the ileum through the proximal colon, circulates as proguanylin; the latter, expressed principally in the jejunum, circulates in its active form.[92] The two peptides differ in their sensitivity to proteases. Because of a tyrosine residue at the ninth amino acid, guanylin is sensitive to renal inactivation by protease digestion, whereas uroguanylin can be locally activated by the same proteases.[93] After an oral salt load, guanylin and uroguanylin released in the intestine lead to increased intestinal secretion of Cl^-, HCO_3^- and water and to inhibition of Na^+ absorption. In the kidneys, Na^+, K^+, and water excretion is increased, without any change in RBF or GFR and independently of RAAS, AVP, or ANPs.[93] Guanylin signal transduction occurs via binding to and activation of the receptor guanylate cyclase C (GC-C), in the intestinal brush border and increased cGMP, which inhibits Na^+/H^+ exchange and activates protein kinases G II and A. These, in turn, activate the cystic fibrosis transmembrane conductance regulator (CFTR), leading to Cl^- secretion, activation of the Cl^-/HCO_3^- exchanger, and HCO_3^- secretion.[95]

The best evidence for a link between the gut and kidneys comes from mice lacking the uroguanylin gene, which display an impaired natriuretic response to oral salt loading but not to intravenous NaCl infusion.[96] However, because plasma prouroguanylin levels do not rise but urinary uroguanylin levels do increase after a high-salt meal, locally released peptide by the kidneys may play a role in uroguanylin-associated natriuresis.[97,95] In the kidneys, both GC-C–dependent and GC-C–independent signaling pathways for guanylin peptides exist, inasmuch as knockout of GC-C in mice does not affect the high-salt diet–induced increase in uroguanylin.[93]

From experiments on cell lines and isolated tubules, it appears that uroguanylin acts to decrease Na^+ reabsorption in the proximal tubule and principal cells of the cortical collecting duct (CCD).[93] Crosstalk between guanylin peptides and ANPs may also occur in the proximal tubule.[98] In the principal cell, uroguanylin activation of a G protein–coupled receptor results in phospholipase A_2–dependent inhibition of the renal outer medullary potassium (ROMK) channel, which leads to depolarization and a reduced driving force for Na^+ reabsorption.[93] Guanylin may cause cell shrinkage in the inner medullary collecting duct (IMCD), suggestive of water secretion from this segment.[93] Together, the experimental data are highly suggestive of a role for uroguanylin as a natriuretic hormone, in response to NaCl absorbed via the GI tract.[92,93]

More recently, gastrin, secreted from the stomach and duodenum, has been proposed as a second candidate substance mediating natriuresis in response to an oral salt load. Gastrin appears to signal natiuresis via its receptor, cholecystokinin B receptor linked to a dopamine D1-like receptor.[94] However, despite all the evidence from animal studies, recent work has cast substantial doubt on the relevance of a GI renal signaling axis for Na^+ regulation in humans.[99]

A final point is that although multiple receptors are involved in the regulation of EABV, their functions appear to be redundant. In this regard, cardiac or renal denervation and chronic aldosterone administration in nonhuman primates do not significantly affect the maintenance of Na^+ balance.[100,101]

Table 14.2 Major Renal Effector Mechanisms for Regulating Effective Arterial Blood Volume

Glomerular Filtration Rate and Tubular Reabsorption
Tubuloglomerular feedback
Glomerulotubular balance
Peritubular capillary Starling forces
Luminal composition
Physical factors beyond proximal tubule
Medullary hemodynamics (pressure natriuresis)
Neural Mechanisms
Sympathetic nervous system
Renal nerves
Humoral Mechanisms
Renin-angiotensin-aldosterone system
Vasopressin
Prostaglandins
Natriuretic peptides
Endothelium-derived factors
Endothelins
Nitric oxide
Others (see text)

EFFERENT LIMB: EFFECTOR MECHANISMS FOR MAINTAINING EFFECTIVE ARTERIAL BLOOD VOLUME

The maintenance of Na^+ homeostasis is achieved by adjustments of renal Na^+ excretion according to the body's needs. The adjustments are made by integrated changes in GFR and tubular reabsorption, so that changes in one component lead to appropriate changes in the other to maintain Na^+ homeostasis. In addition, tubular reabsorption is regulated by local peritubular and luminal factors as well as by neural and humoral mechanisms (Table 14.2).

INTEGRATION OF CHANGES IN GLOMERULAR FILTRATION RATE AND TUBULAR REABSORPTION

In humans, normal GFR leads to the delivery of approximately 24,000 mmol of Na^+/day to the tubules where more than 99% of the filtrate is reabsorbed. Therefore, even minute changes in the relationship between filtered load and fraction of Na^+ absorbed can profoundly influence net Na^+ balance. However, even marked perturbations in GFR are not necessarily associated with drastic alterations in U_{Na} excretion and overall Na^+ balance is usually preserved. Such preservation results from adjustments in two important protective mechanisms—TGF, in which changes in tubular fluid Na^+ inversely affect GFR, and glomerulotubular balance, whereby changes in tubular flow rate resulting from changes in GFR directly affect tubular reabsorption.[68,102,103]

Tubuloglomerular Feedback

A remarkable feature of nephron architecture is that after emerging from Bowman's capsule and descending deep into the medulla, each tubule returns to its parent glomerulus.

The functional counterpart of this anatomic relationship is TGF[104,105] (see also Chapter 3). TGF is constructed as a negative feedback loop in which an increase in NaCl concentration at the macula densa (the point of contact between the specialized tubular cells of the cortical thick ascending limb of Henle, cTALH, adjacent to the extraglomerular mesangium) leads to increases in afferent arteriolar resistance and a consequent fall in the GFR. This, in turn, leads to an increase in proximal reabsorption and a reduction in distal delivery of solute, whereby NaCl delivery to the distal nephron is maintained within narrow limits.[105]

The complexities of TGF were initially unraveled by micropuncture, imaging and electrophysiologic techniques in isolated perfused tubule/glomerulus preparations. Subsequently, the signaling mechanisms linking changes in tubular composition with altered glomerular arteriolar tone became evident through experiments in gene-manipulated mice.[105] The primary detection mechanism of TGF is uptake of salt by means of the NKCC2, located in the apical membrane of macula densa cells. The evidence comes from TGF inhibition by inhibitors of the cotransporter, furosemide and bumetanide,[106] and by deletions in mice of the A or B isoform of NKCC2, both of which are expressed in macula densa cells.[69,105] In fact, complete inactivation of the NKCC2 gene leads to the severe salt-losing phenotype of antenatal Bartter syndrome.[107] Similarly, inhibition or deletion of the ROMK channel in mice abolishes TGF.[105]

The next step in the juxtaglomerular cascade is less clear. One possibility is direct coupling of NKCC2-dependent NaCl uptake to the mediation step. Results of studies in the isolated perfused rabbit JGA have indicated that depolarization, alkalinization, and various ionic compositional changes occur after increased NaCl uptake; thus, one or more of these changes could trigger the signal.[108] A second possibility is that signal propagation is the consequence of transcellular NaCl transport and Na^+-K^+-ATPase–dependent basolateral extrusion. Experiments using double-knockout mice, in which the α_1-subunit of Na^+-K^+-ATPase was made sensitive and the α_2-subunit resistant to the pump inhibitor, ouabain, clearly indicate an important role for Na^+-K^+-ATPase in supporting TGF and that adenosine triphosphate (ATP) consumption is required for the process.[108]

In contrast, other studies have yielded strong evidence that ATP release and degradation, rather than consumption, may be the link connecting NaCl changes in the macula densa with alteration of glomerular arteriolar tone. According to the current working model, after NaCl uptake and transcellular transport, ATP is released from macula densa cells and undergoes stepwise hydrolysis and dephosphorylation by ecto-ATPases and nucleotidases to adenosine diphosphate, adenosine monophosphate and then adenosine. Adenosine, in a paracrine manner, then causes A1AR–dependent afferent arteriolar constriction. Although the evidence for ATP breakdown is as yet incomplete, evidence for adenosine as a mediator of TGF is very strong. For example, isolated perfused mouse afferent arterioles exposed to adenosine display vigorous vasoconstriction, an effect not seen in A1AR–deficient mice.[109,110] As shown by overexpression[111] and conditional knockout of the receptor, this A1AR effect is primarily on afferent arteriolar smooth muscle cells, although A1AR effects on extravascular, perhaps mesangial, cells appear to contribute to the TGF response.[112] The response is mediated by inhibitory G protein (G_i)–dependent activation of phospholipase C, release of Ca^{2+} from intracellular stores and subsequent entry of Ca^{2+} through L-type Ca^{2+} channels.[109,113] Cellular adenosine uptake is likely to be involved in the TGF response because targeted deletion of the type 1 equilibrative nucleoside transporter (ENT1) led to significant attenuation of the response.[114] Also, vasodilatory adenosine A_2 receptor is more abundant than the A1AR in the renal vasculature and continuous exogenous application of adenosine to mouse kidneys is indeed vasodilatory.[115] However, the generation of adenosine in the confines of the juxtaglomerular interstitium and its exclusive delivery to the afferent arteriole, where A1AR expression predominates, ensures the appropriate response for TGF.

Other factors, both coconstrictors and modulators, appear to be involved in TGF. Ang II is an important cofactor in the vasoconstrictive action of adenosine, since deletions of the Ang II receptor or angiotensin converting enzyme (ACE) in mice were found to abolish TGF. The effect may result from nonresponsiveness to adenosine in the absence of an intact RAS.[111] By contrast, aldosterone appears to blunt TGF through superoxide-mediated activation of mineralocorticoid receptors on macula densa cells.[116,117] In turn, superoxide may also be upregulated by Ang II via the NOX2 and NOX4 isoforms of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.[118] The high levels of neuronal NO synthase (nNOS) expression in macula densa cells are thought to counterbalance Ang II–induced efferent arteriolar vasoconstriction and to modulate renin secretion by the JGA.[119–121] In contrast, chronic absence of functional nNOS in macula densa cells is associated with enhanced vasoconstriction in the subnormal flow range, probably as a result of proportional increases in preglomerular and postglomerular tone. In addition, increased delivery of fluid to the macula densa induces NO release from these cells.[120]

Inhibition of the NO system by nonselective blockers of NOS results in an exaggerated TGF response that leads to even further renal vasoconstriction, Na^+ and water retention, and arterial hypertension.[121] Also, TGF responses are absent in mice with concurrent deficiencies in nNOS and the A1AR, which implies that nNOS deficiency does not overcome deficient A1AR signaling. Moreover, NO modulation of TGF can be mediated by ecto 5′-nucleotidase, the enzyme responsible for adenine formation.[122] Furthermore, NO, via eNOS, modulates the afferent arteriolar myogenic response.[67] Finally, aldosterone-induced modulation of TGF appears to involve interactions between NO and superoxide.[123] Together, these data suggest that A1AR signaling is primary and that nNOS and eNOS and superoxide, play modulatory roles in TGF.

Apart from the RAAS, other hormonal systems and secondary messengers appear to be involved in TGF. For example, stimulation of the glucagon-like peptide 1 receptor leads to an increased GFR and reduced proximal tubular reabsorption.[124] Moreover, high salt intake–induced activation of AMP-activated protein kinase leads to an enhanced TGF response and increased delivery of Na^+ to the end of the proximal tubule.[66] Furthermore, acute saline expansion leads to an increased single-nephron glomerular filtration rate (SNGFR) and distal nephron flow rate, independently of the Ang II receptor.[125]

The afferent arteriolar A1AR may not be the sole mediator of TGF. Activation by adenosine of the low-affinity adenosine

A_{2b} receptor,[115] via increased levels of eNOS,[126] has been shown to dilate mouse cortical efferent receptors. This highly specific effect occurs despite the presence of A1AR in the efferent arteriole. Apparently, therefore, the relative abundance of the various adenosine receptor subtypes in afferent and efferent arterioles ultimately allows fine-tuning of TGF by concerted changes in glomerular vascular tone.[127] On the other hand, purine receptors do not seem to be involved in TGF.[128]

Connexin 40, which plays a predominant role in the formation of gap junctions in the vasculature, also participates in the autoregulation of RBF by the afferent arteriole and, therefore, in TGF.[129] Connexin 40 knockout mice displayed impaired steady-state autoregulation to a sudden stepped increase in RPP, likely due to a marked reduction in TGF. Connexin 40–mediated RBF autoregulation occurred by paracrine signaling between tubular cells and afferent arteriolar vascular cells,[130] independently of NO.[131] Other endogenous modulators of TGF include the eicosanoid 20-hydroxyeicosatetraenoic acid (20-HETE), which modifies the myogenic afferent arteriolar and TGF responses;[132] also heme oxygenase, via carbon monoxide and cGMP generation, blocks TGF through inhibition of depolarization and Ca^{2+} entry into macular densa cells.[133,134]

Sex hormones also appear to regulate TGF. Testosterone in rats leads to upregulation of TGF by generation of superoxide dismutase,[135] whereas enhanced Ang II receptor activity attenuates Ang II–dependent resetting of TGF activity in female rats.[136] A final point in the complexity of TGF is that there is evidence for three sites in addition to the macula densa that are in contact with the efferent arteriole—the terminal cTALH, the early distal tubule and the connecting tubule. In particular, perimacular cells and oscillatory cells of the early distal tubule may be involved in the intracellular Ca^{2+} signaling required for adenosine-induced afferent vasoconstriction. On the other hand, the effect of the connecting tubule on the afferent arteriolar tone appears to be modulatory in that elevations in luminal NaCl and cellular Na^+ entry via the epithelial sodium channel (ENaC) lead to afferent arteriolar dilation[137] through the release of prostaglandins (PG) and epoxyeicosatrienoic acids.[138,139] Moreover, connecting tubule glomerular feedback has been shown to antagonize TGF,[140] at least in the acute setting.[140]

Glomerulotubular Balance

Several factors are involved in glomerulotubular balance (GTB), which describes the ability of proximal tubular reabsorption to adapt proportionally to changes in filtered load.

Peritubular Capillary Starling Forces. Following acute, but probably not chronic, changes in ECFV, alterations in hydraulic and oncotic pressures (Starling forces) in the peritubular capillary seem to play an important role in the regulation of Na^+ and water transport, especially in the proximal nephron. The peritubular capillary network is anatomically connected in series with the capillary bed of cortical glomeruli through the efferent arteriole; thus, changes in the physical determinants of GFR critically influence Starling forces in the peritubular capillaries.

Of importance is that about 10% of glomeruli, mainly those at the corticomedullary junction, are connected in series to the vasa recta of the medulla. In the proximal tubule—whose peritubular capillaries receive 90% of blood flow from glomeruli—the relationship of hydraulic and oncotic driving forces to the transcapillary fluid flux is given by the Starling equation, as follows:

$$Rate_{abs} = K_r[(\pi_c - \pi_i) - (P_c - P_i)]$$

where $Rate_{abs}$ is the absolute rate of reabsorption of proximal tubule reabsorbate by the peritubular capillary, K_r is the capillary reabsorption coefficient (the product of capillary hydraulic conductivity and absorptive surface area), π_c and P_c are the local capillary colloid osmotic (oncotic) and hydraulic pressures, respectively, and π_i and P_i are the corresponding interstitial pressures. Whereas π_i and P_c oppose fluid absorption, π_c and P_i tend to favor uptake of the reabsorbate. As a consequence of the anatomic relationship of the postglomerular efferent arteriole to the peritubular capillary, P_c is significantly lower in the peritubular than in the glomerular capillary. Also, because the peritubular capillary receives blood from the glomerulus, P_c is high at the outset as a result of prior filtration of protein-free fluid. It follows that the greater the GFR in relation to plasma flow rate, the higher the protein concentration in the efferent arteriolar plasma and the lower P_c in the proximal peritubular capillary. Consequently, proximal fluid reabsorption is enhanced (Fig. 14.4). Therefore, in contradistinction to the glomerular and peripheral capillary, the peritubular capillary is characterized by high values of $\pi_c - \pi_i$ that greatly exceed $P_c - P_i$, which results in net reabsorption of fluid. Changes in peritubular capillary Starling forces secondary to reduced RBF, may contribute to Na^+-retaining and edema-forming states, such as HF (see Fig. 14.4).

From studies using micropuncture, microperfusion, and the isolated perfused tubule model,[141–145] the role of peritubular forces in the setting of increased ECF volume can be summarized as follows:

1. Acute saline expansion results in dilution of plasma proteins and reduction in efferent arteriolar π_c. SNGFR and peritubular P_c may be increased as well, but the decrease in peritubular π_c by itself results in a decreased net peritubular capillary reabsorptive force and decreased $Rate_{abs}$. GTB is disrupted because $Rate_{abs}$ falls, despite the tendency for SNGFR to rise, thereby allowing the excess Na^+ to be excreted and plasma volume to be restored.
2. Iso-oncotic plasma infusions tend to raise SNGFR and peritubular P_c but lead to relative constancy of efferent arteriolar π_c. $Rate_{abs}$ may, therefore, decrease slightly, resulting in less disruption of GTB and less natriuresis than that observed with saline expansion.
3. Hyperoncotic expansion usually increases both SNGFR (because of volume expansion) and efferent arteriolar π_c. As a result, $Rate_{abs}$ is enhanced and GTB, therefore, tends to be better preserved than with iso-oncotic plasma or saline expansion.
4. Changes in π_i can directly alter proximal tubular reabsorption, independently of the peritubular capillary bed.

The alterations in proximal peritubular Starling forces that modulate fluid and solute movement across the peritubular basement membrane into the surrounding capillary bed appear to be accompanied by corresponding changes in

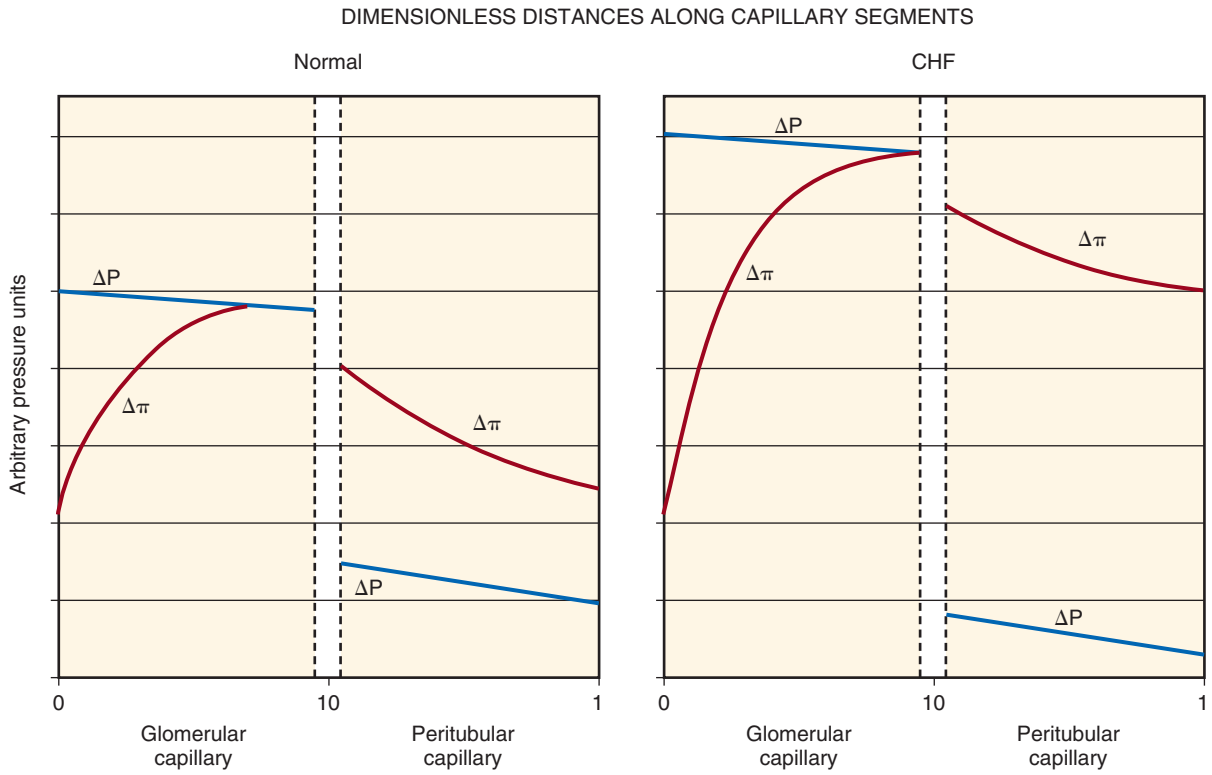

Fig. 14.4 The glomerular and peritubular microcirculations. *Left,* Approximate transcapillary pressure profiles for the glomerular and peritubular capillaries in normal humans. Vessel lengths are given in normalized nondimensional terms, with 0 being the most proximal portion of the capillary bed and 1 the most distal portion. Thus, for the glomerulus, 0 corresponds to the afferent arteriolar end of the capillary bed and 1 corresponds to the efferent arteriolar end. The transcapillary hydraulic pressure difference (ΔP) is relatively constant with distance along the glomerular capillary and the net driving force for ultrafiltration (ΔP – Δπ) diminishes primarily as a consequence of the increase in the opposing COP difference (Δπ), the latter resulting from the formation of an essentially protein-free ultrafiltrate. As a result of the drop in pressure along the efferent arteriole, the net driving pressure in the peritubular capillaries (ΔP – Δπ, in which Δπ is the change in transcapillary oncotic pressure) becomes negative, favoring reabsorption. *Right,* Hemodynamic alterations in the renal microcirculation in congestive heart failure (CHF). The fall in renal plasma flow (RPF) rate in heart failure is associated with a compensatory increase in ΔP for the glomerular capillary, which is conducive to a greater than normal rise in the plasma protein concentration and, hence, in Δπ along the glomerular capillary. This increase in Δπ by the distal end of the glomerular capillary also translates to an increase in Δπ in the peritubular capillaries, resulting in an increased net driving pressure for enhanced proximal tubule fluid absorption, believed to take place in heart failure. The increased peritubular capillary absorptive force in heart failure also probably results from the decline in ΔP, a presumed consequence of the rise in renal vascular resistance. (From Humes HD, Gottlieb M, Brenner BM. *The kidney in congestive heart failure: contemporary issues in nephrology*. Vol 1, New York: Churchill Livingstone 1978: pp 51–72.)

structure of the peritubular interstitial compartment. Ultrastructural data from rats have suggested that the peritubular capillary wall is in tight apposition to the tubule basement membrane for about 60% of the tubule basolateral surface. However, over the other 40%, irregularly shaped wide portions of peritubular interstitium intercede between the tubule and peritubular capillaries. Alterations in the physical properties of the interstitial compartment could conceivably modulate net fluid transport in the proximal tubule via changes in P_i. Also, Starling forces in the peritubular capillary are thought to regulate the rate of fluid entry from the peritubular interstitium into the capillary. Any change in this rate of flux could also lead to changes in Pi that secondarily modify proximal tubule solute transport. This formulation could explain why raising P_i (e.g., by infusion of renal vasodilators, renal venous constriction, renal lymph ligation) were associated with a natriuretic response, whereas the opposite effect was seen with renal decapsulation, which lowers P_i (see section, "Medullary Hemodynamics and Interstitial Pressure in the Control of Sodium Excretion: Pressure Natriuresis").

Because of the relatively high permeability of the proximal tubule, changes in interstitial Starling forces are likely to be transduced mainly through alterations in passive bidirectional paracellular flux through the tight junctions.[146] These changes might be mediated by the claudin family of adhesion molecules present in the tight junctions.[147–149] Among the 24 known mammalian claudin family members, at least two—claudin-2 and claudin-10—are located in the proximal nephron of the mouse.[148,150] In particular, claudin-2 is selectively expressed in the proximal nephron.[151,152] However, the exact role of claudins in the influence of Starling forces on fluid reabsorption remains to be elucidated.[152]

Luminal Composition. Even when the native peritubular environment is kept constant while the rate of perfusion of proximal tubular segments with native tubular fluid is changed, GTB can still be fully expressed.[153] A transtubular anion gradient, normally present in the late portion of the proximal nephron, is necessary for this flow dependence to occur.[154] Establishment of this anion gradient depends on

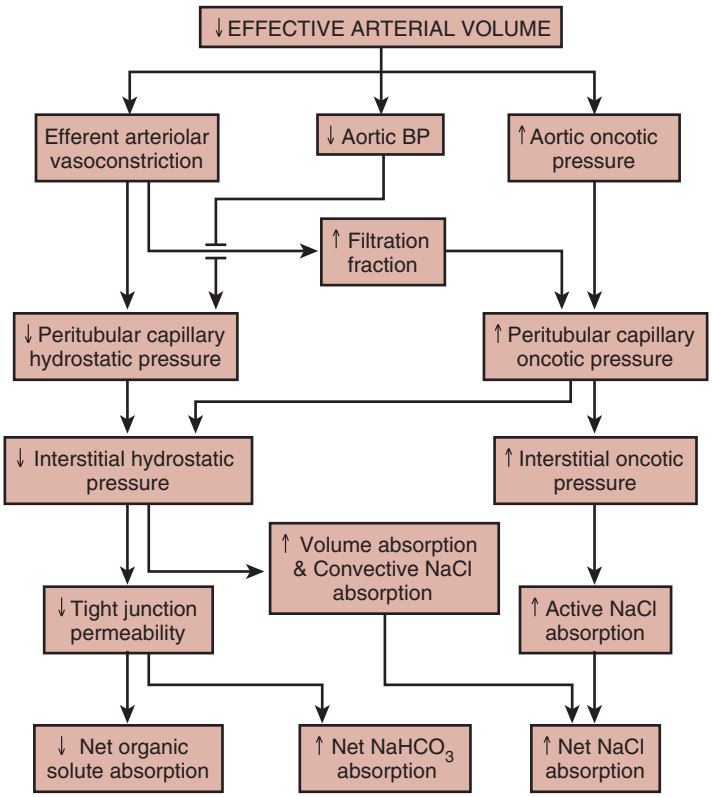

Fig. 14.5 Effects of hemodynamic changes on proximal tubule solute transport. *BP*, Blood pressure. (From Seldin DW, Preisig PA, Alpern RJ. Regulation of proximal reabsorption by effective arterial blood volume. *Semin Nephrol*. 1991;11:212–219.)

the close coupling of Na^+ transport with the cotransport of glucose, amino acids and other organic solutes. The increased delivery of organic solutes that accompanies increases in GFR, together with the preferential reabsorption of Na^+ with bicarbonate in the early proximal tubule, would lead to increased delivery of both Cl^- and organic solutes to the late proximal tubule. The resulting transtubular anion gradient would then facilitate the "passive" reabsorption of the organic solutes and NaCl in this segment, although overall net reabsorption would be reduced.

In summary, regardless of the exact mechanism, ECFV expansion impairs the integrity of GTB, allowing increased delivery of salt and fluid to more distal parts of the nephron. The major factors acting on the proximal nephron during a decrease in ECF and effective arterial circulating volume are outlined schematically in Fig. 14.5.

Physical Factors Beyond the Proximal Tubule. The final urinary excretion of Na^+, in response to volume expansion or depletion, can be dissociated from the amount delivered out of the superficial proximal nephron. This is achieved by appropriate modulation of Na^+ and water excretion in the loop of Henle, distal tubule, cortical and papillary collecting ducts. However, direct evidence that these transport processes are mediated by changes in Starling forces per se is lacking (see Jamison et al.[155] for a detailed review of experimental evidence).

In summary, if ECFV is held relatively constant, an increase in GFR leads to little or no increase in salt excretion because of a close coupling between GFR and intrarenal physical forces acting at the peritubular capillary to control $Rate_{abs}$. In addition, changes in the filtered load of small organic solutes and perhaps other, as yet uncharacterized, substances in tubular fluid may influence $Rate_{abs}$. Any changes in the Na^+ load delivered to more distal segments are accompanied by parallel changes in distal reabsorptive rates, thereby ensuring a high overall degree of GTB. Conversely, ECFV expansion leads to large increases in Na^+ excretion, even in the presence of a reduced GFR. Changes in Na^+ reabsorption in the proximal tubule partially account for this natriuresis, but suppression of more distal Na^+ reabsorption is also likely.

Medullary Hemodynamics and Interstitial Pressure in the Control of Sodium Excretion: Pressure Natriuresis. First proposed in the 1960s and elucidated in the 1970s and 1980s,[156,157] ECFV expansion (or elevation in systemic BP) was shown to elicit an increase in RPP that is transmitted as enhanced medullary plasma flow; this leads to a subsequent loss of medullary hypertonicity, elimination of the medullary osmotic gradient ("medullary washout"), thereby, decreasing water reabsorption in the thin descending loop of Henle. This decrease in water reabsorption lowers the Na^+ concentration in the fluid entering the ascending loop of Henle, thus decreasing the transepithelial driving force for salt transport in this nephron segment, particularly in juxtamedullary nephrons. The result is an increase in Na^+ and water excretion, thereby decreasing circulating blood volume and restoring arterial pressure.[158,159] The phenomenon, known as "pressure natriuresis,"[158–162] occurred in the absence of changes in total RBF, GFR, or filtered load of Na^+ and seems to be triggered by changes in medullary and papillary circulation.[156,161,163–165]

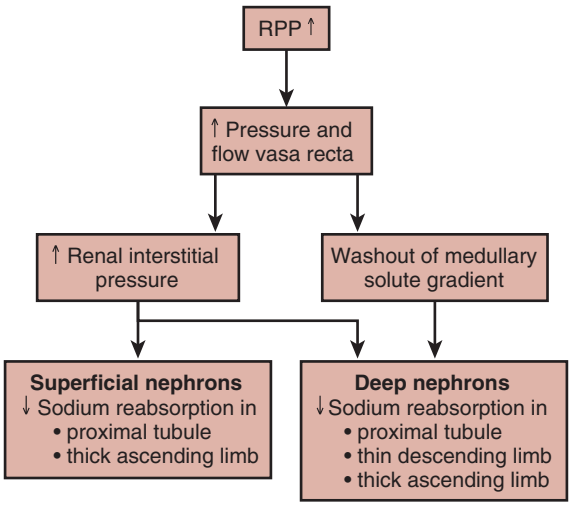

Fig. 14.6 Role of the renal medulla in modulating tubular reabsorption of sodium in response to changes in renal perfusion pressure (RPP). (Modified from Cowley AW, Jr. Role of the renal medulla in volume and arterial pressure regulation. *Am J Physiol*. 1997;273:R1–R15.)

An increase in medullary plasma flow leads not only to medullary washout and a consequent reduction in Na^+ reabsorption in the ascending loop of Henle but also to a rise in P_i. In fact, increasing P_i by ECFV expansion by infusion of renal vasodilatory agents, long-term mineralocorticoid escape, or hilar lymph ligation results in a significant increase in Na^+ excretion.[166,167] Prevention of the increase in P_i by removal of the renal capsule attenuates, but does not completely block, the natriuretic response to elevations in RPP. Thus as depicted in Fig. 14.6, elevation in RPP is associated with an increase in medullary plasma flow and increased vasa recta capillary pressure, which results in an increase in medullary P_i. This increase of P_i is thought to be transmitted to the renal cortex in the encapsulated kidneys and to provide a signal that inhibits Na^+ reabsorption along the nephron. In that regard, the renal medulla may be viewed as a sensor that detects changes in RPP and initiates pressure natriuresis.

To explain how changes in systemic BP are transmitted to the medulla in the presence of efficient RBF and GFR autoregulation, it has been suggested that shunt pathways connect preglomerular vessels of juxtamedullary nephrons directly to the postglomerular capillaries of the vasa recta.[156] Alternatively, autoregulation of RBF might lead to increased shear stress in the preglomerular vasculature, triggering the release of NO and perhaps cytochrome P450 products of arachidonic acid metabolism (see later), thereby driving the cascade of events that inhibit Na^+ reabsorption.[168,169] The mechanisms by which changes in P_i decrease tubular Na^+ reabsorption and increase U_{Na} excretion, as well as the nephron sites responding to the alterations in P_i, have not been fully clarified.[167] As noted earlier, elevations in P_i may increase passive backleak or paracellular pathway hydraulic conductivity, with a resultant increase in back flux of Na^+ through this pathway.[166] However, the absolute changes in P_i, in the range of 3 to 8 mm Hg in response to increments of about 50 to 90 mm Hg in RPP, are probably not sufficient to account for the decrease in Na^+ reabsorption, even in the proximal tubule, the nephron segment with the highest transepithelial hydraulic conductivity.[160] The decreased proximal tubular Na^+ reabsorption may also be explained by redistribution of the apical Na^+/H^+ exchanger from the brush border into intracellular compartments and concomitantly decreased basolateral Na^+-K^+-ATPase activity in response to increased RPP.[170] The reduction in proximal fluid reabsorption is especially marked in deep nephrons, leading to enhanced delivery to the loop of Henle, inhibition of Na^+ reabsorption in the thin descending limb and reduced blood flow in the vasa recta.[166] Pressure-induced changes in tubular reabsorption may also occur in the ascending loop of Henle, distal tubule, and collecting duct.[171]

The demonstration of pressure natriuresis in states of volume expansion and renal vasodilation and its significant attenuation in states of volume depletion[166] suggested that changes in P_i may be amplified by hemodynamic, hormonal and paracrine factors.[156,161,163,166] For example, RAAS inhibition potentiates whereas cyclooxygenase inhibitors attenuate the pressure natriuretic response.[166,172] Moreover, blockade of the Ang II type 2 receptor allows the same amount of Na^+ to be excreted at lower arterial pressure.[173] However, RAAS blockade does not completely eliminate pressure natriuresis, which indicates that the RAAS act as modulators and not as mediators of the phenomenon.

Pressure natriuresis is also modulated by endothelium-derived NO and P450 eicosanoids.[174–176] NO, generated in large amounts in the renal medulla, plays a critical role in the regulation of medullary blood flow and Na^+ excretion.[174–176] In this regard, inhibition of intrarenal NO production reduces Na^+ excretion and markedly suppresses pressure natriuresis,[177] whereas administration of an NO agonist normalizes the defect in the pressure natriuresis response in Dahl salt-sensitive rats.[178] Similarly, a positive correlation between urinary excretion of nitrites and nitrates (metabolites of NO) and changes in renal arterial pressure or U_{Na} excretion was observed both in dogs[174,179,180] and rats.[174] Hydrogen peroxide (H_2O_2) has also been invoked in the mediation of RPP-induced changes in outer medullary blood flow and natriuresis. The response appears to be localized to the medullary thick ascending limb of Henle (mTALH), in contrast to the NO effect, which occurs in the vasa recta.[174] Other factors involved in the regulation of medullary blood flow and pressure natriuresis include superoxide, heme oxygenase,[175,180] and the cytochrome P450 eicosanoid 20-HETE.[168,169,181,182]

One mechanism by which acute elevations in RPP in the autoregulatory range lead to increased endothelial release of NO and reactive oxygen species could be via increased blood flow velocity and shear stress. Enhanced renal production of these molecules may then increase U_{Na} excretion by acting directly on tubular Na^+ reabsorption or through a renal vasodilatory effect. ATP is another paracrine factor involved in pressure natriuresis by inhibiting salt and water reabsorption. ATP release appears to be mediated by mechanosensitive connexin 30 hemichannels.[183] The mechanisms of these cellular events have not been fully elucidated, but they may be related directly to changes in P_i or to changes in the intrarenal paracrine agents described previously.

A major assumption of the pressure natriuresis theory is that changes in systemic and RPP mediate the natriuretic response by the kidneys. As noted in comprehensive reviews, acute regulatory changes in renal salt excretion may occur

without measurable elevation in arterial BP.[48,184–186] In many of these studies, natriuresis was accompanied by a decrease in the activity of the RAAS, without changes in plasma ANP levels.[48,74,185,186] Thus, whereas increases in arterial BP can drive renal Na^+ excretion, other pressure-independent control mechanisms must also operate to mediate the natriuresis.[48]

Neural Mechanisms: Renal Nerves and Sympathetic Nervous System

Extensive sympathetic innervation, predominantly adrenergic, occurs at all segments of the renal vasculature and tubule.[187] Only the basolateral membrane separates the nerve endings from the tubular cells. The greatest innervation is found at the level of the afferent arterioles, followed by the efferent arterioles and outer medullary descending vasa recta.[188] However, high-density tubular innervation is found in the ascending limb of the loop of Henle, with the lowest density in the collecting duct, inner medullary vascular elements and papilla.[189,190] The magnitude of the tubular response to renal nerve activation may thus be proportional to the differential density of innervation.

In accordance with these anatomic observations, stimulation of the renal nerve results in vasoconstriction of afferent and efferent arterioles[190,191] mediated by activation of postjunctional α_1-adrenoreceptors.[192] With respect to tubular function, α_1-adrenergic receptors and most α_2-adrenergic receptors are localized in the basolateral membranes of the proximal tubule.[193] In the rat, β_1-adrenoreceptors have been found in the cTALH.[194] The predominant neurotransmitters in renal sympathetic nerves are noradrenaline and, to a lesser extent, dopamine and acetylcholine.[190] Consistent with their location, changes in RSNA play an important role in controlling body fluid homeostasis and BP.[73,187,191] Renal sympathetic nerve activity can influence renal function and Na^+ excretion through several mechanisms: 1. changes in renal and glomerular hemodynamics; 2. effect on renin release from juxtaglomerular cells, with increased formation of Ang II; and 3. direct effect on renal tubular fluid and electrolyte reabsorption.[73] Graded direct electrical stimulation of renal nerves produces frequency-dependent changes in RBF and GFR, reabsorption of renal tubular Na^+ and water and secretion of renin.[73,187] The lowest frequency (0.5–1.0 Hz) stimulates renin secretion and frequencies of 1.0 to 2.5 Hz increase renal tubule Na^+ and water reabsorption. Increasing the frequency of stimulation to 2.5 Hz and higher results in decreased RBF and GFR.[73,191]

The decrease in SNGFR in response to enhanced RSNA results from a combination of increases in afferent and efferent glomerular resistance, as well as decreases in glomerular capillary hydrostatic pressure (ΔP) and ultrafiltration coefficient (Kf).[187,191] In Munich-Wistar rats, renal nerve stimulation at different frequencies revealed that the effector loci for vasomotor control by renal nerves were in the afferent and efferent arteriole. In addition, although urine flow and Na^+ excretion declined with renal nerve stimulation, there was no change in absolute proximal fluid reabsorption rate, which suggests that reabsorption is increased in the more distal segments of the nephron.

The SNS also has a role in regulating the renal response to varying Na^+ loads. In response to isotonic saline volume expansion and furosemide-induced volume contraction, a low-Na^+ diet resulted in a reduction in right atrial pressure and an increase in RSNA. Conversely, a high-Na^+ diet resulted in increased right atrial pressure and a reduction in RSNA.[191] Other studies using HWI and left atrial balloon inflation have similarly yielded evidence of the importance of reflex regulation of RSNA.[73]

Collectively, these studies demonstrated the reciprocal relationship between ECFV and RSNA and the role of central cardiopulmonary mechanoreceptors governing RSNA. Moreover, efferent RSNA is of special significance during conditions of dietary Na^+ restriction, when the need for renal Na^+ conservation is maximal. Indeed, when the linkage between the renal SNS and excretory renal function is defective, abnormalities in the regulation of ECF volume and BP may develop.[187,195] Thus, in euvolemic animals, acute denervation of the kidneys is associated with increased urine flow and Na^+ excretion, but without alteration in any of the determinants of SNGFR. However, absolute proximal reabsorption was significantly reduced in the absence of changes in peritubular capillary oncotic pressure, hydraulic pressure and renal interstitial pressure. Na^+ and water reabsorption was also reduced in the loop of Henle and more distal segments.[191] In rats with HF, in contrast, denervation ameliorated both renal vasoconstriction, by decreasing afferent and efferent arteriolar resistance and natriuresis, by reduced Na^+ reabsorption.[191]

Human studies, using the renal NE spillover technique, have confirmed that a true increase of efferent RSNA and a fall in U_{Na} excretion occurs secondary to Na^+ restriction, with no change in cardiac NE uptake.[196] Similarly, low-dose NE in salt-replete volunteers resulted in antinatriuresis with a significant decline in lithium (Li^+) clearance, indicating enhanced proximal tubule reabsorption. The reduced Na^+ excretion occurred without change in GFR.[197]

The cellular mechanisms mediating the tubular actions of NE include stimulation of Na^+-K^+-ATPase activity and Na^+/H^+ exchange in proximal tubular epithelium.[191] This occurs via α_1-adrenoreceptor stimulation, mediated by phospholipase C, causing an increase in intracellular Ca^{2+} that activates the Ca^{2+} calmodulin-dependent calcineurin phosphatase. Calcineurin converts Na^+-K^+-ATPase from its inactive phosphorylated form to its active dephosphorylated form.[198] The stimulatory effect of renal nerves on Na^+/H^+ exchange is mediated through the α_2-adrenoreceptor.[191]

In addition to the direct action of Na^+ on epithelial cell transport and renal hemodynamics, interactions of renal nerve input with other effector mechanisms may contribute to the regulation of renal handling of Na^+. Efferent sympathetic nerve activity influences the rate of renal renin secretion both directly and via the macula densa and vascular baroreceptors.[191] The increase in renin secretion is mediated primarily by β_1-adrenergic receptors located on juxtaglomerular granular cells and is augmented during RPP reduction.[191] Intrarenal generation of Ang II facilitates NE release during renal nerve stimulation,[191] but the physiologic significance of this remains unclear.[199]

Sympathetic activity is also a stimulus for the production and release of renal PG, coupled to adrenergic-mediated renal vasoconstriction.[191] Renal vasodilatory PG attenuate the renal hemodynamic vasoconstrictive response to renal adrenergic activation.[191] The primary factor responsible for the reduction in glomerular Kf during renal nerve stimulation may be Ang II rather than NE and endogenously produced

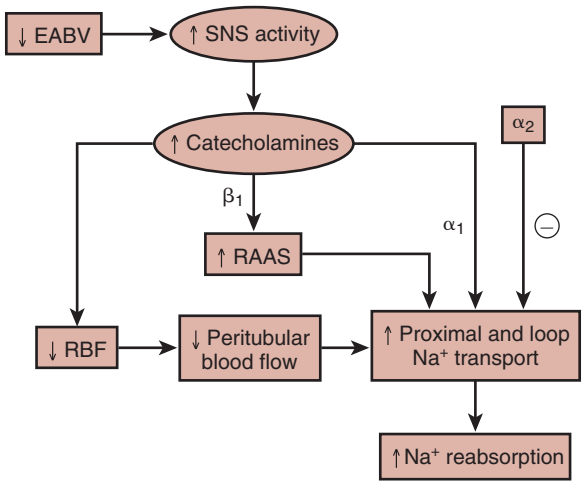

Fig. 14.7 Sympathetic nervous system (SNS)–mediated effects of decreased effective arterial blood volume (EABV) on the kidneys. α_1, α_2, β_1, α_1-, α_2-and β_1-Adrenergic receptors, respectively. *RAAS*, Renin-angiotensin-aldosterone system; *RBF*, renal blood flow; –, inhibitory effect.

PG neutralize the vasoconstrictive effects of renal nerve stimulation at the intraglomerular rather than arteriolar level.

The renal SNS also interacts with AVP, which exerts a dose-related effect on the arterial baroreflex. Low doses of AVP sensitize the central baroreflex neurons to afferent input, whereas higher doses cause direct excitation of these neurons, resulting in reduced renal sympathetic outflow.[191] This response depends on the number of afferent inputs from baroreceptors.[207] Conversely, renal nerve stimulation results in elevated plasma AVP levels and arterial pressure in conscious, baroreceptor-intact rats.[200] Many studies have demonstrated in normal and pathologic situations that increased RSNA can antagonize the natriuretic/diuretic response to ANP and that removal of the influence of sympathetic activity enhances the natriuretic action of the peptide.[191] Conversely, renal denervation in Wistar rats increased ANP receptors and cGMP generation in glomeruli, resulting in increased Kf after ANP infusion.[201]

In summary, renal sympathetic nerves regulate U_{Na} and water excretion by changing renal vascular resistance, by influencing renin release from the juxtaglomerular granular cell and through a direct effect on tubular epithelial cells (Fig. 14.7). These effects may be modulated by other hormonal systems, including ANP, PG, and AVP.

Humoral Mechanisms

Renin-Angiotensin-Aldosterone System. The RAAS plays a central role in the regulation of ECF volume, Na^+ homeostasis and cardiac function.[202] The system is activated in situations that compromise hemodynamic stability, such as blood loss, reduced EABV, low Na^+ intake, hypotension and increase in sympathetic nerve activity. The RAAS is comprised of a coordinated hormonal cascade whose synthesis is initiated by the release of renin from the JGA in response to reduced renal perfusion or decrease in arterial pressure.[203] Renin acts on its circulating substrate, angiotensinogen, which is produced and secreted mainly by the liver, but also by the kidneys.[202] ACE1, which cleaves Ang I to Ang II, exists in large amounts in the microvasculature of the lungs but also on endothelial cells of other vascular beds and cell membranes of the proximal nephron brush border, heart, and brain.[202] Ang II is the principal effector of the RAAS, although other smaller metabolic products of Ang II also have biologic activities.[204,205] Nonrenin (cathepsin G, plasminogen-activating factor, tonin) and non-ACE pathways (chymase, cathepsin G) also exist in these tissues and may contribute to tissue Ang II synthesis.[202]

In addition to its important function as a circulating hormone, Ang II produced locally acts as a paracrine growth-promoting agent in the cardiovascular system and kidneys.[202] For example, proximal tubular epithelial cells, which abundantly express the mRNA for angiotensinogen, synthesize and secrete Ang II into the lumen;[206] this leads to approximately 1000 times higher local concentrations in proximal tubular fluid, as well as higher levels in interstitial fluid and medulla than in the plasma.[207] Moreover, the mechanisms regulating intrarenal levels of Ang II appear to be dissociated from those controlling the systemic concentrations of the peptide.[206]

The biologic actions of Ang II are mediated through activation of AT_1 and AT_2 receptors, encoded by different genes residing on different chromosomes.[208,209] Both receptors are G protein–coupled, seven-transmembrane polypeptides containing approximately 360 amino acids.[202,210] The AT_1 receptor mediates most of the biologic activities of Ang II, whereas the AT_2 receptor appears to have a vasodilatory and antiproliferative effect.[204,211] AT_1 is expressed in the vascular poles of glomeruli, JGA and mesangial cells, whereas the quantitatively lower expression of AT_2 is confined to renal arteries and tubular structures.[209] In addition to their functional distinction, the two receptor types use different downstream pathways. Stimulation of the AT_1 receptor activates phospholipases A_2, C and D, resulting in increased cytosolic Ca^{2+} and inositol triphosphate and inhibition of adenylate cyclase. In contrast, activation of the AT_2 receptor results in increases in NO and bradykinin levels, which lead to elevation in cGMP concentrations and vasodilation.[208]

In addition to being an important source of several components of the RAAS, the kidney is a major target organ for Ang II and aldosterone. The direct effect of Ang II is mediated via AT_1 receptors to induce renal vasoconstriction, stimulation of tubular epithelial Na^+ reabsorption, augmentation of TGF sensitivity, modulation of pressure natriuresis, and stimulation of mitogenic pathways.[202] Moreover, circulating levels of Ang II in the picomolar range are highly effective in modulating renal hemodynamic and tubular function, in comparison with the 10- to 100-fold higher concentrations required for its extrarenal effects. Thus the kidneys appear to be uniquely sensitive to the actions of Ang II. Furthermore, the synergistic interactions between the renal vascular and tubular actions of Ang II significantly amplify the influence of Ang II on Na^+ excretion.[206] Ang II elicits a dose-dependent decrease in RBF but slightly augments GFR as a result of its preferential vasoconstrictive effect on the efferent arteriole, thereby increasing filtration fraction. In turn, this may further modulate peritubular Starling forces, possibly by decreasing interstitial hydraulic and increasing COP. These peritubular changes eventually lead to enhanced proximal Na^+ and water reabsorption. Of importance, however, is that changes in preglomerular resistance have also been described during

Ang II infusion or blockade.[212] These may be secondary to changes in systemic arterial pressure (myogenic reflex) or to increased sensitivity of TGF because Ang II does not alter preglomerular resistance when RPP is clamped or adjustments in TGF are prevented.[212]

In addition, Ang II may affect GFR by reducing Kf, thereby altering the filtered load of Na^+.[213] This effect is likely mediated by mesangial cell contractility and increasing permeability to macromolecules.[212] Finally, Ang II leads to reduced cortical and medullary blood flow and decreased Na^+ and water excretion.[212,214] As noted earlier, changes in medullary blood flow may affect medullary tonicity, which determines the magnitude of passive salt reabsorption in the loop of Henle and may also modulate pressure natriuresis through alterations in renal interstitial pressure.[214]

The other well-characterized renal effect of Ang II is a direct action on proximal tubular epithelial transport, independently of changes in renal or systemic hemodynamics.[202,215] Ang II exerts a dose-dependent biphasic effect on proximal Na^+ reabsorption. Peritubular capillary infusion with solutions containing low concentrations of Ang II (10^{-12} to 10^{-10} mol) stimulate, whereas higher concentrations ($>10^{-7}$ mol) inhibit proximal Na^+ reabsorption. Addition of ACE inhibitors (ACEIs) or angiotensin II receptor blockers (ARB) directly into the luminal fluid results in a significant decrease in proximal Na^+ reabsorption, indicative of tonic regulation of proximal tubule transport by endogenous Ang II.[216]

The specific mechanisms by which Ang II influences proximal tubule transport include increases in reabsorption of Na^+ and HCO_3^- by stimulation of the apical Na^+-H^+ antiporter, Na^+/H^+-exchanger isoform 3 (NHE3), basolateral Na^+-$3HCO_3^-$ symporter and Na^+-K^+-ATPase.[217,218] Thus, Ang II can affect NaCl absorption by two mechanisms:

1. Activation of NHE3 can directly increase NaCl absorption.
2. Increasing the rate of $NaHCO_3$ absorption can stimulate passive NaCl absorption by increasing the concentration gradient for passive Cl^- diffusion.[219]

Na^+ reabsorption is further promoted by the action of Ang II on NHE3 and Na^+-K^+-ATPase in the mTALH.[202]

In the early and late portions of the distal tubule, as well as the connecting tubule, Ang II regulates Na^+ and HCO_3^- reabsorption by stimulating NHE3 and the amiloride-sensitive Na^+ channel.[220–222] Two additional mechanisms may amplify the antinatriuretic effects of Ang II. The first concerns the increased sensitivity of TGF in the presence of Ang II and the second is related to the effect of Ang II on pressure natriuresis. The decrease in distal delivery produced by the action of Ang II on renal hemodynamics and proximal fluid reabsorption could elicit afferent arteriolar vasodilation by means of the TGF mechanism, which, in turn, could antagonize the Ang II–mediated increase in proximal reabsorption. This effect, however, is minimized because Ang II increases the responsiveness of the TGF mechanism, thus maintaining GFR at a lower delivery rate to the macula densa.[75] The second mechanism by which the antinatriuretic effects of Ang II may be amplified is blunting of the pressure natriuresis mechanism so that higher pressures are needed to induce a given amount of Na^+ excretion.[168,202] This shift to the right in the pressure natriuresis curve may be an important Na^+-conserving mechanism in situations of elevated arterial pressure.

As already stated, most of the known intrarenal effects of Ang II in the regulation of renal hemodynamics and proximal tubule reabsorption of Na^+ and HCO_3^- are mediated by the AT_1 receptor.[209] However, activation of the AT_2 receptor may play a counterregulatory protective role against the AT_1 receptor–mediated antinatriuretic and pressor actions of Ang II.[209]

Ang I is converted not only to Ang II but also to angiotensin-(1–7) (Ang 1–7)[223] either directly by the homolog of ACE, ACE2, or indirectly, via angiotensin-(1–9), followed by ACE-mediated conversion to Ang 1–7.[223] Ang 1–7, through its G protein–coupled receptor, Mas, may play a significant role as a regulator of cardiovascular and renal function by opposing the effects of Ang II, through vasodilation, diuresis, and an antihypertrophic action.[223] Thus, the RAAS can currently be envisioned as a dual-function system in which the vasoconstrictor/proliferative or vasodilator/antiproliferative actions are driven primarily by the ACE/ACE2 balance. According to this model, an increased ACE/ACE2 activity ratio leads to increased generation of Ang II and increased catabolism of Ang 1–7, which is conducive to vasoconstriction; conversely, a decreased ACE/ACE2 ratio reduces Ang II and increases Ang 1–7 levels, facilitating vasodilation. The additional effect of Ang 1–7/Mas to antagonize the actions of Ang II directly adds a further level of counterregulation.[223]

The final component of the RAAS, aldosterone, is produced via Ang II stimulation of the adrenal cortex and also plays an important physiologic role in the maintenance of ECFV and Na^+ homeostasis.[224] The primary sites of aldosterone action are the principal cells of the cortical collecting tubule and distal convoluted tubule, in which the hormone promotes the reabsorption of Na^+ and the secretion of K^+ and protons.[224,225] Aldosterone may also enhance electrogenic Na^+ transport, but not K^+ secretion, in the IMCD[226] and proximal tubule.[227] Aldosterone exerts its effects by increasing the number of open Na^+ and K^+ channels in the luminal membrane and the activity of Na^+-K^+-ATPase in the basolateral membrane.[228] The effect of aldosterone on Na^+ permeability appears to be the primary event because blockade of the ENaC with amiloride prevents the initial increase in Na^+ permeability and Na^+-K^+-ATPase activity.[228] This effect on Na^+ permeability is mediated by changes in intracellular Ca^{2+}, intracellular pH,[229] trafficking via protein kinase D1-phosphatidylinositol 4-kinaseIIIβ trans Golgi signaling,[230] and methylation of channel proteins, thus increasing the mean open probability of ENaC.[229] However, the long-term effect of aldosterone on Na^+-K^+-ATPase activity involves de novo protein synthesis, which is regulated at the transcriptional level by serum- and glucocorticoid-induced kinase-1.[229]

Aldosterone specifically regulates the α-subunit of ENaC and changes in expression of a variety of genes are important intermediates in this process. Microarray analysis in a mouse IMCD line showed that the most prominent transcript induced acutely by aldosterone was period homolog 1 (Per1), an important component of the circadian clock and that disruption of the Per1 gene led to attenuated expression of mRNA encoding for the α-subunit and increased U_{Na} excretion. mRNA encoded by the α-subunit was also expressed in an apparent circadian pattern that was dramatically altered in mice lacking functional Per1 genes.[231] These results imply

that the circadian clock has a role in the control of Na^+ balance and provide molecular insight into how the circadian cycle directly affects Na^+ homeostasis.

The Na^+-retaining effect of aldosterone in the collecting tubule induces an increase in the transepithelial potential difference, which is conducive to K^+ excretion. In terms of overall body fluid homeostasis, the actions of aldosterone in the defense of ECF result from the net loss of an osmotically active particle confined primarily to the intracellular compartment (K^+) and its replacement with a corresponding particle confined primarily to the ECF (Na^+). The effect of a given circulating level of aldosterone on overall Na^+ excretion depends on the volume of filtrate reaching the collecting duct and the composition of luminal and intracellular fluids. As noted earlier, this delivery of filtrate is, in turn, determined by other effector mechanisms (Ang II, sympathetic nerve activity and peritubular physical forces) acting at more proximal nephron sites.

Na^+ balance can be regulated over a wide range of intake, even in subjects without adrenal glands and despite fixed low or high supplemental doses of mineralocorticoids. Under these circumstances, other effector mechanisms predominate in controlling urinary Na^+ excretion, although often in a setting of altered ECF volume or K^+ concentration. In this regard, how renal Na^+ reabsorption and K^+ excretion are coordinately regulated by aldosterone has long been a puzzle. In states of EABV depletion, aldosterone release stimulated by Ang II induces maximal Na^+ reabsorption without significantly affecting plasma K^+ levels. Conversely, hyperkalemia-induced aldosterone secretion stimulates maximum K^+ excretion without major effects on renal Na^+ handling.

Elegant studies on the intracellular signaling pathways involved in renal Na^+ and K^+ transport have shed light on this puzzle. The key elements in this transport regulation are the Ste20/SPS1-related proline/alanine-rich kinase (SPAK), oxidative stress-related kinase (OSR1) the with-no-lysine kinases (WNKs) and their effectors, the thiazide-sensitive NaCl cotransporter and the K^+ secretory channel ROMK. According to the proposed model, when EABV is reduced or dietary salt intake is low, Ang II, mediated by the AT_1 receptor, leads to phosphorylation of WNK4, which stimulates phosphorylation of SPAK and OSR1. In turn, SPAK and OSR1 phosphorylate the NaCl cotransporter, inducing Na^+ transport and conservation. Simultaneous phosphorylation of the full-length isoform of WNK1, WNK1-L, causes endocytosis of the ROMK channel, thereby enabling K^+ conservation, despite high aldosterone levels. In contrast, in the presence of hyperkalemia or low dietary salt, Ang II levels are low so that WNK4 cannot be activated, SPAK, OSR1 and the NaCl cotransporter are not phosphorylated and NaCl cotransporter trafficking to the apical membrane is inhibited. At the same time, K^+-induced kidney-specific WNK1 leads to suppression of WNK1-L, which allows ROMK trafficking to the apical membrane and maximal K^+ secretion.[232] For further details, the reader is referred to Chapter 6.

For BP maintenance, systemic vasoconstriction—another major extrarenal action of Ang II—is the appropriate response to perceived ECF volume contraction. As mentioned previously, higher concentrations of Ang II are needed to elicit this response than those governing the antinatriuretic actions of Ang II. Transition from an antinatriuretic to a natriuretic action of Ang II at high infusion rates is almost entirely due to a concomitant BP rise.[233]

In addition to the adrenal glomerulosa, aldosterone, similar to Ang II, is also produced by the heart and vasculature, exerting powerful mitogenic and fibrogenic effects on blood vessels, independently of regulation of salt and water balance.[234] It directly increases the expression and production of transforming growth factor-β (TGF- β) and thus is involved in the development of glomerulosclerosis, hypertension and cardiac injury/hypertrophy.[202,224,235]

In summary, Ang II, the principal effector of the RAAS, regulates extracellular volume and renal Na^+ excretion through intrarenal and extrarenal mechanisms. The intrarenal hemodynamic and tubular actions of the peptide and its main extrarenal actions (systemic vasoconstriction and aldosterone release) act in concert to adjust U_{Na} excretion under a variety of circumstances associated with alterations in ECF volume. Many of these mechanisms are synergistic and tend to amplify the overall influence of the RAAS. However, additional counterregulatory mechanisms, induced directly or indirectly by Ang II, provide a buffer against the unopposed actions of the primary components of the RAAS.

Vasopressin. AVP is a nonapeptide hormone, synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and secreted from the posterior pituitary gland into the circulation in response to an increase in plasma osmolality (through osmoreceptor stimulation) or a decrease in EABV and BP (through baroreceptor stimulation).[236] AVP acts through at least three different G protein–coupled receptors. Two of these, V_{1A} and V_2, are abundantly expressed in the cardiovascular system and the kidneys; V_{1B} receptors are expressed on the surfaces of corticotropic cells of the anterior pituitary gland, in the pancreas and adrenal medulla. V_{1A} and V_2 receptors mediate the two main biologic actions of the hormone, vasoconstriction and increased water reabsorption by the kidneys, respectively. V_{1A} and V_{1B} receptors operate through the phosphoinositide signaling pathway, causing release of intracellular Ca^{2+}. The V_{1A} receptor, found in vascular smooth muscle cells (VSMC), hepatocytes and platelets, mediates vasoconstriction, glycogenolysis and platelet aggregation, respectively. The V_2 receptor, found mainly in renal collecting duct epithelial cells, is linked to the adenylate cyclase/cAMP pathway. A V_3 receptor is not involved in the regulation of ECFV and is not discussed further.[237]

Under physiologic conditions, AVP functions primarily to regulate body water content by adjusting reabsorption in the collecting duct according to plasma tonicity. A change in plasma tonicity by as little as 1% causes a parallel change in AVP release. AVP activates V_2 receptors in the basolateral membrane of the principal cells, leading to increased cytosolic cAMP, which stimulates the activity of protein kinase A. The latter triggers a series of phosphorylation events that promotes the translocation of AQP2 from intracellular stores to the apical membrane,[238] as well as the synthesis of AQP2 mRNA and protein;[239] this allows the reabsorption of water from lumen to cells. Water then exits the cell to the hypertonic interstitium via aquaporin-3 and aquaporin-4 channels at the basolateral membrane.[240] (See Chapter 10 for further details).

The second major effect of AVP on the collecting duct is to increase the permeability of the IMCD to urea through activation of the urea transporter UT-A1, enabling the accumulation of urea in the interstitium; there, along with Na^+, it contributes to the hypertonicity of the medullary interstitium, a prerequisite for maximum urine concentration and water reabsorption.[241] AVP also mediates an increase in Na^+ reabsorption from the mTAL, distal and connecting tubule and collecting duct, by activation of the segment-specific Na^+ transporters; this effect is important for maintenance of the axial corticomedullary osmotic gradient necessary for maximal water reabsorption (see reviews in Kortenoeven et al.[236] and Knepper et al.[242]).

In addition, AVP, via the V_{1A} receptor, reduces RBF, especially to the inner medulla, an effect modulated by local release of NO and PG.[243] At higher concentrations, AVP may also decrease total RBF and GFR as part of the generalized vasoconstrictor effect.[239,244] Experiments in V_{1A} receptor–deficient ($V_{1A}R^{-/-}$) mice have shown decreases in plasma volume, BP, GFR, U_{Na} excretion, AVP-dependent cAMP generation, levels of V_2 receptor and renal AQP2 expression with concomitantly increased urine volume compared with wild type mice; the data imply impaired urinary concentration in $V_{1A}R^{-/-}$ mice. Moreover, plasma renin and Ang II levels were decreased, as was renin expression in granule cells. In addition, the expression of renin stimulators such as nNOS and cyclooxygenase-2 (COX-2) in macula densa cells, where $V_{1A}R$ is specifically expressed, was decreased in $V_{1A}R^{-/-}$ mice. Thus, AVP regulates body fluid homeostasis and GFR through the $V_{1A}R$ in macula densa cells by activating the RAAS and subsequently the V_2 receptor–AQP2 system.[244,245]

In addition to its renal effects, AVP also regulates extrarenal vascular tone through the V_{1A} receptor. Stimulation of this receptor by AVP results in a potent arteriolar vasoconstriction in various vascular beds, with a significant increase in SVR.[242] However, physiologic increases in AVP do not usually cause a significant increase in BP because AVP also potentiates the sinoaortic baroreflexes that subsequently reduce heart rate and cardiac output.[246] Nevertheless, at supraphysiologic concentrations, such as those seen when EABV is severely compromised (e.g., in shock or HF), AVP plays an important role in supporting arterial pressure and maintaining adequate perfusion to vital organs such as the brain and myocardium. AVP also has a direct, V_1 receptor–mediated, inotropic effect in the isolated heart, but, in vivo, leads to decreased myocardial function; this effect is attributed to AVP-induced cardioinhibitory reflexes or coronary vasoconstriction.[247] Of more importance is that AVP, similar to Ang II and catecholamines, has been shown to stimulate cardiomyocyte hypertrophy and protein synthesis in neonatal rat cardiomyocytes and intact myocardium through a V_1-dependent mechanism.[247] This effect may contribute to the induction of cardiac hypertrophy and remodeling in HF.[247]

To summarize, regardless of the effects of AVP on Na^+ excretion, the predominant influence of the hormone is indirectly through water accumulation or vasoconstriction. In fact, the vasoconstrictive V_1 receptor effect of AVP overrides the osmotically driven effect in the presence of an ECF volume deficit of 20% or more (see Chapters 10 and 15). Nevertheless, the vasoconstrictive and water-retaining effects of AVP are modulated, respectively, by concomitant increases in baroreflex-mediated sympathoinhibition or PGE_2 and direct or PGE_2-mediated suppression of V_2 receptor activation.[248]

Prostaglandins. Prostaglandins (see also Chapter 16), or cyclooxygenase–derived prostanoids, possess diverse regulatory functions in the kidneys, including hemodynamic, renin secretion, growth response, tubular transport, and immune responses.[249,250] Two principal isoforms of cyclooxygenase, COX-1 and COX-2, catalyze the synthesis of prostaglandin H_2 (PGH_2) from arachidonic acid, released from membrane phospholipids. PGH_2 is then metabolized to the five major prostanoids—PGE_2, PGD_2, $PGF_2\alpha$, and thromboxane A_2 (TXA_2)—through specific synthases (see also Chapter 16).

Prostanoids are rapidly degraded, so their effect is localized strictly to their site of synthesis, which accounts for their predominant autocrine and paracrine modes of action. Each prostanoid has a location-specific cell surface G protein–coupled receptor that determines the function of the PG in the given cell type.[250] The major sites for PG production (and hence for local actions) are the renal arteries, arterioles, and glomeruli in the cortex and interstitial cells in the medulla, with additional contributions from epithelial cells of the cortical and medullary collecting tubules.[251,252] COX-1 is constitutively and abundantly expressed in the kidneys, especially in the collecting duct but also in medullary interstitial, mesangial, and arteriolar endothelial cells.[250] In contrast, COX-2 is inducible, cell type–specific, and prominently expressed in cells of the medullary interstitium, cTALH and macula densa, in which expression is regulated in response to salt intake.[250] PGE_2 and PGI_2 are the main products in the cortex of normal kidneys, whereas PGE_2 predominates in the medulla.[250] PGF_2 and TXA_2 are also produced in smaller amounts.[250] In addition, the metabolism of arachidonic acid by other pathways (e.g., lipoxygenase, epoxygenase) leads to products involved in crosstalk with COX.[249]

The two major roles for PG in volume homeostasis are modulation of 1. RBF and GFR and 2. tubular handling of salt and water. PGI_2 and PGE_2 have predominantly vasodilating and natriuretic activities, modulate the action of AVP and tend to stimulate renin secretion. TXA_2 causes vasoconstriction, although the importance of the physiologic effects of TXA_2 on the kidneys is still controversial. The end results of the stimulation of renal PG secretion in the kidneys are vasodilation, increased renal perfusion, natriuresis, and facilitation of water excretion.

The cellular targets of vasodilatory PG are the afferent and efferent arteriolar VSMC, glomerular mesangial cells peritubular capillaries, and vasa recta to modulate renal vascular resistance and glomerular function. Intrarenal infusions of PGE_2 and PGI_2 cause vasodilation and increased RBF.[251] In isolated renal microvessels, PGE_2 and PGE_1 attenuate Ang II–induced afferent and PGI_2 antagonizes Ang II–induced efferent arteriolar vasoconstriction.[253] Similarly, PGE_2 can counteract renal nerve stimulation[254] and Ang II–induced contraction of isolated glomeruli and cultured mesangial cells.[255] Furthermore, in volume-contracted states, COX-2 expression and PGE_2 release in the macula densa and cTALH dramatically increases in response to decreased luminal Cl^- delivery. In addition to its direct vasodilatory effect on afferent arterioles, PGE_2 leads to increased renin release from the macula densa.[250] The resulting rise in Ang II and

consequent efferent arteriolar constriction also ensures maintenance of GFR.

In volume-replete states, the renal vasoconstrictive influences of Ang II and NE are mitigated by their simultaneous stimulation of vasodilatory renal PG, so that RBF and GFR are maintained.[256] However, in the setting of heightened vasoconstrictor input from the RAAS, SNS and AVP, as in states of EABV depletion, the vasorelaxant action of PGE_2 and PGI_2 is overwhelmed, with the concomitant risk for the development of acute kidney injury.[250] Similarly, when this PG-mediated counterregulatory mechanism is suppressed by nonselective or COX-2–selective inhibitors, the unopposed actions of Ang II and NE can lead to a rapid deterioration in renal function.[257] Moreover, COX-2–derived prostanoids also promote natriuresis and stimulate renin secretion.[250] Therefore, during states of volume depletion, low Na^+ intake or the use of loop diuretics, COX-2 inhibitors and nonselective COX inhibitors can cause Na^+ and K^+ retention, edema, HF and hypertension.[252]

Besides modulating glomerular vasoreactivity, PGE_2-induced renal vasodilation affects U_{Na} excretion, by inducing medullary interstitial solute washout.[251] The natriuretic response to PGE_2 may also be attenuated by preventing an increase in renal interstitial hydraulic pressure, even in the presence of a persistent increase in RBF.[258] In addition, the natriuresis accompanying direct expansion of renal interstitial volume is significantly attenuated by inhibition of PG synthesis.[258] PGE_2 also affects U_{Na} excretion by direct effects on epithelial transport processes.[251] In the mTALH and collecting tubule, PGE_2 causes a decrease in the reabsorption of water, Na^+ and Cl^- which correlates with reduced Na^+-K^+-ATPase activity. In contrast, in the distal convoluted tubule, PGE_2 causes increased Na^+-K^+-ATPase activity.[259] The net effect of PG on tubular Na^+ handling is probably inhibitory because complete blockade of PG synthesis by indomethacin in rats receiving a normal or salt-loaded diet increases fractional Na^+ reabsorption and enhances medullary Na^+-K^+-ATPase activity.[260] In addition, PGE_2 inhibits AVP-stimulated NaCl reabsorption in the mTALH and AVP-stimulated water reabsorption in the collecting duct.[261,262] Both these effects tend to antagonize the overall hydroosmotic response to AVP. However, the cTALH, which can augment NaCl reabsorption in response to an increased delivered load, is not affected by PG; also, the effects of PG on solute transport in the collecting tubule remain unresolved. Therefore, the contribution of direct epithelial effects of PG to overall Na^+ excretion is unclear.[261]

Not only does renal COX expression influence salt handling, but also changes in Na^+ intake affect the renal expression of these enzymes. COX-2 expression in the macula densa and TALH is increased by a low-salt diet, RAAS inhibition and renal hypoperfusion. Conversely, a high-salt diet leads to decreased COX-2, but unchanged COX-1, expression in the renal cortex.[249,250] In the medulla, whereas a low-salt diet downregulated both COX-1 and COX-2, a high-salt diet enhanced their expression.[249,250] High osmolarity of the medium of cultured IMCD cells induced COX-2 expression whereas infusion of a selective COX-2 inhibitor into anesthetized dogs on a normal Na^+ diet reduced U_{Na} excretion and urine flow rate, without affecting renal hemodynamics or systemic BP.[252]

Collectively, the differential regulation of COX-2 in the renal cortex and medulla can be integrated into a physiologically relevant model in which upregulation of COX-2 in the cTALH and macula densa is induced in a volume-contracted or vasoconstrictor state. In the cTALH, the effect is by direct inhibition of Na^+ excretion, whereas in the macula densa, COX-2 stimulates renin release, which leads to Ang II–mediated Na^+ retention. In contrast, medullary COX-2 is induced by a high-salt diet, which leads to net Na^+ excretion.[250]

Finally, in addition to their hemodynamic and direct epithelial effects, PG may mediate the physiologic responses to other hormonal agents. The intermediacy of PG in renin release responses has already been cited. As another example, some, but not all, of the physiologic effects of bradykinin are mediated by PG production (e.g., inhibition of AVP-stimulated osmotic water permeability in the cortical collecting tubule).[261] In addition, the renal and systemic actions of Ang II appear to be differentially regulated by COX-1 and COX-2. For example, COX-2 deficiency in mice, induced by inhibitors or gene knockout, dramatically augmented the systemic pressor effect of Ang II, whereas COX-1 deficiency abolished this effect. Similarly, Ang II infusion reduced medullary blood flow in COX-2–deficient, but not COX-1–deficient animals, which suggests that COX-2–dependent vasodilators are synthesized in the renal medulla. Moreover, the diuretic and natriuretic effects of Ang II were absent in COX-2–deficient but remained in COX-1–deficient animals. Thus, COX-1 and COX-2 seem to exert opposite effects on systemic BP and renal function.[263]

Natriuretic Peptides. There are four NPs: ANP, BNP, CNP, and DNP.[264] Although encoded by different genes, their structures, gene regulation and degradation pathways are very similar, and they exert various actions on renal, cardiac, and vascular tissues.[265] ANP, a 28-amino acid peptide, plays an important role in BP and volume homeostasis through its natriuretic/diuretic and vasodilatory responses.[264,265] BNP has an amino acid sequence similar to that of ANP, with an extended NH_2-terminus. In humans, BNP is produced from pro–brain NP (proBNP), which contains 108 amino acids and, by proteolysis, releases a mature, 32–amino acid molecule and N-terminal fragment into the circulation. Although BNP was originally cloned from the brain, it is now considered a circulating hormone produced mainly in the cardiac ventricles.[266,267] CNP, produced mostly by endothelial cells, shares the ring structure common to all NP members; however, it lacks the C-terminal tail. DNP is released by the kidney and is a more effective activator of renal functions than ANP.[268]

The biologic effects of the natriuretic peptides (NPs) are mediated by binding to specific membrane receptors localized to numerous tissues, including the vasculature, renal arteries, glomerular mesangial and epithelial cells, collecting ducts, adrenal zona glomerulosa, and CNS.[264] At least three different, subtypes of NP receptors have been identified—NP-A, NP-B and NP-C. NP-A and NP-B, single-transmembrane proteins with molecular weights of approximately 120 to 140 kDa, mediate most of the biologic effects of NPs. Both are coupled to guanylate cyclase (GC) in their intracellular portions.[269] After binding to their receptors, all three NP isoforms markedly increase cGMP in target tissues and plasma. Therefore analogs of cGMP or inhibitors of its degradation mimic the vasorelaxant and renal effects of NPs. NP-C (molecular weight, 60 to 70 kDa), the most abundant NP receptor in many key

target organs, is believed to be a clearance receptor because it is not coupled to any known second-messenger system.[270]

Two additional routes for the removal of NPs should be noted. The first is the enzymatic degradation by neutral endopeptidase (NEP) 24.11, a metalloproteinase located mainly in the lungs and the kidneys.[270] Extensive research into this pathway culminated in the development of a specific NEP inhibitor, which leads to enhanced NP activity and improved HF outcomes (see section, "Specific Treatments Based on the Pathophysiology of Congestive Heart Failure"). The second route involves the negative regulation of ANP by microRNA-425 (miRNA-425). In this context, carriers of the rs5068 minor G allele of the gene encoding ANP, *NPPA*, have ANP levels 50% higher than those with two copies of the major A allele and have a 15% lower risk of hypertension. miRNA-425, expressed in human atria and ventricles, is predicted to bind the sequence spanning rs5068 in the 3′ untranslated region of the A, but not G, allele. Only the A allele was silenced by miRNA-425, whereas possession of the G allele conferred resistance to miR-425. The results raise the possibility that miR-425 antagonists could be used to treat disorders of salt overload, such as hypertension and HF.[271]

Atrial Natriuretic Peptide. Studies in humans and experimental animals have established the role of ANP in the regulation of ECFV and BP by acting on all organs and tissues involved in the homeostasis of Na^+ and BP (Table 14.3).[267] Therefore, it is not surprising that ANP and NH_2-terminal ANP levels are increased in: 1. conditions associated with enhanced atrial pressure; 2. systolic or diastolic cardiac dysfunction; 3. cardiac hypertrophy/remodeling; and 4. severe myocardial infarction.[38] In the kidneys, ANP exerts hemodynamic/glomerular effects that increase Na^+ and water delivery to the tubule and inhibits tubular Na^+ and water reabsorption, leading to significant natriuresis and diuresis.[267]

Table 14.3 Physiologic Actions of the Natriuretic Peptides

Target Organ	Biologic Effects
Kidney	Increased GFR
	Afferent arteriolar vasodilation
	Efferent arteriolar vasoconstriction
	Natriuresis
	Inhibition of Na^+/H^+ exchanger (proximal tubule)
	Inhibition of Na^+-Cl^- cotransporter (distal tubule)
	Inhibition of Na^+ channels (collecting duct)
	Diuresis
	Inhibition of AVP-induced AQP2 incorporation into CD-AM
Cardiac	Reduction in preload, leading to reduced cardiac output
	Inhibition of cardiac remodeling
Hemodynamic	Vasorelaxation
	Elevation of capillary hydraulic conductivity
	Decreased cardiac preload and afterload
Endocrine	Suppression of RAAS
	Suppression of sympathetic outflow
	Suppression of AVP
	Suppression of endothelin
Mitogenesis	Inhibition of mitogenesis in VSMC
	Inhibition of growth factor–mediated hypertrophy of cardiac fibroblasts

AQP2, Aquaporin 2; *AVP*, arginine vasopressin; *CD-AM*, collecting duct apical membrane; *GFR*, glomerular filtration rate; *RAAS*, renin-angiotensin-aldosterone system.

In addition, ANP relaxes vascular smooth muscle and leads to vasodilation by antagonizing the concomitant vasoconstrictive influences of Ang II, endothelin (ET), AVP, and α_1-adrenergic input.[267] This vasodilation reduces preload, resulting in a fall in cardiac output.[267] ANP also reduces cardiac output by shifting fluid from the intravascular to extravascular compartment, an effect mediated by increased capillary hydraulic conductivity.[272] Studies in endothelial-restricted, GC-A knockout mice have found that ANP, through GC-A, enhances albumin permeability in the microcirculation of the skin and skeletal muscle. This effect is mediated by caveolae[273] and is critically involved in the hypovolemic and hypotensive actions of ANP in vivo.[274]

ANP also inhibits proliferation of cultured mesangial, vascular smooth muscle and endothelial cells.[275] ANP causes afferent vasodilation, efferent vasoconstriction and mesangial relaxation, which leads to increased glomerular capillary pressure, GFR and filtration fraction.[267] In combination with increased medullary blood flow, these hemodynamic effects enhance diuresis and natriuresis, although the natriuretic effect of ANP infusion does not usually require these changes in glomerular function. In the tubules, ANP inhibits the stimulatory effect of Ang II on the luminal Na^+/H^+ exchanger of the proximal tubule, the thiazide-sensitive NaCl cotransporter in the distal tubule, ENaC and AVP-induced AQP2 incorporation into the apical membrane of the collecting duct (see Table 14.3).[267]

Brain Natriuretic Peptide. BNP is produced by activated satellite cells in ischemic skeletal muscle or by cardiomyocytes, mainly by the ventricles but also, in small amounts, by the atrium, in response to volume or pressure load, as seen in HF and hypertension. BNP, like ANP, induces natriuretic, endocrine, and hemodynamic responses.[267] BNP levels rise with age, more than doubling from age 55 to 64 years to 75 years and older. However, in HF and other chronic volume-expanded conditions much greater elevations occur in BNP levels.[276]

Studies in animals and humans have demonstrated the natriuretic effects of pharmacologic doses of BNP. The combination with ANP produced no further effect.[267] Moreover, like ANP, BNP exerts a hypotensive effect in animals and humans. For example, transgenic mice, overexpressing either the BNP or ANP gene exhibit lifelong hypotension.[267] Therefore, it is clear that BNP induces its biologic actions through mechanisms similar to those of ANP.[267]

This notion is supported by several findings: 1. both ANP and BNP act through the same receptors and induce similar renal, cardiovascular and endocrine actions in association with an increase in cGMP production (see Table 14.3); and 2. BNP suppresses ACTH-induced aldosterone generation both in cell culture and following infusion. The latter action may be attributed to inhibition of renin secretion in dogs, although apparently not in humans.[267] At high, but not low,

doses, BNP caused a profound fall in systolic BP in humans.[267] In the clinical setting, the effects of BNP are used routinely to monitor volume overload in HF (see section, "Specific Treatments Based on the Pathophysiology of Congestive Heart Failure").

C-Type Natriuretic Peptide. Although CNP is considered a neurotransmitter in the CNS, considerable amounts are produced by endothelial cells, where it plays a role in the local regulation of vascular tone.[270] Smaller amounts of CNP are produced by the kidneys, heart ventricles and intestines[270] and can be detected in human plasma.[277] CNP is produced in response to hypoxia, cytokines, shear stress, and fibrotic growth factors[278] and enhanced expression of CNP mRNA has been reported after volume overload.[270] Infusion of CNP decreases BP, cardiac output, urinary volume and Na^+ excretion; the effects are less pronounced than those of ANP and BNP, despite strong stimulation of cGMP and inhibition of vascular smooth muscle cell proliferation.

All three NPs inhibit the RAAS, although CNP does not induce significant changes in cardiac output, BP or plasma volume in sheep.[270] This finding supports the widely-accepted concept that ANP and BNP are the major circulating NPs, whereas CNP is a local regulator of vascular structure and tone.

D-Type Natriuretic Peptide. DNP infused into rabbits increased urine volume and electrolyte excretion. The effects were more pronounced than those of ANP, possibly because of DNP resistance to degradation by endogenous peptidases. DNP, via the NP-A receptor, preferentially induced cGMP production in glomeruli compared with cortical, outer medullary and inner medullary tubules. Thus, DNP may play a regulatory role in the kidney, via specific NP receptors with a GC domain.[268]

Together, the various biologic actions of NPs lead to reduction of EABV, in response to perceived overfilling of the central intrathoracic circulation. Furthermore, all NPs counteract the adverse effects of the RAAS, suggesting that the two systems act in opposite directions in the regulation of body fluid and cardiovascular homeostasis.

Endothelial-Derived Factors. The endothelium is a major source of factors that regulate vascular tone in health and disease.[300] These factors regulate the perfusion pressure of multiple organ systems involved in water and Na^+ balance, such as the kidneys, heart and vasculature. This section summarizes some concepts regarding actions of ET and NO relevant to volume homeostasis.

Endothelin. The ET system consists of three vasoactive peptides—endothelin 1 (ET-1), endothelin 2 (ET-2) and endothelin 3 (ET-3), which act in a paracrine and autocrine manner.[279] Endothelins are synthesized by proteolytic cleavage from specific preproendothelins that are further cleaved to form 37– to 39–amino acid precursors called "big ET". Big ET is then converted into the biologically active 21–amino acid peptide by a highly specific endothelin-converting enzyme (ECE), a phosphoramidon-sensitive, membrane-bound metalloprotease. There are two main isoforms of ECE, ECE-1 and ECE-2. ECE-1 itself has four isoforms.[279] ECE-2 is localized mainly to VSMC and is probably intracellular. Both chymase[280] and carboxypeptidase A[281] are also involved in mature ET production.

Endothelins bind to two distinct receptors, types A and B (ET-A and ET-B).[279] The ET-A receptor shows a higher affinity for ET-1 than for ET-2 or ET-3. The ET-B receptor shows equal affinity for all three ETs. ET-A receptors are found mainly on VSMC, on which their activation leads to vasoconstriction through an increase in cytosolic Ca^{2+}. ET-B receptors are also found on VSMC, where they can mediate vasoconstriction, but are found predominantly on vascular endothelium, where their activation results in vasodilation through prostacyclin and NO.[279] Endothelin is detectable in the plasma of humans and experimental animals and, therefore, can also act as a circulating vasoactive hormone.[279]

Selective ET-A receptor antagonism is associated with vasodilation and a reduction in BP, whereas selective ET-B antagonism is accompanied by vasoconstriction and a rise in BP.[279] These data suggest complementary roles for the ET receptor subtypes in the maintenance of vascular tone. In addition to its vasoconstrictive action, ET has a variety of renal effects.[282–284] The kidney (mainly the inner medulla) is both a source and target of ET. ET-1, the principal subtype involved in renal functional regulation, is synthesized by vascular endothelial cells, whereas ET-1 and ET-3 are produced by various renal cell types, but at a rate one to two orders of magnitude lower than ET-1.[279]

In relation to volume homeostasis, ET-1 acts in a paracrine or autocrine manner to regulate: 1. renal and intrarenal blood flow; 2. glomerular hemodynamics; and 3. tubular salt and water transport. Both ET-A and ET-B receptors are present in the glomerulus, renal vessels and tubular epithelial cells, but most ET-B receptors are found in the medulla.[285] The renal vasculature is exquisitely sensitive to the vasoconstrictor action of ET-1. Infusion of ET-1 into the renal artery of anesthetized rabbits was found to decrease RBF, GFR, natriuresis, and urine volume.[286] ET-1 increases afferent and efferent arteriolar resistance (afferent > efferent), which results in reduced glomerular plasma flow. In addition, Kf is reduced because of mesangial cell contraction, resulting in a diminished SNGFR.

The profound reduction of RBF and concomitant lesser reduction in GFR should result in a rise in filtration fraction, but this is not evident in all models. Infusion of ET-1 for 8 days into conscious dogs increased plasma levels of ET by two- to threefold and resulted in increased renal vascular resistance and decreased GFR and RBF.[287] Interestingly, the effect of ET on regional intrarenal blood flow is not homogeneous. As measured by laser Doppler flowmetry, administration of ET-1 to rats produced sustained cortical vasoconstriction but only transient medullary vasodilation.[288] These results are in line with the medullary predominance of ET-B receptors and the high density of ET-A–binding sites in the cortex.[279]

The effect of ET on Na^+ and water excretion varies, depending on the dose and source of ET. Systemic infusion of ET in high doses results in profound antinatriuresis and antidiuresis, apparently secondary to a decrease in GFR and RBF. However, in low doses, or when produced locally in tubular epithelial cells, ET decreases salt and water reabsorption, consistent with tubular ET-1 target sites.[289] Also, administration of big ET has been shown to cause natriuresis, supporting the notion of direct autocrine inhibition of ET on tubular salt reabsorption.[290]

The natriuretic and diuretic actions of big ET-1 are significantly reduced by ET-B–specific blockade.[291] Furthermore, ET-B knockout rats have salt-sensitive hypertension reversed by luminal ENaC blockade with amiloride, by a Ca^{2+}-dependent effect, which suggests that collecting duct ET-B tonically inhibits ENaC activity.[292] Similarly, mice with collecting duct–specific knockout of the ET-1 gene have impaired Na^+ excretion in response to Na^+ load and develop hypertension with a high salt intake.[279] These mice also have heightened sensitivity to AVP and reduced ability to excrete an acute water load. These findings are in line with observations that ET-B mediates the inhibitory effects of ET-1 on Na^+ and water transport in the collecting duct and TALH.[283] Thus if vascular and mesangial ET exert a greater physiologic effect than tubule-derived ET, then RBF is diminished and net fluid retention occurs. Whereas if the tubule-derived ET effect predominates, salt and water excretion are increased.

Renal endothelin production is regulated differently than in the vasculature. Whereas vascular (and mesangial) ET generation is controlled by thrombin, Ang II and TGF–β, tubular ET production seems to particularly depend on medullary tonicity. For example, a high-salt diet, by raising medullary tonicity, stimulates ET-1 release, which in turn leads to increased eNOS (NOS3) expression and natriuresis.[293] (see "Nitric Oxide" section, later). The signaling mechanisms for these phenomena, as well other renal actions of ET-1, continue to be a subject of intensive research and the interested reader is referred to a recent review summarizing the current state of knowledge.[279]

Nitric Oxide. Nitric oxide (NO) is a diffusible gaseous molecule produced from L-arginine by the enzyme NOS, which exists in three distinct isoforms—nNOS (NOS1), inducible NOS (iNOS, or NOS2) and eNOS (NOS3).[291] NOS is expressed in renal vascular endothelial cells (mainly eNOS), tubular epithelial and mesangial cells and macula densa (mainly nNOS). There is controversy regarding iNOS expression in normal kidneys, but upregulation is clearly seen in pathologic conditions such as ischemia-reperfusion injury.[294]

Selective NOS inhibitors and NOS knockout mice have been used to elucidate the role of NOS isoforms in the regulation of renal function.[294] The action of NO is mediated by activation of a soluble GC, thereby increasing intracellular levels of cGMP.[295] In the kidneys, the physiologic roles of NO include the regulation of glomerular hemodynamics, attenuation of TGF, mediation of pressure natriuresis, maintenance of medullary perfusion, inhibition of tubular Na^+ reabsorption and modulation of RSNA.[291,296] Renal NOS activity is regulated by several factors, such as Ang II (see earlier section, "Tubuloglomerular Feedback") and salt intake.[120]

The role of NO in the regulation of renal hemodynamics and excretory function is best illustrated by the fact that inhibition of intrarenal NO production results in increased BP and impaired renal function.[294] Infusion of the NOS inhibitor, N^G-monomethyl-L-arginine (L-NMMA), into one kidney in anesthetized dogs resulted in a dose-dependent decrease in urinary cGMP levels, decreases in RBF and GFR, Na^+ and water retention in the ipsilateral compared with the contralateral kidney.[297] In addition, acute NO blockade amplified the renal vasoconstrictive action of Ang II in isolated microperfused rabbit afferent arterioles and in conscious rats.[120,294] Also L-NMMA–induced vasoconstriction led to decreased RBF and Kf, effects reversed by RAAS blockade. Thus, a major effect of NO is to counterbalance the efferent vasoconstrictive action of Ang II as well as modulate renin secretion by the JGA (see section, "Tubuloglomerular Feedback").

The involvement of NO in promoting diuresis and natriuresis in normal and increased salt intake/volume-expanded states is well characterized.[291] In conscious dogs on a normal Na^+ diet, NO inhibition induced a significant decrease in natriuresis and diuresis with no change in arterial pressure. On a high-Na^+ diet, treatment with the NO inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), increased arterial pressure and cumulative Na^+ balance.[298] Similarly, rats on a high salt intake for 2 weeks had increased Na^+ excretion and proportionally elevated urinary concentrations of the NO metabolites, NO_2 and NO_3. The increase in urinary NO metabolites is attributed to enhanced expression of all three NOS isoforms in the renal medulla.[121]

NO exerts a vasodilatory effect on the renal medullary circulation and promotes Na^+ excretion.[164] Consistent with these data are the high levels of eNOS in the renal medulla and the inhibitory effect of NO on collecting duct Na^+-K^+-ATPase.[298] In contrast, in salt-sensitive hypertension, NOS activity (mainly nNOS) is significantly lower in salt-sensitive than salt-resistant rats maintained on a high-salt diet.[299,300] Also, L-arginine-induced NO production prevented the development of salt-induced hypertension in Dahl salt-sensitive rats.[301] These findings suggest that nNOS plays an important role in Na^+ handling and decreased nNOS activity may be involved in the mechanism of salt-sensitive hypertension.

The involvement of NO in salt retention and subsequent hypertension could result from an inadequate direct effect on proximal and distal tubular Na^+ reabsorption. However, attenuated NO inhibition of renin secretion and TGF may also contribute. In this context, macula densa-derived NO could blunt the TGF-mediated vasoconstriction during high salt intake in salt-resistant but not salt-sensitive rats.[302] As noted, the medullary and other effects of NO occur in response to local ET production.[291] For example, the inhibition of NOS by L-NAME or the highly selective ET-B antagonist A-192621 abolished the diuretic and natriuretic effects of big ET-1 in anesthetized rat kidneys.[303] In addition, ET-1 acutely activated eNOS in the isolated mTALH and nNOS in isolated IMCD cells, via ET-B activation, an effect dependent on increased NOS protein, but not mRNA, expression.[291] These data suggest that nNOS and eNOS activation occur by posttranscriptional pathways. NO also reduces Cl^- absorption in CCD through an ENaC-dependent mechanism.[304]

Activation of eNOS, by a paracrine effect in the IMCD, where the highest renal NOS activity is found,[305] is also associated with inhibition of Na^+ reabsorption in the mTALH through phosphatidylinositol-3-kinase (PI3K)–stimulated Akt activity, leading to eNOS phosphorylation at Ser1177.[305] However, the functional corollary of nNOS activation in the IMCD remains to be determined. NO also reduces paracellular Na^+ reabsorption in the mTALH, and the magnitude of this effect is equal to that due to NO inhibition of transcellular transport.[306] A further action of NO is inhibition of AVP-enhanced Na^+ reabsorption and hydroosmotic water permeability of the CCD.[307] A mouse model of specific collecting duct NOS1 gene deletion should be a valuable tool to study

the signaling mechanisms involved in the NO effects on AVP-enhanced Na^+ reabsorption, as well as salt-dependent BP mechanisms.[296] The role of NO in pressure natriuresis and RSNA has been discussed in the relevant sections.

Kinins. The kallikrein-kinin system (KKS) is a complex cascade responsible for the generation and release of vasoactive kinins. The active peptides bradykinin (BK) and kallidin are formed from precursors (kininogens) that are cleaved by tissue and circulatory kinin-forming enzymes.[229] Kinins are produced by many cell types and can be detected in urine, saliva, sweat, interstitial fluid and, rarely, venous blood. Circulating BK is almost undetectable because of rapid metabolism by kininases, particularly kininase II/ACE1. The renal KKS can produce local BK concentrations much higher than those in blood. In the kidney, bradykinin is metabolized by NEP.[308]

Kinins play an important role in hemodynamic and excretory processes through their G protein–coupled receptors, BK-B_1 and BK-B_2. BK-B_2 receptors mediate most of the actions of kinins and are located mainly in the kidneys, although they are also detectable in the heart, lungs, brain, uterus and testes.[308] Activation of BK-B_2 receptors results in vasodilation, probably through an NO– or arachidonic acid metabolite–dependent mechanism.[309] BK selectively increases medullary perfusion, especially to the inner layer, via activation of NO and Ca^{2+}-activated K^+ channels.[310] BK also has multiple effects on the cardiovascular system, particularly vasodilation and plasma extravasation.[309]

In the kidney, kinins induce diuresis and natriuresis through activation of BK-B_2 receptors, increased RBF and secondary inhibition of Na^+ and water reabsorption via ENaC in the distal nephron.[229] Unlike many vasodilators, BK increases RBF without significantly affecting GFR or proximal tubular Na^+ reabsorption.

Studies with transgenic animals have elaborated the physiologic role of kinins and the interaction between the KKS and the RAAS.[309] In the kidney, Na^+ depletion-induced Ang II, acting via the AT_2 receptor, stimulates a vasodilator cascade of BK, NO and cGMP.[297] In the absence of the AT_2 receptor, pressor and antinatriuretic hypersensitivity to Ang II is associated with BK and NO deficiency.[297] Furthermore, renal kinins are involved in pressure natriuresis.[311] BK also mediates Ang 1–7–mediated diuresis and natriuresis, as shown in rats transgenic for the kallikrein gene.[312] Because ACE is involved in kinin degradation, ACE inhibitors not only attenuate Ang II formation but may also lead to kinin accumulation. The latter effect may be responsible in part for the beneficial effects of ACE inhibitors in HF, but also for their troublesome side effect of cough.[313] In summary, the KKS seems to play a pivotal role as a counterregulatory modulator of vasoconstrictor and Na^+-retaining mechanisms.

Adrenomedullin. Human adrenomedullin (AM) is a 52-amino acid peptide, discovered in 1993 in extracts of human pheochromocytoma cells and approximately 30% homologous in structure with calcitonin gene–related peptide and amylin.[314] AM is produced from a 185–amino acid prepro-hormone that contains a unique NH_2-terminus 20–amino acid sequence, "proadrenomedullin NH_2-terminal 20 peptide," with biologic activity similar to that of AM. AM mRNA is expressed in endothelial cells, glomeruli, distal and medullary collecting tubules. Synthesis and secretion of AM are stimulated by Ang II, NE, ET, bradykinin, and shear stress.[314] AM acts through a 395–amino acid G protein-like–coupled receptor, belonging to the calcitonin receptor–like receptor and a family of receptor activity–modifying proteins.[314] Receptor activation in VSMC increases intracellular cAMP and calcium-activated potassium channel activity,[314] leading to prolonged dose-dependent generalized vasodilation and hypotension, accompanied by increases in heart rate and cardiac output caused by positive inotropic effects.[314] The vasodilating effect of AM is also stimulated by calcium-dependent NO synthesis in endothelial cells.[314]

In addition to its hypotensive action, AM increases RBF through preglomerular and postglomerular arteriolar vasodilation,[315,316] accompanied by dose-dependent diuresis and natriuresis.[316,317] These effects result from a decrease in tubular Na^+ reabsorption, despite the AM-induced hyperfiltration[315] and may be mediated partially by locally released NO[318,319] and PG.[320] In addition, NEP inhibition potentiates exogenous AM-induced natriuresis without affecting GFR.[321] Like NPs, AM suppresses aldosterone secretion in response to Ang II and high potassium levels.[317] Furthermore, in cultured VSMC, AM inhibits ET production induced by various stimuli.[317] In the hypothalamus, AM inhibits AVP secretion, which may also contribute to its diuretic and natriuretic actions.[317]

Two other members of the AM family, AM-2 (intermedin) and AM-5, have cardiovascular effects similar to those of AM-1. AM-2, but not AM-5, also has renal effects similar to those of AM-1.[322,323]

Together, these findings show that AM may be involved in the physiologic control of vascular tone and cardiac function; in the kidney, AM may modulate sodium and water excretion in a paracrine fashion.[324]

Urotensin. Urotensin II (UT II) is a highly-conserved peptide that binds to the human orphan G protein–coupled receptor GPR14, or UT II receptor. The parent peptide, prepro–UT II, is widely expressed in human tissues, including the kidney where it can be detected in tubular epithelial cells, especially of the distal tubule, and endothelial cells of renal capillaries.[325] The C-terminus of the prohormone is cleaved to produce UT II, an 11–amino acid residue peptide. Human UT II includes a cyclic hexapeptide sequence fundamental for the action of the peptide. Substantial local UT II has been demonstrated in the heart, liver, and kidneys.[326]

Infusion of UT II leads to local forearm vasoconstriction, no effect, or cutaneous vasodilation, according to species variation, site and modality of injection, dose, vascular bed and experimental model.[327] The vasoconstrictive action is probably direct, whereas the vasodilatory response is likely mediated by factors such as cyclooxygenase products and NO.

The involvement of the UT II system in the regulation of renal function in mammals is unclear and the data are as contradictory as those for vascular tone. In normal rats, intravenous boluses in the nanomolar range caused minor reductions in GFR and no effect on Na^+ excretion.[325] However, bolus injections in the picomolar range produced a dose-dependent decrease in GFR associated with reduced urine flow and Na^+ excretion.[328] In contrast, continuous infusion of UT II in the picomolar range elicited increases

in GFR and NO–dependent diuresis and natriuresis.[325] Thus, the effect of UT II on renal function seems dependent on the mode of administration and experimental condition.

The variability in renal and vascular responses to UT II may also depend on regulation at the receptor level. The binding density of UT II is correlated with vasoconstrictor response in rats and small changes in receptor density may result in pathophysiologic effects. Under normal conditions, most UT II receptors are already occupied by UT II. Changes in unoccupied receptor reserve—perhaps in response to alterations in UT II levels in experimental models or in disease states—might explain, at least in part, the observed variability in renal and vascular actions.[325]

Selective UT II receptor antagonists have been developed and, in normal rats, can increase GFR, urine flow and Na^+ excretion.[329] However, in light of the complex renal effects of UT II, it is unlikely that these antagonists will have a role in the management of sodium disorders.[325]

Digitalis-Like Factors. The existence of endogenous digitalis-like factors was hypothesized in the 1960s and initially reported in the late 1970s.[330] Among these factors, also known as endogenous cardiotonic steroids, two have been characterized extensively in humans, cardenolide (or ouabain) and bufadienolide (marinobufagenin). The main site of synthesis of these compounds is the adrenal cortex.[331] Cardiotonic steroids act by inhibiting Na^+-K^+-ATPase, leading to attenuation of tubular Na^+ transport and increased vascular resistance via enhanced cytosolic Ca^{2+} in VSMC.[330] The latter mechanism has been implicated in the pathogenesis of hypertension.[330]

Neuropeptide Y. Neuropeptide Y (NPY), a 36–residue peptide, is a sympathetic cotransmitter stored and released together with NE by adrenergic nerve terminals of the SNS.[332,333] Although originally isolated from the brain and highly expressed in the CNS, the peptide exhibits a wide spectrum of biologic activities in the cardiovascular system, GI tract and kidneys through multiple $G_{i/o}$ protein–coupled receptors.[333,334] Experimentally, NPY can reduce RBF and increase renal vascular resistance without significantly affecting GFR in various species, including humans.[333] Similarly, the peptide may exert a natriuretic or antinatriuretic action, depending on experimental conditions and species studied.[333] However, overall, NPY appears to have no significant role in the physiological regulation of sodium.

Apelin. Apelin is the endogenous ligand of the APJ receptor, a G protein–coupled receptor involved in water homeostasis, regulation of cardiovascular tone and cardiac contractility.[335] Apelin and its receptor are widely expressed in the CNS, endothelial cells (systemic and renal), VSMC of glomerular arterioles and, to a lesser extent, in other nephron segments.[336]

AJP activation leads to inhibition of cAMP production and activation of the Na^+/H^+ exchanger type 1 (NHE1). Through the former pathway, apelin enhances vascular dilation via induction of eNOS, whereas NHE1 activation in cardiomyocytes leads to a dose-dependent increase in myocardial contractility.[336] With regard to the renal effects of apelin, direct injection into the hypothalamus of lactating rats inhibited AVP release and reduced circulating AVP. Conversely, water deprivation led to increased systemic AVP and decreased apelin levels.[335] Moreover, APJ expression in the rat collecting duct occurs near the vasopressin V_2 receptor and apelin directly counteracts the V_2 receptor-mediated antidiuretic effect of AVP.[337] Thus, AVP and apelin have a reciprocal relationship in controlling water diuresis.

With respect to vascular tone, the apelin/APJ system acts as a counterregulator of the RAS. For example, intravenous apelin caused an NO–dependent fall in arterial pressure, whereas apelin receptor knockout mice displayed an enhanced vasopressor response to systemic Ang II.[336] Intravenous injection of apelin also induced vasorelaxation of Ang II–preconstricted efferent and afferent arterioles, as well as a significant diuresis.[338] Activation of endothelial apelin receptors caused release of NO, which inhibited the Ang II–induced rise in intracellular Ca^{2+} levels.[336] Furthermore, apelin had a direct receptor-mediated vasoconstrictive effect on vascular smooth muscle.[336] These results indicate that apelin has complex effects on the preglomerular and postglomerular microvasculature regulating renal hemodynamics.

Glucagon-Like Peptide-1

The incretin hormone glucagon-like peptide-1 (GLP-1) is released from the gut in response to fat or carbohydrate and contributes to negative feedback control of blood glucose by stimulating insulin secretion, inhibiting glucagon and slowing gastric emptying. GLP-1 receptors (GLP-1Rs) are also expressed in the proximal tubule and the GLP-1 agonist, exenatide, has natriuretic effects by inhibiting NHE-3, thereby reducing proximal tubular Na^+ reabsorption; this effect may be mediated by Ang II inhibition.[339] Exenatide also increased SNGFR by 33% to 50%, doubled early distal flow rate and increased urine flow rate sixfold without altering GTB, TGF responsiveness, or the tonic influence of TGF. This implies that exenatide is a proximal diuretic and a renal vasodilator.[339] Because the natural agonist for the GLP-1 receptor is regulated by intake of fat and carbohydrate, but not by salt or fluid, the control of salt excretion by the GLP-1R system departs from the usual negative-feedback paradigm for regulating salt balance.[340]

Novel Factors

A novel intrarenal paracrine mechanism for sodium regulation in mice was recently described. Early studies showed that changes in dietary acid-base load could reverse the direction of apical transport of the tricarboxylic acid intermediate, α-ketoglutarate (α-KG), in the proximal tubule and loop of Henle from reabsorption following an acid load to secretion following a base load.[341] Work on isolated microperfused CCDs from *Oxgr1*$^{-/-}$ mice indicated that the concentration of α-KG is sensed by the α-KG receptor, OXGR1, expressed in type B and non-A, non-B intercalated cells of the connecting tubule and CCD. Addition of 1 mM α-KG to the tubular lumen strongly stimulated Cl-dependent HCO_3^- secretion and electroneutral NaCl reabsorption in tubules of wild type but not *Oxgr1*$^{-/-}$ mice. *Oxgr1*$^{-/-}$ mice also displayed significantly increased ENaC activity, without changes in plasma aldosterone.[341] In contrast, α-KG inhibited amiloride-sensitive sodium reabsorption in principal cells independently of OXGR1 activation. This effect is possibly related to increased ATP production, inducing autocrine activation of P2Y2 receptors and, thereby, inhibition of ENaC.[342] It appears that

receptor-dependent and receptor-independent effects of α-KG converge to compensate for an alkalosis-induced decrease in proximal tubular reabsorption of NaCl by favoring NaCl reabsorption over Na^+/K^+ exchange along the connecting tubule and CCD.[341] Taken together, the data indicate that α-KG acts as a paracrine mediator involved in the functional coordination of proximal and distal parts of the tubule in the adaptive regulation of HCO_3^- secretion and NaCl reabsorption in the presence of acid-base disturbances.[341]

Members of the epidermal growth factor (EGF) family have been shown to be important for maintaining transepithelial Na^+ transport. For example, a high salt diet was shown to decrease cortical EGF levels promoting ENaC-mediated Na^+ reabsorption in the collecting duct and the development of hypertension. Conversely, intravenous EGF decreased ENaC activity, prevented the development of hypertension and attenuated glomerular and renal tubular damage in the Dahl salt-sensitive rat.[343] The inhibitory effect of EGF on ENaC-dependent Na^+ absorption appears to be mediated via the H-Ras/c-Raf, MEK/ERK signaling pathway, and Cav-1 is an essential component of this EGF-activated signaling mechanism.[344] The physiological implications of these observations are eagerly awaited.

The role of obesity in the pathogenesis of hypertension and renal dysfunction has led to the exploration of appetite-related hormones in salt and water retention. In this context, the orexigenic hormone, ghrelin, secreted by the stomach, has been shown to stimulate Na^+ absorption through cAMP-dependent trafficking of ENaC in the CCD.[345] The effect appears to be mediated via ghrelin receptor upregulation of Sirt-1 and was also shown in rats fed a low-salt diet.[346] These data indicate that the ghrelin-Sirt1 system may participate in regulating sodium reabsorption in the distal nephron, but its physiologic and pathologic roles in sodium homeostasis remain to be clarified.[347]

Another novel development is the growing interest in the circadian rhythmicity of many basic physiologic functions. These functional rhythms are driven, in part, by the circadian clock, a ubiquitous molecular mechanism allowing cells and tissues to anticipate and prepare for regular environmental events. This clock has been shown to play a role in the regulation and maintenance of RPF, GFR, tubular reabsorption, and secretion of Na^+, Cl^- and K^+.[348] Studies in clock-deficient mice have identified the 20-HETE synthesis pathway as one of the clock's principal renal targets to mediate Na^+ excretion.[349] By exerting dynamic control over renal sodium handling, the circadian clock could affect BP, at least in part.

Rhythmicity of salt regulation seems to occur not only at the circadian level but also on a longer periodic basis (so-called infradian rhythms). In a fascinating study on men involved in space flight simulations, Rakova and colleagues[350] have shown that even on fixed salt diets (6, 9, or 12 g/day), daily Na^+ excretion exhibited aldosterone-dependent, weekly (circaseptan) rhythms, resulting in periodic Na^+ storage. Changes in total-body Na^+ (±200 to 400 mmol) exhibited monthly or longer period lengths, without parallel changes in body weight and extracellular water. These changes were directly related to urinary aldosterone excretion and inversely to urinary cortisol, suggesting rhythmic hormonal control. These findings suggest the existence of rhythmic Na^+ excretory and retention patterns independent of BP or body water and irrespective of salt intake.[350]

SODIUM BALANCE DISORDERS

HYPOVOLEMIA

DEFINITION

Hypovolemia is the condition in which the volume of the ECF compartment is reduced in relation to its capacitance. As noted, the reduction may be absolute or relative. In states of absolute hypovolemia, Na^+ balance is truly negative, reflecting past or ongoing losses. Hypovolemia is described as "relative" when there is no Na^+ deficit but the capacitance of the ECF compartment is increased. In this situation of reduced EABV, the ECF intravascular and extravascular (interstitial) compartments may vary in the same or opposite directions. ICF volume, reflected by measurements of plasma Na^+ or osmolality, may or may not be concomitantly disturbed; thus, hypovolemia may be classified as normonatremic, hyponatremic or hypernatremic.

ETIOLOGY

The causes of hypovolemia are summarized in Table 14.4. Absolute and relative hypovolemia, in turn, can have extrarenal or renal causes. Absolute hypovolemia results from massive blood loss or fluid loss from the skin, GI or respiratory

Table 14.4 Causes of Absolute and Relative Hypovolemia

Absolute
Extrarenal
Gastrointestinal fluid loss
Bleeding
Skin fluid loss
Respiratory fluid loss
Extracorporeal ultrafiltration
Renal
Diuretics
Obstructive uropathy/postobstructive diuresis
Hormone deficiency
Hypoaldosteronism
Adrenal insufficiency
Na^+ wasting tubulopathies
Genetic
Acquired tubulointerstitial disease
Relative
Extrarenal
Edematous states
Heart failure
Cirrhosis
Generalized vasodilation
Sepsis
Drugs
Pregnancy
Third-space loss
Renal
Severe nephrotic syndrome

system, or kidneys. Relative hypovolemia results from states of vasodilation, generalized edema or third-space loss. In both absolute and relative hypovolemia, the perceived reduction in EABV prompts the compensatory hemodynamic changes and renal responses described earlier (see section, "Physiology").

PATHOPHYSIOLOGY

Absolute Hypovolemia

Extrarenal. The commonest causes of absolute hypovolemia are persistent diarrhea, vomiting and massive bleeding, either gastrointestinal or as a result of trauma. The reduction in ECF volume is isotonic inasmuch as there is a proportionate loss of water and plasma. The consequent fall in systemic BP leads to compensatory tachycardia and vasoconstriction and the ensuing altered transcapillary Starling hydraulic forces enable a shift of fluid from the interstitial to intravascular compartment. In addition, the neural and hormonal responses to hypovolemia (see section, "Physiology") result in renal Na^+ and water retention, with the aim of restoring intravascular volume and hemodynamic stability.

Similar compensatory mechanisms become activated after fluid losses from the skin, GI system and respiratory system. Because of the large surface area of the skin, large amounts of fluid can be lost from this tissue as a result of burns or excessive perspiration. Severe burns allow the loss of large volumes of plasma and interstitial fluid and can lead rapidly to profound hypovolemia. Without medical intervention, hemoconcentration and hypoalbuminemia supervene. As occurs after massive bleeding, the fluid loss is isotonic, so plasma Na^+ concentration and osmolality remain normal. In contrast, excessive sweating, induced by exertion in a hot environment, leads to hypotonic fluid loss as a result of the relatively low Na^+ concentration (20 to 50 mmol/L) in sweat. The resulting hypovolemia may therefore be accompanied by hypernatremia and hyperosmolality and the type of fluid replacement must be tailored accordingly (see Chapter 15).

In addition to oral intake, the GI tract is characterized by the entry of approximately 7 L of isotonic fluid, the overwhelming majority of which is reabsorbed in the large intestine. Hence, in normal conditions, fecal fluid loss is minimal. However, in the presence of pathologic conditions, such as vomiting, diarrhea, colostomy and ileostomy secretions, especially those caused by infection, considerable or even massive fluid loss may occur. The ionic composition, osmolality and pH of secretions vary according to the part of the GI tract involved; therefore, the resulting hypovolemia is associated with a large spectrum of electrolyte and acid–base abnormalities (see Chapters 16 and 17 for further discussion).

In contrast to the massive losses that can occur from the skin and GI system, fluid loss from the respiratory tract—as occurs in febrile states and in patients who receive mechanical ventilation with inadequate humidification—is usually modest and hypovolemia ensues only in the presence of accompanying causes. Finally, a special situation in which hypovolemia can occur is after excessive ultrafiltration in dialysis patients (see Chapter 63).

Renal. Even when GFR is markedly impaired, the amount of filtered Na^+ far exceeds the dietary intake, and all but 1% of the filtered load is reabsorbed. However, if one or more of the tubular reabsorptive mechanisms is impaired, serious Na^+ deficit and absolute volume depletion can occur. The causes of absolute renal Na^+ losses include pharmacologic agents and renal structural, endocrine, and systemic disorders (see Table 14.4). All diuretics used to treat hypervolemic states may induce hypovolemia if administered in excess or inappropriately. Particularly, the powerful loop diuretics, furosemide, bumetanide, torsemide, and ethacrynic acid, are often given in combination with diuretics acting on other tubular segments (e.g., thiazides, aldosterone antagonists, distal ENaC blockers, and carbonic anhydrase inhibitors). Patients receiving these combinations need to be carefully monitored and fluid balance scrupulously adjusted to prevent hypovolemia. Patients commonly at risk are those with HF or underlying hypertension who develop intercurrent infections.

In patients with hypertension, diuretic treatment appreciably increases the risk of volume depletion. Osmotic diuretics, endogenous or exogenous, may also reduce tubular Na^+ reabsorption. Endogenous agents include urea, the principal molecule involved in the polyuric recovery phase of acute kidney injury and postobstructive diuresis, and glucose in hyperglycemia. In patients with increased intracranial pressure, exogenous agents, such as mannitol or glycerol, may be used to induce translocation of fluid from the ICF to the ECF compartment and decrease brain swelling. The resulting polyuria may be associated with electrolyte and acid-base disturbances, the nature of which depends on the complex interplay between fluid intake and intercompartmental fluid shifts.

Na^+ reabsorption may also be disrupted in inherited and acquired tubular disorders. Inherited disorders of the proximal tubules (e.g., Fanconi syndrome) and distal tubules (e.g., Bartter and Gitelman syndromes) may lead to salt-wasting states in association with other electrolyte or acid-base disturbances. Acquired disorders of Na^+ reabsorption may be acute, as in nonoliguric acute kidney injury, the period immediately after renal transplantation, the polyuric recovery phase of acute kidney injury and postobstructive diuresis (see relevant chapters for further details), or they may be chronic as a result of tubulointerstitial diseases with a propensity for salt wasting. Chronic kidney disease stages 3 to 5 of any cause is associated with heightened vulnerability to Na^+ losses because the ability to match tubular reabsorption with the sum of filtered load minus dietary intake is impaired.

In addition to intrinsic tubular disorders, endocrine and other systemic disturbances may lead to impaired Na^+ reabsorption. The principal endocrine causes are mineralocorticoid deficiency and resistance states. A controversial cause is the systemic disturbance known as cerebral salt wasting (CSW). In this condition, salt wasting is thought to occur in response to an as yet unidentified factor released in the setting of acute head injury or intracranial hemorrhage.[351,352] CSW is usually diagnosed because of concomitant hyponatremia and signs of volume depletion in contrast to the normovolemia characteristic of the syndrome of inappropriate antidiuresis.[353] However, CSW remains an enigmatic and not universally accepted clinical entity.[354]

An underappreciated, but not uncommon, clinical setting for renal Na^+ loss is after the administration of large volumes

of intravenous saline to patients over several days after surgery or after trauma. In this situation, tubular reabsorption of Na^+ is downregulated. If intravenous fluids are stopped before full reabsorptive capacity is restored, volume depletion may ensue. The phenomenon can be minimized by graded reduction in the infusion rate, which allows Na^+ reabsorptive pathways to be restored gradually.

In the context of volume depletion, diabetes insipidus should be mentioned. However, because this results from a deficiency of or tubular resistance to AVP, water loss is the main consequence and the impact on ECF volume is only minor. AVP-related disorders are considered in Chapter 15

Relative Hypovolemia

Extrarenal. As outlined previously, the principal causes of relative hypovolemia are edematous states, vasodilation and third-space loss (see Table 14.4). Vasodilation may be physiologic, as in normal pregnancy, or induced by drugs (hypotensive agents, such as hydralazine or minoxidil, that cause arteriolar vasodilation), or it may occur in sepsis during the phase of peripheral vasodilation and consequent low SVR.[355]

Edematous states in which the EABV and, hence, tissue perfusion, are reduced include HF, decompensated cirrhosis with ascites and nephrotic syndrome. In severe HF, low cardiac output and resulting low systemic BP lead to a fall in RPP. As in absolute hypovolemia, the kidneys respond by retaining Na^+. Because the increased venous return cannot raise the cardiac output, a vicious cycle is created in which edema is further exacerbated and the persistently reduced cardiac output leads to further Na^+ retention. In decompensated cirrhosis, splanchnic venous pooling leads to decreased venous return, a consequent fall in cardiac output and compensatory renal Na^+ retention. The pathophysiology of edematous states is discussed later (see section, "Hypervolemia"). Third-space loss occurs when fluid is sequestered into compartments not normally perfused with fluids, as in states of GI obstruction, after trauma, burns, or in pancreatitis, peritonitis, or malignant ascites. The result is that, even though total body Na^+ is markedly increased, the EABV is severely reduced.

Renal. Approximately 10% of patients with the nephrotic syndrome—especially children with minimal change disease, but also any patient with a serum albumin level lower than 2 g/dL—manifest clinical signs of hypovolemia. The low plasma oncotic pressure is conducive to movement of fluid from the ECF compartment to the interstitial space, thereby leading to reduced EABV.[356]

CLINICAL MANIFESTATIONS

The clinical manifestations of hypovolemia depend on the magnitude and rate of volume loss, solute composition of the net fluid loss (i.e., the difference between input and output) and vascular and renal responses. The clinical features are related to the underlying pathophysiologic process, hemodynamic consequences and electrolyte and acid-base disturbances accompanying the renal response to hypovolemia. A detailed history usually reveals the cause of volume depletion (bleeding, vomiting, diarrhea, polyuria, diaphoresis, medications).

The symptoms and physical signs of hypovolemia appear only when intravascular volume is decreased by 5% to 15% and are often related to tissue hypoperfusion. Symptoms include generalized weakness, muscle cramps, and postural light-headedness. Thirst is prominent if concomitant hypertonicity is present (hypertonic hypovolemia). Physical signs are related to the hemodynamic consequences of hypovolemia and include tachycardia, hypotension (postural, absolute or relative to the usual BP) and low central or jugular venous pressure. Elevated jugular venous pressure, however, does not rule out hypovolemia, because of the possible confounding effects of underlying HF or lung disease. When volume depletion exceeds 10% to 20%, circulatory collapse is liable to occur, with severe supine hypotension, peripheral cyanosis, cold extremities and impaired consciousness, extending even to coma. This is especially likely if fluid loss is rapid or occurs against a background of comorbid conditions. When the source of volume loss is extrarenal, oliguria also occurs. The traditional signs—reduced skin turgor, sunken eyes, and dry mucous membranes—are inconstant findings and their absence does not rule out hypovolemia. Reduction in the EABV, as manifested by relative hypotension, may also be observed in generalized edematous states, even though there is an overall excess of Na^+ and water; however, this excess is maldistributed between the extracellular and interstitial spaces.

DIAGNOSIS

The diagnosis of hypovolemia is based essentially on the clinical findings. Nevertheless, when these are equivocal, various laboratory parameters may be helpful for confirming the diagnosis or for elucidating other changes that may be associated with volume depletion.

Laboratory Findings

Hemoglobin and Plasma Albumin. Hemoglobin may decrease if significant bleeding has occurred or is ongoing, but the change, which is caused by hemodilution owing to fluid translocation from the interstitial to intravascular compartment, may take up to 24 hours. Therefore, stable hemoglobin does not rule out significant bleeding. Moreover, the adaptive response of hemodilution may moderate the severity of hemodynamic compromise and resulting physical signs. In hypovolemic situations that do not arise from bleeding, hemoconcentration is often, but not universally, seen, inasmuch as underlying anemia of chronic disease may mask the differential loss of plasma.

Hemoconcentration may also be manifested as a rise in plasma albumin concentration if albumin-free fluid is lost from the skin, GI tract, or kidneys. On the other hand, when albumin is lost, either in parallel with other extracellular fluids (as in proteinuria, hepatic disease, protein-losing enteropathy, or catabolic states) or in protein-rich fluid (third-space sequestration, burns), significant hypoalbuminemia is observed.

Plasma Na^+ Concentration. This may be low, normal, or high, depending on the solute composition of the fluid lost and the replacement solution administered by the patient or physician. For example, the hypovolemic stimulus for AVP release may lead to preferential water retention and hyponatremia, especially if hypotonic replacement fluid is used. In contrast, the fluid content of diarrhea may be hypotonic or hypertonic, resulting in hypernatremia or hyponatremia,

respectively. The plasma Na^+ concentration reflects the tonicity of plasma and provides no direct information about volume status, which is a clinical diagnosis.

Plasma K^+ and Acid-Base Parameters. These can also change in hypovolemic conditions. After vomiting and after some forms of diarrhea, loss of K^+ and Cl^- may lead to alkalosis. More often, the principal anion lost in diarrhea is bicarbonate, which leads to hyperchloremic (nonanion gap) acidosis. When diuretics or Bartter and Gitelman syndromes (the inherited tubulopathies; see Chapter 44) are the cause of hypovolemia, hypokalemic alkalosis is again typically seen. On the other hand, U_{Na} loss that occurs in adrenal insufficiency or due to aldosterone hyporesponsiveness is accompanied by a tendency for hyperkalemia and metabolic acidosis. Finally, when hypovolemia is sufficiently severe to impair tissue perfusion, high anion gap acidosis caused by lactic acid accumulation may be observed.

Blood Urea and Creatinine Levels. These frequently rise in hypovolemic states and reflect impaired renal perfusion. If tubular integrity is preserved, then the rise in urea levels is typically disproportionate to that of creatinine, so-called prerenal azotemia (see Chapter 28). This results mainly from AVP-enhanced urea reabsorption in the medullary collecting duct (MCD), but also from augmented proximal tubular reabsorption due to increased filtration fraction.[357] In critically ill patients, an increased urea generation rate (from exogenous or endogenous protein catabolism) or low creatinine generation rate due to muscle wasting may lead to an erroneous diagnosis of prerenal azotemia.[358] In the presence of severe hypovolemia, acute kidney injury may ensue, leading to loss of the differential rise in urea level. Proportional rises in urea and creatinine are also observed when hypovolemia occurs against a background of underlying renal functional impairment, as in chronic kidney disease, stages 3 to 5.

Urine Biochemical Parameters. In hypovolemia due to extrarenal fluid losses, the intact kidney will respond to hypoperfusion by enhanced tubular reabsorption of Na^+ and water. The ensuing oliguria will be characterized by urine-specific gravity greater than 1.020, Na^+ concentration less than 10 mmol/L and osmolality greater than 400 mOsm/kg. When urine Na^+ concentration is 20 to 40 mmol/L, the finding of a fractional excretion of Na^+ $\dfrac{\text{urine } Na^+ \times \text{plasma creatinine}}{\text{plasma } Na^+ \times \text{urine creatinine}} \times 100$ of less than 1%, in the presence of oliguria, may be helpful. However, in a patient on previous diuretic therapy, especially with loop diuretics, these indices may merely reflect U_{Na} losses. In that case, fractional excretion of urea of less than 30% to 35% may help in the diagnosis of hypovolemia, although the specificity of this test is rather low.[359,360]

Other Laboratory Parameters. When hypovolemia occurs in the presence of arterial vasodilation, as observed in sepsis, some, but not all, of the clinical manifestations of hypovolemia are observed. Thus, tachycardia and hypotension are usually present, but the extremities are warm, suggesting that perfusion is maintained. This finding is misleading because vital organs, particularly the brain and kidneys, are underperfused as a result of the hypotension. The presence of lactic acidosis helps establish the correct diagnosis.

TREATMENT

Absolute Hypovolemia

General Principles. The goals of treatment of hypovolemia are to restore normal hemodynamic status and tissue perfusion. These goals are achieved by reversal of the clinical symptoms and signs, described previously. Treatment can be divided into three stages: 1. initial replacement of the immediate fluid deficit; 2. maintenance of the restored ECF volume in the presence of ongoing losses; and 3. treatment of the underlying cause, whenever possible. The main strategies to be addressed by the clinician are the route, volume, rate of administration, and composition of the replacement and maintenance fluids. These are liable to change according to the patient's response.

In general, when hypovolemia is associated with a significant hemodynamic disturbance, intravenous rehydration is required. (The use of oral electrolyte solutions in the management of infants and children is discussed in Chapter 73.) The volume of fluid and rate of administration should be determined on the basis of the urgency of the threat to circulatory integrity, adequacy of the clinical response, and underlying cardiac function. Older patients are especially vulnerable to aggressive fluid challenge and careful monitoring is required, particularly to prevent acute left ventricular failure and pulmonary edema from overzealous correction.

Sometimes the clinical signs do not point unequivocally to the diagnosis of hypovolemia, even though the history is strongly suggestive. Invasive monitoring of central venous and pulmonary venous pressures has not been shown to improve outcomes in this situation[361,362]; monitoring preload by stroke volume variation may improve outcomes, at least after major abdominal surgery.[363] However, in case of doubt, a diagnostic fluid challenge should be performed. If the patient improves clinically, BP and urine output increase and no overt signs of HF appear over the succeeding 6 to 12 hours, then the diagnosis is substantiated, and fluid therapy can be cautiously continued. Conversely, if overt signs of fluid overload appear, the fluid challenge can be stopped, and diuretic therapy reinstituted.

The initial calculations for replacing the fluid deficit are based on hemodynamic status. These deficits are notoriously difficult to calculate; therefore, good clinical judgment is necessary for successful management. Patients with life-threatening circulatory collapse and hypovolemic shock require rapid intravenous replacement through the cannula with the widest bore possible. Replacement should continue until BP and tissue perfusion are restored. In the second stage, the rate of fluid replacement should be reduced to maintain BP and tissue perfusion. In older patients and those with underlying cardiac dysfunction, the risk of overrapid correction and precipitating pulmonary edema is heightened; therefore slower treatment is preferable, to allow gradual filling of the ECF volume rather than causing pulmonary edema and the threat of mechanical ventilation associated with adverse outcomes.[364]

Composition of Replacement Fluids. The composition of replacement fluid may also affect outcomes. The two main

categories of replacement solution are crystalloid and colloid solutions. Crystalloid solutions are based largely on NaCl of varying tonicity or dextrose. Isotonic (0.9%) saline, containing 154 mmol of Na^+/L, is the mainstay of volume replacement therapy being confined to the ECF compartment in the absence of deviations in Na^+ concentration. One L of isotonic saline increases plasma volume by approximately 300 mL; the rest is distributed to the interstitial compartment. In contrast, 1 L of 5% dextrose in water (D_5W), which is also isosmotic (277 mOsm/L), is eventually distributed throughout all the body fluid compartments so that only 10% to 15% (100 to 150 mL) remains in the ECF. Therefore, D_5W should not be used for volume replacement.

Administration of 1 L of .45% saline (77 mmol of Na^+/L) in D_5W is equivalent to giving 500 mL of isotonic saline and the same volume of solute-free water. The distribution of the solute-free compartment throughout all the fluid compartments would result in plasma dilution and reduction in the plasma Na^+. Therefore, this solution should be reserved for the management of hypernatremic hypovolemia. Even in that situation, it must be remembered that volume replacement is less efficient than with isotonic saline and, early in the treatment course, may cause plasma tonicity to fall too rapidly.

When hypovolemia is accompanied by severe metabolic acidosis (pH <7.10; plasma HCO_3^- <10 mmol/L), bicarbonate supplementation may be indicated. (For a discussion of bicarbonate balance, see Chapter 16.) Because this anion is manufactured as 8.4% sodium bicarbonate (1000 mmol/L) for use in cardiac resuscitation, appropriate dilution is required for the treatment of acidosis associated with hypovolemia. Two convenient methods are suggested. Either 75 mL (75 mmol) of 8.4% $NaHCO_3^-$ can be added to 1 L of .45% saline, or 150 mL of concentrated bicarbonate can be added to 1 L of D_5W. Although the latter is hypertonic in the short term, it is unlikely to be harmful.

In the presence of accompanying hypokalemia, especially if metabolic alkalosis is also present, volume replacement solutions should be supplemented with K^+. Commercially available 1-L solutions of isotonic saline supplemented with 10 or 20 mmol of KCl make this option safe and convenient. (For details, see Chapter 17.) On the other hand, newer, commercially available crystalloid solutions containing lactate (converted by the liver to bicarbonate) and low concentrations of KCl may offer advantages over isotonic saline. In a recent large prospective observational study performed in the intensive care unit setting, two periods were compared; in the control period, all patients received isotonic saline as fluid replacement, whereas during the intervention period, Hartmann solution (lactate-containing), Plasma-Lyte 148 (a balanced salt solution), or chloride-poor 20% albumin solution was administered. The chloride-poor solutions were associated with a significantly lower risk of subsequent acute kidney injury, even after adjustment for covariates.[365] Clearly, these provocative results indicate the need for randomized controlled trials comparing chloride-rich with more balanced salt solutions for fluid resuscitation.[366,367]

Colloid solutions include plasma, albumin and high-molecular-weight carbohydrate molecules, such as hydroxyethyl starch and dextrans, at concentrations that exert COPs equal to or greater than that of plasma. Because the transcapillary barrier is impermeable to these large molecules, in theory they expand the intravascular compartment more rapidly and efficiently than crystalloid solutions. Colloid solutions may be useful in the management of burns and severe trauma when plasma protein losses are substantial and rapid plasma expansion with relatively small volumes is efficacious. However, when capillary permeability is increased, as in states of multiorgan failure or the systemic inflammatory response syndrome, colloid administration is ineffective. Moreover, randomized controlled studies, in which crystalloid solutions were compared with colloid solutions, have shown no survival benefit and even harm with some colloid solutions, particularly hydroxyethyl starch.[367] Therefore, the much cheaper and more readily available crystalloid solutions should remain the mainstay of therapy.

Relative Hypovolemia

Treatment of relative hypovolemia is more difficult than that of absolute hypovolemia because there is no real fluid deficit. If the relative hypovolemia is caused by peripheral vasodilation, as in sepsis, it may be necessary to administer cautiously a crystalloid solution, such as isotonic saline, to maintain ECF volume until the SVR and venous capacitance return to normal; the excess volume administered can then be excreted by the kidneys. When vasodilation is more severe, vasoconstrictor agents may be needed to maintain systemic BP. In severe HF, advanced cirrhosis with portal hypertension and severe nephrotic syndrome, when EABV is low but there is an overall excess of Na^+ and water, treatment may be extremely challenging. Crystalloid solution will, likely, lead to worsening interstitial edema without significantly affecting EABV. In these situations, prognosis is determined by whether the underlying condition can be reversed.

HYPERVOLEMIA

DEFINITION

Hypervolemia occurs when the volume of the ECF compartment is expanded relative to its capacitance. Normally, increments in Na^+ intake are matched by corresponding changes in Na^+ excretion as elaborated earlier (see section, "Physiology"). However, in the approximately 20% of the population who are salt sensitive, the upward shift in ECF volume induced by high salt intake leads to a persistent rise in systemic arterial pressure, albeit without other signs of fluid retention (see Chapter 46). Here, the discussion is confined to hypervolemia, in which Na^+ retention is ongoing and inappropriate for the prevailing ECF volume, with the appearance of clinical signs of volume overload.

ETIOLOGY

Hypervolemia may result from either primary renal Na^+ retention or can be secondary to disease in other major organs (Table 14.5).

Primary Renal Na^+ Retention

This can be subclassified as caused by intrinsic kidney disease or primary mineralocorticoid excess. Of the primary renal diseases causing Na^+ retention, oliguric renal failure limits the ability to excrete Na^+ and water and affected patients are at risk for rapidly developing ECF volume overload (see Chapter 28). In contrast, in chronic kidney disease, renal tubular adaptation to salt intake is usually efficient until late

Table 14.5 Causes of Renal Sodium Retention

Primary
Oliguric acute kidney injury
Chronic kidney disease
Glomerular disease
Severe bilateral renal artery stenosis
Na^+-retaining tubulopathies (genetic)
Mineralocorticoid excess
Secondary
Heart failure
Cirrhosis
Idiopathic edema

stage 4 and stage 5. However, in some primary glomerular diseases, especially in the presence of nephrotic range proteinuria, significant Na^+ retention may occur, even when GFR is close to normal (see section, "Pathophysiology," and Chapter 30). Primary mineralocorticoid excess leads to transient Na^+ retention. However, because of "mineralocorticoid escape," the dominant clinical feature is hypertension (see Chapters 12 and 46).

Secondary Renal Na^+ Retention

This occurs in low- and high-output cardiac failure with systolic and/or diastolic dysfunction. Nephrotic syndrome and hepatic cirrhosis with portal hypertension are also accompanied by renal Na^+ retention. In this chapter, only HF and cirrhosis are considered. Nephrotic syndrome is discussed in Chapter 28.

PATHOPHYSIOLOGY

Primary renal Na^+ retention is caused by disruption of normal renal function. In contrast, secondary renal Na^+ retention occurs because of reduced EABV in the presence of total ECF volume expansion or in response to factors secreted by the heart or liver that signal the kidneys to retain Na^+ (Fig. 14.8). In secondary Na^+ retention, the renal effector mechanisms that normally operate to conserve Na^+ and protect against a Na^+ deficit are exaggerated and maintained, despite subtle or overt ECF volume expansion. The pathophysiology of hypervolemia involves local mechanisms of edema formation and stimulation of renal Na^+ retention by reduced EABV either directly or indirectly, via abnormalities of the afferent volume sensing mechanisms.

Local Mechanisms of Edema Formation

Peripheral interstitial fluid accumulation, which is common to all conditions causing hypervolemia, results from disruption of the normal balance of transcapillary Starling forces. Transcapillary fluid and solute transport consists of both convective and diffusive flow. Bulk water movement occurs via convective transport induced by hydraulic and osmotic pressure gradients. Capillary hydraulic pressure (P_c) is under the influence of several factors, including systemic arterial and venous BPs, local blood flow and precapillary and postcapillary resistance. Systemic arterial BP, in turn, is determined by cardiac output, intravascular volume and SVR; systemic venous pressure is determined by right atrial pressure, intravascular volume and venous capacitance. Na^+ balance is a key determinant of these latter hemodynamic parameters. Also, massive accumulation of fluid in the peripheral interstitial compartment (anasarca) can itself diminish venous compliance and, thereby, alter overall cardiovascular performance.[368]

The balance of Starling forces prevailing at the arteriolar end of the capillary ($\Delta P > \Delta\pi$, in which $\Delta\pi$ is the change in transcapillary oncotic pressure) favors net filtration of fluid into the interstitium. Net outward movement of fluid along the length of the capillary is associated with an axial decrease in P_c and an increase in π_c. Nevertheless, the local ΔP continues to exceed the opposing $\Delta\pi$ throughout the length of the capillary bed in several tissues; thus, filtration occurs along its entire length.[369] In such capillary beds, a substantial volume of filtered fluid must, therefore, return to the circulation via lymphatic vessels. Hence, to minimize edema formation, the lymphatic vessels must be able to expand and proliferate and lymphatic flow increase in response to increased interstitial fluid formation.

Several other mechanisms for minimizing edema formation have been identified. First, precapillary vasoconstriction tends to lower P_c and diminish the filtering surface area in a given capillary bed. Indeed, in the absence of appropriate regulation of the microcirculatory myogenic reflex, as occurs with some Ca^{2+} channel blockers, excessive precapillary vasodilation may lead to lower extremity interstitial edema.[370] Second, increased net filtration itself is associated with dissipation of P_c, dilution of interstitial fluid protein concentration and a corresponding rise in intracapillary plasma protein concentration. The resulting change in the balance of Starling forces will tend to mitigate further interstitial fluid accumulation.[371] Finally, interstitial fluid hydraulic pressure (P_i) is normally subatmospheric; however, even small increases in interstitial fluid volume tend to augment P_i, again opposing further transudation of fluid into the interstitial space.[372] The appearance of generalized edema in association with expansion of the ECF volume therefore implies the presence of one or more disturbances in microcirculatory hemodynamics—increased venous pressure transmitted to the capillary, unfavorable adjustments in precapillary and postcapillary resistances, and/or inadequacy of lymphatic flow for draining the interstitial and replenishing the intravascular compartment.

For the clinical detection of generalized edema, the volume of accumulated interstitial fluid required (>2 to 3 L) necessitates expansion of ECF volume and, hence, body exchangeable Na^+ content. Since continued net accumulation of interstitial fluid without renal Na^+ retention might result in serious intravascular volume contraction and cessation of interstitial fluid formation, generalized edema must indicate substantial renal Na^+ retention.

Systemic Factors Stimulating Renal Sodium Retention

Reduced Effective Arterial Blood Volume. Renal Na^+ (and water retention) in edematous disorders occurs due to reduced EABV, despite an increase in total blood and ECF volumes and normal intrinsic renal function.[19] If the underlying stimulus for hypervolemia is removed, as dramatically seen after heart[373] or liver transplantation,[374] Na^+ excretion is restored to normal. Conversely, when kidneys from patients with end-stage liver disease are transplanted into patients with normal liver function, Na^+ retention no longer occurs.[374]

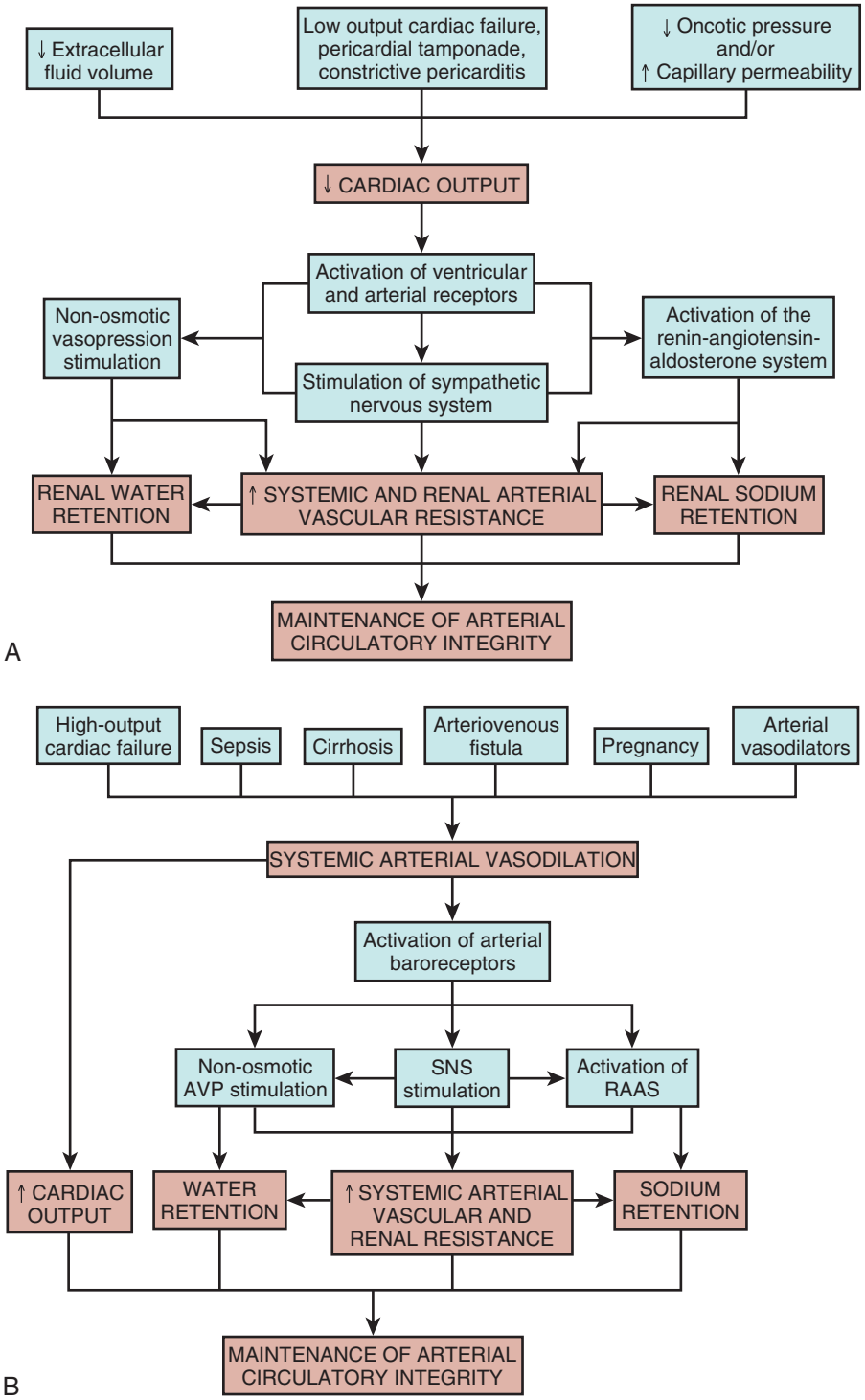

Fig. 14.8 Sensing mechanisms that initiate and maintain renal sodium and water retention in various clinical conditions in which arterial underfilling, with resultant neurohumoral activation and renal sodium and water retention, is caused by a decrease in cardiac output (A) and by systemic arterial vasodilation (B). In addition to activating the neurohumoral axis, adrenergic stimulation causes renal vasoconstriction and enhances sodium and fluid transport by the proximal tubule epithelium. (From Schrier RW. Decreased effective blood volume in edematous disorders: what does this mean? *J Am Soc Nephrol*. 2007;18:2028–2031.)

Because 85% of blood circulates in the venous compartment, expansion of that compartment leads to overall ECF volume excess that could occur concurrently with arterial underfilling. The latter could result from low cardiac output, peripheral arterial vasodilation, or a combination of the two. In turn, low cardiac output could result from true ECF volume depletion (see earlier discussion), cardiac failure, or decreased π_c, with or without increased capillary permeability. All these stimuli would cause activation of ventricular and arterial sensors. Similarly, conditions such as high-output cardiac

failure, sepsis, cirrhosis and normal pregnancy lead to peripheral arterial vasodilation and activation of arterial baroceptors. Activation of these afferent mechanisms would then induce the neurohumoral mechanisms that result in renal Na^+ and water retention (see Fig. 14.8).[375]

Although the mechanisms leading to Na^+ retention in HF and cirrhosis are similar, specific differences between the two conditions have been observed and are discussed separately in the following sections.

Renal Sodium Retention in Heart Failure

Abnormalities of Sensing Mechanisms in Heart Failure. Both the cardiopulmonary and baroceptor reflexes are blunted in HF, so that they cannot exert an adequate tonic inhibitory effect on sympathetic outflow.[376] The resulting SNS activation triggers renal Na^+ retention, as already described. A variety of models of HF have shown marked attenuation of atrial receptor firing and loss of nerve ending arborization in HF.[377] Similarly, altering central cardiac filling pressures in response to postural stimuli (e.g., head-up tilt, LBNP) in HF patients, in contrast to normal subjects, usually do not demonstrate significant alterations in limb blood flow, circulating catecholamines, AVP, or renin activity.[378,379] This diminished reflex responsiveness is proportionate to the severity of ventricular dysfunction.

Arterial baroceptor reflex impairment has been observed in HF. High baseline values of muscle sympathetic activity were found in patients with HF who failed to respond to activation and deactivation of arterial baroreceptors by infusion of phenylephrine and Na^+ nitroprusside, respectively.[377] Carotid and aortic baroreceptor function were also depressed in experimental models of HF.[377] These changes were associated with upward resetting of receptor threshold and a reduced range of pressures over which the receptors functioned.

Multiple abnormalities have been described in cardiopulmonary and arterial baroreceptor control of RSNA in HF. Thus, rats with coronary ligation displayed an increased basal level of efferent RSNA that failed to decrease normally during volume expansion.[73,254] Similarly, in sinoaortic denervated dogs with pacing-induced HF, or following left atrial baroreceptor stimulation, the cardiopulmonary baroreflex control of efferent RSNA was markedly attenuated.[380]

The abnormal regulation of efferent RSNA was caused by impaired function of aortic and cardiopulmonary baroreflexes; the latter defect was functionally more important.[254] Mechanisms implicated in the pathogenesis of these abnormal baroreflexes include loss of compliance in the dilated hearts, gross changes in receptor structure and augmented Na^+-K^+-ATPase activity in the baroreceptor membranes.[377] Increased activity of Ang II through the AT_1 receptor also contributes to depressed baroreflex sensitivity. Thus RAAS inhibition in rats or rabbits with HF significantly improved arterial baroreflex control of RSNA or heart rate, respectively.[377,380] This effect of Ang II could also be blocked by central α_1-adrenoreceptor stimulation.[381]

More recent studies have indicated that Ang II in the paraventricular nucleus potentiates—and AT_1 receptor antisense mRNA normalizes—the enhanced cardiac sympathetic afferent reflex in rats with chronic HF.[377] AT_1 receptors in the nucleus tractus solitarii are thought to mediate the interaction between the baroreflex and cardiac sympathetic afferent reflex.[382] Consistent with this notion, Ang II generation is enhanced and its degradation reduced in central sympathoregulatory neurons, as shown by upregulation of ACE1 and downregulation of ACE2.[383] AT_2 receptors in the rostral ventrolateral medulla inhibited sympathetic outflow, an effect mediated at least partly by an arachidonic acid metabolic pathway.[384] These studies indicated that a downregulation in the AT_2 receptor was a contributory factor in the sympathetic neural excitation in HF.[377]

Together, these data provide evidence of the role of high endogenous levels of Ang II, acting through the AT_1 receptor in concert with downregulation of the AT_2 receptor, in the impaired baroreflex sensitivity observed in HF, both in the afferent limb of the reflex arch and at more central sites. The central effect may be mediated through a central α_1-adrenoreceptor. The blunted cardiopulmonary and arterial baroreceptor sensitivity in HF may also lead to an increase in AVP release and renin secretion.[377]

The disturbances in sensing mechanisms that initiate and maintain renal Na^+ retention in HF are summarized in Fig. 14.8A. As indicated, a decrease in cardiac output or a diversion of systemic blood flow diminishes the blood flow to the critical sites of the arterial circuit with pressure- and flow-sensing capabilities. The responses to diminished blood flow culminate in renal Na^+ retention, mediated by the effector mechanisms. An increase in systemic venous pressure promotes the transudation of fluid from the intravascular to interstitial compartment by increasing the peripheral transcapillary ΔP. These processes augment the perceived loss of volume and flow in the arterial circuit. In addition, distortion of the pressure-volume relationships as a result of chronic dilation in the cardiac atria attenuates the normal natriuretic response to central venous congestion. This attenuation is manifested predominantly as a diminished neural suppressive response to atrial stretch, which results in increased sympathetic nerve activity and augmented release of renin and AVP.

Abnormalities of Effector Mechanisms in Heart Failure. The adaptive changes in the efferent limb of the volume control system in HF are generally similar to those seen in states of true Na^+ depletion. These include adjustments in glomerular hemodynamics and tubular transport, brought about by alterations in the neural, humoral and paracrine systems. However, in contrast to true Na^+ depletion, HF is also associated with activation of vasodilatory/natriuretic agents, which tend to oppose the effects of the vasoconstrictor/antinatriuretic systems. The final effect on urinary Na^+ excretion is determined by the dynamic balance among these antagonistic effector systems.

Alterations in Glomerular Hemodynamics

HF is characterized not only by increased renal vascular resistance and reduced GFR, but also by an even greater reduction in RPF, so that the filtration fraction is increased.[385] As shown in rat models of HF, these changes seem to result from diminished Kf and elevated afferent and efferent arteriolar resistances. The rise in filtration fraction is probably caused by a disproportionate increase in efferent arteriolar resistance.[385]

In Fig. 14.9, a comparison of the glomerular capillary hemodynamic profile in the normal versus the HF state is illustrated on the left graph of each panel. First, ΔP declines

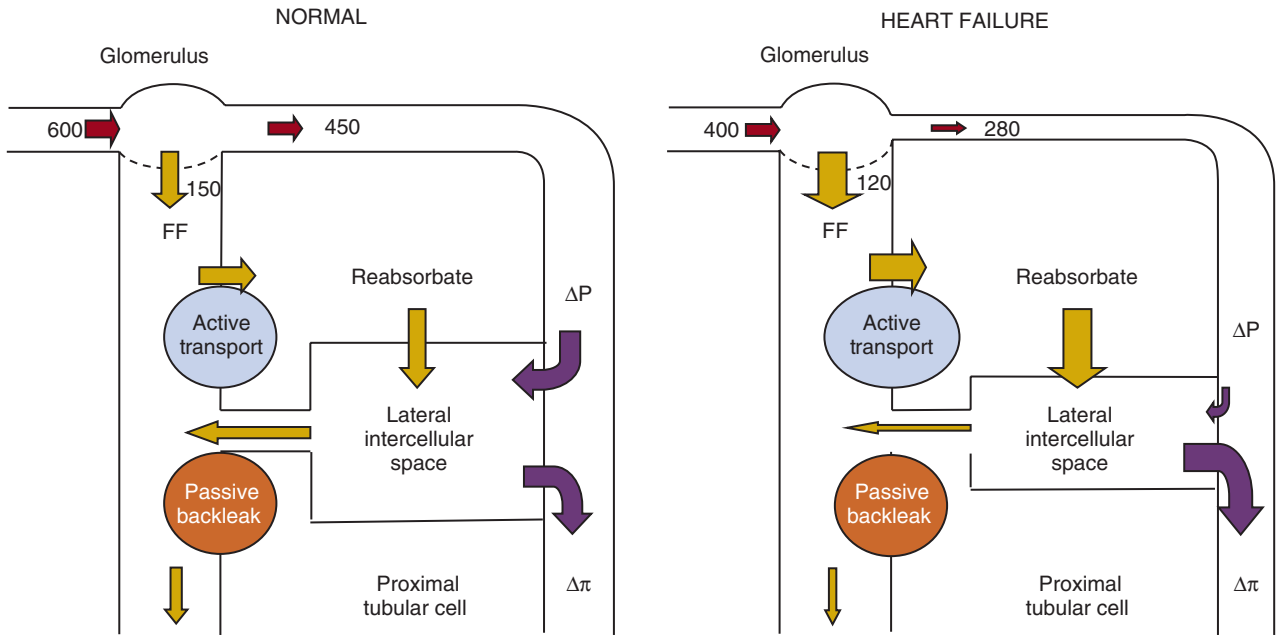

Fig. 14.9 Peritubular control of proximal tubule fluid reabsorption. Fluid reabsorption in the normal state *(left)* and in patients with heart failure *(right)* is shown. Increased postglomerular arteriolar resistance in heart failure is depicted as narrowing. The thickness and font size of the *block arrows* depict relative magnitude of effect. The increase in filtration fraction (FF) in heart failure causes Δπ to rise. The increase in renal vascular resistance in heart failure is believed to reduce ΔP. Both the increase in Δπ and the fall in ΔP enhance peritubular capillary uptake of proximal reabsorbate and thus increase absolute Na^+ reabsorption by the proximal tubule. Numbers and *red block arrows* depict blood flow in preglomerular and postglomerular capillaries; ΔP and Δπ are the transcapillary hydraulic and oncotic pressure differences across the peritubular capillary, respectively; *yellow block arrows* indicate transtubular transport; *purple block arrows* represent the effect of peritubular capillary Starling forces on uptake of proximal reabsorbate. (Modified from Humes HD, Gottlieb M, Brenner BM. *The kidney in congestive heart failure: contemporary issues in nephrology.* Vol 1. New York; Churchill Livingstone; 1978, pp 51–72.)

along the length of the glomerular capillary in normal and HF states, but much more so in HF because of the increased efferent arteriolar resistance. Second, Δπ increases over the length of the glomerular capillary in both states as fluid is filtered into Bowman's space, but again to a greater extent in HF because of the increased filtration fraction. As outlined below (see section, "Renin-Angiotensin-Aldosterone System"), the preferential increase in efferent arteriolar resistance is mediated principally by Ang II and is critical for the preservation of GFR in the presence of reduced RPF. Because of the intense efferent arteriolar vasoconstriction, further compensation is not possible if RPP falls as a result of systemic hypotension, causing a sharp decline in GFR. This phenomenon is dramatically illustrated by HF patients whose Ang II drive is removed by RAAS inhibitors, particularly those with preexisting renal failure, massive diuretic treatment and limited cardiac reserve.[385] In these patients, BP may fall below the level necessary to maintain renal perfusion.

Enhanced Tubular Reabsorption of Sodium. A direct consequence of the glomerular hemodynamic alterations and augmented single-nephron filtration fraction is an increase in the fractional reabsorption of filtered Na^+ in the proximal tubule. In Fig. 14.9, the peritubular capillary hemodynamic profile of the normal state is compared with that of HF on the right graph of each panel. In HF, in comparison with the normal state, the average value of Δπ along the peritubular capillary is increased and that of ΔP is decreased. These values favor fluid movement into the capillary and may also help reduce paracellular backleak of fluid into the tubule, promoting overall net reabsorption.

The peritubular control of proximal fluid reabsorption in normal and HF states is illustrated schematically in Fig. 14.9. A critical mediator of the enhanced tubular reabsorption of Na^+ is Ang II, which, by increasing efferent arteriolar resistance, increases the filtration fraction and augments proximal epithelial transporter activities directly, thereby amplifying the overall increase in proximal Na^+ reabsorption. This is clearly illustrated by the favorable effects of RAAS blockers in HF to modulate single-nephron filtration fraction and normalize proximal peritubular capillary Starling forces and Na^+ reabsorption.[385]

Enhanced reabsorption of Na^+ in HF has been shown in the loop of Henle, probably due to altered renal hemodynamics, as in the proximal tubule.[156] In the distal tubule and collecting duct, elevated Ang II and aldosterone levels, respectively, enhance activities of the NaCl cotransporter and ENaC.[386]

Neurohumoral Mediators. The primary vasoconstrictor/antinatriuretic (and antidiuretic) systems mediating Na^+ and water retention in HF include the RAAS, SNS, AVP, and ETs. The antagonistic vasodilator/natriuretic substances include NO, PG, AM, UT II, and NPY. The development of positive Na^+ balance and edema in HF occurs at the point when the vasoconstrictor/antinatriuretic forces predominate (Fig. 14.10). The dominant activity of Na^+-retaining systems in HF is clinically important, as it is associated with globally-impaired

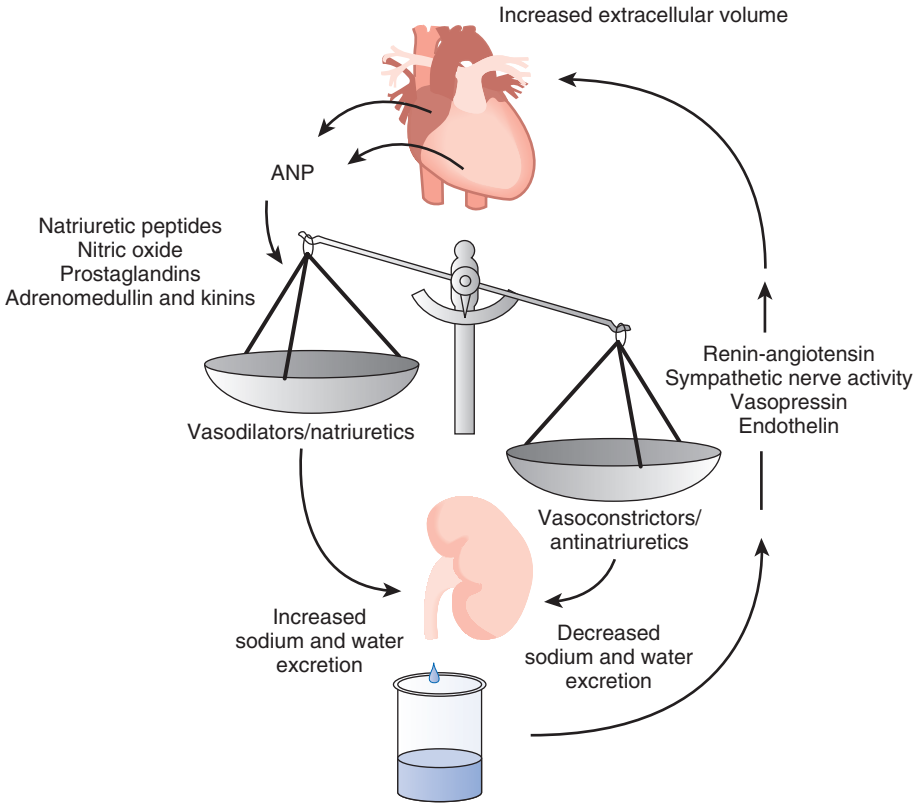

Fig. 14.10 Efferent limb of extracellular fluid volume control in heart failure. Volume homeostasis in heart failure is determined by the balance between natriuretic and antinatriuretic forces. In decompensated heart failure, enhanced activities of the Na^+-retaining systems overwhelm the effects of the vasodilatory/natriuretic systems, which leads to a net reduction in Na^+ excretion and an increase in ECF volume. ANP, Atrial natriuretic peptide. (Modified from Winaver J, Hoffman A, Abassi Z, et al. Does the heart's hormone, ANP, help in congestive heart failure? *News Physiol Sci*. 1995;10:247–253.)

renal function, a strong predictor of mortality[385]; moreover, reversal of neurohumoral impairment is associated with improved outcomes.[385]

VASOCONSTRICTOR/ANTINATRIURETIC (ANTIDIURETIC) SYSTEMS

Renin-Angiotensin-Aldosterone System

The activity of the RAAS is enhanced in most patients with HF in correlation with the severity of cardiac dysfunction[234] and provides a prognostic index for HF patients. Initially, RAAS activation is beneficial by inducing direct systemic vasoconstriction and activating other neurohormonal systems such as AVP, which contribute to maintaining adequate intravascular volume.[234] However, numerous studies in patients and in experimental models of HF have established that continued activation of the RAAS leads to maladaptive myocardial remodeling[234] and progression of cardiovascular and renal dysfunction.[364]

The kidneys in particular are highly sensitive to the action of Ang II, and a decrease in RPF and SNGFR, as well as elevations in efferent arteriolar resistance and filtration fraction, are observed in both clinical and experimental HF. These changes are completely reversed by ACE inhibitors, as well as a low-salt diet.[387]

Activation of Ang II in response to the decreased pumping capacity of the failing myocardium also promotes systemic vasoconstriction and mesangial cell contraction.[202] In addition, Ang II reduces renal cortical circulation in rats with HF and increases tubular Na^+ reabsorption directly and by augmenting aldosterone release.[202]

Local RAAS in the heart and kidney is also important in maintaining Na^+ retention in HF. The phenomenon explains the presence of positive Na^+ balance as well as the maintained efficacy of RAAS inhibition in chronic HF, in the absence of elevated systemic levels of the component hormones.[388] In general, it appears that systemic RAAS activation is most pronounced in acute decompensated HF, whereas local renal RAAS activation may dominate in chronic stable HF.

In the heart, local RAAS activation has a number of effects. In addition to the mechanical stress exerted on the myocardium due to systemic Ang II–mediated increased afterload, pressure overload activates local Ang II production as a result of upregulation of angiotensinogen and tissue ACE.[389] Local Ang II acts through AT_1 in a paracrine/autocrine manner, leading to cell swelling and cardiac hypertrophy, remodeling and fibrosis (mediated by TGF-β) and reduced coronary flow, hallmarks of severe HF.[388] These observations explain the improved cardiac function, prolonged survival, prevention of end-organ damage, and prevention or regression of cardiac hypertrophy in HF treated with RAAS inhibitors.[203,389] In addition, these drugs may improve endothelial dysfunction, vascular remodeling and potentiation of the vasodilatory effects of kinins.[309]

Like Ang II, aldosterone is produced locally by and acts directly on the myocardium in HF, inducing structural remodeling of the interstitial collagen matrix.[390] These adverse effects of aldosterone were elegantly illustrated using eplerenone, a specific aldosterone antagonist, which prevented progressive left ventricular systolic and diastolic dysfunction by reducing interstitial fibrosis, cardiomyocyte hypertrophy and left ventricular chamber sphericity in dogs with HF. Similarly, eplerenone attenuated ventricular remodeling and reactive (but not reparative) fibrosis after myocardial infarction in rats.[391,392] These findings have been translated into the now routine clinical use of aldosterone antagonists in HF (see section, "Specific Treatments Based on the Pathophysiology of Heart Failure").[393]

As noted, in addition to its renal and cardiovascular hemodynamic effects, the RAAS is involved directly in the exaggerated tubular Na^+ reabsorption in HF. Ang II, produced systemically and locally, directly stimulates proximal tubular Na^+ reabsorption.[394] In contrast, in the cortical and MCD, enhanced Na^+ reabsorption is mediated largely by aldosterone, as outlined previously. The pivotal role of aldosterone in HF is amply illustrated by elevated plasma and urine levels and the natriuretic effects of aldosterone antagonists in HF, despite further activation of other antinatriuretic systems.[395]

The importance of RAAS action in the Na^+ retention of HF varies with the stage and severity of disease and, in more severe HF, positive Na^+ balance is associated with blunted renal and hemodynamic responses to ANP; this response to ANP is restored by RAAS inhibition (for further details, see later section, "Natriuretic Peptides").[396] Also, despite low plasma osmolality, patients with HF display increased thirst, probably because of the high Ang II concentrations, which stimulate thirst center cells in the hypothalamus.[397] This phenomenon may contribute to the positive water balance and hyponatremia often seen in advanced HF (see section, "Arginine Vasopressin").

Sympathetic Nervous System

As mentioned earlier, patients with HF experience progressive activation of the SNS with declining cardiac function[234,376] and the adverse influence of sympathetic overactivity on the progression and outcome of patients with HF is abundantly clear.[398,399] Thus, plasma NE levels are frequently elevated and correlate with increased neural traffic. SNS activity is also significantly correlated with intracardiac pressures, cardiac hypertrophy and left ventricular ejection fraction (LVEF).[398,399] Activation of the SNS not only precedes the appearance of congestive symptoms but also is preferentially directed toward the heart and kidneys, as seen in patients with mild HF who have higher NE levels in the coronary sinus than in the renal veins.[399] In early HF, increased SNS activity ameliorates the hemodynamic abnormalities, including hypoperfusion, diminished plasma volume and impaired cardiac function, via vasoconstriction and avid Na^+ reabsorption.[376] However, chronic SNS activation induces several long-term adverse myocardial effects, including apoptosis and hypertrophy, with overall reduction in cardiac contractility. Some of these effects may be mediated by RAAS activation which, in turn, can augment sympathetic activity and create a vicious cycle.[234]

Basal sympathetic outflow to the kidneys is significantly increased in patients with HF[376] and increased efferent RSNA contributes to the increased renal vasoconstriction, avid Na^+ and water retention, renin secretion, and attenuation of the renal actions of ANP.[264] In rats with experimental HF caused by coronary artery ligation, renal denervation resulted in increased RPF and SNGFR and decreased afferent and efferent arteriolar resistance.[398] In the same model, the decrease in RSNA in response to an acute saline load was less than that of control rats.[254] Conversely, bilateral renal denervation restored the natriuretic response to volume expansion. Similarly, in dogs with low cardiac output induced by vena caval constriction, administration of a ganglion blocker resulted in a marked increase in Na^+ excretion.[398] Also, in dogs with high-output HF induced by aortocaval fistula, total postprandial urinary Na^+ excretion was approximately twofold higher in dogs with renal denervation than in those with intact nerves.[398] In line with these observations, administration of the α-adrenoreceptor blocker dibenamine to patients with HF caused an increase in fractional Na^+ excretion, without a change in RPF or GFR. Treatment with ibopamine, an oral dopamine analog, resulted in vasodilation and positive inotropic and diuretic effects in these patients.[400] Moreover, for a given degree of cardiac dysfunction, the concentration of NE is significantly higher in patients with abnormal than in those with preserved renal function.[401] These findings suggest that the association between renal function and prognosis in patients with HF is linked by both systemic and CNS neurohormonal activation.

RSNA may also affect renal hemodynamics and Na^+ excretion in HF by an antagonistic interaction with ANP. On the one hand, ANP has sympathoinhibitory effects;[402,403] on the other, the SNS-induced salt and water retention in HF may reduce renal responsiveness to ANP. For example, the blunted diuretic/natriuretic response to ANP in rats with HF could be restored by prior renal denervation[404] or clonidine,[405] a centrally acting α_2-adrenoreceptor agonist, which decreases RSNA in HF. These examples illustrate the complexity of interactions between the SNS and other humoral factors involved in the pathogenesis of Na^+ retention in HF.

In summary, the SNS plays an important role in the regulation of Na^+ excretion and glomerular hemodynamics in HF, either by a direct renal action or by a complex interplay between the SNS itself and other neurohumoral mechanisms that act on the glomeruli and renal tubules. The recent introduction of renal denervation as a potential therapeutic treatment for HF should facilitate further elucidation of these neurohumoral interactions.

Vasopressin

Numerous studies have demonstrated elevated plasma levels of AVP in HF, mostly in advanced HF with hyponatremia, but also in asymptomatic patients with left ventricular dysfunction.[406] These high levels are related to nonosmotic factors such as attenuated left atrial compliance, hypotension and RAAS activation, and are reversed by RAAS inhibition or α-blockade (prazosin).[407]

The high circulating levels of AVP adversely affect the kidneys and cardiovascular system. In fact, raised levels of the C-terminal portion of the AVP prohormone (copeptin) at the time of diagnosis of acute decompensated HF are highly predictive of 1-year mortality.[408] The prognostic power of an increased copeptin level in HF is similar to that of BNP levels (see section, "Brain Natriuretic Peptide"). The most

recognized renal effect of AVP in HF is the development of hyponatremia, especially in advanced stages of the disease, which most probably results from impaired solute-free water excretion, independent of plasma osmolality. In accordance with this notion, animal models of HF have demonstrated increased collecting duct expression of AQP2.[409] In addition, administration of specific V_2 receptor antagonists (VRAs) is associated with improvement in plasma Na^+ levels in animals and patients with hyponatremia.[410,411] The improvement is associated with correction of the impaired urinary dilution in response to acute water load,[412] increased plasma osmolarity and downregulation of renal AQP2 expression, but with no effect on RBF, GFR or Na^+ excretion.[413]

The adverse effects of AVP on cardiac function[414] occur through its V_{1A} receptor to increase SVR (i.e., cardiac afterload), as well as by V_2-receptor–mediated water retention, which leads to systemic and pulmonary congestion (increased preload). In addition, AVP, through its V_{1A} receptor, causes a direct rise in cardiomyocyte intracellular Ca^{2+} and activation of mitogen-activated kinases and protein kinase C. These signaling mechanisms appear to mediate the observed cardiac remodeling, dilation and hypertrophy. The remodeling might be further exacerbated by the abnormalities in preload and afterload.

In summary, the data suggest that AVP is involved in the pathogenesis of water retention and hyponatremia that characterize HF; and that AVP receptor antagonists result in remarkable diuresis in experimental and clinical models of HF. Treatment of HF with VRA will be discussed further (see section, "Specific Treatments Based on the Pathophysiology of Heart Failure").

Endothelin

ET-1 is involved in both the development and progression of HF as well as in the associated reduced renal function by inducing renal remodeling, interstitial fibrosis, glomerulosclerosis, hypoperfusion, hypofiltration and positive salt and water balance.[279] The pathophysiologic role of ET-1 in HF is supported by two major lines of evidence: 1. The ET system is activated in HF; and 2. ET-1 receptor antagonists modify this pathophysiologic process.[279] The first line of evidence is based on elevated plasma ET-1 and big ET-1 concentrations in clinical HF and experimental models of HF; these levels correlate with hemodynamic severity and symptoms.[415] Also, the degree of pulmonary hypertension was the strongest predictor of plasma ET-1 level in patients with HF.[416] Moreover, plasma levels of big ET and ET-1 are especially high in patients with moderate to severe HF and are independent markers of mortality and morbidity.[415] The increase in plasma ET-1 levels may be the result of enhanced synthesis in the lungs, heart and circulation by stimuli such as Ang II and thrombin or decreased pulmonary clearance.[416] In parallel to ET-1 levels, ET-A receptors are upregulated, whereas ET-B receptors are downregulated in the failing human heart.[417]

A cause-and-effect relationship between these hemodynamic abnormalities and ET-1 in HF was demonstrated using selective and highly specific ET receptor antagonists.[416] In this regard, acute administration of the mixed ET-A/ET-B receptor antagonists, bosentan and tezosentan significantly improved renal cortical perfusion, reversed the profoundly increased renal vascular resistance and increased RBF and Na^+ excretion in rats with severe decompensated HF.[418] In addition, chronic blockade of ET-A by selective or dual ET-A/ET-B receptor antagonists attenuated the magnitude of Na^+ retention and prevented the decline in GFR in experimental HF.[419] These data are in line with earlier observations that rats with decompensated HF, as compared with normal rats, displayed severely blunted cortical vasoconstriction but significantly prolonged medullary vasodilation in response to ET-1 infusion. These effects could have resulted from activation of vasodilatory systems such as PG and NO, as exemplified by higher medullary immunoreactive eNOS levels in rats with HF than controls.[420] Taken together, the data indicate a role for ET in the pathogenesis of renal cortical vasoconstriction and Na^+ retention in HF.

VASODILATORY/NATRIURETIC SYSTEMS

Natriuretic Peptides

In decompensated HF, renal Na^+ and water retention occur, despite ECF volume expansion, even when the NP system is activated. Many clinical and experimental studies have implicated both ANP and BNP in the pathophysiology of the deranged cardiorenal axis in HF.

Atrial Natriuretic Peptide. Plasma levels of ANP and NH_2-terminal ANP are frequently elevated in HF and are correlated positively with the severity of cardiac failure, elevated atrial pressure and left ventricular dysfunction.[264] Hence, circulating ANP level was proposed as a diagnostic marker of cardiac dysfunction and as a predictor of survival in HF.[44] However, in this context, ANP has since been superseded by BNP. There is also evidence that midregional (MR) proANP may perform similarly to BNP as a biomarker of ADHF (see section, "Brain Natriuretic Peptide").[473]

The high plasma ANP levels are attributed to increased production rather than to decreased clearance. Although volume-induced atrial stretch is the main source for the elevated circulating ANP levels in HF, enhanced synthesis and release of the hormone by ventricular tissue in response to Ang II and ET also contribute to the elevated levels.[264] Despite the high levels, patients and experimental animals with HF retain salt and water because renal responsiveness to NPs is attenuated.[421] However, infusion of ANP to patients with HF does lead to hemodynamic improvement and inhibition of activated neurohumoral systems. These data are in line with findings that ANP is a weak counterregulator of the vasoconstriction mediated by the SNS, RAAS, and AVP.[422] However, despite the blunted renal response to ANP in HF, elimination of ANP production by atrial appendectomy in dogs with HF aggravated the activation of the vasoconstrictive factors and resulted in marked Na^+ and water retention.[423] These data suggest that ANP plays a critical role in suppressing Na^+-retaining systems and as an important adaptive or compensatory mechanism aimed at reducing pulmonary vascular resistance and hypervolemia.

Brain Natriuretic Peptide. Plasma levels of BNP and N-terminal (NT)–proBNP are elevated in severe HF in proportion to the degree of myocardial systolic and diastolic dysfunction and New York Heart Association (NYHA) classification.[264,424] The extreme elevation of plasma BNP in severe HF stems mainly from increased synthesis by the hypertrophied ventricular tissue, but the atria also contribute.[264,424]

Although echocardiography remains the gold standard for the evaluation of left ventricular dysfunction, plasma levels of BNP and NT-proBNP are reliable markers and, in fact, superior to ANP and NT-proANP for the diagnosis and prognosis of HF.[276] NT-proBNP in particular has high sensitivity, specificity and negative predictive value in patients with an ejection fraction less than 35%. Similar high predictive values are found in patients with concomitant left ventricular hypertrophy, either in the absence of or after myocardial infarction.[276] The added presence of renal dysfunction appears to enhance these predictive values,[425,426] and graded increases in mortality throughout each quartile of BNP levels have been shown in several clinical trials.[276] In addition, elevated plasma BNP (or NT-proBNP) levels and LVEF lower than 40% are complementary independent predictors of death, HF and new myocardial infarction at 3 years after a first infarction. Moreover, risk stratification with the combination of LVEF lower than 40% and high levels of NT-proBNP is substantially better than that provided by either alone.[427] However, even though BNP levels tend to be lower in patients with preserved LVEF than in HF patients with reduced LVEF, the prognosis in patients with preserved LVEF is as poor as in those with reduced LVEF for a given BNP level.[427]

In asymptomatic patients with preserved LVEF, elevated BNP levels are correlated with diastolic abnormalities on Doppler studies. Conversely, a reduction in BNP levels with treatment is associated with a reduction in left ventricular filling pressures, lower readmission rates and a better prognosis; thus, monitoring of BNP levels may provide valuable information regarding treatment efficacy and expected patient outcomes.[428]

Another diagnostic role for BNP is in the distinction of dyspnea caused by HF from that caused by noncardiac diseases. Using specific cut points, NT-proBNP levels are highly sensitive and specific for the diagnosis of acute HF. Levels lower than 300 pg/mL rule out acute HF, with a negative predictive value of 99%. An increased level of NT-proBNP is the strongest independent predictor of a final diagnosis of acute HF. NT-proBNP testing alone was superior to clinical judgment, the National Health and Nutrition Examination score and Framingham clinical parameters alone for diagnosing acute HF; NT-proBNP plus clinical judgment was superior to NT-proBNP or clinical judgment alone.[276]

There is also evidence, albeit of rather low quality, that circulating BNP and NT-proBNP levels can be useful as a guide to therapeutic efficacy of drugs typically prescribed in HF, including RAAS inhibitors, diuretics, digitalis and β-blockers.[429,430] Also, BNP, but not NT-proBNP, levels at 24 and 48 hours after admission for acute decompensated HF (ADHF) predicted both 30-day and 1-year mortality. Predischarge levels of both peptides were predictive of 30-day and 1-year mortality but not 1-year readmission due to HF.[431] In contrast, BNP levels were not helpful in reducing length of hospital stay and costs.[429]

Together, these findings suggest that a simple and rapid determination of plasma levels of BNP or NT-proBNP in HF patients, together with clinical and echocardiographic measures, can be used to assess cardiac dysfunction, serve as a diagnostic and prognostic marker and possibly assist in titrating relevant therapy.[276] However, it should be emphasized that plasma NP levels are affected by age, salt intake, gender, obesity, hemodynamic status and renal function leading to considerable overlap among diagnostic groups.[276,432] Measurement of a panel may enhance the ability of biomarkers to distinguish between cardiac and noncardiac causes of dyspnea.[433]

C-Type Natriuretic Peptide. Like those of ANP and BNP, plasma CNP levels are increased in HF and are directly correlated with NYHA classification, levels of BNP, ET-1, and AM and with pulmonary capillary wedge pressure, ejection fraction, and left ventricular end-diastolic diameter.[434] CNP is synthesized mainly in the kidney,[278] but it is also processed by the myocardium. Overexpression of CNP in the myocardium during HF may be involved in counteracting cardiac remodeling.[434] On the other hand, renal CNP secretion is blunted in HF.[435] In contrast to the diminished physiologic responses to ANP and BNP in animals with HF, CNP elicited twice as much sGC activity as ANP, due to dramatic reductions in NP receptor A (NPR-A) but not NPR-B activity.[434] These findings imply a significant role for NPR-B–mediated NP activity in HF and may explain the modest effects of the NPR-A–selective nesiritide (BNP) treatment in HF.[436]

Overall, current evidence points to a role of CNP either in the peripheral vascular compensatory response in HF or in mitigating the cardiac remodeling characteristic of HF. Elaboration of the exact role of CNP in HF appears crucial for the design of more effective NP analogs than those currently available for the management of HF.

Overall Relationship Between Natriuretic and Antinatriuretic Factors in Heart Failure

The maintenance of Na^+ balance in the initial compensated phase of HF is at least in part due to the elevated ANP and BNP levels.[264] This notion is supported by the findings that, in experimental HF, inhibition of NP receptors by specific antibodies increased renal vascular resistance and decreased GFR, RBF, urine flow, Na^+ excretion and RAAS activation.[437] In addition, NPs inhibited the Ang II–induced systemic vasoconstriction, proximal tubule Na^+ reabsorption, and secretion of aldosterone and ET.[438]

In view of the remarkable activation and ability of NPs to counter the vasoconstrictor/antinatriuretic neurohormonal effects, why then do salt and water retention occur in overt HF? Several mechanisms could explain this paradox:

1. Appearance of abnormal circulating peptides and inadequate secretory reserves in comparison with the degree of HF. Using an extremely sensitive mass spectrometry–based method, altered processing of proBNP1-108 and/or BNP1-32 has been demonstrated, resulting in very low levels of BNP1-32, despite markedly elevated levels of immunoreactive (i.e., total) BNP.[439] Moreover, proBNP1-108 has a lower affinity for the GC-A receptor, which would reduce effector function of BNP.[440]
2. Decreased availability of NPs by downregulation of corin[441] or upregulation of NEP and clearance receptors.[437] With respect to corin, lower plasma levels have been observed in HF patients in parallel with elevated levels of proANP. On the other hand, circulating cGMP levels are elevated in HF, implying enhanced activity of NPs. These apparently contradictory findings could be reconciled by the demonstration of low intracardiac corin levels in an experimental model of HF caused by dilated cardiomyopathy. Moreover,

transfection of the gene encoding for corin into these animals led to a reduction in cardiac fibrosis, improvement in contractility and reduced mortality.[441] Regarding clearance receptors, there is no convincing evidence to date of upregulation in the renal tissue of HF animals or patients, although increased abundance of clearance receptors for NPs in platelets of patients with advanced HF has been reported.[442] In contrast, enhanced expression and activity of NEP in experimental HF is well documented[264] and NEP inhibitors improve the vascular and renal response to NPs in HF (see section, "Specific Treatments Based on the Pathophysiology of Heart Failure").

3. Activation of vasoconstrictor/antinatriuretic factors and renal hyporesponsiveness to ANP. Renal resistance to ANP may be present, even in the early presymptomatic stage of the disease and progresses proportionately as HF worsens.[437] In advanced HF, when RPF is markedly impaired, the ability of NPs to antagonize the renal effects of the maximally activated RAAS is limited.[264] The mechanisms underlying the attenuated renal effects of ANP in HF include Ang II–induced afferent and efferent vasoconstriction, mesangial cell contraction, activation of cGMP phosphodiesterases that attenuate the accumulation of the second messenger of NPs in target organs and stimulation of Na^+-H^+-exchanger and Na^+ channels in the proximal tubule and collecting duct, respectively.[443]
4. Activation of the SNS also can overwhelm the renal effects of ANP. As described earlier, overactivity of the SNS leads to vasoconstriction of the peripheral circulation and of the afferent and efferent arterioles, which causes reduction of RPF and GFR. These actions, together with the direct stimulatory effects of SNS on Na^+ reabsorption in the proximal tubule and loop of Henle, contribute to the attenuated renal responsiveness to ANP in HF. Moreover, the SNS-induced renal hypoperfusion/hypofiltration stimulates renin secretion, thereby aggravating the positive Na^+ and water balance. In rat models of HF, the natriuretic responses to ANP were increased after sympathetic inhibition by low-dose clonidine[405] or bilateral renal denervation.[444] The beneficial effects of renal denervation could be attributed to upregulation of NP receptors and cGMP production.[201]

In summary, the development of renal hyporesponsiveness to NPs is paralleled closely by overreactivity of the RAAS and SNS and represents a critical point in the development of positive salt balance and edema formation in advanced HF.

Nitric Oxide

NO is implicated in the increased vascular resistance and impaired endothelium-dependent vascular responses characteristic of HF.[445,446] The impaired activity is mediated by reduced shear stress associated with the decreased cardiac output, downregulation or uncoupling of eNOS, decreased availability of the NO precursor L-arginine caused by increased activity of arginase and increased levels of the endogenous NOS inhibitor asymmetric dimethyl arginine (ADMA). In addition, inactivation of NO by superoxide ion and alteration of the redox state of sGC through oxidative stress lead to reduced levels of the NO-sensitive form of sGC and, thereby, of its second messenger cGMP.[445,446] Oxidative stress may be further exacerbated by overactivity of counterregulatory neurohumoral systems, such as the RAAS and the release of proinflammatory messengers.[445,446]

Altered activity of the NO-sGC-cGMP system also underlies the regional vasomotor dysregulation of the renal circulation in HF and Ang II may be involved in mediating the impaired NO–dependent renal vasodilation.[447] The resulting imbalance between NO and excessive activation of the RAAS and ET systems could explain some of the beneficial effects of RAAS inhibition.[448] Support for this imbalance concept came from a model of experimental HF in rats overexpressing eNOS in the renal medulla and, to a lesser extent, in the cortex.[420] This eNOS might play a role in the preservation of intact medullary perfusion and could attenuate the severe cortical vasoconstriction. Accumulation of ADMA as exemplified by elevated plasma levels in normotensive HF could also account for the impaired renal hemodynamics in HF. In fact, in a multiple regression analysis, ADMA levels independently predicted reduced RBF.[449]

Locally generated NO by the myocardium is also believed to modulate cardiac function and, thereby, lead to the impaired renal function in HF.[445,446] Alterations in the expression of cardiac NOS isoforms in HF are complex and the functional consequences of these changes depend on a balance among various factors, including disruption of the unique subcellular localization of each isoform and nitroso-redox imbalance.[445,446]

In summary, endothelium-dependent vasodilation is attenuated in various vascular beds in HF. This attenuation may occur as a result of decreased NO levels and downregulation or inhibition of downstream NO signal transduction pathways. These effects may occur directly or via counterregulatory vasoconstrictor neurohumoral mechanisms.

Protaglandins

PG play an important role in maintaining renal function in the setting of the impaired RBF in HF. Renal hypoperfusion, directly or by RAAS activation, stimulates the release of PG that exert a vasodilatory effect, predominantly on the afferent arteriole, and promote Na^+ excretion by inhibiting reabsorption in the TALH and the MCD.[450,451] Evidence for the compensatory role of PG in experimental and clinical HF comes from two sources. First, plasma levels of PGE_2, PGE_2 metabolites, and 6-keto-PGF_1 were higher in HF patients than in normal subjects.[452] Moreover, in both experimental and human HF there is a direct relationship between PRA/Ang II and plasma and urinary PGE_2 and PGI_2 metabolite concentrations.[453] This correlation probably reflects both Ang II–induced stimulation of PG synthesis and PG-mediated increased renin release. A similar counterregulatory role of PG regarding the other vasoconstrictors (e.g., catecholamines, AVP) may also be inferred.

The second approach, which established the protective role of renal and vascular PG in HF, was by using nonsteroidal antiinflammatory drugs (NSAIDs) to inhibit PG synthesis. In various experimental models of HF, this maneuver was associated with elevated urinary PGE_2 excretion, increase in body weight and renal vascular resistance with resultant decrease in RBF, mainly due to afferent arteriolar constriction.[452,454] Urine flow rate declined significantly and serum creatinine and urea rose.[454] Similarly, in patients with HF and hyponatremia, in whom extreme activation of the SNS and RAAS occurred, significant decreases in RBF and GFR

accompanied by reduced urinary Na^+ excretion, followed NSAID treatment.[452,455] These effects were prevented by intravenous PGE_2. Moreover, pretreatment with indomethacin attenuated the captopril-induced increase in RBF.[455] Thus ACEI-associated improvement in renal hemodynamics is mediated in part by increased PG synthesis.

Selective COX-2 inhibitors also lead to a significant worsening of chronic HF and renal function, especially in older patients taking diuretics.[456–458] These deleterious effects are predictable given the relative abundance of COX-2 in renal tissue and, to a lesser extent, in the myocardium of HF patients.[450,459]

In summary, HF can be viewed as a PG-dependent state, in which elevated Ang II levels and enhanced RSNA stimulate renal synthesis of PGE_2 and PGI_2, to counteract the vasoconstrictor neurohumoral stimuli and maintain GFR and RBF. Both COX-2 and nonselective COX inhibitors should be avoided in HF, as they leave the vasoconstrictor systems unopposed, leading to hypoperfusion, hypofiltration, and Na^+ and water retention.[450]

Adrenomedullin

AM seems to play a role in the pathophysiology of HF. HF patients have up to fivefold elevations in plasma levels of AM, in proportion to the severity of cardiac, hemodynamic, and neurohumoral derangements, including pulmonary arterial and capillary wedge pressure, NE, ANP, BNP levels, and PRA.[317,460] Plasma levels of AM decreased with effective anti-HF treatment, such as carvedilol.[461] High levels of midregional proAM are also strong predictors of mortality in HF.[462–464] The origin of the increased circulating AM appears to be the failing ventricular and, to a lesser extent, atrial myocardium.[461,465]

Not only cardiac but also renal AM levels are significantly increased in some, although not all, experimental models of HF.[466,467] The renal upregulation is consistent with the favorable acute and more prolonged (4 days) effects of AM on creatinine clearance, Na^+ and water excretion, as well as on the hemodynamic abnormalities of experimental HF.[461] In contrast, acute administration of AM to HF patients increased forearm blood flow but less so than in normal subjects. Stroke index and dilation of resistance arteries were increased, and plasma aldosterone reduced, but Na^+ and water excretion were unaffected.[461] Collectively, the data suggest that AM acts to balance the elevation in SVR and volume expansion in HF.[317]

Because the favorable effects of AM alone are rather modest, combination therapy with other vasodilatory/natriuretic substances has been attempted. Combinations with BNP, ACEIs, NEP inhibitors and epinephrine resulted in hemodynamic and renal benefits greater than those achieved by each agent alone.[461,468] A small long-term clinical trial of combined ANP and AM in acute decompensated HF demonstrated a significant increase in cardiac output, reductions in MAP, pulmonary arterial pressure, systemic and pulmonary vascular resistance without changing heart rate. In addition, levels of aldosterone, BNP and free radical metabolites fell and Na^+ and water excretion rose.[469]

With the advent of NEP inhibitors, which enhance the activities of vasodilatory/natriuretic peptides, including AM, HF outcomes have been significantly improved. (See section "Specific treatments based on the pathophysiology of HF".)

Urotensin

A role for UT II and its receptor, GPR14, in the pathogenesis of HF has been suggested. First, some, but not all, studies revealed that plasma levels of UT II are elevated in patients with HF in correlation with levels of other markers, such as NT-proBNP and ET-1.[470] Second, strong myocardial expression of UT II in end-stage HF correlates with the degree of cardiac impairment.[470] The upregulated UT II in HF may also have a role in the regulation of renal function in HF. In rat models of HF, UT II acted primarily as a renal vasodilator, apparently by an NO–dependent mechanism.[325] RPF and GFR, but not urinary Na^+ excretion, were also increased. On the other hand, UT II in control rats led to intense renal vasoconstriction, a fall in GFR and Na^+ retention.[325] In light of the contradictory effects of UT II in different conditions, the clinical application of these data will be challenging.

Neuropeptides

Because NPY co-localizes and is released with adrenergic neurotransmitters, high circulating NE levels in HF are accompanied by excessive co-release of NPY and plasma levels are correlated with disease severity in HF patients.[334] In contrast, local myocardial levels, like those of NE, were lower than normal in association with decreased Y1 and increased Y2 receptor expression.[334] Because Y1 receptor activation is associated with cardiomyocyte hypertrophy and Y2 receptor activation with angiogenesis, the data in this model suggest that NPY may simultaneously attenuate the maladaptive cardiac remodeling observed in HF and stimulate angiogenesis in the ischemic heart.[334] Similar patterns of receptor expression change were observed in the kidneys and these were proportional to the degree of renal failure and Na^+ retention.[334] In contrast, administration of NPY in experimental models of HF led to diuresis and natriuresis, probably by increasing ANP release and inhibiting the RAAS.[471] Therefore, in HF, the higher circulating levels, together with the reduced tissue levels of NPY, could be a counterregulatory mechanism to modulate the vasoconstrictive and Na^+ retaining, as well as the cardiac remodeling, effects of the RAAS and SNS. In addition, the downregulation of Y1 receptors, by reducing vasoconstriction, could contribute to reduced coronary and renal vascular resistance. However, once the stage of decompensated HF is reached, the RAAS and SNS effects likely dominate, thereby overwhelming any favorable effects of NPY.

Levels of other neuropeptides, such as catestatin, may be elevated in HF and have been investigated as potential biomarkers, but did not improve diagnostic accuracy over BNP.[471] In summary, laboratory data on neuropeptides in HF have not translated into clinical application.

Apelin

The expression of apelin and its receptor in the kidney and heart and the involvement of the system in the maintenance of water balance suggested a potential role in HF. Circulating levels rise in early HF but decline in later stages of the disease.[472,473] However, this decline correlates poorly with severity of HF, making apelin not useful as a biomarker of HF progression.[474]

The fact that activation of the apelin receptor induces aquaresis, vasodilation and a positive inotropic effect suggested the receptor as a potential therapeutic target in HF. Along these lines, acute IV injection of apelin to

rats with HF following induced myocardial function led to improved systolic and diastolic function. Moreover, more chronic infusion (3 weeks) decreased Ang II–induced cardiac fibrosis and remodeling.[472] In HF patients, acute intravenous apelin increased cardiac output, reduced BP and vascular resistance.[472] No data are yet available on the direct renal effects of apelin in HF, although, by reducing AVP levels and improving the renal microcirculation, apelin might increase aquaresis. In addition, the favorable effects on cardiac function are likely to increase renal perfusion and hence promote diuresis.[475] Stable apelin analogs are currently in development.[476]

Peroxisome Proliferator-Activated Receptors

Peroxisome proliferator–activated receptors (PPARs) are nutrient-sensing nuclear transcription factors, of which PPARγ is of special interest in the context of Na^+ and water retention because of its ligands, the thiazolidinediones (TZD). TZD, by increasing insulin sensitivity, are used for the management of type 2 diabetes mellitus. TZD also decrease circulating free fatty acids and triglycerides, lower BP, reduce levels of inflammatory markers and reduce atherosclerosis. Moreover, they have a beneficial effect on cardiac remodeling in myocardial ischemia.[477] However, a troubling side effect of TZD is fluid retention mainly resulting from PPARγ-induced Na^+ reabsorption mediated by increased ENaC expression in collecting duct epithelium.[478] However, TZD may also augment proximal tubular Na^+ reabsorption by upregulation of apical NEH3, basolateral Na^+-HCO_3^- cotransporter, and Na^+-K^+-ATPase. These effects are mediated by PPARγ-induced nongenomic transactivation of the epidermal growth factor receptor and downstream extracellular signal-regulated kinases.[479] Moreover, by reducing SVR, TZD might lead to higher capillary perfusion pressures and fluid extravasation[478];TZD are also potent VEGF inducers, leading to increased vascular permeability. In clinical terms, the Na^+-retaining effect of TZD translates into an increased incidence of HF[478] and, therefore, they are contraindicated in advanced HF.

Because of the Na^+-retaining and fluid-retaining effects, as well as other concerns related to increased cardiovascular events on the one hand and favorable effects on the myocardium on the other, the exact role of TZD in HF remains a hotly debated subject.[480]

In summary, alterations in the efferent limb of volume regulation in HF include both enhanced activities of vasoconstrictor/Na^+-retaining systems and counterregulatory vasodilatory/natriuretic systems. The magnitude of Na^+ excretion by the kidneys and, therefore, the disturbance in volume homeostasis in HF are largely determined by the balance between these antagonistic systems. In the early stages of HF, the vasodilatory/natriuretic systems are important in the maintenance of circulatory and renal function. However, with the progression of HF, the balance shifts toward dysfunction of the vasodilatory/natriuretic systems and enhanced activation of the vasoconstrictor/antinatriuretic systems. The net result is renal circulatory and tubular alterations that result in avid retention of salt and water, and edema formation.

Renal Sodium Retention in Cirrhosis With Portal Hypertension. Avid Na^+ and water retention commonly occurs in cirrhosis with portal hypertension, leading eventually to ascites, a major cause of morbidity and mortality, with the occurrence of spontaneous bacterial peritonitis, variceal bleeding, and development of the HRS.[481,482] As in HF, the pathogenesis of renal Na^+ and water retention in cirrhosis is related to extrarenal regulation of renal Na^+ and water handling.

A *sine qua non* for the Na^+ and water retention in cirrhosis is the development of intrasinusoidal portal hypertension, with values of portal pressure above 12 mm Hg generally being required. In contrast, presinusoidal hypertension alone, as observed in portal vein thrombosis, is not associated with fluid retention. The hallmark of fluid retention in cirrhosis is peripheral arterial vasodilation, in association with renal vasoconstriction. In the early stages of cirrhosis, vasodilation occurs in the splanchnic vascular bed, with arterial pressure maintained through increases in plasma volume and cardiac output, leading to the so-called "hyperdynamic circulation" (overfilling). At this stage, renal Na^+ and water retention is already evident and aids in the maintenance of EABV.[483] However, as cirrhosis progresses, vasodilation in the systemic and pulmonary circulations becomes prominent and cardiac output can no longer compensate for the progressive decrease in SVR.[484] The resulting relative arterial underfilling[483] and reduced EABV leads to unloading of the arterial high-pressure baroreceptors and other volume receptors, in turn, stimulating the classical compensatory neurohumoral response. This response manifests itself as renal, brachial, femoral, and cerebral vasoconstriction and further Na^+ and fluid retention.[485]

Peripheral Arterial Vasodilation. The initial trigger for splanchnic arterial vasodilation is hepatic tissue damage itself, which leads to venous outflow obstruction, reduced portal venous and increased hepatic arterial blood flow. Moreover, the lower the portal venous flow, the higher the hepatic arterial flow (Fig. 14.11A). These changes lead to increased intrahepatic vascular resistance and sinusoidal pressure.[483] Increased hepatic resistance to portal flow causes the gradual development of portal hypertension, collateral vein formation and shunting of blood to the systemic circulation. As portal hypertension develops, local production of vasodilators—mainly NO, but also carbon monoxide, glucagon, prostacyclin, AM, and endogenous opiates—increases, leading to splanchnic vasodilation.[485] Other contributing factors to splanchnic vasodilation include intestinal bacterial translocation, proinflammatory cytokines and mesenteric angiogenesis.[486,487]

The decreases in SVR associated with low arterial BP and high cardiac output account for the well-known clinical manifestations of the hyperdynamic circulation commonly seen in patients with cirrhosis. These include warm extremities, cutaneous vascular spiders, wide pulse pressure, capillary pulsations in the nail bed[488] and pulmonary vasodilation, associated with the hepatopulmonary syndrome.[489]

Abnormalities of Sensing Mechanisms in Cirrhosis

Nitric Oxide. The NO system is integrally involved in the pathogenesis of the hyperdynamic circulation and Na^+ and water retention in cirrhosis, as well as in hepatic encephalopathy, hepatopulmonary syndrome, and cirrhotic cardiomyopathy.[490] NO is produced in excess by the vasculature of different animal models of portal hypertension, as well as in cirrhotic patients.[490] In animal models, the increased production of NO can be detected at the onset of Na^+ retention

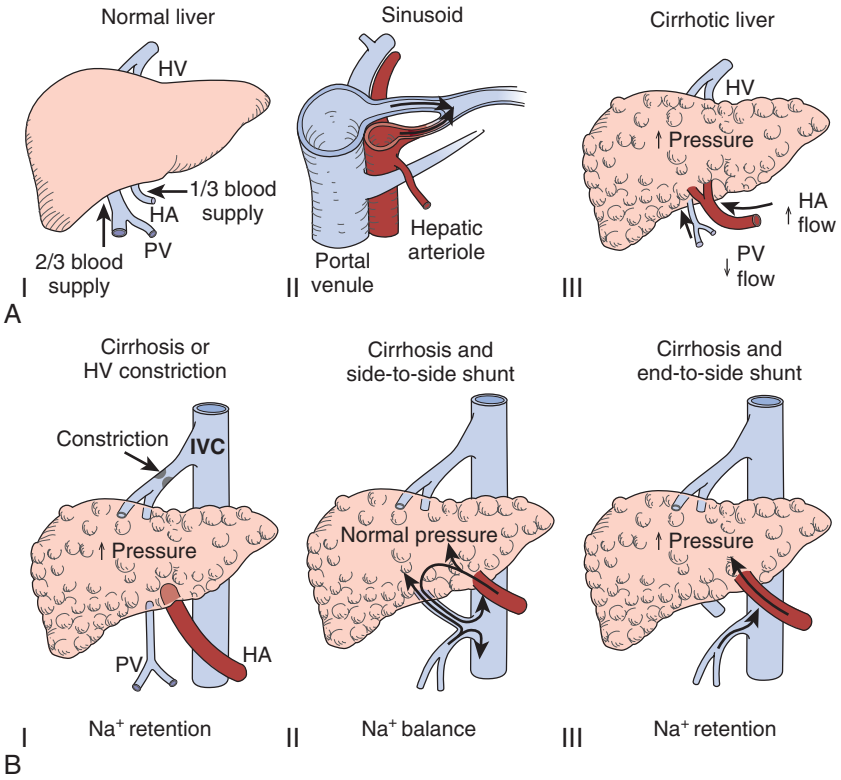

Fig. 14.11 Characteristics of hepatic blood flow. A, Hepatic circulation. I, The normal liver receives two thirds of its blood flow from the portal vein (PV) and the remaining third from the hepatic artery (HA). II, Both the portal venules and hepatic arterioles drain into hepatic sinusoids, but the exact arrangement that allows forward flow of the mixed venous and arterial blood remains unclear. III, Cirrhosis increases intrahepatic vascular resistance and sinusoidal pressure. In addition, PV flow is markedly decreased, and HA flow is unchanged or increased. B, Hepatic vascular hemodynamics and sodium balance. I, Cirrhosis or restriction of HV flow increases intrahepatic vascular resistance and sinusoidal pressure, markedly decreasing PV flow and increasing HA flow. Changes in the physical forces or in the composition of the hepatic blood trigger Na^+ retention and edema formation. II, Insertion of a side-to-side portocaval shunt decreases sinusoidal pressure and maintains mixing of PV and HA blood, irrigating the liver. Under these conditions and despite cirrhosis, there is no Na^+ retention. III, Insertion of an end-to-side portocaval shunt only partially decreases the elevated sinusoidal pressure and prevents mixing of PV and HA blood supplies, inasmuch as the PV blood is diverted to the inferior vena cava (IVC). Under these conditions and, despite normalization of PV pressure, Na^+ retention continues unabated. (Modified from Oliver JA, Verna EC. Afferent mechanisms of sodium retention in cirrhosis and HRS. *Kidney Int*. 2010;77:669–680.)

and before the appearance of ascites and NO has been implicated in the impaired vascular responsiveness to vasoconstrictors.[491] Moreover, removal of the vascular endothelial layer abolishes the difference in vascular reactivity between cirrhotic and control vessels.[488]

Inhibition of NOS has beneficial effects in experimental models of cirrhosis and in cirrhotic patients. By reducing the high NO production to control levels, the hyperdynamic circulation in cirrhotic rats with ascites was corrected and accompanied by a marked increase in Na^+ and water excretion and regression of ascites. Concomitant decreases in PRA, aldosterone and vasopressin concentrations were also observed.[492,493] In cirrhotic patients, the vascular hyporesponsiveness of the forearm circulation to NE could be reversed by NOS inhibition.[494] Inhibition of NO production also corrected the hypotension and hyperdynamic circulation, led to improved renal function and Na^+ excretion and to a decrease in plasma NE levels in these patients. However, in patients with established ascites, NOS inhibition did not improve renal function.[495]

The main enzymatic isoform responsible for the increased systemic vascular NO generation in cirrhosis appears to be eNOS in the systemic and splanchnic circulations.[490] Upregulation of eNOS appears, at least in part, to be caused by increased shear stress as a result of portal venous hypertension with increased splanchnic blood flow.[490] Increased NO release, as well as eNOS upregulation, in the superior mesenteric arteries was found to precede the development of the hyperdynamic splanchnic circulation.[490] In accord with this concept, upregulation of hepatic eNOS (or nNOS) expression in rats with experimental cirrhosis was associated with a decrease in portal hypertension.[496] However, mice with targeted deletion of eNOS alone or combined deletions of eNOS and iNOS, can still develop a hyperdynamic circulation in association with portal hypertension.[493] This suggests that activation of other vasodilatory agents such as PGI_2, endothelium-derived hyperpolarizing factor, carbon monoxide, and AM may participate in the pathogenesis of the hyperdynamic circulation in experimental cirrhosis.[491]

In addition to eNOS, other isoforms may be involved in the generation of the hyperdynamic circulation and fluid retention in experimental cirrhosis. Increased expression of nNOS in mesenteric nerves may compensate partially for eNOS deficiency in eNOS knockout mice and reduce

intrahepatic venous resistance and portal hypertension.[496] Also, splanchnic vasodilation is modestly promoted, possibly by modulating neurogenic NE release.[496] In contrast, the role of iNOS remains controversial; some researchers have shown increased iNOS in the superior mesenteric arteries of animals with experimental biliary cirrhosis but not in other forms of experimental cirrhosis.[496] Specific iNOS inhibition led to peripheral vasoconstriction, but had no effect on portal hypertension.[496] iNOS is primarily regulated at the transcription level by many proinflammatory factors, principally nuclear factor-kappaB (NF-κB), which could be induced by endotoxin, from translocated intestinal bacteria. Interestingly, there is also an interaction between eNOS and iNOS in the vasculature in cirrhosis. Overexpression of eNOS in large arteries results in systemic hypotension and increased blood flow. These effects can be abrogated by activated iNOS in the small splanchnic vessels.[496] Thus, overall, available data indicate a predominant role for eNOS deficiency, with possible modulation by both nNOS and iNOS.

In marked contrast to the increased NO generation in the splanchnic and systemic circulation, NO production and endothelial function in the intrahepatic microcirculation are impaired in cirrhotic rats.[496] The resulting paradoxical increase in intrahepatic vascular resistance is likely to result from contraction of myofibroblasts and stellate cells and mechanical distortion of the vasculature by fibrosis.[496] A second mechanism for the increased intrahepatic vascular resistance could be that the locally decreased NO production shifts the balance in favor of local vasoconstrictors (ET, leukotrienes, TXA_2, Ang II).[497] The increased vascular resistance may also play a role in the pathogenesis of intrahepatic thrombosis and collagen synthesis in cirrhosis.[497]

Several cellular mechanisms have been implicated in the upregulation of splanchnic eNOS and in the downregulation of intrahepatic eNOS. Elevation in shear stress as a result of the hyperdynamic circulation and portal hypertension has already been mentioned and is generally consistent with this well-documented mechanism for upregulating eNOS gene transcription. However, eNOS activity is not only regulated transcriptionally, but also posttranscriptionally, by tetrahydrobiopterin (THB_4) and direct phosphorylation of eNOS protein.[496] Furthermore, circulating endotoxins may increase the enzymatic production of THB_4, thereby enhancing mesenteric vascular eNOS activity.[496]

Potential contributors to intrahepatic eNOS downregulation include interactions with caveolin, calmodulin, heat shock protein 90, eNOS trafficking inducer,[496] disorders of GC activity,[498] and increased levels of the NO inhibitor, ADMA.[498] In fact, ADMA levels correlate with the severity of portal hypertension during hepatic inflammation and levels are higher in patients with decompensated than compensated cirrhosis.[499] Raised ADMA levels have been linked to reduced activity of dimethylarginine dimethylaminohydrolases (DDAHs) that metabolize ADMA to citrulline.[500] Similarly, patients with alcoholic cirrhosis and superimposed alcoholic hepatitis have higher plasma and tissue levels of ADMA, higher portal venous pressures and decreased DDAH expression.[499] However, attempts at pharmacological or genetic upregulation of DDAH to reduce ADMA levels and increase NO in experimental cirrhosis have not translated into improved management of decompensated portal hypertension.[501]

In the final analysis, the relative importance of the various mechanisms involved in the reduced intrahepatic and increased splanchnic and systemic NOS activity in cirrhosis remains to be determined.

Endocannabinoids. Endogenous cannabinoids are lipid-signaling molecules that mimic the activity of Δ9-tetrahydrocannabinol, the main psychotropic constituent of marijuana. *N*-arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol are the most widely studied endocannabinoids that bind the two specific receptors, CB_1 and CB_2. Anandamide also interacts with the vanilloid receptor.[502]

In animal models of cirrhosis, both CB_1 and CB_2 receptors and endocannabinoid production are greatly upregulated and anandamide caused a dose-dependent increase in intrahepatic vascular resistance, especially in the isolated perfused cirrhotic liver. The effect appeared to be mediated by CB_1 receptor-enhanced production of COX-derived vasoconstrictive eicosanoids. Also, the CB_1 receptor antagonist, rimonabant, in cirrhotic rats reversed arterial hypotension, increased hepatic vascular resistance, decreased mesenteric arterial blood flow and portal venous pressure, and prevented ascites formation. The reduction in splanchnic blood flow was enhanced by the vanilloid receptor antagonist, capsazepine. These findings indicate that the transient receptor potential vanilloid type 1 protein and the CB_1 receptor have a dual role in the splanchnic vasodilation characteristic of cirrhosis.[502]

Endotoxin was found to be a major stimulus for endocannabinoid generation in monocytes and platelets of cirrhotic animals. This pathway could operate in patients with advanced cirrhosis in whom elevated circulating endotoxin levels are frequently found. Endocannabinoid production could then trigger splanchnic and peripheral vasodilation, arterial hypotension, and intrahepatic vasoconstriction through activation of CB_1 receptors in the vascular wall and perivascular nerves.[502] The potentially favorable effect of CB_1 receptor blockade on Na^+ excretion opens the possibility of pharmacologic modification of human HRS.

In summary, afferent sensing of volume in cirrhosis is characterized by increased intrahepatic vascular resistance and sinusoidal pressure, decreased portal venous blood flow, and increased hepatic arterial flow. Either changes in intrahepatic physical forces or in the composition of the mixed intrahepatic blood could initiate abnormal Na^+ retention and edema formation (see Fig. 14.11B). Side-to-side portocaval shunt (currently performed by transjugular intrahepatic portovenous shunt insertion) prevents (if inserted before induction of cirrhosis) or corrects (if inserted after induction of cirrhosis) renal Na^+ retention. This outcome could result from decreases in sinusoidal pressure or maintenance of the mixing of portal venous and hepatic arterial blood perfusing the liver. In contrast, it diverts blood to the inferior vena cava (IVC), but only partially decreases sinusoidal pressure and prevents mixing of portal venous and arterial hepatic blood supplies. Although portal venous pressure is normalized, Na^+ retention continues unabated (see Fig. 14.11B). Consequently, end-to-side shunting is no longer used clinically.

Afferent Sensing of Intrahepatic Hypertension. Available data are most consistent with the view that the putative EABV sensor(s) in the hepatic circulation is pathologically activated in cirrhosis, failing to respond to the expanded ECF volume.[87]

The sensing mechanisms likely respond specifically to elevated hepatic venous pressure with increased hepatic afferent nerve activity. The relays for these impulses consist of two autonomic nerve plexuses, surrounding the hepatic artery and portal vein, respectively.[503] These neural networks connect hepatic venous congestion to enhanced renal and cardiopulmonary sympathetic activity.

Occlusion of the IVC at the diaphragm was associated with increases in hepatic, portal and renal venous pressures and resulted in markedly increased hepatic afferent nerve traffic and renal and cardiopulmonary sympathetic efferent nerve activity. Section of the anterior hepatic nerves eliminated the reflex increase in renal efferent nerve activity[503] and hepatic denervation in dogs with IVC constriction increased urinary Na^+ excretion.[89] This effect of hepatic denervation was shown to be mediated by the adenosine A1 receptor in cirrhotic rats.[504]

Apart from the adenosine-mediated hepatorenal reflex, other currently undefined humoral pathways could provide an anatomic or physiologic basis for the primary effects of alterations in intrahepatic hemodynamics on renal function. Despite the wealth of information on hepatic volume sensing, the molecular identity, cellular location of the sensor, and what is sensed remain elusive.

Arterial Underfilling. Several mechanisms have been proposed to account for the development of relative hypovolemia. The first is disruption in normal Starling relationships governing fluid movement in the hepatic sinusoids. Unlike other capillaries, these are highly permeable to plasma proteins. As a result, partitioning of ECF between the intravascular (intrasinusoidal) and interstitial (space of Disse) and lymphatic compartments of the liver is determined predominantly by the ΔP along the length of the hepatic sinusoids. Obstruction of hepatic venous outflow promotes enhanced efflux of a protein-rich filtrate into the space of Disse and results in augmented hepatic lymph formation.[486,505] In parallel, vastly increased hepatic lymph formation is accompanied by increased flow through the thoracic duct.[506] When the rate of enhanced hepatic lymph formation exceeds the capacity for return to the intravascular compartment via the thoracic duct, hepatic lymph accumulates as ascites and the intravascular compartment is further compromised. As liver disease progresses, fibrosis around Kupffer cells lining the sinusoids renders the sinusoids less permeable to serum proteins. Under such circumstances, termed "capillarization of sinusoids," a decrease in oncotic pressure also promotes transudation of ECF within the hepatic lymph space, as in other vascular beds.[507]

Another consequence of intrahepatic hypertension is transmission of elevated intrasinusoidal pressures to the portal vein. This leads to expansion of the splanchnic venous system, collateral vein formation and portosystemic shunting, resulting in increased vascular capacitance and diversion of blood flow from the arterial circuit.[508] Not only splanchnic but also systemic vasodilation occurs and this has been attributed to refractoriness to the vasoconstrictive effects of hormones such as Ang II and catecholamines, although the mechanism remains unknown.[509] Along with diminished hepatic reticuloendothelial cell function, portosystemic shunting allows various products of intestinal metabolism and absorption to bypass the liver and escape hepatic elimination. Among these products, endotoxins are thought to contribute to perturbations in renal function in cirrhosis, either due to intestinal bacterial translocation, stimulating the release of proinflammatory cytokines (e.g., tumor necrosis factor-α [TNF-α] and interleukin-6), secondary to the hemodynamic consequences of endotoxemia, or through direct renal effects.[486]

Levels of conjugated bilirubin and bile acids may become elevated as a result of intrahepatic cholestasis or extrahepatic biliary obstruction. Bile acids directly decrease proximal tubular reabsorption of Na^+, tending to promote natriuresis.[510] This diuretic effect might contribute to the underfilling state in advanced cirrhosis.[510,511]

Hypoalbuminemia in advanced cirrhosis, either as a result of decreased synthesis by the liver or dilution caused by ECF volume expansion, could also contribute to the development of hypovolemia by diminishing COP in the systemic capillaries and hepatic sinusoids.[507] In addition, tense ascites might reduce venous return (preload) to the heart, leading to reduced cardiac output and diminished arterial BP.[484]

Other factors that may also adversely affect cardiac performance include diminished β-adrenergic receptor signal transduction, cardiomyocyte cellular plasma membrane dysfunction, and increased activity or levels of cardiodepressants, such as cytokines, endocannabinoids, and NO. Although the cardiac dysfunction, termed "cirrhotic cardiomyopathy," usually is clinically mild or silent, overt HF can be precipitated by stresses such as liver transplantation or transjugular intrahepatic portosystemic shunt (TIPS) insertion.[484] Finally, intravascular volume depletion in cirrhotic patients may be aggravated by vomiting, occult variceal bleeding and excessive diuretic use, leading to cardiovascular collapse.

Table 14.6 summarizes the various causative factors contributing to underfilling of the circulation in patients with advanced liver disease. In summary, the early stages of compensated cirrhosis are characterized by increased plasma volume, which frequently antedates ascites formation.[512] However, as cirrhosis progresses, EABV decreases, leading to increased neurohumoral activity (RAAS, SNS, and AVP) and severe Na^+ and water retention.

Abnormalities of Effector Mechanisms in Cirrhosis. The efferent limb of volume regulation in cirrhosis is similar to that in HF, consisting of adjustments in glomerular hemodynamics and tubular transport mediated by vasoconstrictor/antinatriuretic forces (RAAS, SNS, AVP, and ET) and

Table 14.6 Factors Causing Underfilling of the Circulation in Cirrhosis

Peripheral vasodilation and blunted vasoconstrictor response to reflex, chemical and hormonal influences
Arteriovenous shunts, particularly in portal circulation
Increased vascular capacity of portal and systemic circulation
Hypoalbuminemia
Impaired left ventricular function, so-called cirrhotic cardiomyopathy
Diminished venous return secondary to advanced tense ascites
Occult gastrointestinal bleeding from ulcers, gastritis, or varices
Volume losses caused by vomiting and excessive use of diuretics

counterbalanced by vasodilator/natriuretic systems (NPs and PG). Tilting the balance toward Na^+ retaining forces leads to renal Na^+ and water retention, as in HF.[485]

VASOCONSTRICTOR AND ANTINATIURETIC (ANTIDIURETIC) SYSTEMS

Renin-Angiotensin-Aldosterone System

As in HF, the RAAS plays a central role in mediating renal Na^+ retention in cirrhosis. Although positive Na^+ balance may already be evident in the preascitic phase of the disease, PRA and aldosterone levels remain within the normal range or may even be depressed at this stage.[513] With progression of the disease, RAAS activation increases in parallel and aldosterone levels are inversely correlated with renal Na^+ excretion in preascitic cirrhotic patients, particularly in the upright position.[513] Moreover, treatment with the ARB, losartan, at a dosage that did not affect systemic and renal hemodynamics or GFR, was associated with a significant natriuresis,[514] likely due to inhibition of the local intrarenal RAAS.[513,514] Indeed, activation of the intrarenal RAAS may precede systemic activation.[515] In addition, losartan caused a decrease in portal venous pressure in cirrhotic patients with portal hypertension.[516] The postural-induced RAAS activation and the beneficial effects of low-dose losartan treatment in preascitic cirrhosis may be explained by splanchnic venous compartmentalization of the expanded blood volume on standing and translocation toward the central and arterial circulatory beds during recumbency.[513]

In contrast, in Na^+-retaining cirrhotic patients with ascites, Ang II inhibition, even at low doses, resulted in decreased GFR and Na^+ excretion.[517] At this stage of the disease, RAAS activation serves to maintain arterial pressure and adequate circulation. Therefore, RAAS blockade may lead to a profound decrease in RPP. This scenario might be important in the pathogenesis of the HRS, which is regularly preceded by Na^+ retention and may be precipitated by a hypovolemic insult. Abnormalities of the renal circulation in HRS include marked diminution of RPF with renal cortical ischemia and increased renal vascular resistance, abnormalities consistent with the known actions of Ang II on the renal microcirculation.[518] In this regard, RAAS activation correlates with worsening hepatic hemodynamics and decreased survival in patients with cirrhosis.[519] Therefore ACEIs and ARBs should be avoided in patients with cirrhosis and ascites.

Evolving knowledge on the ACE2, Ang 1–7, Mas receptor pathway has shed new light on the role of the RAAS in the pathogenesis of Na^+ retention in cirrhosis. In this regard, exogenous Ang 1–7 elicited a marked NO-dependent vasodilatory effect on the Ang-II-evoked vasoconstrictive response in the portal vein of isolated perfused cirrhotic rat liver.[520,521] The data raise the possibility of reducing intrahepatic resistance and portal pressure by targeted upregulation of the alternate RAAS pathway in the liver.[522]

Sympathetic Nervous System

Activation of the SNS is characteristic of cirrhosis and ascites.[523] Circulating NE levels, as well as urinary excretion of catecholamines and their metabolites, are elevated in patients with cirrhosis and usually are correlated with the severity of the disease. Moreover, high levels of plasma NE in patients with decompensated cirrhosis are predictive of increased mortality.[523] The increased NE levels stem from enhanced SNS activity, rather than reduced dissipation, with nerve terminal spillover from hepatic, cardiac, renal, muscular, and cutaneous innervation.[524] Elevated plasma NE levels were correlated closely with Na^+ and water retention in cirrhotic patients.[525] In addition, increased efferent renal sympathetic tone, perhaps due to defective arterial and cardiopulmonary baroreflex control, was observed in experimental cirrhosis.[526,527] This scenario could explain why volume expansion does not suppress enhanced RSNA in cirrhosis.

Concomitant with the increase in NE release, cardiovascular responsiveness to reflex autonomic stimulation may be impaired in patients with cirrhosis.[525] This impairment could be explained partially by increased occupancy of endogenous catecholamine receptors, downregulation of adrenergic receptors, or a defect in postreceptor signaling.[524] Excessive NO-dependent vasodilation alone could, in fact, account for the vascular hyporesponsiveness in cirrhosis.[528,529] Also, enhanced release of NPY may be a compensatory mechanism to counteract splanchnic vasodilation by restoring the vasoconstrictor efficacy of endogenous catecholamines.[524]

The increase in RSNA and plasma NE levels could contribute to the antinatriuresis of cirrhosis by decreasing total RBF, or its intrarenal distribution, or by acting directly on the tubular epithelium to enhance Na^+ reabsorption. Patients with compensated cirrhosis may have decreased RBF and, as the disease progresses, RBF tends to decline further, concomitantly with increased sympathetic activity.[523] Indeed, SNS activation in cirrhotic patients is associated with a rightward and downward shift of the RBF-RPP autoregulatory curve such that RBF becomes critically dependent on RPP. This phenomenon was found to contribute to the development of the HRS. Furthermore, insertion of TIPS to reduce portal venous pressure in patients with HRS leads to a fall in plasma NE levels and to an upward shift in the RBF-RPP curve.[530]

Reflex activation of the splenic afferent and renal sympathetic nerves also controls renal microvascular tone. In portal hypertension, the splenorenal reflex–mediated reduction in renal vascular conductance exacerbates Na^+ and water retention and may eventually contribute to renal dysfunction. Also increased splenic venous outflow pressure resulting from, but independent of, portal hypertension, reflexly activates adrenergic-angiotensinergic and vasodilator mesenteric nerves, and the RAAS. Finally, the spleen itself may be the source of a vasoactive factor.[531,532]

The centrality of SNS overactivity in cirrhosis has been illustrated by the finding that in patients with cirrhosis and increased SNS activity, addition of clonidine or guanfacine to diuretic treatment induces an earlier and enhanced diuretic response, with fewer complications.[533,534] In advanced cirrhosis, increased SNS activity parallels increases in RAAS and AVP activities.[534] This marked neurohumoral activation probably reflects a shift toward decompensation, characterized by a severe decrease in EABV depletion.[535] Overall, the three pressor systems might be activated by the same mechanisms and operate in concert to counteract the low arterial BP and decrease in EABV.[483]

Arginine Vasopressin

Impaired water excretion as a result of nonosmotic release of AVP secondary to decreased EABV is frequent in advanced cirrhosis, leading to water retention with hyponatremia.[536]

Affected patients also have higher PRA and aldosterone levels and lower urinary Na^+ excretion.[483] In rats with experimental cirrhosis, plasma levels of AVP were elevated in association with overexpression of hypothalamic AVP mRNA and diminished pituitary AVP content.[537] Concomitantly increased expression of AQP2, the AVP-regulated water channel in the collecting duct, was significantly diminished by the AVP receptor antagonist, terlipressin, indicating the important role of AQP2 in the water retention associated with hepatic cirrhosis.[538]

As noted earlier, AVP supports arterial BP through its action on the V_1 receptors on VSMC, whereas the V_2 receptor is responsible for water transport in the collecting duct.[539] The availability of selective blockers of these receptors has provided clear evidence for the dual roles of AVP in pathogenesis of cirrhosis.[412,539] Thus, administration of a V_2 receptor antagonist to cirrhotic patients, as well as to rats with experimental cirrhosis, increases urine volume, decreases urine osmolality, and corrects hyponatremia[412,539] (see further discussion in section, "Specific Treatments Based on the Pathophysiology of Sodium Retention in Cirrhosis").

AVP also increases the synthesis of the vasodilatory PGE_2 and PGI_2 in renal and other vascular beds, as well as in the collecting duct. This increase may offset the vasoconstrictive, as well as the hydroosmotic effect of AVP in cirrhosis.[540]

Endothelin

Levels of ET-1 and big ET-1 in plasma, splanchnic, and renal venous beds are markedly elevated in patients with cirrhosis and ascites, as well as in the HRS.[541,542] Levels correlate positively with portal venous pressure and cardiac output and inversely with central blood volume.[542] The rise in ET-1 is accompanied by a reduction in ET-3 levels and the consequently elevated ET-1/ET-3 ratio is associated with a poor outcome of portal hypertension.[543] In animal models of cirrhosis with portal hypertension, ET-A receptor activation and attenuated ET-B receptor repression on the portal vein has been reported.[544] ET-B receptor blockade led to sinusoidal constriction and hepatotoxicity,[545] whereas the dual ET-A and B receptor blocker, tezosentan, had no effect on hepatic blood flow.[546]

In humans with HRS, ET-1 and big ET-1 levels were significantly reduced in portal and renal veins 1 to 2 months after TIPS insertion, with a parallel increase in creatinine clearance and urinary Na^+ excretion.[541] Similar improvements have been observed within 1 week after successful orthotopic liver transplantation.[547] Conversely, temporary occlusion of TIPS by angioplasty balloon inflation led to a transient increase in portal venous pressure, increased plasma ET-1, marked reduction of RPF, and increased intrarenal generation of ET-1.[548]

The importance of the intrarenal ET system has been demonstrated in a rat model of HRS.[549] Plasma ET-1 increased twofold after the onset of liver and renal failure and the ET-A receptor was upregulated in the renal cortex. Bosentan, a nonselective ET receptor antagonist, prevented the development of renal failure when given before or 24 hours after onset of liver injury.[549]

Increased intrahepatic production of ET probably also contributes to the development of portal (and pulmonary) hypertension in cirrhosis through contraction of the stellate cells and a concomitant decrease in sinusoidal blood flow.[550]

To summarize, the hemodynamic changes in cirrhosis with refractory ascites could be related to local ET-1 production by the splanchnic and renal vascular beds. After TIPS and orthotopic liver transplantation, there are improvements in both ET and other vasoconstrictive factors (e.g., RAAS and vasopressin).[551] Therefore, the contribution of the intrarenal ET system relative to other vasoconstrictor hormones in the pathogenesis of the HRS remains speculative.

Apelin

The possible involvement of apelin in the pathogenesis of cirrhosis was suggested by the raised plasma levels[552] and enhanced expression of its receptor in proliferated arterial capillaries directly connected with sinusoids.[553,554] In addition, an apelin receptor antagonist led to a reduction in the raised cardiac index, reversal of the increased total peripheral resistance, and improvement in Na^+ and water excretion in rats with experimental cirrhosis.[552] However, to date no therapeutic role of apelin antagonism in the management of severe HRS has been elucidated, possibly owing to the complex effects of apelin on glomerular hemodynamics.[338]

VASODILATORS/NATIURETICS

Apart from their role in the hyperdynamic circulation characteristic of advanced cirrhosis, vasodilators play an important part in the pathogenesis of renal Na^+ retention. The principal vasodilators involved are NPs and PGs.

Natriuretic Peptides

Atrial Natriuretic Peptide. In recent years, measurements of BNP and NT-proBNP have largely superseded ANP as a biomarker of cirrhosis and portal hypertension. Nevertheless, the role of NPs in the pathogenesis of HRS was largely elucidated through studies on ANP and these are summarized here. Plasma ANP is elevated in cirrhosis at all stages, irrespective of the EABV.[555,556] In the preascitic stage, increased plasma ANP may be important for the maintenance of Na^+ homeostasis, but with progression of the disease, resistance to the natriuretic action of the peptide develops.[555,556] The high levels of ANP mostly reflect increased cardiac release rather than impaired clearance.[557] The stimulus for increased cardiac ANP synthesis and release in early cirrhosis is likely increased left atrial size caused by overfilling of the circulation, secondary to intrahepatic hypertension–related renal Na^+ retention.[558]

In addition to elevated ANP, preascitic patients also had significantly elevated left and right pulmonary volumes, despite normal BP, PRA, aldosterone, and NE levels.[559] High Na^+ intake in these patients resulted in weight gain and positive Na^+ balance for 3 weeks, followed by a return to normal Na^+ balance, thereby preventing fluid retention and the development of ascites.[560] The factors responsible for maintaining relatively high levels of ANP during the later stages of cirrhosis, in association with arterial underfilling, may be related to a futile cycle of mutual interactions between vasoconstrictor/Na^+-retaining and vasodilatory/natriuretic forces. The fact that ANP levels do not increase further as patients proceed from early to late decompensated stages of cirrhosis would be consistent with this explanation. Furthermore, infusion of Ang II mimicked the nonresponder state by causing patients with cirrhosis, who still responded to ANP,

to become unresponsive.[561] This Ang II effect occurred at proximal (decreased distal delivery of Na^+) and distal nephron sites to abrogate ANP-induced natriuresis and was reversible. The importance of distal solute delivery was confirmed using mannitol, which also resulted in an improved natriuretic response to ANP in responders but not nonresponders.[562,563] ANP resistance was also ameliorated by endopeptidase inhibitors, renal sympathetic denervation, peritoneovenous shunting and orthotopic liver transplantation.[564–567]

To summarize, ANP resistance is best explained by an effect of decreased delivery of Na^+ to ANP-responsive distal nephron sites (glomerulotubular imbalance caused by abnormal systemic hemodynamics and activation of the RAAS) combined with an overriding effect of more powerful antinatriuretic factors to overcome the natriuretic action of ANP in the medullary collecting tubule.[568] The latter effect could result from decreased delivery as well as permissive paracrine/autocrine cofactors, such as PGs and kinins.

Brain Natriuretic Peptide and C-Type Natriuretic Peptide. BNP levels are also elevated in cirrhosis with ascites and, like ANP, its natriuretic effect is blunted in these patients.[569–572] Plasma BNP levels may be correlated with cardiac dysfunction[570] and severity of disease and may be of prognostic value in the progression of cirrhosis.[572,573] Plasma CNP levels in cirrhotic preascitic patients, although normal, were directly correlated with natriuresis and urine volume[574] and inversely correlated with arterial compliance but not SVR.[575] These data suggested that compensatory downregulation of CNP occurs in cirrhosis when vasodilation persists and that regulation of large and small arteries by CNP may differ.

In contrast with the preascitic stage, patients with more advanced disease and impaired renal function had lower plasma and higher urinary CNP levels than those with intact renal function. Moreover, urinary CNP was correlated inversely with urinary Na^+ excretion. In patients with refractory ascites or HRS treated with terlipressin infusion or TIPS (see later section, "Specific Treatments Based on the Pathophysiology of Sodium Retention in Cirrhosis"), urinary CNP declined and urinary Na^+ excretion increased 1 week later.[574,665] Thus CNP may have a significant role in renal Na^+ handling in cirrhosis.

Finally, *Dendroaspis* NP levels were found to be increased in cirrhotic patients with ascites, but not in those without and levels were correlated with disease severity.[576] The significance of these findings remains unknown.

Prostaglandins

As noted, PG modulate the hydroosmotic effect of AVP and protect RPF and GFR when the activity of endogenous vasoconstrictor systems is increased. These properties of PG appear to be critical in decompensated cirrhosis with ascites but no renal failure. Such patients excrete more vasodilatory PG than healthy subjects, suggesting that renal production of PG is increased.[577] Similarly, in experimental cirrhosis, there is increased synthesis and activity of renal and vascular PG as well as upregulation of COX-2.[577,578] PGE_2 upregulation in the thick ascending limb of preascitic cirrhotic rats is mediated by downregulation of calcium-sensing receptors (CaSRs). This maneuver resulted in increased expression of the NKCC2, increased Na^+ reabsorption in this segment, and augmented free water reabsorption in the collecting duct. The effects were reversed by the CaSR agonist poly-L-arginine.[579]

Not surprisingly, nonselective COX inhibitors resulted in a significant decrease in GFR and RPF in cirrhotic patients, with or without ascites. The decrement in renal hemodynamics varied directly with the degree of Na^+ retention and neurohumoral activation, so that patients with high PRA and NE levels were particularly sensitive to these adverse effects.[577,578] These negative effects of COX- inhibition appear to be solely COX-1-dependent because selective COX-2 antagonists spare renal function in both human and experimental cirrhosis, even with ascites.[577,578] The favorable renal effect of selective COX-2 antagonists may be indirect and related to hepatic upregulation of COX-2 in that celecoxib can ameliorate portal hypertension by hepatic anti-angiogenic and antifibrotic actions.[580]

In contrast with nonazotemic patients with cirrhosis and ascites, patients with HRS have reduced renal synthesis of vasodilatory PG.[581] However, treatment with intravenous PGE_2 or its oral analogue, misoprostol, did not improve renal function in HRS patients.[582] This PGE_2 resistance may be related to overwhelming neurohumoral vasoconstrictive/antinatriuretic effects and may be crucial in the pathogenesis of HRS.[577]

Integrated View of the Pathogenesis of Sodium Retention in Cirrhosis

Portal hypertension leads to increased intestinal permeability, bacterial translocation, endotoxemia and exposure to bacterial DNA. In turn, hepatic NO and PG increase, leading to splanchnic, then systemic vasodilation, with increased cardiac output, decreased SVR, and arterial pressure ("hyperdynamic circulation"). The resulting imbalance between vascular capacity and plasma volume induces baroceptor and subsequent neurohumoral activation (RAAS, SNS) leading to systemic and renal vasoconstriction, Na^+ retention, and maintained circulation (compensated cirrhosis). In addition, a yet elusive hepatic-derived factor may also act directly to induce renal Na^+ retention (overflow theory). If there is enough compensatory increase in systemic natriuretic factors and renal synthesis of PG and NO, renal function is also maintained. However, as cirrhosis advances and portal hypertension worsens, features of the HRS appear: EABV falls (underfilling), vasoconstrictive/antinatriuretic factors dominate over vasodilatory/natriuretic factors, GFR declines, and Na^+ retention is further exacerbated leading to edema and ascites. Also, at this stage nonosmotic release of AVP becomes prominent, leading to impaired water excretion and hyponatremia.

CLINICAL MANIFESTATIONS OF HYPERVOLEMIA

Apart from the clinical manifestations of the underlying disease, the symptoms and signs of hypervolemia per se depend on the amount and relative distribution of fluid between the intravascular and interstitial spaces. Arterial volume overload is manifested as hypertension, whereas venous overload is characterized by raised jugular venous pressure (JVP). Interstitial fluid accumulation appears as peripheral edema, effusions in the pleural or peritoneal cavity (ascites) or in the alveolar space (pulmonary edema), or a combination of these. If cardiac and hepatic functions are normal and transcapillary Starling forces intact, the excess volume is distributed proportionately throughout the ECF compartments. In this situation, the signs of hypervolemia

will be hypertension and raised JVP. Peripheral edema appears only when interstitial volume overload exceeds 3 L, usually due to ongoing renal Na^+ retention.

When cardiac systolic function is impaired, as a result of myocardial, valvular, or pericardial disease, pulmonary and systemic venous hypertension predominate, and systemic BP may be low as a result of disproportionate fluid accumulation in the venous rather than the arterial circulation. Disruption in transcapillary Starling forces, as found in advanced cardiac and hepatic disease, may lead to fluid transudation into the pleural and peritoneal spaces, manifested as pleural effusions and ascites, respectively.

As already mentioned, advanced cirrhosis or fulminant hepatic failure lead to ascites and oliguric renal failure in the absence of diuretics, nephrotoxins, shock, and without evidence of significant intrarenal pathology. This is referred to as hepatorenal syndrome (HRS). Two subtypes of HRS have been defined. Type 1 is characterized by a rapid decline in renal function (doubling of serum creatinine level to >2.5 mg/dL, or 50% reduction in creatinine clearance to <20 mL/min) over a 2-week period. Typically, an acute precipitating factor can be identified. Type 2 develops spontaneously and progressively over months (serum creatinine level >1.5 mg/dL, or creatinine clearance <40 mL/min). More recently the International Club of Ascites has adopted the AKIN criteria for AKI, a rise of 0.3 mg/dL and/or 50% or more rise from baseline for the diagnosis of HRS. This more sensitive definition allows the possibility for earlier treatment of HRS but has yet to be tested in terms of prognostic significance.[483,535] HRS is discussed in detail in Chapter 28.

DIAGNOSIS

The diagnosis of hypervolemia is usually evident from the clinical history and physical examination. Any combination of peripheral edema, raised JVP, pulmonary crepitations, and pleural effusions is likely to be diagnostic. In the presence of these findings, high BP would suggest renal failure as the cause of hypervolemia, whereas relatively low BP would point to severe HF or advanced cirrhosis as the precipitating factor. In more enigmatic cases, in which dyspnea is the sole complaint and clinical findings are minimal, measurement of plasma biomarkers such as BNP, NTproBNP, and MR-proANP may help distinguish between cardiac and pulmonary causes of the dyspnea.[583,584]

Simple laboratory tests may aid in confirming the clinical diagnosis. An elevated cardiac troponin level is consistent with myocardial damage and high levels may be observed in acute decompensated HF.[585,586] Transaminase levels may be raised in hepatic disease and hypoalbuminemia would be consistent with hepatic cirrhosis or nephrotic-range proteinuria caused by glomerular disease. The latter, of course, is confirmed by appropriate urine testing.

When BP is low, evidence of prerenal azotemia (increased ratio of blood urea nitrogen [BUN] to creatinine) may be found, as in advanced cardiac or hepatic failure (cardiorenal syndrome and HRS, respectively); intrinsic renal failure—proportionate increases in BUN and creatinine—may also ensue (see Chapter 28 for detailed discussion). Low EABV in the presence of hypervolemia is confirmed by a low urine Na^+ concentration or low fractional excretion of Na^+, indicative of secondary renal Na^+ retention.

TREATMENT

Therapy for volume overload can be divided into management of the volume overload itself and prevention or minimization of its recurrence and associated morbidity and mortality. The critical first step is recognition and treatment of the underlying cause of hypervolemia. Thus, when the EABV is significantly reduced, as in cardiac and hepatic failure (or severe nephrotic syndrome), hemodynamic parameters should be optimized. Otherwise, therapy to induce negative Na^+ balance is associated with an enhanced risk for worsening hemodynamic compromise.

Once the EABV is restored, negative Na^+ balance can be induced by dietary Na^+ restriction, diuretics, and extracorporeal ultrafiltration. The degree of hypervolemia and the clinical urgency for Na^+ removal determine which modality should be used. Therefore, in a patient with life-threatening pulmonary edema, immediate intravenous loop diuretics are indicated; if high doses of these drugs do not induce significant diuresis, then extracorporeal ultrafiltration may be life-saving. At the other extreme, a hypertensive patient with mild volume overload and preserved renal function may require only dietary salt restriction and a thiazide diuretic.

Once acute hypervolemia has been controlled, therapy is directed toward the prevention or minimization of further acute episodes and improvement in prognosis. In addition to maintenance diuretic treatment, several strategies, based on the pathophysiologic process of Na^+ retention, are available clinically or are under experimental development.

Sodium Restriction

Until recently, the prevailing belief was that effective management of hypervolemia of any cause must include Na^+ restriction. Without this intervention, the success of diuretic therapy was thought to be limited because the relative hypovolemia induced by diuretics led to compensatory Na^+ retention, increased diuretic dosage, further reduction in EABV and still more renal Na^+ retention. However, this view was challenged by a randomized controlled trial in patients with acute decompensated HF. In this study, no difference in weight loss or clinical stability was observed at 3 days between the group restricted to 800 mg sodium/day and those with a more liberal sodium intake. Moreover, those on the severely sodium-restricted diet were significantly thirstier than their less restricted counterparts.[587] In light of these new data, a reasonable goal is to restrict Na^+ intake to 50 to 80 mmol (approximately 3 to 5 g of salt/day).[588] Because of the generally poor palatability of salt-restricted diets, salt substitutes may be used; however, these preparations usually contain high concentrations of potassium and must be used with caution by patients with renal impairment or those taking potassium-retaining RAAS antagonists.

In hospitalized patients, extra attention must be paid to amounts and types of intravenous fluids administered. A frequent scenario encountered by nephrologists consulted in internal medicine departments is a patient receiving intravenous saline together with high-dose diuretics. The usual rationale offered for this combination is that the saline will expand the intravascular volume while the diuretic will mobilize excess interstitial volume. This logic has no sound physiologic or therapeutic basis, since both modalities operate principally on the intravascular space. Moreover,

the combination of saline and furosemide in HF is associated with adverse outcomes.[589] Furthermore, water restriction is also inappropriate, except in the presence of accompanying hyponatremia (plasma Na^+ <135 mmol/L). On the other hand, intravenous infusion of small volumes of hypertonic saline during diuretic dosing and liberalizing dietary salt intake while continuing to limit water consumption may improve fluid removal in HF patients. Furthermore, less deterioration in renal function, shorter hospitalizations, reduced readmission rates, and even reductions in mortality have been observed.[590,591]

Diuretics

Diuretics are classified according to their sites of action along the nephron and are discussed in detail in Chapter 50. The reader is also referred to a comprehensive review.[592] Diuretics are described briefly here in relation to the treatment of hypervolemia.

Proximal Tubule Diuretics. The prototype proximal tubular diuretic is acetazolamide, a carbonic anhydrase inhibitor that inhibits the reabsorption of sodium bicarbonate. Prolonged use may cause hyperchloremic metabolic acidosis and the drug is usually used in the management of chronic glaucoma rather than for reducing volume overload. Another proximally acting diuretic is the thiazide-like metolazone, which also inhibits the NaCl cotransporter in the distal tubule. The proximal action of metolazone may be associated with phosphate loss greater than that seen with traditional thiazides.[593] In general, metolazone is used as an adjunct to loop diuretics in resistant HF.[594] Mannitol, which also inhibits proximal tubular reabsorption,[595] can be used in combination with furosemide for the management of acute decompensated HF.[596]

Loop Diuretics. This group includes the most powerful diuretics, furosemide, bumetanide, torsemide, and ethacrynic acid. They act by inhibiting transport via the NKCC2 in the apical membrane of the thick ascending limb of the loop of Henle (see Chapter 6).[592] They are used for the treatment of severe hypervolemia and hypertension, especially in stages 4 and 5 of chronic kidney disease. Loop diuretics may lead to hypokalemia, intravascular volume depletion, and worsening prerenal azotemia, especially in older patients and those with reduced EABV. They are also hypercalciuric.[597]

Distal Tubule Diuretics. Diuretics in this segment block the apical NaCl cotransporter and include hydrochlorothiazide, chlorthalidone and metolazone (see also earlier section, "Proximal Tubule Diuretics"). They are typically used as adjuncts to loop diuretics in resistant HF, particularly metolazone. Inhibition of Na^+ reabsorption by diuretics in the proximal tubule (except for carbonic anhydrase inhibitors), loop of Henle, and distal tubule leads to increased solute delivery to the collecting duct. Consequently, potassium and proton secretion are enhanced, which may lead to hypokalemia and metabolic alkalosis.[598] Thiazides are also hypocalciuric.[597]

Collecting Duct Diuretics. Collecting duct (K^+-sparing) diuretics operate by competing with aldosterone for occupation of the mineralocorticoid receptor (spironolactone, eplerenone) or by direct inhibition of the ENaC (amiloride and triamterene).[592] As their alternative name implies, important side effects of this group are hyperkalemia and metabolic acidosis, resulting from concomitant suppression of K^+ and proton secretion. Therefore, they are widely used in combination with thiazide and loop diuretics to minimize hypokalemia. Aldosterone antagonists are especially useful in the management of disorders characterized by secondary hyperaldosteronism, such as cirrhosis with ascites. Moreover, these drugs are cardioprotective and renoprotective via non-epithelial mineralocorticoid receptor blockade (see sections, "Pathophysiology" and "Specific Treatments Based on the Pathophysiology of Heart Failure" in this chapter; also see Chapter 12).

Diuretic Resistance. As noted, when Na^+ retention is severe and resistant to conventional doses of loop diuretics, combinations of diuretics acting at different nephron sites may produce effective natriuresis. Another method for overcoming diuretic resistance is the administration of a bolus dose of loop diuretic to yield a high plasma level, followed by high-dose continuous infusion. Alternately, high doses given intermittently may be successful in reversing diuretic resistance. There appears to be no difference between continuous infusion and repeated bolus administration of high or low doses of furosemide in improvement of shortness of breath or volume of diuresis at 72 hours after admission for acute decompensated HF.[599]

Whichever method is used to treat diuretic resistant hypervolemia, plasma Na^+, K^+, Mg^{2+}, Ca^{2+}, phosphate, BUN, and creatinine should be monitored carefully, and any deviations appropriately corrected. Rarer side effects of diuretics include cutaneous allergic reactions, acute interstitial nephritis (see Chapter 28), pancreatitis, and, rarely, blood dyscrasias.[600]

Extracorpreal Ultrafiltration

On occasion, extreme resistance to diuretics occurs, often accompanied by renal functional impairment. In such cases, effective removal of volume excess may be achieved by ultrafiltration using hemofiltration, hemodialysis, peritoneal dialysis (see Chapter 65), or small devices designed for isolated ultrafiltration (UF).[599] Chronic ambulatory peritoneal dialysis may also reduce hospitalization rates in patients with resistant HF who are not candidates for surgical intervention.[601] However, a randomized controlled trial comparing intensive diuretic therapy with UF for the management of acute decompensated HF with worsened renal function (CARRESS-HF) was halted early because of lack of benefit of UF and an excess of early and late (60 days) adverse events.[602] Moreover, UF was inferior to diuretic therapy in terms of the bivariate primary endpoint of body weight and rise in serum creatinine level at 96 hours after commencement of therapy. Therefore, UF should currently be reserved for those patients with unequivocal diuretic resistance and as part of an algorithmic stepped approach to HF management.[599]

SPECIFIC TREATMENTS BASED ON THE PATHOPHYSIOLOGY OF HEART FAILURE

Because the clinical situation of an HF patient at any given time depends on the delicate balance between vasoconstrictor/antinatriuretic and vasodilator/natriuretic factors, any treatment that can tip the balance in favor of the latter should be efficacious. Thus, treatment is aimed at

either pharmacologically increasing natriuretic or reducing antinatriuretic mechanisms. The principal approaches include reducing RAAS or SNS activity or increasing NP activity.

INHIBITION OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

Based on the maladaptive actions of locally produced or circulatory Ang II, numerous studies have shown that ACE inhibition and ARBs improve renal function, cardiac performance and life expectancy of HF patients.[234,603] A small decline in GFR is occasionally observed as a result of blockade of Ang II–induced preferential efferent arteriolar constriction, which leads to a sharp fall in glomerular capillary pressure, but this is usually not clinically significant. Because patients with HF cannot overcome the Na^+-retaining action of aldosterone, blockade of the latter by spironolactone or eplerenone induces substantial natriuresis in these patients.[603]

Overall, the effect of RAAS inhibition on renal function in HF depends on a multiplicity of interacting factors. On the one hand, RBF may improve as a result of lower efferent arteriolar resistance. Systemic vasodilation may be associated with a rise in cardiac output. Under such circumstances, reversal of the hemodynamically mediated effects of Ang II on Na^+ reabsorption would promote natriuresis. Moreover, RAAS inhibition could facilitate the action of NPs to improve GFR and enhance Na^+ excretion. On the other hand, Ang II–induced elevation of the single-nephron filtration fraction facilitates preservation of GFR in the presence of diminished RPF. In patients with precarious renal hemodynamics, a fall in systemic arterial pressure below the autoregulatory range, combined with removal of the Ang II effect on glomerular hemodynamics, may cause severe deterioration of renal function. The net result depends on the integrated sum of these physiologic effects, which, in turn, depends on the severity of HF (Table 14.7).

Table 14.7 Renal Effects of Renin-Angiotensin-Aldosterone System Inhibition in Heart Failure

Factors Favoring Improvement in Renal Function
Maintenance of Na^+ balance
Reduction in diuretic dosage
Increase in Na^+ intake
Mean arterial pressure >80 mm Hg
Minimal neurohumoral activation
Intact counterregulatory mechanisms
Factors Favoring Deterioration in Renal Function
Evidence of Na^+ depletion or poor renal perfusion
Large doses of diuretics
Increased urea/creatinine ratio
Mean arterial pressure <80 mm Hg
Evidence of maximal neurohumoral activation
AVP-induced hyponatremia
Interruption of counterregulatory mechanisms
Coadministration of prostaglandin inhibitors
Adrenergic dysfunction (e.g., diabetes mellitus)

AVP, Arginine vasopressin.

In addition to promoting Na^+ retention, the RAAS contributes to vascular and cardiac remodeling by inducing perivascular and interstitial fibrosis in HF.[234,604] In accordance with this mechanism, the addition of small doses of aldosterone inhibitors to standard therapy, including ACEIs or ARBs, substantially reduces mortality rate and morbidity in HF patients.[234,603] In contrast, combinations of ACEI and ARB are not useful and, often, are detrimental, owing to increased risk of hypotension, AKI, and hyperkalemia.[604] However, the combination of RAAS inhibitor, aldosterone inhibitor β-blocker, and low-dose loop diuretics is associated with a better prognosis than chronic use of increasing loop-diuretic dose alone.[605]

Finally, all patients receiving one or more RAAS inhibitors require careful follow-up for detection and management of hyperkalemia, particularly those with renal dysfunction.[604]

β-BLOCKADE

Insofar as β-blockade is now the standard of care in the management of HF, this review would not be complete without mention of these drugs. However, because their effect in HF is not directly related to Na^+ and water, this important therapy is not elaborated further in this chapter. The reader is referred to a recent meta-analysis for up-to-date information.[606]

NITRIC OXIDE DONOR AND REACTIVE OXYGEN SPECIES/PEROXYNITRATE SCAVENGERS

Because NO signaling is disrupted in HF, achieving NO balance by NO donors or selective NOS inhibitors could be important for correcting the pathophysiologic process of HF.[607] In this regard, the beneficial effects of combined isosorbide dinitrate (NO donor) and hydralazine (reactive oxygen species and peroxynitrite scavenger) therapy, particularly in African-American patients, are well-established.[608] Newer methods for enhancing NO activity include NOS and GC stimulators and inhibitors of GC breakdown.[607] Relaxin-mediated and waon-mediated NOS stimulation, in particular, are currently being tested in a phase 3 clinical trial. Relaxin mediates NO production both immediately via stimulation of eNOS and chronically via increased expression of iNOS. Treatment of acute HF with serelaxin, recombinant relaxin-2, was associated with dyspnea relief and reduced 180-day but had no effect on hospital readmission. Serelaxin treatment was well tolerated and safe.[609] Further trials are in the pipeline.

Waon or "soothing warm" therapy, which causes vasodilation via eNOS stimulation, is a thermal treatment developed in Japan and shown in a recent clinical trial to improve NYHA class and exercise tolerance in chronic HF.[610] Waon also enhanced the favorable actions of RAAS inhibition and reduced SNS activity.[611]

An additional strategy exploits upregulation of nNOS in the paraventricular nucleus and a reduction in sympathetic outflow. This observation raised the potential for nNOS upregulation by ACE2 therapy in HF.[612,613]

ENDOTHELIN ANTAGONISTS

Acute ET nonselective antagonists decrease vascular resistance and increase cardiac index and cardiac output in HF. However,

both short- and long-term clinical trials with ET-A receptor antagonists have demonstrated no benefits, but serious adverse events, including fluid retention, increased need for hospitalization, hepatic dysfunction, and mortality in HF patients.[614] These disappointing results may be explained by the observation that ET-A receptor antagonism in experimental HF further activates the RAAS in association with sustained Na^+ retention.[279] Moreover, the increased cardiac, pulmonary, and renal ET-1 production in HF, together with the marked vasoconstrictor and mitogenic properties of the molecule, suggest that ET-1 exacerbates Na^+ retention directly and indirectly by aggravating renal and cardiac functions, respectively.[279] In addition, nonspecific ET-B receptor blockade could have exacerbated cardiac remodeling. Given the antiproliferative effect of ET-B1–receptor activation and the detrimental vasoconstrictor effect of ET-B2 stimulation, combined ET-B1 stimulation and ET-B2 blockade was recently proposed for control of the remodeling associated with postischemic HF.[417] Concomitant ET-A blockade would help to prevent the undesired effects of unopposed ET-1 activity and exploit the established therapeutic effects of ET-A antagonists.[417] In this regard, a recent report that dual ET-A/ET-B receptor inhibition improves echocardiographic parameters in a mouse model of HF with preserved ejection fraction (HfpEF) is encouraging.[615] The favorable effects occurred by abrogating adverse cardiac remodeling and reducing stiffness. Additional studies on the role of dual ET-1 receptor antagonists in patients with HfpEF are eagerly awaited.

NATRIURETIC PEPTIDES

As noted previously, circulating NP levels are elevated in HF in proportion to disease severity, but renal actions of NPs are attenuated in severe HF. Nevertheless, elimination of NP action using NPR-A blockers or surgical removal of the atrium disrupted renal function and cardiac performance in experimental HF.[616] Conversely, increasing circulating levels of NPs (BNP > ANP) by intravenous administration improved general clinical status,[617] reduced pulmonary arterial and capillary wedge pressures, right atrial pressure and SVR, with improved cardiac output, systemic BP and diuresis. In parallel, plasma NE and aldosterone levels were suppressed.[618] However, these beneficial effects have not translated into clinical efficacy, with several controlled studies showing minimal natriuretic effects of BNP (nesiritide) compared with placebo.[264] Furthermore, serious adverse events of nesiritide treatment were seen, including dose-related hypotension, worsening renal function, and possible increased risk of death.[264] Finally, in a randomized controlled trial comparing nesiritide or low-dose dopamine as add-on therapy to intravenous furosemide in patients with HF and renal dysfunction, no improvement in decongestion or renal function was observed with either combination therapy over furosemide alone.[619] Similarly, ularitide, the pharmacological equivalent of the NP, urodilatin, as add-on therapy did not affect a clinical composite endpoint or reduce long-term cardiovascular mortality, despite significant reductions in NT-proBNP levels (TRUE-AHF trial). Therefore, the therapeutic role of NP in HF remains unclear and novel approaches are being explored. These include strategies to convert the prohormone into its active form might be efficacious, CNP analogs and designer chimeric peptides such as CD-NP, consisting of CNP and part of DNP.[620]

NEUTRAL ENDOPEPTIDASE INHIBITORS AND VASOPEPTIDASE INHIBITORS

Correcting the imbalance between the RAAS and NP systems can also be achieved by inhibiting the enzymatic degradation of NPs by NEP. In both experimental models and clinical trials NEP inhibitors enhanced plasma ANP and BNP levels in association with vasodilation, natriuresis, diuresis, reduced cardiac preload and afterload.[439] However, initial favorable hemodynamic, neurohormonal, and renal effects of NEP inhibitors in HF patients[621,622] were not confirmed in later studies. These showed enhanced RAAS activation, increased ET-1 levels and attenuated renal and hemodynamic improvement.[439] On the basis of these findings, combined RAAS and NEP (vasopeptidase) inhibitors were designed and, indeed, initially shown to have more favorable hemodynamic and renal effects, such as preserved GFR, than each treatment alone.[439]

However, randomized clinical trials indicated that neither vasopeptidase inhibitors nor NEP inhibitors as add-on therapy to RAAS inhibition were more effective than RAAS inhibitors alone in the treatment of HF. Furthermore, the combination was associated with a significantly increased incidence of severe angioedema.[439] Possible reasons for the failure of NEP inhibitors include disproportionate increase in RAAS and ET activity over time, the development of tolerance with chronic treatment and downregulation of NP receptors in response to degradation of NEP inhibitors. The increased incidence of angioedema with vasopeptidase inhibition may result from excessive accumulation of bradykinin or inhibition of aminopeptidase P.[439]

The challenge of preventing angioedema led to the development of combined ARB-NEP inhibition since ARBs do not disrupt bradykinin metabolism. Three trials in HF with reduced EF, IMPRESS, OVERTURE and PARADIGM-HF, compared combined NEP/RAS inhibition with RAS inhibition alone and reported clinical outcomes. The pooled hazard ratio (HR) for all-cause death or HF hospitalization and all-cause mortality was significantly reduced in patients receiving combined NEP/RAS inhibition in all three trials. Combined NEP/RAS inhibition compared with ACE inhibition was associated with more hypotension, but less renal dysfunction and hyperkalaemia.[623] These results pave the way for a paradigm shift in the management of severe HF.[624]

VASOPRESSIN RECEPTOR ANTAGONISTS

AVP receptor antagonists (vaptans) are small, orally active, nonpeptide molecules that lack agonist effects and display high affinity for and specificity to their corresponding receptors.[412,625] Highly selective and potent antagonists for the V_{1A}, V_2, and V_{1B} receptor subtypes and mixed V_{1A}/V_2 receptor antagonists are now available.[412] In clinical trials and experimental models of compensated and decompensated HF with hyponatremia, V_2 receptor–specific vaptans produced hemodynamic improvement, with transient decrease in SVR and increased cardiac output. Water diuresis caused decreases in body weight and edema, hyponatremia was corrected, and RBF and renal function stabilized.[412,625] Dyspnea lessened in some but not all patients. Both tolvaptan, a V_2 selective

receptor antagonist, and conivaptan, a nonselective V_{1A}/V_2 receptor antagonist (both US Food and Drug Administration [FDA]-approved for use in HF) induced these favorable effects after treatment for up to 60 days. Similar results were reported for tolvaptan in stable class II or III HF.[626,627] Positive effects were observed, regardless of whether LVEF was less or greater than 40%.[412,625] In patients with LVEF less than 40%, a single dose of conivaptan reduced pulmonary capillary wedge and right atrial pressure in comparison with placebo; cardiac index, pulmonary arterial pressure, systemic, and pulmonary vascular resistance, systemic arterial pressure and heart rate were unaffected. Urine output rose and osmolarity fell significantly.[628,629] However, functional capacity, exercise tolerance, and overall quality of life were unchanged.[627]

The effect of vaptans on survival is unclear. In one study, mortality rate two months after discharge was halved in patients treated with tolvaptan who had an increase in serum Na^+ of 2 mmol/L or more, compared with those with no increase in serum Na^+.[630] In contrast, in the other trial, tolvaptan given for 60 days had no effect on survival after 9 months of follow-up.[627] Lixivaptan, another V_2 selective antagonist, was associated with an excessive mortality rate, despite favorable clinical and laboratory effects similar to those of tolvaptan.[631] Hence, the FDA did not approve lixivaptan for use in HF.[632]

Tolvaptan did not adversely affect cardiac remodeling or LVEF after 1 year of treatment in patients receiving optimal, background therapies for HF (RAAS and β-blockers).[633] In contrast, in a murine model of HF, tolavaptan was associated with increased remodeling and mortality, which was prevented by concomitant furosemide therapy.[634] Therefore, vigilance will need to be exercised in the long-term use of tolvaptan in HF.

In view of the impressive diuretic effect of vaptans, there is considerable interest in a potential loop diuretic–sparing effect, as mentioned previously. To summarize results to date, tolvaptan compared with furosemide alone or in combination, was not associated with improvement in dyspnea 24 hours after admission for acute HF, with or without preserved LVEF, despite greater diuresis and weight loss.[635–638] Tolvaptan was associated with better preserved renal function and serum electrolytes.[635–637] A Japanese multicenter clinical effectiveness of tolvaptan in patients with acute decompensated heart failure and renal failure (AQUAMARINE) study, in which tolvaptan is being compared with standard furosemide therapy in HF patients with renal impairment is ongoing (University Medical Information Network [UMIN] clinical trial registry number, UMIN000007109).[639]

The adverse effects of vaptans appear to be relatively few and, overall, minor. Thirst and dry mouth are not unexpected; hypokalemia occurs in less than 10% of recipients similar to loop diuretics. In the largest study to date, involving more than 4000 patients, there was a small but significant increase in strokes but a small but significant reduction in myocardial infarctions.[406]

In summary, AVP receptor antagonists appear to have important short-term benefits in the treatment of advanced HF. However, the question of improvement in longer term prognosis, especially the high mortality among HF patients already receiving optimal treatment with RAAS inhibitors (±NEP inhibition) and β-blockers remains open.[640]

SODIUM-GLUCOSE TRANSPORTER-2 INHIBITORS

Sodium-glucose transporter-2 (SGLT-2) inhibitors decrease proximal tubular Na^+ and Cl^- reabsorption, leading to a reset of TGF. This induces plasma volume contraction without activation of the SNS, decreases glomerular hyperfiltration leading to better long-term renal preservation, and improves diuretic and natriuretic responses to other diuretics. Moreover, SGLT-2 inhibitors might improve the efficiency of myocardial energetics by offering β-hydroxybutyrate as an attractive fuel for oxidation and increase hematocrit improving oxygen transport. Finally, decreased vascular stiffness and improved endothelial function are observed with the use of SGLT-2 inhibitors in diabetes. These multiple nonglycemic effects make SGLT-2 inhibitors preferred glucose-lowering drugs in diabetic patients with HF and raise the possibility for use in HF without diabetes.[641]

SPECIFIC TREATMENTS BASED ON THE PATHOPHYSIOLOGY OF SODIUM RETENTION IN CIRRHOSIS

The prognosis of type 1 HRS is dismal. The mortality rate, which rises with increased AKI stage, is as high as 80% in the first 2 weeks and only 10% of patients survive longer than 3 months. Therefore specific aggressive therapy in these patients is usually indicated in preparation for liver transplantation.[483,535] Type 2 HRS has a better prognosis. Median survival is approximately 6 months.[483,535] Aggressive management for HRS includes pharmacologic therapy, TIPS insertion, renal replacement therapy (RRT), and liver transplantation. The management of precipitating factors is discussed elsewhere.

PHARMACOLOGIC TREATMENT

The goals of pharmacologic therapy are to reverse the functional renal failure and serve as a bridge to liver transplantation. Earlier attempts to reverse renal vasoconstriction with low-dose dopamine, the PGE_1 analog misoprostol, RAAS and ET inhibitors were unsuccessful and even detrimental.[483,485,519] Therefore current treatment is based on reversal of splanchnic and systemic arterial vasodilation, AVP-induced water retention and hyponatremia.

SYSTEMIC VASOCONSTRICTORS

Three groups of vasoconstrictors have been studied—vasopressin V_1 receptor analogs, a somatostatin analog, and α-adrenergic agonists.[483,535]

Vasopressin V_1 Receptor Analogs

These agents cause marked vasoconstriction through their action on V_1 receptors in the arterial smooth muscle wall. They are used for the management of acute variceal bleeding in cirrhosis and portal hypertension. Ornipressin infusion in combination with volume expansion led to a remarkable increase in RPF, GFR, and Na^+ excretion in almost 50% of treated patients. However, significant ischemic adverse effects in 30% of patients led to the abandonment of this drug.[642]

Terlipressin, without significant adverse ischemic reactions, is now the vasoconstrictor drug of choice. Terlipressin and albumin in type 1 HRS is associated with a significant improvement in GFR, increase in arterial pressure, near-normalization of neurohumoral levels, and reduction of serum creatinine level in 40% to 50% of cases.[483,535,643] Response rates to terlipressin in type 2 HRS are better than those in type 1, with more than 80% survival at 3 months.[481,538] Despite relapses in 50% of cases, reintroduction of therapy produces a further response.[481,538]

The results of several randomized controlled trials, 2 meta-analyses and several Cochrane reviews, have shown that the combination of terlipressin and albumin is clearly superior to albumin or terlipressin alone in improving renal function and survival in both types 1 and 2 HRS.[644–647] No difference in efficacy between boluses and infusions was found, although the latter was associated with fewer adverse effects.[648] The optimum duration of terlipressin therapy is not clear, but it is usually given until serum creatinine levels decrease to less than 1.5 mg/dL or for a maximum of 15 days. Whether extending therapy beyond 15 days will add any benefit is unknown. A key prognostic target after combined terlipressin-albumin therapy may be a rise in MAP of 10 mm Hg or more; these patients required less dialysis and were more likely to attain liver transplantation than those with smaller responses in MAP. This response was associated with better short-term, long-term, and transplant-free survival.[649] Moreover, pretransplantation normalization of renal function by terlipressin resulted in similar posttransplantation outcomes to those of patients with normal renal function.[650]

In patients with HRS undergoing living related donor liver transplantation, terlipressin intra- and postoperatively is associated with significant increases in MAP, SVR, and renal function. Significantly decreased heart rate, cardiac output, hepatic and renal arterial resistive indices, portal venous blood flow, and use of vasoconstrictor drugs were seen during reperfusion.[651,652] To summarize, terlipressin and albumin infusion may be appropriate only for patients awaiting or already undergoing liver transplantation and a favorable hemodynamic response is associated with a better overall prognosis both before and after liver transplantation.

Unfortunately, terlipressin is currently unavailable in the United States and Canada and the results of a phase 3 clinical trial, comparing terlipressin with placebo (mannitol) in these countries, are, therefore, eagerly awaited (CONFIRM study, ClincialTrials.gov NCT 02770716).

Somatostatin Analogs and α-Adrenergic Agonists

Octreotide is an α-adrenergic agonist that inhibits the release of glucagon and other vasodilator peptides. Octreotide with albumin infusion or midodrine had no effect on renal function in HRS. However, both agents in combination with albumin infusion led to a significant improvement in renal function and survival in types 1 and 2 HRS in comparison with controls.[653–655] However, no effect of this combination on outcomes of subsequent liver transplantation was observed.[656] Attempts to prevent relapse of type 2 HRS with midodrine after terlipressin-induced improvement were also unsuccessful.[657] Moreover, terlipressin plus albumin was significantly more effective than octreotide, midodrine and albumin, in terms of improved renal function, complete reversal of HRS and 90-day survival.[658] Finally, NE was compared with terlipressin in the treatment of type 1 HRS in three randomized controlled trials. Responses to both agents were similar in terms of MAP and renal function. Cumulative survival and adverse event rates were not significantly different between the two drugs. NE is less expensive than terlipressin, but, unlike terlipressin, NE administration requires cardiac monitoring. Therefore, total costs might be similar for the two therapies.[483,535]

VASOPRESSIN V_2 RECEPTOR ANTAGONISTS

As noted, hyponatremia, caused by persistent nonosmotic AVP-induced water retention, is often seen in HRS and is a marker of poor prognosis.[659] Therefore, attaining a water diuresis and reversing hyponatremia using V_2 receptor antagonists are potentially important therapeutic goals. Several controlled studies, using lixivaptan, tolvaptan, and satavaptan have shown modest improvements in serum Na^+, but without effect on mortality or adverse events.[659–661] Satavaptan in combination with diuretics was associated with a higher mortality rate than either agent alone, leading to early termination of one study.[662] The specific role of satavaptan in the increased mortality was uncertain, given that most deaths were due to complications of cirrhosis. Overall, the effects of vaptans in HRS appear to be modest; this may be explained by avid proximal tubular solute reabsorption leading to reduced distal delivery or by V_2 receptor–independent pathways of water retention.[410]

TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT

The efficacy of TIPS in the reduction of portal venous pressure in patients with cirrhosis and refractory ascites with type 1 or 2 HRS has been demonstrated in several small cohort and controlled studies.[481,663] Significant improvement in renal hemodynamics, GFR, and vasoconstrictive neurohumoral factors were observed in most patients.[519] Liver transplant-free survival also was markedly improved[663] and might be better than that reported for terlipressin and albumin infusion.[664] Some patients who were dialysis dependent were able to discontinue this treatment after TIPS insertion. Moreover, liver transplantation was performed in two patients when the medical condition that precluded transplantation had resolved after TIPS insertion.[665] However, TIPS seems to increase wait-time for liver transplantation and days in hospital after transplantation, although without effect on 30-day mortality.[666]

TIPS appears to exert its favorable effects by reducing sinusoidal hypertension with suppression of the putative hepatorenal reflex (see earlier), improvement of EABV by shunting portal venous blood into the systemic circulation, or amelioration of cardiac dysfunction.[551]

TIPS is currently recommended in the setting of refractory ascites, particularly in type-2 HRS, but is contraindicated in severe liver failure, severe intrinsic renal failure (serum creatinine >3 mg/dL), HF, severe porto-pulmonary hypertension, recurrent or persistent severe hepatic encephalopathy despite adequate treatment, and uncontrolled sepsis.[667]

RENAL AND LIVER REPLACEMENT THERAPY

The benefits of conventional hemodialysis and continuous RRT, in terms of prolonging survival, are dubious,[668] and

morbidity resulting from these therapies is high.[669] In oliguric patients awaiting liver transplantation who do not respond to vasoconstrictors or TIPS and who develop diuretic-resistant volume overload, hyperkalemia, or intractable metabolic acidosis, RRT may be considered. In view of the dismal prognosis of HRS, especially type 1, RRT should probably be used solely as a bridge to transplantation.

In contrast with conventional RRT, albumin-dialysis offers the potential advantage of removing albumin-bound, water-soluble vasoactive agents, toxins, and proinflammatory cytokines. Molecules relevant to the pathogenesis of advanced cirrhosis include bile acids, TNF-α, interleukin-6, and NO.[485] Three albumin dialysis systems, molecular adsorbent recirculating system (MARS), fractionated plasma separation, adsorption and hemodialysis (Prometheus system), and single-pass albumin dialysis, have been examined in randomized trials for supportive treatment of liver failure. These therapies enable partial recovery of hepatic function and provide simultaneous RRT. They lead to a decrease in renal vascular resistance and improvement in the splenic resistance index, a parameter related to portal resistance. The hemodynamic effects are thought to be mediated by clearance of vasoactive substances.[670] Bilirubin levels, encephalopathy grade, serum creatinine, and serum Na^+ all improved.[671] In a recent meta-analysis, albumin dialysis achieved a significant net decrease in serum total bilirubin level relative to standard medical therapy but not in serum ammonia or bile acids. Albumin dialysis achieved an improvement in hepatic encephalopathy relative to standard medical therapy but had no effect on survival. Bearing in mind the limited reporting of adverse events, no major safety concerns were detected. Extracorporeal nonbiologic liver support system should at present probably be considered only as a bridge to transplantation.[672]

LIVER TRANSPLANTATION

Liver transplantation is the treatment of choice for HRS because it offers a cure for the liver disease and renal dysfunction. Large case series indicate that survival is significantly lower in transplant recipients with HRS (types 1 and 2) both immediately postoperatively and long-term than in those without the syndrome (3-year survival rates of 60% vs. 70% to 80%) but may be improved by the bridging therapies described previously.[673,674]

With respect to renal function after transplantation in patients with HRS, GFR decreases in the first month owing to the stress of surgery, infections, immunosuppressive therapy, and other factors. Despite the prompt correction of hemodynamic and neurohumoral parameters, GFR recovers incompletely (30 to 40 mL/min at 1 to 2 months) and often remains impaired over the long term. Although dialysis requirement in the first month is greater in patients with HRS than without HRS (35% vs. 5%), duration of dialysis pretransplantation does not influence renal recovery posttransplantation. Predictors of renal recovery included younger recipient and donor, nonalcoholic liver disease, and low posttransplantation serum bilirubin.[675] Overall, the rate of posttransplantation reversal of HRS has been estimated to be no greater than 58%.

Unsurprisingly, the 5-year risk of ESRD posttransplantation in patients with HRS is significantly higher than in those without HRS. Moreover, combined simultaneous liver and kidney transplantation (SLKT) apparently offers no greater benefit over liver transplantation alone with respect to early posttransplantation kidney function. However, better 5-year survival rates are more likely after SLKT than after liver transplantation alone in patients with pretransplantation serum creatinine levels greater than 2.2 mg/dL, irrespective of dialysis requirement.[674] More studies are needed to enable a rational decision about who should receive SLKT, rather than liver transplants alone. At present, SLKT should probably be reserved for patients who are dialysis dependent for 8 weeks or more pretransplantation.[485,674,676]

The introduction of Model of End-stage Liver Disease (MELD) scores for the allocation of livers has increased the number of transplantations in patients with impaired renal function, but more SLKTs are also being performed.[677] On the other hand, a favorable response to vasoactive therapy reduces the MELD score and may lead to a paradoxical delay in liver transplantation.[678] Therefore, only pretreatment MELD scores should be used to predict potential outcomes of liver transplantation in HRS.[481] A further paradox is that patients with type 2 HRS have lower MELD scores than those with type 1 HRS, resulting in the former being ascribed a lower priority for transplantation and a longer time on the waiting list. This delay is associated with higher mortality, especially in the presence of hyponatremia and persistent ascites.[677] Therefore, the criteria for donor allocation need to be modified to incorporate these factors into the final score for prioritization.[481]

For a general summary of all aspects of liver transplantation, the reader is referred to several excellent recent reviews.[679,680]

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15 Disorders of Water Balance

Joseph G. Verbalis

CHAPTER OUTLINE

Disorders of body fluids are among the most commonly encountered problems in clinical medicine. This is, in large part, because many different disease states can disrupt the finely balanced mechanisms that control the intake and output of water and solute. Because body water is the primary determinant of the osmolality of the extracellular fluid, disorders of water metabolism can be broadly divided into hyperosmolar disorders, in which there is a deficiency of body water relative to body solute, and hypoosmolar disorders, in which there is an excess of body water relative to body solute. Because sodium is the main constituent of plasma osmolality, these disorders are typically characterized by hypernatremia and hyponatremia, respectively. Before discussing specific aspects of these disorders, this chapter will first review the regulatory mechanisms underlying water metabolism, which, in concert with sodium metabolism, maintains body fluid homeostasis.

BODY FLUIDS: COMPARTMENTALIZATION, COMPOSITION, AND TURNOVER

Water constitutes approximately 55% to 65% of body weight, varying with age, gender, and amount of body fat, and therefore constitutes the largest single constituent of the body. Total body water (TBW) is distributed between the intracellular fluid (ICF) and extracellular fluid (ECF) compartments. Estimates of the relative sizes of these two pools differ significantly, depending on the tracer used to measure the ECF volume, but most studies in animals and humans have indicated that 55% to 65% of TBW resides in the ICF, and 35% to 45% is in the ECF. Approximately 75% of the ECF compartment is interstitial fluid, and only 25% is intravascular fluid (blood volume).[1,2] Fig. 15.1 summarizes the estimated body fluid spaces of an average weight adult.

The solute composition of the ICF and ECF differs considerably because most cell membranes possess multiple transport systems that actively accumulate or expel specific solutes. Thus, membrane-bound Na^+-K^+-ATPase maintains Na^+ in a primarily extracellular location and K^+ in a primarily intracellular location.[3] Similar transporters effectively result in confining Cl^- largely to the ECF, and Mg^{2+}, organic acids, and phosphates to the ICF. Glucose, which requires an insulin-activated transport system to enter most cells, is present in significant amounts only in the ECF because it is rapidly converted intracellularly to glycogen or metabolites. HCO_3^- is present in both compartments but is approximately three times more concentrated in the ECF. Urea is unique among the major naturally occurring solutes in that it diffuses freely across most cell membranes[4]; therefore, it is present in similar concentrations in almost all body fluids, except in the renal medulla, where it is concentrated by urea transporters (see Chapter 10).

Despite very different solute compositions, both the ICF and ECF have an equivalent osmotic pressure,[5] which is a function of the total concentration of all solutes in a fluid compartment. This is because most biologic membranes are semipermeable (i.e., freely permeable to water but not to all aqueous solutes). Thus, water will flow across membranes into a compartment with a higher solute concentration until a steady state is reached and the osmotic pressures have equalized on both sides of the cell membrane.[6] An important consequence of this thermodynamic law is that the volume of distribution of body Na^+ and K^+ is actually the TBW rather than just the ECF or ICF volume, respectively.[7] For example, any increase in ECF sodium concentration ($[Na^+]$) will cause water to shift from the ICF to ECF until the ICF and ECF osmotic pressures are equal, thereby effectively distributing the Na^+ across extracellular and intracellular water.

Osmolality is defined as the concentration of all of the solutes in a given weight of fluid. The total solute

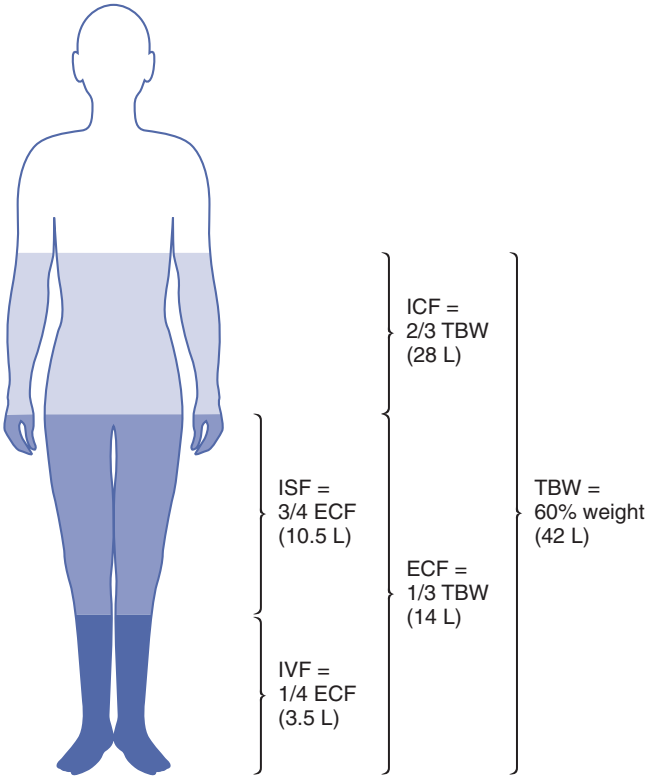


Fig. 15.1 Schematic representation of body fluid compartments in humans. The *shaded areas* depict the approximate size of each compartment as a function of body weight. The *numbers* indicate the relative sizes of the various fluid compartments and the approximate absolute volumes of the compartments (in liters) in a 70-kg adult. *ECF,* Extracellular fluid; *ICF,* intracellular fluid; *ISF,* interstitial fluid; *IVF,* intravascular fluid; *TBW,* total body water. (From Verbalis JG: Body water and osmolality. In Wilkinson B, Jamison R, eds. *Textbook of Nephrology.* London: Chapman & Hall; 1997:89–94.)

concentration of a fluid can be determined and expressed in several different ways. The most common method is to measure its freezing point or vapor pressure because these are colligative properties of the number of free solute particles in a volume of fluid.[8] The result is expressed relative to a standard solution of known concentration using units of osmolality (milliosmoles of solute per kilogram of water, mOsm/kg H_2O), or osmolarity (milliosmoles of solute per liter of water, mOsm/L H_2O). Plasma osmolality (P_{osm}) can be measured directly, as described earlier, or can be calculated by summing the concentrations of the major solutes present in the plasma:

$$P_{osm}\,(\text{mOsm/kg } H_2O) = 2 \times \text{plasma } [Na+]\,(\text{mEq/L}) + \frac{\text{glucose (mg/dL)}}{18} + \frac{\text{BUN (mg/dL)}}{2.8}$$

where BUN = blood urea nitrogen.

Both methods produce comparable results under most conditions (the value obtained using this formula is generally within 1% to 2% of that obtained by direct osmometry), as will simply doubling the plasma [Na^+], because sodium and its accompanying anions are the predominant solutes present in plasma. However, the total osmolality of plasma is not always equivalent to the effective osmolality, often referred to as the "tonicity of the plasma," because the latter is a function of the relative solute permeability properties of the membranes separating the two compartments. Solutes that are impermeable to cell membranes (e.g., Na^+, mannitol) are restricted to the ECF compartment. They are effective solutes because they create osmotic pressure gradients across cell membranes, leading to the osmotic movement of water from the ICF to ECF compartments. Solutes that are permeable to cell membranes (e.g., urea, ethanol, methanol) are ineffective solutes because they do not create osmotic pressure gradients across cell membranes and therefore are not associated with such water shifts.[9] Glucose is a unique solute because, at normal physiologic plasma concentrations, it is taken up by cells via active transport mechanisms and therefore acts as an ineffective solute but, under conditions of impaired cellular uptake (e.g., insulin deficiency), it becomes an effective extracellular solute.[10]

The importance of this distinction between total and effective osmolality is that only the effective solutes in plasma are determinants of whether clinically significant hyperosmolality or hypoosmolality is present. An example of this is uremia; a patient with BUN concentration that has increased by 56 mg/dL will have a corresponding 20-mOsm/kg H_2O elevation in plasma osmolality, but the effective osmolality will remain normal because the increased urea is proportionally distributed across the ECF and ICF. In contrast, a patient whose plasma [Na^+] has increased by 10 mEq/L will also have a 20-mOsm/kg H_2O elevation of plasma osmolality because the increased cation must be balanced by an equivalent increase in plasma anions. However, in this case, the effective osmolality will also be elevated by 20 mOsm/kg H_2O because the Na^+ and accompanying anions will largely remain restricted to the ECF due to the relative impermeability of cell membranes to Na^+ and other ions. Thus, elevations of solutes such as urea, unlike elevations of sodium, do not cause cellular dehydration and consequently do not activate mechanisms that defend body fluid homeostasis by increasing body water stores.

Both body water and solutes are in a state of continuous exchange with the environment. The magnitude of the turnover varies considerably, depending on physical, social, and environmental factors, but, in healthy adults, it averages 5% to 10% of the total body content each day. For the most part, daily intake of water and electrolytes is not determined by physiologic requirements but is more a function of dietary preferences and cultural influences. Healthy adults have an average daily fluid ingestion of approximately 2 to 3 L, but with considerable individual variation; approximately one-third of this is derived from food or the metabolism of fat and the rest from discretionary ingestion of fluids. Similarly, of the 1000 mOsm of solute ingested or generated by the metabolism of nutrients each day, nearly 40% is intrinsic to food, another 35% is added to food as a preservative or flavoring, and the rest is mostly urea. In contrast to the largely unregulated nature of basal intakes, the urinary excretion of water and solute is highly regulated to preserve body fluid homeostasis. Thus, under normal circumstances, almost all ingested Na^+, Cl^-, and K^+, as well as ingested and metabolically generated urea, are excreted in the urine under the control of specific regulatory mechanisms. Other ingested solutes, such as divalent minerals, are excreted primarily by the gastrointestinal tract. Urinary excretion of water is also tightly

regulated by the secretion and renal effects of arginine vasopressin (AVP; vasopressin, antidiuretic hormone), discussed in greater detail in Chapter 10 and in the following section ("Water Metabolism").

WATER METABOLISM

Water metabolism is responsible for the balance between the intake and excretion of water. Each side of this balance equation can be considered to consist of a regulated and unregulated component, the magnitudes of which can vary markedly under different physiologic and pathophysiologic conditions. The unregulated component of water intake consists of the intrinsic water content of ingested foods, consumption of beverages primarily for reasons of palatability or desired secondary effects (e.g., caffeine), or for social or habitual reasons (e.g., alcoholic beverages), whereas the regulated component of water intake consists of fluids consumed in response to a perceived sensation of thirst. Studies of middle-aged subjects have shown mean fluid intakes of 2.1 L/24 hours, and analyses of the fluids consumed have indicated that the vast majority of the fluid ingested is determined by influences such as meal-associated fluid intake, taste, or psychosocial factors, rather than by true thirst.[11]

The unregulated component of water excretion occurs via insensible water losses from a variety of sources (e.g., cutaneous losses from sweating, evaporative losses in exhaled air, gastrointestinal losses), as well as the obligate amount of water that the kidneys must excrete to eliminate solutes generated by body metabolism, whereas the regulated component of water excretion is comprised of the renal excretion of free water in excess of the obligate amount necessary to excrete metabolic solutes. Unlike solutes, a relatively large proportion of body water is excreted by evaporation from the skin and lungs. This amount varies markedly, depending on several factors, including dress, humidity, temperature, and exercise.[12] Under the sedentary and temperature-controlled indoor conditions typical of modern urban life, daily insensible water loss in healthy adults is minimal, approximately 8 to 10 mL/kg body weight (BW; ≈0.5–0.7 L in a 70-kg adult man or woman). However, insensible losses can increase to twice this level (20 mL/kg BW) simply under conditions of increased activity and temperature and, if environmental temperature or activity is even higher, such as in an arid environment, the rate of insensible water loss can even approximate the maximal rate of free water excretion by the kidney.[12] Thus, in quantitative terms, insensible loss and the factors that influence it can be just as important to body fluid homeostasis as regulated urine output.

Another major determinant of unregulated water loss is the rate of urine solute excretion, which cannot be reduced below a minimal obligatory level required to excrete the solute load. The volume of urine required depends not only on the solute load, but also on the degree of antidiuresis. At a typical basal level of urinary concentration (urine osmolality = 600 mOsm/kg H_2O) and a typical solute load of 900 to 1200 mOsm/day, a 70-kg adult would require a total urine volume of 1.5 to 2.0 L (21–29 mL/kg BW) to excrete the solute load. However, under conditions of maximal antidiuresis (urine osmolality = 1200 mOsm/kg H_2O), the same solute load would require a minimal obligatory urine output of only 0.75 to 1.0 L/day and, conversely, a decrease in urine concentration to minimal levels (urine osmolality = 60 mOsm/kg H_2O) would obligate a proportionately larger urine volume of 15 to 20 L/day to excrete the same solute load.

The earlier discussion emphasizes that water intake and water excretion have very substantial unregulated components, and these can vary tremendously as a result of factors unrelated to the maintenance of body fluid homeostasis. In effect, the regulated components of water metabolism are those that act to maintain body fluid homeostasis by compensating for whatever perturbations have resulted from unregulated water losses or gains. Within this framework, the major mechanisms responsible for regulating water metabolism are pituitary secretion and the renal effects of vasopressin and thirst, each of which will be discussed in greater detail in the following sections.

VASOPRESSIN SYNTHESIS AND SECRETION

The primary determinant of free water excretion in animals and humans is the regulation of urinary water excretion by circulating levels of AVP in plasma. The renal effects of AVP are covered extensively in Chapter 10. This chapter will focus on the regulation of AVP synthesis and secretion.

STRUCTURE AND SYNTHESIS

Before AVP was biochemically characterized, early studies used the general term "antidiuretic hormone" (ADH) to describe this substance. Now that AVP is known to be the only naturally occurring antidiuretic substance, it is more appropriate to refer to it by its correct hormonal designation. AVP is a nine–amino acid peptide that is synthesized in the hypothalamus. It is composed of a six–amino acid, ringlike structure formed by a disulfide bridge, with a three–amino acid tail, at the end of which the terminal carboxyl group is amidated. Substitution of lysine for arginine in position 8 yields lysine vasopressin, the antidiuretic hormone found in pigs and other members of the suborder Suina. Substitution of isoleucine for phenylalanine at position 3 and of leucine for arginine at position 8 yields oxytocin (OT), a hormone found in all mammals and in many submammalian species.[13] OT has weak antidiuretic activity[14] but is a potent constrictor of smooth muscle in mammary glands and uterus. As implied by their names, arginine and lysine vasopressin also cause the constriction of blood vessels, which was the property that led to their original discovery in the late 19th century,[15] but this pressor effect occurs only at concentrations many times higher than those required to produce antidiuresis. This is probably of little physiologic or pathologic importance in humans except under conditions of severe hypotension and hypovolemia, where it acts to supplement the vasoconstrictive actions of angiotensin II (Ang II) and the sympathetic nervous system.[16] The multiple actions of AVP are mediated by different G protein–coupled receptors, designated V_{1a}, V_{1b}, and V_2[17] (see Chapter 10).

AVP and OT are produced by the neurohypophysis, often referred to as the "posterior pituitary gland," because the neural lobe is located centrally and posterior to the adenohypophysis, or anterior pituitary gland, in the sella turcica. However, it is important to understand that the posterior pituitary gland consists only of the distal axons of the

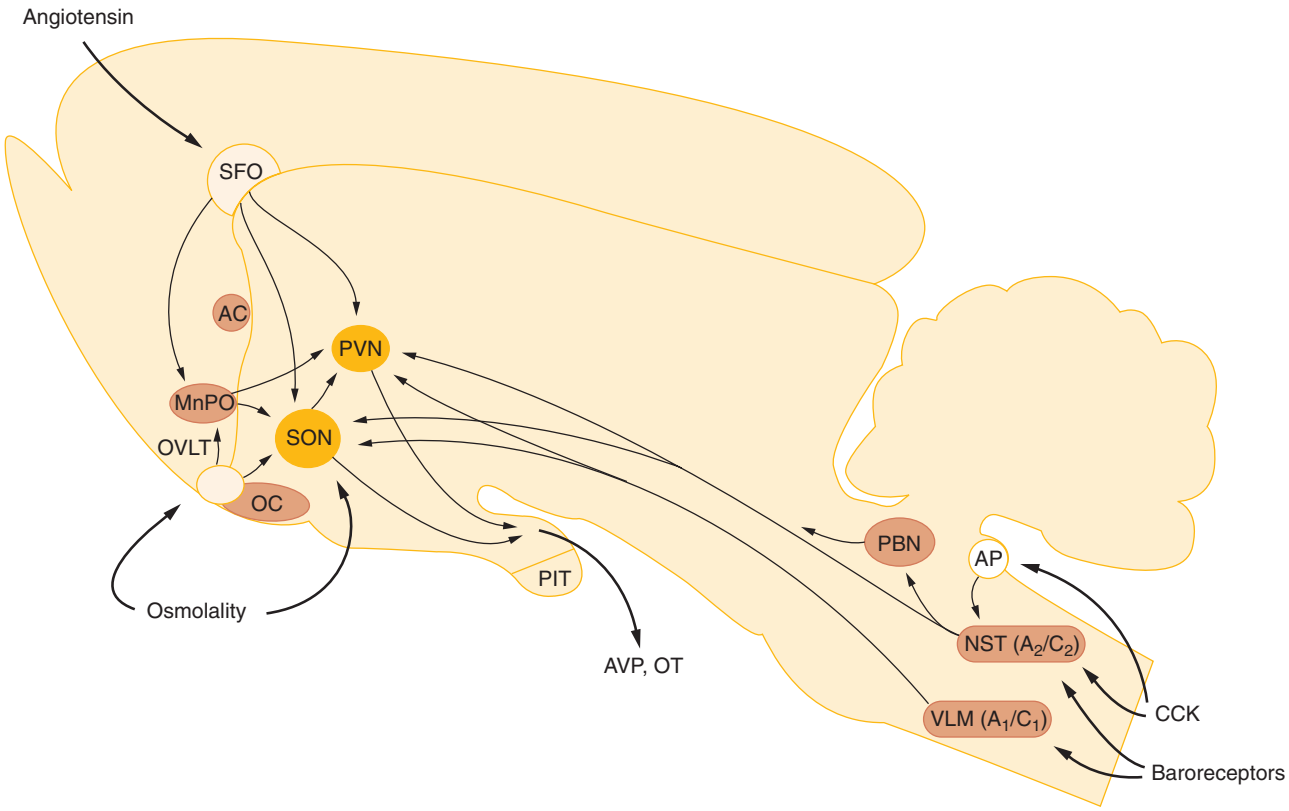


Fig. 15.2 Summary of the main anterior hypothalamic pathways that mediate secretion of arginine vasopressin (AVP) and oxytocin (OT). The vascular organ of the lamina terminalis (OVLT) is especially sensitive to hyperosmolality. Hyperosmolality also activates other neurons in the anterior hypothalamus, such as those in the subfornical organ (SFO) and median preoptic nucleus (MnPO), and magnocellular neurons, which are intrinsically osmosensitive. Circulating angiotensin II (Ang II) activates neurons of the SFO, an essential site of Ang II action, as well as cells throughout the lamina terminalis and MnPO. In response to hyperosmolality or Ang II, projections from the SFO and OVLT to the MnPO activate excitatory and inhibitory interneurons that project to the supraoptic nucleus (SON) and paraventricular nucleus (PVN) to modulate direct inputs to these areas from the circumventricular organs. Cholecystokinin (CCK) acts primarily on gastric vagal afferents that terminate in the nucleus of the solitary tract (NST) but, at higher doses, it can also act at the area postrema (AP). Although neurons are apparently activated in the ventrolateral medulla (VLM) and NST, most neurohypophyseal secretion appears to be stimulated by monosynaptic projections from A_2-C_2 cells, and possibly also noncatecholaminergic somatostatin-inhibin B cells, of the NST. Baroreceptor-mediated stimuli, such as hypovolemia and hypotension, are more complex. The major projection to magnocellular AVP neurons appears to arise from A_1 cells of the VLM that are activated by excitatory interneurons from the NST. Other areas, such as the parabrachial nucleus (PBN), may contribute multisynaptic projections. Cranial nerves IX and X, which terminate in the NST, also contribute input to magnocellular AVP neurons. *AC,* Anterior commissure; *OC,* optic chiasm; *PIT,* anterior pituitary. (From Stricker EM, Verbalis JG: Water intake and body fluids. In Squire LR, Bloom FE, McConnell SK, et al, eds. *Fundamental Neuroscience,* San Diego: Academic Press; 2003:1011–1029.)

magnocellular neurons that comprise the neurohypophysis. The cell bodies of these axons are located in specialized (magnocellular) neural cells located in two discrete areas of the hypothalamus, the paired supraoptic nuclei (SON) and paraventricular nuclei (PVN; Fig. 15.2). In adults, the posterior pituitary is connected to the brain by a short stalk through the diaphragm sellae. The neurohypophysis is supplied with blood by branches of the superior and inferior hypophysial arteries, which arise from the posterior communicating and intracavernous portion of the internal carotid artery. In the posterior pituitary, the arterioles break up into localized capillary networks that drain directly into the jugular vein via the sellar, cavernous, and lateral venous sinuses. Many of the neurosecretory neurons that terminate higher in the infundibulum and median eminence originate in parvicellular neurons in the PVN; they are functionally distinct from the magnocellular neurons that terminate in the posterior pituitary because they primarily enhance secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary. AVP-containing neurons also project from parvicellular neurons of the PVN to other areas of the brain, including the limbic system, nucleus tractus solitarius, and lateral gray matter of the spinal cord. The full extent of the functions of these extrahypophysial projections are still under study.

The genes encoding the AVP and OT precursors are located in close proximity on chromosome 20, but are expressed in mutually exclusive populations of neurohypophyseal neurons.[18] The AVP gene consists of approximately 2000 base pairs and contains three exons separated by two intervening sequences or introns (Fig. 15.3). Each exon encodes one of the three functional domains of the preprohormone, although small parts of the nonconserved sequences of neurophysin are located in the first and third exons that code for AVP and the C-terminal glycoprotein, called copeptin, respectively. The untranslated 5′-flanking genomic region, which regulates expression of the gene, shows extensive sequence homology

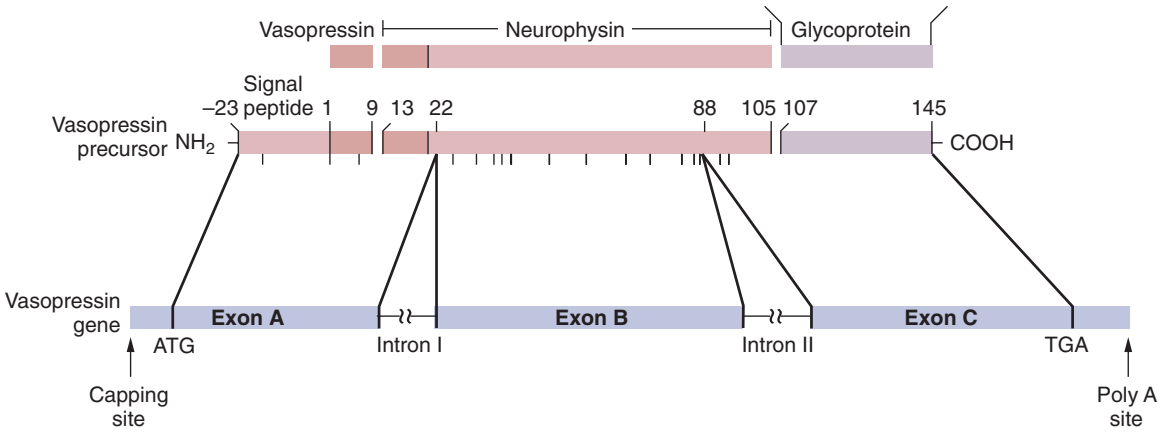


Fig. 15.3 The arginine vasopressin (AVP) gene and its protein products. The three exons encode a 145–amino acid prohormone with an NH_2-terminal signal peptide. The prohormone is packaged into neurosecretory granules of magnocellular neurons. During axonal transport of the granules from the hypothalamus to the posterior pituitary, enzymatic cleavage of the prohormone generates the final products—AVP, neurophysin, and a COOH-terminal glycoprotein called copeptin. When afferent stimulation depolarizes the AVP-containing neurons, the three products are released into capillaries of the posterior pituitary in equimolar amounts. (Adapted from Richter D, Schmale H: The structure of the precursor to arginine vasopressin, a model preprohormone. *Prog Brain Res.* 1983;60:227–233.)

across several species but is markedly different from the otherwise closely related gene for OT. This regulatory or promoter region of the AVP gene in the rat contains several putative regulatory elements, including a glucocorticoid response element, cyclic adenosine monophosphate (cAMP) response element, and four activating protein-2 (AP-2) binding sites.[19] Experimental studies have suggested that the DNA sequences between the AVP and OT genes, the intergenic region, may contain critical sites for cell-specific expression of these two hormones.[20]

The gene for AVP is also expressed in a number of other neurons, including but not limited to the parvicellular neurons of the PVN and SON. AVP and OT genes are also expressed in several peripheral tissues, including the adrenal medulla, ovary, testis, thymus, and certain sensory ganglia.[21] However, the AVP mRNA in these tissues appears to be shorter (620 bases) than its hypothalamic counterpart (720 bases), apparently because of tissue-specific differences in the length of the polyA tails. More importantly, the levels of AVP in peripheral tissues are generally two or three orders of magnitude lower than in the neurohypophysis, suggesting that AVP in these tissues likely has paracrine rather than endocrine functions. This is consistent with the observation that destruction of the neurohypophysis essentially eliminates AVP from the plasma, despite the presence of these multiple peripheral sites of AVP synthesis.

The secretion of AVP and its associated neurophysin and copeptin peptide fragments occurs by a calcium-dependent exocytotic process, similar to that described for other neurosecretory systems. Secretion is triggered by propagation of an electrical impulse along the axon that causes depolarization of the cell membrane, an influx of Ca^{2+}, fusion of secretory granules with the cell membrane, and extrusion of their contents. This view is supported by the observation that AVP, neurophysin, and the glycoprotein copeptin are released simultaneously by many stimuli.[22] However, at the physiologic pH of plasma, there is no binding of AVP or OT to their respective neurophysins so, after secretion, each peptide circulates independently in the bloodstream.[23]

Stimuli for secretion of AVP or OT also stimulate transcription and increase the mRNA content of both prohormones in the magnocellular neurons. This has been well documented in rats, in which dehydration, which stimulates secretion of AVP, accelerates transcription and increases the levels of AVP (and OT) mRNA,[24,25] and hypoosmolality, which inhibits the secretion of AVP, produces a decrease in the content of AVP mRNA.[26] These and other studies have indicated that the major control of AVP synthesis most likely resides at the level of transcription.[27]

Antidiuresis occurs via interaction of the circulating hormone with AVP V_2 receptors in the kidney, which results in increased water permeability of the collecting duct through the insertion of the aquaporin-2 (AQP2) water channel into the apical membranes of collecting tubule principal cells (see Chapter 10). The importance of AVP for maintaining water balance is underscored by the fact that the normal pituitary stores of this hormone are very large, allowing more than 1 week's supply of hormone for maximal antidiuresis under conditions of sustained dehydration.[27] Knowledge of the different conditions that stimulate pituitary AVP release in humans is therefore essential for understanding water metabolism.

OSMOTIC REGULATION

AVP secretion is influenced by many different stimuli, but since the pioneering studies of ADH secretion by Ernest Basil Verney, it has been clear that the most important stimulus under physiologic conditions is the osmotic pressure of plasma. With further refinement of radioimmunoassays for AVP, the unique sensitivity of this hormone to small changes in osmolality, as well as the corresponding sensitivity of the kidney to small changes in plasma AVP levels, have become apparent. Although the magnocellular neurons themselves have been found to have intrinsic osmoreceptive properties,[28] research over the last several decades has clearly shown that the most sensitive osmoreceptive cells that can sense small changes in plasma osmolality and transduce these changes into AVP secretion are located in the anterior hypothalamus,

likely in or near the circumventricular organ termed the "organum vasculosum of the lamina terminalis" (OVLT; see Fig. 15.2).[29] Perhaps the strongest evidence for location of the primary osmoreceptors in this area of the brain are the multiple studies that have demonstrated that destruction of this area disrupts osmotically stimulated AVP secretion and thirst, without affecting the neurohypophysis or its response to nonosmotic stimuli.[30,31]

Although some debate still exists with regard to the exact pattern of osmotically stimulated AVP secretion, most studies to date have supported the concept of a discrete osmotic threshold for AVP secretion, above which there is a linear relationship between plasma osmolality and AVP levels (Fig. 15.4).[32] At plasma osmolalities below a threshold level, AVP secretion is suppressed to low or undetectable levels; above this point, AVP secretion increases linearly in direct proportion to plasma osmolality. The slope of the regression line relating AVP secretion to plasma osmolality can vary significantly across individual human subjects, in part because of genetic factors,[33] but also in relation to other factors. In general, each 1-mOsm/kg H_2O increase in plasma osmolality causes an increase in the plasma AVP level, ranging from 0.4 to 1.0 pg/mL. The renal response to circulating AVP is similarly linear, with urinary concentration that is directly proportional to AVP levels from 0.5 to 4 to 5 pg/mL, after which urinary osmolality is maximal and cannot increase further, despite additional increases in AVP levels (Fig. 15.5). Thus, changes of as little as 1% in plasma osmolality are sufficient to cause significant increases in plasma AVP levels, with proportional increases in urine concentration, and maximal antidiuresis is achieved after increases in plasma osmolality of only 5 to 10 mOsm/kg H_2O (2%–4%) above the threshold for AVP secretion.

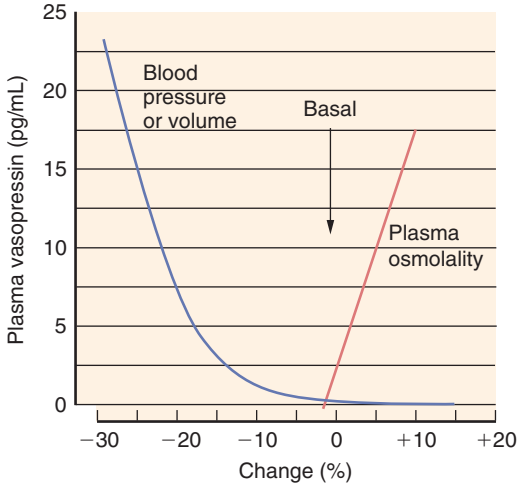


Fig. 15.4 Comparative sensitivity of arginine vasopressin (AVP) secretion in response to increases in plasma osmolality versus decreases in blood volume or blood pressure in human subjects. The *arrow* indicates the low plasma AVP concentrations found at basal plasma osmolality. Note that AVP secretion is much more sensitive to small changes in blood osmolality than to small changes in volume or pressure. (Adapted from Robertson GL: Posterior pituitary. In Felig P, Baxter J, Frohman LA, eds. *Endocrinology and Metabolism,* New York: McGraw-Hill; 1986:338–386.)

However, even this analysis underestimates the sensitivity of this system to regulate free water excretion. Urinary osmolality is directly proportional to plasma AVP levels as a consequence of the fall in urine flow induced by the AVP, but urine volume is inversely related to urine osmolality (see Fig. 15.5). An increase in plasma AVP concentration from 0.5 to 2 pg/mL has a much greater relative effect to decrease urine flow than a subsequent increase in AVP concentration from 2 to 5 pg/mL, thereby magnifying the physiologic effects of small changes in lower plasma AVP levels. Furthermore, the rapid response of AVP secretion to changes in plasma osmolality, coupled with the short half-life of AVP in human plasma (10–20 minutes), allows the kidneys to respond to changes in plasma osmolality on a minute to minute basis. The net result is a finely tuned osmoregulatory system that adjusts the rate of free water excretion accurately to the ambient plasma osmolality, primarily via changes in pituitary AVP secretion.

The set point of the osmoregulatory system also varies from person to person. In healthy adults, the osmotic threshold for AVP secretion ranges from 275 to 290 mOsm/kg H_2O (averaging ≈280–285 mOsm/kg H_2O). Similar to sensitivity, individual differences in the set point of the osmoregulatory system are relatively constant over time and appear to be genetically determined.[33] However, multiple factors can alter the sensitivity and/or set point of the osmoregulatory system for AVP secretion, in addition to genetic influences.[33] Foremost among these are acute changes in blood pressure, effective blood volume, or both (discussed in the following section). Aging has been found to increase the sensitivity of the osmoregulatory system in multiple studies.[34,35] Metabolic factors, such as serum Ca^{2+} levels and various drugs, can alter the slope of the plasma AVP-osmolality relationship as well.[36] Lesser degrees of shifting of the osmosensitivity and set point for AVP secretion have been noted with alterations in gonadal hormones. Some studies have found increased osmosensitivity in women, particularly during the luteal phase of the menstrual cycle,[37] and in estrogen-treated men,[38] but these effects were relatively minor, and others have found no significant gender differences.[33] The set point of the osmoregulatory system is reduced more dramatically and reproducibly during pregnancy.[39] Evidence has suggested the possible involvement of the placental hormone relaxin,[40] rather than gonadal steroids or human chorionic gonadotropin hormone in pregnancy-associated resetting of the osmostat for AVP secretion. Both the changes in volume and in osmolality have been reproduced by the infusion of relaxin into virgin female and normal rats and reversed in pregnant rats by immunoneutralization of relaxin.[41] Increased nitric oxide (NO) production by relaxin has been reported to increase vasodilation, and estrogens also increase NO synthesis.[42] That multiple factors can influence the set point and sensitivity of osmotically regulated AVP secretion is not surprising because AVP secretion reflects a balance of bimodal inputs—inhibitory and stimulatory[43]—from multiple different afferent inputs to the neurohypophysis (Fig. 15.6).[44]

Understanding the osmoregulatory mechanism also requires addressing the observation that AVP secretion is not equally sensitive to all plasma solutes. Sodium and its anions, which normally contribute more than 95% of the osmotic pressure of plasma, are the most potent solutes in terms of their capacity to stimulate AVP secretion and thirst, although certain

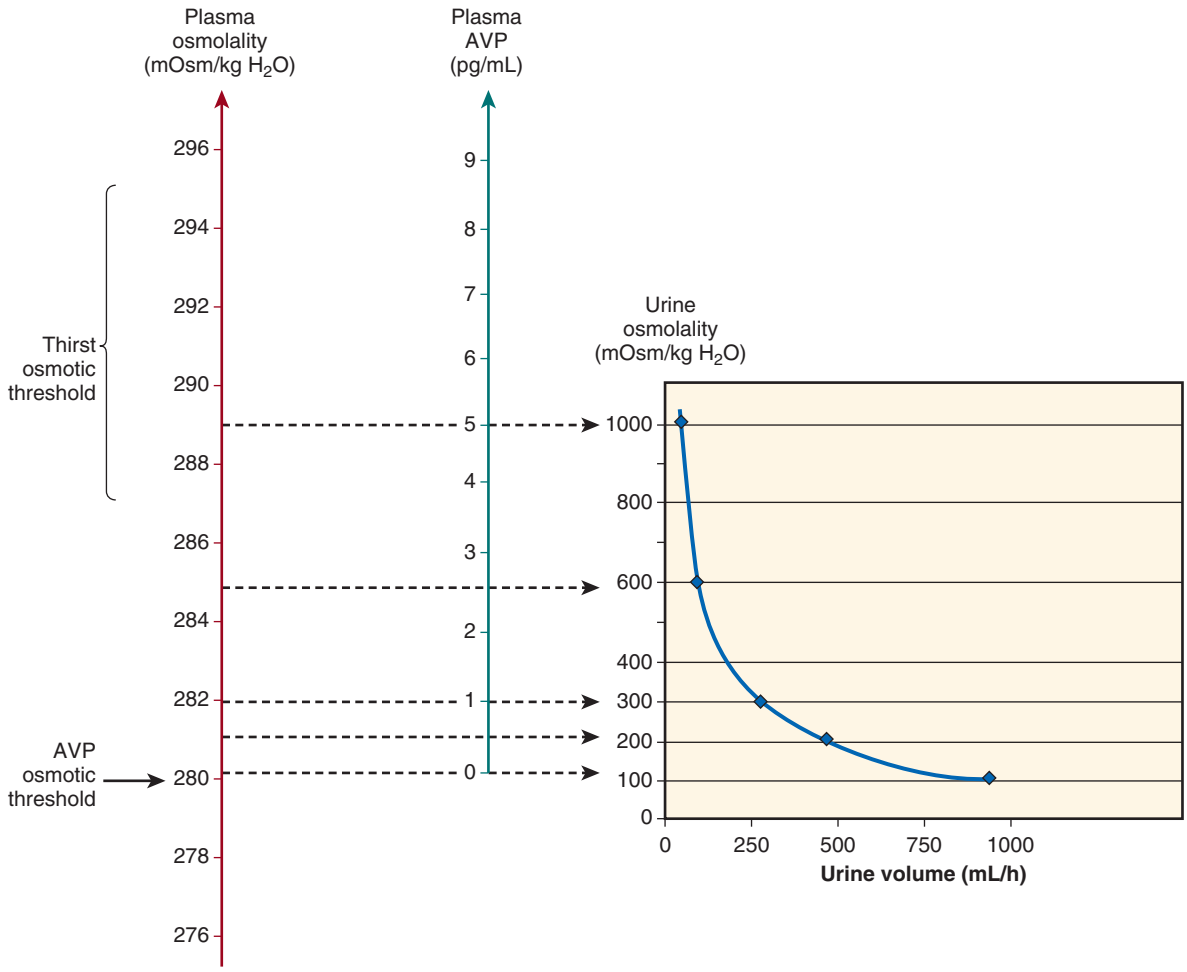


Fig. 15.5 Relationship of plasma osmolality, plasma arginine vasopressin (AVP) concentrations, urine osmolality, and urine volume in humans. The osmotic threshold for AVP secretion defines the point at which urine concentration begins to increase, but the osmotic threshold for thirst is significantly higher and approximates the point at which maximal urine concentration has already been achieved. Note also that because of the inverse relation between urine osmolality and urine volume, changes in plasma AVP concentrations have much larger effects on urine volume at low plasma AVP concentrations than at high plasma AVP concentrations. (Adapted from Robinson AG: Disorders of antidiuretic hormone secretion. *J Clin Endocrinol Metab.* 1985;14:55–88.)

sugars such as mannitol and sucrose are also equally effective when infused intravenously.[9] In contrast, increases in plasma osmolality caused by noneffective solutes such as urea or glucose result in little or no increase in plasma AVP levels in nondiabetic humans or animals.[9,45] These differences in response to various plasma solutes are independent of any recognized nonosmotic influence, indicating that they are a property of the osmoregulatory mechanism itself. According to current concepts, the osmoreceptor neuron is stimulated by osmotically induced changes in its water content. In this case, the stimulatory potency of any given solute would be an inverse function of the rate at which it moves from the plasma to the inside of the osmoreceptor neuron. Solutes that penetrate slowly, or not at all, create an osmotic gradient that causes an efflux of water from the osmoreceptor, and the resultant shrinkage of the osmoreceptor neuron activates a stretch-inactivated, noncationic channel that initiates depolarization and firing of the neuron.[46] Conversely, solutes that penetrate the cell readily create no gradient and thus have no effect on the water content and cell volume of the osmoreceptors. This mechanism agrees well with the observed relationship between the effect of certain solutes on AVP secretion, such as Na^+, mannitol, and glucose, and the rate at which they penetrate the blood-brain barrier.[29]

Many neurotransmitters have been implicated in mediating the actions of the osmoreceptors on the neurohypophysis. The supraoptic nucleus is richly innervated by multiple pathways, including acetylcholine, catecholamines, glutamate, gamma-aminobutyric acid (GABA), histamine, opioids, Ang II, and dopamine.[47] Studies have supported a potential role for all of these and others in the regulation of AVP secretion, as has local secretion of AVP into the hypothalamus from dendrites of the AVP-secreting neurons.[48] Although it remains unclear which of these are involved in the normal physiologic control of AVP secretion, in view of the likelihood that the osmoregulatory system is bimodal and integrated with multiple different afferent pathways (see Fig. 15.6), it seems likely that magnocellular AVP neurons are influenced by a very complex mixture of neurotransmitter systems, rather than only a few.

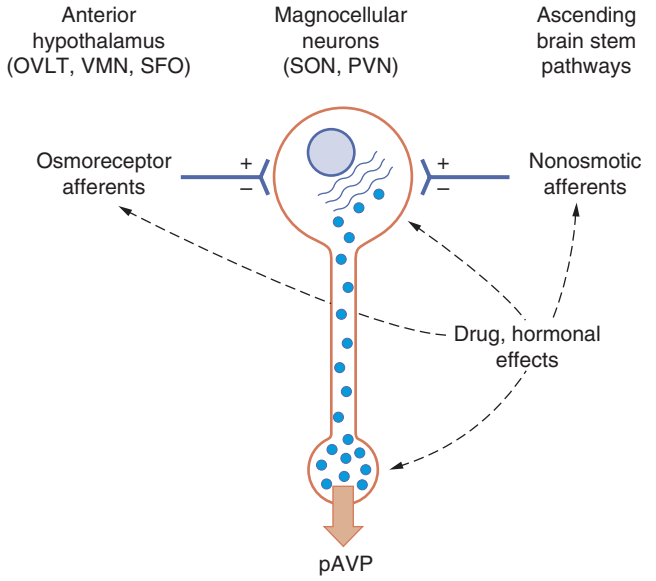


Fig. 15.6 Schematic model of the regulatory control of the neurohypophysis. The secretory activity of individual magnocellular neurons is determined by an integration of the activities of excitatory and inhibitory osmotic and nonosmotic afferent inputs. Superimposed on this are the effects of hormones and drugs, which can act at multiple levels to modulate the output of the system. *OVLT,* Organum vasculosum of the lamina terminalis; *PVN,* paraventricular nucleus; *SFO,* subfornical organ; *SON,* supraoptic nucleus; *VMN,* ventromedial nucleus. (Adapted from Verbalis JG: Osmotic inhibition of neurohypophyseal secretion. *Ann N Y Acad Sci.* 1983;689:227–233.)

Exactly how cells sense volume changes is a critical step for all the mechanisms activated to achieve osmoregulation. Some of the most exciting new data have come from studies of brain osmoreceptors.[29] The cellular osmosensing mechanism used by the OVLT cells is an intrinsic depolarizing receptor potential. This potential is generated in these cells via a molecular transduction complex. Studies have suggested that this likely includes members of the transient receptor potential vanilloid (TRPV) family of cation channel proteins. These channels are generally activated by cell membrane stretch to cause a nonselective conductance of cations, with a preference for Ca^{2+}. Multiple studies have characterized various members of the TRPV family as cellular mechanoreceptors in different tissues.[49] Both in vitro and in vivo studies of the TRPV family of cation channel proteins have provided evidence supporting the roles of TRPV1, TRPV2, and TRPV4 proteins in the transduction of osmotic stimuli in mammals that are important for sensing cell volume.[50] Moreover, genetic variation in the TRPV4 gene affects TRPV4 function and may influence water balance on a population-wide basis.[51] The details of exactly how and where various members of the TRPV family of cation channel proteins participate in osmoregulation in different species remains to be ascertained by additional studies. However, a strong case can already be made for their involvement in the transduction of osmotic stimuli in the neural cells in the OVLT and surrounding hypothalamus that regulate osmotic homeostasis, which appears to have been highly conserved throughout evolution.[50]

NONOSMOTIC REGULATION

Hemodynamic Stimuli

Not surprisingly, hypovolemia also is a potent stimulus for AVP secretion in humans[32,52] because an appropriate response to volume depletion should include renal water conservation. In humans and many animal species, lowering blood pressure suddenly by any of several methods increases plasma AVP levels by an amount proportional to the degree of hypotension achieved.[32,53] This stimulus-response relationship follows an exponential pattern, so that small reductions in blood pressure, of the order of 5% to 10%, usually have only small effects on plasma AVP levels, whereas blood pressure decreases of 20% to 30% result in hormone levels many times higher than those required to produce maximal antidiuresis (see Fig. 15.4). The AVP response to acute reductions in blood volume appears to be quantitatively and qualitatively similar to the response to blood pressure. In rats, plasma AVP increases as an exponential function of the degree of hypovolemia. Thus, little increase in plasma AVP can be detected until blood volume falls by 5% to 8%; beyond that point, plasma AVP increases at an exponential rate in relation to the degree of hypovolemia and usually reaches levels 20 to 30 times normal when blood volume is reduced by 20% to 40%.[54,55] The volume-AVP relationship has not been as thoroughly characterized in other species, but it appears to follow a similar pattern to that in humans.[56] Conversely, acute increases in blood volume or pressure suppress AVP secretion. This response has been characterized less well than that of hypotension or hypovolemia but seems to have a similar quantitative relationship (i.e., relatively large changes, ≈10%–15%, are required to alter hormone secretion appreciably).[57]

The minimal to absent effect of small changes in blood volume and pressure on AVP secretion contrasts sharply with the extraordinary sensitivity of the osmoregulatory system (see Fig. 15.4). Recognition of this difference is essential for understanding the relative contribution of each system to control AVP secretion under physiologic and pathologic conditions. Because daily variations of total body water rarely exceed 2% to 3%, their effect on AVP secretion must be mediated largely, if not exclusively, by the osmoregulatory system. Nonetheless, modest changes in blood volume and pressure do, in fact, influence AVP secretion indirectly, even though they are weak stimuli by themselves. This occurs via shifting the sensitivity of AVP secretion to osmotic stimuli so that a given increase in osmolality will cause a greater secretion of AVP during hypovolemic conditions than during euvolemic states (Fig. 15.7).[58,59] In the presence of a negative hemodynamic stimulus, plasma AVP continues to respond appropriately to small changes in plasma osmolality and can still be fully suppressed if the osmolality falls below the new (lower) set point. The retention of the threshold function is a vital aspect of the interaction because it ensures that the capability to regulate the osmolality of body fluids is not lost, even in the presence of significant hypovolemia or hypotension. Consequently, it is reasonable to conclude that the major effect of moderate degrees of hypovolemia on AVP secretion and thirst is to modulate the gain of the osmoregulatory responses, with direct effects on thirst and AVP secretion occurring only during more severe degrees of hypovolemia (e.g., >10% to 20% reduction in blood pressure or volume).

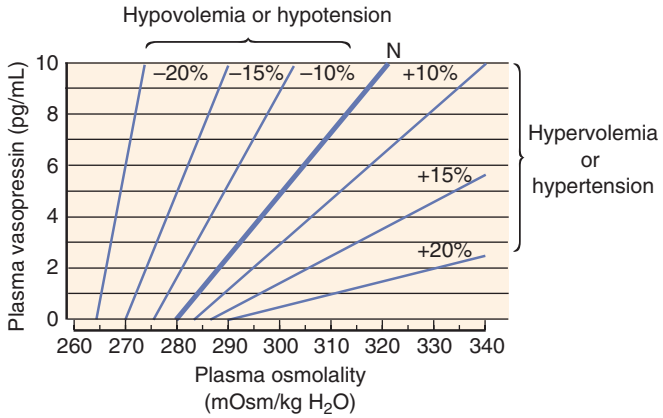


Fig. 15.7 The relationship between the osmolality of plasma and concentration of arginine vasopressin (AVP) in plasma is modulated by blood volume and pressure. The line labeled *N* shows plasma AVP concentration across a range of plasma osmolalities in an adult with normal intravascular volume (i.e., euvolemic) and normal blood pressure (i.e., normotensive). The lines to the left of *N* show the relationship between plasma AVP concentration and plasma osmolality in adults whose low intravascular volume (i.e., hypovolemia) or blood pressure (i.e., hypotension) is 10%, 15%, and 20% below normal, respectively. Lines to the right of *N* indicate volumes and blood pressures 10%, 15%, and 20% above normal, respectively. Note that hemodynamic influences do not disrupt the osmoregulation of AVP but rather raise or lower the set point, and possibly also the sensitivity, of AVP secretion in proportion to the magnitude of the change in blood volume or pressure. (Adapted from Robertson GL, Athar S, Shelton RL: Osmotic control of vasopressin function. In Andreoli TE, Grantham JJ, Rector FC, Jr, eds. *Disturbances in Body Fluid Osmolality,* Bethesda, MD: *Am Physiol Soc* 1977: 125.)

Table 15.1 Drugs and Hormones That Affect Vasopressin Secretion

Stimulatory	Inhibitory
Acetylcholine	Norepinephrine
Nicotine	Fluphenazine
Apomorphine	Haloperidol
Morphine (high doses)	Promethazine
Epinephrine	Oxilorphan
Isoproterenol	Butorphanol
Histamine	Opioid agonists
Bradykinin	Morphine (low doses)
Prostaglandin	Ethanol
β-Endorphin	Carbamazepine
Cyclophosphamide IV	Glucocorticoids
Vincristine	Clonidine
Insulin	Muscimol
2-Deoxyglucose	Phencyclidine
Angiotensin II	Phenytoin
Lithium	
Corticotropin-releasing factor	
Naloxone	
Cholecystokinin	

These hemodynamic influences on AVP secretion are mediated, at least in part, by neural pathways that originate in stretch-sensitive receptors, generally termed "baroreceptors," in the cardiac atria, aorta, and carotid sinus (see Fig. 15.2; reviewed in detail in Chapter 14). Afferent nerve fibers from these receptors ascend in the vagus and glossopharyngeal nerves to the nuclei of the tractus solitarius (NTS) in the brain stem.[60] A variety of postsynaptic pathways from the NTS then project, directly and indirectly via the ventrolateral medulla and lateral parabrachial nucleus, to the PVN and SON in the hypothalamus.[61] Early studies have suggested that the input from these pathways is predominantly inhibitory under basal conditions because interrupting them acutely results in large increases in plasma AVP levels, as well as in arterial blood pressure.[62] However, as for most neural systems, including the neurohypophysis, innervation is complex and consists of excitatory and inhibitory inputs. Consequently, different effects have been observed under different experimental conditions.

The baroreceptor mechanism also appears to mediate a large number of pharmacologic and pathologic effectors of AVP secretion (Table 15.1). Among them are diuretics, isoproterenol, nicotine, prostaglandins, nitroprusside, trimethaphan, histamine, morphine, and bradykinin, all of which stimulate AVP, at least in part by lowering blood volume or pressure,[52] and norepinephrine, which suppresses AVP by raising blood pressure.[63] In addition, an upright posture, sodium depletion, congestive heart failure, cirrhosis, and nephrosis likely stimulate AVP secretion by reducing the effective circulating blood volume.[64,65] Symptomatic orthostatic hypotension, vasovagal reactions, and other forms of syncope stimulate AVP secretion more markedly via greater and more acute decreases in blood pressure, with the exception of the orthostatic hypotension associated with the loss of afferent baroregulatory function.[66] Almost every hormone, drug, or condition that affects blood volume or pressure will also affect AVP secretion but, in most cases, the degree of change of blood pressure or volume is modest and will result in a shift of the set point and/or sensitivity of the osmoregulatory response, rather than marked stimulation of AVP secretion (see Fig. 15.7).

Drinking

Peripheral neural sensors other than baroreceptors also can affect AVP secretion. In humans, as well as dogs, drinking lowers plasma AVP before there is any appreciable decrease in plasma osmolality or serum [Na^+]. This is clearly a response to the act of drinking itself because it occurs independently of the composition of the fluid ingested,[67,68] although it may be influenced by the temperature of the fluid because the degree of suppression appears to be greater in response to colder fluids.[69] The pathways responsible for this effect have not been delineated, but likely include sensory afferents originating in the oropharynx and transmitted centrally via the glossopharyngeal nerve.

Nausea

Among other nonosmotic stimuli to AVP secretion in humans, nausea is the most prominent. The sensation of nausea, with or without vomiting, is the most potent stimulus to AVP secretion known in humans. Although 20% increases in osmolality will typically elevate plasma AVP levels to the range of 5 to 20 pg/mL, and 20% decreases in blood pressure to 10 to 100 pg/mL, nausea has been described to cause AVP elevations in excess of 200 to 400 pg/mL.[70] The pathway

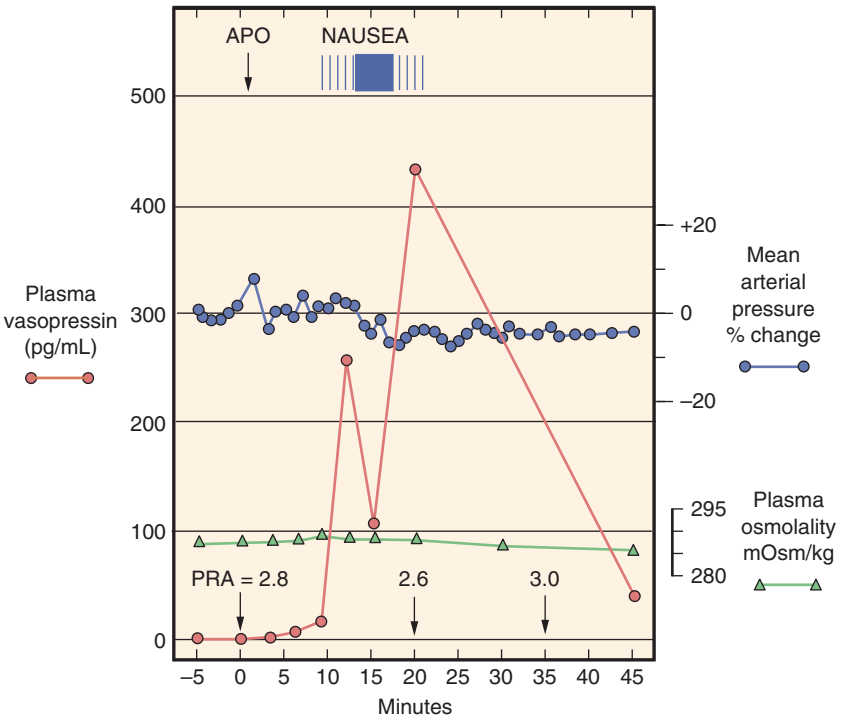


Fig. 15.8 Effect of nausea on arginine vasopressin (AVP) secretion. Apomorphine (APO) was injected at the point indicated by the *vertical arrow*. Note that the rise in plasma AVP coincided with the occurrence of nausea and was not associated with detectable changes in plasma osmolality or blood pressure. *PRA,* Plasma renin activity. (Adapted from Robertson GL: The regulation of vasopressin function in health and disease. *Recent Prog Horm Res.* 1977; 33:333–385.)

mediating this effect has been mapped to the chemoreceptor zone in the area postrema of the brain stem in animal studies (see Fig. 15.2). It can be activated by a variety of drugs and conditions, including apomorphine, morphine, nicotine, alcohol, and motion sickness. Its effect on AVP is instantaneous and extremely potent (Fig. 15.8), even when the nausea is transient and not accompanied by vomiting or changes in blood pressure. Pretreatment with fluphenazine, haloperidol, or promethazine in doses sufficient to prevent nausea completely abolishes the AVP response. The inhibitory effect of these dopamine antagonists is specific for emetic stimuli because they do not alter the AVP response to osmotic and hemodynamic stimuli. Water loading blunts, but does not abolish, the effect of nausea on AVP release, suggesting that osmotic and emetic influences interact in a manner similar to that for osmotic and hemodynamic pathways. Species differences also affect emetic stimuli. Whereas dogs and cats appear to be even more sensitive than humans to the emetic stimulation of AVP release, rodents have little or no AVP response but release large amounts of OT instead.[71]

The emetic response probably mediates many pharmacologic and pathologic effects on AVP secretion. In addition to the drugs and conditions already noted, it may be responsible at least in part for the increase in AVP secretion that has been observed with vasovagal reactions, diabetic ketoacidosis, acute hypoxia, and motion sickness. Because nausea and vomiting are frequent side effects of many other drugs and diseases, many additional situations likely occur as well. The reason for this profound stimulation is not known (although it has been speculated that the AVP response assists evacuation of stomach contents via the contractions of gastric smooth muscle, AVP is not necessary for vomiting to occur), but it is responsible for the intense vasoconstriction that produces the pallor often associated with nausea.

Hypoglycemia

Acute hypoglycemia is a less potent but reasonably consistent stimulus for AVP secretion.[72,73] The receptor and pathway that mediate this effect are unknown; however, they appear separate from those of other recognized stimuli because hypoglycemia stimulates AVP secretion, even in patients who have selectively lost the capacity to respond to hypernatremia, hypotension, or nausea.[73] The factor that actually triggers the release of AVP is likely intracellular deficiency of glucose or ATP because 2-deoxyglucose is also an effective stimulus to AVP secretion.[74] Generally, more than 20% decreases in glucose are required to increase plasma AVP levels significantly; the rate of decrease in the glucose level is probably the critical stimulus, however, because the rise in plasma AVP is not sustained with persistent hypoglycemia.[72] However, glucopenic stimuli are of unlikely importance in the physiology or pathology of AVP secretion because there are probably few drugs or conditions that lower plasma glucose rapidly enough to stimulate release of the hormone and because this effect is transient.

Renin-Angiotensin-Aldosterone System

The renin-angiotensin-aldosterone system (RAAS) has also been intimately implicated in the control of AVP secretion.[75] Animal studies have indicated dual sites of action. Bloodborne Ang II stimulates AVP secretion by acting in the brain at the circumventricular subfornical organ (SFO),[76] a small structure

located in the dorsal portion of the third cerebral ventricle (see Fig. 15.2). Because circumventricular organs lack a blood-brain barrier, the densely expressed Ang II receptor type 1 (AT_1R) of the SFO can detect very small increases in blood levels of Ang II.[77] Neural pathways from the SFO to the hypothalamic SON and PVN mediate AVP secretion and also appear to use Ang II as a neurotransmitter.[78] This accounts for the observation that the most sensitive site for angiotensin-mediated AVP secretion and thirst is intracerebroventricular injection into the cerebrospinal fluid. Further evidence in support of Ang II as a neurotransmitter is that the intraventricular administration of angiotensin receptor antagonists inhibits the AVP response to osmotic and hemodynamic stimuli.[79] The level of plasma Ang II required to stimulate AVP release is quite high, leading some to argue that this stimulus is active only under pharmacologic conditions. This is consistent with observations that even pressor doses of Ang II increase plasma AVP only about two- to fourfold[75] and may account for the failure of some investigators to demonstrate stimulation of thirst by exogenous angiotensin. However, this procedure may underestimate the physiologic effects of angiotensin because the increased blood pressure caused by exogenously administered Ang II appears to blunt the thirst induced via activation of inhibitory baroreceptive pathways.[80]

Stress

Nonspecific stress caused by factors such as pain, emotion, or physical exercise has long been thought to cause AVP secretion, but it has never been determined whether this effect is mediated by a specific pathway or is secondary to the hypotension or nausea that often accompanies stress-induced vasovagal reactions. In rats[81] and humans,[82] a variety of noxious stimuli capable of activating the pituitary-adrenal axis and sympathetic nervous system do not stimulate AVP secretion unless they also lower blood pressure or alter blood volume. The marked rise in plasma AVP levels elicited by the manipulation of the abdominal viscera in anesthetized dogs has been attributed to nociceptive influences,[83] but mediation by emetic pathways cannot be excluded in this setting. Endotoxin-induced fever stimulates AVP secretion in rats, and studies have supported the possible mediation of this effect by circulating cytokines, such as interleukin-1 (IL-1) and IL-6.[84] Clarification of the possible role of nociceptive and thermal influences on AVP secretion is particularly important in view of the frequency with which painful or febrile illnesses are associated with osmotically inappropriate secretion of antidiuretic hormone.

Hypoxia and Hypercapnia

Acute hypoxia and hypercapnia also stimulate AVP secretion.[85,86] In conscious humans, however, the stimulatory effect of moderate hypoxia (arterial partial pressure of oxygen [PaO_2] > 35 mm Hg) is inconsistent and seems to occur mainly in subjects who develop nausea or hypotension. In conscious dogs, more severe hypoxia (PaO_2 < 35 mm Hg) consistently increases AVP secretion without concomitant reductions in arterial pressure.[87] Studies of anesthetized dogs have supported these studies and suggested that the AVP response to acute hypoxia depends on the level of hypoxemia achieved. At a PaO_2 of 35 mm Hg or lower, plasma AVP increases markedly, even though there is no change or even an increase in arterial pressure, but less severe hypoxia (PaO_2 > 40 mm Hg) has no effect on AVP levels.[88] These results indicate that there is likely a hypoxemic threshold for AVP secretion and suggest that severe hypoxemia alone may also stimulate AVP secretion in humans. If so, it may be responsible, at least in part, for the osmotically inappropriate AVP elevations noted in some patients with acute respiratory failure.[89] In conscious or anesthetized dogs, acute hypercapnia, independent of hypoxia or hypotension, also increases AVP secretion.[87,88] It has not been determined whether this response also exhibits threshold characteristics or otherwise depends on the degree of hypercapnia, nor is it known whether hypercapnia has similar effects on AVP secretion in humans or other animals. The mechanisms whereby hypoxia and hypercapnia release AVP remain undefined, but they likely involve peripheral chemoreceptors and/or baroreceptors because cervical vagotomy abolishes the response to hypoxemia in dogs.[90]

Drugs

As will be discussed more extensively in the section on clinical disorders, a variety of drugs also stimulates AVP secretion, including nicotine (see Table 15.1). Drugs and hormones can potentially affect AVP secretion at many different sites. As already discussed, many excitatory stimulants such as isoproterenol, nicotine, high doses of morphine, and cholecystokinin act, at least in part, by lowering blood pressure and/or producing nausea. Others, such as substance P, prostaglandin, endorphin, and other opioids, have not been studied sufficiently to define their mechanism of action, but they may also work by one or both of the same mechanisms. Inhibitory stimuli similarly have multiple modes of action. Vasopressor drugs such as norepinephrine inhibit AVP secretion indirectly by raising the arterial pressure. In low doses, a variety of opioids of all subtypes, including morphine, met-enkephalin, and κ-agonists, inhibit AVP secretion in rats and humans.[91] Endogenous opioid peptides interact with the magnocellular neurosecretory system at several levels to inhibit basal and stimulated secretion of AVP and oxytocin. Opioid inhibition of AVP secretion has been found to occur in isolated posterior pituitary tissue, and the action of morphine and of several opioid agonists such as butorphanol and oxilorphan likely occurs via activation of κ-opioid receptors located on nerve terminals of the posterior pituitary.[92] The well-known inhibitory effect of ethanol on AVP secretion may be mediated, at least in part, by endogenous opiates because it is due to an elevation in the osmotic threshold for AVP release[93] and can be partially blocked by treatment with naloxone.[94] Carbamazepine inhibits AVP secretion by diminishing the sensitivity of the osmoregulatory system; this effect occurs independently of changes in blood volume, blood pressure, and/or blood glucose levels.[95] Other drugs that inhibit AVP secretion include clonidine, which appears to act via central and peripheral adrenoreceptors[96]; muscimol,[97] which acts as a GABA antagonist; and phencyclidine,[98] which probably acts by raising blood pressure. However, despite the importance of these stimuli during pathologic conditions, none of them is a significant determinant of the physiologic regulation of AVP secretion in humans.

DISTRIBUTION AND CLEARANCE

Plasma AVP concentration is determined by the difference between the rates of secretion from the posterior pituitary

gland and removal of the hormone from the vascular compartment via metabolism and urinary clearance. In healthy adults, intravenously injected AVP distributes rapidly into a space equivalent in size to the ECF compartment. This initial, or mixing, phase has a half-life of 4 to 8 minutes and is virtually complete in 10 to 15 minutes. The rapid mixing phase is followed by a second slower decline that corresponds to the metabolic clearance of AVP. Most studies of this phase have yielded mean values of 10 to 20 minutes by steady-state and non–steady-state techniques,[32] consistent with the observed rates of change in urine osmolality after water loading and injection of AVP, which also support a short half-life.[99] In pregnant women, the metabolic clearance rate of AVP increases nearly fourfold,[100] which becomes significant in the pathophysiology of gestational diabetes insipidus (see later discussion). Smaller animals such as rats clear AVP much more rapidly than humans because their cardiac output is higher relative to their BW and surface area.[99]

Although many tissues have the capacity to inactivate AVP, metabolism in vivo appears to occur largely in the liver and kidney.[99] The enzymatic processes whereby the liver and kidney inactivate AVP involve an initial reduction of the disulfide bridge, followed by aminopeptidase cleavage of the bond between amino acid residues 1 and 2 and cleavage of the C-terminal glycinamide residue. Some AVP is excreted intact in the urine, but there is disagreement about the amount and factors that affect it. For example, in healthy, normally hydrated adults, the urinary clearance of AVP ranges from 0.1 to 0.6 mL/kg/min under basal conditions and has never been found to exceed 2 mL/kg/min, even in the presence of solute diuresis.[32] The mechanisms involved in the excretion of AVP have not been defined with certainty, but the hormone is probably filtered at the glomerulus and variably reabsorbed at sites along the nephron. The latter process may be linked to the reabsorption of Na^+ or other solutes in the proximal nephron because the urinary clearance of AVP has been found to vary by as much as 20-fold in direct relation to the solute clearance.[32] Consequently, measurements of urinary AVP excretion in humans do not provide a consistently reliable index of changes in plasma AVP and should be interpreted cautiously when glomerular filtration or solute clearance is inconstant or abnormal.

THIRST

Thirst is the body's defense mechanism to increase water consumption in response to perceived deficits of body fluids. It can be most easily defined as a consciously perceived desire for water. True thirst must be distinguished from other determinants of fluid intake such as taste, dietary preferences, and social customs, as discussed previously. Thirst can be stimulated in animals and humans by intracellular dehydration caused by increases in the effective osmolality of the ECF or by intravascular hypovolemia caused by losses of ECF.[101,102] As would be expected, these are many of the same variables that provoke AVP secretion. Of these, hypertonicity is clearly the most potent. Similar to AVP secretion, substantial evidence to date has supported mediation of osmotic thirst by osmoreceptors located in the anterior hypothalamus of the brain,[30,31] whereas hypovolemic thirst appears to be stimulated via activation of low- and/or high-pressure baroreceptors[103] and circulating Ang II.[104] Regardless of the origin of the stimulus to thirst, the actual perception of thirst occurs in higher brain centers, specifically the anterior cingulate cortex (ACC) and insular cortex (IC), which receive information from circumventricular organs such as the organum OVLT and SFO (see Fig. 15.2) via relay nuclei in the thalamus.[105]

OSMOTIC THIRST

In healthy adults, an increase in effective plasma osmolality of only 2% to 3% above basal levels produces a strong desire to drink.[106] This response is not dependent on changes in ECF or plasma volume because it occurs similarly whether plasma osmolality is raised by the infusion of hypertonic solutions or by water deprivation. The absolute level of plasma osmolality at which a person develops a conscious urge to seek and drink water is termed the "osmotic thirst threshold." It varies appreciably among individuals, likely as a result of genetic factors,[33] but in healthy adults it averages approximately 295 mOsm/kg H_2O. Of physiologic significance is the fact that this level is above the osmotic threshold for AVP release and approximates the plasma osmolality at which maximal concentration of the urine is normally achieved (see Fig. 15.5).

The brain pathways that mediate osmotic thirst have not been well defined, but it is clear that the initiation of drinking requires osmoreceptors located in the anteroventral hypothalamus and OVLT in the same area as the osmoreceptors that control osmotic AVP secretion.[30,31] Whether the osmoreceptors for AVP and thirst are the same cells or are simply located in the same general area remains unknown.[29] However, the properties of the osmoreceptors are very similar. Ineffective plasma solutes such as urea and glucose, which have little or no effect on AVP secretion, are equally ineffective at stimulating thirst, whereas effective solutes such as NaCl and mannitol can stimulate thirst.[9,107] The sensitivities of the thirst and AVP osmoreceptors cannot be compared precisely, but they are also probably similar. Thus, in healthy adults, the intensity of thirst increases rapidly in direct proportion to serum $[Na^+]$ or plasma osmolality and generally becomes intolerable at levels only 3% to 5% above the threshold level.[108] Water consumption also appears to be proportional to the intensity of thirst in humans and animals and, under conditions of maximal osmotic stimulation, can reach rates as high as 20 to 25 L/day. The dilution of body fluids by ingested water complements the retention of water that occurs during AVP-induced antidiuresis, and both responses occur concurrently when drinking water is available.

As with AVP secretion, the osmoregulation of thirst appears to be bimodal because a modest decline in plasma osmolality induces a sense of satiation and reduces the basal rate of spontaneous fluid intake.[108,109] This effect is sufficient to prevent hypotonic overhydration, even when antidiuresis is fixed at maximal levels for prolonged periods, suggesting that osmotically inappropriate secretion of AVP (syndrome of inappropriate antidiuretic hormone secretion [SIADH]) should not result in the development of hyponatremia unless the satiety mechanism is impaired or fluid intake is inappropriately high for some other reason, such as the unregulated components of fluid intake discussed earlier.[109] Also similar to AVP secretion, thirst can be influenced by oropharyngeal or upper gastrointestinal receptors that respond to the act of drinking itself.[68] In humans, however, the rapid relief of thirst provided by this mechanism lasts only a matter

of minutes, and thirst quickly recurs until enough water is absorbed to lower plasma osmolality to normal. Therefore, although local oropharyngeal sensations may have a significant short-term influence on thirst, it is the hypothalamic osmoreceptors that ultimately determine the volume of water intake in response to dehydration.

HYPOVOLEMIC THIRST

In contrast, the threshold for producing hypovolemic or extracellular thirst is significantly higher in animals and humans. Studies in several species have shown that sustained decreases in plasma volume or blood pressure of at least 4% to 8%, and in some species 10% to 15%, are necessary to stimulate drinking consistently.[110,111] In humans, the degree of hypovolemia or hypotension required to produce thirst has not been precisely defined, but it has been difficult to demonstrate any effects of mild to moderate hypovolemia to stimulate thirst independently of osmotic changes occurring with dehydration. This blunted sensitivity to changes in ECF volume or blood pressure in humans probably represents an adaptation that occurred as a result of the erect posture of primates, which predisposes them to wider fluctuations in blood and atrial filling pressures as a result of the orthostatic pooling of blood in the lower body. Stimulation of thirst (and AVP secretion) by such transient postural changes in blood pressure might lead to overdrinking and inappropriate antidiuresis in situations in which the ECF volume was actually normal but only transiently maldistributed. Consistent with a blunted response to baroreceptor activation, studies have also shown that the systemic infusion of Ang II to pharmacologic levels is a much less potent stimulus to thirst in humans than in animals,[112] in whom it is one of the most potent dipsogens known. Nonetheless, this response is not completely absent in humans, as demonstrated by rare cases of polydipsia in patients with pathologic causes of hyperreninemia.[113] The pathways whereby hypovolemia or hypotension produces thirst have not been well defined, but probably involve the same brain stem baroreceptive pathways that mediate hemodynamic effects on AVP secretion,[103] as well as a likely contribution from circulating levels of Ang II in some species.[114]

ANTICIPATORY THIRST

Recent studies of the neural circuitry underlying drinking behavior have identified a new type of thirst that precedes physiological challenges to osmotic and volume homeostasis, which has been termed "anticipatory thirst." The best studied example of this is the increase in drinking that occurs in animals a few hours at the end of their awake period, which serves to maintain hydration during the sleep period when there is no fluid intake. This drinking behavior appears to be mediated by vasopressin-containing neurons in the suprachiasmatic nucleus (SCN), which is the brain nucleus that controls diurnal rhythms. SCN vasopressin neurons project to the OVLT, where they excite thirst-activating neurons, thereby enabling maintenance of osmotic homeostasis during sleep.[115]

INTEGRATION OF VASOPRESSIN SECRETION AND THIRST

A synthesis of what is presently known about the regulation of AVP secretion and thirst in humans leads to a relatively simple but elegant system to maintain water balance. Under normal physiologic conditions, the sensitivity of the osmoregulatory system for AVP secretion accounts for the maintenance of plasma osmolality within narrow limits by adjusting renal water excretion to small changes in osmolality. Stimulated thirst does not represent a major regulatory mechanism under these conditions, and unregulated fluid ingestion supplies adequate water in excess of true "need," which is then excreted in relation to osmoregulated pituitary AVP secretion. However, when unregulated water intake cannot adequately supply body needs in the presence of plasma AVP levels sufficient to produce maximal antidiuresis, plasma osmolality rises to levels that stimulate thirst (see Fig. 15.5), and water intake increases proportionally to the elevation of osmolality above this thirst threshold.

In such a system, thirst essentially represents a backup mechanism that becomes active when pituitary and renal mechanisms prove insufficient to maintain plasma osmolality within a few percentage points of basal levels. This arrangement has the advantage of freeing humans from frequent episodes of thirst. These would require a diversion of activities toward behavior oriented to seeking water when water deficiency is sufficiently mild to be compensated for by renal water conservation, but would stimulate water ingestion once water deficiency reaches potentially harmful levels. Stimulation of AVP secretion at plasma osmolalities below the threshold for subjective thirst acts to maintain an excess of body water sufficient to eliminate the need to drink whenever slight elevations in plasma osmolality occur. This system of differential effective thresholds for thirst and AVP secretion nicely complements many studies that have demonstrated excess unregulated (or need-free) drinking in humans and animals. Only when this mechanism becomes inadequate to maintain body fluid homeostasis does thirst-induced regulated fluid intake become the predominant defense mechanism for the prevention of severe dehydration.

DISORDERS OF INSUFFICIENT VASOPRESSIN OR VASOPRESSIN EFFECT

Disorders of insufficient AVP or AVP effect are associated with inadequate urine concentration and increased urine output, termed "polyuria." If thirst mechanisms are intact, this is accompanied by compensatory increases in fluid intake ("polydipsia") as a result of stimulated thirst to preserve body fluid homeostasis. The net result is polyuria and polydipsia, with preservation of normal plasma osmolality and serum electrolyte concentrations. However, if thirst is impaired, or if fluid intake is insufficient for any reason to compensate for the increased urine excretion, then hyperosmolality and hypernatremia can result, with the consequent complications associated with these disorders. The quintessential disorder of insufficient AVP is diabetes insipidus (DI), which is a clinical syndrome characterized by excretion of abnormally large volumes of urine (diabetes) that is dilute (hypotonic) and devoid of taste from dissolved solutes (e.g., insipid), in contrast to the hypertonic, sweet-tasting urine characteristic of diabetes mellitus (from the Greek, meaning honey).

Several different pathophysiologic mechanisms can cause hypotonic polyuria (Box 15.1). Central DI (also called hypothalamic, neurogenic, or neurohypophyseal DI) is due to inadequate secretion and usually deficient synthesis of

Box 15.1 Causes of Hypotonic Polyuria

Central (Neurogenic) Diabetes Insipidus

Congenital (congenital malformations; autosomal dominant, arginine vasopressin [AVP] neurophysin gene mutations)
Drug- or toxin-induced (ethanol, diphenylhydantoin, snake venom)
Granulomatous (histiocytosis, sarcoidosis)
Neoplastic (craniopharyngioma, germinoma, lymphoma, leukemia, meningioma, pituitary tumor; metastases)
Infectious (meningitis, tuberculosis, encephalitis)
Inflammatory, autoimmune (lymphocytic infundibuloneurohypophysitis)
Traumatic (neurosurgery, deceleration injury)
Vascular (cerebral hemorrhage or infarction, brain death)
Idiopathic

Osmoreceptor Dysfunction

Granulomatous (histiocytosis, sarcoidosis)
Neoplastic (craniopharyngioma, pinealoma, meningioma, metastases)
Vascular (anterior communicating artery aneurysm or ligation, intrahypothalamic hemorrhage)
Other (hydrocephalus, ventricular or suprasellar cyst, trauma, degenerative diseases)
Idiopathic

Increased AVP Metabolism

Pregnancy

Nephrogenic Diabetes Insipidus

Congenital (X-linked recessive, AVP V_2 receptor gene mutations; autosomal recessive or dominant, aquaporin-2 water channel gene mutations)
Drug-induced (demeclocycline, lithium, cisplatin, methoxyflurane)
Hypercalcemia
Hypokalemia
Infiltrating lesions (sarcoidosis, amyloidosis)
Vascular (sickle cell anemia)
Mechanical (polycystic kidney disease, bilateral ureteral obstruction)
Solute diuresis (glucose, mannitol, sodium, radiocontrast dyes)
Idiopathic

Primary Polydipsia

Psychogenic (schizophrenia, obsessive-compulsive behaviors)
Dipsogenic (downward resetting of thirst threshold, idiopathic or similar lesions, as with central DI)

AVP in the hypothalamic neurohypophyseal system. Lack of AVP-stimulated activation of the V_2 subtype of AVP receptors in the kidney collecting tubules (see Chapter 10) causes the excretion of large volumes of dilute urine. In most cases, thirst mechanisms are intact, leading to compensatory polydipsia. However, in a variant of central DI, osmoreceptor dysfunction, thirst is also impaired, leading to hypodipsia. DI of pregnancy is a transient disorder due to an accelerated metabolism of AVP as a result of increased activity of the enzyme oxytocinase or vasopressinase in the serum of pregnant women, again leading to polyuria and polydipsia. Accelerated metabolism of AVP during pregnancy may also cause a patient with subclinical DI from other causes to shift from a relatively asymptomatic state to a symptomatic state as a result of the more rapid AVP degradation. Nephrogenic DI is due to inappropriate renal responses to AVP. This produces excretion of dilute urine, despite normal pituitary AVP secretion and secondary polydipsia, similar to central DI. The final cause of hypotonic polyuria, primary polydipsia, differs significantly from the other causes because it is not due to deficient AVP secretion or impaired renal responses to AVP, but rather to excessive ingestion of fluids. This can result from an abnormality in the thirst mechanism, in which case it is sometimes called "dipsogenic DI," or from psychiatric disorders, in which case it is generally referred to as "psychogenic polydipsia."

CENTRAL DIABETES INSIPIDUS

CAUSES

Central diabetes insipidus (CDI) is caused by inadequate secretion of AVP from the posterior pituitary in response to osmotic stimulation. In most cases, this is due to destruction of the neurohypophysis by a variety of acquired or congenital anatomic lesions that destroy or damage the neurohypophysis by pressure or infiltration (see Box 15.1). The severity of the resulting hypotonic diuresis depends on the degree of destruction of the neurohypophysis, leading to complete or partial deficiency of AVP secretion.

Despite the wide variety of lesions that can potentially cause CDI, it is much more common not to have CDI in the presence of such lesions than actually to produce the syndrome. This apparent inconsistency can be understood by considering several common principles of neurohypophyseal physiology and pathophysiology that are relevant to all these causes.

First, the synthesis of AVP occurs in the hypothalamus (see Fig. 15.2); the posterior pituitary simply represents the site of storage and secretion of the neurosecretory granules that contain AVP. Consequently, lesions contained within the sella turcica that destroy only the posterior pituitary generally do not cause CDI because the cell bodies of the magnocellular neurons that synthesize AVP remain intact, and the site of release of AVP shifts more superiorly, typically into the blood vessels of the median eminence at the base of the brain. Perhaps the best examples of this phenomenon are large pituitary macroadenomas that completely destroy the anterior and posterior pituitary. DI is a distinctly unusual presentation for such pituitary adenomas because destruction of the posterior pituitary by such slowly enlarging intrasellar lesions merely destroys the nerve terminals, but not the cell bodies, of the AVP neurons. As this occurs, the site of release of AVP shifts more superiorly to the pituitary stalk and median eminence. Sometimes this can be detected on noncontrast magnetic resonance imaging (MRI) scans as a shift of the pituitary bright spot more superiorly to the level of the infundibulum or median eminence,[116] but this process is often too diffuse to be detected in this manner. The development of DI from a pituitary adenoma is so uncommon, even with macroadenomas that completely obliterate sellar contents sufficiently to cause panhypopituitarism, that its presence

should lead to consideration of alternative diagnoses, such as craniopharyngioma. This often causes damage to the median eminence because of adherence of the capsule to the base of the hypothalamus, more rapidly enlarging sellar or suprasellar masses that do not allow sufficient time for shifting the site of AVP release more superiorly (e.g., metastatic lesions, acute hemorrhage), or granulomatous disease, with more diffuse hypothalamic involvement (e.g., sarcoidosis, histiocytosis). With very large pituitary adenomas that produce ACTH deficiency, it is actually more likely that patients will present with hypoosmolality from an SIADH-like picture as a result of the impaired free water excretion that accompanies hypocortisolism, as will be discussed later.

A second general principle is that the capacity of the neurohypophysis to synthesize AVP is greatly in excess of the body's daily needs for the maintenance of water homeostasis. Carefully controlled studies of surgical section of the pituitary stalk in dogs have clearly demonstrated that destruction of 80% to 90% of the magnocellular neurons in the hypothalamus is required to produce polyuria and polydipsia in these species.[117] Thus, even lesions that cause destruction of the AVP magnocellular neuron cell bodies must result in a large degree of destruction to produce DI. The most illustrative example of this is surgical section of the pituitary stalk in humans. Necropsy studies of these patients have revealed atrophy of the posterior pituitary and loss of the magnocellular neurons in the hypothalamus.[118] This loss of magnocellular cells presumably results from the retrograde degeneration of neurons whose axons were cut during surgery. As is generally true for all neurons, the likelihood of retrograde neuronal degeneration depends on the proximity of the axotomy, in this case section of the pituitary stalk, to the cell body of the neuron. This was shown clearly in studies of human subjects in whom section of the pituitary stalk at the level of the diaphragm sellae (a low stalk section) produced transient but not permanent DI, whereas section at the level of the infundibulum (a high stalk section) was required to cause permanent DI in most cases.[119]

Several genetic causes of AVP deficiency have also been characterized. Prior to the application of techniques for the amplification of genomic DNA, the only experimental model to study the mechanism of hereditary hypothalamic DI was the Brattleboro rat, a strain that was found serendipitously to have CDI.[120] In this animal, the disease demonstrates a classic pattern of autosomal recessive inheritance in which DI is expressed only in the homozygotes. The hereditary basis of the disease has been found to be a single base deletion producing a translational frameshift beginning in the third portion of the neurophysin coding sequence. Because the gene lacks a stop codon, there is a modified neurophysin, no glycopeptide, and a long polylysine tail.[121] Although the mutant prohormone accumulates in the endoplasmic reticulum, sufficient AVP is produced by the normal allele that the heterozygotes are asymptomatic. In contrast, almost all families with genetic CDI in humans that have been described to date demonstrate an autosomal dominant mode of inheritance.[122–124] In these cases, DI is expressed, despite the expression of one normal allele, which is sufficient to prevent the disease in the heterozygous Brattleboro rats. Numerous studies have been directed at understanding this apparent anomaly. Two potentially important clues about the cause of the DI in familial genetic CDI are the following:

1. Severe to partial deficiencies of AVP and overt signs of DI do not develop in these patients until several months to several years after birth and then gradually progress over the ensuing decades,[122,125] suggesting adequate initial function of the normal allele, with later decompensation.
2. A limited number of autopsy studies have suggested that some of these cases are associated with gliosis and a marked loss of magnocellular AVP neurons in the hypothalamus,[126] although other studies have shown normal neurons, with decreased expression of AVP or no hypothalamic abnormality. In most of these cases, the hyperintense signal normally emitted by the neurohypophysis in T1-weighted MRI scans (see later discussion) is also absent, although some exceptions have been reported.[127]

Another interesting, but as yet unexplained, observation is that some adults in these families have been described in whom DI was clinically apparent during childhood but who went into remission as adults, without evidence that their remissions could be attributed to renal or adrenal insufficiency or to increased AVP synthesis.[128]

The autosomal dominant form of familial CDI is caused by diverse mutations in the gene that codes for the AVP-neurophysin precursor (Fig. 15.9). All the mutations identified to date have been in the coding region of the gene and affect only one allele. They are located in all three exons and are predicted to alter or delete amino acid residues in the signal peptide, AVP, and neurophysin moieties of the precursor. Only the C-terminus glycopeptide, or copeptin moiety, has not been found to be affected. Most are missense mutations, but nonsense mutations (premature stop codons) and deletions also occur.[129] One feature shared by all the mutations is that they are predicted to alter or delete one or more amino acids known, or reasonably presumed, to be crucial for processing, folding, and oligomerization of the precursor protein in the endoplasmic reticulum.[122,124] Because of the related functional effects of the mutations, the common clinical characteristics of the disease, the dominant-negative mode of transmission, and the autopsy and hormonal evidence of postnatal neurohypophyseal degeneration, it has been postulated that all the mutations act by causing the production of an abnormal precursor protein that accumulates and eventually kills the neurons because it cannot be correctly processed, folded, and transported out of the endoplasmic reticulum. Expression studies of mutant DNA from several human mutations in cultured neuroblastoma cells have supported this misfolding-neurotoxicity hypothesis by demonstrating abnormal trafficking and accumulation of mutant prohormone in the endoplasmic reticulum with low or absent expression in the Golgi apparatus, suggesting difficulty with packaging into neurosecretory granules.[130] However, cell death may not be necessary to decrease available AVP. Normally, proteins retained in the endoplasmic reticulum are selectively degraded but, if excess mutant is produced and the selective normal degradative process is overwhelmed, an alternate, nonselective, degradative system (autophagy) is activated. As more and more mutant precursor builds up in the endoplasmic reticulum, the normal wild-type protein becomes trapped with the mutant protein and degraded by the activated nonspecific degradative system. In this case, the amount of AVP that matures and is packaged would be markedly reduced.[131,132] This explanation is consistent with

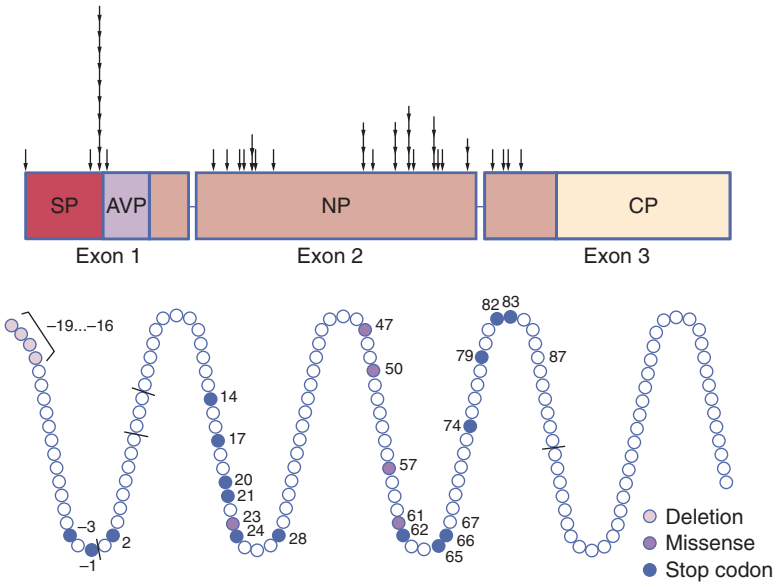


Fig. 15.9 Location and type of mutations in the gene that codes for the arginine vasopressin (AVP)–neurophysin precursor in kindreds with the autosomal dominant form of familial central diabetes insipidus (CDI). The location of the mutation in a different kindred is indicated by the *arrows.* The various portions of the precursor protein are designated by AVP (vasopressin), CP (copeptin), NP (neurophysin), and SP (signal peptide). Deletion and missense mutations are those expected to remove or replace one or more amino acid residues in the precursor. Those designated stop codons are expected to cause premature termination of the precursor. Note that none of the mutations causes a frameshift or affects the part of the gene that encodes the copeptin moiety, all the stop codons are in the distal part of the neurophysin moiety, and only one of the mutations affects the AVP moiety. All these findings are consistent with the concept that a mutant precursor is produced but cannot be folded properly because of interference with the binding of AVP to neurophysin, formation of intrachain disulfide bonds, or extreme flexibility or rigidity normally required at crucial places in the protein. (Adapted from Rittig S, Robertson GL, Siggaard C, et al: Identification of 13 new mutations in the vasopressin-neurophysin gene in 17 kindreds with familial autosomal dominant neurohypophyseal DI. *Am J Hum Genet.* 1996;58:107–117; and Hansen LK, Rittig S, Robertson GL: Genetic basis of familial neurohypophyseal diabetes insipidus. *Trends Endocrinol Metab.* 1997;8:363–372.)

cases in which little pathology is found in the magnocellular neurons and also with DI in which a small amount of AVP can still be detected.

Wolfram syndrome is a rare autosomal recessive disease with DI, diabetes mellitus, optic atrophy, and deafness (DIDMOAD). The genetic defect is the protein wolframin, which is found in the endoplasmic reticulum and is important for folding proteins.[133] Wolframin is involved in beta cell proliferation, intracellular protein processing, and calcium homeostasis, producing a wide spectrum of endocrine and central nervous system (CNS) disorders. DI is usually a late manifestation associated with decreased magnocellular neurons in the paraventricular and supraoptic nuclei.[134]

Idiopathic forms of AVP deficiency represent a large pathogenic category in adults and children. A study in children has revealed that over half (54%) of all cases of CDI were classified as idiopathic.[135] These patients do not have historic or clinical evidence of any injury or disease that can be linked to their DI, and MRI of the pituitary-hypothalamic area generally reveals no abnormality other than the absence of the posterior pituitary bright spot and sometimes varying degrees of thickening of the pituitary stalk. Several lines of evidence have suggested that many of these patients may have had an autoimmune destruction of the neurohypophysis to account for their DI. First, the entity of lymphocytic infundibuloneurohypophysitis has been documented to be present in a subset of patients with idiopathic DI.[136] Lymphocytic infiltration of the anterior pituitary, lymphocytic hypophysitis, has been recognized as a cause of anterior pituitary deficiency for many years, but it was not until an autopsy called attention to a similar finding in the posterior pituitary of a patient with DI that this pathology was recognized to occur in the neurohypophysis as well.[137] Since that initial report, a number of similar cases have been described, including cases in the postpartum period, which is characteristic of lymphocytic hypophysitis.[138] With the advent of MRI, lymphocytic infundibuloneurohypophysitis has been diagnosed based on the appearance of a thickened stalk and/or enlargement of the posterior pituitary, mimicking a pituitary tumor. In these cases, the characteristic bright spot on MRI T1-weighted images is lost. The enlargement of the stalk can mimic a neoplastic process, resulting in some of these patients undergoing surgery based on the suspicion of a pituitary tumor.

Since then, a number of patients with a suspicion of infundibuloneurohypophysitis and no other obvious cause of DI have been followed and have shown regression of the thickened pituitary stalk over time.[135,136,139] Several cases have been reported with the coexistence of CDI and adenohypophysitis; these presumably represent cases of combined lymphocytic infundibuloneurohypophysitis and hypophysitis.[140–143] A second line of evidence supporting an autoimmune cause in many cases of idiopathic DI is based on the finding of AVP antibodies in the serum of as many as one-third of patients with idiopathic DI and two-thirds of those with Langerhans cell histiocytosis X, but not in patients with DI caused by tumors.[144] A recently recognized form of infundibuloneurohypophysitis occurs in middle-aged

to older men and is associated with immunoglobulin G4 (IgG4)–related systemic disease.[145,146] Various organs, especially the pancreas, are infiltrated with IgG4 plasma cells, and neurohypophysitis is only one manifestation of a multiorgan disease that may include other endocrine glands. This cause should be considered as a cause of DI based on age and gender at presentation and evidence of other systemic disease. The diagnosis can be established by elevated serum IgG4 levels and characteristic histology of biopsies. Response to steroids or other immunosuppressive drugs is characteristic.

PATHOPHYSIOLOGY

The normal inverse, and nonlinear, relationship between urine volume and urine osmolality (see Fig. 15.5) means that initial decreases in maximal AVP secretion will not cause an increase in urine volume sufficient to be detected clinically by polyuria. In general, basal AVP secretion must fall to less than 10% to 20% of normal before basal urine osmolality decreases to less than 300 mOsm/kg H_2O and urine flow increases to symptomatic levels (i.e., >50 mL/kg BW/day). This resulting loss of body water produces a slight rise in plasma osmolality that stimulates thirst and induces a compensatory polydipsia. The resultant increase in water intake restores balance with urine output and stabilizes the osmolality of body fluids at a new, slightly higher but still normal level. As the AVP deficit increases, this new steady-state level of plasma osmolality approximates the osmotic threshold for thirst (see Fig. 15.5). It is important to recognize that the deficiency of AVP need not be complete for polyuria and polydipsia to occur; it is only necessary that the maximal plasma AVP concentration achievable at or below the osmotic threshold for thirst is inadequate to concentrate the urine.[147] The degree of neurohypophyseal destruction at which such failure occurs varies considerably from person to person, largely because of individual differences in the set point and sensitivity of the osmoregulatory system.[33] In general, functional tests of AVP levels in patients with DI of variable severity, duration, and cause have indicated that AVP secretory capacity must be reduced by at least 75% to 80% for significant polyuria to occur. This also agrees with neuroanatomic studies of cell loss in the supraoptic nuclei of dogs with experimental pituitary stalk section[117] and of patients who had undergone pituitary surgery.[118]

Because renal mechanisms for sodium conservation are normal in patients with impaired or absent AVP secretion, there is no accompanying sodium deficiency. Although untreated DI can lead to hyperosmolality and volume depletion, until the water losses become severe, volume depletion is minimized by osmotic shifts of water from the ICF compartment to the more osmotically concentrated ECF compartment. This phenomenon is not as evident following increases in ECF $[Na^+]$ because such osmotic shifts result in a slower increase in the serum $[Na^+]$ than would otherwise occur. However, when non–sodium solutes such as mannitol are infused, this effect is more obvious due to the progressive dilutional decrease in serum $[Na^+]$ caused by the translocation of intracellular water to the ECF compartment. Because patients with DI do not have impaired urine Na^+ conservation, the ECF volume is generally not markedly decreased, and regulatory mechanisms for the maintenance of osmotic homeostasis are primarily activated—stimulation of thirst and

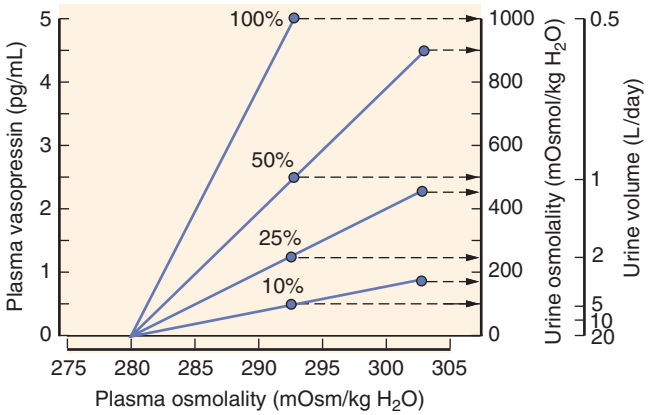


Fig. 15.10 Relationship between plasma arginine vasopressin (AVP) levels, urine osmolality, and plasma osmolality in subjects with normal posterior pituitary function (100%) compared with patients with graded reductions in AVP-secreting neurons (to 50%, 25%, and 10% of normal). Note that the patient with a 50% secretory capacity can achieve only half the plasma AVP level and half the urine osmolality of normal subjects at a plasma osmolality of 293 mOsm/kg H_2O. However, with increasing plasma osmolality, this patient can nonetheless eventually stimulate sufficient AVP secretion to reach a near-maximal urine osmolality. In contrast, patients with more severe degrees of AVP-secreting neuron deficits are unable to reach maximal urine osmolalities at any level of plasma osmolality. (Adapted from Robertson GL: Posterior pituitary. In Felig P, Baxter J, Frohman LA, eds. *Endocrinology and Metabolism.* New York: McGraw-Hill; 1986:338–386.)

AVP secretion (to whatever degree the neurohypophysis is still able to secrete AVP). In cases in which AVP secretion is totally absent (complete DI), patients are dependent entirely on water intake for the maintenance of water balance. However, in cases in which some residual capacity to secrete AVP remains (partial DI), plasma osmolality can eventually reach levels that allow for moderate degrees of urinary concentration (Fig. 15.10).

The development of DI following surgical or traumatic injury to the neurohypophysis represents a unique situation and can follow any of several different, well-defined patterns. In some patients, polyuria develops 1 to 4 days after injury and resolves spontaneously. Less often, the DI is permanent and continues indefinitely (see previous discussion on the relationship between the level of pituitary stalk section and development of permanent DI). Most interestingly, a triphasic response can occur as a result of pituitary stalk transection (Fig. 15.11).[119] The initial DI (first phase) is due to axon shock and lack of function of the damaged neurons. This phase lasts from several hours to several days and is followed by an antidiuretic phase (second phase) that is the result of the uncontrolled release of AVP from the disconnected and degenerating posterior pituitary or from the remaining severed neurons.[148] Overly aggressive administration of fluids during this second phase does not suppress the AVP secretion and can lead to hyponatremia. The antidiuresis can last from 2 to 14 days, after which DI recurs following depletion of the AVP from the degenerating posterior pituitary gland (third phase).[149]

Transient hyponatremia without preceding or subsequent DI has been reported following transsphenoidal surgery for pituitary microadenomas,[150] which generally occurs 5 to 10

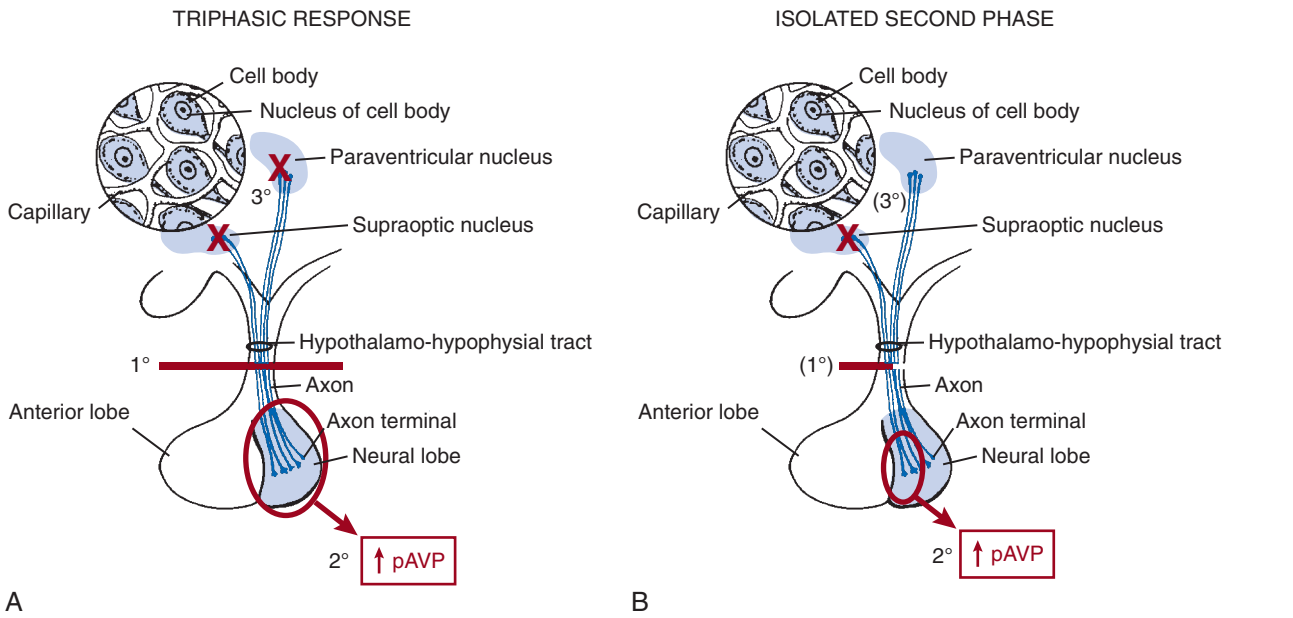


Fig. 15.11 Mechanisms underlying the pathophysiology of the triphasic pattern of diabetes insipidus (DI) and the isolated second phase. (A) In the triphasic response, the first phase of DI is initiated following a partial or complete pituitary stalk section, which severs the connections between the AVP neuronal cell bodies in the hypothalamus and nerve terminals in the posterior pituitary gland, thus preventing stimulated AVP secretion (1 degree). This is followed in several days by the second phase of syndrome of inappropriate antidiuretic hormone secretion (SIADH), which is caused by uncontrolled release of AVP into the bloodstream from the degenerating nerve terminals in the posterior pituitary (2 degrees). After all the AVP stored in the posterior pituitary gland has been released, the third phase of DI returns if more than 80% to 90% of the AVP neuronal cell bodies in the hypothalamus have undergone retrograde degeneration (3 degrees). (B) In the isolated second phase, the pituitary stalk is injured, but not completely cut. Although the maximum AVP secretory response will be diminished as a result of the stalk injury, DI will not result if the injury leaves intact at least 10% to 20% of the nerve fibers connecting the AVP neuronal cell bodies in the hypothalamus to the nerve terminals in the posterior pituitary gland (1 degree). However, this is still followed in several days by the second phase of SIADH, which is caused by the uncontrolled release of AVP from the degenerating nerve terminals of the posterior pituitary gland that have been injured or severed (2 degrees). Because a smaller portion of the posterior pituitary is denervated, the magnitude of AVP released as the pituitary degenerates will be smaller and of shorter duration than with a complete triphasic response. After all the AVP stored in the damaged part of the posterior pituitary gland has been released, the second phase ceases, but clinical DI will not occur if less than 80% to 90% of the AVP neuronal cell bodies in the hypothalamus undergo retrograde degeneration (3 degrees). (From Loh JA, Verbalis JG: Disorders of water and salt metabolism associated with pituitary disease. *Endocrinol Metab Clin North Am*. 2008;37:213–234.)

days postoperatively. The incidence may be as high as 30% when these patients are carefully followed, although most cases are mild and self-limited.[151,152] This is due to inappropriate AVP secretion via the same mechanism as in the triphasic response, except that in these cases only the second phase occurs (isolated second phase) because the initial neural lobe or pituitary stalk damage is not sufficient to impair AVP secretion enough to produce clinical manifestations of DI (see Fig. 15.11).[153]

Once a deficiency of AVP secretion has been present for more than a few weeks, it rarely improves, even if the underlying cause of the neurohypophyseal destruction is eliminated. The major exception to this is in patients with postoperative DI, for whom spontaneous resolution is the rule. Although recovery from DI that persists more than several weeks postoperatively is less common, well-documented cases of long-term recovery have nonetheless been reported.[149] The reason for amelioration and resolution is apparent from the pathologic and histologic examination of neurohypophyseal tissue following pituitary stalk section.[154,155] Neurohypophyseal neurons that have intact perikarya are able to regenerate axons and form new nerve terminal endings capable of releasing AVP into nearby capillaries. In animals, this may be accompanied by a bulbous growth at the end of the severed stalk, which represents a new, albeit small, neural lobe. In humans, the regeneration process appears to proceed more slowly, and formation of a new neural lobe has not been noted. Nonetheless, histologic examination of a severed human stalk from a patient 18 months after hypophysectomy has demonstrated reorganization of neurohypophyseal fibers, with neurosecretory granules in close proximity to nearby blood vessels, closely resembling the histology of a normal posterior pituitary.[155]

Recognition of the fact that almost all patients with CDI retain a limited capacity to secrete some AVP allows for an understanding of some otherwise perplexing features of the disorder. For example, in many patients, restricting water intake long enough to raise plasma osmolality by only 1% to 2% induces sufficient AVP secretion to concentrate the urine (Fig. 15.12). As the plasma osmolality increases further, some patients with partial DI can even secrete enough AVP to achieve near-maximal urine osmolality (see Fig. 15.10). However, this should not cause confusion about the diagnosis of DI because, in these patients, the urine osmolality will still be inappropriately low at a plasma osmolality within a normal range, and they will respond to exogenous AVP administration with further increases in urine osmolality. These responses to dehydration illustrate the relative nature

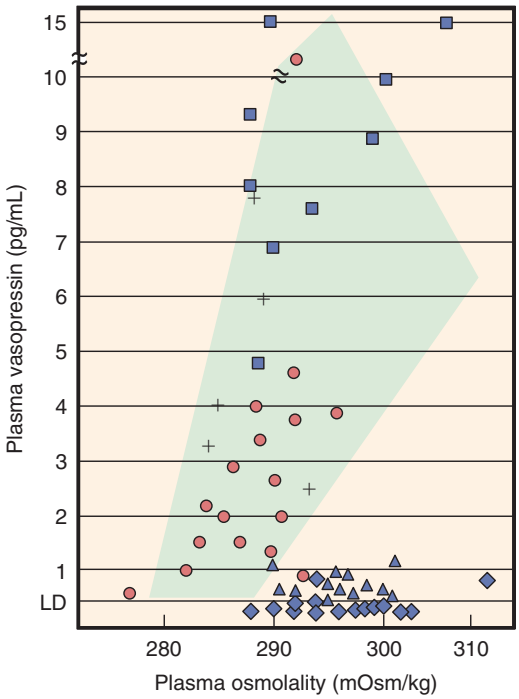


Fig. 15.12 Relationship between plasma arginine vasopressin (AVP) and concurrent plasma osmolality in patients with polyuria of diverse causes. All measurements were made at the end of a standard dehydration test. *Shaded area,* Range of normal. In patients with severe (◆) or partial (▲) central diabetes insipidus (DI), plasma AVP was almost always subnormal relative to plasma osmolality. In contrast, the values from patients with primary polydipsia (●) or nephrogenic DI (■) were consistently within or above the normal range. (From Robertson GL: Diagnosis of diabetes insipidus. In Czernichow AP, Robinson A, eds. *Diabetes Insipidus in Man: Frontiers of Hormone Research.* Basel, Switzerland: S. Karger; 1985:176.)

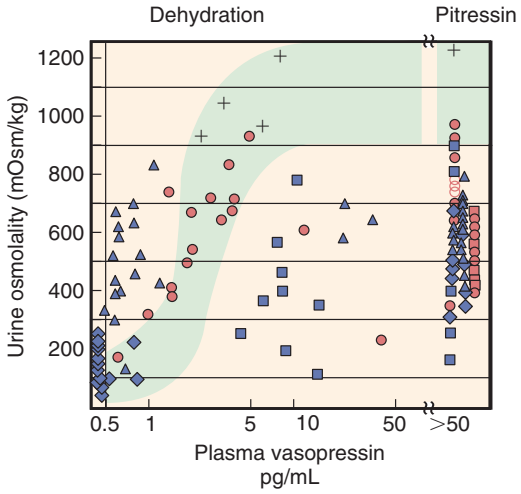


Fig. 15.13 Relationship between urine osmolality and concurrent plasma arginine vasopressin (AVP) in patients with polyuria of diverse causes. All measurements were made at the end of a standard dehydration test. *Shaded area,* Range of normal. In patients with severe (◆) or partial (▲) central diabetes insipidus (DI), urine osmolality is normal or supranormal relative to plasma AVP when the latter is submaximal. In patients with nephrogenic DI (■), urine osmolality is always subnormal for plasma AVP. In patients with primary polydipsia (●), the relationship is normal at submaximal levels of plasma AVP but usually subnormal when plasma AVP is high. (From Robertson GL: Diagnosis of diabetes insipidus. In Czernichow AP, Robinson A, eds. *Diabetes Insipidus in Man: Frontiers of Hormone Research.* Basel, Switzerland: S. Karger; 1985:176.)

of the AVP deficiency in most cases and underscore the importance of the thirst mechanism to restrict the use of residual secretory capacity under basal conditions of ad libitum water intake.

CDI is also associated with changes in the renal response to AVP. The most obvious change is a reduction in maximal concentrating capacity, which has been attributed to washout of the medullary concentration gradient caused by the chronic polyuria in combination with decreased synthesis of AQP2 in the renal collecting duct principal cells. The severity of this defect is proportional to the magnitude of the polyuria and is independent of its cause.[147] Because of this, the level of urinary concentration achieved at maximally effective levels of plasma AVP is reduced in all types of DI. In patients with CDI, this concentrating abnormality is offset to some extent by an apparent increase in renal sensitivity to low levels of plasma AVP (Fig. 15.13). The cause of this supersensitivity is unknown, but it may reflect upward regulation of AVP V_2 receptor expression or function secondary to a chronic deficiency of the hormone.[156]

OSMORECEPTOR DYSFUNCTION

CAUSES

There is extensive literature in animals indicating that the primary osmoreceptors controlling AVP secretion and thirst are located in the anterior hypothalamus; lesions of this region in animals, called the "AV3V area," cause hyperosmolality through a combination of impaired thirst and impaired osmotically stimulated AVP secretion.[30,31] Initial reports in humans described this syndrome as essential hypernatremia,[157] and subsequent studies used the term "adipsic hypernatremia" in recognition of the profound thirst deficits found in most of the patients.[158] Based on the known pathophysiology, all these syndromes can be grouped together, termed "disorders of osmoreceptor dysfunction."[159] Although the pathologies responsible for this condition can be quite varied, all the cases reported to date have been due to various degrees of osmoreceptor destruction associated with a variety of different brain lesions, as summarized in Box 15.1. Many of these are the same types of lesions that can cause CDI but, in contrast to CDI, these lesions usually occur more rostrally in the hypothalamus, consistent with the anterior hypothalamic location of the primary osmoreceptor cells (see Fig. 15.2). One lesion that is unique to this disorder is an anterior communicating cerebral artery aneurysm. Because the small arterioles that feed the anterior wall of the third ventricle originate from the anterior communicating cerebral artery, an aneurysm in this region[160]—but more often following surgical repair of such an aneurysm that typically involves ligation of the anterior communicating artery[161]—produces infarction of the part of the hypothalamus containing the osmoreceptor cells.

PATHOPHYSIOLOGY

The cardinal defect of patients with this disorder is lack of the osmoreceptors that regulate thirst. With rare exceptions, osmoregulation of AVP is also impaired, although the

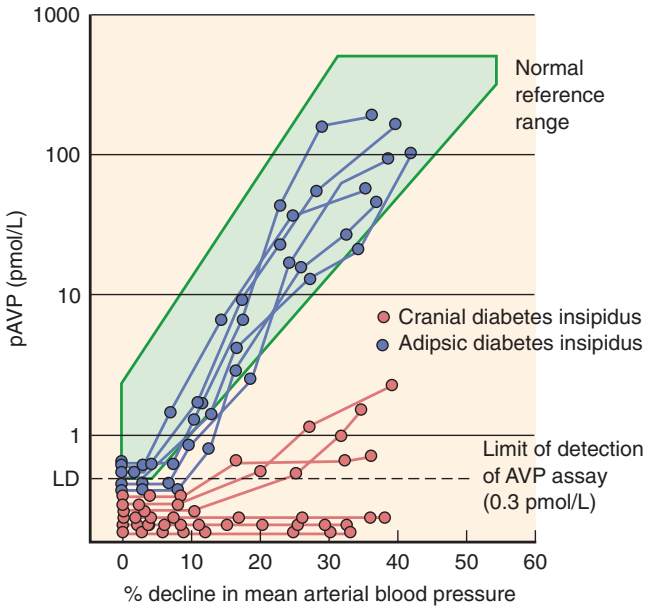


Fig. 15.14 Plasma arginine vasopressin (AVP) responses to arterial hypotension produced by the infusion of trimethaphan in patients with central DI (cranial diabetes insipidus) and osmoreceptor dysfunction (adipsic diabetes insipidus). Normal responses in healthy volunteers are shown by the *shaded area.* Note that despite absent or markedly blunted AVP responses to hyperosmolality, patients with osmoreceptor dysfunction respond normally to baroreceptor stimulation induced by hypotension. (From Baylis PH, Thompson CJ: Diabetes insipidus and hyperosmolar syndromes. In Becker KL, ed. *Principles and Practice of Endocrinology and Metabolism,* Philadelphia: JB Lippincott; 1995:257.)

hormonal response to nonosmotic stimuli remains intact (Fig. 15.14).[162,163] Four major patterns of osmoreceptor dysfunction have been described, and each is characterized by defects in thirst and/or AVP secretory responses, as follows: (1) upward resetting of the osmostat for both thirst and AVP secretion (normal AVP and thirst responses but at an abnormally high plasma osmolality); (2) partial osmoreceptor destruction (blunted AVP and thirst responses at all plasma osmolalities); (3) total osmoreceptor destruction (absent AVP secretion and thirst, regardless of plasma osmolality); and (4) selective dysfunction of thirst osmoregulation with intact AVP secretion.[159] Regardless of the actual pattern, the hallmark of this disorder is an abnormal thirst response in addition to variable defects in AVP secretion. Thus, such patients fail to drink sufficiently as their plasma osmolality rises and, as a result, the new set point for plasma osmolality rises far above the normal thirst threshold. Unlike patients with CDI, whose polydipsia maintains their plasma osmolality within a normal range, patients with osmoreceptor dysfunction typically have osmolality in the range of 300 to 340 mOsm/kg H_2O. This again underscores the critical role played by normal thirst mechanisms in maintaining body fluid homeostasis; intact renal function alone is insufficient to maintain plasma osmolality within normal limits in such cases.

The rate of the development and severity of hyperosmolality and hypertonic dehydration in patients with osmoreceptor dysfunction is influenced by a number of factors. First is the ability to maintain some degree of osmotically stimulated thirst and AVP secretion, which will determine the new set point for plasma osmolality. Second are environmental influences that affect the rate of water output. When physical activity is minimal, and the ambient temperature is not elevated, the overall rates of renal and insensible water loss are low, and the patient's diet may be sufficient to maintain a relatively normal balance for long periods of time. Anything that increases perspiration, respiration, or urine output greatly accelerates the rate of water loss and thereby uncovers the patient's inability to mount an appropriate compensatory increase in water intake.[12] Under these conditions, severe and even fatal hypernatremia can develop relatively quickly. When the dehydration is only moderate (plasma osmolality = 300 to 330 mOsm/kg H_2O), the patient is usually asymptomatic, and signs of volume depletion are minimal, but if the dehydration becomes severe, the patient can exhibit symptoms and signs of hypovolemia, including weakness, postural dizziness, paralysis, confusion, coma, azotemia, hypokalemia, hyperglycemia, and secondary hyperaldosteronism (see later, "Clinical Manifestations of Diabetes Insipidus"). In severe cases, there may also be rhabdomyolysis, with marked serum elevations in muscle enzyme levels and occasionally acute renal failure.

However, a third factor also influences the degree of hyperosmolality and dehydration present in these patients. For all cases of osmoreceptor dysfunction, it is important to remember that afferent pathways from the brain stem to the hypothalamus remain intact; therefore, these patients will usually have normal AVP and renal concentrating responses to baroreceptor-mediated stimuli, such as hypovolemia and hypotension (see Fig. 15.14),[163] or to other nonosmotic stimuli, such as nausea (see Fig. 15.8).[158,162] This has the effect of preventing severe dehydration because, as hypovolemia develops, this will stimulate AVP secretion via baroreceptive pathways through the brain stem (see Fig. 15.2). Although protective, this effect often causes confusion, because sometimes these patients appear to have DI yet at other times they can concentrate their urine quite normally. Nonetheless, the presence of refractory hyperosmolality with absent or inappropriate thirst should alert clinicians to the presence of osmoreceptor dysfunction, regardless of occasional apparent normal urine concentration.

In a few patients with osmoreceptor dysfunction, forced hydration has been found to lead to hyponatremia in association with inappropriate urine concentration.[157,158] This paradoxic defect resembles that seen in SIADH and has been postulated to be caused by two different pathogenic mechanisms. One is continuous or fixed secretion of AVP because of loss of the capacity for osmotic inhibition and stimulation of hormone secretion. These observations, as well as electrophysiologic data,[43] have strongly suggested that the osmoregulatory system is bimodal (i.e., it is composed of inhibitory and stimulatory input to the neurohypophysis; see Fig. 15.6). The other cause of the diluting defect appears to be hypersensitivity to the antidiuretic effects of AVP because, in some patients, urine osmolality may remain elevated, even when the hormone is undetectable.[158]

Hypodipsia is also a common occurrence in older adults in the absence of any overt hypothalamic lesion.[164] In such cases, it is not clear whether the defect is in the hypothalamic osmoreceptors, in their projections to the cortex, or in some other regulatory mechanism. However, in most cases, the osmoreceptor is likely not involved because basal and stimulated plasma AVP levels have been found to be normal, or even hyperresponsive, in relation to plasma osmolality in

older adults, with the exception of only a few studies that showed decreased plasma levels of AVP relative to plasma osmolality.[165]

GESTATIONAL DIABETES INSIPIDUS

CAUSES

A relative deficiency of plasma AVP can also result from an increase in the rate of AVP metabolism.[100,166] This condition has been observed only in pregnancy and therefore is generally termed "gestational diabetes insipidus."[167,168] It is due to the action of a circulating enzyme called cysteine aminopeptidase (oxytocinase or vasopressinase) that is normally produced by the placenta to degrade circulating oxytocin and prevent premature uterine contractions.[169] Because of the close structural similarity between AVP and OT, this enzyme degrades both peptides.[170] There are two types of gestational diabetes insipidus.[169] In the first type, the activity of cysteine aminopeptidase is extremely and abnormally elevated. This syndrome has been referred to as vasopressin-resistant diabetes insipidus of pregnancy.[171] It can occur in association with preeclampsia, acute fatty liver, and coagulopathies (e.g., HELLP syndrome [*h*emolysis, *e*levated *l*iver enzymes, and *l*ow *p*latelet count]). These patients have decreased metabolism of vasopressinase by the liver.[172] Usually, in subsequent pregnancies, these women have neither diabetes insipidus nor acute fatty liver. In the second type, the accelerated metabolic clearance of vasopressin produces DI in a patient with borderline vasopressin function from a specific disease (e.g., mild nephrogenic DI or partial CDI). AVP is rapidly destroyed, and the neurohypophysis is unable to keep up with the increased demand. Labor and parturition usually proceed normally, and patients have no trouble with lactation. Severe dehydration can occur if DI is unrecognized, which may pose a threat to a pregnant woman and her fetus. The relationship of this disorder to the transient nephrogenic DI (NDI) of pregnancy is not clear.[171]

PATHOPHYSIOLOGY

The pathophysiology of gestational DI is similar to that of CDI. The only exception is that the polyuria is usually not corrected by the administration of AVP because this is rapidly degraded, just as is endogenous AVP, but it can be controlled by treatment with desmopressin, the AVP V_2 receptor agonist that is more resistant to degradation by oxytocinase or vasopressinase.[169] It should be remembered that patients with partial CDI in whom only low levels of AVP can be maintained, or patients with compensated NDI in whom the lack of response of the kidney to AVP may not be absolute, can be relatively asymptomatic with regard to polyuria. However, with accelerated destruction of AVP during pregnancy, the underlying DI may become manifest. Consequently, patients presenting with gestational DI should not be assumed simply to have excess oxytocinase or vasopressinase; rather, these patients should be evaluated for other possible underlying pathologic diagnoses (see Box 15.1).[173]

NEPHROGENIC DIABETES INSIPIDUS

CAUSES

Resistance to the antidiuretic action of AVP is usually due to some defect within the kidney, and is commonly referred to as "nephrogenic diabetes insipidus" (NDI). It was first recognized in 1945 in several patients with the familial, sex-linked form of the disorder. Subsequently, additional kindreds with the X-linked form of familial NDI were identified. Clinical studies of NDI have indicated that symptomatic polyuria is present from birth, plasma AVP levels are normal or elevated, resistance to the antidiuretic effect of AVP can be partial or almost complete, and the disease affects mostly males and is usually, although not always,[174] mild or absent in carrier females. More than 90% of cases of congenital NDI are caused by mutations of the AVP V_2 receptor[175,176] (see Chapter 44). Most mutations occur in the part of the receptor that is highly conserved among species and/or is conserved among similar receptors, such as homologies with AVP V_{1A} or OT receptors. The effect of some of these mutations on receptor synthesis, processing, trafficking, and function has been studied by in vitro expression.[177,178]

These types of studies have shown that the various mutations cause several different defects in cellular processing and function of the receptor but can be classified into four general categories based on differences in transport to the cell surface and AVP binding and/or stimulation of adenylyl cyclase, as follows: (1) the mutant receptor is not inserted in the membrane; (2) the mutant receptor is inserted in the membrane but does not bind or respond to AVP; (3) the mutant receptor is inserted in the membrane and binds AVP but does not activate adenylyl cyclase; or (4) the mutant protein is inserted into the membrane and binds AVP but responds subnormally in terms of adenylyl cyclase activation. Two studies have shown a relationship between the clinical phenotype and genotype and/or cellular phenotype.[177,179] Approximately 10% of the V_2 receptor defects causing congenital NDI are thought to be de novo. This high incidence of de novo cases, coupled with the large number of mutations that have been identified, hinders the clinical use of genetic identification because it is necessary to sequence the entire open reading frame of the receptor gene rather than short sequences of DNA. Nonetheless, the use of automated gene sequencing techniques in selected families has been shown to identify mutations in patients with clinical disease and in asymptomatic carriers.[180] Although most female carriers of the X-linked V_2 receptors defect have no clinical disease, some have been reported with symptomatic NDI.[174] Carriers can have a decreased maximum urine osmolality in response to plasma AVP levels, but are generally asymptomatic because of the absence of overt polyuria. In one study, a girl manifested severe NDI due to a V_2 receptor mutation, which was likely due to skewed inactivation of the normal X chromosome.[181]

Congenital NDI can also result from mutations of the autosomal gene that codes for AQP2, the protein that forms the water channels in renal medullary collecting tubules. When the proband is a girl, it is likely the defect is a mutation of the *AQP2* gene on chromosome 12, region q12-q13.[182] More than 25 different mutations of the *AQP2* gene have been described[183] (see Chapter 44). The patients may be heterozygous for two different recessive mutations[184] or homozygous for the same abnormality from both parents.[185] Because most of these mutations are recessive, the patients usually do not present with a family history of DI unless consanguinity is present. Functional expression studies of these mutations have shown that all of them result in varying degrees of reduced water transport because the mutant

aquaporins are not expressed in normal amounts, are retained in various cellular organelles, or simply do not function as effectively as water channels. Regardless of the type of mutation, the renal phenotype of NDI from AQP2 mutations is identical to that produced by V_2 receptor mutations. Of interest, and sometimes helpful clinically, an AVP V_2 receptor agonist still stimulates the release of von Willebrand factor (vWF) from the Weibel-Palade bodies of endothelial cells in patients with AQP2 mutations. Some of the defects in cellular routing and water transport can be reversed by treatment with chemicals that act like chaperones,[186] suggesting that misfolding of the mutant AQP2 may be responsible for misrouting. Similar salutary effects of chaperones have been found to reverse defects in cell surface expression and function of selected mutations of the AVP V_2 receptor.[187]

NDI can also be caused by a variety of drugs, diseases, and metabolic disturbances, including lithium, hypokalemia, and hypercalcemia (see Box 15.1). Some of these disorders (e.g., polycystic kidney disease) act to distort the normal architecture of the kidney and interfere with the normal urine concentration process. However, experimental studies in animal models have suggested that many have in common a downregulation of AQP2 expression in the renal collecting tubules (Fig. 15.15; see also Chapter 10).[188,189] The polyuria associated with potassium deficiency develops in parallel with decreased expression of kidney AQP2, and repletion of potassium reestablishes the normal urinary concentrating mechanism and normalizes the renal expression of AQP2.[190] Similarly, hypercalcemia has also been found to be associated with downregulation of AQP2.[191] A low-protein diet diminishes the ability to concentrate the urine, primarily by a decreased delivery of urea to the inner medulla, thus decreasing the medullary concentration gradient, but rats on a low- protein diet also appear to downregulate AQP2, which could be an additional component of the decreased ability to concentrate the urine.[192] Bilateral urinary tract obstruction causes an inability to produce a maximum concentration of the urine, and rat models have demonstrated a downregulation of AQP2, which persists for several days after release of the obstruction.[193] However, it is not yet clear which of these effects on AQP2 expression are primary or secondary and which cellular mechanism(s) are responsible for the downregulation of AQP2 expression.

The administration of lithium to treat psychiatric disorders is the most common cause of drug-induced NDI and illustrates the multiple mechanisms likely involved in producing this disorder. As many as 10% to 20% of patients on chronic lithium therapy develop some degree of NDI.[194] Lithium is known to interfere with the production of cAMP[195] and produces a dramatic (95%) reduction in kidney AQP2 levels in animals.[196] The defect of aquaporins is slow to correct in experimental animals and humans and, in some cases, it can be permanent,[197] in association with glomerular or tubulointerstitial nephropathy.[198] Several other drugs that are known to induce renal concentrating defects have also been associated with abnormalities of AQP2 synthesis.[199]

PATHOPHYSIOLOGY

Similar to CDI, renal insensitivity to the antidiuretic effect of AVP also results in the excretion of an increased volume of dilute urine, decrease in body water, and rise in plasma osmolality, which by stimulating thirst induces a compensatory increase in water intake. As a consequence, the osmolality of body fluid stabilizes at a slightly higher level, which approximates the osmotic threshold for thirst. As in patients with CDI, the magnitude of polyuria and polydipsia varies greatly depending on a number of factors, including the degree of renal insensitivity to AVP, individual differences in the set points and sensitivity of thirst and AVP secretion, and total solute load. It is important to note that the renal insensitivity to AVP need not be complete for polyuria to occur; it is only necessary that the defect is great enough to prevent the concentration of the urine at plasma AVP levels achievable under ordinary conditions of ad libitum water intake (i.e., at plasma osmolalities near the osmotic threshold for thirst). Calculations similar to those used for states of AVP deficiency indicate that this requirement is not met until the renal sensitivity to AVP is reduced by more than

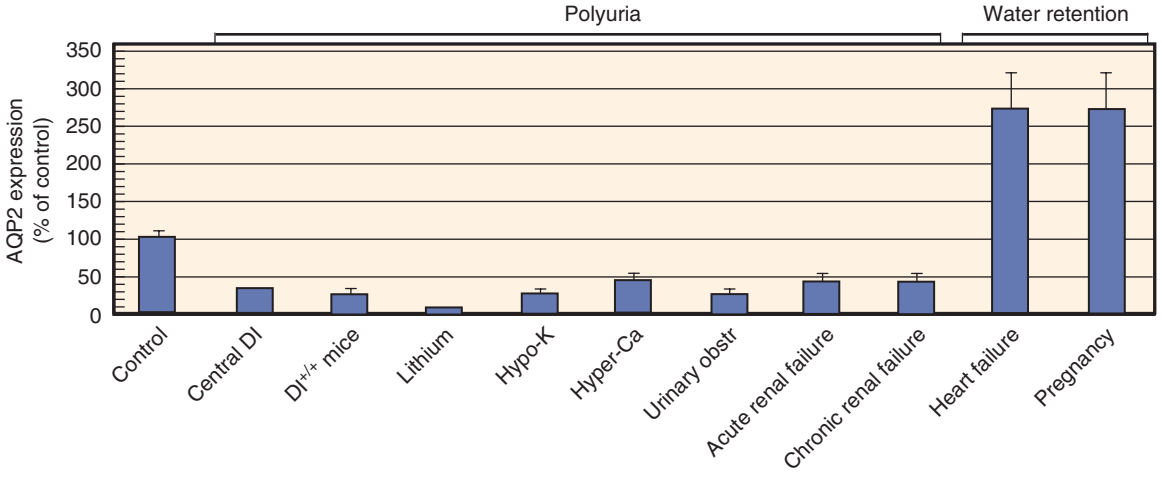


Fig. 15.15 Kidney expression of the water channel aquaporin-2 in various animal models of polyuria and water retention. Note that kidney aquaporin-2 expression is uniformly downregulated relative to levels in controls in all animal models of polyuria, but upregulated in animal models of inappropriate antidiuresis. *DI+/+,* Genetic diabetes insipidus; *Hyper-Ca,* hypercalcemia; *Hypo-K,* hypokalemia; *Urinary obstr,* ureteral obstruction. (From Nielsen S, Kwon TH, Christensen BM, et al: Physiology and pathophysiology of renal aquaporins. *J Am Soc Nephrol.* 1999;10:647–663.)

10-fold. Because renal insensitivity to the hormone is often incomplete, especially in cases of acquired rather than congenital NDI, many patients with NDI are able to concentrate their urine to varying degrees when they are deprived of water or given large doses of desmopressin.

Information about the renal concentration mechanism from studies of AQP2 expression in experimental animals (see Chapter 10) has suggested that a form of NDI is likely associated with all types of DI, as well as with primary polydipsia. Brattleboro rats have been found to have low levels of kidney AQP2 expression compared with Long-Evans control rats; AQP2 levels are corrected by treatment with AVP or desmopressin, but this process takes 3 to 5 days, during which time urine concentration remains subnormal, despite pharmacologic concentrations of AVP.[200] Similarly, physiologic suppression of AVP by chronic overadministration of water produces a downregulation of AQP2 in the renal collecting duct.[200] Clinically, it is well known that patients with both CDI and primary polydipsia often fail to achieve maximally concentrated urine when they are given desmopressin during a water deprivation test to differentiate among the various causes of DI. This effect has long been attributed to a washout of the medullary concentration gradient as a result of the high urine flow rates in polyuric patients; however, based on the results of animal studies, it seems certain that at least part of the decreased response to AVP is due to a downregulation of kidney AQP2 expression. This also explains why it takes time, typically several days, to restore normal urinary concentration after patients with primary polydipsia and CDI are treated with water restriction or antidiuretic therapy.[201]

PRIMARY POLYDIPSIA

CAUSES

Excessive fluid intake also causes hypotonic polyuria and, by definition, polydipsia. Consequently, this disorder must be differentiated from the various causes of DI. Furthermore, it is apparent that despite normal pituitary and kidney function, patients with this disorder share many characteristics of both CDI (AVP secretion is suppressed as a result of the decreased plasma osmolality) and NDI (kidney AQP2 expression is decreased as a result of the suppressed plasma AVP levels). Many different names have been used to describe patients with excessive fluid intake, but the term "primary polydipsia" remains the best descriptor because it does not presume any particular cause for the increased fluid intake. Primary polydipsia is often due to a severe mental illness, such as schizophrenia, mania, or an obsessive-compulsive disorder,[202] in which case it is termed "psychogenic polydipsia." These patients usually deny true thirst and attribute their polydipsia to bizarre motives, such as a need to cleanse their body of poisons. Studies of a series of polydipsic patients in a psychiatric hospital have shown an incidence as high as 42% of patients with some form of polydipsia and, in most reported cases, there was no obvious explanation for the polydipsia.[203]

However, primary polydipsia can also be caused by an abnormality in the osmoregulatory control of thirst, in which case it has been termed "dipsogenic diabetes insipidus."[204] These patients have no overt psychiatric illness and invariably attribute their polydipsia to a nearly constant thirst. Dipsogenic DI is usually idiopathic but can also be secondary to organic structural lesions in the hypothalamus identical to any of the disorders described as causes of CDI, such as neurosarcoidosis of the hypothalamus, tuberculous meningitis, multiple sclerosis, or trauma. Consequently, all polydipsic patients should be evaluated with an MRI scan of the brain before concluding that excessive water intake has an idiopathic or psychiatric cause. Primary polydipsia can also be produced by drugs that cause a dry mouth or by any peripheral disorder causing pathologic elevations of renin and/or angiotensin levels.[113]

Finally, primary polydipsia is sometimes caused by physicians, nurses, or the lay press who advise a high fluid intake for valid (e.g., recurrent nephrolithiasis) or unsubstantiated reasons of health.[205] These patients lack overt signs of mental illness but also deny thirst and usually attribute their polydipsia to habits acquired from years of adherence to their drinking regimen.

PATHOPHYSIOLOGY

The pathophysiology of primary polydipsia is essentially the reverse of that in CDI—the excessive intake of water expands and slightly dilutes body fluids, suppresses AVP secretion, and dilutes the urine. The resultant increase in the rate of water excretion balances the increase in intake, and the osmolality of body water stabilizes at a new, slightly lower level that approximates the osmotic threshold for AVP secretion. The magnitude of the polyuria and polydipsia vary considerably, depending on the nature or intensity of the stimulus to drink. In patients with abnormal thirst, the polydipsia and polyuria are relatively constant from day to day. However, in patients with psychogenic polydipsia, water intake and urine output tend to fluctuate widely and, at times, can be quite large.

Occasionally, fluid intake rises to such extraordinary levels that the excretory capacity of the kidneys is exceeded, and dilutional hyponatremia develops.[206] There is little question that excessive water intake alone can sometimes be sufficient to override renal excretory capacity and produce severe hyponatremia. Although the water excretion rate of normal adult kidneys can generally exceed 20 L/day, maximum hourly rates rarely exceed 1000 mL/h. Because many psychiatric patients drink predominantly during the day or during intense drinking binges,[207] they can transiently achieve symptomatic levels of hyponatremia, with the total daily volume of water intake less than 20 L if ingested rapidly enough. This likely accounts for many of the patients who present with maximally dilute urine, accounting for as many as 50% of patients in some studies, and are corrected quickly via free water diuresis.[208] The prevalence of this disorder, based on hospital admissions for acute symptomatic hyponatremia, may have been underestimated because studies of polydipsic psychiatric patients have shown a marked diurnal variation in serum [Na^+], from 141 mEq/L at 7 AM to 130 mEq/L at 4 PM, suggesting that many such patients drink excessively during the daytime but then correct themselves via water diuresis at night.[209] This and other considerations have led to defining this disorder as the "psychosis, intermittent hyponatremia, and polydipsia" (PIP) syndrome.[207]

However, many other cases of hyponatremia with psychogenic polydipsia have been found to meet the criteria for a diagnosis of SIADH, suggesting the presence of nonosmotically stimulated AVP secretion. As might be expected, in the presence of much higher than normal water intake, almost

any impairment of urinary dilution and water excretion can exacerbate the development of a positive water balance and thereby produce hypo-osmolality. Acute psychosis itself can also cause AVP secretion,[210] which often appears to take the form of a reset osmostat.[202] It is therefore apparent that no single mechanism can completely explain the occurrence of hyponatremia in polydipsic psychiatric patients, but the combination of higher than normal water intake plus modest elevations of plasma AVP levels from a variety of potential sources appears to account for a significant portion of these cases.

CLINICAL MANIFESTATIONS OF DIABETES INSIPIDUS

The characteristic clinical symptoms of DI are the polyuria and polydipsia that result from the underlying impairment of urine-concentrating mechanisms, which have been described earlier in the sections on the pathophysiology of specific types of DI. Interestingly, patients with DI typically describe a craving for cold water, which appears to quench their thirst better.[69] Patients with CDI also typically describe a precipitous onset of their polyuria and polydipsia, which simply reflects the fact that urinary concentration can be maintained fairly well until the number of AVP-producing neurons in the hypothalamus decreases to 10% to 15% of normal, after which plasma AVP levels decrease to the range at which urine output increases dramatically.

However, patients with DI, particularly those with osmoreceptor dysfunction syndromes, can also present with varying degrees of hyperosmolality and dehydration, depending on their overall hydration status. It is therefore also important to be aware of the clinical manifestations of hyperosmolality. These can be divided into the signs and symptoms produced by dehydration, which are largely cardiovascular, and those caused by the hyperosmolality itself, which are predominantly neurologic and reflect brain dehydration as a result of osmotic water shifts out of the CNS. Cardiovascular manifestations of hypertonic dehydration include hypotension, azotemia, acute tubular necrosis secondary to renal hypoperfusion or rhabdomyolysis, and shock.[211,212] Neurologic manifestations range from nonspecific symptoms, such as irritability and cognitive dysfunction, to more severe manifestations of hypertonic encephalopathy, such as disorientation, decreased level of consciousness, obtundation, chorea, seizures, coma, focal neurologic deficits, subarachnoid hemorrhage, and cerebral infarction.[211,213] One study has also suggested an increased incidence of deep venous thrombosis in hyperosmolar patients.[214]

The severity of symptoms can be roughly correlated with the degree of hyperosmolality, but individual variability is marked and, for any single patient, the level of serum [Na^+] at which symptoms will appear cannot be accurately predicted. Similar to hypoosmolar syndromes, the length of time over which hyperosmolality develops can markedly affect the clinical symptomatology. The rapid development of severe hyperosmolality is frequently associated with marked neurologic symptoms, whereas gradual development over several days or weeks generally causes milder symptoms.[211,215] In this case, the brain counteracts osmotic shrinkage by increasing the intracellular content of solutes. These include electrolytes such as potassium and a variety of organic osmolytes, which previously had been termed "idiogenic osmoles"; for the most part, these are the same organic osmolytes that are lost from the brain during adaptation to hypoosmolality.[216] The net effect of this process is to protect the brain against excessive shrinkage during sustained hyperosmolality. However, once the brain has adapted by increasing its solute content, rapid correction of the hyperosmolality can produce brain edema because it takes a finite length of time (24–48 hours in animal studies) to dissipate the accumulated solutes and, until this process has been completed, the brain will accumulate excess water as plasma osmolality is normalized.[217] This effect is usually seen in dehydrated pediatric patients who can develop seizures with rapid rehydration,[218] but has been described only rarely in adults, including the most severely hyperosmolar patients with nonketotic hyperglycemic hyperosmolar coma.

DIFFERENTIAL DIAGNOSIS OF POLYURIA

Before beginning involved diagnostic testing to differentiate among the various forms of DI and primary polydipsia, the presence of true hypotonic polyuria should be established by measurement of a 24-hour urine for volume and osmolality. Generally accepted standards are that the 24-hour urine volume should exceed 50 mL/kg BW, with an osmolality lower than 300 mOsm/kg H_2O.[219] Simultaneously, there should be a determination of whether the polyuria is due to an osmotic agent such as glucose or intrinsic renal disease. Routine laboratory studies and the clinical setting will usually distinguish these disorders; diabetes mellitus and other forms of solute diuresis usually can be excluded by the history, routine urinalysis for glucose, and/or measurement of the solute excretion rate (urine osmolality × 24-hour urine volume [in liters] > 15 mOsm/kg BW/day). There is general agreement that the diagnosis of DI requires stimulating AVP secretion osmotically and then measuring the adequacy of the secretion by direct measurement of plasma AVP levels or indirect assessment by urine osmolality.

In a patient who is already hyperosmolar, with submaximally concentrated urine (i.e., urine osmolality < 800 mOsm/kg H_2O), the diagnosis is straightforward and simple; primary polydipsia is ruled out by the presence of hyperosmolality,[219] confirming a diagnosis of DI. CDI can then be distinguished from NDI by evaluating the response to the administration of AVP (5 units subcutaneously) or, preferably, of the AVP V_2 receptor agonist desmopressin (1-deamino-8-D-arginine vasopressin [DDAVP], 1–2 μg subcutaneously or intravenously). A significant increase in urine osmolality within 1 to 2 hours after injection indicates insufficient endogenous AVP secretion, and therefore CDI, whereas an absent response indicates renal resistance to AVP effects, and therefore NDI. Although conceptually simple, interpretational difficulties can arise because the water diuresis produced by AVP deficiency in CDI produces a washout of the renal medullary concentrating gradient and downregulation of the kidney AQP2 water channels (see earlier), so that initial increases in urine osmolality in response to administered AVP or desmopressin are not as great as would be expected. Generally, increases of urine osmolality of more than 50% reliably indicate CDI, and responses of less than 10% indicate NDI, but responses between 10% and 50% are indeterminate.[147] Therefore, plasma AVP levels should be measured to aid in this distinction; hyperosmolar patients with NDI will have clearly elevated plasma AVP levels, whereas those with CDI will have absent (complete) or blunted (partial) AVP responses

relative to their plasma osmolality (see Fig. 15.11). Because it will not be known beforehand which patients will have diagnostic versus indeterminate responses to AVP or desmopressin, a plasma AVP level should be determined prior to AVP or desmopressin administration in patients with hyperosmolality and inadequately concentrated urine without a solute diuresis.

Patients with DI have intact thirst mechanisms, so they usually do not present with hyperosmolality, but rather with a normal plasma osmolality and serum [Na^+] and symptoms of polyuria and polydipsia. In these cases, it is most appropriate to perform a fluid deprivation test. The relative merits of the indirect fluid deprivation test (Miller-Moses test[220]) versus direct measurement of plasma AVP levels after a period of fluid deprivation[147] has been debated in literature, with substantial pros and cons in support of each of these tests. On the one hand, the standard indirect test has a long track record of making an appropriate diagnosis in the large majority of cases, generally yields interpretable results by the end of the test, and does not require sensitive assays for the notoriously difficult measurement of plasma AVP levels.[221,222] However, maximum urine-concentrating capacity is well known to be variably reduced in all forms of DI, as well as primary polydipsia[147] and, as a result, the absolute levels of urine osmolality achieved during fluid deprivation and after AVP administration are reduced to overlapping degrees in patients with partial CDI, partial NDI, and primary polydipsia.

Measurements of basal plasma osmolality or serum [Na^+] are of little use because they also overlap considerably among these disorders.[219] Although association with certain diseases, surgical procedures, or family history often helps differentiate among these disorders, sometimes the clinical setting may not be helpful because certain disorders (e.g., sarcoidosis, tuberculous meningitis, other hypothalamic pathologies) can cause more than one type of DI (see Box 15.1). Consequently, a simpler approach that has been proposed is to measure plasma or urine AVP before and during a suitable osmotic stimulus, such as fluid restriction or hypertonic NaCl infusion, and plot the results as a function of the concurrent plasma osmolality or plasma [Na^+] (see Figs. 15.12 and 15.13).[223,224]

Using a highly sensitive and validated research assay for plasma AVP determinations, this approach has been shown to provide a definitive diagnosis in most cases, provided that the final level of plasma osmolality or sodium achieved is above the normal range (>295 mOsm/kg H_2O or 145 mmol/L, respectively). The diagnostic effectiveness of this approach derives from the fact that the magnitude of the AVP response to osmotic stimulation is not appreciably diminished by chronic overhydration[202] or dehydration. Hence, the relationship of plasma AVP to plasma osmolality is usually within or above normal limits in NDI and primary polydipsia. In most cases, these two disorders can then be distinguished by measuring urine osmolality before and after the dehydration test and relating these values to the concurrent plasma AVP concentration (see Fig. 15.12). However, because maximal concentrating capacity can be severely blunted in patients with primary polydipsia, it is often better to analyze the relationship under basal nondehydrated conditions, when the plasma AVP level is not elevated. Because of the solute diuresis that often ensues following infusion of hypertonic NaCl, measurements of urine osmolality or AVP excretion are unreliable indicators of changes in hormone secretion and are of little or no diagnostic value when this procedure is used to increase osmolality to more than 295 mOsm/kg H_2O. Given the proven usefulness of the indirect and direct approaches, a combined fluid deprivation test that synthesizes the crucial aspects of both tests can easily be performed (Box 15.2). In many cases, this will allow interpretation of the plasma AVP levels and response to an AVP challenge.

Because measurement of vasopressin in plasma is difficult, recent studies have suggested that the C-terminal fragment of the vasopressin prohormone copeptin (see Fig. 15.3) may

Box 15.2 Fluid Deprivation Test for the Diagnosis of Diabetes Insipidus (DI)

Procedure

1. Initiation of the deprivation period depends on the severity of the DI; in routine cases, the patient should be made NPO after dinner, whereas in patients with more severe polyuria and polydipsia, this may be too long a period without fluids, and the water deprivation should be started early on the morning (e.g., 6 AM) of the test.
2. Obtain plasma and urine osmolality and serum electrolyte and plasma AVP or copeptin levels at the start of the test.
3. Measure urine volume and osmolality hourly or with each voided urine.
4. Stop the test when body weight decreases by ≥3%, the patient develops orthostatic blood pressure changes, the urine osmolality reaches a plateau (i.e., <10% change over two or three consecutive measurements), or the serum Na^+ > 145 mmol/L.
5. Obtain plasma and urine osmolality and serum electrolyte and plasma AVP or copeptin levels at the end of the test, when the plasma osmolality is elevated, preferably >300 mOsm/kg H_2O.
6. If serum Na^+ < 146 mmol/L or plasma osmolality < 300 mOsm/kg H_2O when the test is stopped, then consider a short infusion of hypertonic saline (3% NaCl at a rate of 0.1 mL/kg/min for 1 to 2 hours) to reach these end points.
7. If hypertonic saline infusion is not required to achieve hyperosmolality, administer AVP (5 U) or desmopressin (DDAVP; 1 μg) subcutaneously and continue following urine osmolality and volume for an additional 2 hours.

Interpretation

1. An unequivocal urine concentration after AVP or DDAVP (>50% increase) indicates central diabetes insipidus (CDI); an unequivocal absence of urine concentration (<10%) strongly suggests nephrogenic DI (NDI) or primary polydipsia (PP).
2. Differentiating between NDI and PP, as well as cases in which the increase in urine osmolality after AVP or DDAVP administration is more equivocal (e.g., 10%–50%), is best done using the relationship between plasma AVP or copeptin levels and plasma osmolality obtained at the end of the dehydration period and/or hypertonic saline infusion and the relationship between plasma AVP levels and urine osmolality determined under basal conditions (see Figs. 15.12, 15.13, and 15.16).

be a reliable and more convenient surrogate measure of vasopressin secretion.[225] Copeptin is released with AVP in a 1:1 stoichiometric ratio because both peptides are part of the same prohormone, and several studies have demonstrated high correlations between plasma AVP and copeptin levels (Fig. 15.16).[226] This has led to studies that indicate a higher accuracy of the correct diagnosis of the cause of polyuria using plasma copeptin levels at the end of water deprivation than by indirect measurement of the urine osmolality response to AVP or desmopressin.[227,228] Consequently, it seems likely that the measurement of copeptin will replace the measurement of AVP to establish a diagnosis of DI in the future, although successful interpretation of either measure will require measurement during hyperosmolality via water deprivation or hypertonic saline challenge.[229]

With use of the fluid deprivation test for plasma AVP or copeptin determinations, most cases of polyuria and polydipsia can be diagnosed accurately. A useful approach in the remaining indeterminate cases is to conduct a closely monitored trial with standard therapeutic doses of desmopressin. If this treatment abolishes thirst and polydipsia, as well as polyuria, for 48 to 72 hours without producing water intoxication, the patient most likely has uncomplicated CDI. On the other hand, if the treatment abolishes the polyuria but has no or a lesser effect on thirst or polydipsia and results in the development of hyponatremia, it is more likely that the patient has some form of primary polydipsia. If desmopressin has no effect over this time interval, even when given by injection, it is almost certain that the patient has some form of NDI. However, if this approach is used, serum sodium levels must be checked within several days to avoid the development of severe hyponatremia in patients with primary polydipsia.

MRI has also proved to be useful for diagnosing DI. In normal subjects, the posterior pituitary produces a characteristic bright signal in the posterior part of the sella turcica that is similar on T1-weighted images, usually best seen in sagittal views.[230] This was originally thought to represent fatty tissue, but more recent evidence has indicated that the bright spot is actually due to the stored hormone in neurosecretory granules.[231] An experimental study done in rabbits subjected to dehydration for varying periods of time has shown a linear correlation between pituitary AVP content and the signal intensity of the posterior pituitary by MRI.[232] As might be expected from the fact that destruction of more than 85% to 90% of the neurohypophysis is necessary to produce clinical symptomatology of DI, this signal has been found to be almost always absent in patients with CDI in multiple studies.[233]

However, as with any diagnostic test, its clinical usefulness is dependent on the sensitivity and specificity of the test. Although earlier studies using small numbers of subjects demonstrated the presence of the bright spot in all normal subjects, subsequent larger studies reported an age-related absence of a pituitary bright spot in up to 20% of normal subjects.[234] Conversely, some studies have reported the presence of a bright spot in patients with clinical evidence of DI.[235] This may be because some patients with partial CDI have not yet progressed to the point of depletion of all neurohypophyseal reserves of AVP, or because a persistent bright spot in patients with DI might be due to the pituitary content of OT rather than AVP. In support of this, it is known that oxytocinergic neurons are more resistant to destruction by trauma than vasopressinergic neurons in rats[236] and in humans.[22] The presence of a positive posterior pituitary bright spot has been variably reported in other polyuric disorders. In primary polydipsia, the bright spot is usually seen,[233] consistent with studies in animals in which even prolonged lack of secretion of AVP caused by hyponatremia did not cause a decreased content of AVP in the posterior pituitary.[26] In NDI, the bright spot has been reported to be absent in some patients, but present in others.[127] Consequently, specificity is lacking in regard to using MRI routinely as a diagnostic screening test for DI. Nonetheless, the sensitivity is sufficient to allow a high probability that a patient with a bright spot on MRI does not have CDI. Thus, MRI is more useful for ruling out than for ruling in a diagnosis of CDI.

Additional useful information can be gained through MRI via assessment of the pituitary stalk. Enlargement of the stalk beyond 2 to 3 mm is generally considered to be pathologic[237] and can be caused by multiple disease processes.[238] Consequently, when MRI scans reveal thickening of the stalk, especially with absence of the posterior pituitary bright spot, systemic diseases should be searched for diligently, including cerebrospinal fluid (CSF), plasma β-human chorionic gonadotropin (β-hCG), and alpha-fetoprotein measurements for the evaluation of suprasellar germinoma, chest imaging and CSF and plasma angiotensin-converting enzyme (ACE) levels for the evaluation of sarcoidosis, and bone and skin surveys for the evaluation of histiocytosis. When a diagnosis is still in doubt, MRI should be repeated every 3 to 6 months. Continued enlargement, especially in children over the first 3 years of follow-up, suggests a germinoma and mandates a biopsy, whereas a decrease in the size of the stalk over time is more indicative of an inflammatory process, such as lymphocytic infundibuloneurohypophysitis.[239]

TREATMENT OF DIABETES INSIPIDUS

The general goals of treatment of all forms of DI are a correction of any preexisting water deficits and a reduction in the ongoing excessive urinary water losses. The specific therapy required (Box 15.3) will vary according to the type of DI present and clinical situation. Awake ambulatory patients with normal thirst have relatively little body water deficit, but benefit greatly by alleviation of the polyuria and polydipsia that disrupt their normal daily activities. In contrast, comatose patients with acute DI after head trauma are unable to drink in response to thirst and, in these patients, progressive hyperosmolality can be life-threatening.

Box 15.3 Therapies for the Treatment of Diabetes Insipidus

Water
Antidiuretic agents
Arginine vasopressin (Pitressin)
1-Deamino-8-D-arginine vasopressin (desmopressin; DDAVP)
Antidiuresis-enhancing agents
Chlorpropamide
Prostaglandin synthetase inhibitors (e.g., indomethacin, ibuprofen, tolmetin)
Natriuretic agents
Thiazide diuretics
Amiloride

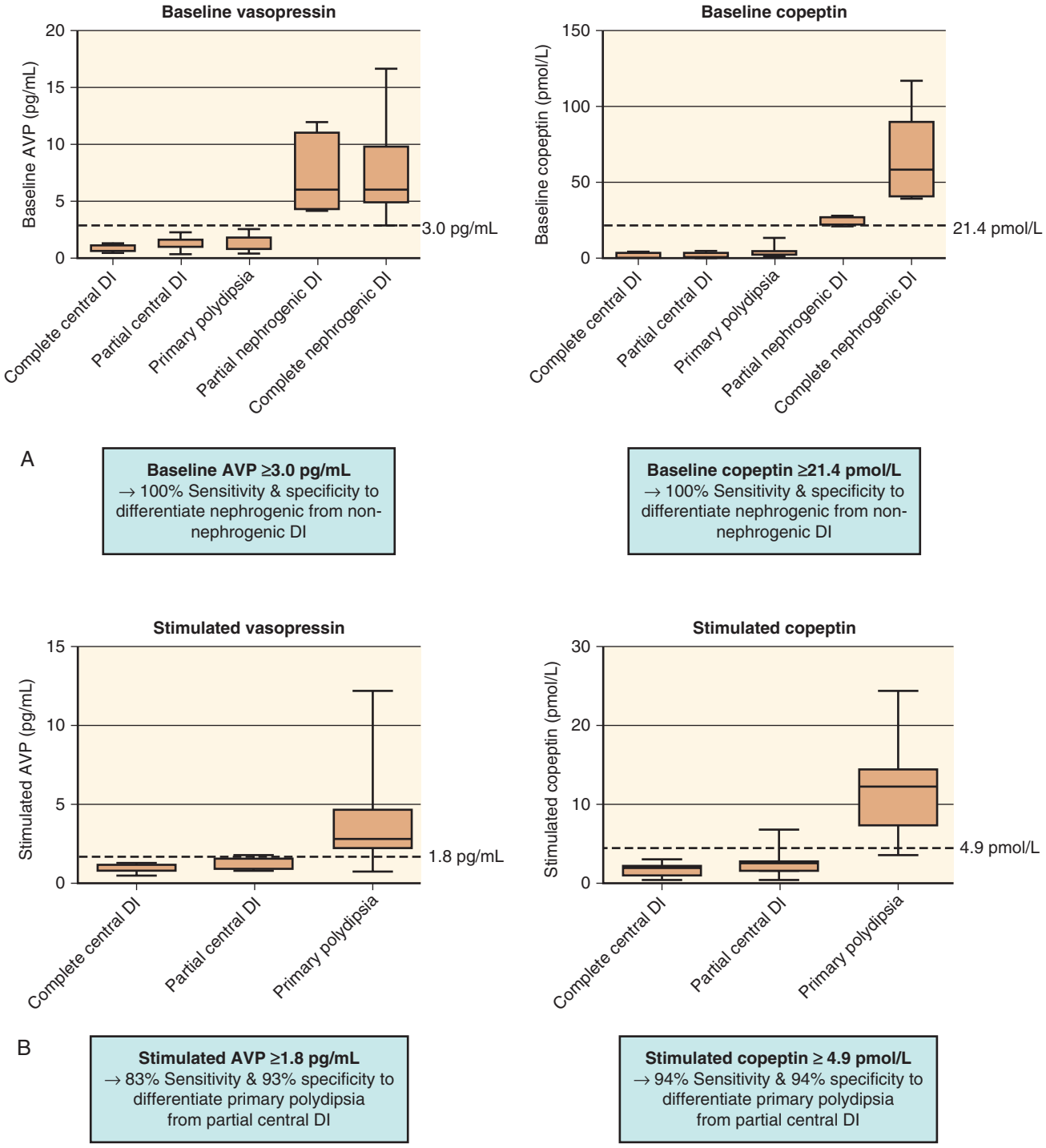


Fig. 15.16 (A) Baseline arginine vasopressin (AVP) and copeptin plasma levels in the differential diagnosis of the polyuria and polydipsia. Box plots depict interquartile ranges, with medians and whiskers depicting minimal and maximal values for baseline AVP and copeptin levels without prior thirsting in patients with complete and partial central diabetes insipidus (DI), primary polydipsia, and complete and partial nephrogenic diabetes insipidus. Cutoffs for best discrimination between nephrogenic versus nonnephrogenic diabetes insipidus for AVP and copeptin are shown. (B) Osmotically stimulated AVP and copeptin plasma levels in the differential diagnosis of polyuria and polydipsia. Box and whisker plots with medians and minimal and maximal values for stimulated AVP and copeptin plasma values at a plasma sodium level >147 mmol/L are depicted for patients with complete and partial central diabetes insipidus and for patients with primary polydipsia. Osmotic stimulation was provided by a combined water deprivation and saline infusion test. The cutoffs for best discrimination between primary polydipsia versus central diabetes insipidus for AVP and copeptin are depicted. *DI,* Diabetes insipidus; *PP,* primary polydipsia. (From Christ-Crain M, Moganthaler NG, Fenske W: Copeptin as a biomarker and a diagnostic tool in the evaluation of patients with polyuria-polydipsia and hyponatremia. *Best Pract Res Clin Endocrinol Metab.* 2016;30:235–247.)

The total body water deficit in a hyperosmolar patient can be estimated using the following formula:

$$\text{Total body water deficit} = (0.6 \times \text{premorbid weight}) \times (1 - 140/[Na^+])$$

where $[Na^+]$ is the serum sodium concentration in millimoles per liter and weight is in kilograms. This formula is dependent on three assumptions: (1) total body water is approximately 60% of the premorbid body weight; (2) no body solute was lost as the hyperosmolality developed; and (3) the premorbid serum $[Na^+]$ was 140 mEq/L.

To reduce the risk of CNS damage from protracted exposure to severe hyperosmolality, in most cases the plasma osmolality should be rapidly lowered in the first 24 hours to the range of 320 to 330 mOsm/kg H_2O, or by approximately 50%. Plasma osmolality may be most easily estimated as twice the serum $[Na^+]$ if there is no hyperglycemia, and measured osmolality may be substituted if azotemia is not present. As discussed earlier, the brain increases intracellular osmolality by increasing the content of a variety of organic osmolytes as protection against excessive shrinkage during hyperosmolality.[216] Because these osmolytes cannot be immediately dissipated, further correction to a normal plasma osmolality should be spread over the next 24 to 72 hours to avoid producing cerebral edema during treatment.[217] This is especially important in children,[240] in whom several studies have indicated that limiting correction of hypernatremia to a maximal rate of no greater than 0.5 mmol/L/h prevents the occurrence of symptomatic cerebral edema with seizures.[218,241] In addition, the possibility of associated thyroid or adrenal insufficiency should also be kept in mind because patients with CDI caused by hypothalamic masses can have associated deficiencies of anterior pituitary function.

The earlier formula does not take into account ongoing water losses and is, at best, a rough estimate. Frequent serum and urine electrolyte determinations should be made, and the administration rate of oral water, or IV 5% dextrose in water, should be adjusted accordingly. Note, for example, that the estimated deficit of a 70-kg patient whose serum $[Na^+]$ is 160 mEq/L is 5.25 L of water. In such an individual, administration of water at a rate greater than 200 mL/h would be required simply to correct the established deficit over 24 hours. Additional fluid would be needed to keep up with ongoing losses until a definitive response to treatment has occurred.

THERAPEUTIC AGENTS

The therapeutic agents available for the treatment of DI are shown in Box 15.3. Water should be considered a therapeutic agent because, when ingested or infused in sufficient quantity, there is no abnormality of body fluid volume or composition.

As noted previously, in most patients with DI, thirst remains intact, and the patient will drink sufficient fluid to maintain a relatively normal fluid balance. A patient with known DI should therefore be treated to decrease the polyuria and polydipsia to acceptable levels that allow him or her to maintain a normal lifestyle. Because the major goal of therapy is improvement in symptomatology, the therapeutic regimen prescribed should be individually tailored to each patient to accommodate his or her needs. The safety of the prescribed agent and use of a regimen that avoids potential detrimental effects of overtreatment are primary considerations because of the relatively benign course of DI in most cases and the potential adverse consequences of hyponatremia. Available treatments are summarized later; their use is discussed separately for different types of DI.

Arginine Vasopressin

Arginine vasopressin (Pitressin) is a synthetic form of naturally occurring human AVP. The aqueous solution contains 20 units/mL. Because of the drug's relatively short half-life (2- to 4-hour duration of antidiuretic effect) and propensity to cause acute increases in blood pressure when given as an IV bolus, this route of administration should generally be avoided. This agent is mainly used for acute situations, such as postoperative DI. However, repeated dosing is required unless a continuous infusion is used, and the frequency of dosing or infusion rate must be titrated to achieve the desired reduction in urine output (see subsequent discussion of postoperative DI).

Desmopressin

DDAVP is an agonist of the AVP V_2 receptor that was developed for therapeutic use because it has a significantly longer half-life than AVP (8- to 20-hour duration of antidiuretic effect) and is devoid of the latter's pressor activity because of the absence of activation of AVP V_{1A} receptors on vascular smooth muscle.[242] As a result of these advantages, it is the drug of choice for acute and chronic administration in patients with CDI.[243] Several different preparations are available. The intranasal form is provided as an aqueous solution containing 100 µg/mL in a bottle with a calibrated rhinal tube, which requires specialized training to use appropriately, or as a nasal spray delivering a metered dose of 10 µg in 0.1 mL. An oral preparation is also available in doses of 0.1 or 0.2 mg. Rather recently, a sublingual preparation, called Minirin Melt, has been introduced in doses of 60 to 120 µg.[244]

Neither the intranasal or oral preparations should be used in an acute emergency setting, in which it is essential that the patient achieve a therapeutic dose of the drug. In this case, the parenteral form should always be used. This is supplied as a solution containing 4 µg/mL and may be given by the intravenous, intramuscular, or subcutaneous route. The parenteral form is approximately 5 to 10 times more potent than the intranasal preparation, and the recommended dosage of DDAVP is 1 to 2 µg every 8 to 12 hours. For intranasal and parenteral preparations, increasing the dose generally has the effect of prolonging the duration of antidiuresis for several hours rather than increasing its magnitude; consequently, altering the dose can be useful to reduce the required frequency of administration.

Chlorpropamide

Chlorpropamide (Diabinese) is primarily used as an oral hypoglycemic agent; this sulfonylurea also potentiates the hydroosmotic effect of AVP in the kidney. Chlorpropamide has been reported to reduce polyuria by 25% to 75% in patients with CDI. This effect appears to be independent of the severity of the disease and is associated with a proportional rise in urine osmolality, correction of dehydration, and elimination of the polydipsia, similar to that caused by small doses of AVP or desmopressin.[219]

The major site of action of chlorpropamide appears to be at the renal tubule to potentiate the hydroosmotic action of circulating AVP, but there is also evidence of a pituitary effect to increase the release of AVP; the latter effect may account for the observation that chlorpropamide can produce significant antidiuresis, even in patients with severe CDI and presumed near-total AVP deficiency.[219] The usual dose is 250 to 500 mg/day, with a response noted in 1 or 2 days and a maximum antidiuresis in 4 days. It should be remembered that this is an off-label use of chlorpropamide; it should not be used in pregnant women or in children, it should never be used in an acute emergency setting in which achieving rapid antidiuresis is necessary, and it should be avoided in patients with concurrent hypopituitarism because of the increased risk of hypoglycemia. Other sulfonylureas share chlorpropamide's effect but generally are less potent. In particular, the newer generation of oral hypoglycemic agents, such as glipizide and glyburide, are almost devoid of any AVP-potentiating effects.

Prostaglandin Synthase Inhibitors

Prostaglandins have complex effects in the CNS and kidney, many of which are still incompletely understood due to the variety of different prostaglandins and their multiplicity of cellular effects. In the brain, intracerebroventricular infusion of E prostaglandins stimulates AVP secretion,[245] and administration of prostaglandin synthase inhibitors attenuates osmotically stimulated AVP secretion.[246] However, in the kidney, prostaglandin E2 (PGE2) has been reported to inhibit AVP-stimulated generation of cAMP in the cortical collecting tubule by interacting with inhibitory G protein (G_i).[247] Thus, the effect of prostaglandin synthase inhibitors to sensitize AVP effects in the kidney likely result from enhanced cAMP generation on AVP binding to the V_2 receptor. The predominant renal effects of these agents has been demonstrated by the fact that clinically these agents successfully reduce urine volume and free water clearance, even in patients with NDI of different causes.[248]

Natriuretic Agents

Thiazide diuretics have a paradoxic antidiuretic effect in patients with CDI.[249] However, given that better antidiuretic agents are available for the treatment of CDI, its main therapeutic use is in NDI. Hydrochlorothiazide at doses of 50 to 100 mg/day usually reduces urine output by approximately 50%, and its efficacy can be further enhanced by restricting sodium intake. Unlike desmopressin or the other antidiuresis-enhancing drugs, these agents are equally effective for treating most forms of NDI (see later).

TREATMENT OF DIFFERENT TYPES OF DIABETES INSIPIDUS

Central Diabetes Insipidus

Patients with CDI should generally be treated with intranasal or oral desmopressin. Unless the hypothalamic thirst center is also affected by the primary lesion causing superimposed osmoreceptor dysfunction, these patients will develop thirst when the plasma osmolality increases by only 2% to 3%.[219] Severe hyperosmolality is therefore not a risk in the patient who is alert, ambulatory, and able to drink in response to perceived thirst. Polyuria and polydipsia are thus inconvenient and disruptive, but not life-threatening. However, hypoosmolality is largely asymptomatic and may be progressive if water intake continues during a period of continuous antidiuresis. Therefore, treatment must be designed to minimize polyuria and polydipsia but without an undue risk of hyponatremia from overtreatment.

Treatment should be individualized to determine optimal dosage and dosing intervals. Although tablets offer greater convenience and are generally preferred by patients, it is useful to start with the nasal spray initially because of the greater consistency of absorption and physiologic effect, and then switch to oral tablets only after the patient is comfortable with use of the intranasal preparation to produce antidiuresis. Having tried both preparations, the patient can then choose which she or he prefers for long-term usage. Because of variability in response among patients, it is desirable to determine the duration of action of individual doses in each patient.[250] A satisfactory schedule can generally be determined using modest doses, and the maximum dose of desmopressin needed is rarely above 0.2 μg orally or 10 μg (one nasal spray) given two or occasionally three times daily.[251] These doses generally produce plasma desmopressin levels many times more than those required to produce maximum antidiuresis but obviate the need for more frequent treatment. Rarely, once-daily dosing suffices. In a few patients, the effect of intranasal or oral desmopressin is erratic, probably as a result of variable interference with absorption from the gastrointestinal tract or nasal mucosa. This variability can be reduced and the duration of action prolonged by administering the oral agent on an empty stomach[252] or use of the intranasal preparation after thorough cleansing of the nostrils. Resistance caused by antibody production has not been reported.

Hyponatremia is the major complication of desmopressin therapy, and 27% incidences of mild hyponatremia (131–134 mmol/L) and 15% incidences of more severe hyponatremia (≤130 mmol/L) have been reported with long-term follow-up of patients with chronic CDI.[253] This generally occurs if the patient is continually antidiuretic while maintaining a fluid intake sufficient to become volume-expanded and natriuretic.[254] There have been reports of hyponatremia in patients with normal AVP function, and presumably normal thirst, when they are given desmopressin to treat hemophilia and von Willebrand disease[255] and in children treated with desmopressin for primary enuresis.[256] In these cases, the hyponatremia can develop rapidly and is often first noted by the onset of convulsions and coma.[257] Severe hyponatremia in patients with DI who are being treated with desmopressin can be avoided by monitoring serum electrolyte levels frequently during the initiation of therapy. Patients who show a tendency to develop a low serum [Na^+] and do not respond to recommended decreases in fluid intake should then be instructed to delay a scheduled dose of desmopressin once or twice weekly so that polyuria recurs, thereby allowing any excess retained fluid to be excreted.[220]

Acute postsurgical DI occurs relatively frequently following surgery that involves the suprasellar hypothalamic area,[258] but several confounding factors must be considered. These patients often receive stress doses of glucocorticoids, and the resulting hyperglycemia with glucosuria may confuse a diagnosis of DI. Thus, the blood glucose level must first be brought under control to eliminate an osmotic diuresis as

the cause of the polyuria. In addition, excess fluids administered intravenously may be retained perioperatively but then excreted normally postoperatively. If this large output is matched with continued intravenous input, an incorrect diagnosis of DI may be made based on the resulting polyuria. Therefore, if the serum [Na^+] is not elevated concomitantly with the polyuria, the rate of parenterally administered fluid should be slowed, with careful monitoring of serum [Na^+] and urine output to establish the diagnosis.

Once a diagnosis of DI is confirmed, the only acceptable pharmacologic therapy is an antidiuretic agent. However, because many neurosurgeons fear water overload and brain edema after this type of surgery, the patient is sometimes treated only with intravenous fluid replacement for a considerable time before the institution of antidiuretic hormone therapy (see the potential benefits of this approach later). If the patient is awake and able to respond to thirst, he or she can be treated with an antidiuretic hormone, and the patient's thirst can then be the guide for water replacement. However, if the patient is unable to respond to thirst because of a decreased level of consciousness or from hypothalamic damage to the thirst center, fluid balance must be maintained by administering fluid intravenously. The urine osmolality and serum [Na^+] must be checked every several hours during the initial therapy and then at least daily until stabilization or resolution of the DI. Caution must also be exercised regarding the volume of water replacement because excess water administered during the continued administration of AVP or desmopressin can create a syndrome of inappropriate antidiuresis and potentially severe hyponatremia. Studies in experimental animals have indicated that desmopressin-induced hyponatremia markedly impairs the survival of AVP neurons after pituitary stalk compression,[236] suggesting that overhydration with subsequent decreased stimulation of the neurohypophysis may also increase the likelihood of permanent DI.

Postoperatively, desmopressin may be given parenterally in a dose of 1 to 2 µg subcutaneously, intramuscularly, or intravenously. The intravenous route is preferable because it obviates any concern about absorption, is not associated with significant pressor activity, and has the same total duration of action as the other parenteral routes. A prompt reduction in urine output should occur; the duration of the antidiuretic effect is generally 6 to 12 hours. Usually, the patient is hypernatremic, with relatively dilute urine when therapy is started. One should monitor the urine osmolality and urine volume to be certain that the dose was effective, and check the serum [Na^+] at frequent intervals to ensure some improvement of hypernatremia. It is generally advisable to allow some return of the polyuria before the administration of subsequent doses of desmopressin because postoperative DI is often transient, and return of endogenous AVP secretion will become apparent by a lack of return of the polyuria. Also, in some cases, transient postoperative DI is part of a triphasic pattern that has been well described following pituitary stalk transection (see previous discussion and Fig. 15.11). Because of this possibility, allowing a return of polyuria before redosing with desmopressin will allow for the earlier detection of a potential second phase of inappropriate antidiuresis and decrease the likelihood of producing symptomatic hyponatremia by continuing antidiuretic therapy and intravenous fluid administration when it is not required.

Some clinicians have recommended using a continuous intravenous infusion of a dilute solution of AVP to control DI postoperatively. Algorithms for continuous AVP infusion in postoperative and posttraumatic DI in pediatric patients have begun at infusion rates of 0.25 to 1.0 mU/kg/h and titrated the rate using urine specific gravity (goal of 1.010–1.020) and urine volume (goal of 2–3 mL/kg/h) as a guide to the adequacy of the antidiuresis.[259] Although pressor effects have not been reported at these infusion rates, and the antidiuretic effects are quickly reversible in 2 to 3 hours, it should be remembered that use of continuous infusions versus intermittent dosing will not allow assessing when the patient has recovered from transient DI or entered the second phase of a triphasic response. If DI persists, the patient should eventually be switched to maintenance therapy with intranasal or oral preparations of desmopressin for the treatment of chronic DI.

Acute traumatic DI can occur after injuries to the head, usually a motor vehicle accident. DI is more common with deceleration injuries that result in a shearing action on the pituitary stalk and/or cause hemorrhagic ischemia of the hypothalamus and/or posterior pituitary.[260] Similar to the onset of postsurgical DI, posttraumatic DI is usually recognized by hypotonic polyuria in the presence of increased plasma osmolality. The clinical management is similar to that for postsurgical DI, as outlined earlier, except that the possibility of anterior pituitary insufficiency must also be considered in these cases, and the patient should be given stress doses of glucocorticoids (e.g., hydrocortisone, 50–100 mg intravenously every 8 hours) until anterior pituitary function can be definitively evaluated.

Osmoreceptor Dysfunction

Acutely, patients with hypernatremia due to osmoreceptor dysfunction should be treated the same as any hyperosmolar patient by replacing the underlying free water deficit, as described at the beginning of this section. The long-term management of osmoreceptor dysfunction syndromes requires a thorough search for potentially treatable causes (see Box 15.1) in conjunction with the use of measures to prevent recurrence of dehydration. Because the hypodipsia cannot be cured, and rarely if ever improves spontaneously, the mainstay of management is education of the patient and family about the importance of continuously regulating her or his fluid intake in accordance with the hydration status.[261] This is never accomplished easily in such patients, but can be done most efficaciously by establishing a daily schedule of water intake based on changes in BW, regardless of the patient's thirst.[262] In effect, a prescription for daily fluid intake must be written for these patients because they will not drink spontaneously. In addition, if the patient has polyuria, desmopressin should also be given, as for any patient with DI. The success of this regimen should be monitored periodically (weekly at first and later every month, depending on the stability of the patient) by measuring the serum [Na^+]. In addition, the target weight (at which hydration status and the serum [Na^+] concentration are normal) may need to be recalculated periodically to allow for growth in children or changes in body fat in adults.

Gestational Diabetes Insipidus

The polyuria of gestational DI is usually not corrected by the administration of AVP itself because this is rapidly

degraded by high circulating levels of oxytocinase or vasopressinase, just as endogenous AVP is degraded by these enzymes. The treatment of choice is desmopressin because this synthetic AVP V_2 receptor agonist is not destroyed by the cysteine aminopeptidase (oxytocinase or vasopressinase) in the plasma of pregnant women[263] and, to date, appears to be safe for both the mother and child.[264,265] Desmopressin has only 2% to 5% the oxytocic activity of AVP[243] and can be used with minimal stimulation of the OT receptors in the uterus. Doses should be titrated to individual patients because higher doses and more frequent dosing intervals are sometimes required as a result of the increased degradation of the peptide. However, physicians should remember that the naturally occurring volume expansion and reset osmostat that occurs in pregnancy maintains the serum [Na^+] at a lower level during pregnancy.[39] During delivery, these patients can maintain adequate oral intake and continued administration of desmopressin. However, physicians should be cautious about the overadministration of fluid parenterally during delivery because these patients will not be able to excrete the fluid and will be susceptible to the development of water intoxication and hyponatremia. After delivery, oxytocinase and vasopressinase levels decrease in the plasma within several days and, depending on the cause of the DI, the disorder may disappear or the patient may become asymptomatic with regard to fluid intake and urine volume.[266]

Nephrogenic Diabetes Insipidus

By definition, patients with NDI are resistant to the effects of AVP. Some patients with NDI can be treated by eliminating the drug (e.g., lithium) or disease (e.g., hypercalcemia) responsible for the disorder. For many others, however, including those with the genetic forms, the only practical form of treatment at present is to restrict sodium intake and administer a thiazide diuretic alone[249] or in combination with a prostaglandin synthetase inhibitor or amiloride.[267–269] The natriuretic effect of the thiazide class of diuretics is conferred by their ability to block sodium absorption in the renal cortical diluting site. When combined with dietary sodium restriction, these drugs cause modest hypovolemia. This stimulates isotonic proximal tubular solute reabsorption and diminishes solute delivery to the more distal diluting site, at which experimental studies have indicated that thiazides also act to enhance water reabsorption in the inner medullary collecting duct independently of AVP.[270] Together, these effects diminish renal diluting ability and free water clearance, also independently of any action of AVP. Thus, agents of this class are the mainstay of therapy for NDI. Monitoring for hypokalemia is recommended, and potassium supplementation is occasionally required. Any drug of the thiazide class may be used with equal potential for benefit, and clinicians should use the one with which they are most familiar from use in other conditions. Care must be exercised when treating patients taking lithium with diuretics because the induced contraction of plasma volume may increase lithium concentrations and worsen potential toxic effects of the therapy. In the acute setting, diuretics are of no use in NDI, and only free water administration can reverse hyperosmolality.

Indomethacin, tolmetin, and ibuprofen have been used in this setting,[267,271,272] although ibuprofen may be less effective than the others. The combination of thiazides and a nonsteroidal antiinflammatory drug (NSAID) will not increase urinary osmolality above that of plasma, but lessening of the polyuria is nonetheless beneficial to patients. In many cases, the combination of thiazides with the potassium-sparing diuretic amiloride is preferred to lessen the potential side effects associated with long-term use of NSAIDs.[268,269] Amiloride also has the advantage of decreasing lithium entrance into cells in the distal tubule and, because of this, may have a preferable action for the treatment of lithium-induced NDI.[273,274]

Although desmopressin is generally not effective in NDI, a few patients may have receptor mutations that allow partial responses to AVP or desmopressin,[275] with increases in urine osmolality following much higher doses of these agents than those typically used to treat CDI (e.g., 6–10 μg intravenously). It is generally worth a trial of desmopressin at these doses to ascertain whether this is a potential useful therapy in selected patients in whom the responsivity of other affected family members is not already known. Potential therapies involving the administration of chaperones to bypass defects in cellular routing of misfolded aquaporin[186] and AVP V_2 receptor proteins[187] is an exciting, future possibility.[175]

Primary Polydipsia

At present, there is no completely satisfactory treatment for primary polydipsia. Fluid restriction would seem to be the obvious treatment of choice. However, patients with a reset thirst threshold will be resistant to fluid restriction because of the resulting thirst from stimulation of brain thirst centers at higher plasma osmolalities.[276] In some cases, the use of alternative methods to ameliorate the sensation of thirst (e.g., wetting the mouth with ice chips, using sour candies to increase salivary flow) can help reduce fluid intake. Fluid intake in patients with a psychogenic cause of polydipsia is driven by psychiatric factors that have responded variably to behavioral modification and pharmacologic therapy. Several reports have suggested limited efficacy of the antipsychotic drug clozapine as an agent to reduce polydipsia and prevent recurrent hyponatremia in at least a subset of these patients.[277] Administration of any antidiuretic hormone or thiazide to decrease polyuria is hazardous because they invariably produce water intoxication.[219,278] Therefore, if the diagnosis of DI is uncertain, any trial of antidiuretic therapy should be conducted with close monitoring, preferably in the hospital, with frequent evaluation of fluid balance and serum electrolyte levels.

DISORDERS OF EXCESS VASOPRESSIN OR VASOPRESSIN EFFECT

The disorders of the renal concentrating mechanism described in the previous section can lead to water depletion, sometimes in association with hyperosmolality and hypernatremia. In contrast, disorders of the renal diluting mechanism usually present as hyponatremia and hypoosmolality. Hyponatremia is among the most common electrolyte disorders encountered in clinical medicine, with an incidence of 0.97% and a prevalence of 2.48% in hospitalized adult patients when the serum [Na^+] less than 130 mEq/L is the diagnostic criterion[279] and as high as 15% to 30% if the serum [Na^+] less than 135 mEq/L is used as the diagnostic criterion.[280] The prevalence may be somewhat lower in the hospitalized pediatric

population but, conversely, the prevalence is higher than originally recognized in the geriatric population.[280–282]

RELATIONSHIP BETWEEN HYPOOSMOLALITY AND HYPONATREMIA

Because plasma osmolality is usually measured to help evaluate hyponatremic disorders, it is useful to bear in mind the basic relationship of plasma osmolality to the plasma or serum [Na^+]. As reviewed in the introduction to this chapter, Na^+ and its associated anions account for almost all the osmotic activity of plasma. Therefore, changes in serum [Na^+] are usually associated with comparable changes in plasma osmolality. The osmolality calculated from the concentrations of Na^+, urea, and glucose is usually in close agreement with that obtained from a measurement of osmolality.[283] When the measured osmolality exceeds the calculated osmolality by more than 10 mOsm/kg H_2O, an osmolar gap is present.[283] This occurs in two circumstances: (1) with a decrease in the water content of the serum; and (2) with the addition of a solute other than urea or glucose to the serum.

A decrease in the water content of serum is usually due to its displacement by excessive amounts of protein or lipids, which can occur in severe hyperlipidemia or hyperglobulinemia. Normally, 92% to 94% plasma volume is water, with the remaining 6% to 8% being lipids and protein. Because of its ionic nature, Na^+ dissolves only in the water phase of plasma. Thus, when a greater than normal proportion of plasma is accounted for by solids, the concentration of Na^+ in plasma water remains normal, but the concentration in the total volume, as measured by flame photometry, is artificially low. Such a discrepancy can be avoided if [Na^+] is measured with an ion-selective electrode.[284] However, the sample needs to remain undiluted (direct potentiometry) for accurate measurement of the serum [Na^+]. Whereas the flame photometer measures the concentration of Na^+ in the total plasma volume, the ion-selective electrode measures it only in the plasma water. Normally, this difference is only 3 mEq/L but, in the settings under discussion, the difference can be much greater. Because the large lipid and protein molecules contribute only minimally to the total osmolality, the measurement of osmolality by freezing point depression remains normal in these patients.

Hyponatremia associated with normal osmolality has been termed "factitious hyponatremia" or "pseudohyponatremia." The most common causes of pseudohyponatremia are primary or secondary hyperlipidemic disorders. The serum need not appear lipemic because increments in cholesterol alone can cause the same discrepancy.[284] Plasma protein level elevations above 10 g/dL, as seen in multiple myeloma or macroglobulinemia, can also cause pseudohyponatremia. The administration of intravenous immune globulin has been reported to be associated with hyponatremia without hypoosmolality in several patients.[285]

The second setting in which an osmolar gap occurs is the presence in plasma of an exogenous low-molecular-weight substance such as ethanol, methanol, ethylene glycol, or mannitol.[286] Undialyzed patients with chronic renal failure, as well as critically ill patients,[287] also have an increment in the osmolar gap of unknown cause. Whereas all these exogenous substances, as well as glucose and urea, elevate measured osmolality, the effect that they have on the serum [Na^+] and intracellular hydration depends on the solute in question. As noted, in the presence of relative insulin deficiency, glucose does not penetrate cells readily and remains in the ECF. As a consequence, water is drawn osmotically from the ICF compartment, causing cell shrinkage, and this translocation of water commensurately decreases the [Na^+] in the ECF. In this setting, therefore, the serum [Na^+] can be low while plasma osmolality is normal or high. It is generally estimated that for every 100-mg/dL rise in serum glucose, the osmotic shift of water causes serum [Na^+] to fall by 1.6 mEq/L. However, it has been suggested that this may represent an underestimate of the decrease caused by more severe degrees of hyperglycemia, and a 2.4-mEq/L correction factor is recommended in such cases.[288]

Similar "translocational" hyponatremia occurs with mannitol or maltose or with the absorption of glycine during transurethral prostate resection, as well as in gynecologic and orthopedic procedures. A potential toxicity for glycine in this setting also requires consideration.[289] The recent introduction of bipolar retroscopes, which allow for the use of NaCl as an irrigant, should result in the disappearance of this clinical entity. When the plasma solute is readily permeable (e.g., urea, ethylene glycol, methanol, ethanol), it enters cells and so does not establish an osmotic gradient for water movement. There is no cellular dehydration, despite the hyperosmolar state, so the serum [Na^+] remains unchanged. The relationship among plasma osmolality, plasma tonicity, and serum [Na^+] in the presence of various solutes is summarized in Table 15.2.

Table 15.2 Relationship Between Serum Tonicity and Sodium Concentration in the Presence of Other Substances

Condition or Substance	Serum Osmolality	Serum Tonicity	Serum [Na^+]
Hyperglycemia	↑	↑	↓
Mannitol, maltose, glycine	↑	↑	↓
Azotemia (high blood urea)	↑	↔	↔
Ingestion of ethanol, methanol, ethylene glycol	↑	↔	↔
Elevated serum lipid or protein	↔	↔	↓

↑, Increased; ↓, decreased; ↔, unchanged.

VARIABLES THAT INFLUENCE RENAL WATER EXCRETION

In considering clinical disorders that result from excessive or inappropriate secretion of AVP, it is important to remember the many other variables that also influence renal water excretion. These factors fall into four broad categories.

Fluid Delivery From the Proximal Tubule

In spite of the fact that proximal fluid reabsorption is isoosmotic and therefore does not contribute directly to urine dilution, the volume of tubular fluid that is delivered to the distal nephron largely determines the volume of dilute urine that can be excreted. Thus, if glomerular filtration is decreased

or proximal tubule reabsorption is greatly enhanced, the resulting diminution in the amount of fluid delivered to the distal tubule itself limits the rate of renal water excretion, even if other components of the diluting mechanism are intact.

Dilution of Tubular Fluid

The excretion of urine that is hypotonic to plasma requires that some segment of the nephron reabsorb solute in excess of water. The water impermeability of the entire ascending limb of Henle, as well as the capacity of its thick segment to reabsorb NaCl, actively endows this segment of the nephron with the characteristics required by the diluting process. Thus, the transport of NaCl by the Na^+-K^+-$2Cl^-$ cotransporter converts the hypertonic tubule fluid delivered from the descending limb of the loop of Henle to a distinctly hypotonic fluid. Likewise, the distal convoluted tubule is impermeable to water, and reabsorption of NaCl by the thiazide-sensitive NaCl cotransporter further dilutes the luminal fluid (down to an osmolality of ≈100 mOsm/kg H_2O). Interference with the reabsorption of Na^+ and Cl^- in these segments, as occurs with loop and thiazide diuretics, will therefore impair urine dilution.

Water Impermeability of the Collecting Duct

The excretion of urine that is more dilute than the fluid that is delivered to the distal convoluted tubule requires continued solute reabsorption and minimal water reabsorption in the terminal segments of the nephron. Because the water permeability of the collecting duct epithelium is primarily dependent on the presence or absence of AVP, this hormone plays a pivotal role in determining the fate of the fluid delivered to the collecting duct and thus the concentration or dilution of the final urine (see Chapter 10). In the absence of AVP, the collecting duct remains essentially impermeable to water, even though some water is still reabsorbed. The continued reabsorption of solute then results in the excretion of a maximally dilute urine (≈50 mOsm/kg H_2O). Because the medullary interstitium is always hypertonic, the absence of circulating AVP, which renders the collecting duct impermeable to water, is critical to the normal diluting process. This diluting mechanism allows for the intake and subsequent excretion of large volumes of water, without major alterations in the tonicity of body water.[290] Rarely, this limit can be exceeded, causing water intoxication. Much more commonly, however, hyponatremia occurs at lower rates of water intake because of an intrarenal defect in urine dilution or the persistent secretion of AVP in the circulation. Because hypoosmolality normally suppresses AVP secretion,[291] the hypoosmolar state frequently reflects the persistent secretion of AVP in response to hemodynamic or other nonosmotic stimuli.[291]

Solute Excretion Rate

At any fixed urine osmolality, the total osmolar load that needs to be excreted each day determines the daily urine volume and hence the volume of free water that can be excreted.[292] This osmolar load is made up predominantly of salt and urea and is therefore dependent on dietary protein intake. This explains why patients with a very low protein intake can develop hyponatremia,[293] and why increasing the intake of protein, or administration of urea, can correct chronic hyponatremia.

PATHOGENESIS AND CAUSES OF HYPONATREMIA

The plasma or serum $[Na^+]$ is determined by the body's total content of sodium, potassium, and water, as shown by the following equation:

$$\text{Serum } [Na^+] = (\text{total body exchangeable } Na^+ + \text{total body exchangeable } [K^+])/\text{total BW}$$

This formula has been simplified from the observations made by Edelman in the 1950s, which introduced some errors in the prediction of changes in serum $[Na^+]$ and has been subject to reinterpretation by Nguyen and Kurtz.[294] Although this revision of the formula is more accurate, there are so many inaccuracies in the measurements of sodium, potassium, and water losses, as well as intake, that there is no substitute for frequent measurements of the serum $[Na^+]$ in rapidly changing clinical settings.[295] As the previous relationship depicts, hyponatremia can therefore occur by an increase in TBW, a decrease in body solutes (Na^+ or K^+), or any combination of these. In most cases, more than one of these mechanisms is operant. Therefore, a classification system to separate the various causes of hyponatremia should be based on factors other than the level of serum $[Na^+]$ itself. In approaching the hyponatremic patient, the physician's first task is to ensure that hyponatremia actually reflects a hypoosmolar state and is not a consequence of pseudohyponatremia or translocational hyponatremia, as discussed earlier. Thereafter, an assessment of ECF volume (Fig. 15.17) provides the most useful working classification of the cause of the hyponatremia because a low serum $[Na^+]$ can be associated with a decreased, normal, or high total body sodium content.[296,297]

HYPONATREMIA WITH EXTRACELLULAR FLUID VOLUME DEPLETION

Patients with hyponatremia who have ECF volume depletion have sustained a deficit in total body Na^+ that exceeds the deficit in TBW. The decrease in ECF volume is manifested by physical findings such as flat neck veins, decreased skin turgor, dry mucous membranes, orthostatic hypotension, and tachycardia. If sufficiently severe, volume depletion is a potent stimulus to AVP secretion. When the osmoreceptors and volume receptors receive opposing stimuli, the former remains active but the set point of the system is lowered (see Fig. 15.7). Thus, in the presence of hypovolemia, AVP is secreted and water is retained, despite hypoosmolality. Whereas the hyponatremia in this setting clearly involves a depletion of body solutes, the concomitant AVP-mediated retention of water is critical to the pathologic process producing hyponatremia.

As depicted in the flow chart in Fig. 15.17, measurement of the urine $[Na^+]$ concentration is helpful in assessing whether the fluid losses are renal or extrarenal in origin. A urine $[Na^+]$ of less than 30 mEq/L reflects a normal renal response to volume depletion and indicates an extrarenal source of fluid loss. This is usually seen in patients with gastrointestinal disease with vomiting or diarrhea. Other causes include loss of fluid into a third space, such as the abdominal cavity in pancreatitis or the bowel lumen with

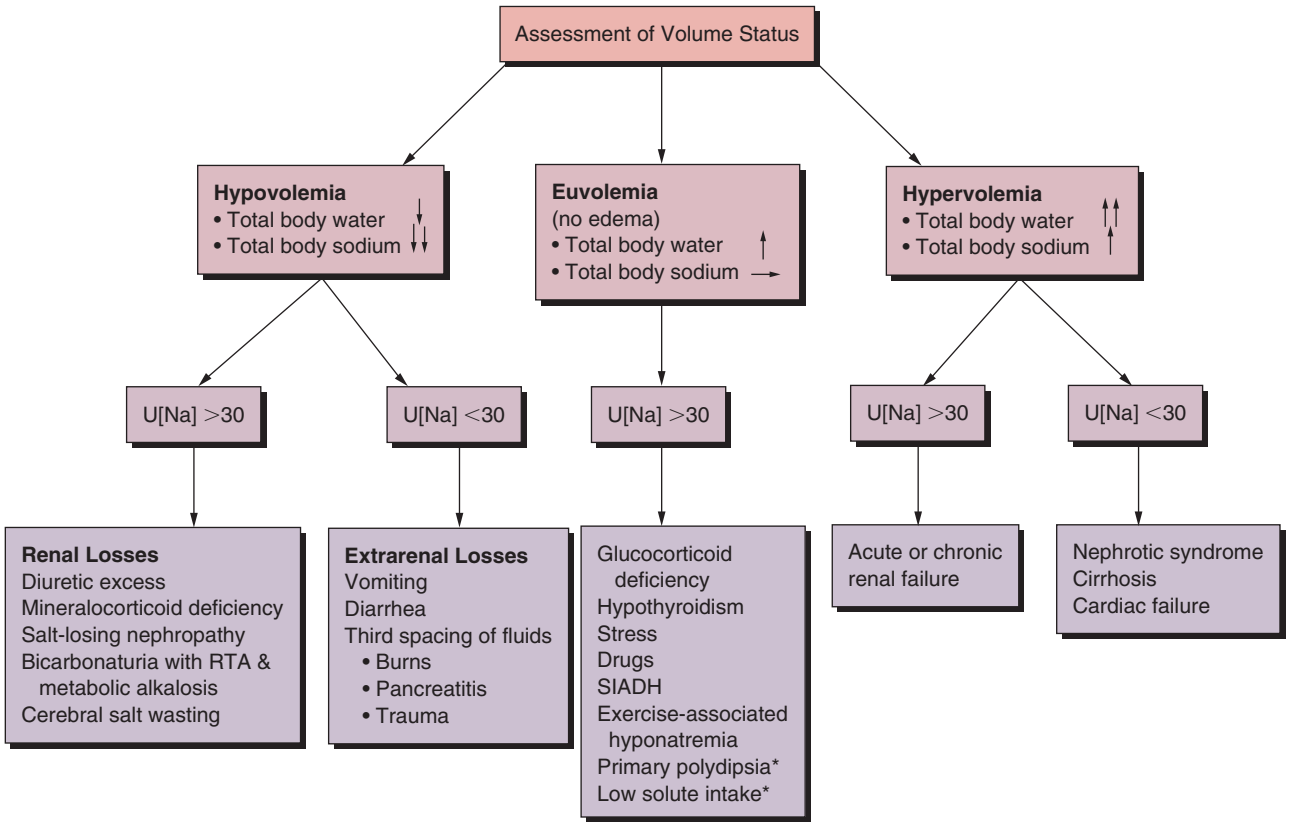


Fig. 15.17 Diagnostic approach to the hyponatremic patient. *SIADH,* Syndrome of inappropriate antidiuretic hormone secretion; *U[Na],* urinary [Na^+]. (Modified from Halterman R, Berl T: Therapy of dysnatremic disorders. In Brady H, Wilcox C, eds. *Therapy in Nephrology and Hypertension,* Philadelphia: WB Saunders; 1999:256.)

ileus. Burns and muscle trauma can also be associated with large fluid and electrolyte losses. Because many of these pathologic states are associated with increased thirst, an increase in orally ingested or parenterally infused free water can lead to hyponatremia.

Hypovolemic hyponatremia in patients whose urine [Na^+] is higher than 30 mEq/L indicates the kidney as the source of the fluid losses. Diuretic-induced hyponatremia, a commonly observed clinical entity, accounts for a significant proportion of symptomatic hyponatremia in hospitalized patients. It occurs almost exclusively with thiazide rather than loop diuretics. This is most likely because, whereas both classes of diuretics can impair urine diluting ability, loop diuretics also impair the generation of the medullary interstitial concentrating gradient, thus limiting the maximal urinary concentration that can be achieved. The hyponatremia is usually evident within 14 days in most patients, but occasionally can occur as late as 2 years after the initiation of therapy.[298] Underweight women appear to be particularly prone to this complication, and advanced age has been found to be a risk factor in some, but not all, studies.[298–300] A careful study of diluting ability in older adults has revealed that thiazide diuretics exaggerate the already slower recovery from hyponatremia induced by water ingestion in this population.[301]

Diuretics can cause hyponatremia by several mechanisms: (1) volume depletion, which results in impaired water excretion by enhanced AVP release and decreased tubular fluid delivery to the diluting segment; (2) a direct effect on the diluting function of the thick ascending limb or distal convoluted tubule; and (3) K^+ depletion that frequently accompanies diuretic use, which contributes to the loss of total body exchangeable solute ($Na^+ + K^+$).[302] The concomitant administration of potassium-sparing diuretics does not prevent the development of hyponatremia. Although the diagnosis of diuretic-induced hyponatremia is frequently obvious, surreptitious diuretic abuse should always be considered in patients in whom other electrolyte abnormalities and high urinary Cl^- excretion suggest this possibility. Recent genetic and phenotyping studies have suggested that an inherited defect in PGE2 uptake in the collecting duct may confer an increased risk of thiazide-induced hyponatremia, which raises the possibility that patients at risk of this adverse effect of thiazides may be able to be identified before exposure to the drug.[303,304]

Salt-losing nephropathy occurs in some patients with advanced renal insufficiency. In most of these patients, the Na^+-wasting tendency is not one that manifests itself at normal rates of sodium intake; however, some patients with interstitial nephropathy, medullary cystic disease, polycystic kidney disease, or partial urinary obstruction develop sufficient Na^+ wasting to exhibit hypovolemic hyponatremia.[305] Patients with proximal renal tubular acidosis exhibit renal Na^+ and K^+ wasting, despite modest renal insufficiency, because bicarbonaturia obligates these cation losses.

It has long been recognized that adrenal insufficiency is associated with impaired renal water excretion and hyponatremia. This diagnosis should be considered in the volume-contracted hyponatremic patient whose urine [Na^+] is not low, particularly when the serum [K^+], urea, and creatinine levels are elevated. Separate mechanisms for mineralocorticoid and glucocorticoid deficiency have been defined.[306] Observations in glucocorticoid-replete adrenalectomized experimental animals have provided evidence to support a role of mineralocorticoid deficiency in the abnormal water excretion. Conscious adrenalectomized dogs given physiologic doses of glucocorticoids develop hyponatremia. Saline or physiologic doses of mineralocorticoids corrected the defect in association with ECF volume repletion and improvement in renal hemodynamics. Immunoassayable AVP levels were elevated in a similarly treated group of mineralocorticoid-deficient dogs, despite hypoosmolality.[307] The decreased ECF volume thus provides a nonosmotic stimulus of AVP release. More direct evidence for the role of AVP has been provided in studies using an AVP receptor antagonist. When glucocorticoid-replete, adrenally insufficient rats were given an AVP antagonist, minimal urine osmolality was significantly lowered but urine dilution was not fully corrected, in contrast to the mineralocorticoid-replete rats, thereby supporting a role for an AVP-independent mechanism.[308] This is in agreement with studies of adrenalectomized homozygous Brattleboro rats, which also have a defect in water excretion that can be partially corrected by mineralocorticoids or by the normalization of volume. In summary, therefore, the mechanism of the defect in water excretion associated with mineralocorticoid deficiency is mediated by AVP and by AVP-independent intrarenal factors, both of which are activated by decrements of ECF volume, more so than by a deficiency of the hormone per se.

The presence in the urine of an osmotically active, nonreabsorbable or poorly reabsorbable solute causes the renal excretion of Na^+ and culminates in volume depletion. Glycosuria secondary to uncontrolled diabetes mellitus, mannitol infusion, or urea diuresis after relief of obstruction are common causes of this disorder. In patients with diabetes, the Na^+ wasting caused by the glycosuria can be aggravated by ketonuria because hydroxybutyrate and acetoacetate also cause urinary electrolyte losses. In fact, ketonuria can contribute to the renal Na^+ wasting and hyponatremia seen in states of starvation and alcoholic ketoacidosis. Na^+ and water excretion are also increased when a nonreabsorbable anion appears in the urine. This is observed principally with the metabolic alkalosis and bicarbonaturia that accompany severe vomiting or nasogastric suction. In these patients, the excretion of HCO_3^- requires the concomitant excretion of cations, including Na^+ and K^+, to maintain electroneutrality. Whereas the renal losses in such clinical settings is often hypotonic, the volume contraction–stimulated thirst and water intake can result in the development of hyponatremia.

Cerebral salt wasting is a rare syndrome described primarily in patients with subarachnoid hemorrhage, but also with other types of CNS lesions, which can lead to renal salt wasting and volume contraction.[309] Although hyponatremia is frequently reported in these patients, true cerebral salt wasting is probably less common than reported.[310] One critical review has found no conclusive evidence for volume contraction or renal salt wasting in any of these patients,[311] as has a more recent study of patients with subarachnoid hemorrhage.[312] The mechanism of this natriuresis is unknown, but an increased release of brain natriuretic peptides has been suggested.[313]

HYPONATREMIA WITH EXCESS EXTRACELLULAR FLUID VOLUME

In advanced stages, the edematous states listed in Fig. 15.17 are associated with a decrease in serum [Na^+]. Patients generally have an increase in total body Na^+ content, but the rise in TBW exceeds that of Na^+. With the exception of renal failure, these states are characterized by avid Na^+ retention (urine Na^+ concentration often < 10 mEq/L). This avid retention may be obscured by the concomitant use of diuretics, which are frequently used in treating these patients.

CONGESTIVE HEART FAILURE

The common association between congestive heart failure and Na^+ and water retention is well established. A mechanism mediated by decreased delivery of tubule fluid to the distal nephron and/or increased release of AVP has been proposed. In an experimental model of low cardiac output, both AVP and diminished delivery to the diluting segment were found to be important in mediating the abnormality in water excretion. It thus appears that the decrement in effective blood volume and decrease in arterial filling are sensed by aortic and carotid sinus baroreceptors, which stimulate AVP secretion.[314]

This stimulation must supersede the inhibition of AVP release that accompanies acute distention of the left atrium. In fact, there is evidence that chronic distention of the atria blunts the sensitivity of this baroreceptor, so high-pressure baroreceptors can act in an uninhibited manner to stimulate AVP release. The importance of AVP in the abnormal dilution in experimental models of heart failure has been underscored by correction of the water excretory defect by an AVP antagonist in rats with inferior vena cava constriction.[315]

High plasma AVP levels have been demonstrated in patients with congestive heart failure in the presence and absence of diuretics.[316] Similarly, the hypothalamic mRNA message for the AVP preprohormone is elevated in rats with chronic cardiac failure.[317] Although these studies did not exclude a role for intrarenal factors in the pathogenesis of the abnormal water retention, they complement the experimental observations that demonstrate a critical role for AVP in the pathologic process. It is most likely that nonosmotic pathways, whose activation is suggested by the increase in sympathetic activity seen in congestive heart failure,[318] are the mediators of AVP secretion in edema-forming states. These neurohumoral factors further contribute to the hyponatremia by decreasing the glomerular filtration rate (GFR) and enhancing tubular Na^+ reabsorption, thereby decreasing fluid delivery to the distal diluting segments of the nephron. The degree of neurohumoral activation correlates with the clinical severity of left ventricular dysfunction.[319] Hyponatremia is a powerful prognostic factor in these patients.[320]

The role of the AVP-regulated water channel (AQP2) has also been examined in heart failure. Two studies have described an upregulation of this water channel in rats with heart failure.[321,322] In the latter study, the nonpeptide V_2 receptor antagonist OPC31260 reversed the upregulation, suggesting that a receptor-mediated function, most likely

enhanced cAMP generation, is responsible for the process.[321] Consistent with these observations, a selective V_2 receptor antagonist decreased AQP2 excretion and increased urine flow in patients with heart failure.[323]

HEPATIC FAILURE

Patients with advanced cirrhosis and ascites frequently present with hyponatremia as a consequence of their inability to excrete a water load.[324] The classic view suggests that a decrement in effective arterial blood volume leads to avid Na^+ and water retention in an attempt to restore volume toward normal. In this regard, a number of the pathologic derangements in cirrhosis, including splanchnic venous pooling, diminished plasma oncotic pressure secondary to hypoalbuminemia, and decrease in peripheral resistance, could all contribute to a decrease in effective arterial blood volume.[325] This theory was challenged by observations that suggested primary renal Na^+ retention, termed the "overflow hypothesis."[326] A proposal that unifies these views has been presented—that is, Na^+ retention occurs early in the pathologic process, but is a consequence of the severe vasodilation-mediated arterial underfilling.[327]

As with cardiac failure, the relative roles of intrarenal versus extrarenal factors in impaired water excretion has been a matter of some controversy. The observation that expansion of intravascular volume with saline, mannitol, ascites fluid, water immersion, or peritoneovenous shunting improves water excretion in cirrhosis could be interpreted as implicating an intrarenal mechanism in the impaired water excretion. This is because these maneuvers increase the GFR and improve distal delivery. Such maneuvers could also suppress baroreceptor-mediated AVP release and cause an osmotic diuresis, which would also improve water excretion.[323] Experimental models of deranged liver function, including acute portal hypertension by vein constriction, bile duct ligation, and chronic cirrhosis produced by the administration of carbon tetrachloride, have demonstrated a predominant role for AVP secretion in the pathogenesis of the disorder. In this latter model, an increment in hypothalamic AVP mRNA has also been demonstrated.[328] A study using an AVP antagonist also has indicated a central role for AVP in the process.[329] As was the case in heart failure, increased expression of AQP2 has also been reported in the cirrhotic rat,[330] but dysregulation of AQP1 and AQP3 is also present in carbon tetrachloride (CCl_4)–induced cirrhosis.[331] In contrast, in the common bile duct model of cirrhosis, no increase in AQP2 was observed.[332]

Although patients with cirrhosis who have no edema or ascites excrete a water load normally, those with ascites usually do not. Several studies have demonstrated elevated AVP levels in these patients.[324] Patients who had a defect in water excretion had higher levels of AVP, plasma renin activity, plasma aldosterone, and norepinephrine,[333] as well as lower rates of PGE2 production. Similarly, their serum albumin level was lower, as was their urinary excretion of Na^+, all suggesting a decrease in effective arterial blood volume. As is the case in heart failure, sympathetic tone is high in cirrhosis.[334] In fact, the plasma concentration of norepinephrine, a good index of baroreceptor activity in humans, appears to correlate well with the levels of AVP and excretion of water. These studies, therefore, offer strong support for the view that effective arterial blood volume is contracted, rather than expanded, in decompensated cirrhosis.[327] This concept is further strengthened by observations of subjects during head-out water immersion. This maneuver, which translocates fluid to the central blood volume, caused a decrease in AVP levels and improved water excretion[335] but, in this study, peripheral resistance decreased further. By combining head-out water immersion with norepinephrine administration in an effort to increase systemic pressure and peripheral resistance, water excretion was completely normalized.[336] Such observations underline the critical role of peripheral vasodilation in the pathologic process. The observation that the inhibition of nitric oxide corrects the arterial hyporesponsiveness to vasodilators and the abnormal water excretion in cirrhotic rats provides strong evidence of a role for nitric oxide in the vasodilation.[337–339]

NEPHROTIC SYNDROME

The incidence of hyponatremia in the nephrotic syndrome is lower than in congestive heart failure or cirrhosis, most likely as a consequence of the higher blood pressure, higher GFR, and more modest impairments in Na^+ and water excretion than in the other groups of patients.[340] Because lipid levels are frequently elevated, a direct measurement of plasma osmolality should always be done. Diminished excretion of free water was first noted in children with the nephrotic syndrome and, since then, other investigators[341] have noted elevated plasma levels of AVP in these patients. In view of the alterations in Starling forces that accompany hypoalbuminemia and allow the transudation of salt and water across capillary membranes to the interstitial space, patients with the nephrotic syndrome are thought to have intravascular volume contraction. Increased levels of neurohumoral markers of decreased effective arterial blood volume also support this underfilling theory.[342] The possibility that this nonosmotic pathway stimulates AVP release has been suggested by studies in which head-out water immersion and blood volume expansion[342] increases water excretion in nephrotic subjects. However, these pathogenic events may not be applicable to all patients with the disorder. Some patients with the nephrotic syndrome have increased plasma volumes, with suppressed plasma renin activity and aldosterone levels.[343] The cause of these discrepancies is not immediately evident, but this overfill view has been subject to some criticism.[344] It is most likely that the underfilling mechanism is operant in patients with a normal GFR and with the histologic lesion of minimal change disease, and that hypervolemia may be more prevalent in patients with underlying glomerular pathology and decreased renal function. In such patients, an intrarenal mechanism probably causes Na^+ retention, as has been described in an experimental model of nephrotic syndrome.[345] Also, in contrast to the increase in AQP2 found in the previously described Na^+- and water-retaining states, the expression of AQP2 was decreased in two models of nephrotic syndrome induced with puromycin aminonucleoside[346] or doxorubicin.[347] The animals were not hyponatremic and most likely had expanded ECF volumes to explain the discrepancy.

RENAL FAILURE

Hyponatremia with edema can occur with acute or chronic renal failure. It is clear that in the setting of experimental

or human renal disease, the ability to excrete free water is maintained better than the ability to reabsorb water. Nonetheless, the patient's GFR still determines the maximal rate of free water formation. Thus, if minimal urine osmolality is limited to 150 to 250 mOsm/kg H_2O and fractional water excretion approaches 20% to 30% of the filtered load, a uremic patient with a GFR of 2 mL/min/1.73 m^2 is estimated to excrete only ~300 mL of free water/day. Intake of more fluid than this will result in hyponatremia. Thus, in most cases, a decrement in GFR with an increase in thirst underlies the hyponatremia of patients with renal insufficiency.[348]

HYPONATREMIA WITH NORMAL EXTRACELLULAR FLUID VOLUME

Fig. 15.17 lists the clinical entities that have to be considered in patients with hyponatremia whose volume is neither contracted nor expanded and who are, at least by clinical assessment, euvolemic. These are considered individually here.

SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION

SIADH is the most common cause of hyponatremia in hospitalized patients.[279] As first described by Schwartz and associates[349] in two patients with bronchogenic carcinoma, and later characterized further by Bartter and Schwartz,[350] patients with this syndrome have serum hypoosmolality when excreting urine that is less than maximally dilute (>100 mOsm/kg H_2O). Thus, a diagnostic criterion for this syndrome is the presence of inappropriate urine concentration. The development of hyponatremia with a dilute urine (<100 mOsm/kg H_2O) should raise suspicion of a primary polydipsic disorder. Although large volumes of fluid need to be ingested to overwhelm the normal water excretory ability, this volume need not be excessively high if there are concomitant decreases in solute intake.[293] In SIADH, the urinary Na^+ concentration is dependent on intake, because Na^+ balance is well maintained. As such, urinary Na^+ concentration is usually high, but it may be low in patients with the syndrome who are receiving a low-sodium diet. The presence of Na^+ in the urine is helpful in excluding extrarenal causes of hypovolemic hyponatremia, but a low urinary Na^+ concentration does not exclude SIADH. Before the diagnosis of SIADH is made, other causes for a decreased diluting capacity, such as renal, pituitary, adrenal, thyroid, cardiac, or hepatic disease, must be excluded. In addition, nonosmotic stimuli for AVP release, particularly hemodynamic derangements (e.g., caused by hypotension, nausea, or drugs), need to be ruled out.

Another clue to the presence of SIADH is the finding of hypouricemia. In one study, 16 of 17 patients with a diagnosis of SIADH had levels below 4 mg/dL, whereas in 13 patients with hyponatremia of other causes, the level was higher than 5 mg/dL. Hypouricemia appears to occur as a consequence of increased urate clearance.[351] Measurement of an elevated level of AVP can confirm the clinical diagnosis, but is not necessary. It should be noted that most patients with SIADH have AVP levels in the normal range (2–10 pg/mL); the presence of any measurable AVP is, however, abnormal in the hypoosmolar state. Plasma AVP levels have never been a requirement for the diagnosis of SIADH, in part because they are elevated in states of hypovolemic hyponatremia as well as SIADH, and therefore have little differential diagnostic value. For similar reasons, the measurement of copeptin levels has been found to be of little diagnostic value in the differential diagnosis of hyponatremia.[352] Because the presence of hyponatremia is itself evidence for abnormal dilution, a formal urine-diluting test need not be performed in most cases. The water loading test is helpful in determining whether an abnormality remains in a patient whose serum [Na^+] has been corrected by water restriction. Because Brattleboro rats receiving AVP[353] have displayed upregulation of AQP2 expression, the excretion of AQP2 has been investigated as a marker for the persistent secretion of AVP. The excretion of the water channel remains elevated in patients with SIADH, but this is not specific to this entity because a similar pattern was observed in patients with hyponatremia due to hypopituitarism.[354]

Pathophysiology

In 1953, Leaf and associates[355] described the effects of chronic AVP administration on Na^+ and water balance. They noted that high-volume water intake was required for the development of hyponatremia. Concomitant with the water retention, an increment in urinary Na^+ excretion was observed. The relative contributions of the water retention and Na^+ loss to the development of hyponatremia were subsequently investigated. Acute water loading causes transient natriuresis but, when water intake is increased more slowly, no significant negative Na^+ loss can be documented. These studies have clearly demonstrated that the hyponatremia is mainly a consequence of water retention; however, it must be noted that the net increase in water balance fails to account entirely for the decrement in serum [Na^+].[355]

In a carefully studied model of SIADH in rats, the retained water was found to be distributed in the intracellular space and in equilibrium with the tonicity of the ECF.[356] The natriuresis and kaliuresis that occur early in the development of this model contribute to a decrement of body solutes and, in part, account for the observed hyponatremia.[357] Studies involving analysis of whole-body water and electrolyte content have demonstrated that the relative contributions of water retention and solute losses vary with the duration of induced hyponatremia; the former is central to the process but, with more prolonged hyponatremia, Na^+ depletion becomes predominant.[358] In this regard, it has even been suggested that the natriuresis and volume contraction are important components of the syndrome that maintains the secretion of AVP,[359] with atrial natriuretic peptide as a potential mediator of the Na^+ loss.[360] Therefore, although natriuresis frequently accompanies the syndrome, nonosmotically stimulated AVP secretion is essential. Finally, patients with the syndrome must also have abnormal thirst regulation, whereby the osmotic inhibition of water intake is not operant. The mechanism of this failure to suppress thirst is not fully understood, but may simply reflect the continued ingestion of beverages for reasons other than true thirst.

After the initial retention of water, loss of Na^+, and development of hyponatremia, continued administration of AVP is accompanied by reestablishment of Na^+ balance and a decline in the hydroosmotic effect of the hormone. The integrity of renal regulation of Na^+ balance is manifested by the ability to conserve Na^+ during Na^+ restriction and by the normal excretion of a Na^+ load. Thus, the mechanisms that regulate Na^+ excretion are intact. Loss of the hydroosmotic effect of AVP, albeit to varying degrees, has been evident in many

studies[355,357] because urine flow increases and urine osmolality decreases, despite continued administration of the hormone. This effect has been termed "vasopressin escape."[361] Several studies have demonstrated that hypotonic ECF volume expansion, rather than chronic administration of AVP per se, is needed for escape to occur because the escape phenomenon is seen only when a positive water balance is achieved.[361]

The cellular mechanisms responsible for vasopressin escape have been the subject of some investigation. Studies of broken epithelial cell preparations of the toad urinary bladder have revealed downregulation of AVP receptors,[362] as well as vasopressin binding in the inner medulla.[363] Post-cAMP mechanisms are probably also operant. In this regard, a decrease in the expression of AQP2 has been reported in the process of escape from desmopressin-induced antidiuresis, without a concomitant change in basolateral AQP3 and AQP4.[364,365] The decrement in AQP2 was associated with decreased V_2 responsiveness.[364] The distal tubule also has an increase in sodium transporters, including the alpha and gamma subunits of the epithelial sodium channel and the thiazide-sensitive Na^+-Cl^- cotransporter.[366] In addition to a renal mechanism, it appears that chronic hyponatremia causes a decrement in hypothalamic mRNA production, a process that could ameliorate the syndrome in the clinical setting.[27]

Clinical Settings

It is now apparent that the previously described pathophysiologic sequence occurs in a variety of clinical settings characterized by persistent AVP secretion. Since the original report of Schwartz and coworkers,[349] the syndrome has been described in an increasing number of clinical settings (Table 15.3). These fall into four general categories[367]: (1) malignancies; (2) pulmonary disease; (3) CNS disorders; and (4) drug effects. In addition, an increasing number of patients with acquired immunodeficiency syndrome have been reported to have hyponatremia. The frequency may be as high as 35% of hospitalized patients with the disease and, in as many as two-thirds, SIADH may be the underlying cause.[368] As noted previously, hyponatremia caused by excessive water repletion can occur after moderate and severe exercise.[369–372] Finally, it has been increasingly recognized that an idiopathic form is common in older adults.[373–376] In one study, as many as 25% of older patients admitted to a rehabilitation center had a serum [Na^+] lower than 135 mEq/L.[374] In a significant proportion of these patients, no underlying cause was discovered.

A material with antidiuretic properties has been extracted from some of the tumors or metastases of patients with malignancy-associated SIADH. However, not all patients with the syndrome have AVP in their tumors. Of the tumors that cause SIADH secretion, bronchogenic carcinoma, and particularly small cell lung cancer, is the most common, with a reported incidence of 11%.[377] It appears that patients with bronchogenic carcinoma have higher plasma AVP levels in relation to plasma osmolality, even if they do not manifest full-blown SIADH; however, in patients with the syndrome, levels of the hormone are higher. The possibility that the hormone could serve as a marker of bronchogenic carcinoma has been suggested, and SIADH has been reported occasionally to precede diagnosis of the tumor by several months.[378] Recent epidemiologic analyses have strongly implicated hyponatremia as a predictor of subsequent malignancies. Among 625,114 Danish subjects, all-cause mortality was increased in mild, moderate, and severe hyponatremia compared with normonatremic individuals. Of special note, the incident rate ratio of a subsequent diagnosis of head and neck or pulmonary cancer was markedly increased in patients at all levels of hyponatremia; 1893 of 14,517 of all hyponatremic patients (13%) subsequently had a diagnosis

Table 15.3 Disorders Associated With Syndrome of Inappropriate Antidiuretic Hormone Secretion

Carcinomas	Pulmonary Disorders	Central Nervous System Disorders	Other Disorders
Bronchogenic carcinoma	Viral pneumonia	Encephalitis (viral or bacterial)	AIDS
Carcinoma of the duodenum	Bacterial pneumonia	Meningitis (viral, bacterial, tuberculous, fungal)	Prolonged exercise
Carcinoma of the ureter	Pulmonary abscess	Head trauma	Idiopathic (in older adults)
Carcinoma of the pancreas	Tuberculosis	Brain abscess	Nephrogenic
Thymoma	Aspergillosis	Guillain-Barré syndrome	Acute intermittent porphyria
Carcinoma of the stomach	Positive pressure breathing	Subarachnoid hemorrhage or subdural hematoma	
Lymphoma	Asthma	Cerebellar and cerebral atrophy	
Ewing sarcoma	Pneumothorax	Cavernous sinus thrombosis	
Carcinoma of the bladder	Mesothelioma	Neonatal hypoxia	
Prostatic carcinoma	Cystic fibrosis	Shy-Drager syndrome	
Oropharyngeal tumor		Rocky Mountain spotted fever	
		Delirium tremens	
		Cerebrovascular accident (cerebral thrombosis or hemorrhage)	
		Acute psychosis	
		Peripheral neuropathy	
		Multiple sclerosis	

Adapted from Berl T, Schrier RW: Disorders of water metabolism. In Schrier RW, ed. *Renal and Electrolyte Disorders.* 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2003.

of cancer, leading to the conclusion that hyponatremia is linked to an increased risk of being subsequently diagnosed with any cancer, particularly pulmonary and head and neck cancers.[379] In view of the potential to treat patients with this tumor, it is important that patients with unexplained SIADH be fully investigated and evaluated for the presence of this malignancy. Head and neck malignancies are the second most common tumors associated with SIADH, which occurs in approximately 3% of these patients.

The mechanism whereby AVP is produced in other pulmonary disorders is not known, but the associated abnormalities in blood gases could act as mediators of the effect. Antidiuretic activity has also been assayed in tuberculous lung tissue. The syndrome can also occur in the setting of miliary rather than only lung-limited tuberculosis.[380] In CNS disorders, AVP is most likely released from the neurohypophysis. Studies of monkeys have shown that elevations of intracranial pressure cause AVP secretion, which may be the mechanism that mediates the syndrome in at least some CNS disorders. The magnocellular AVP-secreting cells in the hypothalamus are subject to numerous excitatory inputs (see Fig. 15.6), and therefore it is conceivable that a large variety of neurologic disorders can stimulate the secretion of AVP.

Although SIADH as typically described is associated with inappropriate secretion of AVP, hyponatremia has been described in two infants who met all the criteria for a diagnosis of SIADH but had undetectable AVP levels. Genetic analysis revealed a gain-of-function mutation at the X-linked AVP V_2 receptor, where in codon 137 a missense mutation resulted in the change from arginine to cysteine or leucine. The authors termed this "nephrogenic syndrome of inappropriate antidiuresis" (NSIAD).[381]

Zebra and colleagues have studied the osmoregulation of AVP secretion in a large group of patients with SIADH.[382] In the great majority, the plasma AVP concentration was inadequately suppressed relative to the hypotonicity present. In most patients, the plasma AVP concentration ranged from 1 to 10 pg/mL, the same range as in normally hydrated healthy adults. Inappropriate secretion, therefore, can often be demonstrated only by measuring AVP under hypotonic conditions. Even with this approach, however, abnormalities in plasma AVP were not apparent in almost 10% of patients with clinical evidence of SIADH. To define the nature of the osmoregulatory defect in these patients better, plasma AVP was measured during infusion of hypertonic saline. When this method of analysis was applied to 25 patients with SIADH, four different types of osmoregulatory defects were identified.

As shown in Fig. 15.18, infusion of hypertonic saline in the type A osmoregulatory defect was associated with large and erratic fluctuations in plasma AVP, which bore no relation to the rise in plasma osmolality. This pattern was found in 6 of 25 patients studied who had acute respiratory failure, bronchogenic carcinoma, pulmonary tuberculosis, schizophrenia, or rheumatoid arthritis. This pattern indicates that the secretion of AVP had been totally divorced from osmoreceptor control or was responding to some periodic nonosmotic stimulus.

A completely different type of osmoregulatory defect is exemplified by the type B response (see Fig. 15.18). The infusion of hypertonic saline resulted in prompt and progressive rises in plasma osmolality. Regression analyses have shown that the precision and sensitivity of this response are essentially

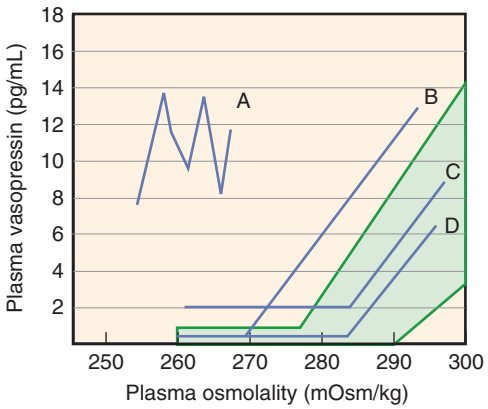


Fig. 15.18 Plasma vasopressin as a function of plasma osmolality during the infusion of hypertonic saline in four groups of patients with the clinical syndrome of inappropriate antidiuretic hormone (SIADH). *Shaded area,* Range of normal values. See text for description of each group. (From Zerbe R, Stropes L, Robertson G: Vasopressin function in the syndrome of inappropriate antidiuresis. *Annu Rev Med.* 1980;31:315–327.)

the same as those in healthy subjects, except that the intercept or threshold value at 253 mOsm/kg is well below the normal range. This pattern, which reflects the resetting of the osmoreceptor, was found in 9 of 25 patients who had a diagnosis of bronchogenic carcinoma, cerebrovascular disease, tuberculous meningitis, acute respiratory disease, or carcinoma of the pharynx. Another patient was reported with hyponatremia and acute idiopathic polyneuritis who reacted in an identical manner to the hypertonic saline infusion and was determined to have had resetting of the osmoreceptor. Because their threshold function is retained when they receive a water load, this patient and others with reset osmostats have been able to dilute their urine maximally and sustain a urine flow sufficient to prevent a further increase in body water. Thus, an abnormality in AVP regulation can exist, in spite of the ability to dilute the urine maximally and excrete a water load at some lower level of plasma osmolality.

In the type C response (see Fig. 15.18), plasma AVP was elevated initially but did not change during the infusion of hypertonic saline until plasma osmolality reached the normal range. At that point, plasma AVP began to rise appropriately, indicating a normally functioning osmoreceptor mechanism. This response was found in 8 of 25 patients with the diagnosis of CNS disease, bronchogenic carcinoma, carcinoma of the pharynx, pulmonary tuberculosis, or schizophrenia. Its pathogenesis is unknown, but the authors speculated that it may be due to a constant, nonsuppressible leak of AVP, despite otherwise normal osmoregulatory function.[382] Unlike type B, the resetting type of defect, the type C response results in impaired urine dilution and water excretion at all levels of plasma osmolality.

In the type D response (see Fig. 15.18), the osmoregulation of AVP appears to be completely normal, despite a marked inability to excrete a water load. The plasma AVP is appropriately suppressed under hypotonic conditions and does not rise until plasma osmolality reaches the normal threshold level. When this procedure is reversed by water loading, plasma osmolality and plasma AVP again fall normally, but urine dilution does not occur, and the water load is not

excreted. This defect was present in 2 of 25 patients diagnosed with bronchogenic carcinoma, indicating that in these patients, the antidiuretic defect is caused by some abnormality other than SIADH. It could be due to increased renal tubule sensitivity to AVP or the presence of an antidiuretic substance other than AVP. Alternatively, it is possible that currently available assays are not sensitive enough to detect significant levels of AVP. It is intriguing to speculate that some of these subjects have NSIAD, as described previously,[381] but only a small number of adult kindreds with this diagnosis have been described.[383]

It is of interest that patients with bronchogenic carcinoma, which was generally believed to be associated with ectopic production of AVP, manifest every category of osmoregulatory defect, including the reset osmostat. It has been suggested that many of these tumors probably cause SIADH secretion not by producing the hormone ectopically, but by interfering with the normal osmoregulation of AVP secretion from the neurohypophysis through direct invasion of the vagus nerve, metastatic implants in the hypothalamus, or some other more generalized neuropathic change.

GLUCOCORTICOID DEFICIENCY

Considerable evidence exists for an important role for glucocorticoids in the abnormal water excretion of adrenal insufficiency.[384] The water excretory defect of anterior pituitary insufficiency, and particularly ACTH deficiency, is associated with elevated AVP levels[385,386] and corrected by physiologic doses of glucocorticoids. Similarly, adrenalectomized dogs receiving replacement of mineralocorticoids still have abnormal water excretion. The relative importance of intrarenal factors and AVP in defective water excretion has been a matter of controversy. Studies using a sensitive radioimmunoassay for plasma AVP and the Brattleboro rat with hypothalamic DI have provided evidence that both factors are involved. Support for a role for AVP has been obtained in studies of conscious adrenalectomized, mineralocorticoid-replaced dogs[387] and rats[388] and with the use of an inhibitor of the hydroosmotic effect of AVP.[308] Because the plasma AVP level was elevated despite a fall in plasma osmolality, the hormone's release was likely nonosmotically mediated. Although ECF volume was normal in both these studies, a decrease in systemic pressure and cardiac function[387,388] could have provided the hemodynamic stimulus for AVP release. In addition, there may be a direct effect of glucocorticoids that inhibits AVP secretion. In this regard, AVP gene expression is increased in glucocorticoid-deficient rats.[389] The presence of a glucocorticoid-responsive element on the AVP gene promoter may be responsible for the inhibition of AVP gene transcription by glucocorticoids.[390] Also, glucocorticoid receptors are present in magnocellular neurons and are increased during hypoosmolality.[391]

A role for AVP-independent intrarenal factors was defined in the antidiuretic-deficient, adrenalectomized Brattleboro rat[388] and with the use of AVP receptor antagonists.[308] It appears that prolonged glucocorticoid deficiency (14–17 days) is accompanied by decreases in renal hemodynamics that impair water excretion. A direct effect of glucocorticoid deficiency that enhances water permeability of the collecting duct has been proposed, but such a concept has not been supported by studies of anuran membranes, suggesting that glucocorticoids enhance rather than inhibit water transport. Also, in vitro perfusion studies of the collecting duct of adrenalectomized rabbits have shown an impaired rather than an enhanced AVP response,[392] a defect that may be related to enhanced cAMP metabolism.[393] AQP2 and AQP3 abundance appears not to be sensitive to glucocorticoids.[394]

In summary, the defect in glucocorticoid deficiency is primarily AVP-dependent, but an AVP-independent mechanism becomes evident with more prolonged hormone deficiency. It appears likely that alterations in systemic hemodynamics account for the nonosmotic release of AVP, but a direct effect of glucocorticoid hormone on AVP release has not been entirely excluded. The AVP-independent renal mechanism is probably caused by alterations in renal hemodynamics and not by a direct increase in collecting duct permeability. It should be remembered that secondary hypoadrenalism, as occurs in hypopituitarism, can also be associated with hyponatremia.[395,396]

HYPOTHYROIDISM

Patients and experimental animals with hypothyroidism often have impaired water excretion and sometimes develop hyponatremia.[384,397] The dilution defect is reversed by treatment with thyroid hormone. Decreased delivery of filtrate to the diluting segment and persistent secretion of AVP, alone or in combination, have been proposed as mechanisms responsible for the defect.

Hypothyroidism has been shown to be associated with decreases in GFR and renal plasma flow.[397] In the AVP-deficient Brattleboro rat, the decrement in maximal free water excretion can be entirely accounted for by the decrease in GFR. The osmotic threshold for AVP release appears not to be altered in hypothyroidism.[398] The normal suppression of AVP release with water loading and the normal response to hypertonic saline,[399] coupled with the failure to observe upregulation of hypothalamic AVP gene expression in hypothyroid rats,[400] supports an AVP-independent mechanism. There is, however, also evidence for a role of AVP in impairing water excretion in hypothyroidism. In experimental animals[401] and humans with advanced hypothyroidism,[397] elevated AVP levels were measured in the basal state and after a water load. Although increased sensitivity to AVP in hypothyroidism has been proposed, experimental evidence has suggested the contrary because urine osmolality is relatively low for the circulating levels of the hormone,[401] and AVP-stimulated cyclase is impaired in the renal medulla of hypothyroid rats,[402] possibly leading to decreased AQP2 expression.[403] However, the predominant defect is one of water excretion with increased AQP2 expression and reversal with a V_2 receptor antagonist.[404] It appears, therefore, that diminished distal fluid delivery and persistent AVP release mediate the impaired water excretion in this disorder, but the relative contributions of these two factors remain undefined and may depend on the severity of the endocrine disorder.

PRIMARY POLYDIPSIA

It has long been recognized that patients with psychiatric disease demonstrate increased water intake. Although such polydipsia is normally not associated with hyponatremia, it has been observed that these patients are at increased risk of developing hyponatremia when they are acutely psychotic.[405] Most of these patients have schizophrenia, but some have psychotic depression. The frequency of hyponatremia in this

population of patients is unknown, but in a survey conducted in one large psychiatric hospital, 20 polydipsic patients with a serum [Na^+] lower than 124 mEq/L were reported,[406] and another survey found hyponatremia in 8 of 239 patients.[407] Elucidation of the mechanism of the impaired water excretion has been confounded by antipsychotic drug treatment (see later). The relative contributions of the pharmacologic agent and the psychosis are therefore difficult to define, because thiazides and carbamazepine are also frequently implicated.[408] Nonetheless, there have been reports of psychotic patients who suffered water intoxication, even when free of medication.[409]

The mechanism responsible for the hyponatremia in psychosis appears to be multifactorial. In a comprehensive study of water metabolism in eight psychotic hyponatremic patients and seven psychotic, normonatremic control subjects, no unifying defect emerged. The investigators found a small defect in osmoregulation that caused AVP to be secreted at plasma osmolalities somewhat lower than those of the control group, but they did not observe a true resetting of the osmostat. Also, the hyponatremic patients had a mild urine dilution defect, even in the absence of AVP. When AVP was present, the renal response was somewhat enhanced, suggesting increased renal sensitivity to the hormone. Psychotic exacerbations appear to be associated with increased AVP levels in schizophrenic patients with hyponatremia.[410] Finally, thirst perception is also increased, because excessive water intake that exceeds excretory capacity is responsible for most episodes of hyponatremia in these patients. However, concurrent nausea caused increased AVP levels in some of the subjects.[411] Although each of these derangements by itself would remain clinically unimportant, it is possible that during exacerbation of the psychosis the defects are more pronounced, and that in combination they can culminate in hyponatremia.[412]

Hyponatremia occurs in beer drinkers (so-called "beer potomania"). Although this has been ascribed to an increase in fluid intake in the setting of very low solute intake,[413] such patients may also have sustained significant solute losses.[414] A similar presentation can occur in patients who do not drink beer excessively, but have very low solute intake, either due to patient preference for a specific diet (e.g., ovolactovegetarian), poor appetite, or limited access to food ("tea and toast" diet).[293]

POSTOPERATIVE HYPONATREMIA

The incidence of hospital-acquired hyponatremia is high in adults[214] and children[415] and is particularly prevalent in the postoperative stage[416,417] (incidence ≅ 4%). Most affected patients appear clinically euvolemic and have measurable levels of AVP in their circulation.[416,418] Although this occurs primarily as a consequence of administration of hypotonic fluids,[419] a decrease in serum [Na^+] can occur in this high-AVP state, even when isotonic fluids are given.[420] Hyponatremia has also been reported following cardiac catheterization in patients receiving hypotonic fluids.[421] Although the presence of hyponatremia is a marker for poor outcomes, this is likely a consequence not of the hyponatremia per se but of the severe underlying disease associated with it. There is, however, a subgroup of postoperative hyponatremic patients, almost always premenstrual women, who develop catastrophic neurologic events, frequently accompanied by seizures and hypoxia.[422,423]

ENDURANCE EXERCISE

There has been increasing recognition that strenuous endurance exercise, such as military training[424] and marathons and triathlons,[369] can cause hyponatremia that is frequently symptomatic (often referred to as "exercise-associated hyponatremia"). A prospective study of 488 runners in the Boston Marathon revealed that 13% of the runners had a serum [Na^+] lower than 130 mEq/L. A multivariate analysis has revealed that weight gain, presumably related to excessive fluid intake, is the strongest single predictor of the hyponatremia. Longer racing times and a very low body mass index (BMI) were also predictors.[425] Composition of the fluids consumed and use of NSAIDs was not predictive. Symptomatic hyponatremia is even more frequent in ultraendurance events.[426] In addition to excessive intake of hypotonic fluids, patients with exercise-associated hyponatremia generally have inappropriately elevated AVP levels, suggesting that nonosmotic AVP secretion contributes to its pathogenesis.[427]

PHARMACOLOGIC AGENTS

Many drugs have been associated with water retention. Some of the more clinically important agents are discussed here.

Desmopressin

Because desmopressin is a selective agonist of the AVP V_2 receptors, it would be expected that patients treated with desmopressin are at increased risk of developing hyponatremia. The reported incidence in patients treated with desmopressin for DI is relatively low because they generally do not drink excessive amounts of fluid. However, a recent study has reported a 27% incidence of mild hyponatremia (131–134 mmol/L) and a 15% incidence of more severe hyponatremia (≤130 mmol/L) with long-term follow-up of patients with chronic CDI.[226] Patients who receive desmopressin at higher doses for indications such as von Willebrand disease,[428] or older patients with decreased renal function who receive desmopressin for nocturnal enuresis,[429,430] are also at risk to develop symptomatic hyponatremia.

Chlorpropamide

The incidence of mild hyponatremia in patients taking chlorpropamide may be as high as 7%, but severe hyponatremia (<130 mEq/L) occurs in 2% of patients so treated.[431] As noted earlier, the drug exerts its action primarily by potentiating the renal action of AVP.[432] Studies of the toad urinary bladder have demonstrated that although chlorpropamide alone has no effect, it enhances AVP- and theophylline-stimulated water flow but decreases cAMP-mediated flow. The enhanced response may be due to the upregulation of AVP V_2 receptors.[433] Alternatively, studies of chlorpropamide-treated animals have suggested that the drug enhances solute reabsorption in the medullary ascending limb (thereby increasing interstitial tonicity and the osmotic drive for water reabsorption), rather than a cAMP- mediated alteration in collecting duct water permeability.[434]

Antiepileptic Drugs

The anticonvulsant drug carbamazepine is well known to have antidiuretic properties. The incidence of hyponatremia in carbamazepine-treated patients was believed to be as high as 21%, but a survey of patients with mental retardation has

reported a lower incidence of 5%.[435] Cases continue to be reported.[436] The antiepileptics oxcarbazepine and eslicarbazapine, both of the same class as carbamazepine, have been reported to cause hyponatremia at even higher rates than carbamazepine (43% and 33% vs. 16% incidences).[437] Evidence exists for a mechanism mediated by AVP secretion and for renal enhancement of the hormone's action[438] to explain carbamazepine's antidiuretic effect. The drug also appears to decrease the sensitivity of the AVP response to osmotic stimulation.[439]

Psychotropic Drugs

An increasing number of psychotropic drugs have been associated with hyponatremia, and they are frequently implicated to explain the water intoxication in psychotic patients. Among the agents implicated are the phenothiazines, the butyrophenone haloperidol, and the tricyclic antidepressants.[440–442] An increasing number of cases of amphetamine (ecstasy)-related hyponatremia have been described.[443,444] Similarly, the widely used antidepressants fluoxetine,[445] sertraline,[446] and paroxetine[447] have been associated with hyponatremia. In this latter study, involving 75 patients, 12% developed hyponatremia (serum $[Na^+] < 135$ mmol/L). Older adults appear to be particularly susceptible, with an incidence as high as 22% to 28%.[448–451] The tendency for these drugs to cause hyponatremia is further compounded by their anticholinergic effects; by drying the mucous membranes, they can stimulate water intake. The role of these drugs in impaired water excretion has not, in most cases, been dissociated from the role of the underlying disorder for which the drug was given. Furthermore, evaluation of the effect of the drugs on AVP secretion has frequently revealed a failure to increase levels of the hormone, particularly if the mean arterial pressure remained unaltered. Therefore, although a clinical association between antipsychotic drugs and hyponatremia is frequently encountered, the pharmacologic agents themselves may not be the principal factors responsible for the water retention.

Antineoplastic Drugs

Several drugs used in cancer therapy cause antidiuresis. The effect of vincristine may be mediated by the drug's neurotoxic effect on the hypothalamic microtubule system, which then alters normal osmoreceptor control of AVP release.[452] A retrospective survey has suggested that this may be more common in Asians who were given the drug.[453] Cyclophosphamide administration also causes hyponatremia. The mechanism of the diluting defect that results from cyclophosphamide is not fully understood. It may act, at least in part, to enhance action, because the drug does not increase hormone levels.[454] It is known that the antidiuresis has its onset 4 to 12 hours after injection of the drug, lasts as long as 12 hours, and seems to be temporally related to excretion of a metabolite. The importance of anticipating potentially severe hyponatremia in cyclophosphamide-treated patients who are vigorously hydrated to prevent urologic complications cannot be overstated. The synthetic analogue of cyclophosphamide, ifosfamide, has also been associated with hyponatremia and AVP secretion.[455]

Narcotics

Since the 1940s, it has been known that the administration of opioid agonists, such as morphine, reduces urine flow by causing the release of an antidiuretic substance. The possibility that endogenous opioids could serve as potential neurotransmitters has been suggested by the finding of enkephalins in nerve fibers projecting from the hypothalamus to the neurohypophysis. However, the reported effects vary; they range from stimulation to no change and even to inhibition of AVP secretion. The reasons for these diverse observations may be that the opiates and their receptors are widely distributed in the brain, implying that the site of action of the opiate can differ markedly, depending on the route of administration. Also, there are multiple opiate peptides and receptor types. It has now been determined that agonists of μ-receptors have antidiuretic properties, whereas δ-receptors have the opposite effect.

Miscellaneous Agents

Several case reports have suggested an association between the use of ACE inhibitors and hyponatremia.[456–458] Of interest is that all three reported patients were women in their 60s. The use of ACE inhibition was also a concomitant risk factor for the development of hyponatremia in a survey of veterans who received chlorpropamide.[431] However, given the widespread use of these agents, the true incidence of hyponatremia must be vanishingly low. Similarly, an association with angiotensin receptor blockers has not been reported to date. Rare patients have been reported to develop hyponatremia during amiodarone loading.[459] There have been increasing reports of hyponatremia associated with the use of tacrolimus, mostly in patients after organ transplantation.[460,461] This seems to be more frequent with tacrolimus than cyclosporin, suggesting that it is not a class effect of calcineurin inhibitors.[462,463]

HYPONATREMIA: SYMPTOMS, MORBIDITY, AND MORTALITY

Symptoms of hyponatremia correlate with the degree of decrease in the serum $[Na^+]$ and with the chronicity of the hyponatremia. Most clinical manifestations of hyponatremia usually begin at a serum $[Na^+]$ lower than 130 mEq/L, but mild neurocognitive symptoms can begin at any sodium level that is low (Table 15.4). Although gastrointestinal complaints often occur early, most of the manifestations of hyponatremia are neurologic, including lethargy, confusion, disorientation, obtundation, and seizures, designated as "hyponatremic encephalopathy."[464] Many of the more marked symptoms of hyponatremic encephalopathy are caused by cerebral edema, which may, at least in part, be mediated by AQP4.[465] In its most severe form, the cerebral edema can lead to tentorial herniation; in such cases, death can occur as a result of brainstem compression with respiratory arrest. The cerebral edema can also cause a neurogenic pulmonary edema and hypoxemia,[466] which can in turn increase the severity of brain swelling.[467] The most severe life-threatening clinical features of hyponatremic encephalopathy are generally seen in cases of acute hyponatremia, currently defined as shorter than 48 hours in duration. A few acutely hyponatremic patients have been reported to develop rhabdomyolysis as well.[359]

The development of neurologic symptoms depends on the age, gender, and magnitude and acuteness of the process. Older persons and young children with hyponatremia are most likely to develop symptoms. Neurologic complications

Table 15.4 Classification of Hyponatremia According to Severity of Presenting Symptoms

Severity	Serum Sodium Level	Neurologic Symptoms	Typical Duration of Hyponatremia
Severe	Generally <125 mmol/L	Vomiting, seizures, obtundation, respiratory distress, coma	Acute (<24–48 hours)
Moderate	Generally <130 mmol/L	Nausea, confusion, disorientation, altered mental status, unstable gait, falls	Intermediate or chronic (>24–48 hours)
Mild	<135 mmol/L	Headache, irritability, difficulty concentrating, altered mood, depression	Chronic (several days to many weeks or months)

From Verbalis JG: Emergency management of acute and chronic hyponatremia. In Matfin G, ed. *Endocrine and metabolic emergencies,* Washington DC: Endocrine Press; 2014:352.

may occur more frequently in menstruating women. In a case-control study, Ayus and colleagues have noted that despite an approximately equal incidence of postoperative hyponatremia in males and females, 97% of those with permanent brain damage were women, and 75% of them were menstruating.[423] However, this view is not universally held, because others have not found increased postoperative hyponatremia in this population.[468]

The degree of clinical impairment is not as much related to the absolute measured level of lowered serum [Na^+] as it is to the rate and extent of the decrease in ECF osmolality. In a survey of hospitalized hyponatremic patients (serum [Na^+] < 128 mEq/L), 46% had CNS symptoms, and 54% were asymptomatic.[469] It is notable, however, that the authors thought that the hyponatremia was the cause of the symptoms in only 31% of the symptomatic patients. In this subgroup of symptomatic patients, the mortality was no different from that of asymptomatic patients (9%–10%). In contrast, the mortality of patients whose CNS symptoms were not caused by hyponatremia was high (64%), suggesting that the mortality of these patients is more often due to the associated comorbidity than to the electrolyte disorder itself. This is in agreement with an earlier study of Anderson,[279] which noted a 60-fold increase in mortality in hyponatremic patients over that of normonatremic control subjects. However, in the hyponatremic patients death frequently occurred after the plasma [Na^+] was returned to normal and was generally thought to be due to the progression of severe underlying disease. These studies suggested that hyponatremia is an indicator of severe underlying disease and poor prognosis rather than a cause of the observed increased mortality of such patients. In contrast to this point of view, a recent meta-analysis of studies in which some patients had correction of their hyponatremia indicated that improvement in serum [Na^+] was associated with a 50% reduction in mortality of the corrected groups compared with patients in whom the hyponatremia remained uncorrected, suggesting that hyponatremia may, in fact, be causally related to increased mortality.[470]

Other studies have further indicated that even mild hyponatremia is an independent predictor of higher mortality across a wide variety of disorders, including patients with acute ST-elevation myocardial infarction, heart failure, and liver disease.[471,472] A large study of more than 55,000 electronic heath records from a single Boston hospital has shown that the association of hyponatremia with inpatient mortality is significant across all levels of hyponatremia and even began at serum [Na^+] levels in the lower part of the normal range.[473] These findings were corroborated in studies of 249,000 Danish patients hospitalized over a 5-year period that showed increased 30-day and 1-year mortality associated with all levels of hyponatremia, including the range of 130 to 134.9 mmol/L,[474] and analyses of 2.3 million hospitalized patients enrolled in the Cerner Health Facts database.[475] The mortality associated with chronic hyponatremia is much less well studied, but results of the Rotterdam Longitudinal Aging Study noted significantly decreased survival of older patients with hyponatremia over a 12-year period of observation.[476]

The observed severe CNS symptoms are most likely related to the cellular swelling and cerebral edema that result from acute lowering of ECF osmolality, which leads to the movement of water into cells. In fact, such cerebral edema occasionally causes herniation, as has been noted in postmortem examinations of humans and experimental animals. The increase in brain water is, however, much less marked than would be predicted from the decrease in tonicity were the brain to operate as a passive osmometer. The volume regulatory responses that protect against cerebral edema, and which probably occur throughout the body, have been extensively studied and reviewed.[477] Studies of rats have demonstrated a prompt loss of solutes from cells, both electrolytes and organic osmolytes, after the onset of hyponatremia.[478] Some of the solute losses occur very quickly within 24 hours[479] and, in experimental animals, most of the brain solute loss is completed by 48 hours (Fig. 15.19).

The rate at which the brain restores the lost electrolytes and osmolytes when hyponatremia is corrected is also of pathophysiologic importance. Na^+, K^+, and Cl^- recover quickly and even overshoot normal brain contents.[480] However, the reaccumulation of organic osmolytes is considerably delayed (see Fig. 15.19). This process is likely to account for the more marked cerebral dehydration that accompanies the correction in previously adapted animals,[481] which predisposes them to the development of myelinolysis as a result of disruption of the blood-brain barrier.[482,483] It has been observed that urea may prevent the myelinolysis associated with this pathology. This may be due to the more rapid reaccumulation of organic osmolytes, particularly inositol in the azotemic state,[484] and is consistent with a dearth of reports of the osmotic demyelination syndrome (OSD) in patients with chronic kidney disease, despite wide swings in serum [Na^+] during dialysis.

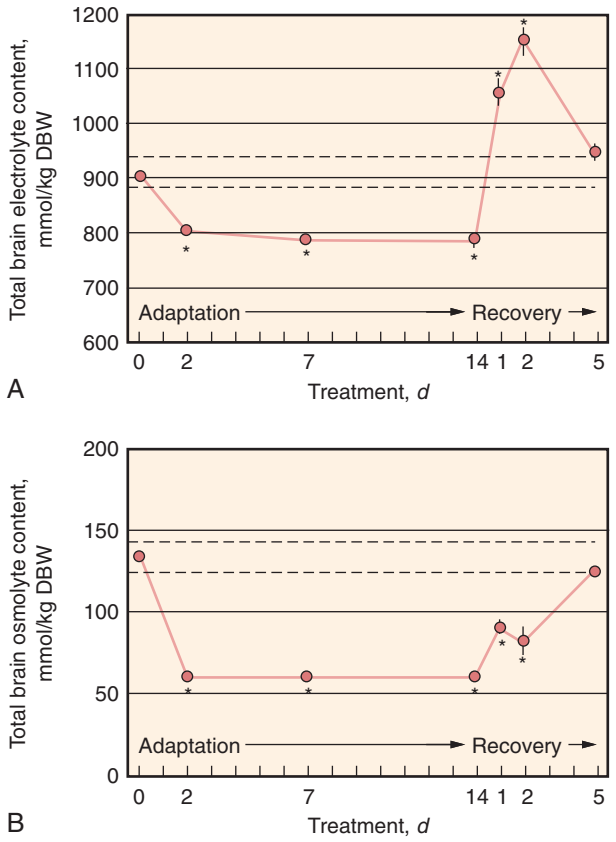


Fig. 15.19 Comparison of changes in brain electrolyte (A) and organic osmolyte (B) content during adaptation to hyponatremia and after rapid correction of hyponatremia in rats. Electrolytes and organic osmolytes are lost quickly after the induction of hyponatremia, beginning on day (d) 0. The brain content of both solutes remains depressed during maintenance of hyponatremia from days 2 through 14. After rapid correction of the hyponatremia on day 14, electrolytes reaccumulate rapidly and overshoot normal brain contents on the first 2 days after correction, before returning to normal levels by the fifth day after correction. In contrast, brain organic osmolytes recover much more slowly and do not return to normal brain contents until the fifth day after correction. The *dashed lines* indicate ± SEM (standard error of mean) from the mean values of normonatremic rats on day 0; $P < .01$ compared with brain contents of normonatremic rats. *DBW,* Dry brain weight. (Data from Verbalis JG, Gullans SR: Hyponatremia causes large sustained reductions in brain content of multiple organic osmolytes in rats. *Brain Res.* 1991;567:274–282; and Verbalis JG, Gullans SR: Rapid correction of hyponatremia produces differential effects on brain osmolyte and electrolyte re-accumulation in rats. *Brain Res.* 1993;606:19–27.)

In contrast to acute hyponatremia, chronic hyponatremia is much less symptomatic. The reason for the profound differences between the symptoms of acute and chronic hyponatremia is now well understood to be caused by the process of brain volume regulation, described earlier.[485] Despite this powerful adaptation process, chronic hyponatremia is frequently associated with neurologic symptomatology, albeit milder and more subtle in nature (see Table 15.4). One report has found a fairly high incidence of symptoms in 223 patients with chronic hyponatremia as a result of thiazide administration—49% had malaise-lethargy, 47% had dizzy spells, 35% had vomiting, 17% had confusion-obtundation, 17% experienced falls, 6% had headaches, and 0.9% had seizures.[486] Although dizziness can potentially be attributed to a diuretic-induced hypovolemia, symptoms such as confusion, obtundation, and seizures are more consistent with hyponatremic symptomatology. Because thiazide-induced hyponatremia can be readily corrected by stopping the thiazide and/or administering sodium, this represents an ideal situation in which to assess improvement in hyponatremia symptomatology with normalization of the serum [Na^+]; in this study, all these symptoms improved with correction of the hyponatremia. This represents one of the best examples demonstrating reversal of the symptoms associated with chronic hyponatremia by correction of the hyponatremia because the patients in this study did not in general have severe underlying comorbidities that might complicate interpretation of their symptoms, as is often the case in patients with SIADH.

Even in patients adjudged to be "asymptomatic" by virtue of a normal neurologic examination, accumulating evidence has suggested that there may be previously unrecognized adverse effects as a result of chronic hyponatremia. In one study, 16 patients with hyponatremia secondary to SIADH, in the range of 124 to 130 mmol/L, demonstrated a significant gait instability that normalized after correction of the hyponatremia to a normal range.[487] The functional significance of the gait instability was illustrated in a study of 122 Belgian patients with a variety of levels of hyponatremia, all judged to be asymptomatic at the time of visiting an emergency department (ED). These patients were compared with 244 age-, gender-, and disease-matched controls also presenting to the ED during the same time period. Researchers found that 21% of the hyponatremic patients came to the ED because of a recent fall, compared with only 5% of the controls; this difference was highly significant and remained so after multivariable adjustment.[487] Analogous results were found in a study of admissions to a US hospital geriatric trauma unit over a 3-year period; when patients admitted because of a fall ($n = 1841$) were analyzed for risk factors associated with falls, the odds ratio for serum [Na^+] < 135 mmol/L was 1.81 ($P < .001$), which was greater than all other risk factors except age older than 85 years.[488] Consequently, these studies have clearly documented an increased incidence of falls in so-called asymptomatic hyponatremic patients. Recent studies in both experimental animals[489] and humans[490] have demonstrated decreases in nerve conduction associated with hyponatremia as a potential cause of gait disturbances.

The clinical significance of the gait instability and fall data have been indicated by multiple independent international studies that have demonstrated increased rates of bone fractures in patients with hyponatremia.[491–494] Other studies have shown that hyponatremia is associated with increased bone loss in experimental animals and with a significantly increased odds ratio for osteoporosis of the femoral neck in those older than 50 years in the Third National Health and Nutrition Examination Survey (NHANES III) database.[495] These findings have been corroborated by multiple epidemiologic studies that demonstrated decreased bone mineral density in human subjects.[496,497] In the largest epidemiologic analysis to date (2.9 million independent electronic health records), the odds ratios for osteoporosis and fractures were significantly greater for hyponatremia (3.99 and 3.05, respectively) than for any other diseases or medications associated with increased bone loss and fracture risk.[498] Of particular

note, the odds ratios for both osteoporosis and fractures were the highest in patients with chronic persistent hyponatremia, indicating that the duration of hyponatremia represents an important risk factor for bone disease and fractures.[498] These findings were supported by a subsequent clinical study of hip fractures in Argentina.[499]

Recent studies of a cohort of 5435 community-dwelling men older than 65 years of age in the in the United States have found that hyponatremia is independently associated with greater cognitive impairment and cognitive decline in this population,[500] and a retrospective study of 4900 hyponatremic patient in Taiwan has found that the hyponatremic patients have a 2.36-fold higher hazard ratio of developing dementias compared with matched controls, which increased to a hazard ratio of 4.29 for patients with more severe hyponatremia. Thus, the major clinical significance of chronic hyponatremia may lie in the increased morbidity and mortality associated with falls, fractures, neurocognitive impairments, and dementias in our older population, as well as potential adverse effects not yet studied in humans.[501]

HYPONATREMIA TREATMENT

Correction of hyponatremia is associated with markedly improved neurologic outcomes in patients with severely symptomatic hyponatremia. In a retrospective review of patients who presented with severe neurologic symptoms and a serum [Na^+] lower than 125 mmol/L, prompt therapy with isotonic or hypertonic saline resulted in a correction of approximately 20 mEq/L over several days and neurologic recovery in almost all cases. In contrast, in patients who were treated with fluid restriction alone, there was very little correction over the study period (<5 mEq/L over 72 hours), and the neurologic outcomes were much worse, with most of these patients dying or entering a persistent vegetative state.[502] Consequently, based on this and many similar retrospective analyses, prompt therapy to increase the serum [Na^+] rapidly represents the standard of care for treatment of patients presenting with severe life-threatening symptoms of hyponatremia.

Brain herniation, the most dreaded complication of hyponatremia, is seen almost exclusively in patients with acute hyponatremia (usually <24 hours) or in patients with intracranial pathology.[503–505] In postoperative patients, and in patients with self-induced water intoxication associated with marathon running, psychosis, or use of ecstasy (3,4-methylenedioxymethamphetamine [MDMA]), nonspecific symptoms such as headache, nausea and vomiting, or confusion can rapidly progress to seizures, respiratory arrest, and ultimately death or to a permanent vegetative state as a complication of severe cerebral edema.[506] Hypoxia from noncardiogenic pulmonary edema and/or hypoventilation can exacerbate brain swelling caused by the low serum [Na^+].[466,467] Seizures can complicate severe chronic hyponatremia and acute hyponatremia. Although usually self-limited, hyponatremic seizures may be refractory to anticonvulsants.

As discussed earlier, chronic hyponatremia is much less symptomatic as a result of the process of brain volume regulation. Because of this adaptation process, chronic hyponatremia is arguably a condition that clinicians think they may not need to be as concerned about, which has been reinforced by common usage of the descriptor "asymptomatic hyponatremia" for many of these patients. However, as noted, it is clear that many such patients very often do have neurologic symptoms, even if milder and more subtle in nature, including headaches, nausea, mood disturbances, depression, difficulty concentrating, slowed reaction times, unstable gait, increased falls, confusion, and disorientation.[487] Consequently, all patients with hyponatremia who manifest any neurologic symptoms that could possibly be related to the hyponatremia should be considered as candidates for treatment, regardless of the chronicity of the hyponatremia or the level of serum [Na^+]. An additional reason for treating even asymptomatic hyponatremia effectively is to prevent a lowering of the serum [Na^+] to more symptomatic and dangerous levels during treatment of underlying conditions (e.g., increased fluid administration via parenteral nutrition, treatment of heart failure with loop diuretics).

CURRENT THERAPIES

Conventional management strategies for hyponatremia range from saline infusion and fluid restriction to pharmacologic measures to adjust fluid balance. Although there are many available treatments for hyponatremia, some are not appropriate for the correction of symptomatic hyponatremia because they work too slowly or inconsistently to be effective in hospitalized patients (e.g., demeclocycline, mineralocorticoids). Consideration of treatment options should always include an evaluation of the benefits and potential toxicities of any therapy and must be individualized for each patient.[507] It should always be remembered that sometimes simply stopping treatment with an agent associated with hyponatremia is sufficient to correct a low serum [Na^+], which is especially true for thiazide-induced hyponatremia.[508]

HYPERTONIC SALINE

Acute hyponatremia presenting with severe neurologic symptoms is life-threatening and should be treated promptly with hypertonic solutions, typically 3% NaCl ([Na^+] = 513 mmol/L), because this represents the most reliable method to raise the serum [Na^+] quickly. A continuous infusion of hypertonic NaCl is generally used in inpatient settings. Various formulas have been suggested for calculating the initial rate of infusion of hypertonic solutions,[503] but there has been no consensus regarding optimal infusion rates of 3% NaCl. One of the simplest methods to estimate an initial 3% NaCl infusion rate uses the following relationship[507]:

$$\frac{\text{Patient's weight (in kg)} \times \text{desired correction rate (in mEq/L/h)}}{\text{infusion rate of 3\% NaCl (in mL/h)}}$$

This may not achieve the desired correction rate, but frequent monitoring of the serum [Na^+] will inform the clinician about whether the rate should be increased or decreased, similar to using the measurement of the serum glucose level to guide the infusion rate of insulin drips. Depending on individual hospital policies, the administration of a hypertonic solution may require special considerations (e.g., placement in the intensive care unit [ICU], sign-off by a consultant), which each clinician needs to be aware of to optimize patient care. One barrier to the use of hypertonic saline that appears to be overstated and unfounded is the frequent requirement

for a central intravenous catheter for chronic infusion. A recent study has demonstrated a low rate of complications using peripheral infusions of 3% NaCl (6% infiltration and 3% thrombophlebitis) and concluded that the peripheral administration of 3% NaCl carries a low risk of minor, nonlimb, or life-threatening complications.[509]

An alternative option for more emergent situations is the administration of a 100-mL bolus of 3% NaCl, repeated once if there is no clinical improvement in 30 minutes. This was recommended by a consensus conference organized to develop guidelines for the prevention and treatment of exercise-induced hyponatremia, an acute and potentially lethal condition,[510] and adopted as a general recommendation by an expert panel.[511] Injecting this amount of hypertonic saline intravenously raises the serum [Na^+] by an average of 2 to 4 mmol/L, which is well below the recommended maximal daily rate of change of 10 to 12 mmol/24 hours or 18 mmol/48 hours.[512] Because the brain can only accommodate an average increase of approximately 8% to 10% in brain volume before herniation occurs, quickly increasing the serum [Na^+] by as little as 2 to 4 mmol/L in acute hyponatremia can effectively reduce brain swelling and intracranial pressure.[513]

ISOTONIC SALINE

The treatment of choice for depletional hyponatremia (hypovolemic hyponatremia) is isotonic saline ([Na^+] = 154 mmol/L) to restore ECF volume and ensure adequate organ perfusion. This initial therapy is appropriate for patients who have clinical signs of hypovolemia or in whom a spot urine [Na^+] is lower than 20 to 30 mEq/L.[511] However, this therapy is usually ineffective for dilutional hyponatremias such as SIADH,[514] and continued inappropriate administration of isotonic saline to a euvolemic patient may worsen the hyponatremia[515] and/or cause fluid overload. Although isotonic saline may improve the serum [Na^+] in some patients with hypervolemic hyponatremia, their volume status will generally worsen with this therapy, so unless the hyponatremia is profound, isotonic saline should be avoided.

FLUID RESTRICTION

For patients with chronic hyponatremia, fluid restriction has been the most popular and most widely accepted treatment. When SIADH is present, fluids should generally be limited to 500 to 1000 mL/24 hours. Because fluid restriction increases the serum [Na^+] largely by underreplacing the excretion of fluid by the kidneys, some have advocated an initial restriction to 500 mL less than the 24-hour urine output.[516] When instituting a fluid restriction, it is important for the nursing staff and patient to understand that this includes all fluids that are consumed, not just water (Box 15.4). Generally, the water content of ingested food is not included in the restriction because this is balanced by insensible water losses (e.g., perspiration, exhaled air, feces), but caution should be exercised with foods that have a high fluid concentration (e.g., fruits, soups). Restricting fluid intake can be effective when properly applied and managed in select patients, but the serum [Na^+] is generally increased only slowly (1–2 mmol/L/day), even with severe fluid restriction.[514] In addition, this therapy is often poorly tolerated because of an associated increase in thirst, leading to poor compliance with long-term therapy. However, it is economically favorable, and some patients do respond well to this option.

Box 15.4 General Recommendations for Using Fluid Restriction and Predictors of Its Increased Likelihood of Failure

General Recommendations

- Restrict all intake that is consumed by drinking, not just water.
- Aim for a fluid restriction that is 500 mL/day below the 24-hour urine volume.
- Do not restrict sodium or protein intake unless indicated.

Predictors of Likely Failure of Fluid Restriction

- High urine osmolality (>500 mOsm/kg H_2O)
- Sum of urine Na^+ and K^+ concentrations exceeds serum Na^+ concentration
- 24-hour urine volume < 1500 mL/day
- Increase in serum Na^+ sodium concentration < 2 mmol/L/day in 24 hours on fluid restriction ≤ 1 L/day

From Verbalis JG, Goldsmith SR, Greenberg A, et al: Diagnosis, evaluation, and treatment of hyponatremia: expert panel recommendations. *Am J Med.* 2013;126(Suppl 1):S1–S42.

Fluid restriction should not be used with hypovolemic patients and is particularly difficult to maintain in hospitalized patients with very elevated urine osmolalities secondary to high AVP levels (e.g., >500 mOsm/kg H_2O) because urine solute-free water excretion is very low. Also, if the sum of the urine Na^+ and K^+ concentrations exceeds the serum [Na^+], most patients will not respond to a fluid restriction because an electrolyte-free water clearance will be difficult to achieve.[292,517,518] These and other predictors of failure of fluid restriction have been confirmed in clinical studies[519–521] and are summarized in Box 15.4. The presence of any of these factors in hospitalized patients with symptomatic hyponatremia makes this less than ideal as an initial therapy. In addition, fluid restriction is not practical for some patients, particularly patients in an ICU setting who often require the administration of significant volumes of fluids as part of their therapy. Consequently, such patients are candidates for more effective pharmacologic or saline treatment strategies.

ARGININE VASOPRESSIN RECEPTOR ANTAGONISTS

Conventional therapies for hyponatremia, although effective in specific circumstances, are suboptimal for many different reasons, including variable efficacy, slow responses, intolerable side effects, and serious toxicities. However, perhaps the most striking deficiency of most conventional therapies is that most of these therapies do not directly target the underlying cause of most dilutional hyponatremias—namely, inappropriately elevated plasma AVP levels. A newer class of pharmacologic agents, vasopressin receptor antagonists, also known as vaptans, which directly block AVP-mediated receptor activation, have been approved for the treatment of euvolemic hyponatremia (in the United States and European Union) and hypervolemic hyponatremia (in the United States).[522]

Conivaptan has been approved by the US Food and Drug Administration (FDA) for euvolemic and hypervolemic hyponatremia in hospitalized patients. It is available only as

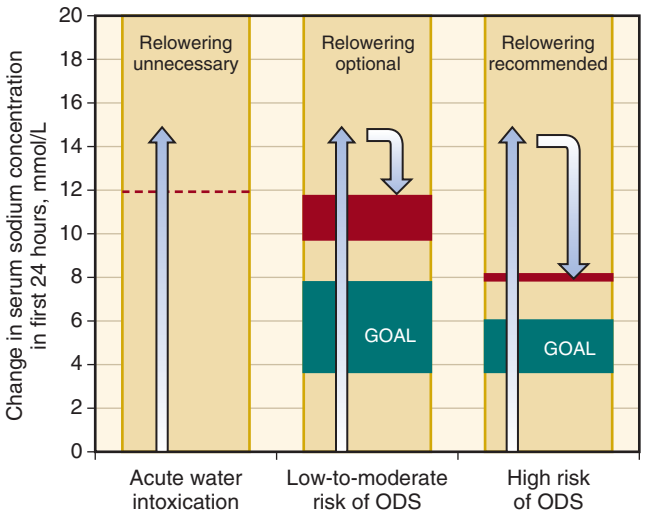


Fig. 15.20 Recommended goals *(green)* and limits *(red)* for correction of hyponatremia based on the risk of producing osmotic demyelination syndrome (ODS). Also shown are recommendations for relowering of the serum [Na^+] to goals for patients presenting with serum [Na^+] < 120 mmol/L who exceed the recommended limits of correction in the first 24 hours. (From Verbalis JG, Goldsmith SR, Greenberg A, et al: Diagnosis, evaluation, and treatment of hyponatremia: expert panel recommendations. *Am J Med.* 2013;126(Suppl 1):S1–S42.)

Box 15.5 Factors Increasing Risk of Osmotic Demyelination Syndrome[a]

- Serum sodium concentration ≤ 105 mmol/L
- Hypokalemia[b]
- Alcoholism[b]
- Malnutrition[b]
- Advanced liver disease[b]

[a]Requiring slower correction of chronic hyponatremia.
[b]Unlike the rate of increase in serum [Na^+], neither the precise level of the serum potassium concentration nor the degree of alcoholism, malnutrition, or liver disease that alters the brain's tolerance to an acute osmotic stress have been rigorously defined.
From Verbalis JG, Goldsmith SR, Greenberg A, et al: Diagnosis, evaluation, and treatment of hyponatremia: expert panel recommendations. *Am J Med.* 2013;126(Suppl 1):S1–S42.

an intravenous preparation and is given as a 20-mg loading dose over 30 minutes, followed by a continuous infusion of 20 or 40 mg/day.[523] Generally, the 20-mg continuous infusion is used for the first 24 hours to gauge the initial response. If the correction of serum [Na^+] is thought to be inadequate (e.g., <5 mmol/L), the infusion rate can be increased to 40 mg/day. Some clinical studies have supported the efficacy of bolus infusions of conivaptan rather than continuous infusions.[524] Therapy is limited to a maximum duration of 4 days because of drug interaction effects with other agents metabolized by the CYP3A4 hepatic isoenzyme.

Importantly, for conivaptan and all other vaptans, it is critical that the serum [Na^+] be measured frequently during the active phase of correction of the hyponatremia, a minimum of every 6 to 8 hours for conivaptan but more frequently in patients with risk factors for OSD.[507] If the correction exceeds 10 to 12 mmol/L in the first 24 hours, the infusion should be stopped and the patient monitored closely. Consideration should be given to administering sufficient water, orally or as intravenous 5% dextrose in water, to avoid a correction of more than 12 mmol/L/day. The maximum correction limit should be reduced to 8 mmol/L over the first 24 hours in patients with risk factors for ODS[511] (Fig. 15.20; Box 15.5). The most common side effects of conivaptan include headache, thirst, and hypokalemia.[525,526]

Tolvaptan, an oral vasopressin receptor antagonist, is also FDA-approved for the treatment of euvolemic and hypervolemic hyponatremia. In contrast to conivaptan, the availability of tolvaptan in tablet form allows short- and long-term use.[527] Similar to conivaptan, tolvaptan treatment must be initiated in the hospital so that the rate of correction can be monitored carefully. In the United States, patients with a serum [Na^+] lower than 125 mmol/L are eligible for therapy with tolvaptan as primary therapy; if the serum [Na^+] is 125 mmol/L or higher, tolvaptan therapy is only indicated if the patient has symptoms that could be attributable to the hyponatremia, and the patient is resistant to attempts at fluid restriction.[528] In the European Union, tolvaptan is approved only for the treatment of euvolemic hyponatremia, but any symptomatic euvolemic patient is eligible for tolvaptan therapy, regardless of the level of hyponatremia or response to previous fluid restriction. The starting dose of tolvaptan is 15 mg on the first day although, in clinical practice, some clinicians recommend starting with a lower dose of 7.5 mg,[529] and the dose can be titrated to 30 and 60 mg at 24-hour intervals if the serum [Na^+] remains lower than 135 mmol/L or the increase in serum [Na^+] has been less than 5 mmol/L in the previous 24 hours. As with conivaptan, it is essential that the serum [Na^+] be measured frequently during the active phase of correction of the hyponatremia at a minimum of every 6 to 8 hours, particularly in patients with risk factors for ODS. Goals and limits for the safe correction of hyponatremia and methods to compensate for overly rapid corrections are the same as described previously for conivaptan (see Fig. 15.20). One additional factor that helps avoid overly rapid correction with tolvaptan is the recommendation that fluid restriction not be used during the active phase of correction, thereby allowing the patient's thirst to compensate for an overly vigorous aquaresis. Common side effects of tolvaptan include dry mouth, thirst, increased urinary frequency, dizziness, nausea, and orthostatic hypotension.[527,528]

Vaptans are not needed for the treatment of hypovolemic hyponatremia because simple volume expansion would be expected to abolish the nonosmotic stimulus to AVP secretion and lead to a prompt aquaresis. Furthermore, inducing increased renal fluid excretion via diuresis or aquaresis can cause or worsen hypotension in these patients. This possibility has resulted in the labeling of these drugs as contraindicated for hypovolemic hyponatremia.[507] Importantly, clinically significant hypotension was not observed in the conivaptan or tolvaptan clinical trials in euvolemic and hypervolemic hyponatremic patients. Although vaptans are not contraindicated with decreased renal function, these agents generally will not be effective if the serum creatinine level is more than 3.0 mg/dL.

The FDA has issued a caution about hepatic injury[530] that was noted in patients who received tolvaptan in a 3-year clinical trial examining the effect of tolvaptan on autosomal dominant polycystic kidney disease, the Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and Its Outcomes (TEMPO) study.[531] An external panel of liver experts found that three cases of reversible jaundice and increased transaminase levels in this trial were probably or highly likely caused by tolvaptan. Additionally, 4.4% of autosomal dominant polycystic kidney disease (ADPKD) patients on tolvaptan (42 of 958) exhibited elevations of alanine aminotransferase (ALT) levels more than three times that of the upper limit of normal (ULN) compared with 1.0% of patients (5 of 484) on placebo.

These findings indicate that tolvaptan has the potential to cause irreversible and potentially fatal liver injury. The doses used in the TEMPO study were up to twice the maximum dose approved for hyponatremia (tolvaptan, 120 mg/day). Also, in clinical trials of tolvaptan at doses approved by the FDA for treatment of clinically significant euvolemic or hypervolemic hyponatremia, liver damage was not reported, including long-term trials longer than 30 days (e.g., SALT-WATER, EVEREST [Efficacy of Vasopressin Antagonism in Heart Failure: Outcome Study with Tolvaptan]).[532,533] Of note, meta-analyses of studies involving vasopressin antagonist use for the treatment of hyponatremia have confirmed increased drug-related adverse events, including rapid sodium level correction, constipation, dry mouth, thirst, and phlebitis in vasopressin antagonist-treated patients, but found no differences in the total number of AEs, discontinuations due to AEs, serious AEs, death, headache, hypotension, nausea, anemia, hypernatremia, urinary tract infection, renal failure, pyrexia, upper gastrointestinal bleeding, diarrhea, vomiting, peripheral edema, and dizziness between the vasopressin antagonist-treated and control groups.[534]

Based largely on the hepatic injury noted in the TEMPO trial, the FDA, on April 30, 2013, recommended that "Samsca [tolvaptan] treatment should be stopped if the patient develops signs of liver disease. Treatment duration should be limited to 30 days or less, and use should be avoided in patients with underlying liver disease, including cirrhosis."[530] The European Medicines Agency (EMA) has approved the use of tolvaptan for SIADH but not for hyponatremia due to heart failure or cirrhosis. Based on the TEMPO trial results, the EMA also issued a warning about the possible occurrence of hepatic injury in patients treated with tolvaptan, but did not recommend any restrictions on the duration of treatment of SIADH patients with tolvaptan.[535]

Accordingly, appropriate caution should be exercised in patients treated with tolvaptan for hyponatremia for extended periods (e.g., >30 days), but this decision should be based on the clinical judgment of the treating physician. Patients who are refractory to or unable to tolerate or obtain other therapies for hyponatremia, and for whom the benefits of tolvaptan treatment outweigh the risks, remain candidates for long-term therapy with tolvaptan. In these patients, liver function tests should be monitored carefully and serially (i.e., every 3 months) and the drug discontinued in the event of significant changes in liver function test results (i.e., twice the ULN of ALT).[510] With rare exceptions, tolvaptan should not be used for patients with underlying liver disease, given the difficulty of attributing causation to any observed deterioration of hepatic function. Such an exception may be hyponatremic patients with end-stage liver disease awaiting imminent liver transplantation who are at little risk of added hepatic injury and will benefit from the correction of hyponatremia prior to surgery to decrease the risk of ODS postoperatively.[536]

An additional barrier to the use of vasopressin antagonists for treatment of hyponatremia is the high cost of the drug. This is true in the United States and European Union, but not in Asian countries. Despite this pronounced geographic disparity, many economic analyses have confirmed the increased economic burden of hyponatremia, which is largely driven by longer hospital and ICU stays.[537,538] An economic analysis of use of tolvaptan compared with fluid restriction has shown favorable cost savings that offset the high cost of tolvaptan, suggesting that selective use of these agents in appropriate inpatients may, in fact, be cost-effective.[539]

UREA

Urea has been described as an alternative oral treatment for SIADH and other hyponatremic disorders. The mode of action is to correct hypoosmolality not only by increasing solute-free water excretion but also by decreasing urinary sodium excretion. Dosages of 15 to 60 g/day are generally effective; the dose can be titrated in increments of 15 g/day at weekly intervals as necessary to achieve normalization of the serum [Na^+]. It is advisable to dissolve the urea in orange juice or some other strongly flavored liquid to camouflage the bitter taste. Even if completely normal water balance is not achieved, it is often possible to allow the patient to maintain a less strict regimen of fluid restriction while receiving urea. The disadvantages associated with the use of urea include poor palatability (although some clinicians believe that this has been exaggerated), the development of azotemia at higher doses, and the unavailability until recently of a convenient or FDA-approved form of the agent. Evidence has suggested that blood urea concentrations may double during treatment,[540] but it is important to remember that this does not represent renal impairment. There is now a product available in the United States (Ure-Na) that has been approved by the FDA as a medical food for the management of euvolemic and hypervolemic hyponatremia.

Reports from retrospective uncontrolled studies have suggested that the use of urea has been effective in treating SIADH in patients with hyponatremia due to subarachnoid hemorrhage and in critical care patients,[541] and case reports have documented success in infants with chronic SIADH[542] and NSIAD.[543] Evidence from a short study in a small cohort of SIADH patients has suggested that urea may have a comparable efficacy to vaptans in reversing hyponatremia due to chronic SIADH.[544] Although these reports suggest that urea might be an acceptable alternative for treatment of chronic hyponatremia, data regarding the efficacy and safety of long-term urea treatment of hyponatremia are lacking at this time.[545]

In patients with hyponatremia caused or exacerbated by low protein ingestion, increasing dietary protein intake may have a similar effect as urea and improve the serum sodium concentration.[293]

FUROSEMIDE AND NACL

The use of furosemide (20 to 40 mg/day), coupled with a high salt intake (e.g., 200 mEq/day, often administered

as salt tablets), which represents an extension of the treatment of acute symptomatic hyponatremia[546] to the chronic management of euvolemic hyponatremia, has also been reported to be successful in select cases.[547] However, the efficacy of this approach to correct symptomatic hyponatremia promptly and within accepted goals limits (see Fig. 15.20) is unknown.

EFFICACY OF DIFFERENT TREATMENTS FOR HYPONATREMIA DUE TO SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION

There have been no adequately powered, randomized controlled trials to compare the efficacy and safety of different treatments used to correct hyponatremia. However, results of a prospective observational study in a large number of hospitalized patients in the United States and European Union have provided useful data about the success rates of different therapies when used as monotherapy in patients with SIADH (Table 15.5).[519,548] In this study, "success" was defined by three different criteria: (1) the least stringent was an increase in serum [Na^+] of at least 5 mmol/L; (2) the next was correction to a serum [Na^+] of 130 mmol/L or higher; and (3) the most stringent was correction to a normal serum [Na^+] of 135 mmol/L or higher. As seen in Table 15.5, only 3% NaCl and tolvaptan had success rates significantly higher than 50% for the least stringent criterion, and only tolvaptan achieved this level for the next most stringent criteria and a significantly higher rate for the most stringent criteria of normalization of the serum [Na^+]. Of particular note, fluid restriction alone, the most frequently prescribed therapy in the Hyponatremia Registry patients, achieved a correction of serum [Na^+] in only 44% of patients treated with this therapy and isotonic saline in only 36% of patients. These data underscore the importance of carefully selecting therapy for individual patients to meet predefined goals for the correction of serum [Na^+].

Table 15.5 Efficacy of Different Treatments Used as Monotherapy for Syndrome of Inappropriate Antidiuretic Hormone Secretion From the Hyponatremia Registry (%)

Treatment	δ [Na^+] ≥ 5 mmol/L	[Na^+] ≥ 130 mmol/L	[Na^+] ≥ 135 mmol/L
No treatment (n = 168)	41	45	20
Fluid restriction (n = 625)	44	29	10
Isotonic saline (n = 384)	36	20	4
Tolvaptan (n = 183)	78	74	40
3% NaCl (n =78)	60	25	13

From Verbalis JG, et al: Diagnosing and treating the syndrome of inappropriate antidiuretic hormone secretion. *Am J Med.* 2016;129:537.e9–537.e23.

HYPONATREMIA TREATMENT GUIDELINES

Although many authors have published recommendations on the treatment of hyponatremia,[503,505,507,511,549–553] no standardized treatment algorithms have yet been universally accepted, and there are still some major differences between the various guidelines and expert recommendations.[554,555] For almost all treatment recommendations, the initial evaluation includes an assessment of the ECF volume status of the patient because treatment recommendations differ for hypovolemic, euvolemic, and hypervolemic hyponatremic patients. Recommendations for hypovolemic and hypervolemic patients have been updated rather recently.[511] Euvolemic patients, mainly including those with SIADH, represent a unique challenge because of the multiplicity of causes and presentations of patients with SIADH. A synthesis of recommendations for the treatment of hyponatremia is illustrated in Fig. 15.21. This algorithm is based primarily on the neurologic symptomatology of hyponatremic patients rather than the serum [Na^+] or the chronicity of the hyponatremia, because the latter is often difficult to ascertain accurately.

LEVELS OF SYMPTOMS

A careful neurologic history and assessment should always be done to identify potential causes for the patient's symptoms other than hyponatremia. However, it will not always be possible to exclude an additive contribution from the hyponatremia to an underlying neurologic condition. In this algorithm, patients are divided into three major groups based on their presenting symptoms (see Table 15.4).

Severe Symptoms

Coma, obtundation, seizures, respiratory distress or arrest, and unexplained vomiting usually imply a more acute onset or worsening of hyponatremia that requires immediate active treatment. Therapies that will quickly raise the serum [Na^+] are necessary to reduce cerebral edema and decrease the risk of potentially fatal brain herniation.

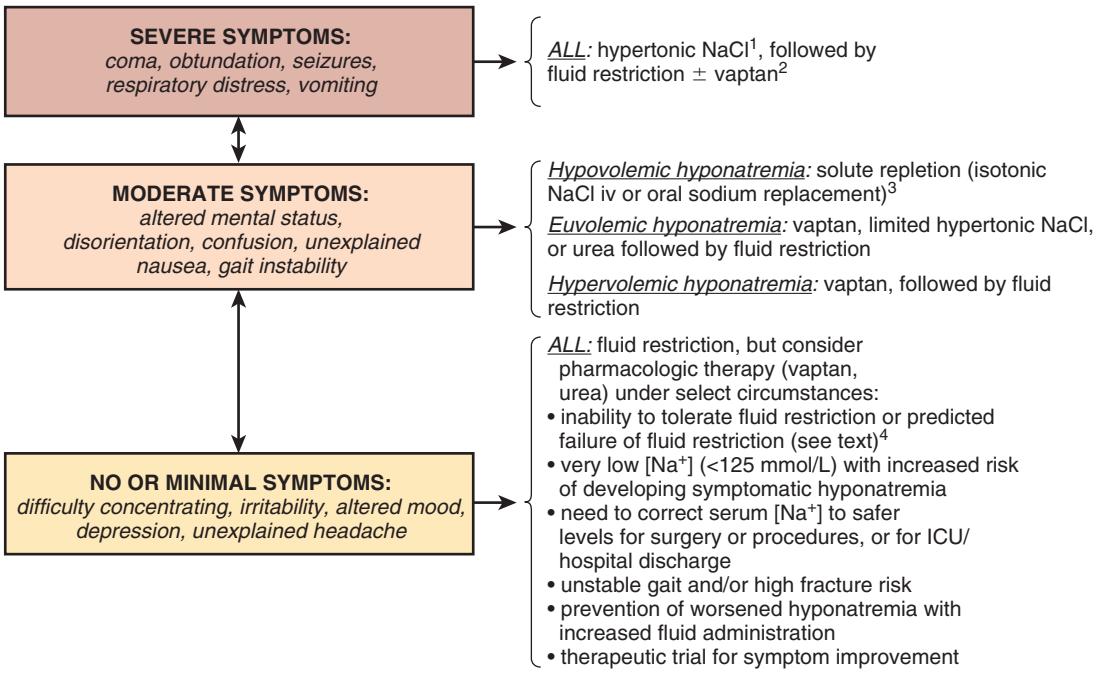
Moderate Symptoms

Altered mental status, disorientation, confusion, unexplained nausea, gait instability, and falls generally indicate some degree of brain volume regulation and absence of clinically significant cerebral edema. These symptoms can be chronic or acute, but allow more time to elaborate a deliberate approach to the choice of therapies.

Mild or Absent Symptoms

Minimal symptoms, such as difficulty concentrating, irritability, altered mood, depression, or unexplained headache, or a virtual absence of discernible symptoms, indicate that the patient may have chronic or slowly evolving hyponatremia. These symptoms necessitate a cautious approach, especially when patients have underlying comorbidities, to prevent worsening of the hyponatremia and overly rapid correction with the production of ODS.

Patients with severe neurologic symptoms should be treated with hypertonic (3%) NaCl as first-line therapy, followed after 24 to 48 hours by fluid restriction and/or vaptan therapy. Because overly rapid correction of the serum [Na^+] occurs in more than 10% of patients treated with hypertonic NaCl,[556] such patients are at risk for ODS unless carefully monitored.



1. Some authors recommend simultaneous treatment with desmopressin to limit speed of correction.
2. No active therapy should be started within 24 hours of hypertonic saline to decrease the risk of overly rapid correction of [Na^+] and risk of ODS.
3. With isotonic NaCl infusion, serum [Na^+] must be followed closely to prevent overly rapid correction and risk of ODS due to secondary water diuresis.

Fig. 15.21 Algorithm for the treatment of patients with euvolemic hyponatremia based on their presenting symptoms. The *arrows* between the symptom boxes indicate movement of patients between different symptom levels. *ALL,* All types of hypotonic hyponatremia; *ICU,* intensive care unit; *iv,* intravenous; *ODS,* osmotic demyelination syndrome. (Modified from Verbalis JG: Emergency management of acute and chronic hyponatremia. In Matfin G, ed. *Endocrine and Metabolic Emergencies.* Washington, DC: Endocrine Press; 2014:359.)

For this reason, some authors have proposed simultaneous treatment with desmopressin to reduce the rate of correction to only that produced by the hypertonic NaCl infusion itself.[557,558] Whether sufficient clinical data eventually prove that this approach is effective and safe in larger numbers of patients remains to be determined.[559,560] Only one case of ODS has been reported in a patient receiving vaptan monotherapy,[561] and two abstracts have reported ODS when vaptans were used directly following hypertonic saline administration within the same 24-hour period.[511] Consequently, no additional active hyponatremia therapy should be administered until at least 24 hours following successful increases in the serum [Na^+] using hypertonic NaCl.

The choice of treatment for patients with moderate symptoms will depend on their ECF volume status (see Fig. 15.21). Hypovolemic patients should be treated with solute repletion via isotonic NaCl infusion or oral sodium replacement.[511] Euvolemic patients, typically with SIADH, will benefit from vaptan therapy, limited hypertonic saline administration or, in some cases, urea, where available. This can be followed by fluid restriction or long-term vaptan therapy when the cause of the SIADH is expected to be chronic.[511] In hypervolemic patients with heart failure, vaptans are usually the best choice because fluid restriction is rarely successful in this group,[562] saline administration can cause fluid retention with increased edema, and urea can lead to ammonia buildup in the gastrointestinal tract if hepatic function is impaired.

Although moderate neurologic symptoms can indicate that a patient is in an early stage of acute hyponatremia, they more often indicate a chronically hyponatremic state with sufficient brain volume adaptation to prevent marked symptomatology from cerebral edema. Most patients with moderate hyponatremic symptoms have a more chronic form of hyponatremia, so guidelines for goals and limits of correction should be followed closely (see Fig. 15.20), and close monitoring of these patients in a hospital setting is warranted until their symptoms improve or stabilize.

Patients with no or minimal symptoms should be managed initially with fluid restriction, although treatment with pharmacologic therapy, such as vaptans or urea, may be appropriate for a wide range of specific clinical conditions (see Fig. 15.21). Foremost of these is a failure to improve the serum [Na^+], despite reasonable attempts at fluid restriction, or the presence of clinical characteristics associated with poor responses to fluid restriction (see Box 15.5).

A special case is when spontaneous correction of hyponatremia occurs at an undesirably rapid rate because of the onset of water diuresis. This can occur following cessation of desmopressin therapy in a patient who has become hyponatremic, replacement of glucocorticoids in a patient with adrenal insufficiency, replacement of solutes in a patient with hypovolemia, cessation of thiazides in diuretic-induced hyponatremia, or spontaneous resolution of transient SIADH. Brain damage from ODS can clearly ensue in this setting if

the preceding period of hyponatremia has been long enough (usually, ≥48 hours) to allow brain volume regulation to occur. If the correction parameters discussed earlier have been exceeded, and the correction is proceeding more rapidly than planned (usually because of continued excretion of hypotonic urine), the pathologic events leading to demyelination can be reversed by the administration of hypotonic fluids, with or without desmopressin. The efficacy of this approach has been suggested from animal studies[563] and case reports in humans,[505,564] even when patients are overtly symptomatic.[565] However, relowering the serum [Na^+] after an initial, overly rapid correction is only strongly recommended for patients at high risk of ODS (see Box 15.5). It is considered optional for patients with a low to moderate risk of ODS and unnecessary for patients with acute water intoxication (see Fig. 15.20).

Although this classification is based on presenting symptoms at the time of initial evaluation, it should be remembered that in some cases, patients initially exhibit more moderate symptoms because they are in the early stages of hyponatremia. In addition, some patients with minimal symptoms are prone to develop more symptomatic hyponatremia during periods of increased fluid ingestion. In support of this, approximately 70% of 31 patients presenting to a university hospital with symptomatic hyponatremia and a mean serum [Na^+] of 119 mmol/L had preexisting asymptomatic hyponatremia as the most common risk factor identified.[566] Consequently, therapy for chronic hyponatremia should also be considered to prevent progression from a lower to higher level of symptomatic hyponatremia, particularly in patients with a past history of repeated presentations for symptomatic hyponatremia.

MONITORING SERUM SODIUM CONCENTRATION IN HYPONATREMIC PATIENTS

The frequency of serum [Na^+] monitoring is dependent on the severity of the hyponatremia and therapy chosen. In all hyponatremic patients, neurologic symptomatology should be carefully assessed very early in the diagnostic evaluation to assess the symptomatic severity of the hyponatremia and determine whether the patient requires more urgent therapy. All patients undergoing active treatment with hypertonic saline for symptomatic hyponatremia should have frequent monitoring of their serum [Na^+] and ECF volume status (every 1–4 hours) to ensure that the serum [Na^+] does not exceed the limits of safe correction during the active phase of correction,[507] because overly rapid correction of serum sodium will increase the risk of ODS.[567] Patients treated with vaptans for mild to moderate symptoms should have their serum [Na^+] monitored every 6 to 8 hours during the active phase of correction, which will generally be the first 24 to 48 hours of therapy. Active treatment with hypertonic saline or vaptans should be stopped when the patient's symptoms are no longer present, a safe serum [Na^+] has been achieved (usually, >120 mmol/L), or the rate of correction has reached maximum limits of 12 mmol/L within 24 hours or 18 mmol/L within 48 hours[507,512] or 8 mmol/L over any 24-hour period in patients at high risk of ODS (see Box 15.5). In patients with a stable level of serum [Na^+] treated with fluid restriction or therapy other than hypertonic saline, measurement of the serum [Na^+] daily is generally sufficient because levels will not change that quickly in the absence of active therapy or large changes in fluid intake or administration.

LONG-TERM TREATMENT OF CHRONIC HYPONATREMIA

Some patients will benefit from continued treatment of hyponatremia following discharge from the hospital. In many cases, this will consist of continued fluid restriction. However, as discussed, long-term compliance with this therapy is poor because of the increased thirst that occurs with more severe degrees of fluid restriction. Thus, for select patients who have responded to tolvaptan in the hospital, consideration should be given to continuing the treatment as an outpatient after discharge. In patients with established chronic hyponatremia, tolvaptan has shown to be effective for maintaining a normal [Na^+] for as long as 3 years of continued daily therapy.[568] However, many patients with inpatient hyponatremia have a transient form of SIADH, without the need for long-term therapy. In the conivaptan open-label study, approximately 70% of patients treated as an inpatient for 4 days had normal serum [Na^+] concentrations 7 and 30 days after the cessation of the vaptan therapy in the absence of chronic therapy for hyponatremia. Selection of which patients with inpatient hyponatremia are candidates for long-term therapy should be based on the cause of the SIADH. Fig. 15.22 shows estimates of the relative probability that patients with different causes of SIADH will have persistent hyponatremia that may benefit from long-term treatment with tolvaptan following hospital discharge. Nonetheless, for any individual patient, this simply represents an estimate of the likelihood of requiring long-term therapy. In all cases, consideration should be given to a trial of stopping the drug 2 to 4 weeks after discharge to determine if hyponatremia is still present. A reasonable period of tolvaptan cessation to evaluate the presence of continued SIADH is 7 days, because this period was found to be sufficient for demonstration of a recurrence of hyponatremia in the tolvaptan SALT trials.[568,569] The serum [Na^+] should be monitored every 2 to 3 days following the cessation of tolvaptan so that the drug can be resumed as quickly as possible in patients with recurrent hyponatremia; the longer the patient is hyponatremic, the greater the risk of subsequent ODS with overly rapid correction of the low serum [Na^+].

Findings of hepatotoxicity in a small number of patients on high doses of tolvaptan in a clinical trial of polycystic kidney disease have led to a recent FDA recommendation that tolvaptan not be used for longer than 30 days.[530] This decision should be based on a risk-benefit analysis individualized for specific patients; if tolvaptan is used for longer than 30 days, liver function should be assessed at regular intervals (e.g., every 3 months).[511]

FUTURE OF HYPONATREMIA TREATMENT

Despite the many advances made in understanding the manifestations and consequences of hyponatremia, and the availability of effective pharmacologic therapies for the treatment of hyponatremia, it is obvious that we do not yet have a uniformly accepted consensus on how and when this disorder should be treated. In particular, indications for the use of vasopressin receptor antagonists by regulatory agencies

Etiology of SIADH	Likely duration of SIADH*	Relative risk of chronic SIADH
Tumors producing vasopressin ectopically (small-cell lung carcinoma, head and neck carcinoma)	Indefinite	High
Drug-induced, with continuation of offending agent (carbamazepine, SSRI)	Duration of drug therapy	
Brain tumors	Indefinite	
Idiopathic (senile)	Indefinite	
Subarachnoid hemorrhage	1–4 weeks	
Stroke	1–2 weeks	
Inflammatory brain lesions	Dependent on response to therapy	Medium
Respiratory failure (chronic obstructive lung disease)	Dependent on response to therapy	
HIV infection	Dependent on response to therapy	
Traumatic brain injury	2–7 days to indefinite	
Drug-induced, with cessation of offending agent	Duration of drug therapy	
Pneumonia	2–5 days	
Nausea, pain, prolonged exercise	Variable, depending on cause	
Postoperative hyponatremia	2–3 days postoperatively	Low
*Time frames are based on clinical experience.		

Fig. 15.22 Estimated probability of need for long-term treatment of SIADH, depending on underlying cause.

differ substantially worldwide, and various treatment guidelines published to date also differ substantially in regard to appropriate hyponatremia management.[511,570,571] There are many reasons for this failure to achieve consensus and, until this occurs via further clinical research studies, physicians must recognize the primary role that clinical judgment must continue to play in making decisions about the management of hyponatremia in individual patients. Their recommendations should take into account appropriate appraisals of evidence by authoritative experts in the field, the decisions of regulatory agencies based on critical reviews of the efficacy and safety data for approved treatments for hyponatremia and, most importantly, the specialized needs of individual hyponatremic patients.[571]

In the meantime, clinical trials using vasopressin receptor antagonists will enable investigators to answer some long-standing questions about the role of vasopressin V_2 receptor activation in various physiologic conditions (e.g., regulation of sweat production),[572] pathophysiologic states (e.g., hyponatremic patients without measurable vasopressin levels), and especially the potential reversibility of long-term adverse effects associated with hyponatremia, such as falls, bone loss, and fractures. This may, in part, account for the increased mortality and morbidity in hyponatremic patients across multiple different comorbidities, as well as in older, community-dwelling subjects without known underlying diseases.

Complete reference list available at ExpertConsult.com.

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