CHAPTER

3 Renal Circulation and Glomerular Hemodynamics

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The capability of the kidneys to achieve their sophisticated homeostatic function is optimized by an intricate microvascular system that adjusts vascular resistance to maintain an appropriate control of the intracapillary and interstitial forces that govern renal blood flow in the cortex and medulla as well as the glomerular filtration rate. A combination of intrinsic and extrinsic regulatory mechanisms are responsible for controlling the one-fifth of the cardiac output that circulates through the kidneys. Essentially all the renal blood flow (RBF) traverses through the glomerular capillaries, where about 20% of the plasma is filtered. The complex glomerular filtration apparatus is truly unique in having both a very high hydraulic conductivity and a remarkably low permeability to plasma proteins. One major function of the renal vasculature is to regulate the intraglomerular forces so that an adequate, yet not excessive, volume is filtered into the urinary tubules. In this chapter, we discuss the characteristics of the filtering and the reabsorbing microcirculatory structures in the normal kidney. Particular emphasis is placed on the dynamic interactions among the intrarenal paracrine and extrarenal homeostatic mechanisms that participate in regulating these processes. To allow a better appreciation of basic mechanisms, some structural relationships and fundamental concepts related to vascular smooth muscle, endothelial cells, and other components of the renal microvascular network are discussed. A detailed discussion of the anatomic features of the kidney is provided in Chapter 1.

THE MAGNITUDE OF RENAL BLOOD FLOW AND GLOMERULAR FILTRATION RATE

The multiple intrarenal parallel arteriolar pathways provide the kidneys with a very low vascular resistance. They normally receive about 20% of the cardiac output. This amounts to a blood flow of 1,000 to 1,200 mL per minute in a 70- to 75-kg person. RBF is even more impressive when considered per unit of kidney weight, because the kidneys account for only 0.5% of the total body weight, or about 300 g. Thus, as shown in Table 3.1, blood flow per gram of kidney weight is about 4 mL per minute, which is 5 to 50 times greater than the flow through other organs and circulatory beds. Based on a total of 1 million glomeruli in each kidney or a glomerular density of 7,000 glomeruli per gram, the average blood flow and glomerular filtration rate (GFR) per glomerulus is 570 nL per minute and 62 nL per minute, respectively. This large flow, coupled with the maintenance of a high hydrostatic pressure in glomerular capillaries, allows the filtration of about 20% of the plasma, which amounts to an average GFR of 120 mL per minute, or 170 L per day.[1,2]

The extraordinarily high RBF is in marked excess of that simply required to provide the renal parenchyma with adequate supplies of oxygen and nutrients. For this reason, it is generally recognized that RBF is regulated primarily to maintain the glomerular and peritubular intrarenal hemodynamic environments at levels compatible with the optimum delivery of filtrate to the nephrons and appropriate reabsorption of fluid back into the systemic vasculature.

The Relationship of Renal Blood Flow to Oxygen Consumption

Although oxygen (O_2) use is not a major determinant of RBF, O_2 consumption by the kidneys is still quite high because of the very high metabolic activity of the tubules. Over 99% of the filtered fluid, electrolytes, and essential organic nutrients are normally reabsorbed by the tubules and returned to the circulation via the peritubular capillaries. The tubular reabsorptive processes depend on the integrity of the epithelial transport systems, in particular the energy requiring Na-K-ATPase. Such tubular enzyme systems account for the majority of the O_2 consumption by the kidneys.

RBF is about 400 mL/min/100 g of tissue, and the arteriovenous O_2 difference is relatively low, only 1 to 2 mL per deciliter of blood. Thus, O_2 consumption by the kidney is about 8 mL of O_2 per minute or 400 μm of O_2/min/100 g, which amounts to 6% to 8% of the whole body O_2 consumption. This level of O_2 use is relatively constant and is not reduced by moderate hypoxemia. Under physiologic conditions, there is a consistent relationship between RBF

3.1 Renal Hemodynamic Function in Humans

	Total	Per Kilogram Body Weight	Per Gram Kidney Weight
Renal blood flow	1,200 mL/min	17 mL/min	4 mL/min
Renal plasma flow	670 mL/min	9.6 mL/min	2.2 mL/min
Glomerular filtration rate	130 mL/min	1.9 mL/min	0.45 mL/min
Number of glomeruli	2 million	28,500	7,000
Oxygen consumption	1,200 μMO_2/min	17 μMO_2/min	4 μMO_2/min

and renal O_2 consumption. However, this relationship is a consequence of the associated changes in GFR and filtered sodium load, reflecting a direct causal relationship between tubular sodium reabsorption and O_2 consumption, as shown in Figure 3.1. The rate of actively transported sodium appears to be the primary determinant of the rate of O_2 consumption. About 20% of the consumption, or approximately 100 μm of O_2/min/100 g of kidney, is used for basal metabolic purposes and continues even in the absence of filtration. Above this basal rate, there is a linear relationship. Approximately 27 to 35 mEq of sodium is reabsorbed per millimole of O_2 consumed, depending on the contribution of passive transport via paracellular pathways, which may be about 40%. In contrast to other organs, the kidneys do not have a hyperemic response to hypoxia, making them more susceptible to hypoxemia.[3,4]

The balance of O_2 consumption for sodium reabsorption and O_2 delivery is reflected by the tissue pressure of O_2 (pO_2). A cortical–medullary gradient of oxygenation exists in the kidney. In the superficial cortex, pO_2 in efferent arterioles is 40 to 45 mm Hg, with values of 40 mm Hg recorded in other cortical structures (proximal tubule, distal tubule, superficial cortical tissue), and 30 mm Hg in the deep cortex. The fact that pO_2 in the renal vein exceeds that of any site in the cortex indicates precapillary shunting of O_2 from artery to vein. Renal medullary pO_2 is about 20 mm Hg, with cells functioning in a climate of constant relative hypoxia. Recent mathematical analysis indicates that arteriovenous O_2 shunting in the cortex is substantial. The shunting contributes to the stabilization of tissue pO_2 levels. Cortical ischemia may exacerbate medullary hypoxia even when medullary perfusion is maintained.[5–8]

In the renal medullary microcirculation, the net amount of O_2 reabsorbed from vasa recta into the interstitium is significantly lower than estimated medullary O_2 requirements based on active sodium reabsorption. Low inner medullary pO_2 results from the countercurrent arrangement of vasa recta and high vascular permeability to O_2, as well as high metabolic needs. Diffusional shunting of O_2 between descending and ascending vasa recta explains why a 20-mm Hg decrease in initial pO_2 at the corticomedullary border only leads to a small drop in pO_2 at the papillary tip (<2 mm Hg with baseline parameter values). In contrast, small decreases in medullary blood flow, hematocrit, and O_2 consumption by tubules markedly reduce interstitial pO_2. Without erythrocytes, papillary tip pO_2 cannot be maintained above 10 mm Hg, even when O_2 consumption is zero. An increase in medullary blood flow during water diuresis improves medullary O_2 delivery.

The renal medulla normally functions in an hypoxic environment. Tissue hypoxia impacts on O_2-regulated genes and leads to the renal production of adrenomedullin and erythropoietin. Hypoxia-inducible factor-1alpha (HIF-1α) is a transcription factor that regulates the O_2-dependent expression of many genes. This transcription factor may contribute to gene expression in renal medullary cells that

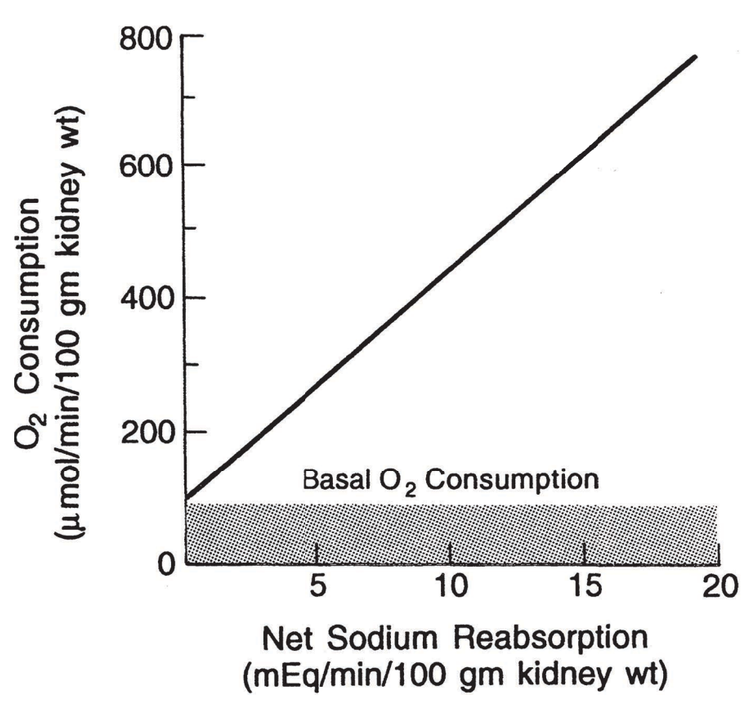

FIGURE 3.1 The relationship between tubular sodium reabsorption and oxygen consumption by the kidney. The primary determinant of oxygen consumption above basal levels is the rate of active sodium transport.

function normally under hypoxic conditions. In this regard, the loop diuretic furosemide markedly increases renal medullary pO_2 levels (20 to 50 mm Hg) in association with the inhibition of reabsorption along the ascending limb of Henle loop and a reduction in HIF-1α.[9,10]

The efficiency of coupling between tubular transport and O_2 consumption is modified by paracrine/autocrine factors. Nitric oxide (NO) normally suppresses O_2 consumption by epithelial mitochondria. The inhibition of NO synthesis increases O_2 extraction and O_2 consumption and reduces the efficiency of sodium transport. The regulation of renal O_2 consumption by NO may become impaired during oxidative stress when superoxide production is excessive. Oxidative stress and decreased availability/activity of NO can lead to reduced intrarenal pO_2 due to enhanced O_2 usage relative to tubular sodium transport. A low sodium intake leads to increased renal medullary oxygenation.[11]

Endothelial-dependent NO plays a role in the regulation of renal O_2 consumption in normal kidneys. Baseline cortical O_2 consumption is about 600 nmol per minute per gram of tissue. Stimulation of NO production by bradykinin or administration of a NO donor reduces O_2 consumption 25% to 30% in the renal cortex and 30% in the medulla. Superoxide scavenging of NO attenuates the stimulatory effect of bradykinin or NO donors.[3,5,8]

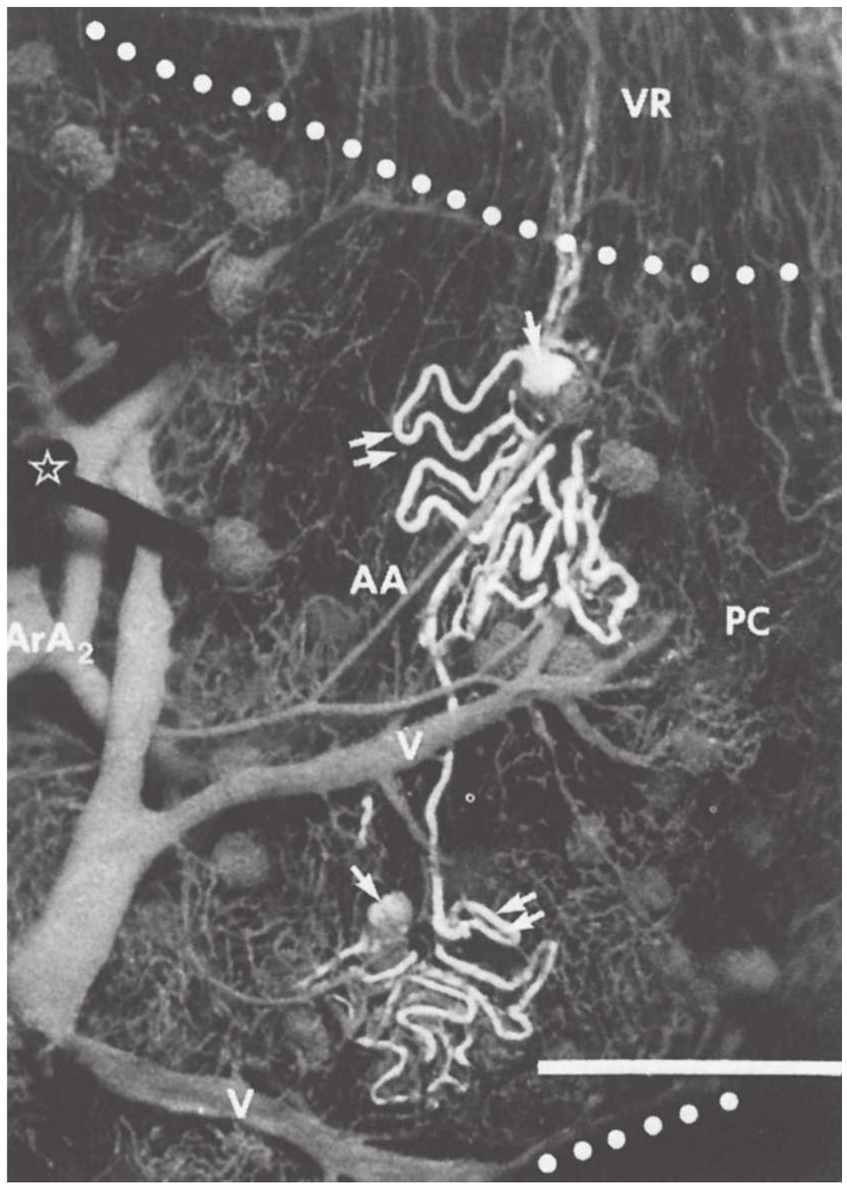

FIGURE 3.2 Renal microcirculation showing branching of afferent arterioles from arcuate arteries, glomerular capillary tufts, efferent arterioles, peritubular capillaries, some initial portions of the vasa recta, and the venous system. The vessels are filled with dark elastic polymer (Microfill), and two tubules are filled with light polymer, showing the Bowman capsule (*single arrow*) and the proximal tubules (*double arrow*) and parts of loop of Henle. *AA*, afferent arteriole; *ArA*, arcuate artery; *PC*, peritubular capillary; *V*, venule; *VR*, vasa recta. (Courtesy of Dr. Daniel Casellas.)

CHARACTERISTICS OF THE CONTRACTILE PROCESS

Structural–Functional Aspects

There are important interactions among various cell types in the microvasculature that determine the caliber of the small diameter resistance vessels. The complex vasculature of the kidney (Fig. 3.2) allows the fine regulation of the intrarenal hemodynamic environment. Smooth muscle cells surround all vascular structures from the main renal artery to the individual afferent and efferent arterioles. The preglomerular vasculature also has extensive innervation and responds to renal nerve stimulation and many different hormonal, paracrine, and physical stimuli. Nevertheless, most of the fine control of preglomerular resistance occurs in the small diameter afferent arterioles. The afferent arterioles vary in length and in the angle at which they branch from the interlobular arteries; those in juxtamedullary portions branch at a much sharper angle. In addition, smooth muscle cells of the afferent arterioles are modified as the vessels approach the glomerulus. The proximal portions of afferent arterioles possess typical elongated smooth muscle cells, whereas cells closer to the glomerulus are more rounded and many possess renin-containing granules.[1,2]

As is shown in Figure 3.3, the magnitude of the hydrostatic pressure drop along the arterial tree is relatively small up to the terminal segments of the afferent arterioles. About 70% of the preglomerular pressure drop occurs in the terminal portion of the afferent arteriole. In rodents, the arteries and the larger arterioles leading to the superficial nephrons contribute more to this pressure drop. Overall, the pressure drop up to the glomerular capillary tuft is much lower than in other vascular beds. This allows for high hydrostatic pressure in the glomerular capillaries, which is much greater than the plasma colloid osmotic pressure and is thus responsible for the ultrafiltration of fluid into the Bowman space.[1]

The terminal portion of an afferent arteriole contains modified granular epithelioid cells that form part of the juxtaglomerular apparatus. The granules contain renin and other components of the renin–angiotensin system; the extent of granulation varies inversely with sodium intake. There is a reciprocal relationship between the amount of renin and actin, and the granular cells may have reduced contractile capability. As is shown in Figure 3.4, the juxtaglomerular granular cells are adjacent to the tubular macula densa segment at the end of the ascending loop of Henle, and they are associated with the nongranular extraglomerular mesangial cells between the afferent and efferent arterioles. The appearance of the macula densa cells with large nuclei along with their close apposition to the glomerular vessels provide the

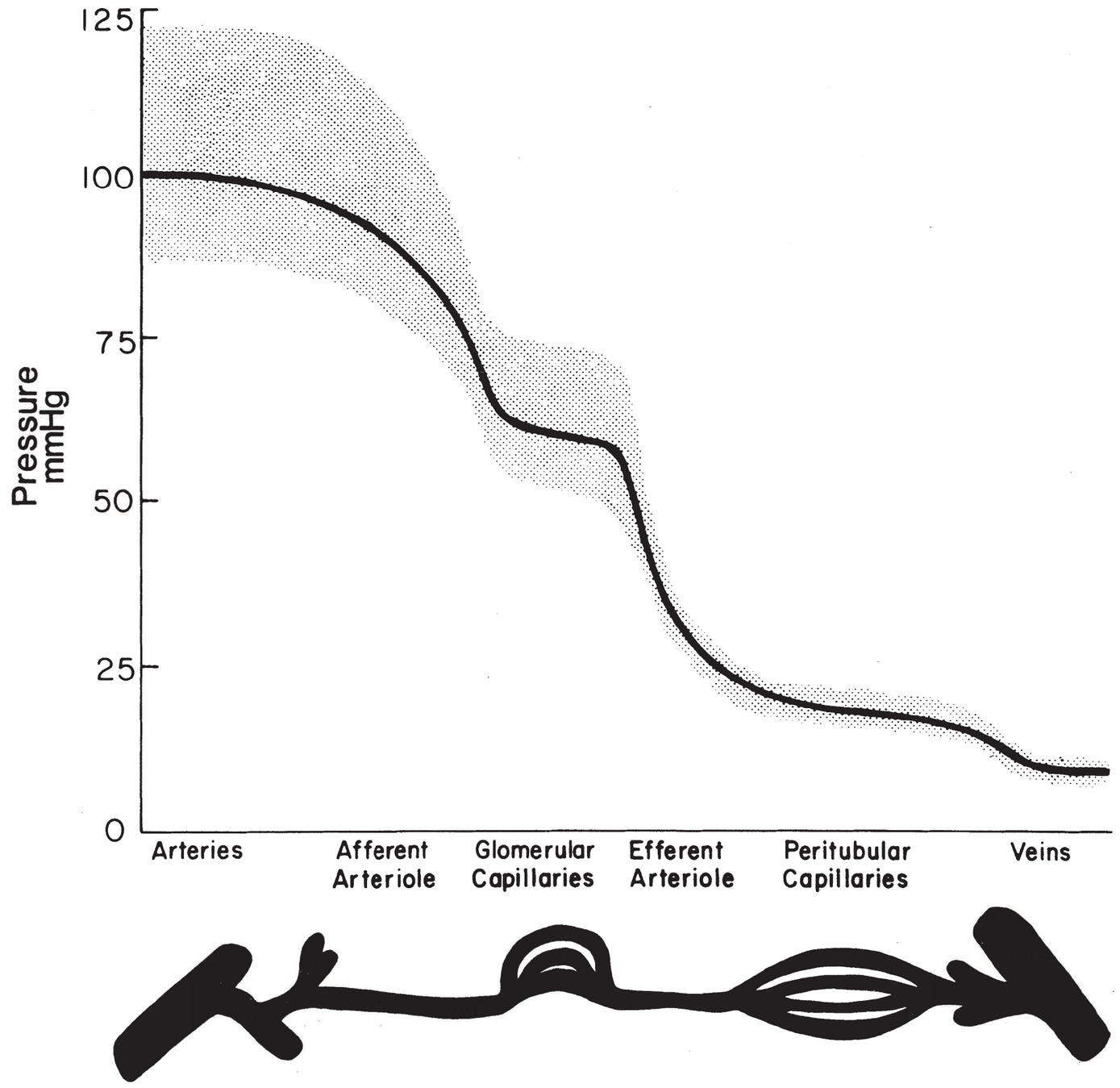

FIGURE 3.3 A representative pressure profile along the renal microvasculature in a normal kidney. The segments are depicted at the bottom of the graph, and the range of ideal pulse pressures is represented by the stippled area.

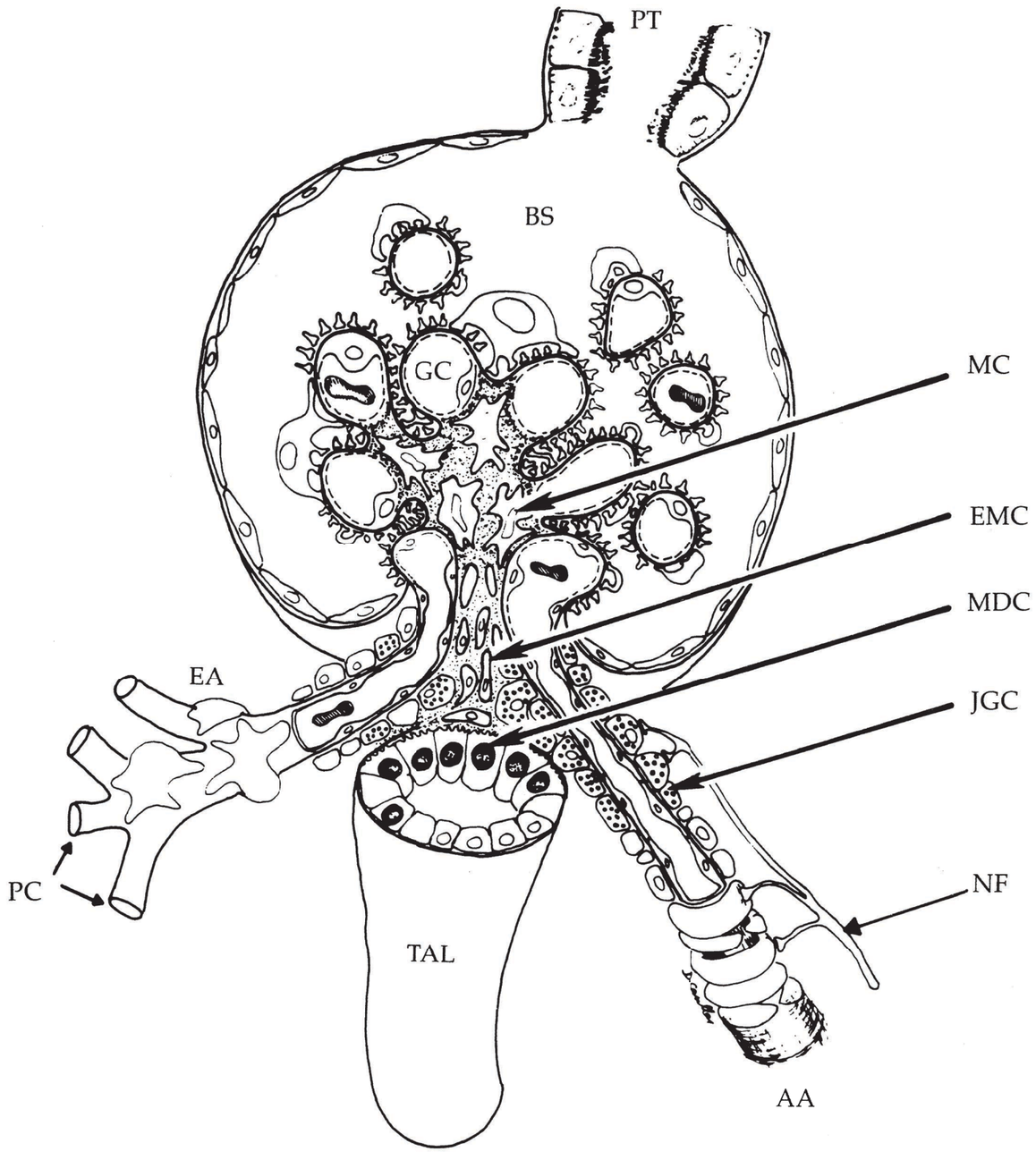

FIGURE 3.4 A drawing of the glomerulus and the juxtaglomerular complex consisting of the afferent arteriole (*AA*) with the granular cells of the juxtaglomerular apparatus (*JGC*), the extraglomerular mesangial cells (*EMC*), the macula densa (*MDC*) segment of the ascending loop of Henle, and the efferent arteriole (*EA*). Also shown are the thick ascending limb (*TAL*), the proximal tubule (*PT*), the Bowman space (*BS*), glomerular capillaries (*GC*), peritubular capillaries (*PC*), mesangial cells (*MC*), and nerve fibers (*NF*). (Drawing by Dr. Daniel Casellas.)

morphologic basis for the influence of alterations in flow or composition of the tubular fluid to generate signals that are transmitted to the afferent arteriole or juxtaglomerular cells to control vascular tone and renin release.[12–14]

As an afferent arteriole approaches a glomerulus, the muscle cells surrounding the vessel intermingle with extraglomerular and intraglomerular mesangial cells. As it enters the glomerulus, the arteriole expands into a manifold lined by endothelial cells, which, in turn, gives rise to a series of glomerular capillary loops. The loops subdivide further into a branching system of exchange channels. Finally, the channels coalesce into a small number of terminal capillaries, which join to form the efferent arteriole. Greater structural detail regarding the glomerular capillaries subserving the filtration process is provided in Chapter 1.

Efferent arterioles originate deep within the glomeruli and vary with regard to length, diameter, and density of smooth muscle cells. In the outer cortex, these vessels are relatively short, have a smaller diameter, and have a less well-developed muscular wall than efferent arterioles in deeper cortical regions. The smooth muscle cells of the superficial efferent arterioles resemble pericytes that often extend onto the peritubular capillaries. In the midcortex, the efferent arterioles are usually longer and have a greater degree of smooth muscle development. In the deeper portion of the cortex, the efferent arterioles are more variable in length. Some efferent arterioles of juxtamedullary nephrons give rise to typical cortical peritubular capillary systems, whereas others are much longer and descend toward the medulla. These two distinct capillary networks subserve the reabsorptive functions of the cortex and medulla, respectively, and may be subject to independent regulation. At the corticomedullary border, the efferent arterioles break up into vascular bundles that branch into numerous descending vasa recta. Vasa recta branch to form a capillary plexus at each level within the medulla. There are three distinct capillary plexuses, with the densest found in the inner stripe of the outer medulla. The ascending vasa recta are morphologically distinct from the descending vasa recta and ascend within vascular bundles to drain into the arcuate veins. The ascending vasa recta are more numerous and have a highly fenestrated, thin endothelium, whereas the descending vasa recta have a continuous thick endothelium. These anatomic differences suggest that the ascending vasa recta have a much greater permeability to macromolecules than the descending vasa recta.[1,2,15,16]

Each glomerular resistance segment contributes to the regulation of glomerular blood flow and pressure in a unique manner because the glomerular capillary is "nested" between the afferent and efferent arterioles. Although a more quantitative analysis of their respective roles is presented in a following section, it should be appreciated that alterations in preglomerular resistance produce changes in glomerular blood flow, pressure, and GFR, which are directionally similar. In contrast, selective changes in efferent arteriolar resistance cause more complex GFR responses because glomerular pressure and blood flow change in opposite directions. The maintenance of appropriate efferent arteriolar tone serves to keep glomerular capillary pressure sufficiently high to provide an adequate hydrostatic pressure for filtration. The efferent arterioles also are responsible for the marked decrease in pressure at the peritubular capillaries, which allows the reabsorptive force of the plasma colloid osmotic pressure to predominate.[1]

There are important regional differences in the circulation within the kidney, which have considerable functional significance. The relative distribution of glomerular and postglomerular blood flow is depicted in Figure 3.5. Glomerular blood flow is proportional to the size of the glomeruli. The larger deep juxtamedullary nephrons have higher flows than the superficial or midcortical glomeruli. These juxtamedullary glomeruli give rise to muscular efferent arterioles that descend toward the medulla and branch into the vasa recta bundles, which are intimately associated with the surrounding concentric rings of loops of Henle and collecting ducts. With regard to the postglomerular blood flow, about 75% to 85% of the total RBF is distributed to the peritubular capillaries in the cortex, whereas 15% to 25% goes to the medullary region. Blood flow throughout the cortex is much higher than in the medulla and is higher in the outer cortex than in the inner cortex. Overall cortical blood flow averages 4 to 6 mL/min/g of tissue. Medullary blood flow ranges from 2.0 to 3.5 mL/min/g in the outer medulla to much lower values of approximately 0.2 to 1.0 mL/min/g of tissue in the inner medulla and papilla. Regional mean transit times of intravascular indicators are 1 to 3 seconds for the cortex,

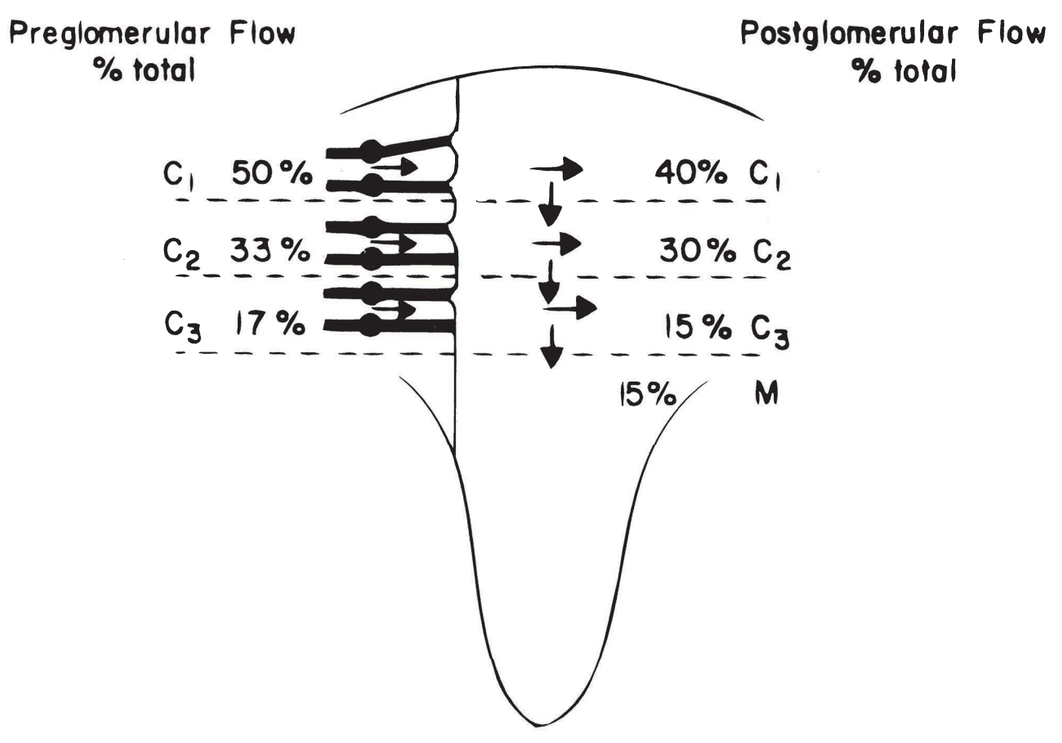

FIGURE 3.5 The distribution of glomerular and postglomerular flow and of cortical (C_1, C_2, C_3) and medullary (M) blood flow. The distribution of glomerular flow is expressed as a percentage of total blood flow; preglomerular flow is presented on the left, and postglomerular flow on the right. The deep cortical flow is subdivided to account for medullary flow distribution. As noted from the *arrows*, there is a general shift of the postglomerular blood flow toward deeper areas.

4 to 6 seconds for the outer medulla, and 10 to 30 seconds for the inner medulla.[15,17–19].

Receptor Activation and Intracellular Signaling

Contractile responses at various sites along the vascular network have different functional characteristics, depending on the expression of receptor populations and/or activation mechanisms. The actions of circulating hormones and neural stimuli combined with local paracrine factors from endothelial and epithelial cells are expressed through different effector mechanisms to provide a highly integrated regulation of the renal microcirculation and the interstitial environment. Many vasoactive agents interact with membrane receptors on the vascular smooth muscle and the endothelial and mesangial cells.

Plasma membrane surface receptors can be subdivided into three main groups in which the receptor is coupled to a guanine nucleotide binding protein (Gα protein), regulates enzyme activity, or serves as part of an ion channel. Examples of the latter two groups are the atrial natriuretic peptide (ANP) receptor, guanylate cyclase, in vascular smooth muscle, and the nicotinic-acetylcholine receptor that directly activates a cation channel at the neuromuscular junction. Almost all known vasoactive agents affect vasomotor tone via receptor coupling to G proteins. G protein-coupled receptors (GPCR) share several common structural features with seven transmembrane domains with three extracellular and three intracellular loops. The extracellular loops act in concert with the transmembrane domains to bind the agonist. The intracellular loops function to activate a G protein. G proteins are heterotrimeric proteins consisting of α, β, and γ subunits. The Gα subunit is unique for each receptor and is responsible for generating a specific intracellular signal. The β and γ subunits share a high degree of homology among G proteins; together they function to modulate the ability of the α subunit to generate the signal.

G proteins undergo a conformational change following agonist binding to a membrane receptor, which in turn enables guanosine triphosphate (GTP) to replace guanosine diphosphate (GDP) on the α subunit. The Gα-GTP complex then dissociates from the β and γ subunits and interacts with an effector such as an enzyme or a channel. The intrinsic GTPase activity of the Gα subunit then hydrolyzes GTP to GDP, and the Gα-GDP complex reassociates with the β and γ subunits, which terminates the response. G proteins linked to adenylate cyclase are classified as $G\alpha_s$ or $G\alpha_i$, depending on whether they stimulate or inhibit adenylate cyclase and cyclic adenosine monophosphate (cAMP) generation. $G\alpha_{q11/12}$ activate membrane-bound phospholipase C (PLC) or activate membrane Ca^{2+} channels, or both. An example of multiple effects an agonist can produce depending on receptor coupling to different G proteins (e.g., $G\alpha q_{11/12}$) is provided by norepinephrine. The binding of norepinephrine to an α_2-adrenoceptor inhibits adenylate cyclase, reduces the formation of cAMP, and attenuates activity of protein kinase A (PKA), whereas binding to a β_1- or β_2-adrenoceptor activates adenylate cyclase to increase cAMP/PKA signaling. Norepinephrine also binds to α_1-adrenoceptors and activates a $G\alpha_q$ protein, which is coupled to PLC, leading to the formation of inositol triphosphate (IP_3) and the release of Ca^{2+} from the sarcoplasmic reticulum. α_1-Adrenoceptors also stimulate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to increase superoxide oxide production and adenosine diphosphate (ADP) ribosyl cyclase generation of cyclic ADP ribose that sensitizes ryanodine receptors to release Ca^{2+} from sarcoplasmic reticular stores.[20–24]

GPCRs and/or their immediate signaling partners (G proteins) are concentrated in caveolae, flask-shaped plasma membrane invaginations (50 to 100 nm in diameter) that are subcellular microdomains of lipid rafts and caveolae, enriched in glycosphingolipids and cholesterol and the protein caveolin. Caveolae are complexes with a high concentration of signaling molecules. Caveolin is a scaffolding protein that anchors receptors (e.g., angiotensin II subtype 1 [AT_1], endothelin [ET-1], epidermal growth factor [EGF]) signaling and trafficking proteins ($G\alpha_{q/11}$) as well as ion channels (K^+ and transient receptor potential [TRP] channels) and enzymes (endothelial nitric oxide synthase [eNOS], protein kinase C [PKC], phospholipase C [PLC], NADPH oxidase, ADP ribosyl cyclase) involved in controlling vasomotor tone. Endothelial cells and fibroblasts are rich in caveolins 1 and 2; smooth muscle cells express all three caveolins (Cav-1, -2, and -3). In vascular smooth muscle cells, Cav-1 serves as a scaffold or chaperone to target AT_1 receptors to caveolae to activate downstream signaling; Cav-1 is required for efficient coupling of $G\alpha_{q/ll}$ proteins and PLC_β to promote Ca^{2+} mobilization from the sarcoplasmic reticulum. Cav-1 links AT_1 receptors to NADPH oxidases and promotes recruitment of Rac1 to activate reactive oxygen species (ROS) formation. Cav-1 couples IP_3 receptors and TRPC3 channels for store-operated Ca^{2+} entry. It also suppresses K_{ATP} channel activity and negatively regulates Ang II–induced EGF receptor transactivation. Most caveolae in vascular smooth muscle cells have nanocontacts with the sarcoplasmic reticulum, providing a direct link to intracellular Ca^{2+} mobilization and excitation–contraction coupling.

In endothelial cells, Cav-1 binds eNOS, limiting the translocation and activation of eNOS. Caveolin-deficient animals exhibit unusual endothelial dysfunction in that NO production is unopposed, leading to marked vasodilation. Cav-1 knockout animals display attenuated vasoconstriction to phenylephrine and a lack of myogenic tone, largely due to excessive NO production. Mice lacking caveolin show that caveolae and caveolins play a prominent role in various pathophysiologic conditions, especially those related to the cardiovascular system. These disease phenotypes include atherosclerosis, cardiac hypertrophy, cardiomyopathy, diabetes, and neointimal hyperplasia (smooth muscle cell proliferation).[25–28]

GPCR desensitization reduces receptor downstream signaling and effector response. G-protein coupled receptor kinases (GRKs), a family of serine/threonine protein

kinases, initiate receptor-specific, homologous desensitization that curtails receptor signaling by phosphorylating the C-terminal tail of agonist-bound GPCR to promote docking with inhibitory β-arrestins. β-Arrestins not only uncouple receptors from heterotrimeric G-proteins, they also target GPCRs to clathrin-coated vesicles for internalization. Endocytosed receptors are either resensitized and recycled to the plasma membrane or degraded in lysosomes. GPCRs internalize as a stable complex with β-arrestin with signaling potential and gene transcription.[22,29–31]

Although GRKs regulate GPCR activity, regulators of G protein signaling (RGS) proteins directly control the activity of Gα-protein subunits, functioning as endogenous negative regulators of GPCR signaling by accelerating GTP hydrolysis by Gα-subunits and thereby attenuating signaling. The most well-characterized RGS proteins (e.g., RGS2, PGS4, PGS5 of the R4 family) determine signaling specificity of $G\alpha_q$- and $G\alpha_i$-coupled receptors. RGS2 is a GTPase activating protein that binds to both $G\alpha_{q/11}$ and $G\alpha_i$ subunits of GPCRs to accelerate their intrinsic GTPase activity. As a result, they are deactivated with a reduced stimulation of PLC_β-mediated Ca^{2+} release and subsequent vasoconstriction. RGS2 and/or RGS5 reduce Ca^{2+} signaling initiated by AT_1 and ET-1 and α1-adrenoceptors and subsequent contraction. RGS5 mRNA, a regulator of vascular remodeling, is expressed in medial smooth muscle of afferent arterioles and the main renal artery in nonhuman primates. RGS proteins may play a role in mechanosensation and stretch-induced myogenic responses, as well as coordinating actions of vasoconstrictor hormones and paracrine agents.

RGS2 activity is acutely stimulated by NO and cGMP signaling to promote vascular relaxation by attenuating Ca^{2+} signaling in response to vasoconstrictor agents. RGS2 expression in vascular smooth muscle is upregulated by Ang II and is downregulated in hypertension. RGS2 knockout mice are hypertensive with enhanced vasoconstriction produced by Ang II and α1-adrenoceptor agonists. The exaggerated $G\alpha_{q/11}$ signaling in mutant animals is associated with renovascular abnormalities, exaggerated vasoconstriction, hypertension, thickening of the vascular wall of the aorta and renal interlobular arteries, and cardiac hypertrophy. Kidney cross-transplantation studies demonstrate that a specific loss of RGS2 in the kidney causes hypertension, whereas the absence of RGS2 from all extrarenal tissues, including the peripheral vasculature, does not affect arterial pressure. Isolated perfused kidneys of RGS4 knockout mice show increased renal vasoconstrictor responses to ET-1.[21,32–34]

Regulation of Microvascular Contractility

Changes in vascular perfusion are mediated by smooth muscle cell contraction or relaxation that elicits a change in vessel radius and in vascular resistance. Multiple steps and enzymatic cascades are involved in the contractile process, and many of these mechanisms interact with one another to modulate the contractile response. A pivotal step in mediating the contractile response in vascular smooth muscle cells is an increase in the cytosolic concentration of free ionized calcium ($[Ca^{2+}]_i$) above its very low basal value of 10^{-7} M. (This is approximately 0.01% of the ionic Ca^{2+} levels of plasma and extracellular fluid, 1 mM). As shown in Figure 3.5, cytosolic Ca^{2+} binds with calmodulin. The Ca^{2+}-calmodulin complex activates myosin light chain (MLC) kinase, leading to the phosphorylation of MLC that interacts with actin and adenosine triphosphate (ATP) to elicit tension development. Increased $[Ca^{2+}]_i$ also phosphorylates CPI-17, a phosphoprotein that inhibits MLC. GPCR activation of PKC enhances Ca^{2+} sensitivity of the contractile apparatus by potentiating the efficiency of Ca^{2+} stimulation of CPI-17. In addition, GPCR couples to the $G\alpha_{q/11}$ signal through the monomeric GTPase RhoA/Rho kinase pathway to decrease the activity of MLC phosphatase, resulting in increased Ca^{2+} sensitivity of myofilaments and enhanced vasoconstriction for a given level of cytosolic Ca^{2+} concentration. Relaxation occurs as a consequence of the removal or sequestration of Ca^{2+} from the cytosol and/or MLC dephosphorylation. Decreases in Ca^{2+} signaling occur following dissociation of ligand from GPCR surface receptor, receptor inactivation, or internalization.[2,35]

Various hormones and drugs activate plasma membrane receptors to induce contraction by eliciting an increase in $[Ca^{2+}]_i$. Cytosolic Ca^{2+} is increased through a combination of Ca^{2+} entry from the extracellular environment and mobilization of Ca^{2+} from internal stores. Ca^{2+} can be released from intracellular stores via release channels, activated by IP_3 or by a ryanodine (Ry)-like ligand. Ca^{2+} is released from a common sarcoplasmic reticular pool, with uptake mediated by a common sarcoplasmic–endoplasmic reticular Ca^{2+} ATPase (SERCA). Many vasoconstrictor agents increase intracellular Ca^{2+} by activating a Gα protein that stimulates PLC to break down membrane-bound phosphatidylinositol 4,5-diphosphate (PIP_2) into IP_3 and 1,2-diacylglycerol (DAG). The soluble IP_3 binds to an IP_3 receptor located on the sarcoplasmic reticulum, leading to the activation of Ca^{2+} channels and the release of Ca^{2+} into the cytoplasm. The lipophilic DAG remains within the membrane environment and activates PKC isoform that phosphorylates various regulatory proteins. DAG may also be generated by the actions of phospholipase D on phosphatidylcholine that is not accompanied by concurrent IP_3 generation and the associated increase in $[Ca^{2+}]_i$. Agents that activate PKC, such as phorbol esters, induce slowly developing, sustained contractions or enhance contractile responsiveness to other stimuli.[2,36]

Activation of cell surface GPCRs also leads to the stimulation of plasma membrane-bound ADP-ribosyl cyclase that converts the substrate nicotinamide adenine dinucleotide (NAD+) to adenosine 5′-cyclic diphosphate (cADP)-ribose, a potent Ca^{2+} mobilizing agent that acts on a RyR to trigger Ca^{2+} release from the sarcoplasmic reticulum. cADP-ribose is a calmodulin-dependent Ca^{2+}-mobilizing second messenger system that acts independently of IP_3, with RyR-mediated Ca^{2+} release to amplify IP_3-mediated

Ca^{2+} mobilization. Ryanodine receptors are extremely sensitive to $[Ca^{2+}]_i$ and exhibit Ca^{2+}-induced Ca^{2+} release (CICR), a form of autopotentiation. Concentrations of Ca^{2+} over a wide range (5 to 100 μM) enhance the open probability of RyR, which contrasts with a narrower range (180 to 220 nM) for the IP_3R.[20]

Calcium entry occurs through a variety of pathways, including voltage-operated channels (VOCs) that are activated upon membrane depolarization and voltage-independent receptor-operated channels (ROCs) and store-operated channels (SOCs). Membrane depolarization downstream of GPCR activation results from the activation of Cl^- channels or the inactivation of K^+ channels. Voltage-independent ROCs and SOCs also contribute to agonist-induced Ca^{2+} entry in both preglomerular and efferent arterioles. ROCs are activated downstream of cell surface GPCR, independent of Ca^{2+} mobilization, via signaling mechanisms involving DAG and calmodulin, and other possible intermediates. SOCs are known to contribute to agonist-induced Ca^{2+} entry in renal vascular smooth muscle cells; they are also termed capacitative as they respond to depletion of Ca^{2+} stores.[37,38]

Among the identified families of TRP proteins, the canonical TRP (TRPC) family (TRPC1 through TRPC7) is thought to be involved in Ca^{2+} entry in vascular smooth muscle cells, most likely functioning as voltage-independent SOCs and/or ROCs. TRPC1 proteins in vascular smooth muscle cells form tetrameric channels in association with TRPC4 or TRPC5. TRPC3, -6, and -7 are activated by DAG and thus are attractive possibilities as ROCs. TRPC6 is an essential component of α1-adrenoceptor–activated cation channels in portal venous smooth muscle cells. Preglomerular arterioles have a predominance of TRPC3 and -6 subunits that may comprise SOCs and/or ROCs in these vessels. Evidence to support TRPC channel function is attenuation of Ca^{2+} responses of the afferent arteriole to norepinephrine by the blockers of voltage-insensitive Ca^{2+} entry Gd^{3+} and SKF 96365. Activation of TRPC6 in the afferent arteriole leads to increased $[Ca^{2+}]_i$. The Ca^{2+} permeable TRPC6 is a key signaling component in a functional slit diaphragm formed by podocytes in the glomerular filtration barrier. Gain-of-function mutations in TRPC6 are the cause for progressive kidney failure with urinary protein loss and focal segmental glomerular sclerosis. SOCs are activated by Ca^{2+} mobilization to specifically restore Ca^{2+} content in sarcoplasmic reticulum. Current interest focuses on stromal interaction molecules (STIM) in the sarcoplasmic reticulum, which sense SR Ca^{2+} depletion and then translocate to junctions near the plasma membrane to organize Orai proteins, the Ca^{2+} release-activated Ca^{2+} channels, to form a pore and increase Ca^{2+} entry.[37,39–47]

Na^+/Ca^{2+} exchange (NCX) contributes to the regulation of $[Ca^{2+}]_i$, working reversibly in the exit mode to extrude Ca^{2+} or in the entry mode to facilitate Ca^{2+} entry. Exchanger activity is normally higher in afferent then in efferent arterioles. Ang II stimulates $[Ca^{2+}]_i$ in afferent arterioles by activating NCX to promote Ca^{2+} entry. Consistent with this observation, genetic deletion of Na^+/Ca^{2+} exchanger NCX1 in smooth muscle cells reduces Ang II–induced renal vasoconstriction in vivo, presumably due to less Ca^{2+} entry into smooth muscle cells. Nevertheless, the pharmacologic blockade of NCX or lowering extracellular Na^+ concentration causes renal vasoconstriction and exaggerated Ang II–induced constriction of the isolated perfused kidney. Smooth muscle Na^+/Ca^{2+} activity responds to Na^+/K^+-ATPase inhibition by an endogenous ouabainlike glycoside to extrude the high cellular Na^+ in exchange for Ca^{2+} entry in hypertensive states.[43,48–50]

Several different K^+ channels have been identified; the most prominent are Ca^{2+}-activated and ATP-dependent K^+ channels, which mediate relaxation by hyperpolarizing the cell membrane and reducing Ca^{2+} entry through voltage-gated Ca^{2+} channels. Importantly, increases in intracellular ATP inhibit K^+ channels, thus causing depolarization. Channel activity is reduced by intracellular ATP concentrations normally present, suggesting that the activity of this channel is quite low in normal cells and may serve a primary protective role during the depletion of energy reserves.[2,51–54]

At the whole kidney level, Rho-kinase inhibition dilates the renal vasculature under basal conditions and attenuates renal vasoconstriction produced by intrarenal infusion of Ang II, arginine vasopressin (AVP), or norepinephrine, as well as increased renal perfusion pressure. Frequency analysis of renal vascular admittance indicates that Rho-kinase strengthens the myogenic response. Ca^{2+} sensitivity of the afferent arteriole is increased by adenosine and norepinephrine. Adenosine increases Ca^{2+} sensitivity by PKC, Rho-kinase, and p38 mitogen activated protein (MAP) kinase signaling pathways. This provides a mechanism by which different vasoactive agents can modulate reactivity to other stimuli. Interestingly, adenosine enhances Ang II–induced afferent arteriolar contraction but does not potentiate the contractile responses to ET-1 or norepinephrine. Reactivity to Ang II is enhanced by the ability of norepinephrine to increase Ca^{2+} sensitivity with increased MLC phosphorylation.

Rho-kinase participates in pressure-induced interlobular arterial and afferent arteriolar myogenic behavior and vasoconstrictor responses evoked by Ang II, adenosine A_1, endothelin ET_B, and purinergic P_2X_1 receptor activation as well as membrane depolarization. Ca^{2+} sensitization due to Rho-kinase also contributes to Ang II–induced constriction of efferent arterioles. Rho-kinase inhibition dilates preglomerular and postglomerular arterioles and attenuates vasoconstriction elicited by adenosine A_1 or endothelin ET_B receptor stimulation. Ang II, AVP, and TxA_2 constrict preglomerular arcuate and interlobular arteries in the hydronephrotic kidney in part via Rho-kinase. The afferent arteriolar myogenic response in this preparation is markedly attenuated by Rho-kinase inhibition.[55–59]

As mentioned earlier, cAMP also activates Ca^{2+} translocation mechanisms that increase extrusion of Ca^{2+} out of the cell, return Ca^{2+} to the sarcoplasmic reticulum, or inhibit IP_3-mediated mobilization of Ca^{2+} from sarcoplasmic

Agents that increase cytosolic calcium:

Ang II, AVP, Epinephrine (α), TXA_2, Leukotrienes, Norepinephrine, Endothelin, ATP

Agents that increase cAMP (or cGMP):

Epinephrine (β), PTH, PGI_2, PGE_2, ANP, Dopamine, Bradykinin, Nitric Oxide, Adenosine (A_2)

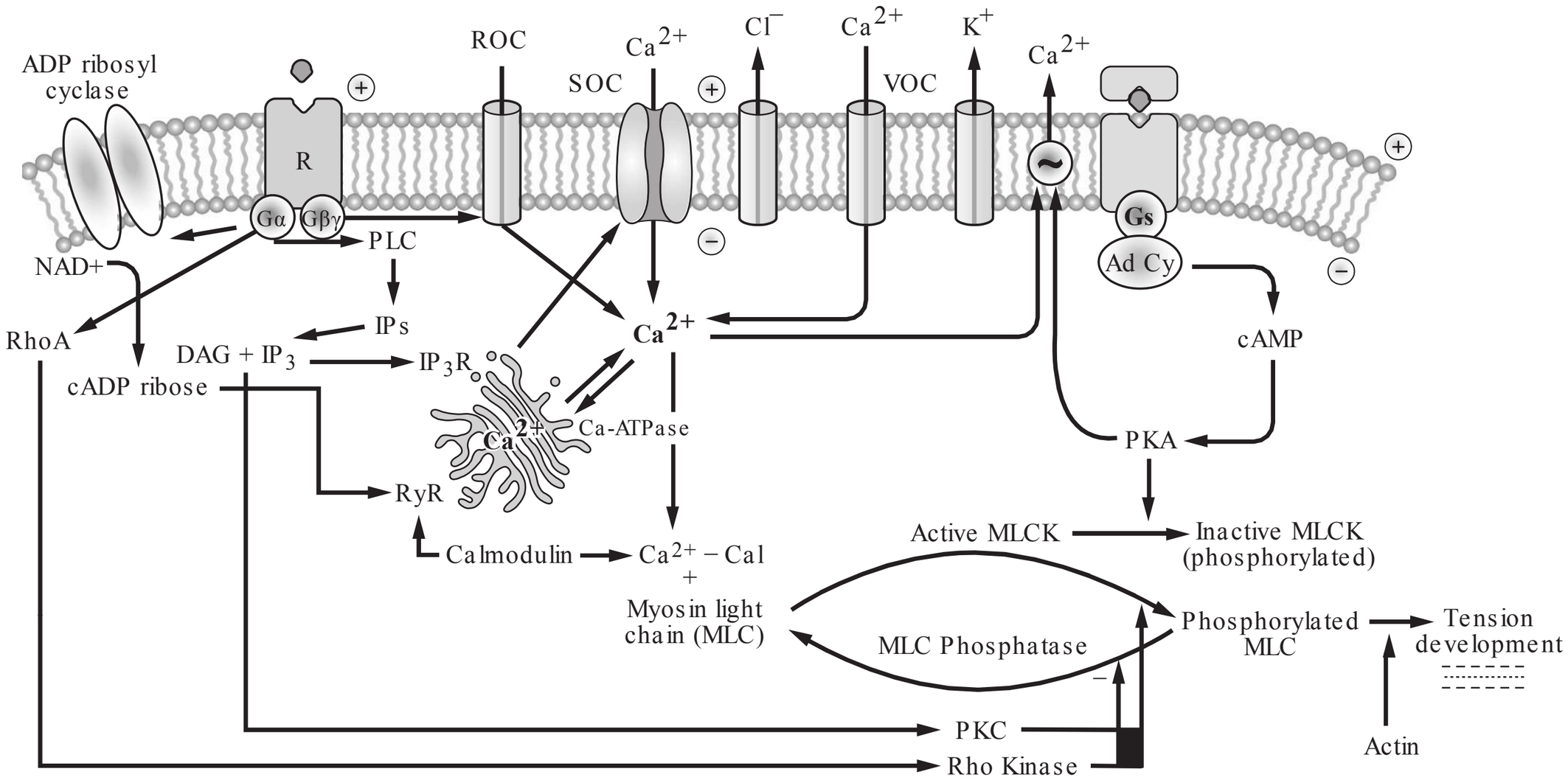

FIGURE 3.6 Primary intracellular signaling systems mediating smooth muscle cell or mesangial cell contraction, with effects of various hormones and vasoactive agents on the two major types of receptor systems. For ease of presentation, only two receptor mechanisms are depicted, but each agent acts on its own receptor system.

reticulum stores (Fig. 3.6). Drugs or agents such as PGE_2 and PGI_2 that increase cAMP via $G\alpha_s$ signaling produce increases in RBF and GFR. Small amounts of such ligands can buffer the action of vasoconstrictor agents without affecting baseline vascular tone. In contrast, ligand-receptor complexes coupled to the inhibitory G protein ($G\alpha_i$) reduce cAMP levels and cause greater contraction for a given level of $[Ca^{2+}]_i$. $G\alpha_i$ proteins are reported to activate PLC and mobilize Ca^{2+} in the afferent arteriole.[60,61]

Another family of receptors operates through the G protein-dependent activation of guanylate cyclase, the generation of cGMP, and the activation of protein kinase C to mediate vasodilation. In addition, two major guanylate cyclase activators are not G protein-dependent. NO derived from endothelial cells directly interacts with soluble guanylate cyclase. Also, ANP directly activates particulate guanylate cyclase in vascular smooth muscle cells. The mechanisms mediating cGMP-dependent vasorelaxation are similar to those used by cAMP. cGMP-dependent kinases lead to the inhibition of voltage-gated L-type Ca^{2+} channels, activation of a Na^+/Ca^{2+} exchanger, stimulation of Ca^{2+}-ATPase, inhibition of IP_3 formation, and phosphorylation of phospholamban, resulting in increased Ca^{2+}-ATPase activity in the sarcoplasmic reticulum. Ca^{2+} desensitization of the contractile machinery, independent of $[Ca^{2+}]_i$ changes, takes place because cGMP/PKG can block RhoA activation and can reduce MLC activity by inhibiting CPI-17 phosphorylation and activating MLC phosphatase. cGMP also may stimulate Ca^{2+}-activated K^+ channels, which leads to hyperpolarization.[33,62]

The arachidonic acid pathways constitute another intracellular signaling system. Increased $[Ca^{2+}]_i$ can activate phospholipase A_2 and can release arachidonic acid from membrane phospholipids, resulting in the production of various metabolites that lead to vasodilation or vasoconstriction. Arachidonic acid metabolites exert effects through multiple pathways, including cAMP, cytosolic Ca^{2+}, and inhibition of K^+ channels. Arachidonic acid itself may increase Ca^{2+} entry via a noncapacitative Ca^{2+} entry channel. These actions will be discussed in detail later in this chapter.

Differences in cellular sites and mechanisms of smooth muscle activation may be partially responsible for the large variety of renal hemodynamic responses produced by different vasoactive agents. There is a major difference in the mechanisms leading to Ca^{2+} activation in the vascular smooth muscle cells of the afferent and efferent arterioles. Preglomerular vessels have a strong dependence on L-type voltage-gated Ca^{2+} channels, whereas their influence is not readily apparent in efferent arterioles. Antagonists of Ca^{2+} influx through L-type, dihydropyridine-sensitive Ca^{2+} channels selectively block agonist-induced constriction of

the preglomerular arterioles, including the afferent arteriole, without affecting efferent arteriolar contraction. This is the case for Ang II, ET-1, norepinephrine, and potassium chloride–induced depolarization. Agents that block L-type Ca^{2+} channels such as nifedipine, diltiazem, and verapamil primarily cause afferent vasodilation and impair autoregulatory responses to changes in renal perfusion pressure, affecting both myogenic and tubuloglomerular feedback (TGF responses). In contrast, T-type Ca^{2+} channels are active at both afferent and efferent arteriolar sites and influence vascular responsiveness. T-type Ca^{2+} channel blockers vasodilate afferent and efferent arterioles and prevent contractile responses to various stimuli at both sites. In afferent arterioles, T-type channels may act cooperatively with L-type channels to bring about membrane depolarization and Ca^{2+} entry. A primary action on the preglomerular vasculature also explains the increases in GFR and glomerular capillary pressure produced by Ca^{2+} entry blockers as well as inhibition of the TGF system. An example of a hormone that exerts its effects through different mechanisms is Ang II. Its effects are mediated by at least two mechanisms. Afferent arteriolar responses are highly dependent on Ca^{2+} entry via L-type channels, whereas the efferent arteriolar response is influenced by Ca^{2+} mobilization and Ca^{2+} entry via T-type channels and through store-operated channels in the absence of any entry through voltage-gated L-type channels. NO may suppress voltage-sensitive Ca^{2+} entry in both afferent and efferent arterioles. Ca^{2+} entry in outer medullary vasa recta is mediated by a combination of L- and T-type Ca^{2+} channels as well as store-operated cation channels.[63–67]

In addition to the smooth muscle cells of the resistance vessels, the mesangial cells within the glomerular tufts possess contractile capability, which may contribute not only to the regulation of blood flow through the glomerulus but also to the filtering capacity. Mesangial contraction is postulated to reduce the glomerular filtration coefficient (K_f) by decreasing the radius of capillaries or the surface area available for filtration, or both, but the precise mechanism is not clear. Many agents, including Ang II and vasopressin, reduce K_f. Low K_f values have also been observed during sodium depletion when plasma and local concentrations of endogenous Ang II are elevated. These responses reflect specific receptor-mediated effects on mesangial cells, because the response can be reversed by selective receptor antagonists in vivo and in cultured mesangial cells. However, podocytes are closely associated with mesangial cells in vivo and also respond to Ang II, suggesting that part of the actions of Ang II on K_f could be due to responses by podocytes. Some mesangial cell receptors exert stabilizing effects on mesangial cell contraction and counteract the influence of excessive levels of vasoconstrictor agents or serve metabolic functions. β-Adrenergic agonists and vasodilator prostaglandins (PGE_2 and PGI_2) increase cAMP in isolated glomeruli and mesangial cells and they directly oppose the apparent contractile effects of Ang II. Nevertheless, the structural mechanism by which mesangial cell contraction actually alters K_f remains unclear.[2,68–70]

Endothelial Interactions with Vascular Smooth Muscle

The vasculature is lined with a continuous layer of endothelial cells, which has many functions including serving as a diffusion barrier and preventing vascular thrombosis. Endothelial cells are dynamic metabolic units having membrane receptors and membrane-bound enzymes, which allow them to respond to and contribute to changes in the concentration of humoral agents. Membrane-bound ectoenzymes form or degrade, circulating vasoactive substances such as Ang II (angiotensin converting enzyme [ACE]), ET-1 (endothelin converting enzyme and metallopeptidase), bradykinin (kininase II), and adenonucleotides (three ectonucleotidases convert ATP, ADP, and AMP). Localization of ACE in preglomerular vessels allows the conversion of systemically delivered Ang I, an inactive decapeptide, to the biologically active octapeptide Ang II, that can then induce vasoconstriction locally or in downstream segments.[2]

The vascular endothelium serves an important paracrine role (Fig. 3.7). Endothelial cells participate in contractile and dilator mechanisms by responding to a variety of stimuli and increasing or decreasing formation of potent vasoactive substances that act locally to modulate the tone of adjacent smooth muscle cells. General classes consist of endothelium-derived relaxing factors (EDRF) and endothelium-derived contracting factors (EDCF). Specific examples of relaxing factors that cause renal vasodilation are NO, PGE_2, and PGI_2 (prostacyclin), carbon monoxide (CO), and an epoxyeicosatrienoic acid (EET), a hyperpolarizing factor that is a metabolite of the cytochrome P450 pathway. Examples of EDCF include Ang II, ET-1, thromboxane (TxA_2), and oxygen-free radicals. These paracrine factors act on smooth muscle cells to modify vasomotor tone, proliferative state, and provide a balance between antioxidant defense mechanisms and excess generation of O_2-derived free radicals. Thus, the endothelial cells are intimately involved in controlling the renal microcirculation.[71,72]

One of the most studied interactions between endothelial cells and smooth muscle cells involves the ability of the endothelium to modify the vascular responses to acetylcholine and other agents. Acetylcholine is a powerful vasodilator in vivo and also in isolated smooth muscle preparations that have an intact endothelium because it stimulates endothelial cells to produce vasodilatory paracrine substances. However, when applied to vascular preparations whose endothelium has been removed, acetylcholine induces vasoconstriction by acting directly on muscarinic receptors on smooth muscle cells. Many other substances have now been shown to stimulate the release of endothelium-derived vasoactive factors.

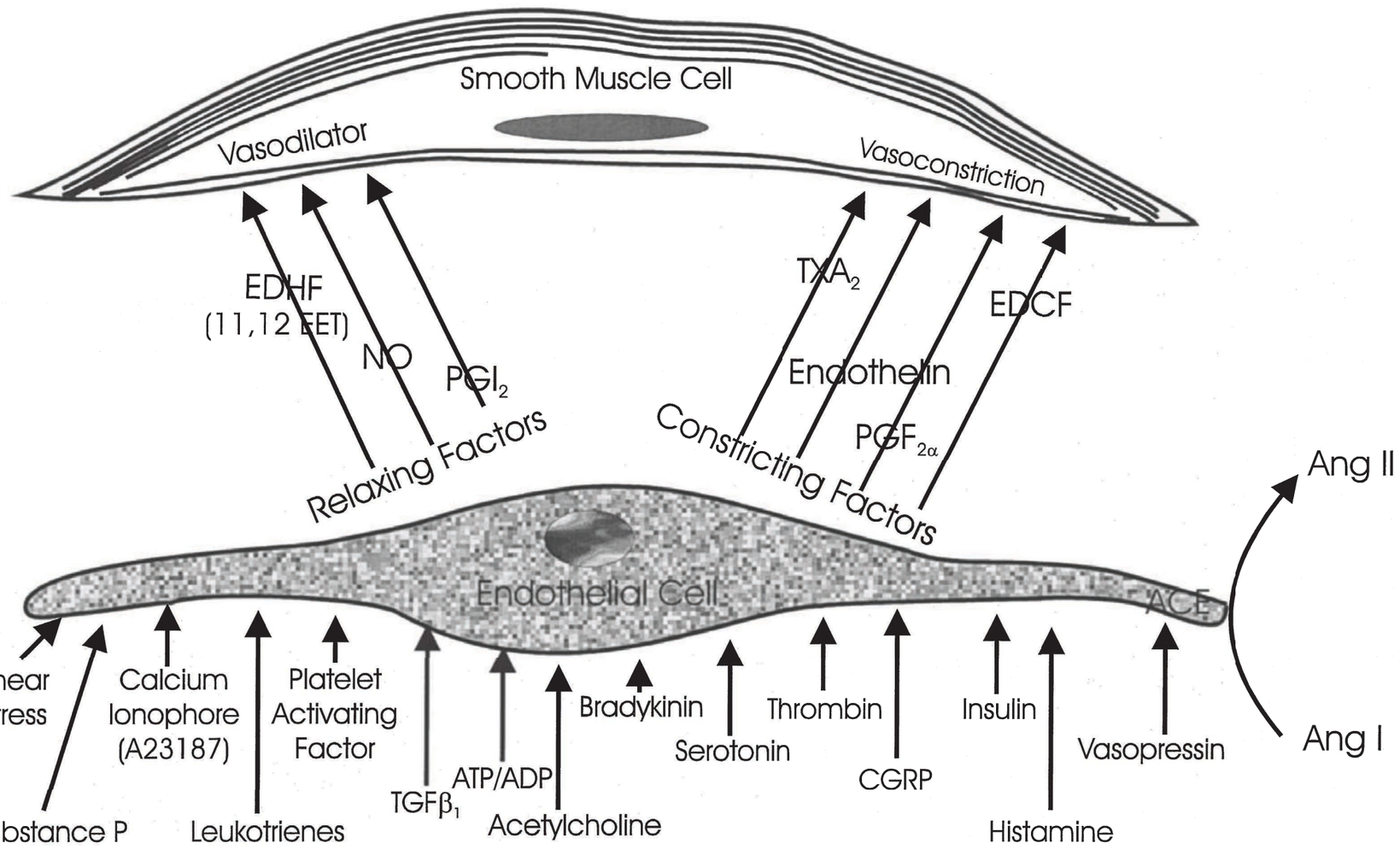

FIGURE 3.7 The interaction of endothelial cells with smooth muscle or mesangial cells. Agents that are known to influence endothelial-derived relaxing factors or nitric oxide (*NO*) and endothelial-derived constricting factors (EDCF) production by endothelial cells are shown. Endothelial cells also produce several vasoconstrictor and vasodilator agents, as shown in the figure and described in the text.

One of the major relaxing factors is NO derived from L-arginine. These paracrine systems will be discussed in later sections.[2,73,74]

Endothelial cells have a remarkable capability to transport substances across their layers through a variety of mechanisms. One of the most impressive features of endothelial cells lining the vasculature is their ability to form fenestrations that serve as extracellular channels. This feature occurs predominantly in capillary structures having large rates of transcapillary volume flux. Both glomerular and peritubular capillary systems have fenestrations. The glomerular capillaries have abundant, well-rounded fenestrations that are 50 to 100 nm in diameter and lack a diaphragm. These fenestrations constitute highly permeable pathways for the large volume of plasma filtrate that continuously traverses from the glomerular capillaries into the Bowman space. Although it is not clear whether subtle changes in the size of the fenestrations contribute to the regulation of the hydraulic conductivity of the glomerular capillary barrier, it is apparent that their integrity is essential for the maintenance of glomerular filtration.[2,75]

The fenestrations of the peritubular capillaries are bridged by a thin diaphragm and are smaller in diameter (20 nm). Considering the total number of capillaries, there is much more peritubular than glomerular capillary surface area. However, because the overall reabsorptive rate by the peritubular capillaries is nearly equal to the GFR, the average hydraulic conductivity of the peritubular capillaries per unit of surface area is estimated to be less than that of glomerular capillaries. Fenestrations also exist in the terminal segments of afferent arterioles, and they may provide a pathway for renin entry into the circulation from juxtaglomerular granular cells.[2,76]

TRANSCAPILLARY EXCHANGE IN RENAL MICROCIRCULATION

Forces Governing Ultrafiltration at the Glomerulus

Bulk movement of fluid across capillary membranes of the renal microcirculation is passive in nature, driven by physical forces. As blood flows from the afferent arterioles into the glomerular capillary tufts, the high hydrostatic pressure predominates over the counteracting forces caused by Bowman space hydrostatic pressure and plasma colloid osmotic pressure. Therefore, fluid is driven from the glomerular capillaries through the endothelial fenestrations, across the basement membrane, and between the podocyte foot processes into the Bowman space. This movement of fluid can be described quantitatively by the Starling filtration-reabsorption principle, which is based on the premises that (1) water and solutes flow through extracellular channels or pathways and (2) the diameters of these channels are large with respect to water molecules, hydrated ions, and solutes of low-molecular weight, such as urea, glucose, and amino acids. Thus, except for the larger solutes, mainly plasma proteins that approach or exceed the size of the channels, the filtrate is translocated without substantive compositional alterations. Detailed consideration of the structure and biochemical composition of the glomerular filtration barrier is provided in Chapter 1.

The physical forces acting across the glomerular membrane are glomerular capillary pressure (P_g), Bowman space

pressure (P_B), glomerular plasma colloid osmotic pressure (π_g), and colloid osmotic pressure of filtrate in the Bowman space (π_B). The filtering capacity of the filtration barrier is expressed as the glomerular filtration coefficient (K_f), which is the product of the hydraulic conductivity of the glomerular membrane (L_p) and the total filtering surface area (S_f). Because the net forces change as fluid is filtered along the length of the glomerular capillaries, total GFR can be expressed by the equation:

$$GFR = K_f \int_0^1 [(P_{g(x)} - P_B) - \sigma (\pi_{g(x)} - \pi_B)]\, dx \qquad (1)$$

where x represents the normalized length of the glomerular capillaries, with 0 designating the afferent end and 1 designating the efferent end; σ (sigma) is the reflection coefficient, which has a range of 0 to 1. When sigma is 1, proteins are completely "reflected" by the capillary wall, and the colloid osmotic pressure is maximally effective. Normal glomerular capillaries are extremely efficient in restricting the passage of macromolecules, and the amount of protein present in the normal filtrate in the Bowman space is less than 0.1% of the plasma protein. For practical considerations, the effective colloid osmotic pressure is considered equivalent to that of the plasma in the glomerular capillaries (π_g). As is shown in Figure 3.8, this value increases progressively along the length of the capillaries as a function of the relative volume of protein-free fluid that is filtered. Because colloid osmotic pressure is the major force retarding glomerular filtration, filtration is greatest in the initial segments of the glomerular capillaries and decreases progressively along the length of the capillaries.[1,2,77]

The exact hydrostatic pressure drop along the glomerular capillaries is uncertain because experimental assessment is not possible. Nevertheless, there are abundant parallel capillaries that collectively have a large cross-sectional area relative to that of the afferent and efferent arterioles; thus, the hydrostatic pressure drop along the glomerular capillaries is small as compared with the pressure drops across the afferent and efferent arterioles. Computations based on the number and dimensions of the glomerular capillaries yield estimates that are in the range of 1 to 4 mm Hg. Thus, P_g is usually treated as a constant value. With these simplifying assumptions and the use of average values for hydrostatic and colloid osmotic pressures in glomerular capillaries, the more commonly used formulation for GFR results:

$$GFR = K_f (P_g - P_B - \pi_g) \qquad (2)$$

The net, or mean, effective filtration pressure (EFP) is calculated as

$$EFP = P_g - P_B - \pi_g \qquad (3)$$

The increase in plasma protein concentration is a direct function of the filtration fraction, defined as the quotient of GFR and renal plasma flow. Because of the nonlinear relationship between plasma protein concentration and colloid osmotic pressure, the rate of increase in colloid osmotic pressure from the afferent to the efferent arteriole increases progressively (Fig. 3.9). Empirically derived relationships allow the prediction of colloid osmotic pressure (π) from the total plasma protein concentration (C) when the albumin-to-globulin (A/G) ratio is known. The commonly used Landis-Pappenheimer relationship

$$\pi = 2.1\, C + 0.16\, C^2 + 0.009\, C^3 \qquad (4)$$

applies to an A/G ratio of about 1.2, which is considered normal for humans.[2]

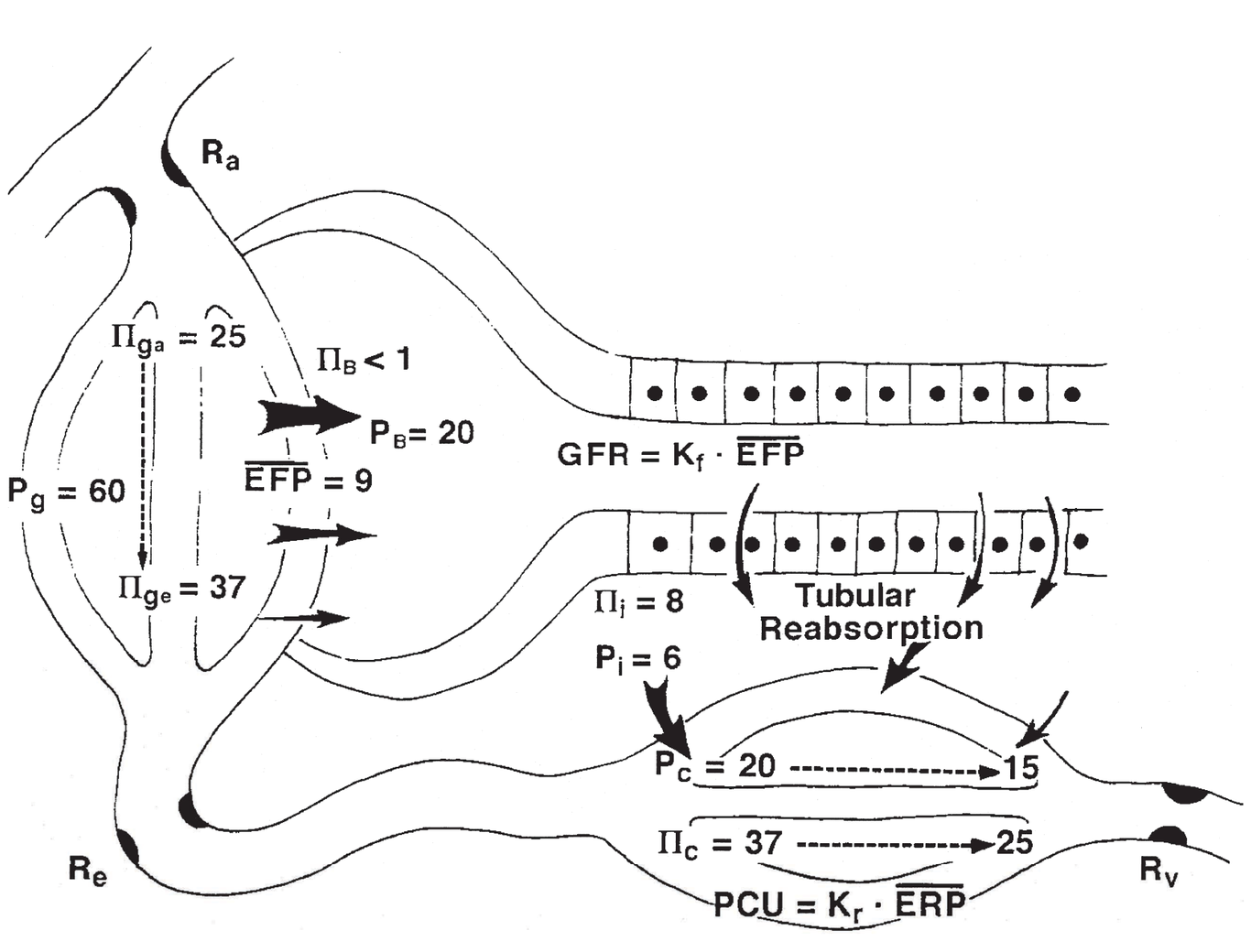

FIGURE 3.8 A schematic diagram of the forces responsible for the filtration of fluid from the glomerular capillaries and the reabsorption of fluid into the peritubular capillaries. The values are considered representative of forces in humans. R_a, afferent arteriole resistance; R_e, efferent arteriole resistance; $P_{g,B,c,i}$, pressure in glomerular capillaries, Bowman's space, peritubular capillaries, and renal interstitium; $\Pi_{g,B,C,i}$, colloid osmotic pressure in glomerular capillaries, Bowman's space, peritubular capillaries, and renal interstitium; EFP, effective filtration pressure; K_f, filtration coefficient; GFR, glomerular filtration rate.

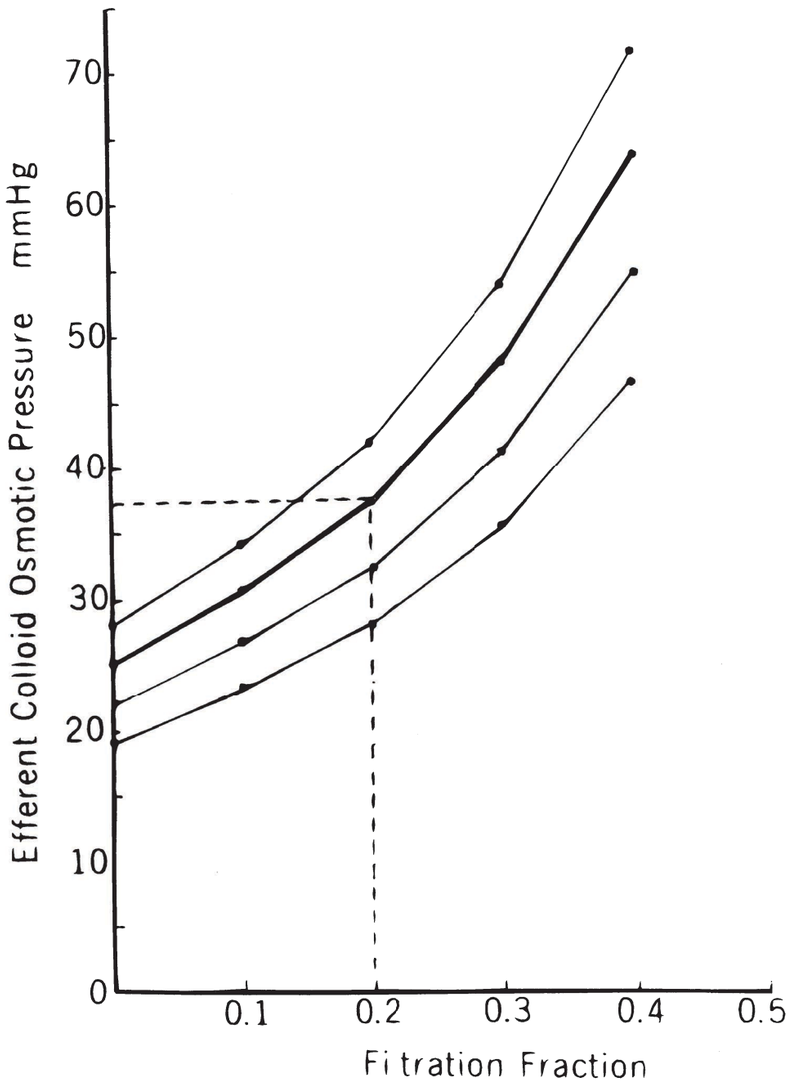

FIGURE 3.9 Anomogram relating the efferent arteriolar colloid osmotic pressure to the initial plasma colloid osmotic pressure and the filtration fraction. Normal afferent arteriolar colloid osmotic pressure, 25 mm Hg, is indicated by the *thicker curve*. An example of how to estimate efferent arteriolar colloid osmotic pressure for any given filtration fraction and plasma colloid osmotic pressure is shown by the *dashed lines*.

The efferent arteriolar colloid osmotic pressure is determined by the initial plasma value and the filtration fraction. The nomogram in Figure 3.9 allows for the estimation of the efferent arteriolar colloid osmotic pressure and is independent of A/G ratios. For example, at a normal filtration fraction of 0.20 and normal plasma colloid osmotic pressure of 25 mm Hg, the predicted value for efferent colloid osmotic pressure is 37 mm Hg.

The hydrostatic pressure in Bowman space (P_B) in humans has not been measured directly. In laboratory animals, P_B is equal to proximal tubular pressure, which ranges from 11 to 15 mm Hg in rats and from 18 to 22 mm Hg in dogs. Also, proximal tubular pressure is slightly higher than the pressure in adjacent peritubular capillaries. Peritubular capillary pressure has also not been measured directly in humans, but it can be estimated from intrarenal venous pressure measurements obtained by retrograde passage of a renal vein catheter. Values obtained in humans are 20 to 25 mm Hg and provide reasonable estimates of proximal tubular pressure. This pressure plus an average efferent colloid osmotic pressure of 37 mm Hg provides a minimal glomerular pressure in humans in the range of 57 to 62 mm Hg; actual values are higher to the extent that there is net filtration pressure at the terminal end of the glomerular capillaries.

Micropuncture studies in animals also indicate that glomerular pressure is 50 to 60 mm Hg and approximately 40 mm Hg greater than the opposing hydrostatic pressure in the Bowman space. From this difference in transglomerular capillary hydrostatic pressure, it can be calculated that EFP ranges from 15 mm Hg at the afferent end of the glomerular capillaries to about 3 mm Hg at the efferent end, yielding an average EFP of 9 mm Hg (Fig. 3.8). Using this value and one of 120 mL per minute for total GFR, a K_f of 13 mL/min/mm Hg for the total nephron population is calculated. Assuming there are 2 million nephrons in both human kidneys, the K_f for a single glomerulus is approximately 6 to 7 nL/min/mm Hg. This value generally agrees with micropuncture measurements, which indicates that K_f for an individual glomerulus is 4 to 5 nL/min/mm Hg in dogs and 2 to 5 nL/min/mm Hg in rats. The large variation in K_f among rats is due in part to differences observed among different strains.[2,77]

The filtration process can operate under one of two conditions. The first condition is the case described previously, in which filtration continues throughout the entire length of the glomerular capillaries and a finite positive EFP remains at the efferent end of the glomerular capillaries. This pattern of disequilibrium is shown by the solid lines in the left panel of Figure 3.10. The second condition occurs when the increase in colloid osmotic pressure is so rapid that the forces favoring and opposing filtration become equal at some point within the capillary system, a condition termed *filtration pressure equilibrium* (Fig. 3.10, solid lines in right panel). Under equilibrium conditions, the latter part of the available filtering surface area is not used and becomes a functional reserve. Studies in some strains of rats have suggested that the normal condition is one of filtration equilibrium. Data from other strains of rats and dogs indicate that, under normal circumstances, glomerular capillary hydrostatic pressure is sufficiently high and the K_f is sufficiently low to prevent the achievement of filtration equilibrium within the glomerular capillaries, and thus filtration occurs along the entire length of the glomerular capillaries.[2]

A physiologic consequence of the equilibrium or disequilibrium of filtration pressures is the influence of plasma flow on GFR. Using a mathematical model presented later, the specific effect of plasma flow can be predicted for both conditions when the transcapillary hydrostatic pressure gradient is kept constant. As shown by the dashed line in Figure 3.10 (right panel), an increase in plasma flow to a system in filtration equilibrium diminishes the rate of increase of colloid osmotic pressure along the length of the glomerular capillaries. The EFP is not dissipated as quickly, and the point of equilibration of hydrostatic and colloid osmotic forces is moved distally, which, in effect, results in the recruitment of additional filtering surface area (S_f) and an increase in the functional K_f. Consequently, increases in plasma flow can increase the GFR proportionately even when glomerular capillary pressure is unchanged. In the case of filtration

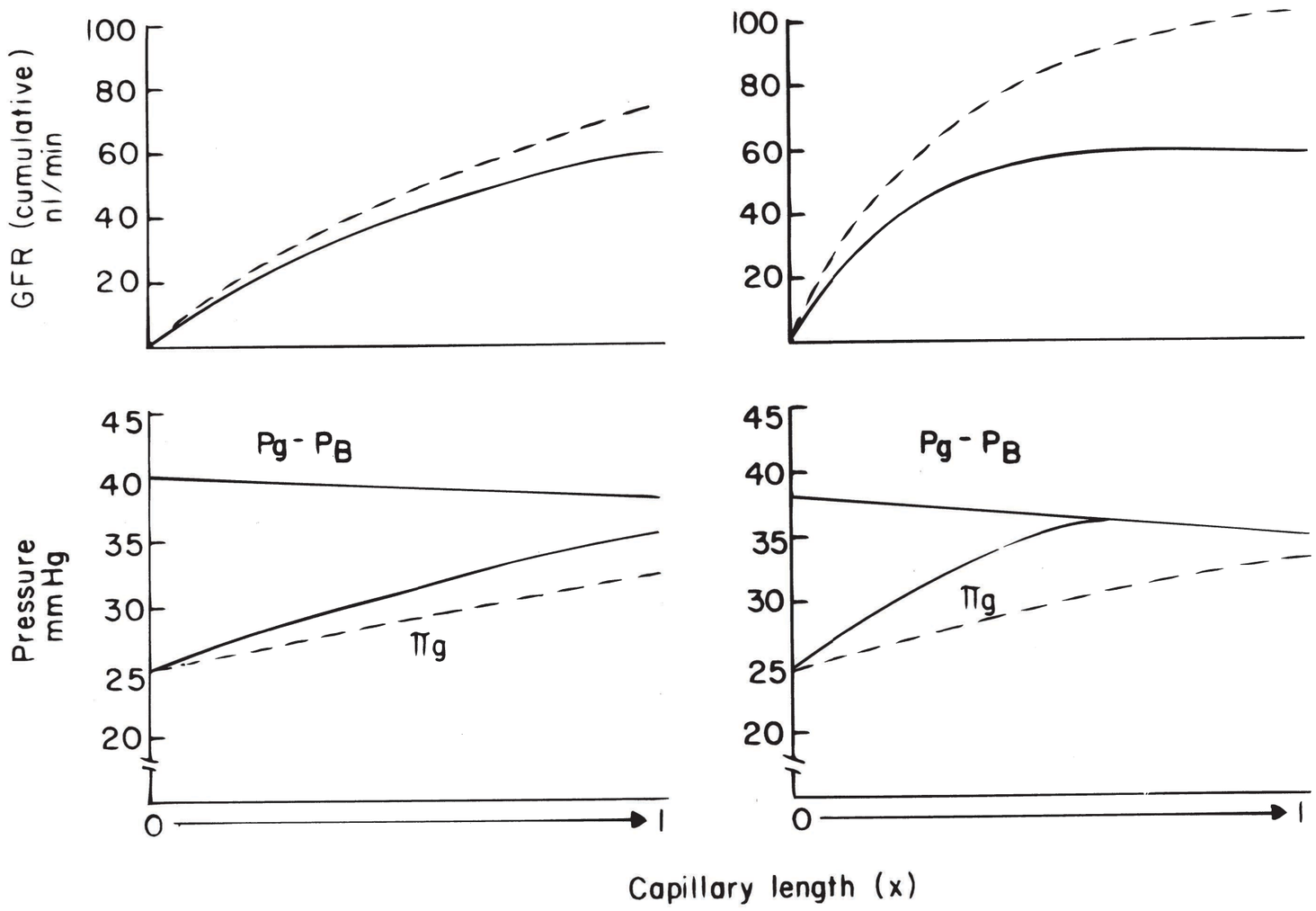

FIGURE 3.10 A comparison of filtration dynamics in conditions of filtration equilibrium (*right*) and disequilibrium when filtration occurs throughout the length of capillary (*left*). The lower panels represent the changes in the transcapillary hydrostatic pressure gradient ($P_g - P_B$) and the glomerular plasma colloid osmotic pressure (π_g), and the upper panels represent the cumulative GFR along the length of the glomerular capillary. The *dashed lines* indicate the changes occurring in response to doubling of plasma flow under both conditions. P_g, glomerular capillary hydrostatic pressure; P_B, hydrostatic pressure in Bowman's space.

pressure disequilibrium, increases in plasma flow increase GFR only modestly as a consequence of a reduced colloid osmotic pressure profile, and there is no net recruitment of previously unused surface area (see Fig. 3.10, dashed lines in left panel). Thus, the magnitude of a selective plasma flow effect is smaller during filtration pressure disequilibrium than during equilibrium. In humans, the low filtration fraction and the relative lack of plasma flow dependence of GFR suggest that the filtration process continues throughout the entire length of the glomerular capillaries (i.e., disequilibrium, as shown in the left panel of Fig. 3.9).[1,2,77]

Glomerular Permeability to Macromolecules

Experiments examining the filterability of test molecules of different sizes, shapes, and charges have been used to characterize the hydrodynamic properties of the filtration barrier. A sieving coefficient (Φ), or fractional clearance of a test molecule, is obtained relative to that of a freely filtered reference molecule such as inulin. Accurate determinations can be made when both substances enter the urine by means of filtration and are not subjected to tubular reabsorption or secretion. Such data have been fitted to various theoretic models based on limiting membrane structures, consisting of an impermeable matrix that is perforated with cylindrical pores, rectangular slitlike openings, or a meshwork of fibrous or granular gel-like structures. An evaluation of molecular sieving or steric restriction in each model, however, is based on the principle of geometric exclusion of large solute molecules from a portion of the membrane that is accessible to water and small solutes. In essence, the larger molecules that approach or exceed the effective size of the channels are restricted or "sieved." Conceptually, the simplest model that is applicable to the glomerular barrier consists of a size-discriminating membrane with a large population of fluid-filled cylindrical pores of about 5 nm in radius, which totals approximately 5% of the total surface area. There may also be a very small population of much larger pores.[2,75]

Studies involving quantitative consideration of macromolecular passage through capillary membranes have relied on the thermodynamic approach developed by Kedem and Katchelsky. Derivations for solute flux (J_s) across a constraining membrane include a convection term, which is the solute flux that occurs as a consequence of the bulk volume flow (J_v), and a diffusion flux, which is a function of the concentration gradient of the solute. In its most elementary form, solute flux due to convection is

$$J_s = J_v C_s (1 - \sigma) \tag{5}$$

and solute flux due to diffusion is

$$J_s = PS (\Delta C_s) \tag{6}$$

where J_v is the volume flow (in this case the GFR), and C_s is the average concentration across the membrane; sigma (σ) is the reflection coefficient previously discussed. ΔC_s is the concentration difference across the capillary wall, and PS is the diffusional, permeability surface-area product coefficient. With small uncharged molecules, such as glucose, sigma approaches zero and thus glucose flux is simply defined by the product of GFR and the plasma glucose concentration. For very large molecules that are restricted with almost complete efficiency, sigma approaches 1 and thus solute flux due to convection is negligible. The most relevant example is for plasma albumin. Using a value of 1 to 3 mg per deciliter for albumin concentration in early tubular fluid and a systemic plasma albumin concentration

of 3,600 mg per deciliter, sigma is greater than 0.99. Furthermore, the PS coefficient is so low (0.001 mL per minute) that solute flux due to diffusion also approaches zero. These quantitative considerations also highlight the difficulty in attempting to evaluate mechanisms of proteinuria. Theoretically, protein passage across the glomerular membrane could increase more than 100-fold, which could be accounted for by a change in sigma from 0.99 to 0.95. Such small changes in membrane permeability would not be expected to be associated with discernible morphologic changes.[2,75]

Passage of macromolecules across capillary membranes is dependent on several factors in addition to the effective radius. These factors include the electrical charge and the structural conformation and rigidity of the molecule. As shown in Figure 3.10, the glomerular sieving coefficient or fractional clearance (usually determined as C_D/C_{IN}) of graded sizes of electrically neutral dextran molecules declines progressively as effective radius and molecular weight increase. Water, electrolytes, and other small, uncharged solute molecules with an effective Stokes-Einstein radius of less than 1.8 nm freely permeate. As the effective radius increases, there is a progressive restriction. The fractional clearance of macromolecules the size of immunoglobulin G (IgG) (5 nm) is essentially zero. For the same equivalent radius, the fractional clearances of albumin (3.6 nm) and negatively charged dextran sulfate are considerably lower than the clearances of uncharged molecules. In addition, polycationic macromolecules are filtered more readily than neutral molecules. These differences in transport of electrically charged macromolecules are due to the membrane-bound polyanionic glycoproteins that are rich in sialic acid and heparin sulfate residues, which set up a negative electrostatic field that repels polyanions. These are associated with the glycoprotein coat that covers the endothelial fenestrations, the basement membrane, and the podocytes. Partial loss of these anionic sites on the glomerular capillary wall can lead to albuminuria in the absence of any gross structural abnormalities and in cases of mild glomerulonephritis. Such a loss has been induced experimentally by neutralization of the electrostatic barrier with the polycation protamine. In more severe glomerular injury–associated proteinuria, a larger fraction of the filtrate appears to pass through a population of large diameter, nonselective pores.

In addition to size and charge, molecular configuration influences the sieving coefficient (Fig. 3.11). Rigid or globular molecules such as horseradish peroxidase or ficoll have lower sieving coefficients for any given molecular size than neutral dextran polymers with highly deformable linear structures. Thus, it is likely that the curve for neutral dextrans in Figure 3.11 overestimates the true permeability characteristics of more rigid, globular-structured macromolecules such as plasma proteins. Because shape, flexibility, and deformability contribute to the quantitative relationship between molecular size and transglomerular solute flux, it is difficult to establish the true dimensions of the extracellular

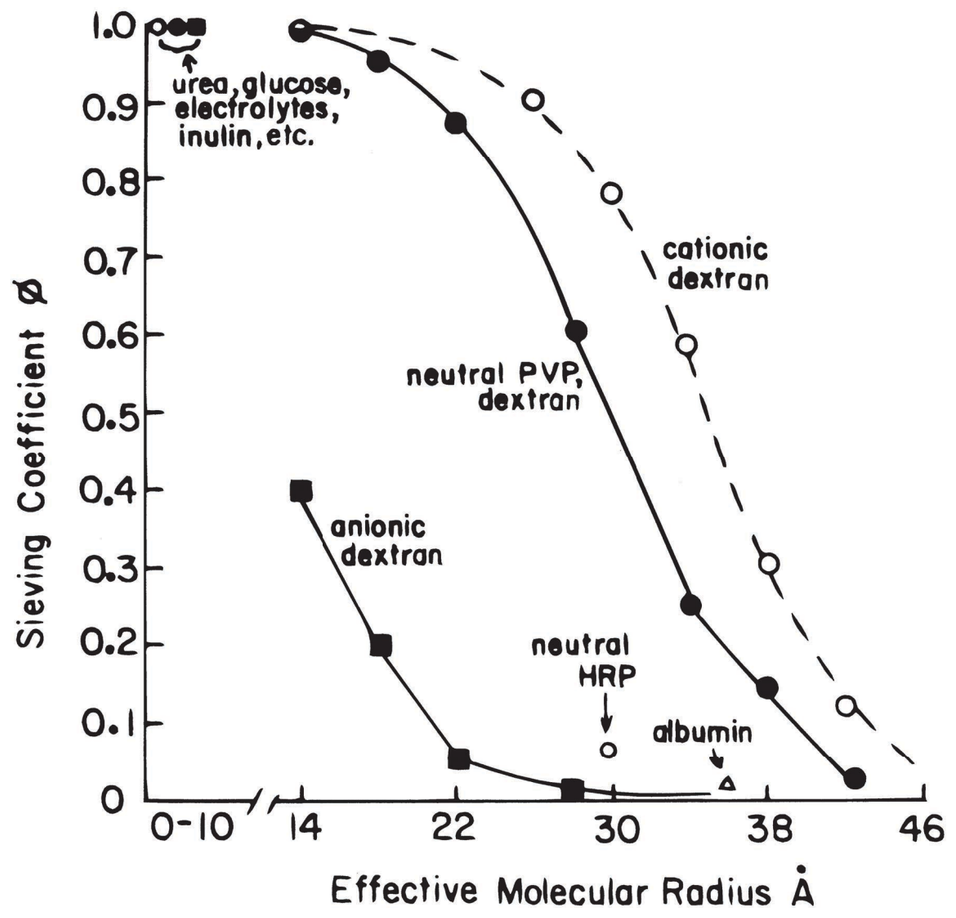

FIGURE 3.11 Representative sieving curves for several test molecules in the glomerular circulation. The curve representing neutral molecules is based on data obtained with the use of polyvinylpyrrolidone (PVP) and neutral dextran. The curves for anionic and cationic molecules are based on studies with charged dextrans. Also shown are the sieving values for neutral horseradish peroxidase (*neutral HRP*) and for albumin. The smaller molecules are shown to have a sieving coefficient of 1.0. See text for details.

channels. Data currently available indicate that the effective radius of the channels in the glomerular membrane is in the range of 4.5 to 6 nm.[2,75]

Recent studies have challenged the generally held notions described previously regarding glomerular permeability and have suggested that much greater amounts of albumin are filtered across the glomerular capillaries and that most of it is then reabsorbed in the proximal tubules by megalin and other macromolecule transporters. Studies based on measurements of glomerular permeability of fluorescent labeled albumin suggest that even normal glomerular capillaries allow passage of albumin that is avidly bound and retrieved by proximal tubules. This concept has been met with substantial resistance and alternative studies have failed to indicate such high levels of albumin passage across normal glomerular capillaries. Advanced multiphoton determinations have yielded a glomerular sieving coefficient of 0.001, a value consistent with the view that the primary determinant of albuminuria is of glomerular origin and that charge does play a significant role.[78–83]

The controversy about glomerular permselectivity highlights important recent findings regarding the extremely complex nature of the glomerular barrier because it consists of three distinct layers in series; each having its own selectivity characteristics. Gene targeted mouse models illustrate the

important role that podocytes have in restricting macromolecules. In particular, studies of the structural and molecular characteristics of the slit diaphragms between adjacent podocytes reveal an extremely complex network of molecular interactions that serve to maintain the normal structure of the podocytes and their close relationship with the basement membranes. The structure of the glomerular membrane is described in detail in Chapter 1. With regard to macromolecular permeability, the podocyte slit-diaphragm layer and its complex array of proteins, including nephrin, podocin, cadherin, and integrins, are clearly identified as the final barrier responsible for the extremely high degree of restriction. Nevertheless, the emerging consensus is that all of the three major restriction sites make a contribution. In essence, when the endothelial glycocalyx is compromised, proteinuria develops. Disorganization of the basement membrane also leads to proteinuria. Furthermore, disruption of the nephrin-actin cytoskeleton complex of the podocyte layer and foot process effacement are associated with the greatest degree of proteinuria. Conceptually, it is attractive to attribute a progressively greater reflection coefficient (sigma) to the three layers in series.[81,84]

Hemodynamics in Peritubular Capillaries and Its Role in Fluid Reabsorption

Virtually all of the peritubular capillary network stems from efferent arterioles. About 85% of the postglomerular blood flow is distributed to peritubular capillaries in the cortex, and the remaining 15% goes to the medulla and papilla (Fig. 3.5). The overall density of peritubular capillaries and the total surface area are considerably greater than those of glomerular capillaries. The peritubular capillary wall consists of a fenestrated endothelial layer covered by a thin basement membrane. Per unit of surface area, it has a lower hydraulic conductivity and a slightly higher permeability to large molecules than the glomerular wall.

In a manner analogous to the process of filtration, the peritubular capillary reabsorption (PR) of interstitial fluid that is reabsorbed by the renal tubules is determined by the imbalance of hydrostatic and osmotic forces between the interstitial space and adjacent peritubular capillaries. If one considers the forces responsible for reabsorption into the capillaries, then

$$PR = K_r [(\pi_c - \pi_i) - (P_c - P_i)] \quad (7)$$

where K_r is the reabsorptive coefficient, π_c and π_i represent the average colloid osmotic pressures in the capillaries and in the interstitial fluid, and P_c and P_i represent the corresponding hydrostatic pressures.

As plasma emerges from the glomerular capillaries, it has a colloid osmotic pressure of 35 to 37 mm Hg (Fig. 3.8). Furthermore, the hydrostatic pressure drops about 40 mm Hg along the efferent arteriole (Fig. 3.3), yielding an initial peritubular capillary pressure of about 20 mm Hg. With regard to the interstitial compartment, π_i and P_i are about 6 to 8 mm Hg and tend to cancel each other out. Thus, the mean effective reabsorption force is 15 mm Hg at the beginning of the peritubular capillary bed. As fluid is reabsorbed into the capillaries, plasma proteins are diluted and the colloid osmotic pressure progressively decreases to the original value entering the kidney. There is also a small decline in capillary hydrostatic pressure along the peritubular capillaries. Thus, there is an effective reabsorptive force over the entire length of the peritubular capillaries, which falls from about 15 mm Hg to about 8 mm Hg (Fig. 3.8). The hydraulic reabsorptive coefficient, K_r, for the peritubular capillaries is about 9 to 10 mL/min/mm Hg, which is slightly lower, overall, than the glomerular K_f. This suggests a lower hydraulic conductivity, which is compensated by the larger surface area of the peritubular capillaries.

With regard to macromolecular permeability, the situation existing in the peritubular circulation contrasts with that in the glomerulus because the convective component is directed inward in association with the continuous fluid reabsorption. Thus, the loss of macromolecules from the postglomerular capillaries occurs only as a consequence of diffusion of macromolecules from the plasma into the interstitial compartment. Although significant amounts of protein accumulate in the interstitium, the actual permeability is still quite low because of the low removal rate by the lymphatics in the renal cortex. Some studies indicate that the postglomerular circulation constrains molecules that can readily pass through the glomerular membrane. There also may be a small population of pores with diameters greater than 5 nm. Nevertheless, most of the channels have a high degree of efficiency in restricting albumin and other plasma proteins, so their reflection coefficients are very close to 1. This occurrence is due, in part, to an electrostatic barrier similar to that found in the glomerular capillaries such that negatively charged macromolecules permeate more slowly than neutral molecules of the same size. Thus, plasma proteins exert almost their full osmotic pressure across the peritubular capillaries. In spite of these high reflection coefficients, the concentration of albumin in the renal lymph, and presumably in the interstitial fluid, is about one-fourth that in systemic plasma. Although this concentration seems rather high, it should be noted that lymph flow is very low and less than 1% of net protein is lost from the plasma flowing through the peritubular capillaries.[2]

Maintaining the high density of peritubular capillaries is of critical importance in providing adequate oxygenation to the surrounding tubules. Renal interstitial inflammation may lead to reduced peritubular capillary density and the impairment of renal function, resulting in salt-sensitive hypertension. Peritubular capillary loss in renal transplant patients is associated with interstitial fibrosis and tubular atrophy, leading to reduced renal function.[85,86]

Lymphatic capillaries, primarily distributed throughout the cortex, are very permeable to protein and fluid. They

serve to return the proteins that leak out of the peritubular capillaries back to the circulation, and it is usually assumed that the protein concentration in the lymph reflects the protein concentration in the interstitial fluid. The normal renal lymph flow in humans is estimated to be about 2 to 5 mL per minute, or less than 1% of the plasma flow. Lymph flow is increased by elevations in interstitial hydrostatic pressure, such as those accompanying diuretic states, ureteral obstruction, or increases in renal venous pressure.[87,88]

Capillary Uptake by the Vasa Recta

Efferent arterioles of juxtamedullary nephrons provide the vascular supply to the medulla. These efferent arterioles branch into long-looped capillaries, termed the vasa recta, which descend into the medulla in vascular bundles. The vasa recta bundles are intimately associated with and are surrounded by concentric rings of loops of Henle and collecting ducts. The medullary circulation has the important function of removing water and solutes reabsorbed from descending and ascending loops of Henle and collecting ducts without disrupting the large longitudinal osmotic gradients that exist in the inner medulla during water conserving states. This delicate balance is achieved by virtue of the low blood flow and an efficient countercurrent diffusion of fluid and small molecular-weight solutes, which occur because of the specialized structures of the hairpin-shaped parallel loops of the descending and ascending vasa recta. The end result is passive equilibration and shunting of fluid across the vasa recta loops, from the descending to the ascending limbs, and the trapping of solute at the bends. The descending vasa recta have a continuous thick endothelium but aquaporin-1 channels allow for water efflux. Because protein permeability is low, the high plasma protein concentration of the efferent arteriolar blood is preserved. In contrast, the ascending vasa recta have a highly fenestrated thin endothelium, which greatly facilitates passive reabsorption. The ascending vasa recta also have a higher permeability to protein. However, the hydrostatic pressure in the ascending vasa recta is relatively low, about 10 mm Hg, and probably not much higher than interstitial hydrostatic pressure. In the face of a very small outward hydrostatic pressure gradient, the transcapillary colloid osmotic pressure gradient provides an important reabsorptive force, favoring capillary fluid uptake throughout these specialized capillaries. Importantly, the outer medullary descending vasa recta are encircled at points by contractile pericytes that provide a means to locally regulate blood flow.[2,15,19,89,90]

A Quantitative Analysis of Filtration and Reabsorption Dynamics

The mechanisms regulating GFR involve complex interactions among the individual determinants. To achieve a better understanding of the singular effects of each determinant, one can examine the theoretical influence of selective changes in an idealized situation where the other determinants are held constant. Such theoretical predictions can be made from the simple mathematical model shown in Figure 3.12, which analyzes fluid flow dynamics along the length of a single filtering capillary and the resistances of the afferent and efferent arterioles.

This model can be used to analyze the effects on GFR of singular perturbations, such as changes in the transcapillary hydrostatic pressure gradient, the systemic plasma protein concentration, the glomerular plasma flow, and the filtration coefficient. As shown in Figure 3.13 (panel A), changes in the transcapillary hydrostatic pressure difference produce striking responses in GFR. An increase of 10% causes a greater relative increase in EFP leading to an increase in GFR of 19%. GFR is inversely related to plasma colloid osmotic pressure; as can be seen in panel B, a 10% increase of the plasma protein concentration reduces GFR by 25%. The influence of changes in K_f and in plasma flow on GFR are more complex because they affect the rate of rise of plasma colloid osmotic pressure along the capillary bed and thus EFP. Panel C in Figure 3.13 shows that GFR is affected more by decreases than by increases in K_f. The reduced effect of increases in K_f above the normal values reflects the achievement of filtration–pressure equilibrium. Once filtration equilibrium is reached, further increases in K_f enhance ultrafiltration in early portions of the capillary, which causes protein concentration to increase more rapidly. However, this effect is offset because the colloid osmotic pressure equilibrates with the

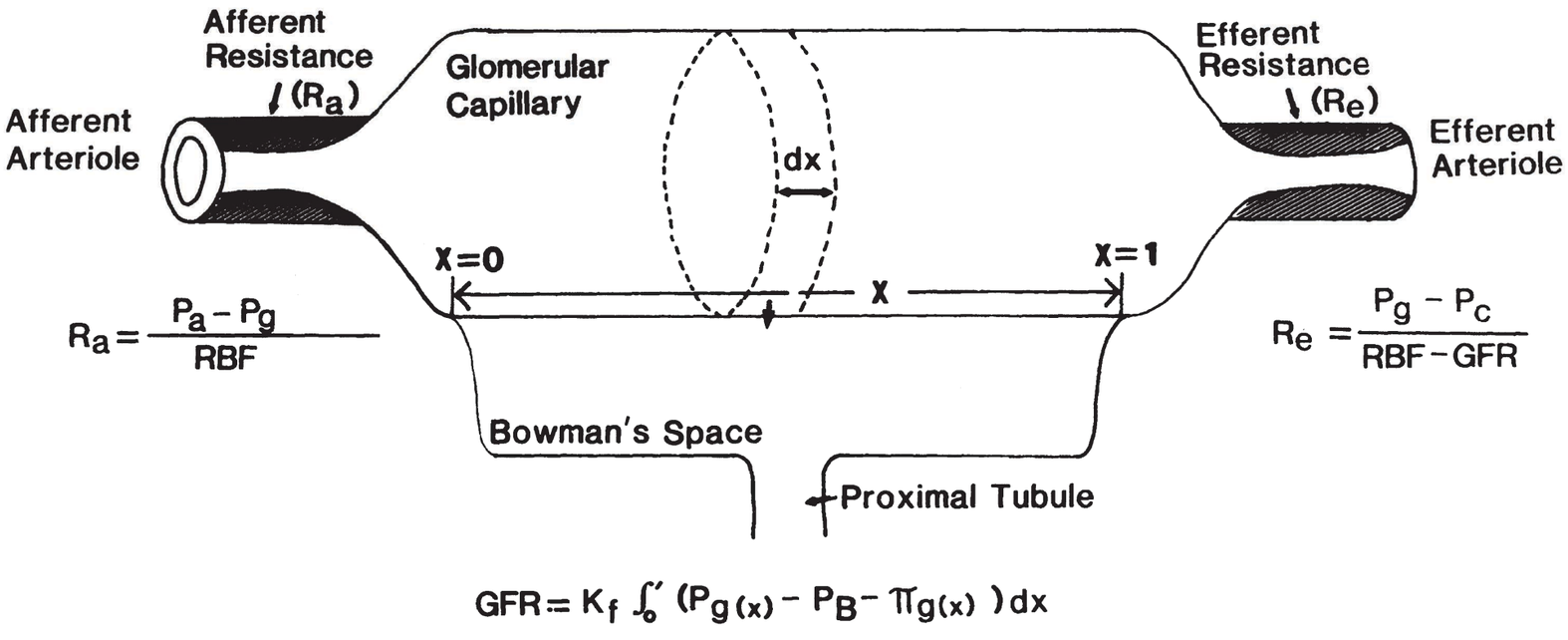

FIGURE 3.12 A "single capillary" model of glomerular filtration dynamics and afferent arteriolar (*R*a) and efferent arteriolar (*R*e) resistances. P_a, arterial pressure; P_g, glomerular capillary pressure; P_c, peritubular capillary pressure; P_B, Bowman's space pressure; *RBF*, renal blood flow; *GFR*, glomerular filtration rate.

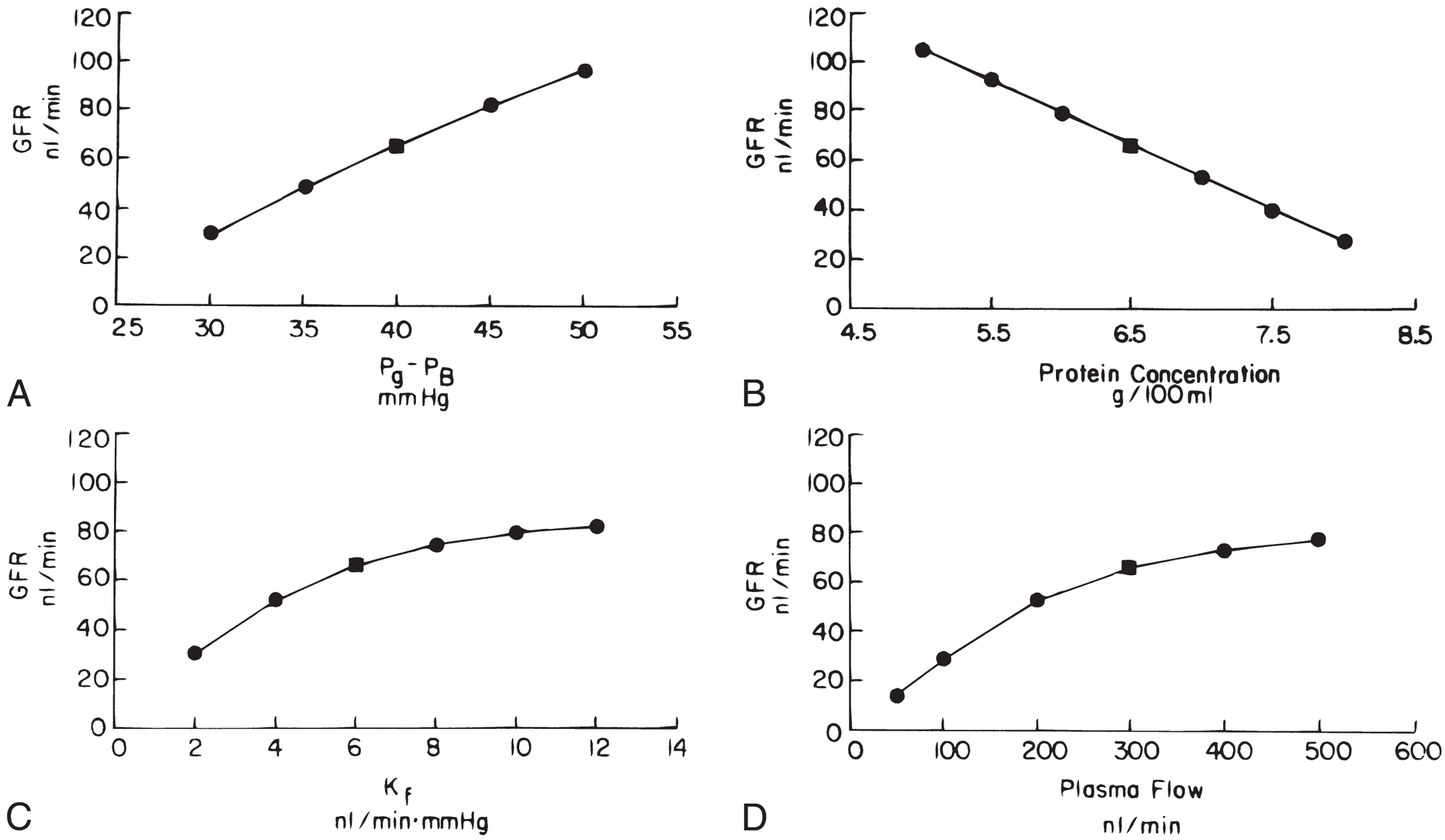

FIGURE 3.13 Theoretical effects of singular perturbations in **(A)** the transcapillary hydrostatic pressure gradient ($P_g - P_B$), **(B)** the plasma protein concentration, **(C)** the filtration coefficient (K_f), and **(D)** the plasma flow at entry to glomeruli. For these simulations, control values (*squares*) estimated to be representative of single nephron function in humans were used: GFR = 65 nL/min; plasma flow = 300 nL/min; ($P_g - P_B$) = 40 mm Hg; plasma protein concentration = 6.5 g/dL. *GFR*, glomerular filtration rate.

hydrostatic pressure difference at a more proximal site along the capillary. Thus, the mean EFP and the total GFR remain the same, although the locus of equilibrium is shifted to an earlier site.

Panel D of Figure 3.13 demonstrates that the effects of glomerular plasma flow on GFR are also nonlinear. In the absence of changes in the other determinants, GFR increases only modestly with increases in plasma flow. On the other hand, decreases in plasma flow below 200 nL per minute produce roughly proportional decrements in GFR. This increase in sensitivity is again due to the attainment of filtration equilibrium at the lower values of plasma flow. Glomerular plasma flow exerts these effects by modifying the intraglomerular profile of colloid osmotic pressure and thus mean EFP. The effects of increases in plasma flow during filtration equilibrium and not in equilibrium were discussed earlier and are shown in Figure 3.10. Changes in plasma flow have a relatively small effect on the colloid osmotic pressure profile during filtration disequilibrium (Fig. 3.10). A decrease in plasma flow increases the fraction of plasma being filtered per unit of capillary length in proximal segments. As a result, the rate of rise of colloid osmotic pressure is increased progressively. During filtration pressure equilibrium, GFR is highly plasma flow dependent. Thus, there are two major functional consequences of filtration pressure equilibrium. GFR is insensitive to increases in K_f and is strongly influenced by changes in plasma flow. In contrast, GFR is directly responsive to K_f and is less plasma flow dependent under disequilibrium conditions. In either situation, glomerular capillary pressure is quantitatively a much more powerful determinant of GFR than plasma flow.

A mathematical analysis that is of more physiologic relevance involves an integrated consideration of changes in preglomerular and efferent arteriolar resistance on glomerular dynamics. The predicted effects of constriction and dilation of either afferent or efferent arterioles under idealized conditions are presented in Figure 3.14, when the other resistances as well as other inputs are maintained at normal values. A selective increase in afferent resistance reduces plasma flow and hydrostatic pressure in glomerular and peritubular capillaries. GFR decreases proportionately more than plasma flow and thus the filtration fraction falls. In contrast, an increase in efferent arteriolar resistance reduces plasma flow but increases glomerular pressure. GFR initially increases slightly but then reaches a plateau. The plateau region of mean EFP is due to the counteracting effects of the increases in glomerular capillary and colloid osmotic pressures. From these quantitative considerations, it is apparent that the preglomerular resistance is ideally suited to control GFR. Efferent arteriolar resistance contributes to subtle alterations in GFR but exerts major effects on the dynamics of the postglomerular circulation. It is important to

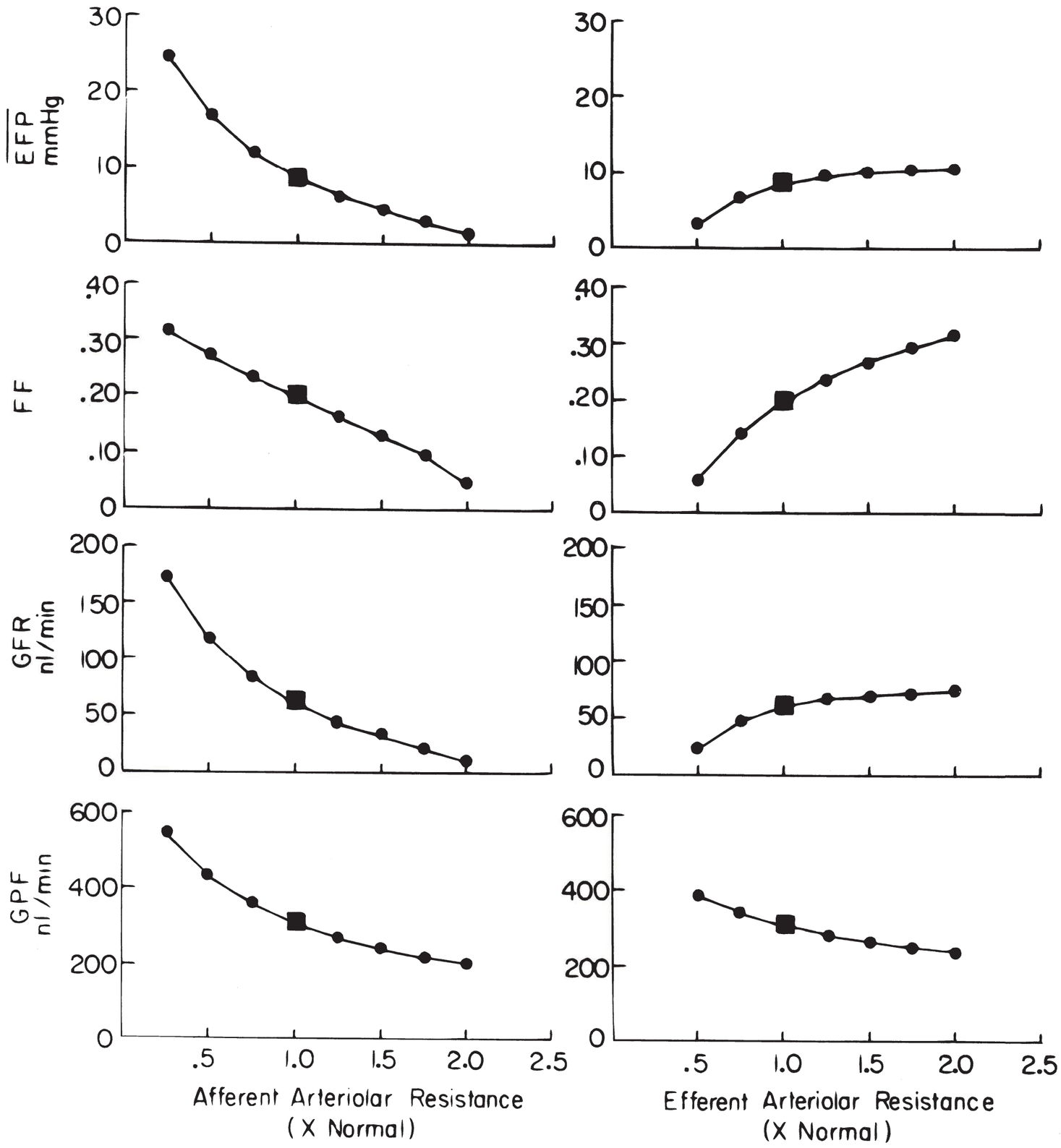

FIGURE 3.14 Effects of increases and decreases in afferent and efferent arteriolar resistance on the glomerular filtration rate (*GFR*), plasma flow (*GPF*), the filtration fraction (*FF*), and the mean effective filtration pressure (*EFP*).

emphasize that changes in filtration fraction alone cannot be used to determine the localization of resistance changes to a specific manipulation, condition, or drug. For example, combined increases in afferent and efferent resistances reduce the plasma flow proportionately more than the GFR, and the filtration fraction increases. Likewise, combined decreases in afferent and efferent arteriolar resistances increases blood flow proportionately more than GFR and filtration fraction falls. These changes in the filtration fraction have often been interpreted incorrectly as being indicative of selective change in efferent arteriolar resistance.

Control of Glomerular Dynamics by the Filtration Coefficient

In addition to the regulation of capillary flow and pressure by resistance changes of the preglomerular and postglomerular vascular segments, K_f and K_r may be influenced by many factors. Alterations in the size of the capillaries or closure of a fraction of the capillaries may reduce the available filtering surface area and thus influence K_f. The hydraulic conductivity may be altered by adjustments in the size and number of endothelial fenestrations, the thickness or permeability of the basement membrane, and the number or structural configuration of the slit pores between the foot processes. Recent attention has been focused on the role of the podocytes in control of GFR. Changes in any of these properties could be manifested as changes in K_f.[91]

Animal studies suggest that vasoconstrictor hormones and some vasodilators are capable of reducing K_f. Also, K_f may be reduced drastically in disease states that involve sclerosis of glomerular capillaries or thickening of the basement membrane. K_f may be increased slightly in certain circumstances, such as increased plasma colloid osmotic pressure. Differences in basal K_f are reported among different species and different strains and colonies of rats. Agents such as Ang II and catecholamines decrease K_f. Blockade of the vascular effects of Ang II negates the K_f lowering effect of prostaglandins E_2 and I_2 and parathyroid hormone (PTH). In addition, several vasodilators (acetylcholine, bradykinin, and histamine) decrease K_f in rats through a mechanism that is not clear. Although the vasodilator actions are primarily due to NO release, it is not apparent how this would decrease K_f. In contrast to the effects observed in rats, vasodilator

agents do not affect K_f appreciably in dogs. The reason for this apparent species difference is not known. Nevertheless, it seems clear that a variety of paracrine agents, including NO and ET-1, can influence K_f and filtration dynamics.[1,36,77]

With regard to the postglomerular vasculature, the major regulator of hydrostatic pressure in the peritubular capillaries is the efferent arteriolar tone. For a given flow, an increase in efferent arteriolar resistance increases the pressure drop along this vessel and thus reduces the pressure in peritubular capillaries. An increase in downstream resistance due to venous obstruction or elevated tubular pressure increases hydrostatic pressure in the capillaries. Interstitial hydrostatic pressure changes in the same direction as pressure in the peritubular capillaries. Colloid osmotic pressure of blood entering the peritubular capillaries is primarily regulated by the filtration fraction. A higher colloid osmotic pressure exerts a greater reabsorptive force in the postglomerular circulation. The colloid osmotic pressure in interstitial fluid is determined by a balance of protein entry from circulating plasma and protein exit by means of the lymphatic circulation. In general, stimuli promoting efferent arteriolar constriction reduce hydrostatic pressure in peritubular capillaries and increase efferent arteriolar colloid osmotic pressure, changes that favor increased fluid reabsorption from the renal interstitium into the peritubular capillaries. Vasodilating stimuli have the opposite effects and are often accompanied by natriuretic and diuretic responses. In all cases, however, there is a very intimate coupling between the rate of fluid reabsorption from the tubules into the interstitium and fluid reabsorption from the interstitial compartment into peritubular capillaries.[87]

THE REGULATION OF RENAL HEMODYNAMICS

The high sensitivity of glomerular and peritubular capillary dynamics to variations in the intrarenal pressures and flows emphasizes the importance of regulatory mechanisms that maintain the intrarenal hemodynamic environment. Overall control is shared by several mechanisms that exert specific effects on various segments of the renal vasculature. Some of these mechanisms are intrinsic to the kidney, whereas others depend on extrarenal signals mediated by neural or hormonal stimuli.

Mechanisms of Autoregulation

Intrinsic paracrine signals can adjust intrarenal vascular resistance in response to a variety of extrarenal perturbations. Alterations in vascular resistance serve to counter the effect of the extrarenal disturbance to stabilize RBF and GFR. The most widely studied manifestation of these intrinsic mechanisms is the phenomenon of renal autoregulation. In response to alterations in renal arterial pressure over a wide range, the kidney adjusts its vascular resistance to maintain, or "autoregulate," RBF. This range encompasses the physiologically relevant arterial pressures, both above and below normal. In response to reductions in arterial pressure, which may occur during situations such as sleep or recumbence, intrarenal mechanisms decrease renal vascular resistance (RVR) to maintain RBF and GFR at optimum levels. Likewise, increases in arterial pressure, which might occur during exercise or acute episodes of stress, elicit intrarenal signals that increase vascular resistance and thus maintain RBF and GFR at or near control levels. In addition to RBF and GFR, the microvascular and tubular pressures exhibit autoregulatory behavior. Because glomerular pressure and GFR are autoregulated, the predominant adjustments of vascular resistance are localized to the preglomerular arterioles. Figure 3.15 shows representative relationships between the renal arterial pressure and RBF, GFR, and segmental vascular resistances. The responses of vascular resistance to changes in perfusion pressure represent the most commonly investigated aspect of the renal autoregulatory mechanism, but other stimuli, such as increases in ureteral or renal venous pressure or changes in plasma colloid osmotic pressure, also elicit autoregulatory responses. The response serves a negative feedback function to counteract the effect of the disturbance and restore RBF or GFR back toward normal.[1,2,92–94]

Although complex interactions with multiple signaling pathways are involved in the total response, the autoregulatory component of the vasculature requires activation of voltage-dependent Ca^{2+} channels and is prevented by L-type Ca^{2+} channel blockers. Much of the research oriented toward understanding this basic response is focused on the mechanisms by which messages are initiated, transmitted to, and received by the smooth muscle cells to affect the requisite alterations in vascular resistance. The two basic mechanisms that contribute to the autoregulation phenomenon are the myogenic and the TGF mechanisms. A third possible contributor has been identified recently, but the underlying mechanism is not known.[2,92–94]

The myogenic mechanism responds to a distending force on the vessel wall caused by an increase in arterial pressure. The actual distending force can be calculated from the law of Laplace that relates tangential wall tension (T) to the inner radius of the vessel (r) and the transmural pressure difference:

$$T = r\,(P_a - P_i) \tag{8}$$

where P_a is intra-arteriolar hydrostatic pressure, and P_i is the interstitial fluid pressure. When the transmural pressure difference increases, wall tension is increased, which activates Ca^{2+} channels and leads to constriction and a reduction of the radius allowing the tension to return to normal. A myogenic response occurs in preglomerular vessels but not postglomerular efferent arterioles. This may be due to the differential activating mechanisms in these vessels because efferent arterioles do not normally have functional L-type Ca^{2+} channels. In addition to the afferent arteriole, the arcuate and interlobular arteries also display myogenic responses.

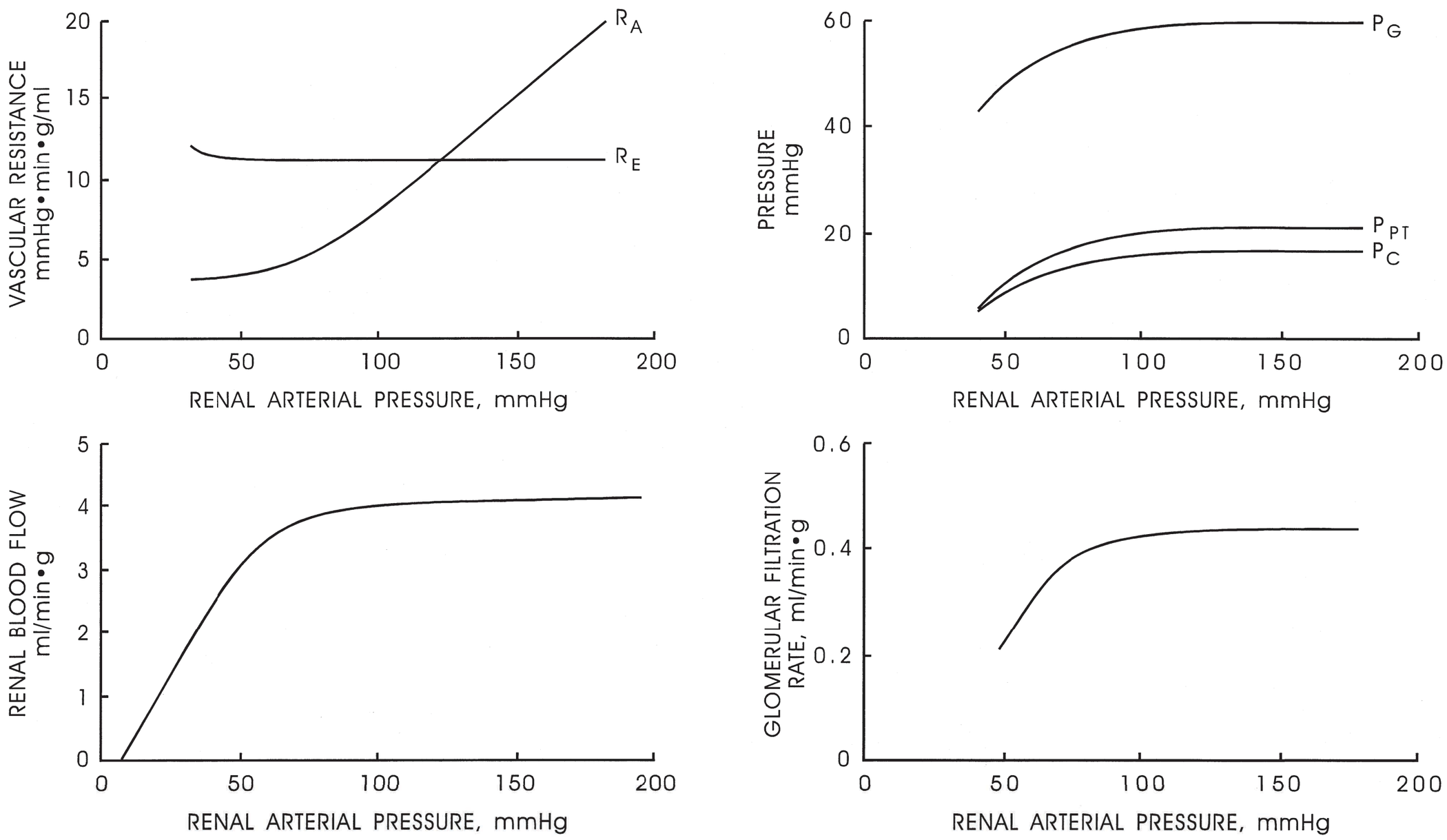

FIGURE 3.15 Representative relationships between renal arterial pressure and renal blood flow (*lower left panel*), glomerular filtration rate (*lower right panel*), and segmental vascular resistance (*upper left panel*) for afferent arteriolar resistance (R_A), efferent arteriolar resistance (R_E), and hydrostatic pressure (*upper right panel*) in glomerular capillary (P_g), proximal tubule (P_{PT}), and peritubular capillary (P_C).

Although a large vessel such as the arcuate artery responds to pressure, its contribution to total resistance is quite small. Thus, the preglomerular arteriolar network has the ability to respond to extrinsic physical or mechanical disturbances by an intrinsic myogenic response of vascular smooth muscle. The initial autoregulatory adjustments in vascular resistance occur rapidly (a few seconds) as a result of a direct local vascular mechanism. Such a fast response is thought to buffer the glomerular capillaries and the tubular network from sudden changes in arterial pressure and protect from high systolic pressures, especially at higher frequencies. Long-standing glomerular hypertension is associated with proteinuria and the development of glomerular sclerosis.[1,93,95–97]

A contraction caused by an increase in intraluminal pressure is elicited by cell membrane depolarization and increased Ca^{2+} entry through voltage-gated L-type Ca^{2+} channels. L-type channel blockers inhibit the myogenic response and the stretch-activated Ca^{2+} channels in the preglomerular microcirculation. T-type Ca^{2+} channels contribute to the myogenic response as a blockade of T-type channels also attenuate autoregulatory responses. In afferent arterioles, the T-type channels may sense relatively small stimuli, which then cause sufficient Ca^{2+} entry and membrane depolarization to activate voltage-gated L-type channels to activate intracellular signaling pathways and to elicit contraction. The increased $[Ca^{2+}]_i$ activates the inositol phosphate cascade to increase IP_3 and DAG and activate PKC. Inhibition of PKC attenuates the autoregulatory constrictor response to a pressure increase. Subconstrictor concentrations of either Ang II or ET-1 potentiate myogenic contraction of afferent arterioles. NO attenuates the rate and strength of the myogenic response. Although endothelial cells play a role in the rate of autoregulation, stretch-induced, steady-state myogenic tone is observed in vessels without a functional endothelium. The actual mechanosensitive transducer on vascular smooth muscle cell membranes has not been completely characterized. Cell surface integrins have been postulated to serve as mechanotransducers by responding to shear, stretch, or tension. Sodium channels have also been shown to respond to mechanical deformation via a degenerin/epithelial sodium channel complex.[2,14,92,93,98–101]

Additional theories to explain the autoregulatory phenomenon evolved because of the recognition that slower acting hemodynamic mechanisms are responsive to the metabolic demands of tubular transport. The existence of structures within the kidney that seem ideally suited to act as communication links between the distal tubular segments and the vasculature provide the morphologic basis for the TGF hypothesis. Indeed, the unique configuration of the juxtaglomerular apparatus (Fig. 3.4) allows the macula densa to sense some aspect of fluid composition at the end of the ascending loop of Henle and transmit a signal(s) to the afferent arteriole of the parent glomerulus. Thus, the juxtaglomerular apparatus provides the anatomic basis for a negative feedback mechanism, operating in each nephron,

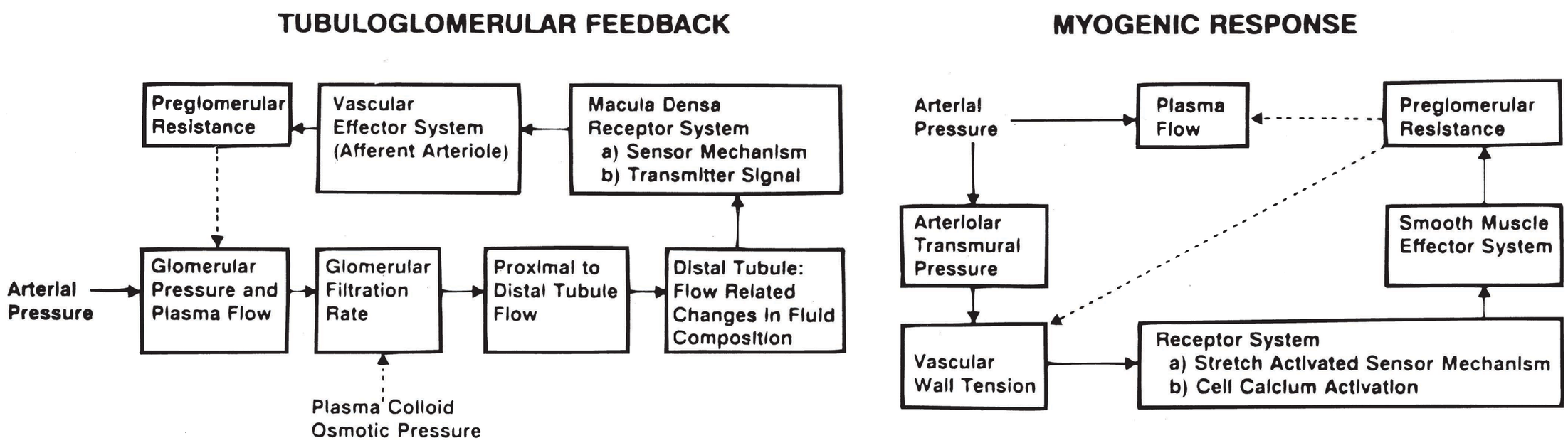

FIGURE 3.16 The macula densa tubuloglomerular feedback hypothesis (*left panel*) and the myogenic response (*right panel*) as mechanisms to explain renal autoregulation. *Solid lines* indicate direct relations; *dashed lines* indicate inverse effects.

that maintains balance between the hemodynamic inputs that control GFR and the filtered load and the metabolically determined reabsorptive function of the tubules.[1,2,102]

Autoregulation mediated by the TGF mechanism is shown in the left panel of Figure 3.16. For example, an increase in arterial pressure initially increases RBF, glomerular pressure, and GFR. The increased filtered load increases fluid and solute delivery from the proximal convoluted tubule into the loop of Henle. Such an effect leads to flow-dependent increases in NaCl concentration and osmolality of the tubular fluid in the ascending loop of Henle. The macula densa cells sense the increased tubular fluid NaCl or total solute concentration and transmit a vasoconstrictor signal(s) to the afferent arteriole and thus restore RBF and GFR to preexisting levels. Conversely, a decrease in arterial pressure causes a reduction in tubular fluid flow that elicits dilation of the afferent arterioles. The presence of primary cilia on the lumen of macula densa cells provides a flow sensing mechanism such that increased flow, per se, activates macula densa signals that elicit afferent arteriolar constriction. The TGF mechanism helps explain vascular responses that occur when the solute load to the distal nephron changes as a consequence of changes in tubular reabsorptive function such as those pharmacologically induced in the proximal reabsorption rate.[2,12,14,103–105]

Studies at the single nephron level indicate that the maintenance of flow to the distal nephron is a requisite for the full manifestation of autoregulation of GFR. As is shown in Figure 3.17, autoregulation of single nephron GFR in

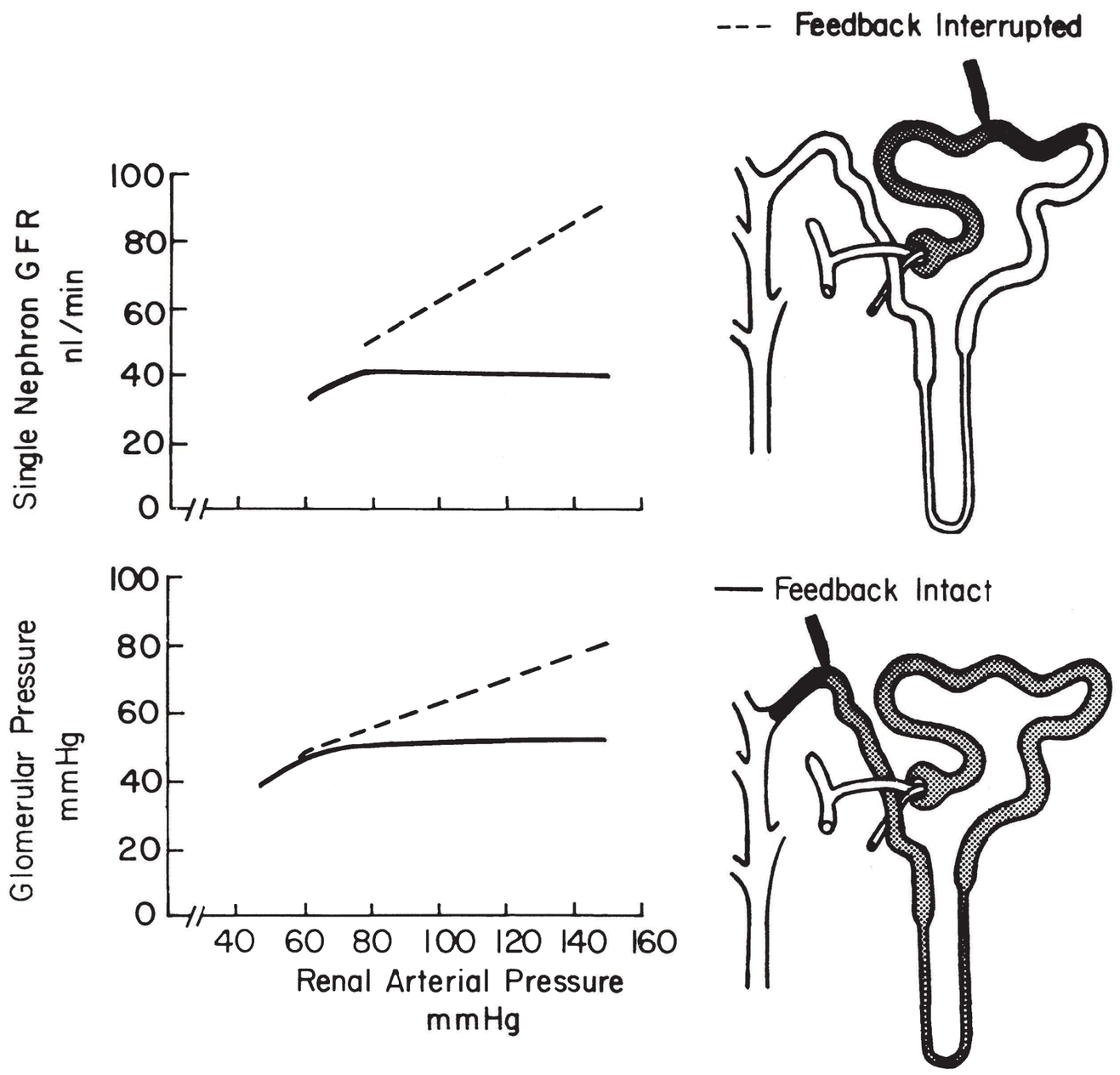

FIGURE 3.17 Responses of single nephron glomerular filtration rate (*GFR*) and glomerular pressure to changes in arterial pressure during conditions of intact flow to the macula densa (*solid lines*) and during interrupted flow conditions (*dashed lines*).

response to acute changes in arterial pressure is highly efficient when tubular fluid flow to the distal nephron is maintained, but it is significantly impaired when flow past macula densa cells is interrupted. Similar responses have been reported for glomerular capillary pressure. Nevertheless, the impairment in GFR autoregulation is not as great as would be predicted for a fully passive mechanism, indicating that the TGF mechanism works in concert with the myogenic mechanism to yield the highly efficient autoregulation characteristic of renal circulation. Recent studies suggest that the interactions are synergistic, in that the presence of active macula densa signals augments the sensitivity of the myogenic response. The afferent arteriole is the effector limb of both mechanisms, and the blockade of Ca^{2+} entry through L-type channels inhibits both the myogenic and TGF responses. The highly localized myogenic component can respond very quickly to a pressure stimulus. The TGF loop is more complex, involving multiple structures and cell types, and its response to a pressure change being transmitted along the tubule is slower, on the order of 15 seconds. The relative importance of these two systems may vary according to experimental conditions. Normally each contributes about 45% to near perfect autoregulation initiated by an acute, single step change in renal perfusion pressure. A putative third component contributes roughly 10% to the final adjustments in RVR that occurs between 25 and 100 seconds.[92,93,96,97,106]

Increases in flow through the loop of Henle elicit the constriction of the parent afferent arteriole with consequent reductions in glomerular pressure and the filtration rate of the same nephron. These responses are represented in Figure 3.18. Note that the response is nonlinear, with the most sensitive region in the physiologic range of tubular flow. Another important feature is that the reactivity or sensitivity of the TGF mechanism can be modified by a variety of paracrine agents, hormones, and pharmacologic agents. Increased sensitivity is generally associated with extracellular fluid volume contraction, and reduced responsiveness has been observed during expansion of extracellular fluid volume.[107]

The macula densa sensing segment is located at the end of the ascending loop of Henle, a nephron segment that is virtually impermeable to water and has a powerful NaCl cotransport mechanism. Such transport characteristics result in the delivery of a hypotonic fluid to the macula densa cells. The nature of the transport processes of the thick ascending limb is discussed in detail in Chapter 4. In essence, increases in fluid delivery from the proximal tubule lead to progressive increases in distal flow, sodium chloride concentration, and osmolality. This coupling between fluid flow through the ascending limb and tubular fluid solute concentration at the macula densa provides the means by which volume delivery out of the proximal tubule is sensed and regulated. The signal sensed by macula densa cells may be a specific constituent of tubular fluid such as sodium or chloride or total solute concentration.[1,107,108]

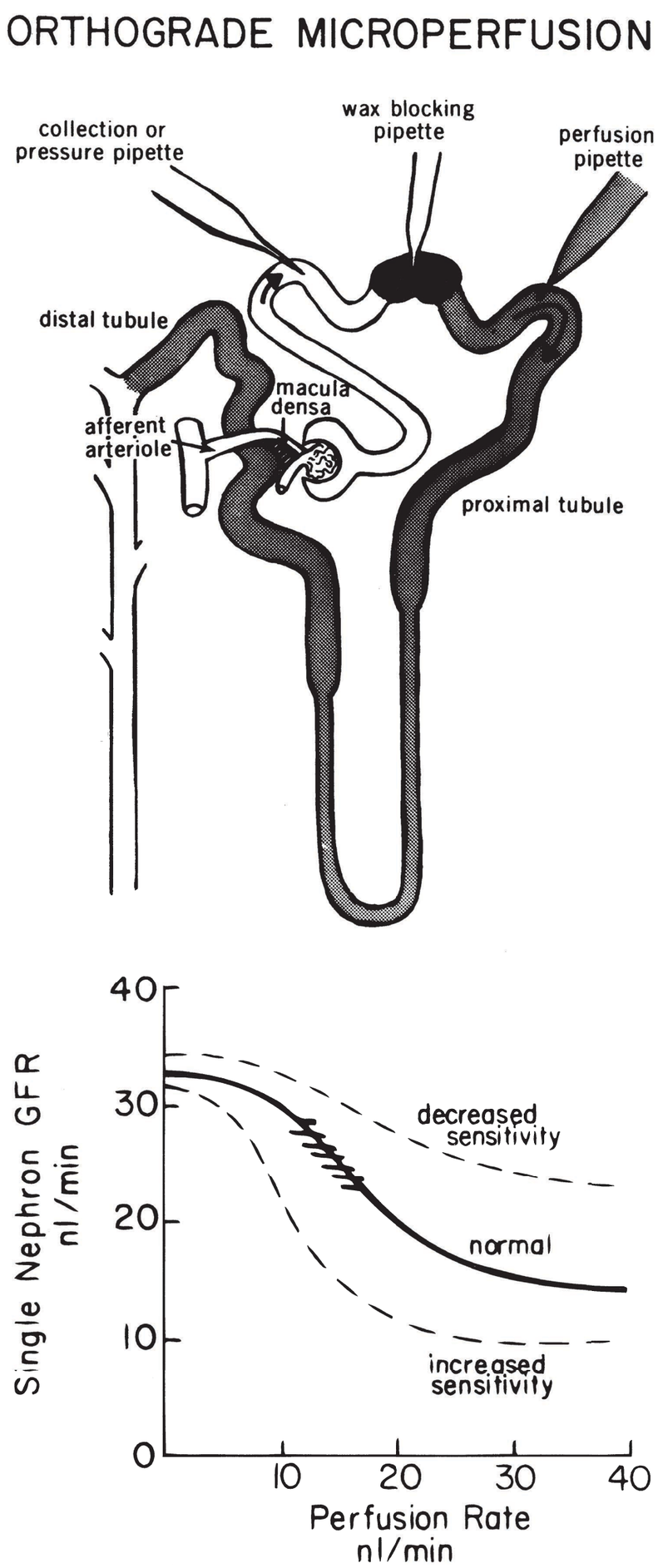

FIGURE 3.18 The relationships between the perfusion rate into a late proximal tubule and a single nephron glomerular filtration rate (GFR). *Dashed lines* show responses during conditions of decreased and increased feedback sensitivity. The *stippled area* shows the physiologic range of single nephron GFR as a function of late proximal flow rate. The technique used to obtain the feedback responses is illustrated in the *top panel*.

The cellular mechanisms responsible for transmitting signals have remained controversial and intriguing. Macula densa cells, like those of the thick ascending limb of Henle, possess a Na-K-2Cl cotransporter, which is sensitive to the diuretic furosemide. This cotransporter must be functional for TGF signals to be transmitted, but it may not be the actual mechanism that activates intracellular signals, which appear to involve increases in Ca^{2+} entry from the basolateral side of the macula densa cells and increased $[Ca^{2+}]_i$ in response to increases in tubular fluid osmolarity or NaCl concentration

of the luminal fluid. The mechanisms by which $[Ca^{2+}]_i$ is related to the formation and release of vasoactive mediators of TGF signals remain unclear. Calcium changes can be counteracted by elevations in cAMP. These intracellular ionic changes signal the macula densa cells to form and secrete constrictor and dilator substances that influence vascular contraction as a function of macula densa transport. The actual final effector messenger between the macula densa cells and the afferent arteriole(s) remain under investigation. Early studies fostered the idea that the effector signal was locally formed Ang II. However, it is now clearly established that the changes in the activity of the renin–angiotensin system modulate the sensitivity of the feedback response but do not directly mediate TGF signaling. The effector agent is thought to operate primarily by activating voltage-dependent Ca^{2+} channels in the afferent arteriole and perhaps the interlobular artery. Attractive candidates that have been considered recently include purinergic agents such as adenosine and ATP or arachidonic acid metabolites. Several recent studies link the secretion of ATP by macula densa cells to afferent arteriolar constriction via the activation of P_2 receptors and associated increases in Ca^{2+} entry via voltage-dependent Ca^{2+} channels. In support of this notion, a defective P_2X receptor function is associated with impaired TGF function of juxtamedullary nephrons.

An alternative view is that the ATP is metabolized to adenosine, which elicits afferent arteriolar vasoconstriction. Consistent with this proposal, mice lacking adenosine A_1 receptors do not exhibit TGF responses in superficial nephrons. Also, mice lacking the nucleotidase involved in degrading ATP to adenosine have impaired TGF. Another possibility is that an arachidonic acid metabolite such as 20-HETE participates in mediating TGF-dependent vasoconstriction. Studies show that increased luminal NaCl concentration leads to NO release, which counteracts the constrictor response. In contrast, PGE_2 release is increased by reduced NaCl concentration.[2,12,103,107,109–112]

The Modulation of Tubuloglomerular Feedback Activity by Vasoactive Agents

Macula densa cells also signal the juxtaglomerular cells to regulate renin synthesis and release. Mechanisms of renin release are discussed in the section on the renin–angiotensin system. In brief, the directional changes in renin release and Ang II formation are opposite to those that are required for Ang II to mediate TGF. For example, high tubular flows and elevated luminal NaCl concentrations are associated with reduced renin release but TGF-mediated afferent arteriolar constriction. Nevertheless, Ang II exerts an important role in modulating TGF activity during changes in salt diet, extracellular fluid volume, and renal perfusion pressure. Tubuloglomerular feedback is absent in mice lacking AT_{1A} receptors, although the renal vasculature is capable of responding to Ang II. Tubuloglomerular feedback is nonresponsive in animals unable to produce Ang II when ACE is mutated.[2,14,104]

The neuronal NO synthase (NOS) isoform localized in macula densa cells produces NO in response to increased tubular flow above the normal range, which modulates TGF activity. A blockade of NO synthesis augments the strength of TGF responses, whereas enhanced NOS levels attenuate the vasoconstrictor response to increased distal nephron flow. Salt uptake across the luminal membrane by a furosemide-sensitive Na-K-2Cl transporter may link increases in cellular cAMP and $[Ca^{2+}]_i$ to NO production. O_2 radicals generated in the vicinity of the juxtaglomerular apparatus can act to scavenge NO, limiting macula densa NO signaling and thereby producing vasoconstriction and enhancing TGF activity. Normal TGF is found in gene knockout animals lacking neuronal NOS.[2,113–115]

Arachidonic acid metabolites also modulate TGF activity and interact with other vasoactive modulators. Cyclooxygenase 2 (COX-2) has been localized to macula densa cells and the surrounding ascending loop of Henle cells; the release of PGE_2 from macula densa cells occurs in response to reduced luminal NaCl. A COX-2 metabolite attenuates the vasoconstrictor autoregulatory and TGF-mediated response of the afferent arteriole to an increase in arterial pressure. Such a dilator agent also appears to contribute to the macula densa production of NO, which inhibits afferent arteriolar responses to pressure. Thromboxane A_2 and a cytochrome P450 metabolite such as 20-HETE are also involved in the constrictor limb of TGF. However, gene targeting rendering the thromboxane receptor nonfunctional has no effect on TGF activity.[104,116–118]

Recent evidence supports the existence of a second TGF loop that links increases in connecting tubule sodium reabsorption via epithelial sodium channels to dilation of the afferent arteriole of the parent glomerulus. It is noteworthy that the response of this positive feedback loop is opposite to the constrictor signal arising from macula densa cells in response to increased salt delivery to the end of the thick ascending limb of Henle. The connecting tubule signal transmitted to the afferent arteriole to increase GFR involves COX-derived prostaglandins and epoxygenase-derived EETs. The magnitude of this feedback response is enhanced by Ang II acting on AT_1 receptors to stimulate epithelial sodium channel activity and sodium reabsorption. The connecting tubule–glomerular feedback circuit may function to dampen the effects of vasoconstrictor stimuli on the afferent arteriole.[119,120]

The Renin–Angiotensin System

The Formation of Ang II

The renin–angiotensin system exerts control of renal hemodynamics via its major vasoactive metabolite Ang II, which acts as both a circulating hormone and a locally generated paracrine agent. Renin is a proteolytic enzyme that cleaves Ang I from renin substrate (angiotensinogen) that is formed primarily by the liver but also in the kidney. Renin is synthesized primarily in epithelioid granular cells of the

juxtaglomerular apparatus and is secreted into the surrounding interstitium; renin is also formed in proximal tubule cells and principal cells of connecting tubule and collecting duct segments. Angiotensinogen availability in the plasma and intrarenal compartments is less than is required to produce maximum reaction velocity, so alterations in substrate levels contribute to the regulation of Ang I production. The inactive decapeptide Ang I is then cleaved by ACE to form the active octapeptide Ang II. There are abundant amounts of endothelium-bound ACE in the lungs and in almost all other tissues. Most of the Ang II in the systemic arterial blood is formed in the lungs. The major sites of ACE localization in the kidney are on the luminal surface of endothelial cells lining arteries and arterioles (in particular, the afferent arterioles), but also the efferent arterioles and the glomerular capillaries, and on the brush border and basolateral membranes of the proximal tubule; extravascular ACE is also present in the interstitial compartment and on the lumen of collecting duct segments.[2,13,121–125]

Several peptidases act on angiotensinogen to form biologically active peptides other than Ang II. Angiotensin with amino acids 1 to 7 is formed by neutral endopeptidase, but appears to have only slight effects on the renal vasculature under physiologic conditions. Elevated levels of Ang 1 to 7 occur during ACE inhibition and may dilate renal and nonrenal vascular beds. Ang III (angiotensin with amino acids 2 to 8) has actions similar to that of Ang II, which can be blocked by Ang II receptor antagonists. Ang IV (angiotensin with amino acids 3 to 8), a hexapeptide, is reported to produce vasodilation by the release of NO or prostanoids from endothelial cells by acting on a specific AT_4 receptor. The recently described enzyme ACE-2 forms Ang (1 to 9) from Ang I and Ang (1 to 7) from Ang II. Thus, ACE-2 can produce more Ang (1 to 7) from Ang II and, in this manner, reduces the available Ang II.[124,126–128]

Ang II is delivered to renal vascular receptors as a circulating hormone or may be formed locally from systemically delivered Ang I by endothelial ACE. About 20% of the circulating Ang I is converted to vasoactive Ang II in the kidney. Ang II is also formed in the interstitial fluid from Ang I, generated as a consequence of enhanced renin release or from Ang I that diffuses from peritubular capillaries into the interstitium. The renal tissue Ang II levels are higher than that of arterial blood, indicating significant amounts of Ang II are generated intrarenally. In addition, Ang I and II may be formed within juxtaglomerular granular cells and coreleased with renin to act on adjacent glomerular arterioles. High concentrations of Ang II also exist in proximal and distal tubular fluid as a result of secretion by proximal tubular cells. Ang II derived from proximal tubular cells also may have vascular effects after traversing the interstitium.[123,129,130]

Renin Production and Release

As Figure 3.19 shows, renin is released in response to several stimuli, including decreases in sodium intake, the contraction of extracellular fluid volume or blood volume, increased sympathetic renal nerve activity, decreased sodium load to the macula densa, and decreased renal arterial perfusion pressure. Ang II, ET-1, NO, vasopressin, prostaglandins, and potassium also influence renin release, acting directly on juxtaglomerular cells. The final effector mechanisms center on changes in the Ca^{2+} and cAMP concentrations

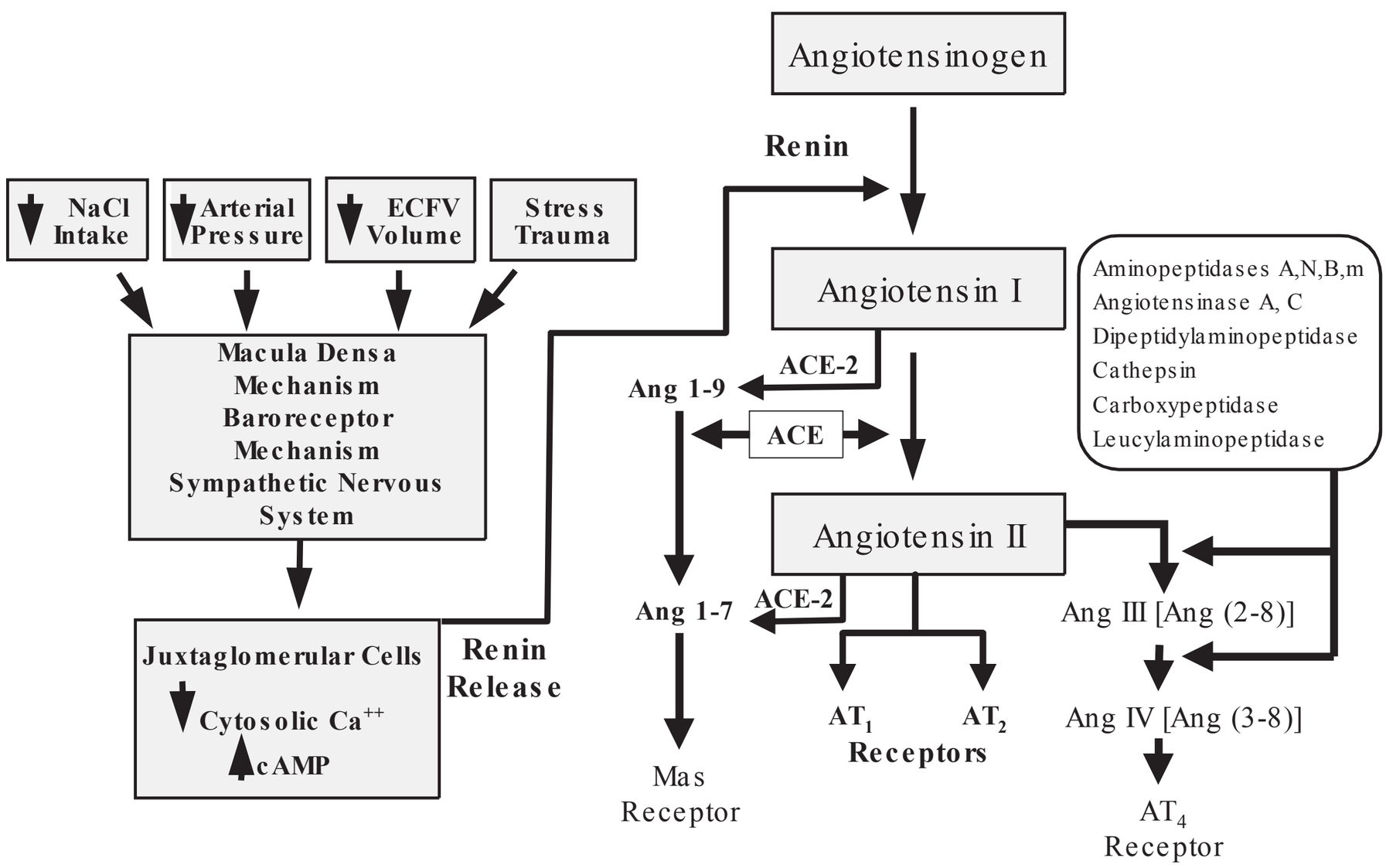

FIGURE 3.19 A schematic representation of the renin–angiotensin system and the mechanisms of renin release.

in juxtaglomerular granular cells. The Ca^{2+} mechanism is unusual for exocytosis of secretory renin granules in that a decrease in $[Ca^{2+}]_i$ functions as a stimulator of renin release, in contrast to the opposite in most other secretory cell types. Renin exocytotic release is elicited by increases in cellular cAMP levels. Cytosolic cAMP concentration is controlled by synthesis via adenylate cyclase and hydrolysis by cyclic nucleotide phosphodiesterases (PDEs). Intracellular Ca^{2+} inhibits PDE to modulate the magnitude of cAMP-mediated renin release. PDE_3 is the major isoform localized to afferent arterioles, and recent evidence indicates that the pharmacologic inhibition of PDE_3 increases cAMP and basal renin secretion and also enhances the renin secretory response to β-adrenergic and PGE_2/EP_4 receptor stimulation.

Exocytosis of secretory granules in the juxtaglomerular cells is also stimulated by cell shrinkage due to high extracellular osmolality. Juxtaglomerular cells have Ca^{2+}-sensitive voltage-gated K channels (BK_{Ca}) that are activated by cAMP to hyperpolarize cells from -32 to -48 mV. Vasoconstrictor G-protein coupled receptor agonists such as Ang II and ET-1 inhibit renin release by activating PLC and mobilizing Ca^{2+} that activates Ca^{2+} entry through store-operated cation channels.[2,13,121,131]

There are several first messenger systems that impact on cAMP or $[Ca^{2+}]_i$ in juxtaglomerular cells to regulate renin release. These are discussed briefly in the following section and in more detail in Chapter 9.

Sympathetic Nervous System and Catecholamines. Juxtaglomerular granular cells are richly innervated and respond to renal sympathetic nerve stimulation and to circulating catecholamines. There are both direct and indirect effects on renin release. Subtle increases in renal nerve traffic or circulating epinephrine activate β_1-adrenoreceptors on juxtaglomerular cells, which activate $G\alpha_s$ proteins to increase cAMP, thereby enhancing renin release. Strong renal nerve activity reduces RBF and GFR through activation of α_1-adrenergic receptors, with subsequent indirect stimulation of renin release secondary to afferent arteriolar vasoconstriction that reduces intrarenal baroreceptor and macula densa stimuli.[13,132]

Renal Vascular Baroreceptor. Decreases in renal afferent arteriolar pressure directly increase renin release independent of the renal nerves and the macula densa mechanism. The juxtaglomerular cells appear to be directly sensitive to intraluminal pressure and stretch such that decreased wall tension decreases cell Ca^{2+} entry and $[Ca^{2+}]_i$, whereas the opposite occurs at elevated arterial pressures. Under some circumstances, the same extrinsic disturbance may influence both the macula densa and the vascular baroreceptor mechanism to increase renin release, but the vascular receptor system can act independently.[2]

Macula Densa. Macula densa cells detect decreases in the NaCl load or concentration emerging from the ascending loop of Henle and send a signal(s) to the juxtaglomerular cells to increase renin secretion. Although the sensing mechanism involving furosemide-sensitive luminal uptake of NaCl is not completely understood, evidence suggests that adenosine can inhibit renin release, and its precursor, ATP, is secreted by macula densa cells in response to increases in tubular NaCl concentration and is converted to adenosine via ectonucleotidases. Circumstances that result in extracellular volume depletion or sodium deprivation stimulate renin release, at least in part, by the macula densa mechanism. Reduced tubular fluid flow to the macula densa leads to increased COX-2 activity and PGE_2 release that directly stimulates renin release via activation of EP_4 receptors and increased cAMP production in juxtaglomerular granular cells.[12,14,116,133]

Other Factors. Renin secretion is inhibited by elevated plasma or local concentrations of Ang II, vasopressin, adenosine, thromboxane A_2, and potassium. The effects of Ang II and other vasoconstrictors appear to be a consequence of an end-product inhibition due to increased $[Ca^{2+}]_i$ in juxtaglomerular cells. PGE_2 and PGI_2 can stimulate renin release through direct effects, which may be related to the stimulation of cellular cAMP levels. The atrial natriuretic peptide increases cellular cGMP production and inhibits renin release. Endothelium-derived factors also modulate renin release, with NO stimulating renin release.[13,116,121,134]

COX-2, nNOS, and renin synthesis often change parallel with changes in salt diet and alterations in tubular fluid NaCl concentration, and their products may mutually determine synthesis and activity of these enzymes. nNOS and COX-2 are coexpressed in macula densa cells and the expression of both enzymes is stimulated in volume contraction and high renin states. Parallel changes are observed during chronic changes in salt in the diet, with low salt stimulating nNOS, COX-2, and renin secretion as compared to the inhibition occurring during a chronic high salt diet. NO derived from nNOS exerts a stimulatory role on COX-2 expression to produce PGE_2 / PGI_2 and to stimulate renin release by acting on EP_4 and IP receptors, respectively. PGE_2 exerts short-loop feedback to inhibit nNOS expression. In some situations, NO appears to play a more indirect permissive role, permitting the macula densa pathway of renin secretion to function normally. NO stimulates renin release by cGMP, inhibiting PDE_3 to increase cell cAMP concentration by reducing cAMP breakdown in juxtaglomerular cells, with the activation of cAMP sensitive PKA.[13,116,121,135]

Although many stimuli increase activity of the renin–angiotensin system, most are related to circumstances that compromise body fluid volume homeostasis. Thus, the stimulation of renin release and the activation of Ang II–dependent mechanisms help to minimize renal fluid and sodium losses and to maintain extracellular fluid volume and arterial blood pressure. The myriad of actions exerted by Ang II all seem to be homeostatically appropriate to achieve this end. Only the renal vascular actions of Ang II will be covered in this chapter, but it should be pointed out

that Ang II is also a potent stimulator of aldosterone release and can directly enhance salt reabsorption by the proximal tubule, the Henle loop, and the distal nephron segments. It also has important effects on the central nervous system such as stimulating thirst, vasopressin release, and sympathetic nerve activity.[2,129]

The macula densa plaque has distinct mechanisms for renin release and the TGF system. The TGF mechanism involves the macula densa by sending a vasoconstrictor signal to the afferent arteriole in response to increases in tubular flow and the accompanying increases in solute and sodium concentration from the Henle loop. Under these conditions, the macula densa signals to decrease renin release and local Ang II activity, which is opposite to that required for Ang II to mediate a TGF-induced contraction of the afferent arteriole. Thus, Ang II clearly does not mediate TGF responses; it does, however, modulate the sensitivity of the TGF mechanism to the macula densa vasoconstrictor signal(s). When tissue Ang II levels are increased, smooth muscle responsiveness is augmented. In contrast, when Ang II levels are suppressed, whether in response to physiologic manipulations or as a consequence of a pharmacologic blockade, the sensitivity of the TGF system is attenuated. Interestingly, sensitivity can be restored by the administration of Ang II but not norepinephrine. In addition to directly affecting the afferent arteriole, Ang II affects TGF activity by altering tubular reabsorption and fluid delivery to the macula densa. The vascular and tubular effects work in concert to reduce GFR when sodium excretion is reduced as observed during volume contraction. As previously mentioned (Fig. 3.18), changes in TGF responsiveness during altered volume states may be largely due to changes in tissue levels of Ang II. The interactions between the renin–angiotensin system and the TGF mechanism are illustrated in Figure 3.20.[1,2]

The precursor of renin, prorenin, is also released by juxtaglomerular granular cells and renin-producing tubular cells. Although prorenin is inactive, it can bind to the prorenin receptor that has been recently discovered and characterized. Binding of prorenin to the prorenin receptor activates prorenin and increases its catalytic efficiency to generate Ang I from angiotensinogen. Thus, increased tissue levels of prorenin or the prorenin receptor may influence the local generation of Ang I, leading to increased Ang II formation. Prorenin also appears to signal via the MAP kinases extracellular signal regulated kinase (ERK) 1/2 to upregulate profibrotic and COX-2 genes independent of Ang II. Prorenin receptors are localized in vascular endothelial and smooth muscle cells, glomerular mesangial cells and podocytes, and collecting duct segments of the nephron.[136,137]

Angiotensin Receptors

There are two major subtypes of receptors for Ang II in the renal circulation. In utero, animals have a larger population of AT_2 than AT_1 receptors, which decreases progressively after birth; in adult life, the major subtype on vascular smooth muscle cells is the AT_1 receptor. The AT_1 receptor is present on preglomerular arteries and arterioles, juxtaglomerular granular cells, glomerular mesangial cells, efferent arterioles, and vasa recta bundles of the inner medullary stripe. Humans have one AT_1 receptor, whereas rodents have two, termed AT_{1A} and AT_{1B}, which are 94% homologous. Currently available pharmacologic antagonists of AT_1 receptors, such as candesartan, losartan, and valsartan, do not distinguish between the two AT_1 subtypes in rodents. Almost all of the vasoconstrictor actions of Ang II on the renal vasculature under physiologic conditions are mediated by AT_1. Both

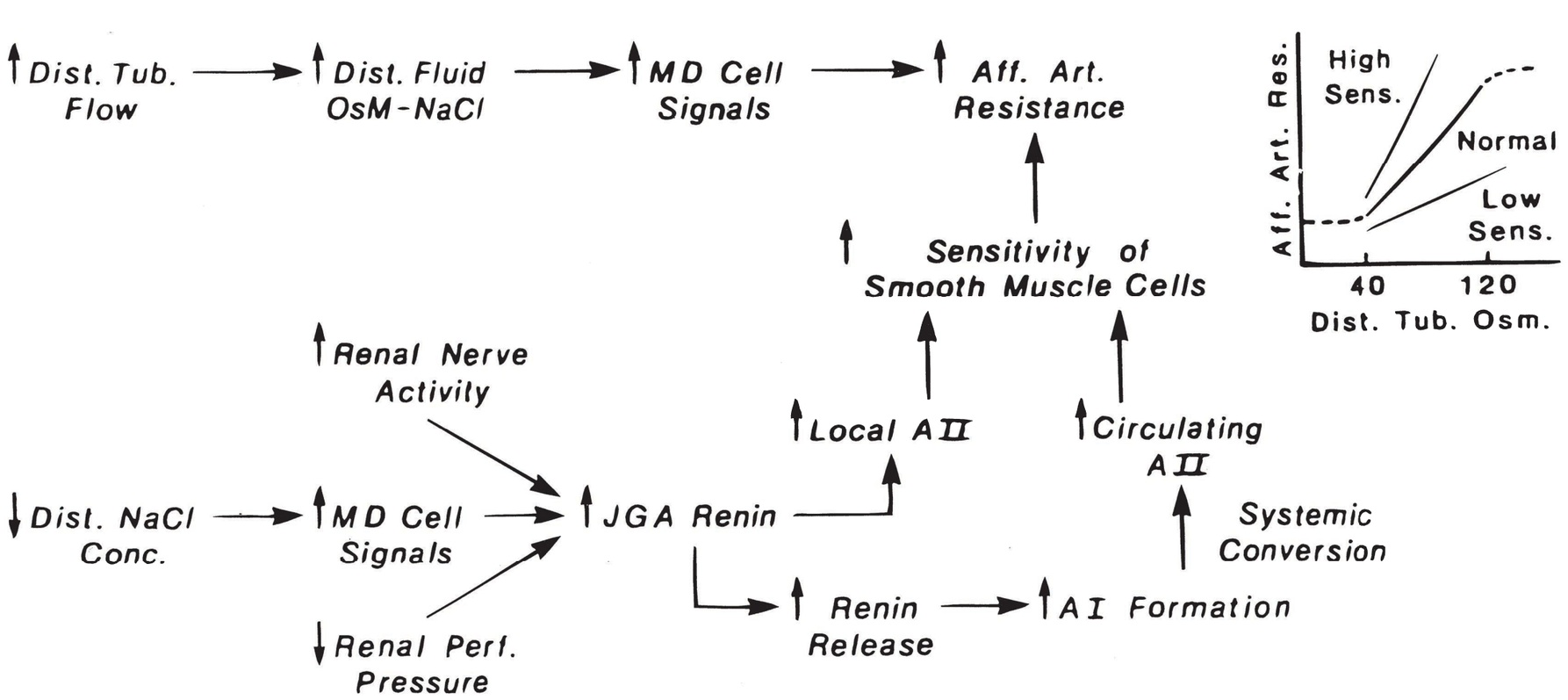

FIGURE 3.20 The modulator hypothesis for postulated interactions between the tubuloglomerular feedback mechanism and the intrarenal renin–angiotensin system. Flow-related changes in the tubular fluid concentration can elicit signals from macula densa (*MD*) cells to vascular contractile cells independent of angiotensin levels. Changes in the angiotensin II (*A II*) concentration influence the sensitivity or responsiveness of macula densa cells or of vascular smooth muscle cells to the signals coming from the macula densa cells. *A I*, angiotensin I; *A II*, angiotensin II; *Aff. Art.*, afferent arteriole; *JGA*, juxtaglomerular apparatus.

the AT_{1A} and AT_{1B} receptor mediate Ang II–induced Ca^{2+} signaling in smooth muscle cells and renal vasoconstriction. Evidence for the role of AT_{1B} receptors derives from mice lacking a functional AT_{1A} receptor.[36,138–141]

The AT_1 receptor is primarily coupled to the GTP-binding protein $G\alpha_{q11/12}$, whose activation leads to the triggering of several signaling pathways, including the stimulation of PLC_β. The receptor activation of protein tyrosine kinases leads to somewhat slower stimulation of phospholipase $C\gamma$. Phospholipases D and A_2 may also be activated, favoring the release of DAG and phosphatidic acid from phosphatidylcholine and arachidonic acid, respectively. The PLCs act on membrane-bound phosphoinositides to yield DAG and IP_3. DAG stimulates protein kinase C, whereas IP_3 diffuses through the cytosol to activate IP_3-sensitive receptors/release channels on membranes of the sarcoplasmic reticulum, triggering Ca^{2+} release to the cytosol. The mobilized Ca^{2+} triggers a complex array of events. Calcium-sensitive chloride channels may be stimulated to promote chloride efflux and depolarization of the plasma membrane. Such a signal will activate voltage-gated L-type calcium channels to allow for influx down a very steep gradient, from 1 to 2 mM in the extracellular fluid to 100 to 200 nM in the cytosol. Calcium release from internal stores also signals store-operated cation channels to open and allow further Ca^{2+} entry. AT_1 receptor activation may also trigger voltage-dependent Ca^{2+} channels independent of Ca^{2+} release from intracellular stores. As discussed earlier, afferent arteriolar contractions induced by Ang II is dependent on Ca^{2+} entry through voltage-gated channels, whereas efferent arterioles are not affected by L-type channel blockers but are influenced by T-type channel blockers. Recent studies indicate that AT_1 receptors rapidly activate NADPH oxidase to produce superoxide anion by the afferent arteriole. This leads to Ca^{2+} mobilization mediated by RyR through a direct action and/or one mediated by ADP ribosyl cyclase and the production of cADP ribose that sensitizes RyR/release channels on the sarcoplasmic reticulum to increase $[Ca^{2+}]_i$.[1,2,36,107,142,143]

The AT_2 receptor is characterized by a high affinity to the nonpeptide antagonists PD123319, CGP 42112, and Compound 21, and has a sequence 33% identical to that of the AT_1 receptor. Its high expression in fetal tissue suggests a role in embryonic development. This receptor has a typical pattern of seven transmembrane domains and is coupled to a G protein. Recent studies suggest that AT_2 receptors exert modulatory actions to partially counteract the effects caused by AT_1 receptor activation. AT_2 receptor activation increases bradykinin and NO levels, leading to increases in cGMP and vasodilation. AT_2 receptor activity may be upregulated during chronic salt deprivation. AT_3 and AT_4 receptors may be selective for angiotensin with amino acids 1 to 7 and Ang IV (angiotensin with amino acids 3 to 8), although they appear to play a minor role in regulating renal hemodynamics.[107,126,138,144]

Ang II receptors are regulated in response to different physiologic conditions. It is noteworthy that glomerular and vascular receptors are regulated differently from proximal tubular receptors. A low salt diet and high levels of Ang II lead to the downregulation of arteriolar and mesangial cell AT_1 receptors and the upregulation of tubular receptors.[140,145,146]

Actions of Ang II on the Renal Microvasculature

Ang II elicits dose-dependent AT_1-mediated decreases in RBF and GFR. The decreases in GFR are often smaller than the decreases in RBF such that filtration fraction increases. The increased vascular resistance is due to both afferent and efferent arteriolar constriction, and glomerular capillary pressure is well maintained. At high concentrations the glomerular filtration coefficient is reduced. Ang II produces more pronounced vasoconstriction when endogenous levels are low, presumably because of receptor upregulation and when vasodilator prostaglandins are blocked by COX inhibitors. Larger Ang II effects are also noted after the endothelial production of NO is blocked. As mentioned earlier, Ang II can potentiate TGF-mediated changes in preglomerular vascular tone. The multiple effects of Ang II are illustrated in Figure 3.21. In addition

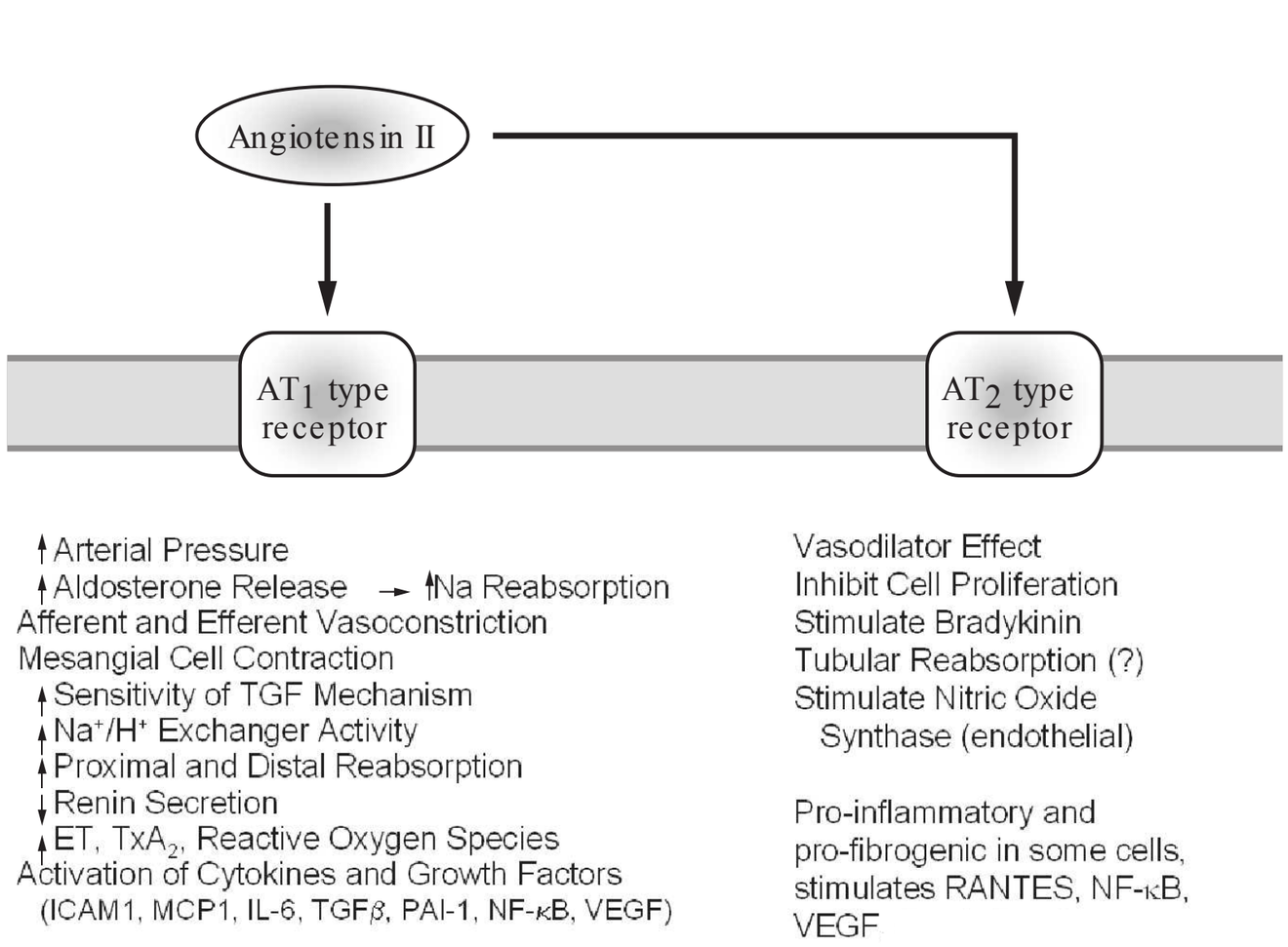

FIGURE 3.21 Multiple actions of angiotensin II on renal function mediated by AT_1 and AT_2 receptors. *ET*, endothelin; T_XA_2, thromboxane; *ICAM1*, intercellular adhesion molecule 1; *MCP1*, monocyte chemotactic protein 1; *IL-6*, interleukin 6; *TGFβ*, transforming growth factor β; *PAI-1*, plasminogen activator inhibitor 1; *NF-κB*, nuclear factor kappa light chain enhancer of activated B cells; *VEGF*, vascular endothelial growth factor.

to these effects, Ang II influences the medullary circulation, notably at concentrations lower than those required to elicit overall vasoconstriction in the cortex. This notion is supported by the finding that the Ang II receptor density is much higher in the medullary vessels and in the interstitial cells than in the postglomerular cortical vasculature.[1,2,36]

The effects of endogenous Ang II are observed using angiotensin receptor antagonists or ACE inhibitors in states when the prevailing Ang II levels are high. During sodium restriction, RBF is increased after the AT_1 receptor blockade, whereas the GFR responses are smaller and more variable; filtration fraction usually declines. Renin inhibition produces similar results, and combined renin and angiotensin inhibition causes decreases in RVR and increases in GFR. Activation of the AT_1 receptor is primarily responsible for the renal vascular effects of the endogenous Ang II on afferent and efferent arterioles and on K_f. Whole-kidney autoregulation is not affected by AT_1 receptor blockade or ACE inhibition, although the contribution of the TGF mechanism may be reduced and the plateau of the autoregulation relationship is elevated.[1,2,36]

Endothelium-Derived Vasoactive Factors

Nitric Oxide

Endothelial cells release NO in response to many stimuli, including mechanical shear stress and hormone, paracrine, and autocrine factors, that increase $[Ca^{2+}]_i$. NO is formed by vascular endothelial cells from the amino acid L-arginine via NOS, which is a soluble $NADPH^-$, and the Ca^{2+}-calmodulin–dependent citrulline-forming enzyme that requires two cosubstrates (O_2 and NADPH) and four cofactors (heme, flavin mononucleotide, flavin adenine dinucleotide, and tetrahydrobiopterin). As shown in Figure 3.7, several agents induce their vascular effects via endothelial cell eNOS activation and the release of NO. Examples include factors that stimulate the M_1-muscarinic receptor (acetylcholine), the B_2-kinin receptor, the α_2-adrenoceptor, the purinergic receptors (ATP, ADP), and the ET_B receptor. The rate of renal NO production is higher in the medulla than in the cortex. NO and nitrosamines have short biologic half-lives of less than 10 seconds, which is especially shortened by hemoglobin scavenging and in oxygenated solutions in the presence of the superoxide anion ($\cdot O_2^-$), by a mechanism involving the production of peroxynitrite (NO_3^-) from NO. In the same manner, NO acts to scavenge $\cdot O_2^-$.[147–149]

The basal release of NO tonically maintains a low RVR at rest, acting to buffer vasoconstriction produced by Ang II, ET-1, catecholamines, and other endogenous factors. Acute inhibition of basal NO production causes renal vasoconstriction, with decreases of 25% to 35% in RBF and reductions in cGMP levels in the interstitium and the urine. GFR responses are smaller and tend to be unchanged or decrease about 10%. Thus, the filtration fraction is increased. Basal NO dilates both the preglomerular vasculature (arcuate and interlobular arteries, afferent arterioles) and the efferent arterioles. NO also appears to contribute to the increased or maintained glomerular filtration coefficient. Endothelial factors mediate or modulate pressure-dependent responses of vascular segments exhibiting autoregulatory behavior. Note however, that NO does not fit a mediator role in this scheme because increased pressure and shear stress cause increased production of NO, a vasodilator, at the same time the responsive vascular elements exhibit vasoconstriction. NO inhibition results in renal vasoconstriction, but the steady-state autoregulatory RBF responses to changes in perfusion pressure are unaffected and remain highly efficient.[2,149,150]

NO-induced renal vasodilation is mediated by cGMP-dependent and perhaps a cGMP-independent system. The latter appears to involve cGMP cross-talk with the cAMP pathway, with cGMP inhibiting cAMP breakdown by PDE_3, more so than the activation of cGMP-protein kinase activity. Part of the dilatory response to NO may be mediated through increased K^+ channel activity and reduced production of 20-HETE.[62,118,151]

Although NO does not affect overall steady-state autoregulatory adjustments in RVR, NO attenuates the strength and rapidity of the myogenic adjustments in resistance. NO also attenuates the strength of TGF responses to increased distal tubular flow. The inhibition of NOS leads to greater relative decreases in medullary blood flow than in cortical blood flow. A NO blockade usually reduces renin release and responsiveness to reductions in perfusion pressure.[92,93,152,153]

Endothelial NO synthase is localized to the endothelial cells all along the renal vasculature (the interlobular arteries, the afferent and efferent arterioles, the glomerular capillaries, and the vasa recta) and the thick ascending limb of the Henle loop. Targeting of eNOS to specialized plasma membrane invaginations termed caveolae is required for maximal eNOS activity. Neuronal NO synthase (nNOS) is present primarily in epithelial cells (thick ascending limb of the Henle loop, the macula densa, the collecting duct). NO inhibits tubular transport along the nephron. An inducible form of NO synthase is normally quiescent but is capable of producing large amounts of NO in vascular smooth cells and mesangial cells during inflammation. An endogenous inhibitor of L-arginine cellular uptake and NOS activity is asymmetric dimethylarginine (ADMA) that is normally inactivated by NG-NG-dimethylarginine dimethylaminohydrolase (DDAH) to form L-citrulline. DDAH expression is found at the same sites as NO synthase. ADMA concentrations are elevated during states of oxidative stress and disease.[154–156]

Endothelin

Endothelin refers to a family of long lasting vasoconstrictor peptides that act locally as paracrine hormones. ET-1, -2, and -3, each a 21 amino-acid peptide, are constitutively released. ET-1 is the major form synthesized and secreted by endothelial cells of the preglomerular vasculature and the vasa recta

and by the medullary collecting ducts. Endothelin-converting enzyme (ECE) is the main enzyme responsible for the genesis of ET-1 from prepro-ET-1 (<200 amino acids); chymase and matrix metalloproteinase II are also involved in the production of ET intermediates. Ang II is a potent stimulus for ET-1 production. Other simulants include bradykinin, ATP, platelet activating factor, thrombin, and shear stress. Neutral endopeptidase 24-11 degrades and inactivates ET-1. Cytokines such as interleukin-1-beta (IL-1β) stimulate ET-1 production. The two known receptor types, ET_A and ET_B, are G-protein coupled and lead to IP_3 and cyclic ADP ribose formation, PKC activation, and Ca^{2+} mobilization, in addition to Ca^{2+} entry. ET_A receptors are predominantly found on vascular smooth muscle cells. ET_B receptors are localized on endothelial and tubular cells as well as vascular smooth muscle cells. The afferent arteriolar constriction produced by ET-1, like that caused by Ang II, is dependent in part on Ca^{2+} entry through voltage-dependent L-type channels. The action of ET-1 on efferent arterioles appears to depend exclusively on the mobilization of intracellular Ca^{2+} and/or entry through voltage-independent cation channels. The highest concentration of ET-1 in the body exists in the renal medulla, where it is synthesized by collecting duct cells and acts in a paracrine/autocrine manner to cause natriuretic and diuretic effects through ET_B receptors via the stimulation of NO. In addition, ET-1 has inotropic, chemotactic, and mitogenic properties. Overall, ET-1 increases blood pressure and vascular tone.[156–158]

The vascular actions of ET-1 reflect a combination of vasoconstrictor ET_A and ET_B receptors on smooth muscle cells and ET_B receptors on endothelial cells, which cause vasodilation mediated by NO. Concurrent ET-1 stimulation of ET_A + ET_B receptors causes net renal vasoconstriction, and the inhibition of ET_B receptor activation leads to more pronounced vasoconstriction. Thus, endothelial ET_B receptors buffer the constriction caused by the stimulation of both ET_A and ET_B receptors on the smooth muscle cells. Nevertheless, selective stimulation of vascular ET_B receptors using a pharmacologic agonist elicits renal vasoconstriction. Thus, there appears to be a complex interaction between receptor signaling. Endothelial ET_B receptors in the kidney and lung also seem to function as nonsignaling clearance receptors, effectively reducing the local concentration of ET-1, and thereby attenuating vasoconstriction.[150,159]

Exogenous ET-1 reduces RBF by stimulating both ET_A and ET_B receptors to cause the constriction of the arcuate and interlobular arteries and the afferent and efferent arterioles. Glomerular capillary pressure is relatively well maintained in the presence of reduced RBF. The decrease in GFR is primarily mediated by reductions in plasma flow and K_f. The reduction in K_f appears to be mediated by a secondary release of Ang II, eicosanoids, or neurotransmitters. The preglomerular vasculature has equal proportions of ET_A and ET_B receptors as compared to a ratio of 2:1 on their smooth muscle cells devoid of endothelial cells. Under basal conditions, ET-1 exerts dual actions on the vasculature that are equal and opposite. The ET_A receptor blockade produces renal vasodilation due to a 5% to 10% increase in RBF, whereas ET_B receptor antagonism leads to constriction of 5% to 10%. Although ET-1 contributes to basal vascular tone, it does not interfere with renal autoregulatory mechanisms.[2,150,160,161]

ET_A and ET_B receptors stimulate $[Ca^{2+}]_i$ in smooth muscle cells of preglomerular arteries/arterioles through a combination of mobilization and entry pathways. Low concentrations of ET-1 activate Ca^{2+} entry channels, with higher concentrations mobilizing intracellular Ca^{2+} via the PLC-IP_3R and NADPH oxidase/cADPR/RyR pathways. Calcium channel blockers reduce the magnitude and duration of ET-1–induced renal vasoconstriction. The afferent arteriolar constriction produced by ET-1, similar to that caused by Ang II, is dependent in part on Ca^{2+} entry through voltage-dependent L-type channels as well as mobilization in intracellular Ca^{2+} stores. The action of ET-1 on efferent arterioles appears to depend exclusively on the mobilization of intracellular Ca^{2+}. ET-1 inhibits renin synthesis and release by ET_A and ET_B receptor stimulation of $[Ca^{2+}]_i$ in juxtaglomerular granular cells.[13,20,162]

ET-1 acts on glomerular mesangial cells to cause the contraction and stimulation of mitogenesis. High concentrations of ET-1 that reduce sodium excretion increase plasma renin activity, presumably via the macula densa mechanism. In addition, ET-1 may affect neurotransmission, eicosanoid synthesis, and ANP synthesis and release. The activation of endothelin receptors causes the release of eicosanoids and NO from endothelial cells and ANP from myocytes.[157,158,163]

Heme Oxygenase and Carbon Monoxide

Heme oxygenases (HO) are microsomal enzymes that catalyze the degradation of heme to form iron, biliverdin, and carbon monoxide (CO). The vascular actions of CO include the direct relaxation of vascular smooth muscle cells and the indirect contraction through the inhibition of NOS. Similar to NO, CO produces vasodilation by stimulating soluble guanylyl cyclase in smooth muscle cells to signal through the cGMP/PKG pathway. CO also may bind directly to Ca^{2+}-activated BK channels to depolarize the plasma membrane. A primary action of CO is to attenuate vasoconstriction produced by agents such as Ang II and catecholamines, with greater buffering effects in the absence rather than in the presence of NO. Nevertheless, CO can dose-dependently increase NADPH oxidase-dependent O_2^- production and constrict interlobar and interlobular arteries via thromboxane thromboxane prostanoid (TP) receptor activation, perhaps involving isoprostanes, effects inhibited by the O_2^- scavenger biliverdin. Thus, the vascular response to CO is mixed in that CO can elicit signaling leading to vasodilation and vasoconstriction, the net effect depending on experimental conditions.[164,165]

HO-2 is constitutively expressed in the kidney, mainly in proximal tubules with relatively weak presence in the renal vasculature. HO-1 is inducible during inflammation

and oxidative stress. The influence of endogenous HO-2 metabolites on renal hemodynamics appears to be minor under physiologic conditions. Pharmacologic inhibition of HO (chromium mesoporphyrin [CrMP]) reduces urinary excretion of sodium and water even under conditions where there are no effects on arterial pressure, RBF, GFR, or plasma renin activity, both in control animals and after NOS inhibition, suggesting a primary tubular effect of endogenous CO. Likewise, HO inhibition with CrMP does not alter afferent arteriolar diameter of juxtamedullary nephrons of normal rats. However, other studies using SnMP to inhibit HO report decreases in RBF with variable responses in GFR. Another study shows that acute inhibition of renal medullary HO activity and CO production reduces medullary blood flow and sodium excretion and blunts pressure natriuresis. Chronic HO inhibition produces hypertension that is salt sensitive. Mice deficient in HO-2 are normotensive with normal RBF during basal conditions or during NOS inhibition, whereas Ang II paradoxically produces less pronounced renal vasoconstriction in the absence of HO-2. Other investigators report that the pressor and renal vasoconstrictor responses to low levels of Ang II are magnified by inhibition of HO activity (tin mesoporphyrin) in normal euvolemic animals.[164,166–169]

Both HO-1 and HO-2 mRNA are expressed in macula densa cells, and HO metabolites inhibit TGF. Increased HO activity attenuates TGF-induced vasoconstriction through macula densa release of CO and biliverdin during increased salt reabsorption. The CO effect on TGF is linked to cGMP pathway and biliverdin to reduce O_2^- levels. Renal HO-1 induction (hemin, $SnCl_2$) dilates afferent arteriolar diameter and attenuates juxtamedullary afferent arteriolar autoregulatory responses to increases in renal perfusion pressure, an effect mimicked by CO acting through cGMP/PKG signaling but not biliverdin. These results are consistent with findings that increasing CO levels directly or with hemin administration increase RBF, urine flow, and sodium excretion. Whether the effects involve myogenic and/or TGF mechanisms await investigation.[164,170,171]

Hypoxia-induced HO upregulation protects renal tissue from acute and chronic injury. Activation of HO has an antioxidant effect by degrading the heme moiety of heme-containing enzymes such as NOS, COX-2, and cytochrome P450 monooxygenase. HO can attenuate the production of reactive oxygen species through its heme degradation and production of CO, biliverdin/bilirubin, and free iron. Excess free heme catalyzes ROS formation, which may lead to endothelial cell dysfunction, vasoconstriction, and tissue damage commonly associated with pathologic cardiovascular–renal conditions. Increased HO-1 activity and the metabolite bilirubin suppress NADPH oxidase activity and O_2^- production in vascular smooth muscle cells.[164,172]

Hydrogen Sulfide

Endogenous hydrogen sulfide (H_2S) is a recently discovered vasoactive gas transmitter (joining NO and CO in this classification). Cystathionine-γ-lyase and -β-synthetase are the main enzymes forming H_2S from L-cysteine or L-homocysteine in endothelial and smooth muscle cells of the vasculature wall and erythrocytes, physiologically activated by Ca^{2+}-calmodulin. H_2S is inactivated by binding to hemoglobin to form sulfhemoglobin, As an endothelial-derived relaxing factor, H_2S produces concentration-dependent dilation of large conduit arteries and small resistance arterioles, primarily acting directly to open K_{ATP} channels to hyperpolarize vascular smooth muscle cells. In contrast to NO and CO, H_2S does not stimulate soluble guanylate cyclase. Being a reducing agent, H_2S appears to alter cellular redox status. Low concentrations of H_2S may cause vasoconstriction reducing NO availability by reacting with NO to form a nitrosothiol compound and inhibit eNOS. H_2S and the H_2S donor NaHS downregulate cAMP production in vascular smooth muscle cells, producing vasoconstriction, and in juxtaglomerular granular cells, inhibiting renin release. Physiologic levels of H_2S are also angiogenic, antiproliferative, and anti-inflammatory. Mice deficient in a synthetic enzyme have reduced H_2S levels, reduced endothelium-mediated vasodilation, and develop hypertension.[173–176]

Little is known about H_2S effects on the renal microcirculation. H_2S is produced in the kidney and the exogenous H_2S donor NaHS exerts diuretic, natriuretic, and kaliuretic effects in association with increases in GFR and RBF. Conversely, acute intrarenal inhibition of H_2S production reduces GFR and sodium excretion without affecting RBF. Chronic inhibition of H_2S synthesis reduces RBF but not GFR because sodium excretion decreases in association with the development of hypertension. The actions of H_2S on afferent and efferent arterioles and TGF activity await investigation.[177,178]

Because H_2S is oxidized in mitochondria in pO_2-dependent manner and ambient pO_2 is lower in the renal medulla than the cortex, H_2S accumulates in medullary regions. High H_2S concentrations in the relatively hypoxic renal medulla function as an oxygen sensor that acts to increase local O_2 by increasing medullary blood flow in combination with the direct inhibition of mitochondrial respiration.[179]

Arachidonic Acid Metabolites

Renal prostaglandins, or eicosanoids, are biologically active fatty acid products of arachidonic acid that contribute to the regulation of renal hemodynamics. They are synthesized intracellularly and immediately released to act locally on the renal vasculature as paracrine/autocrine agents. Free intracellular arachidonic acid can be metabolized on demand via one of three major enzymatic pathways: cyclooxygenase, lipoxygenase, or cytochrome P-450 monooxygenase. Depending on cell types, the net production of the various metabolites may cause vasoconstriction under some conditions and vasodilation during others. The multiple products of this cascade are depicted in Figure 3.22. Phospholipase A_2 (PLA_2) catalyzes the formation of arachidonic

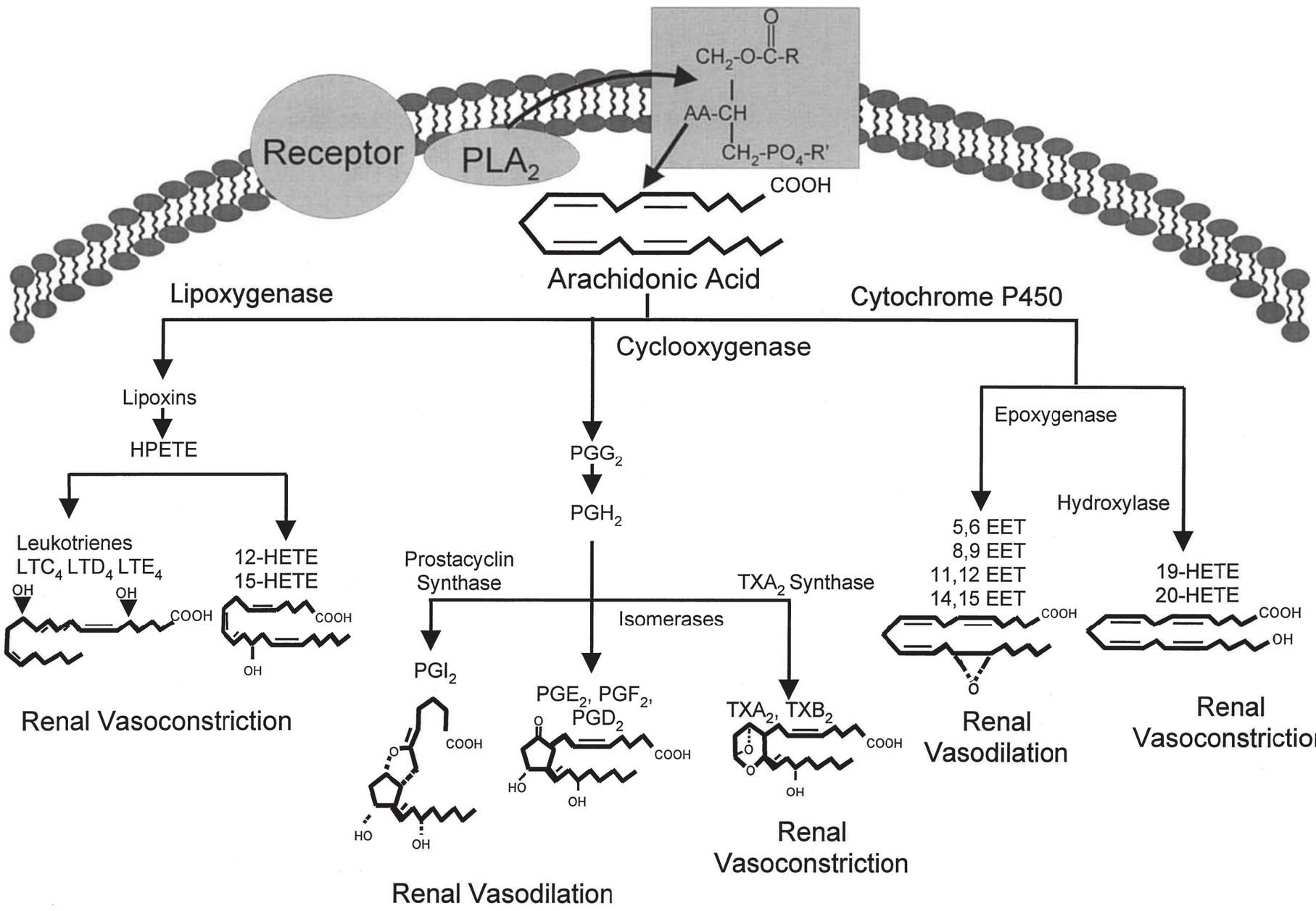

FIGURE 3.22 Three major pathways of eicosanoid synthesis from arachidonic acid involving cyclooxygenase, lipoxygenase, and cytochrome P-450 monooxygenase enzyme systems. Note that only the configuration of the cyclopentane ring is shown. *LT*, leukotriene; *PLA*$_2$, phospholipase A2; *PG*, prostaglandin; *TX*, thromboxane; *EET*, epoxy-eicosatrienoic acid; *HETE*, hydroxyeicosatetraenoic acid.

acid, an unsaturated 20-carbon fatty acid, from membrane phospholipids.[2]

A key regulatory, rate-limiting step for prostaglandin synthesis is the conversion of arachidonic acid to prostaglandin (PG) G_2/H_2 by COX. There are two major COX enzymes. COX-1, a constitutive enzyme, is found in renal arteries and arterioles, glomeruli, cortical and medullary collecting ducts, and medullary interstitial cells. COX-2 is a regulated constitutive enzyme that is important in development, and in adult animals is commonly activated by growth factors, inflammatory states, glucocorticoids, and low salt diet. Renal COX-2 mRNA is localized primarily to epithelial cells of the cortical thick ascending limb that includes the macula densa region and medullary interstitial cells, with greater amounts in the papilla than the cortex. COX-2 expression is noticeably absent from arterioles, glomeruli, and cortical or medullary collecting ducts, sites of COX-1 expression; COX-2 is also expressed in endothelial cells of medullary vasa recta. COX-2 expression in thick ascending limb cells and macula densa cells varies with salt diet, increasing during chronic sodium restriction and decreasing during sodium loading. COX-2 expression in medullary interstitial cells is induced by water deprivation and high interstitial osmolality, high levels of Ang II and AVP, as well as growth factors and cytokines through MAP kinase pathways. Leukotrienes are synthesized by another major pathway involving the enzyme lipoxygenase. Vasoactive metabolites are also formed via the cytochrome P-450 monooxygenase pathway. Medullary tubular and interstitial cells have a larger synthetic capacity than the vasculature in the cortex. Endothelial cells produce PGE_2 and PGI_2, whereas thromboxane A_2 seems to derive from vascular smooth muscle cells and mesangial cells.[118,180–184]

Prostaglandins

General stimuli for renal prostaglandin synthesis are renal vasoconstriction and states of volume depletion and hypoperfusion. Anesthesia, surgery, and associated stress may exacerbate prostaglandin production. The diuretics ethacrynic acid and furosemide also stimulate renal release of prostaglandins. Many vasoactive receptor agonists stimulate phospholipases that promote the release of arachidonic acid from membrane phospholipids. The stimulation of PGE_2 and PGI_2 production by Ang II is well characterized. In turn, the vasodilatory PGE_2 and PGI_2 usually buffer the vasoconstriction elicited by Ang II and stimulate renin release from juxtaglomerular cells. Other stimulants include ET_A receptor agonists. Vasodilators such as acetylcholine and bradykinin stimulate the production of PGE_2 or PGI_2 as well as NO. In addition, acetylcholine may stimulate the production of thromboxane A_2. The vasoactive peptides Ang II, ET-1, vasopressin, acetylcholine, and bradykinin increase the

availability of the substrate, arachidonic acid, secondary to membrane receptor–mediated Ca^{2+} influx and the activation of phospholipase A_2. Phospholipase A_2 is activated by increased activity of the Ca^{2+}-calmodulin complex, increased production of DAG, or phospholipase C-mediated phosphorylation of lipocortin, a membrane-bound enzyme that normally inhibits phospholipase A_2. Thus, there is a common pathway by which many vasoconstrictor agents can increase the production of COX-derived prostaglandins, primarily vasodilatory PGE_2 and PGI_2, which in turn can counteract vasoconstriction. Vasodilatory prostaglandins produced by the endothelium of glomerular arterioles and mesangial cells exert net effects to stimulate adenylate cyclase and the formation of cAMP and the activation of protein kinase A. α-Adrenergic neurotransmission can be inhibited prejunctionally and postjunctionally by prostaglandins.[71,185,186]

Four forms of PGE_2 receptors (termed EP_1 through EP_4) have been identified and cloned. The EP_4 receptor, coupled via $G\alpha_s$-proteins to generate cAMP and activate protein kinase A, predominates along the preglomerular vasculature and mediates the principal vasodilator actions of PGE_2. Similar actions are exerted by the single IP receptor for PGI_2 (prostacyclin). Low concentrations of the prostaglandins PGE_2 and PGI_2 normally formed by the arterial vasculature exert part of their physiologic effects by attenuating the actions of vasoconstrictors, with larger amounts acting as vasodilators that increase RBF. The buffering action of PGE_2 and PGI_2 in the preglomerular vasculature is primarily mediated by the ability of cAMP and protein kinase A activation to inhibit IP_3-induced release of Ca^{2+} from internal stores. A low density of a vasoconstrictor receptor (EP_1 or EP_3) may counteract some of the net dilation. The EP_1 receptor increases Ca^{2+} mobilization. The EP_3 receptor inhibits the production of cAMP via a pertussis-toxin-sensitive $G\alpha_i$ protein. EP_2 receptors, which act through a $G\alpha_s$ protein to increase cAMP in tubules, appear to be absent from the renal vasculature under normal conditions. Arachidonic acid dilates the isolated interlobular artery and afferent arteriole; a smaller response occurs in efferent arterioles. Intrarenal infusions of arachidonic acid, PGE_2 or PGI_2 increase RBF and reduce renal resistance without affecting GFR. $PGF_2\alpha$ has little or no effect on the renal circulation. Local administration of small amounts of PGE_2 or its stable analog cause renal vasodilation and attenuate the constrictor effects of Ang II, thromboxane A_2, and norepinephrine. PGE_2 and PGI_2 dilate both afferent and efferent arterioles such that glomerular capillary pressure is constant when the stimulatory effects of the prostaglandins on Ang II formation are blocked. However, the application of PGE_2 from the interstitial side causes vasoconstriction of juxtamedullary nephrons, a response due to subsequent metabolism to a vasoconstrictor agent or the activation of EP_1 or EP_3 receptors.[60,185,187,188]

Endogenous prostaglandins regulate RBF and GFR by direct effects on vascular smooth muscle and indirectly by modification of the action of other hormones or neural stimuli. The vasodilator prostaglandins serve an important protective function and homeostatically balance the hemodynamic effects of vasoconstrictor substances. Studies of inhibition of COX activity indicate that the major function of COX-derived prostaglandins is to attenuate the influence of vasoconstrictor substances during the activation of the renin–angiotensin system, the sympathetic nervous systems, or both. These counteracting effects provide a balance between the vascular effects of Ang II and those of prostaglandins during variations in plasma volume and sodium intake. As is shown in Figure 3.23, blockade of the compensatory dilator action of prostaglandins promotes vasoconstriction when the renin–angiotensin and sympathetic nervous systems are

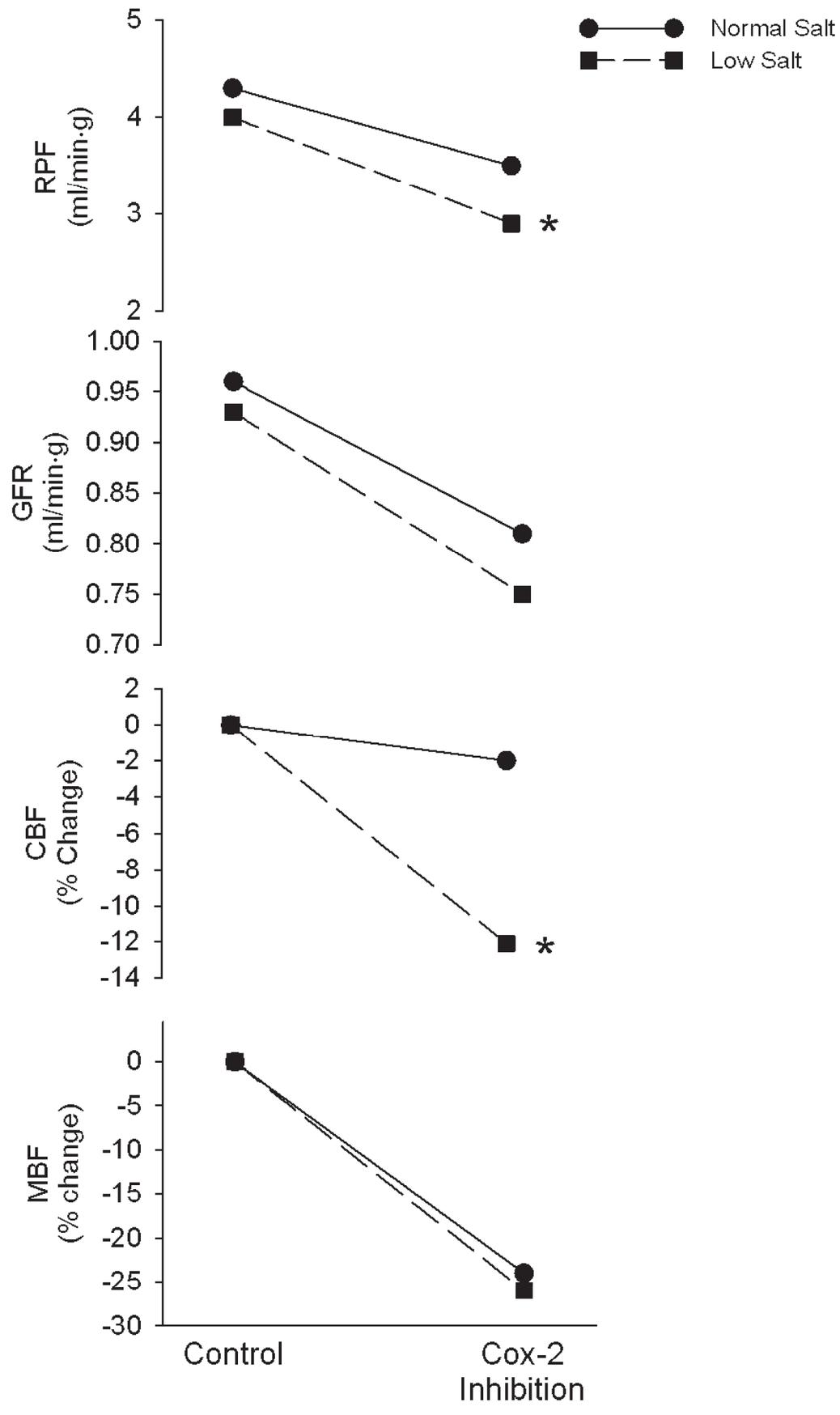

FIGURE 3.23 Renal plasma flow (*RPF*), glomerular filtration rate (*GFR*), cortical blood flow (*CBF*), and medullary blood flow (*MBF*) responses to the inhibition of cyclooxygenase (Cox-2, nimesulide) in anesthetized rats maintained on a normal salt diet (●) or on a low sodium diet (■). * $P < .05$. (Data from Green T, Rodriguez J, Navar LG. Augmented cyclooxygenase-2 effects on renal function during varying states of angiotensin II. *Am J Physiol Renal Physiol.* 2010;299:F954–F962.)

stimulated, such as during sodium depletion. Prostaglandins also antagonize the tendency for high concentrations of vasopressin to produce renal vasoconstriction. In contrast, in a healthy, unstressed individual with a normal plasma volume, the inhibition of prostaglandin synthesis has little or no effect on RBF and GFR (Fig. 3.23). Basic autoregulatory efficiency of whole-kidney RBF and GFR remains high even during the blockade of renal prostaglandin synthesis via the COX pathway. The inhibition of COX-2 produces renal vasoconstriction that may be greater in the medulla than in the cortex. During afferent arteriolar constriction induced by TGF, COX-2 generates vasodilatory metabolites in response to increased nNOS activity that attenuate the strength of feedback-mediated vasoconstriction.[2,116,182]

Renin release is increased by arachidonic acid, endoperoxides, PGE_2, and PGI_2. PGE_2 and PGI_2 act on their respective EP_4 and IP receptors on juxtaglomerular granular cells to stimulate renin release by stimulating adenylate cyclase activity and producing cAMP. COX inhibitors reduce renin release under basal as well as during stimulated conditions. α-Adrenergic neurotransmission can be inhibited prejunctionally and postjunctionally by prostaglandins.[13,116,121,133]

The COX-2 metabolite PGE_2 plays a key role in linking NaCl control of macula densa signaling to juxtaglomerular cells to regulate renin release and to afferent arterioles to regulate TGF. COX-2 activity in the macula densa increases in response to a salt deficient diet and to a loop diuretic, such as furosemide, with increases in PGE_2 production and renin release. nNOS in the same cells may serve as an upstream intermediate responding to salt transport. High levels of nNOS and COX-2 parallel each other, and the inhibition of nNOS causes a fall in COX-2 expression and an uncoupling to transport. On the other hand, high renin states suppress COX-2 expression in the cortical thick ascending limb and macula densa cells via Ang II and AT_1 receptors; ACE inhibition has the opposite effect. The recruitment of renin-containing cells retrograde along the afferent arteriole during chronic salt restriction or ACE inhibition appears to be COX-2 dependent as renin is restricted to juxtaglomerular cells during these stimuli in COX-2–deficient mice. Plasma renin activity is reduced in COX-2–null mice and also in nNOS-deficient mice. The inhibition of COX-2 produces renal vasoconstriction that may be greater in the medulla than the cortex.[117,182,189]

In disease states, endogenous vasodilator prostaglandins serve a protective role in maintaining renal function by means of their effects on vascular resistance, GFR, and renin release. The administration of nonsteroidal anti-inflammatory drugs to patients with clinical disorders such as advanced hepatic cirrhosis, severe congestive heart failure, and sodium depletion often produces deleterious effects with reductions in renal perfusion and GFR. In other pathophysiologic conditions, there may be enhanced production of the vasoconstrictor thromboxane A_2, which may contribute to the deterioration of renal function. Some of the vasoconstriction produced by Ang II may be mediated by thromboxane A_2 in pathophysiologic settings. Administration of a stable thromboxane-receptor agonist reduces RBF and GFR by causing afferent and efferent arteriolar constriction. K_f appears to be unaffected, although it is noteworthy that thromboxane A_2 causes the contraction of isolated glomeruli and cultured mesangial cells. As mentioned in the section on TGF, although very little thromboxane A_2 is produced under normal conditions, thromboxane plays a modulatory role in the preglomerular vasoconstriction associated with the activation of the TGF mechanism and the vasoconstrictor response to chronic Ang II infusion. The thromboxane A_2-PGH_2 receptor is coupled by the $G\alpha_q$ protein to the activation of phospholipase C, increased $[Ca^{2+}]_i$, and possible inhibition of adenylate cyclase. The renal vasoconstriction produced by thromboxane A_2 is primarily mediated by Ca^{2+} influx. Chronic activation of PKC appears to reduce the number of thromboxane A_2 TP receptors. In Ang II–dependent hypertension, the administration of COX-2 inhibitors decreases RBF and GFR due to increased RVR, reflecting a renal vasodilator action of intrarenal COX-2 metabolites; however, systemic vascular resistance is decreased, reflecting a vasoconstrictor role of COX-2 metabolites on the systemic circulation.[116,184,190]

Leukotrienes

Lipoxygenase enzymes convert arachidonic acid to leukotrienes (LTs), HETEs, and lipoxins (see Fig. 3.22). The major lipoxygenase products are monohydroxyeicosatetraenoic acids—12-HETE and 15-HETE—generated by glomeruli, mesangial cells, renal cortical tubules, and vascular tissues. LTs (LTB_4, LTC_4, LTD_4, and LTE_4) are hydroperoxy fatty acid products of the intermediate 5-hydroperoxyeicosatetraenoic acid (HPETE). Stereoisomers of 12-HETE are produced by different enzymatic pathways. The 12(S)-HETE isomer is predominantly produced in the cortex; however, the 12(R)-HETE is a primary biologically active metabolite of the cytochrome P-450 pathway. The renal vasculature constricts in response to 12(S)-HETE and 15-HETE due to the depolarization of smooth muscle cells, increased Ca^{2+} entry, and the activation of PKC. Vascular generation of 12-HETE is increased in pathologic conditions associated with oxidative stress and inflammation.[2,180,183,191]

Receptors for LTC_4 and LTD_4 have been identified on preglomerular arteries, glomeruli and cultured mesangial cells, and efferent arteriole. The infusion of LTC_4 or LTD_4 causes renal vasoconstriction, reduces GFR and filtration fraction, and activates the renin–angiotensin system. Leukotriene C_4 receptors are linked to ion channels, but specific mechanisms are not known. Some of the LT actions may be mediated by endothelial cells. LTD_4 is known to release NO in addition to activating a pertussis-toxin–sensitive G protein. Lipoxin A_4 increases renal plasma flow and GFR, with a small reduction in K_f. Interestingly, the effects are COX dependent and can be completely reversed by the inhibition of this enzyme. On the other hand, the ability of

lipoxin B_4 to decrease RBF and GFR is independent of COX activity.[180,183]

Cytochrome P-450 Metabolites

Vasoactive metabolites of the cytochrome P-450 pathway are produced by vascular smooth muscle cells, endothelial cells, renal tubular cells, and glomeruli. Arachidonic acid is oxygenated via NADPH-dependent microsomal monooxygenases. As is shown in Figure 3.22, epoxygenase enzymes are responsible for the production of epoxides or epoxyeicosatrienoic acids (EETs) (e.g., 11,12-EET and 14,15-EET). A second cytochrome P-450 pathway involves the ω-hydroxylase formation of 19- and 20-HETEs. The major metabolites of this pathway are EETs in the cortex and HETE in the medulla. Salt diet, Ang II and other hormones, and various pathophysiologic settings alter the renal cytochrome P-450 metabolism.[118,183,192–195]

Cytochrome P-450 ω-hydroxylase metabolites, in particular 20-HETE, participate in regulation of cortical and whole-kidney blood flow. Although rodent studies report effects of 20-HETE, studies in dogs and rabbits have failed to show an effect of cytochrome P-450 inhibition on autoregulation and pressure natriuresis. In isolated rat vessels, transmural pressure stimulates ω-hydroxylase activity to produce 20-HETE, which is thought to participate in myogenic vasoconstriction. Cytochrome P450-4A–derived eicosanoids may also participate in the renal hemodynamic effects of Ang II and endothelin. Products of the cytochrome P-450 pathway potentiate control of preglomerular vasomotor tone by the juxtaglomerular apparatus. Inhibition of P-450 metabolites blunts TGF activity, whereas the luminal perfusion of 20-HETE restores TGF responses. 20-HETE causes vasoconstriction by the inhibition of tonically active K^+ channels, thereby causing depolarization and activation of voltage-gated Ca^{2+} channels and increases intracellular Ca^{2+}.[118,181,183]

Epoxygenase metabolites elicit variable vascular responses. 11,12-EET is an endothelial-derived factor distinct from NO that produces vasodilation in interlobular arteries and afferent arterioles mediated by membrane hyperpolarization following the activation of cAMP/protein kinase A and of K^+ channels, actions independent of the vascular endothelium or COX activity. 5,6-EET or 8,9-EET induces vasoconstriction with a decrease in GFR, mediated in part by thromboxane TP receptor activation. However, COX inhibition changes the renal response to these EETs to vasodilation and an increase in GFR. Inhibition of epoxygenase activity enhances afferent arteriolar autoregulation. Not clear is whether epoxygenase/EET actions blunt the pressure-induced myogenic response, or TGF, or both. EETs, in particular 11,12-EET, have now been identified as being an endothelial-derived hyperpolarizing factor and activate potassium channels, thus increasing the membrane potential, leading to renal vasodilation and attenuated responses to vasoconstrictor influences.[183,196,197]

Isoprostanes such as 8-epi-$PGF_{2\alpha}$ are vasoconstrictor metabolites related to prostaglandins. They are stable products of nonenzymatic lipid peroxidation of arachidonic acid, formed in and released from cell membranes and excreted in urine. Isoprostane production is stimulated by peroxynitrate, a reactive oxygen species resulting from NO scavenging by superoxide anion.[114,198,199]

Kallikrein–Kinin System

Plasma and glandular/tissue kallikreins are distinct serine protease enzymes acting on kininogens (inactive α_2-glycoproteins) to form the biologically active nonapeptide bradykinin and also the decapeptide lysyl-bradykinin (kallidin). It is unlikely that circulating kinins affect the renal microcirculation because they are rapidly inactivated enzymatically by endothelial-bound kininase II (ACE) and neutral endopeptidase. Within the kidney, tissue kallikrein and its substrate, kininogen, are located predominantly in the distal convoluted and cortical collecting tubules. The synthesis and the release of kallikrein into the tubular fluid and interstitium are stimulated by prostaglandins, mineralocorticoids, Ang II, increased renal perfusion pressure, and several diuretic drugs. The renal vasculature and tubules contain the constitutive B_2 receptor and the inducible B_1 receptor. Bradykinin B_2 receptors on vascular endothelial cells cause renal vasodilation as a result of stimulation of NO, EET, and prostanoid production. The B_2 receptor density in glomeruli is reduced by a low sodium diet and water deprivation. B_2 receptors on tubular epithelial cells inhibit sodium reabsorption. The bradykinin B_1 receptor is not normally expressed and is silent under physiologic conditions. Its expression is highly inducible by inflammatory mediators and tissue damage, chronic ACE (kininase II) inhibition, or genetic deletion of B_2 receptors.[200–203]

The infusion of bradykinin elicits renal vasodilation characterized by a larger increase in RBF than GFR, and a natriuresis and diuresis. Exogenous kinins also produce an independent stimulation of prostaglandin formation (PGE_2 and PGI_2) and renin release. Kinin-induced vasodilation, however, may be similar in the presence and absence of COX inhibition of prostaglandin synthesis owing to major actions of NO and EETs. As a result of vasodilatorlike actions, exogenous bradykinin reduces the vasoconstrictor responses to Ang II and norepinephrine. The observed decline in glomerular K_f contrasts with the well-known effect of kinins to increase capillary permeability in other tissues. The mechanisms responsible for the reduction in K_f are not known. Additional effects include the enhanced conversion of inactive to active renin and the presynaptic inhibition of adrenergic neurotransmitter release. Isolated vessels and cultured mesangial cells devoid of endothelium exhibit B_2-receptor–dependent constrictor responses to bradykinin. Signal transduction appears to involve a pertussis-toxin–insensitive G protein, a PKC pathway, increased $[Ca^{2+}]_i$, and arachidonic acid metabolites.[1,2]

B_2 receptors reside on endothelial and vascular smooth muscle cells. Vasodilator effects of bradykinin are observed in isolated preparations of afferent and efferent arterioles. In the isolated perfused afferent arteriole, low concentrations of bradykinin ($<10^{-10}$ M) vasodilate by acting on endothelial B_2 receptors to produce NO and prostaglandins. Epoxygenase-dependent EETs also contribute. Higher concentrations ($>10^{-9}$ M) vasoconstrict by acting on B_2 receptors to produce COX-derived thromboxane. In contrast, the vasodilator effect of bradykinin in efferent arterioles (perfused in retrograde direction) via B_2 receptors is mediated by cytochrome P450 metabolites (probably EETs), but not by NO or COX products. Perfusion of bradykinin through glomeruli releases prostaglandins that dilate the efferent arteriole. Bradykinin relaxes pericytes surrounding the outer medullary descending vasa recta. Endogenous bradykinin dilates afferent and efferent arterioles in vivo to a greater extent in the deep versus superficial cortical glomeruli with primary mediation by NO. EETs also participate in vasodilation of afferent arterioles of juxtamedullary nephrons. Isolated vessels and cultured mesangial cells devoid of endothelium exhibit B_2-receptor–dependent constrictor responses to bradykinin. Signal transduction appears to involve a pertussis-toxin–insensitive G protein, a PKC pathway, increased $[Ca^{2+}]_i$, and arachidonic acid metabolites. Endogenous bradykinin, potentiated during ACE inhibition in animals fed a low sodium diet, dilates the renal vasculature with predominant actions in the medulla. During extracellular fluid volume expansion, bradykinin promotes sodium excretion and reduces regional autoregulatory efficiency to increase medullary blood flow, effects largely mediated by B_2 receptor stimulation of NO.[15,204–209]

Early studies evaluated endogenous kinin activity using an infusion of bradykinin-binding antibodies, the suppression of renal kallikrein activity with the serine protease inhibitor aprotinin, and the pharmacologic inhibition of kininase II. The results suggest that locally formed kinins attenuate renal vasoconstriction. Further understanding has been gained by employing more specific receptor antagonists. Use of a specific B_2 receptor antagonist reveals that basal kinin levels do not contribute appreciably to the regulation of renal function during normal conditions. However, when their levels are elevated as during sodium restriction and volume depletion, kinins act as vasodilators in a manner similar to the prostanoids, which buffer the renal vasoconstriction associated with elevated local levels of Ang II, norepinephrine, and vasopressin. A combined blockade of both degrading enzymes (ACE or kininase II and neutral endopeptidase) leads to increases in RBF and GFR in association with an increased urinary excretion of kinins. Kinins may participate in the autoregulation of GFR and the TGF mechanism, although overall steady-state RBF autoregulation is not affected by kinin receptor blockade. Kinins exert larger vasodilatory effects in the medulla than in the cortex.[208]

Animal studies on the role of the renal kallikrein–kinin system using kininogen-deficient rats and also B_2 receptor knockout mice indicate that this system primarily functions to promote a natriuresis that impacts on the pressure–natriuresis relation when there is excess sodium intake or high plasma aldosterone concentration. Genetic dysfunction of the renal kallikrein–kinin system leads to altered functional maturation of the kidney and the development of salt-sensitive hypertension. Rats with a genetic reduction in urinary kallikrein excretion have an altered pressure–natriuresis relationship, with this defect being corrected by an infusion of purified tissue kallikrein. Knockout mice lacking the B_2-receptor gene have elevated arterial pressure under basal conditions, reduced RBF, increased renin mRNA, and enhanced blood pressure sensitivity to salt.[210]

Purinergic Actions on Renal Microcirculation

Adenosine and ATP

Adenosine nucleosides and nucleotides have received considerable attention as regulators of renal hemodynamics and renin release. It is proposed that GFR and filtered sodium load are coupled to tubular transport capacity and O_2 consumption via the hydrolysis of ATP and the resultant adenosine production. Adenosine and other adenine nucleotides are secreted into the interstitial fluid in the extracellular compartment. ATP is released from cells through membrane channels and coreleased with transmitters from nerve terminals. In this fashion, purine-based substances act as paracrine agents to influence renal microcirculation.[1,2,121]

Purinergic Receptors

Renal purinoceptors display different sensitivities for ATP, ADP, AMP, and adenosine. P_1 purinoceptors respond primarily to adenosine and sometimes to AMP, but are relatively insensitive to ADP and ATP. Extracellular ATP predominantly activates P_2 purinoceptors. There are at least two subtypes of adenosine-responsive P_1 receptors in the renal vasculature. P_1-vasoconstrictor A_1 receptors are coupled to a pertussis-toxin–sensitive $G\alpha_i$ protein that inhibits adenylate cyclase and cAMP production. The stimulation of A_1 receptors decreases GFR and RBF. A local application of an A_1 receptor agonist constricts both preglomerular and postglomerular microvessels. A_2 receptors cause vasodilation of afferent and efferent arterioles, stimulating adenylate cyclase through a $G\alpha_s$ protein, and also increase EETs and NO, which contribute to the vasodilation. There are two subtypes (A_2a and A_2b), with A_2b being prevalent in afferent arterioles.

P_2 receptors, present on endothelial and vascular smooth muscle cells, have a greater affinity for ATP and ADP than for adenosine or AMP. Interstitial fluid ATP concentrations are sufficiently high to play a role in regulating the vascular resistance changes responsible for autoregulation and TGF. The receptor subtype P_{2X} increases $[Ca^{2+}]_i$ by increasing Ca^{2+} influx through voltage-gated and receptor-operated

Ca^{2+}channels. The low frequency stimulation of renal sympathetic nerves causes renal vasoconstriction that is mediated, in part, by a nonadrenergic component involving ATP corelease with the neurotransmitter acting on P_{2X} receptors. P_{2Y} receptor activation increases $[Ca^{2+}]_i$ via the PLC-IP_3 cascade. Endothelial cells have a high concentration of P_{2Y} receptors, and their activation by ATP leads to vasorelaxation mediated by NO, or PGI_2, or both. However, ATP causes a rapid, marked constriction of the afferent arteriole when NO synthesis is inhibited. The P_{2U} purinoceptor is termed a nucleotide or 5′-nucleotide receptor because it responds to all nucleotides with a similar potency of ATP and uridine triphosphate. P_{2U} receptor activation increases $[Ca^{2+}]_i$ by a G-protein stimulating the PLC pathway.[1,2,211,212]

Adenosine

The intrarenal administration of adenosine produces a biphasic renal response. Resistance vessels respond initially with transient vasoconstriction, mediated by P_1-A_1 receptors, followed by gradual vasodilation, mediated in part by P_1-A_2 receptors. In isolated preparations, adenosine A_1 receptor stimulation constricts both afferent and efferent arterioles. Endogenous adenosine serves as an important regulator of renal hemodynamics. The blockade of A_1 receptors decreases afferent arteriolar resistance and K_f in anesthetized animals.

Evidence about the importance of adenosine in intrinsic autoregulatory mechanisms is mixed. One set of results suggests that adenosine is not essential for autoregulation. Interstitial fluid concentrations of adenosine are unchanged over the autoregulatory range of arterial pressure, and the administration of adenosine-receptor antagonists does not impair autoregulatory capability. Other results, however, implicate a role of adenosine in the regulation of afferent arteriolar tone by TGF. Salt transport by the ascending limb of the Henle loop or macula densa cells requires metabolic energy and the use of ATP that is linked to control of preglomerular vascular resistance through macula densa signaling. As transport increases, more adenosine is liberated within cells and is available to diffuse to the afferent arteriole, where it elicits vasoconstriction to reduce RBF and GFR. Luminal perfusion of A_1 adenosine-receptor agonists cause TGF-induced vasoconstrictor changes in glomerular capillary pressure. Attenuated TGF-mediated responses of the afferent arteriole are observed when an adenosine antagonist is added to either the luminal fluid or peritubular capillary blood. The effects are insensitive to the salt transport inhibitor furosemide, indicating P_1-A_1 adenosine-receptor–mediated effects by acting on either the macula densa or vascular smooth muscle cells. Moreover, an adenosine-receptor blockade reduces TGF control of preglomerular resistance, and mice without functional A_1 receptors lack TGF activity. However, unopposed A_2 receptor activation may result in substantial vasodilation. Dynamic RBF studies on gene-targeted mice are consistent with A_1 receptors mediating pressure-induced TGF responses participating in RBF autoregulation but not the myogenic response of the preglomerular vasculature. In addition, mice lacking the enzyme NTPDase/CD39, which reduces the formation of adenosine, have impaired TGF activity.[1,104,213–217]

Although adenosine and Ang II can act independently of each other on glomerular arterioles, they also act synergistically. Adenosine enhances afferent arteriolar reactivity to Ang II by increasing Ca^{2+} sensitivity. On the other hand, Ang II amplifies afferent arteriolar constriction to adenosine by a different mechanism, one due to increased $[Ca^{2+}]_i$. In this manner, adenosine can amplify vasoconstriction during high renin states and increased endogenous Ang II. Adenosine causes larger long-term decreases in GFR and the filtration fraction in sodium-depleted animals with increased renin-angiotensin activity than in animals consuming a normal salt diet.[218,219]

Adenosine participates in the regulation of renin secretion. Adenosine infusion inhibits renin release in vivo. The mechanism involves adenosine receptors on juxtaglomerular cells, with A_1 and A_2 receptors having opposite effects. Renin release is inhibited by A_1 purinergic receptors coupled to a $G\alpha_i$ protein and stimulated by A_2 receptors. A tonic inhibitory effect of adenosine is observed in isolated afferent arterioles with and without macula densa cells attached.[220–222]

ATP

P_2 receptors are also involved in mediating autoregulatory adjustments in RVR involving TGF responses. Extracellular ATP, at high levels that saturate P_2 purinoceptors, causes renal vasoconstriction and impairs the autoregulatory ability of the vasculature to dilate in response to decreases in arterial pressure. The effect on autoregulation appears to be specific to ATP because similar studies show that RBF autoregulation is not impaired when the vasoconstrictor is norepinephrine or Ang II.

The renal arterial infusion of ATP increases RBF. The renal vasodilation is mediated by activation of endothelial receptors to release NO. The ATP effect is converted to vasoconstriction during NO synthesis inhibition. P_2 receptors are present on vascular endothelium, with P_{2Y} receptors being responsible for NO-dependent vasodilation. P_{2X} receptors on vascular smooth muscle cells produce vasoconstriction. In considering a paracrine role for ATP, it seems likely that endogenous ATP reaches vascular smooth muscle cells from the interstitium. The vasoconstriction is mediated by Ca^{2+} mobilization and entry through L-type Ca^{2+} channels.[1,2]

ATP acts on P_2 purinoceptors to selectively constrict afferent arterioles in a sustained manner, whereas ATP does not elicit any response in efferent arterioles. This is consistent with autoradiographic and immunohistochemical evidence of abundant P_{2x} receptors limited to the preglomerular vasculature. Pressure-induced TGF–mediated afferent arteriolar constriction is prevented by P_2 receptor saturation or desensitization by high arterial concentrations of ATP. Tubuloglomerular responses also are markedly

blunted during peritubular capillary perfusion with saturating doses of ATP or slowly metabolizable analogs. $P_{2(x)1}$ receptor blockade prevents TGF-initiated responses of afferent arterioles.

Renal interstitial fluid concentrations of ATP measured by microdialysis are closely associated with autoregulatory and TGF-mediated changes in RVR. A direct relationship is observed when TGF is stimulated by increased distal fluid delivery due to a carbonic anhydrase inhibition of proximal tubular reabsorption and when TGF is inhibited by the transport inhibitor furosemide. Mice lacking functional P_{2x} receptors have partially blunted whole kidney RBF autoregulation and a loss of TGF. Macula densa cells secrete ATP through a basolateral maxi-chloride channel in response to increases in luminal NaCl concentration. Although macula densa cells have abundant mitochondria, they have low levels of Na^+-K^+-ATPase, making them a good candidate for a source of extracellular ATP. The activation of macula densa signals trigger the rapid propagation of Ca^{2+} waves and associated afferent arteriolar constriction, which are prevented by blocking P_2 receptor activation or increasing ATP hydrolysis. Collectively, the data support an important role for the ATP-dependent activation of P_2 receptors in the mediation of autoregulation and TGF.[1,2,12,107,211,223,224]

Sympathetic Nervous System and Catecholamines

The renal vasculature is richly innervated with postganglionic adrenergic fibers originating from sympathetic celiac and aorticorenal plexuses that receive inputs from the sixth thoracic through the second lumbar spinal nerves. All arterial segments of the renal vasculature and the large veins are extensively innervated with neuroeffector junctions containing norepinephrine as the primary neurotransmitter. A heavy concentration appears in subadventitial layers of arcuate arteries, with notable innervation of smooth muscle cells of both the afferent and efferent arterioles as well as the juxtaglomerular granular cells and the outer medullary descending vasa recta. The incoming efferent innervation is predominantly adrenergic, although some nerve endings are reported to contain dopamine and neuropeptide Y (NPY) as well as ATP. Alpha$_1$-adrenoceptors, primarily α_{1A} and α_{1D}, increase $[Ca^{2+}]_i$ and Ca^{2+} sensitivity, and mediate nerve stimulation-induced vasoconstriction of glomerular arterioles in the renal cortex and medullary pericytes, an action opposed in part by concurrent activation of α_2-adrenoceptors. Beta-adrenoceptors stimulate renin release from juxtaglomerular granular cells via cAMP/PKA signaling.[132,225]

Renal nerves are divided into two types. Type I almost exclusively innervate afferent arterioles, whereas type II innervate both afferent and efferent arterioles. Type II nerves contain NPY, whereas type I terminals do not. The electrical stimulation of the greater splanchnic or renal efferent nerves produces frequency-dependent renal vasoconstriction that is abolished by α_1-adrenergic receptor antagonists and attenuated by NPY Y_1 receptor blockade. Very low levels of nerve stimulation affect renin release and tubular sodium reabsorption without causing major changes in renal hemodynamics. Intermediate nerve activity elicits renal vasoconstrictor responses in the medullary as well as the cortical circulation, with more pronounced reductions in cortical blood flow. The increased vascular resistance in the cortex is due to the constriction of preglomerular and efferent arteriolar segments. As is shown in Figure 3.24, low levels of stimulation cause an equivalent constriction of afferent and efferent arterioles such that glomerular pressure is unchanged. Similar results are obtained in isolated afferent and efferent arterioles when α_1-adrenoceptors are stimulated. Higher frequencies of nerve stimulation produce predominant constriction of preglomerular vessels and thus reduce glomerular capillary pressure and GFR. Intense stimulation at 10 Hz produces glomerular ischemia.[226]

The renal medullary circulation is less sensitive than the cortical vasculature to renal nerve stimulation, particularly at low stimulus intensities. This is largely due to more effective blunting of vasoconstriction by NO and eicosanoids. The electrical stimulation of renal nerves causes parallel reductions in RBF and cortical blood flow, whereas the decrease in medullary perfusion is approximately 50% less. Low frequency nerve stimulation ($<$2 Hz) increases NO production, which buffers the vasoconstriction in all regions of the kidney. More severe renal vasoconstriction produced by high frequency nerve stimulation ($>$4 Hz) leads to the activation of AT_1 receptors that magnifies nerve-induced constriction in both the renal cortex and the medulla. NO derived from nNOS is critical to protecting the medullary regions.[227,228]

Infusions of norepinephrine, epinephrine, or α_1-adrenergic agonists produce similar dose-related vasoconstrictor effects on the renal microcirculation. Studies on individual vessels indicate that norepinephrine constricts the interlobular artery and the afferent and efferent arterioles. Total RVR responses to norepinephrine are substantially attenuated by Ca^{2+} channel blockers and by the inhibition of IP_3-mediated release of stored Ca^{2+}. Ca^{2+} responses of the afferent arteriole to α-adrenoceptor stimulation are mediated by the mobilization from internal stores in combination with Ca^{2+} entry through voltage-gated L-type channels and channels insensitive to dihydropyridine Ca^{2+} channel blockers. The latter includes voltage-independent receptor-operated Ca^{2+} channels. Ca^{2+} sensitivity is also increased. Recent evidence implicates superoxide anion generation and downstream signaling involving cyclic ADP ribose, ryanodine receptors on the sarcoplasmic reticulum, and Ca^{2+}-induced Ca^{2+} release. Efferent arteriolar responses to norepinephrine appear to be independent of L-type channels.[20,58,143]

The renal nerves are not essential for efficient steady-state autoregulation of RBF and GFR or for the operation of either the myogenic or the TGF mechanism. These conclusions are

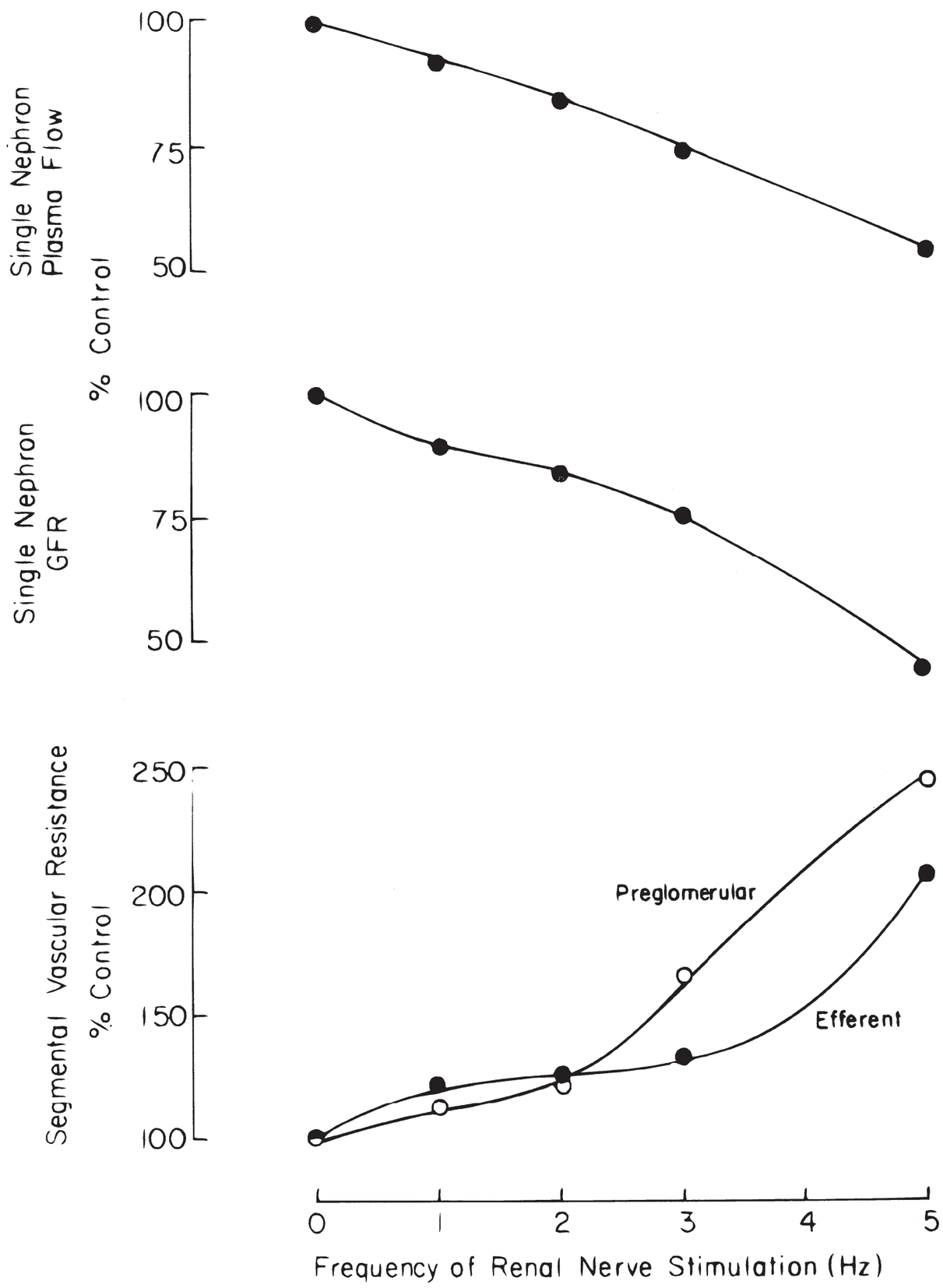

FIGURE 3.24 The effect of electrical stimulation of renal efferent nerves at 1 to 5 Hz on a single nephron glomerular filtration rate (*GFR*), plasma flow, and the resistance of preglomerular vessels and efferent arterioles.

reinforced by a frequency analysis of RBF dynamics indicating that baroreflex-mediated changes in renal sympathetic nerve activity and acute denervation have little impact on intrinsic autoregulatory mechanisms stabilizing renal perfusion in normotensive animals. Under unstressed conditions, while basal efferent sympathetic tone is low, neither acute nor chronic renal denervation affects RBF or GFR. Physiologic changes in renal efferent nerve activity during sleeping, grooming, and movement cause inverse changes in RBF in conscious animals. In anesthetized animals, moderate hypoxia and reflexively induced increases in renal sympathetic nerve activity reduce RBF more than GFR with increases in glomerular capillary pressure as a result of greater constriction of efferent over afferent arterioles. More severe hypoxia reduces RBF and GFR equally as afferent and efferent arteriolar resistances were increased to the same extent. Moderate increases of about 50% in renal nerve activity that are induced reflexively by high- or low-pressure receptors fail to alter renal hemodynamics, although this level of stimulation is capable of affecting tubular sodium reabsorption and renal release of renin and prostaglandins. Hypercapnic acidosis associated with an increase in PCO_2 from 25 to 70 mm Hg activates the renal efferent nerves but the neurally mediated renal vasoconstriction is partially counteracted by enhanced synthesis of vasodilatory prostaglandins. Neurally induced vasoconstriction is more readily demonstrable during more stressful states, such as during dehydration, blood-volume contraction, hemorrhage, and congestive heart failure. The pronounced activation of renal efferent nerve activity (+500%) produced by auditory or emotional stimuli causes intense renal vasoconstriction.[1,132,229]

In addition to a direct effect on vascular smooth muscle, the renal efferent nerves exert secondary hemodynamic effects as a consequence of an intrarenal stimulation of the constrictors Ang II and ET-1 and the formation of dilator prostaglandins and NO. Renal nerve stimulation elicits an increase in renin release from juxtaglomerular cells primarily by the activation of β_1-adrenoceptors signaling through the cAMP/PKA pathway. Prostaglandin synthesis is enhanced by the activation of phospholipase A_2 and the augmented availability of arachidonic acid. Ang II formation accentuates norepinephrine release and renal vasoconstriction

produced by increased renal nerve activity. α_1-Adrenoceptor activation enhances Ang II–mediated afferent arteriolar vasoconstriction by increasing calcium sensitivity. The vasoconstriction caused by moderate renal nerve stimulation is reduced in the presence of the Ang II/AT_1 receptor blockade, suggesting that part of the effect is mediated by increased intrarenal Ang II formation due to the neural stimulation of renin. On the other hand, vasodilatory prostaglandins buffer a significant fraction of the vasoconstriction elicited by nerve stimulation as evidenced by much larger changes in GFR, RBF, and vascular resistance during COX inhibition. Moreover, endothelium-dependent NO release, possibly via the activation of β_3-adrenergic receptors, functions as an inhibitory modulator of vasoconstrictor responses to the sympathetic transmitters norepinephrine and NPY. The effect of renal nerve stimulation on glomerular K_f is controversial. Some investigators report a reduction; others find that K_f is normally unaffected by nerve stimulation but that a reduction of K_f is evident during the inhibition of prostaglandin synthesis.[2]

Afferent renal nerves serve important functions in the neurohumoral control of arterial pressure, vasopressin release, and renal excretion of sodium and water. Afferent nerve endings contain neuropeptides such as calcitonin gene-related peptide, substance P, and vasoactive intestinal peptide. Afferent input from intrarenal sensory receptors in the pelvic wall participates in renorenal reflexes that modulate efferent nerve activity to the contralateral kidney. Baroreceptors or mechanoreceptors are activated by high venous or interstitial pressure. High renal pelvic pressure reduces efferent renal nerve traffic to the opposite kidney, which results in compensatory increases in RBF, GFR, and sodium excretion. Similar functional changes in the opposite kidney are elicited by ischemia-sensitive chemoreceptors responding to increased urinary potassium or very high sodium concentration. Outgoing afferent nerve activity is influenced also by incoming efferent nerve activity such that increased efferent input usually increases afferent nerve activity. The norepinephrine release from efferent nerves activates adrenoceptors on sensory nerves, with α_1-adrenoceptor activation increasing afferent nerve activity and α_2 receptor stimulation reducing afferent firing.[230,231]

Although cholinesterase-containing fibers and β-adrenergic sites have been identified histologically in the renal cortex, there is little functional evidence for neurogenic renal vasodilation mediated by acetylcholine or β-adrenoceptors. The neural release of acetylcholine, however, may exert a small indirect effect at presynaptic sites to inhibit norepinephrine release. Infusions of exogenous acetylcholine increase RBF and reduce RVR, whereas GFR is unaffected. The decline in total resistance is due to parallel reductions in afferent and efferent arteriolar resistance. Acetylcholine relaxes isolated preparations of the interlobular artery and the afferent and efferent arterioles. As pointed out earlier, however, the full vasodilatory effect of acetylcholine requires an intact endothelium and is primarily mediated by a combination of NO as well as prostanoids and EETs.[132]

Dopamine, a sympathomimetic amine precursor of norepinephrine, is a neurotransmitter capable of regulating renal hemodynamics, renin secretion, and sodium excretion. Histofluorescent evidence suggests dopaminergic innervation of the cortical vessels, primarily the glomerular vascular poles. However, dopamine synthesized from L-dopa via L-dopa decarboxylase in proximal tubular cells is clearly the major source of urinary dopamine and its metabolites, with the greatest production during high salt diet and lowest synthesis during sodium restriction. There are two major receptor families: D_1-like (D_1 and D_5 receptors) and D_2-like (D_2, D_3, D_4 receptors). D_1 receptors are postsynaptic receptors located on vascular smooth muscle and tubular cells, but are absent from glomeruli. They cause vasodilation by cAMP/PKA signaling. D_2 receptors are either presynaptic or postsynaptic and are located in glomeruli as well as vessels and tubules in both the cortex and medulla. The presynaptic D_2 receptor indirectly dilates vessels by the inhibition of norepinephrine release. Dopamine is commonly used to evaluate the renal functional reserve—the ability to dilate the renal vasculature—in various pathologic conditions.[232–236]

Although receptor blockers abolish renal effects produced by exogenous dopamine, such antagonists do not consistently affect basal renal hemodynamics, questioning the role of physiologic levels of dopamine. Further, the prejunctional stimulation of dopamine receptors during moderate levels of renal efferent nerve activity has little influence on RVR. Small amounts of exogenous dopamine or a D_1 receptor agonist dilate the renal vasculature. Responses include an increase in RBF and a decrease in filtration fraction; GFR and glomerular capillary pressure are unaffected. Most of the dopamine-induced renal vasodilation is mediated by D_1 receptors, which are coupled to cAMP/PKA signaling. Dopamine and D_1 receptor agonists relax afferent and efferent arterioles and interlobular and arcuate arteries. D_1 receptor stimulation attenuates the constriction of both afferent and efferent arterioles produced by either Ang II or ET-1. The D_1-induced dilation of the preglomerular vasculature does not impair autoregulatory adjustments in vascular resistance to changes in perfusion pressure. D_1-receptor stimulation attenuates tubuloglomerular feedback responsiveness. The vasodilation elicited by dopamine is more pronounced after denervation or pharmacologic blockade of α-adrenoceptors, suggesting that presynaptic D_2-dopamine receptors augment norepinephrine release from nerve terminals. D_2-receptor stimulation is associated with increases in GFR and the attenuation of Ang II–induced contraction of glomerular mesangial cells. D_1 receptors also stimulate a renin release from juxtaglomerular cells. D_1 agonists inhibit fluid and electrolyte transport indirectly via hemodynamic mechanisms and directly by the occupation of D_1 receptors in the proximal tubule, the thick ascending limb of Henle, and the collecting duct. D_1 receptor null mice have elevated proximal tubular salt transport and hypertension.[2,132]

NPY is another neurotransmitter. Nerve endings containing immunoreactive neuropeptides are localized along the interlobular and arcuate arteries extending to glomerular arterioles, innervating both afferent and efferent arterioles. NPY is a cotransmitter of the renal sympathetic nerves that is coreleased with and may potentiate the vascular pressor effects of norepinephrine and ATP. NPY is less potent than norepinephrine. The kidney expresses NPY receptors that can also be activated by peptide YY (PYY), a circulating hormone released from gastrointestinal cells. NPY and PYY produce renal vasoconstriction via the Y_1 receptor. Administered NPY constricts both the afferent and efferent arterioles and inhibits renin release. Nerves innervating the efferent arteriole contain more immunoreactive NPY than those to the afferent arteriole. NPY acts prejunctionally to inhibit norepinephrine release via Y_2 receptors. Despite marked reductions in RBF, systemic NPY infusion elicits a diuresis and natriuresis that is mediated in part by bradykinin. NPY antagonists increase basal RBF but do not alter basal urinary excretion.[226,228,237]

Other Vasoactive Agents

Atrial Natriuretic Peptide

ANP is a 28-amino acid peptide family involved in the physiologic regulation of renal function and sodium excretion. A high molecular weight precursor of ANP is constitutively synthesized in cardiocytes of the atria. ANP is released into the circulation as a function of atrial volume or distention in association with changes in sodium and water balance. In addition to ANP, related peptides include ventricular brain natriuretic peptide (BNP) and renal urodilatin. Studies implicate renal conversion of ANP to urodilatin, which is closely related to sodium excretion under a variety of conditions.[238,239]

ANP receptors are concentrated in glomerular capillaries and the collecting duct; they are also present along the cortical arterioles and the medullary arterioles and the vasa recta; cell types include the collecting duct, the vascular smooth muscle, and the endothelial and mesangial cells. There are three subtypes of natriuretic peptide receptors (NPR), termed NPR-A, NPR-B, and NPR-C. The A- and B-type receptors include a cytoplasmic catalytic domain and the active particulate guanylate cyclase, exerting physiologic effects by increasing cell cGMP. ANP may buffer the action of vasoconstrictors such as Ang II and norepinephrine via an interaction of cGMP with intracellular Ca^{2+}, perhaps secondary to the inhibition of Ca^{2+} mobilization and the stimulation of Ca^{2+} efflux. The renal vasculature also has biologically silent, NPR-C "clearance" receptors, that remove ANP from the circulation with no role in signal transduction or guanylate cyclase activity. [240–242]

ANP is a rapid-acting, potent natriuretic and diuretic peptide that is also capable of lowering arterial pressure by direct vasodilatory effects on the systemic vasculature and reducing cardiac output. Physiologic concentrations of ANP inhibit tubular reabsorption without altering RBF, GFR, or the filtered sodium load. Medullary blood flow is increased during the administration of ANP, but the effect seems to be secondary to the ANP-induced natriuresis. Several tubular sites have been evaluated, including a Na-ATPase of the proximal tubule and an amiloride-sensitive sodium channel in the collecting duct and an angiotensin-sensitive exchanger in the proximal tubule. ANP has a direct inhibitory action on renin release in cultured juxtaglomerular cells that is mediated by cGMP.[243,244]

The renal vascular effects of ANP are mediated by NPR-A and NPR-B receptors. Activation of NPR-A receptors dilates preglomerular resistance vessels, including arcuate and interlobular arteries, afferent arterioles, and efferent arterioles. The NPR-B receptor contributes to the dilation of the preglomerular vasculature. The reported effect of ANP on K_f is variable. Although glomerular capillary pressure is increased, GFR is usually unchanged, presumably because of a decrease in K_f. ANP infusions increase glomerular permeability to macromolecules. The preglomerular vasodilation produced by ANP does not impair autoregulation of either RBF or GFR. ANP exerts both indirect and direct effects on the TGF mechanism. ANP markedly inhibits TGF control of glomerular hemodynamics when feedback activity is evaluated by the perfusion of the Henle loop with artificial fluid. Studies on mice with underexpression and overexpression of the NPR-A receptor highlight the important role ANP has in mediating the renal vascular and tubular changes resulting from blood volume expansion. Overexpression of NPR-A receptors is associated with enhanced renal vasodilatory and natriuretic responses, whereas the responses are markedly attenuated in mice lacking NPR-A receptors. Synthetic ANP (M-ANP), being tested for use in hypertension and congestive heart failure, are potent agents for lowering blood pressure and systemic vascular resistance, while increasing RBF, GFR, and sodium excretion. Transgenic mice with an overexpression of natriuretic peptide receptor A respond to blood volume expansion with marked natriuresis, along with increases in RBF and GFR, whereas mice deficient in the NPR-A fail to show the increases in RBF and GFR or sodium excretion. These data demonstrate the importance of ANP in regulating renal function in response to volume expansion.[78,107,244–247]

Vasopressin

In addition to its antidiuretic properties, AVP is a vasoconstrictor, with predominant actions in the renal medulla. The plasma AVP concentration is directly related to plasma osmolality and inversely with blood volume and pressure. AVP produces effects on renal function by binding to V_{1a} or V_2 membrane receptors. The well-known osmoregulatory function of low plasma AVP concentrations is mediated through a V_2 tubular membrane receptor that signals through cAMP/PKA to alter water permeability and sodium reabsorption. Vasopressin also stimulates the production of medullary NO via the activation of V_2 receptors, which serves a protective

function to prevent excessive vasoconstriction. AVP affects renal hemodynamics by acting on V_1a receptors localized to cortical arteries/arterioles (interlobar, arcuate and interlobular arteries, afferent and efferent arterioles, glomeruli) and outer medullary vasa recta. V_{1a} mRNA and protein in preglomerular resistance arteries/arterioles are upregulated in response to reduced plasma AVP concentration associated with water loading and downregulated as plasma AVP is increased during water deprivation. Desensitization of V_1 receptors and reduced receptor density are related to PKC-mediated phosphorylation. AVP activates vascular V_{1a} receptors to exert pressor effects that contribute to the maintenance of arterial pressure during conditions of chronic water deprivation, graded hemorrhage, and possibly in various forms of hypertension.[107,248,249]

The vascular effects of AVP are due to the activation of V_{1a} receptors on vascular smooth muscle cells, which is coupled to Gα_q protein that leads to increased IP_3 production and PKC activation. In afferent arterioles, $[Ca^{2+}]_i$ is increased by a combination of Ca^{2+} release from the sarcoplasmic reticulum and Ca^{2+} entry through voltage-gated L-type Ca^{2+} channels and voltage-insensitive store-operated cation channels. The contraction of efferent arterioles is more dependent on Ca^{2+} release from sarcoplasmic reticular stores with little Ca^{2+} entry through L-type channels. In addition to increasing $[Ca^{2+}]_i$, AVP increases Ca^{2+} sensitivity via PKC and Rho-kinase signaling. The K_f lowering effect of AVP may be mediated by a direct action on mesangial cells. It was found, however, that K_f could be normalized by a combined blockade of the vascular action of both AVP and Ang II, whereas the selective blockade of either peptide was ineffective. The V_{1A} receptor on endothelial cells mediates AVP-induced NO release. Also, AVP interacts with the renal arachidonic acid and the renin–angiotensin systems. AVP stimulates renal release of PGE_2 in vivo. Prostaglandin production is increased by AVP-receptor interaction with either V_1 or V_2 receptors on medullary interstitial cells or glomerular mesangial cells. Vascular smooth muscle V_{1A} receptor activation stimulates phospholipase A_2 and prostaglandin production. Mice lacking the V_{1A} receptor are hypotensive and have decreased blood volume and sympathetic activity. They also have decreased activity of the renin–angiotensin system and lower aldosterone levels. The V_{1A} receptor is expressed in macula densa cells and colocalizes with COX-2 and nNOS. The reduced RBF and GFR are due, in part, to reduced blood pressure.[1,55,107,142,248,250–252]

The administration of exogenous AVP produces renal vasoconstriction that is buffered more by NO than COX-dependent prostaglandins, effects mediated by V_1 but not V_2 receptors. Vasoconstriction in the renal medulla is more prominent than in the renal cortex. AVP-induced cortical vasoconstriction is effectively buffered by the cytochrome P450 epoxide production of vasodilator EETs such that cortical blood flow is basically unchanged by AVP. In contrast, the medullary vasoconstriction elicited by AVP is unaffected by the inhibition of cytochrome P450 epoxide activity and EET production.[107,253]

In normal, unstressed conscious animals, the antagonism of V_1 receptors has no effect on basal RBF or arterial pressure, indicating minimal pressor activity of AVP when its plasma concentration is low. Physiologic increases in plasma AVP concentration during 48-hour water restriction and maximum urine osmolality reduce blood flow to the inner medulla via V_1 receptors while maintaining a constancy of blood flow to the outer medulla. V_2 receptor antagonism produces a diuresis. Increases in plasma AVP concentrations up to 8 pg per millimeter reduce medullary perfusion selectively and greatly attenuate the arterial pressure–blood flow and pressure–natriuresis relations without affecting total RBF or renal cortical blood flow. AVP-induced vasoconstriction in the renal medulla is normally modulated by AVP-stimulated local release of NO, perhaps mediated by V_2 receptors, reflecting a compensatory response that buffers the magnitude of the vasoconstriction and stabilizes medullary perfusion.[15]

Adrenomedullin

Adrenomedullin, α- and β-calcitonin gene-related peptide (CGRP), calcitonin, and amylin are homologous polypeptides with overlapping biologic actions such as vasodilation, inhibition of bone resorption, and antiproliferative activity. Adrenomedullin is a potent renal vasodilating and natriuretic peptide (52 amino acids with a disulfide ring), which is made in the kidney as well as the adrenal gland. mRNA for adrenomedullin and its receptor are colocalized to renal vessels, glomeruli, and inner medullary collecting ducts. The proximal tubule is abundant in adrenomedullin mRNA, whereas the greatest amounts of receptor mRNA are in the papilla. Changes in the salt diet do not appear to change the expression of the peptide or its receptor in either the cortex or the medulla. This hormone/paracrine agent increases RBF and sodium excretion without affecting GFR. Natriuretic potency increases during the inhibition of neutral endopeptidase, an enzyme that cleaves endogenous peptides with a disulfide ring such as adrenomedullin and ANP.

Adrenomedullin produces vasodilation by cAMP/PKA signaling and by stimulating endothelial NO production, leading to the activation of ATP-sensitive K^+ channels and Ca^{2+}-dependent K channels and the hyperpolarization of vascular smooth muscle cells. Adrenomedullin increases renin release via increasing cAMP in juxtaglomerular granular cells. CGRP exerts NO-dependent as well as cAMP/PKA vasodilation. The administration of adrenomedullin or CGRP increases RBF and to a lesser extent GFR; both are natriuretic and diuretic. However, the renal vasodilator effects of adrenomedullin are not dependent on CGRP. Adrenomedullin and CGRP dilate afferent arterioles and blunt Ang II- and norepinephrine-induced renal vasoconstriction. Increased adrenomedullin levels in diabetic rats are associated with

the early hyperfiltration and may contribute to the afferent arteriolar vasodilation.[107,254–257]

Reactive Oxygen Species

Small amounts of ROS constantly produced by aerobic metabolism have important roles in signal transduction in the vasculature under physiologic conditions. In pathophysiologic states, ROS initiate and amplify deleterious events such as lipid oxidation and tissue/DNA damage associated with glomerular inflammation and proteinuria, and vascular hypertrophy in addition to vasoconstriction. ROS are products of partial reduction of oxygen, generated by enzymatic and nonenzymatic reactions. Common oxidative enzymes include nicotinamide adenine dinucleotide(NADH)/reduced NADPH oxidases (NOX), COX, cytochrome P450, and xanthine and glucose oxidases. The reduction of molecular oxygen ($O_2 + e^-$) produces superoxide anion ($\bullet O_2^-$), which is normally balanced by its degradation. Superoxide dismutases (SOD) catalyze the conversion of $\bullet O_2^-$ to hydrogen peroxide (H_2O_2) that has oxidizing potential. H_2O_2 is subsequently neutralized by glutathione peroxidases and catalase. An alternative pathway for $\bullet O_2^-$ degradation is to rapidly react with NO to form peroxynitrite ($ONOO^-$), which clearly limits the half-life, the diffusion distance, and the biologic activity of NO as well as $\bullet O_2^-$. Vitamins A, E, and C and bilirubin are scavengers of ROS.[199,258–261]

As intracellular signals, ROS may activate or inactivate redox-sensitive protein kinases and phosphatases to modulate GPCR phosphorylation and transcription factors. Reactive nitrogen species such as S-nitrosothiols can modulate GPCR signaling and internalization through S-nitrosylation of β-arrestin and GRK. Normally there is a fine balance between activities of oxidative and antioxidant enzymes, optimizing NO activity and minimizing superoxide anion generation. $\bullet O_2^-$ functions as the counterpart to NO and its antiproliferative and vasodilatory actions. In the kidney, ROS are formed in arteries and arterioles, glomeruli, and juxtaglomerular cells endowed with oxidases such as NOX, NOS, and COX. NOX1, NOX2, and NOX4 are expressed in the kidney. Predominant isoforms in the renal vasculature are NOX1 and NOX2 that primarily produce $\bullet O_2^-$; epithelial NOX4 mainly generates H_2O_2. $\bullet O_2^-$ is degraded by superoxide dismutases, of which the cytosolic or intracellular isoform accounts for ~70% of the enzyme in the kidney, with lesser amounts in extracellular and mitochondrial compartments. In the juxtaglomerular apparatus, endothelial cells produce NO via eNOS and macula densa cells via nNOS. Stimulants of $\bullet O_2^-$ production include vasoconstrictor agents (e.g., Ang II, ET-1, norepinephrine), growth factors, and stretch. Chronic high Ang II and a high salt diet are potent stimulants, increasing the expression of NOX subunits and reducing superoxide dismutase isoforms. Overall renal actions of $\bullet O_2^-$ are vasoconstriction and the enhancement of tubular sodium reabsorption. NOX-derived $\bullet O_2^-$, largely independent of H_2O_2, contributes to GPCR signaling and participates in renal vasoconstriction elicited by Ang II, catecholamines, and ET-1 activation of ET_A and ET_B receptors.[199,262]

$\bullet O_2^-$ exerts tonic renal vasoconstriction in normotensive rats, an action that becomes more pronounced when NO production is inhibited, indicating an interaction between $\bullet O_2^-$ and NO that have opposing actions on vascular resistance. This is evident because the administration of superoxide dismutase and NOX inhibition increases RBF. Moreover, NOX2-deficient mice have an increased basal RBF with normal GFR and arterial pressure, with less renal vasoconstriction in response to NOS inhibition than observed in wild-type mice that are able to produce the vasoconstrictor via NOX2. NOX-derived $\bullet O_2^-$, largely independent of H_2O_2, contributes to GPCR signaling and participates in renal vasoconstriction elicited by Ang II, catecholamines, and ET-1 activation of ET_A and ET_B receptors. Ang II–induced renal vasoconstriction and reduced GFR are magnified during NOS inhibition and weakened during administration of superoxide dismutase to scavenge $\bullet O_2^-$. Increased endogenous $\bullet O_2^-$ activity produced by acute pharmacologic inhibition of superoxide dismutase (diethyldithiocarbamate) reduces total renal, as well as both cortical and medullary blood flow. The vasoconstriction is greater when the buffering afforded by NO is removed by NOS inhibition. This is also the case for renal vasoconstriction produced by norepinephrine, phenylephrine, and ET-1. Other studies show that increased $\bullet O_2^-$ production stimulated by the acute infusion of Ang II produces more pronounced renal vasoconstriction when NADPH oxidase is intact than when NADPH oxidase is rendered nonfunctional in transgenic animals with NOX2 mutated. Ang II elicits less pronounced renal vasoconstriction in the absence of NOX2. Extracellular superoxide dismutase inactivation of the $\bullet O_2^-$ plays an important role in buffering acute Ang II–induced constriction of the afferent arteriole and increased RVR produced by chronic Ang II infusion.[198,263–266]

The mechanism by which $\bullet O_2^-$ causes or modulates renal vasoconstriction in normal kidneys is still unresolved. Ang II stimulates NADPH oxidase activity and $\bullet O_2^-$ production in the renal cortex and medulla. Ang II and ET-1 receptor activation rapidly stimulates NOX2-mediated O_2- release from afferent arterioles that increases cytosolic Ca^{2+} concentration in smooth muscle cells by increasing ADP ribosyl cyclase activity. The metabolite cyclic ADP ribose sensitizes ryanodine receptors on the sarcoplasmic reticulum of smooth muscle to release Ca^{2+}. $\bullet O_2^-$ may also scavenge NO and reduce bioavailability of this vasodilator, a major factor contributing to endothelial dysfunction in disease states. Accordingly, $\bullet O_2^-$ activity is enhanced during NOS inhibition. It should be appreciated that $\bullet O_2^-$-induced acute renal vasoconstriction is observed during NOS inhibition, highlighting a principal direct action on vascular smooth muscle independent of NO.[20,113,160,198,263,264,266–269]

Increased intrarenal $\bullet O_2^-$ produced by infusions of hypoxanthine and xanthine oxidase increases sodium excretion,

while GFR is reduced more than RBF and arterial pressure is unchanged. In these rats, $\bullet O_2^-$ did not affect the efficiency of pressure-induced steady-state RBF autoregulation. However, other evidence convincingly indicates that $\bullet O_2^-$ regulates both myogenic and TGF mechanisms responsible for renal autoregulation. The pressure-induced myogenic constrictor response of isolated afferent arterioles is mediated or enhanced by $\bullet O_2^-$ independent of H_2O_2 and of eNOS production of NO. In addition, $\bullet O_2^-$ produced by NOX2 in macula densa cells plays a role in TGF-induced afferent arteriolar vasoconstriction via direct action on afferent arterioles. This direct action complements quenching NO availability. The effects of $\bullet O_2^-$ are attenuated by increased levels of superoxide dismutase. Thus, ROS are important signaling molecules that participate in intrinsic autoregulatory responses of the preglomerular vasculature to changes in renal perfusion pressure. Local production of NO and ROS modulates reactivity of descending vasa recta pericytes that control medullary perfusion.[114,171,270–274]

eNOS and NADPH oxidase are both expressed in tubular epithelial cells within the renal medulla, particularly the thick ascending limb of the Henle loop, with the production of NO and $\bullet O_2^-$ participating in the regulation of medullary blood flow and influencing the set point of the pressure–natriuresis relation. Sodium retention and hypertension result when the balance of production of these free radicals favors $\bullet O_2^-$ in conditions such as in activation of the renin–angiotensin system, NOS inhibition, and diabetes. For example, during chronic NOS inhibition, renal vascular actions of $\bullet O_2^-$ are largely unopposed. Intrarenal infusion of the superoxide dismutase tempol in hypertensive L-nitro-arginine-methyl-ester (L-NAME) treated rats increases total RBF and blood flow to both the renal cortex and the medulla, and GFR, while urinary excretion of 8-isoprostane is reduced.[275–277]

Peroxynitrite ($ONOO^-$) is formed endogenously by NO reacting with $\bullet O_2^-$, exerting NO-like biologic activity as well as nitrating proteins during oxidative stress. The administration of low $OONO^-$ concentrations causes renal vasodilation as RBF and GFR increase in parallel in a NO-dependent manner, reverting to the constriction during NOS inhibition. High $ONOO^-$ concentrations produce renal vasoconstriction with larger reductions in GFR than in RBF. The reductions in RBF and GFR are magnified during NOS inhibition.[278]

$ONOO^-$ can oxidize arachidonic acid to form the vasoconstrictor 8-iso-$PGF_2\alpha$ (F2-isoprostane), which activates a thromboxane TP receptor to elicit vasoconstriction. Isoprostanes may increase ET-1 release from endothelial cells. Chronic exposure to high levels of Ang II stimulates isoprostane production by the kidney. Oxidative stress and exaggerated isoprostane levels acting on TP receptors enhances the strength of TGF in some models of hypertension. Such signaling may explain the afferent arteriolar vasoconstriction associated with oxidative stress in Ang II–induced hypertension, which appears to be mediated by an endothelial-derived and COX-derived vasoconstrictor that acts on TP receptors on vascular smooth muscle cells.[279]

Endogenous H_2O_2 appears to have little effect on preglomerular resistance vessels in the renal cortex because the combination of catalase with a superoxide dismutase mimetic has no additional effect as compared to superoxide dismutase quenching O_2^- alone. As mentioned in the following, increases in H_2O_2 above basal levels of 100 to 500 nmol per liter cause vasoconstriction in the renal medulla. H_2O_2, at apparent pharmacologic concentrations in micrometer per millimeter range in vitro, is reported to have biphasic effects on nonrenal resistance arterioles, dilating at low concentrations, perhaps due to NO, and constricting at high concentrations (>50 μmol per liter), possibly due to an isoprostane. H_2O_2 inhibits Ang II increases in $[Ca^{2+}]_i$ in afferent arterioles when the concentration is ≥ 1 μm but not less. Electrophysiologic studies of endothelium of freshly isolated porcine renal arteries indicates that a large conductance (300 pS) Ca^{2+}-activated K^+ channel (BK_{Ca}) is inhibited dose dependently by ROS in nanomole per liter range and H_2O_2 in the micromole per liter range. If tonically active, closure of BK_{Ca} channels causes depolarization and leads to vasoconstriction. Consistent with this view, H_2O_2 was found to reduce bradykinin-induced dilation of isolated renal arteries.[272,280,281]

MECHANISMS REGULATING MEDULLARY MICROCIRCULATION

Blood flow to the renal medulla is only about 20% of the total RBF; however, it is of major importance in regulating sodium homeostasis and in the maintenance of the medullary hypertonic environment. Expressed per gram of tissue, medullary blood flow is lower than cortical blood flow. The unique architecture of the renal medullary microcirculation preserves the axial osmotic gradient generated by the countercurrent exchange of water and solutes between the descending and ascending vasa recta while allowing for the removal of the water and solutes reabsorbed from the descending limb of the Henle loop and the medullary collecting ducts. Blood flow to the renal medulla is supplied from efferent arterioles of juxtamedullary nephrons, which give rise to the vascular bundles located in the outer medulla. The descending vasa recta (DVR) gradually transform until the smooth muscle layer, present in the outer medulla, is replaced by the discontinuous rings of pericytes. Thus, the vascular smooth muscle cells of the juxtamedullary nephron arterioles and of the outer medullary DVR are primarily responsible for the differential regulation of the medullary circulation. The outer zone of the inner medulla is partitioned into two distinct compartments with intercluster regions, one consisting of the ascending and the descending vasa recta and another consisting of the ascending vasa recta (AVR) and collecting ducts. Efferent arterioles and the DVR

are the main medullary structures with sympathetic innervation, which terminates when pericytes replace the smooth muscle cells. Extravascular renomedullary interstitial cells also exhibit contractile properties, but their role in regulating medullary blood flow is unclear. It has been more difficult to study the medullary circulation due to its inaccessibility and the fact that it is positioned in series with the glomerular circulation of juxtamedullary nephrons. Although there is considerable discrepancy regarding the absolute values of medullary blood flow obtained using various techniques, most of them provide a reasonable index of relative changes in blood flow.[1,15,18,89,282,283]

Hematocrit

The hematocrit of renal medullary blood is lower than that of systemic blood or blood derived from the renal cortex. Red blood cell (RBC) transit time is shorter than for plasma, and tissue hematocrit varies inversely with the medullary axis. Studies using videomicroscopic techniques and complementary direct measurements of hematocrit with micropuncture have demonstrated low microvessel hematocrit in the renal medulla. Shrinkage of RBCs in the hypertonic medulla also shifts water from the interior of RBCs to plasma and also contribute to the lower medullary microvessel hematocrit.[2,15]

In contrast to the peritubular capillary plexus that arises from efferent arterioles in the cortex to reabsorb massive volumes of tubular fluid, the vasa recta serve different needs specific to the medulla. Through their countercurrent arrangement, the DVR and the AVR trap NaCl and urea deposited to the interstitium by collecting ducts and the loops of Henle and maintain corticomedullary osmotic gradients. However, metabolic substrates, including O_2, that enter the DVR diffuse to the AVR to be shunted back to the cortex, leading to lower O_2 levels in the medulla than in the cortex. Paracrine agents regulate the renal medulla perfusion in a complex manner involving tubular-to-vascular and vascular-to-tubular paracrine signaling cross-talk. Protection of the medulla from hypoxia by these agents involves the local generation of paracrine agents by tubular and vascular structures and trapping by countercurrent exchange to yield axial concentration gradients that exert variable effects on the medullary vessels.[90]

Autoregulation

As already discussed, overall RBF is autoregulated with very high efficiency over a wide range of systemic perfusion pressure. Although cortical blood flow is well autoregulated within the physiologic range, the extent to which medullary blood flow is autoregulated is more controversial, especially in the rat. Some studies suggest that medullary blood flow autoregulation is not as efficient as in the cortex and that changes in medullary blood flow contribute to pressure natriuresis and the regulation of salt and water excretion as perfusion pressure changes. As mentioned previously, however, the renal medulla is largely perfused by the efferent arterioles from juxtamedullary nephrons, which have very high autoregulatory efficiency. Although a small population of shunt vessels bypass glomeruli, and may escape the autoregulatory adjustments, the extent of periglomerular shunting is very small and unlikely to contribute significantly to overall medullary blood flow responses. Both cortical and medullary blood flows are efficiently autoregulated in the dog. This holds for both outer and inner medullary perfusion. The same is true for cortical blood flow in the rat. Inner and outer medullary blood flows are also autoregulated efficiently in hydropenic rats, but not during marked volume expansion. Thus, it appears that loss of medullary blood flow autoregulation occurs primarily during marked volume expansion in rats subjected to increases in perfusion pressure. Recruitment of flow through the previously unperfused vasa recta may also contribute to that process, in particular because individual medullary vessels also exhibit autoregulatory responses. Volume-expanded sodium-replete dogs and rabbits exhibit efficient intact medullary blood flow autoregulation. The in vitro juxtamedullary nephron preparation that evaluates blood flow through nephrons that give rise to the vasa recta clearly exhibits normal autoregulatory behavior of the preglomerular vasculature. Thus, the extent of medullary blood flow autoregulation efficiency as well as the role of medullary perfusion in the generation of pressure natriuresis varies with species and degree of volume expansion. Nevertheless, paracrine factors act within the medulla to exert local control of medullary blood flow. The pressurization of in vitro perfused DVR increases endothelial $[Ca^{2+}]_i$ and the generation of NO. The release of NO by the vasa recta could increase local blood flow as well as inhibit salt reabsorption by adjacent tubules. A role for NO to provide a diffusible signal between the vasculature and nephrons seems likely.[1,2,15,18,107,153,277,284–286]

Vasopressin

Increases in medullary blood flow reduce the efficiency of passive countercurrent exchange, leading to "solute washout" and reductions in the corticomedullary gradients for NaCl and urea. Vasopressin exerts an important role in reducing medullary blood flow which contributes to increased urinary concentrating ability during antidiuresis. Homozygous Brattleboro rats that lack vasopressin have central diabetes insipidus and elevated medullary plasma flow. Vasopressin exerts its actions via subtype specific V_1 (vasoconstrictor) and V_2 (antidiuretic) receptors and reduces vasa recta blood flow through the activation of both V_1 or V_2, indicating roles for both vasoactive and reabsorptive mechanisms. A selective V_1 receptor agonist reduces inner medullary more than outer medullary blood flow. Similarly, elevated circulating vasopressin in water-deprived rats reduces inner medullary blood flow with weaker effects in the cortex and the outer medulla; these effects are blocked

by a V_1 antagonist, supporting V_1-mediated vascular effects of vasopressin to modulate inner medullary blood flow and to promote antidiuresis by maintaining the elevated osmolality in the medullary interstitium. Vasopressin reduces medullary perfusion by constricting juxtamedullary afferent and efferent arterioles. Afferent arteriolar vasopressin–mediated constriction is dependent upon voltage-gated Ca^{2+} entry via voltage-gated L-type and store-operated channels, whereas efferent constriction may be primarily related to Ca^{2+} mobilization from stores. Vasopressin also constricts outer medullary DVR. A vasopressin V_1 agonist reduces medullary blood flow without constricting either afferent or efferent arterioles, suggesting a greater sensitivity of the vasa recta to vasopressin.[15,252,287,288]

In addition to V_1 mediated constrictor effects of vasopressin, vascular V_2 receptors may cause vasodilation via the stimulation of NO release. V_2 agonists dilate preconstricted afferent arterioles and the outer medullary DVR in vitro. The V_2 agonist, dDAVP, stimulates medullary NO release and increases medullary blood flow. AVP-induced V_2 receptor activation in collecting ducts stimulates the phosphoinositide pathway and causes the mobilization of Ca^{2+} to increase NO production in the medulla and to protect the outer medulla from excessive vasoconstriction and ischemia.[1,15,248,286]

Angiotensin

In addition to its actions on the afferent and efferent arterioles, Ang II tonically constricts the medullary microcirculation via effects on the vasa recta. A NOS blockade constricts DVR and intensifies the constriction by Ang II. Similar to NO, PGE_2 and adenosine blunt Ang II constriction of DVR.[15,275,289]

AT_2 receptor activation elicits vasodilation via the generation of NO and the synthesis of vasodilatory cytochrome P450 epoxygenase-derived EETs. AT_2 activation vasodilates DVR where it inhibits reactive oxygen species formation and facilitates endothelium-dependent $[Ca^{2+}]_i$ signaling that leads to the release of vasodilators. There are also mechanisms leading to the Ang II–induced enhancement of medullary perfusion via AT_1 receptors due to the generation of compensatory vasodilators, particularly NO and vasodilator prostaglandins. Interstitial Ang II concentrations in the renal medulla are higher than in the cortex and Ang II receptor density is also higher in this region.[15,129,228,290–292]

Nitric Oxide

NO is particularly important in defending the renal medulla against hypoxia and ischemia. Chronic and acute systemic or intrarenal NOS inhibition reduces medullary blood flow more than cortical blood flow and elicits hypertension. Per volume of tissue, NO production in the renal medulla exceeds that in the cortex. NO production is closely coupled to L-arginine availability and cellular uptake via an amino acid transporter. Accordingly, dietary L-arginine supplementation increases renal medullary interstitial NO and medullary blood flow and reduces blood pressure in hypertensive Dahl rats and spontaneously hypertensive rats (SHR). DVR is dilated by NO donors and endothelium-dependent vasodilators. Similarly, NOS inhibition increases DVR vasomotor tone, producing as much as a 35% decrease in blood flow and blunts the dilation of preconstricted DVR by the endothelium-dependent vasodilators, acetylcholine and bradykinin. NO has a particularly important role to protect against the excessive reduction of medullary perfusion associated with hypoxia and oxidative stress, and NO levels in the medullary interstitium rise in response to Ang II, norepinephrine, and vasopressin. Conversely, medullary inhibition of NOS and NO production increases vascular sensitivity so that infusion of otherwise ineffective doses of vasoconstrictors reduce perfusion and generate tissue hypoxia.[284,293]

NO has both vasodilatory and natriuretic effects and is synthesized by epithelial as well as endothelial cells, suggesting critical tubular–vascular interactions. NO generated by the medullary thick ascending limb influences DVR tone. Conversely, NO generated by the DVR inhibits sodium reabsorption by adjacent nephron segments.[15,284,294]

The bioavailability and actions of NO are modified to a great extent by opposing the generation of ROS. NO levels in the renal medulla are modulated through reactions with oxygen radicals. ROS generation plays a role in the agonist-induced constriction of renal microvessels in the cortex and the medulla and in Na reabsorption by the ascending limb of the loop of Henle. In the DVR, ROS are generated upon stimulation with Ang II and PKC agonists; superoxide anion generation by the medullary thick ascending limb may limit availability of NO delivery and the vasodilation of DVR. The SOD inhibitor, diethyldithiocarbamate, reduces medullary blood flow, indicating a vasoconstrictor action of superoxide anion. Infusion of the SOD mimetic, tempol, increases medullary blood flow and sodium excretion, an effect that is more pronounced when H_2O_2 is simultaneously eliminated with catalase.[228,270,275,294–296]

Arachidonic Acid Metabolites

Prostaglandins, generated from arachidonic acid by COX-1 and COX-2 enzymes, alter intrarenal hemodynamics and increase blood flow toward the juxtamedullary cortex. Medullary blood flow is protected from excessive vasoconstrictors by prostaglandins as well as NO. Nonselective COX blockade decreases vasa recta blood flow by up to 50% and potentiates medullary hypoxia with a relative sparing of cortical perfusion. PGE_2 is generated in large quantities in the renal medulla and blunts Ang II–induced constriction of isolated perfused DVR. Both COX-1 and COX-2 isoforms contribute to renal prostaglandin synthesis and are predominantly expressed in the renal medulla; COX-2 is subject to greater regulation. Renomedullary interstitial cells express receptors and release paracrine substances including PGE_2, EETs, and medullipin, and express both COX-1 and COX-2. Medullary COX-2 expression is

stimulated by tonicity. Genetic deficiency of COX-2, or its chronic inhibition, reduces medullary blood flow and enhances vasoconstrictor responses to Ang II. COX-2–derived prostanoids serve to maintain medullary blood flow under most conditions.[15,180,290,297,298]

Products of arachidonic acid are also generated by cytochrome P450 isoforms to yield EETs, the HETEs, and their products, dihydroxyeicosatetraenoic acids (DHETs). 20-HETE reduces medullary blood flow and the inhibition of 20-HETE with HET0016 enhances medullary blood flow. In contrast, EETs have been linked to endothelial-derived hyperpolarizing factors and cause vasodilation directly and also oppose vasoconstrictor actions of vasopressin.[118,253,299]

Kallikrein–Kinin System

Kallikreins release kinins from kininogens and are expressed by the outer and inner medullary collecting ducts. Kinins exert their actions by activating B_1 and B_2 receptors. B_2 receptors are expressed throughout the outer and inner medulla, and modulate blood flow and sodium reabsorption in the renal medulla. The infusion of a kinin antagonist causes a 20% reduction of papillary blood flow. The enhancement of kinin activity through the infusion of bradykinin or the inhibition of kininases with enalaprilat or phosphoramidon increases both medullary blood flow and sodium excretion. Blocking bradykinin receptors decreases medullary blood flow, an effect that is blocked by NOS inhibition. ACE inhibition in the presence of an AT_1 receptor blockade causes greater increases in MBF than cortical blood flow, and the effects are blocked by the B_2 receptor antagonist, icatibant. Low sodium diets augment the kinin-mediated component elicited by ACE inhibition. In volume-expanded anesthetized rats, a B_2 receptor blockade with icatibant reduces papillary blood flow. Bradykinin, acting through B_2 receptors, generates robust endothelial cytoplasmic calcium $[Ca^{2+}]_i$ responses in isolated DVR leading to marked NO production and EETs leading to vasodilation of Ang II preconstricted vessels.[15,208,300]

Adenosine

As discussed earlier, adenosine exerts its actions on the renal vasculature predominantly through A_1 and A_2 receptor subtypes. Adenosine A_1 receptor activation transiently reduces cortical and medullary blood flow, whereas A_2 receptor stimulation leads to medullary vasodilation and natriuresis. Both A_1 and A_2 receptors are expressed by DVR, and their respective stimulation induces constriction or dilation. Interstitial adenosine concentrations are near the affinity for the A_2 receptor so that changes should modulate vasodilatory and natriuretic effects. A_1-induced constriction is mediated by the pertussis-toxin–sensitive $G\alpha_i$ protein and phospholipase C activation. A_2-mediated dilation is mediated by the stimulation of the $G\alpha_s$ protein and the activation of K_{ATP} channels via enhanced levels of 11,12-EET. During hypoxia, the medullary thick ascending limb of Henle synthesizes adenosine, which serves a paracrine vasodilator function to preserve medullary perfusion and to augment medullary pO_2.[15,61,212,217,301,302]

Endothelin

Medullary ET receptors are present on medullary vascular bundles, medullary interstitial cells, and adjoining collecting duct cells. ET1 concentrations in the renal medulla are regulated by osmolality. ET1 binds to and stimulates both ET_A and ET_B receptors, thus inducing vasoconstriction. Isolated ET_B receptor stimulation, however, mediates vasodilation. Preglomerular smooth muscle cells show $[Ca^{2+}]_i$ responses to ET_A and ET_B agonists. The DVR from the outer medulla constrict in response to ET1, but medullary perfusion is not greatly affected due to the enhanced production of NO from ET_B-receptor stimulation, counteracting the vasoconstriction. ET_B-receptor stimulation of NO production plays a significant role in protecting the medullary circulation from excessive vasoconstriction.[15,150,156,162,303,304]

ET1 treatment selectively reduces cortical blood flow while transiently increasing medullary blood flow. Medullary vasodilation and natriuresis are prevented by blocking ET_B receptors or NO synthesis. Renal hypoxia stimulates ET1 production. The effects of endothelins on medullary blood flow vary with dietary salt intake. Chronic Ang II infusion, combined with salt loading, increases cortical and medullary immunoreactive ET. The administration of ET1 into ET_B-receptor deficient rats or wild-type rats in which the ET_B receptor is blocked fails to increase sodium excretion. Mice with the collecting duct-specific knockout of the ET1 gene have impaired sodium excretion in response to sodium loading and have salt-dependent hypertension.[156,304]

Reactive Oxygen Species

The production of ROS in the kidney is greatest in the outer medulla. eNOS and NADPH oxidase are both expressed in tubular epithelial cells within the renal medulla, particularly the thick ascending limb of the Henle loop, with the production of NO and $\bullet O_2^-$ participating in the regulation of medullary blood flow by acting on pericytes encircling the DVR. $\bullet O_2^-$ and H_2O_2 cause vasoconstriction, effects that are partially offset by the vasodilator NO. The inhibition of superoxide dismutase in the renal medulla increases local $\bullet O_2^-$ levels and reduces medullary perfusion. This is also the case for increased H_2O_2. Chronically increased oxidative stress such as in the activation of the renin–angiotensin system and diabetes, reduces medullary blood flow and sodium excretion, resetting pressure–natriuresis to produce hypertension.[270,275,277,305]

Collectively, the various vasoactive agents selectively regulate medullary blood flow through their actions on the pericytes that surround the vasa recta. Interestingly, the pericytes respond relatively weakly to vasoconstrictors such as Ang II, ET1, norepinephrine, and vasopressin, but

more robustly to the vasodilators including NO, CO, PGE_2, and adenosine. Furthermore, endogenous Ang II enhances, whereas NO reduces, the impact of increased renal sympathetic activity on medullary blood flow. The continuous interactions among these components regulate medullary blood flow and local oxygen supply.[15,18,208,306]

ADAPTATION OF RENAL HEMODYNAMICS TO ALTERED PHYSIOLOGIC CONDITIONS

Changes in Salt Intake

In healthy young adults, chronic changes in salt in the diet cause proportional changes in extracellular fluid volume and sodium excretion while having a subtle or minor influence on renal hemodynamics and arterial pressure. RBF and GFR are usually maintained within normal limits as a result of basically parallel adjustments in vasoconstrictor and dilator systems; this most often is also the case for arterial pressure. Some people, especially the elderly with compromised renal functions, develop salt-sensitive hypertension when consuming a high sodium diet.

As discussed earlier, the sodium diet and the extracellular fluid volume are major regulators of renin synthesis and release from juxtaglomerular granular cells. Renin secretion and Ang II production are stimulated by volume contraction due to macula densa signaling and increased sympathetic activity. A reduced delivery of salt to the macula densa stimulates COX-2 activity and PGE_2 production and, thereby, renin release. Macula densa COX-2 and microsomal PGE synthase mRNA are induced to increase PGE_2 production during a low sodium diet, whereas the EP_4 receptor expression is increased in glomeruli and in renin-secreting juxtaglomerular granular cells. Other important stimuli to renin-containing cells are sympathetic nerve activity and baroreceptor input. High salt intake inhibits renin release and Ang II production by these three major mechanisms of regulating renin. During sodium restriction, the elevated circulating and intrarenal Ang II levels constrict both afferent and efferent arterioles to either maintain or increase glomerular capillary pressure. K_f tends to be reduced. GFR is maintained in the normal range or is slightly reduced, whereas RBF declines to a greater extent, thus increasing the filtration fraction. Single nephron studies indicate that the TGF regulation of the afferent arteriolar tone is augmented during chronic salt restriction and high endogenous Ang II.[13,121,131,182,307]

The adaptive renal vasoconstrictor component is primarily the elevated Ang II acting on vascular AT_1 receptors because the Ang II receptor blockade increases RBF and GFR and reduces arterial pressure, with predominant effects in reducing afferent arteriolar resistance. Intrarenal ACE inhibition increases RBF and GFR in sodium-restricted dogs as a result of equal dilation of afferent and efferent arterioles in the presence of unchanged arterial pressure; K_f increases slightly. It is noteworthy that recent studies of direct renin inhibition with aliskiren in humans on a low salt diet reveal larger increases in RBF and GFR than in inhibition of the renin–angiotensin system using an ACE inhibitor or AT_1 receptor antagonist.[308]

A high salt diet increases the urinary excretion of salt to maintain a steady-state balance. There is a moderate increase in extracellular fluid volume, whereas the arterial pressure and the RBF and GFR usually remain within the normal range in individuals who are not salt sensitive. The stability of renal hemodynamics is achieved by a balance between vasoconstrictor and dilator systems. The vasoconstrictor effects of the renin–angiotensin system are reduced due to multiple mechanisms, including the suppression of renin release and Ang II production. Macula densa nNOS and COX-2 expression and activity are reduced during sodium loading. Reduced PGE_2 production by macula densa cells contributes to a reduced renin release along with reduced β-adrenergic stimulation by sympathetic nerves and increased baroreceptor inhibition in juxtaglomerular granular cells. Accordingly, inhibition of the renin–angiotensin system has little influence on renal hemodynamics and arterial pressure in animals fed a high salt diet. This also is the case for the AT_1-receptor antagonism and for ACE inhibition. Renal efferent sympathetic nerve activity is also suppressed, as is the case for opposing actions of the major vasodilator systems of NO and prostaglandins, which are reduced in parallel. TGF activity is reduced, whereas distal tubular flow is increased during volume expansion. The underlying mechanisms are not known, but low ambient levels of Ang II may contribute to reduced reactivity.[2]

Renal vascular reactivity to exogenous Ang II is enhanced during sodium loading and attenuated during a low salt diet, being related to AT_1-receptor density that is reciprocally associated with endogenous Ang II levels. Thus, Ang II–induced vasoconstriction is less pronounced when Ang II is chronically elevated because of the downregulation of vascular AT_1 receptors. This is not the case for norepinephrine as glomerular hemodynamic responses to renal nerve stimulation are similar whether animals are maintained on a low, normal, or high salt diet.[146] Intrarenal blood flow varies during changes in the sodium diet, with cortical and outer medullary but not inner medullary blood flow paralleling sodium intake. A high salt diet and reductions in the plasma Ang II concentration lead to increased cortical blood flow but not outer or inner medullary perfusion. Medullary blood flow is relatively well maintained during sodium restriction.[111,309]

The renal hemodynamic influence of vasodilator systems such as eNOS/NO, COX/PGE_2-PGI_2, and epoxygenase 11,12-EET are upregulated by a low salt diet, effectively counteracting the exaggerated vasoconstrictor influence of high Ang II and sympathetic nerve activity. In this setting, COX inhibition reduces RBF, renal cortical blood flow, and GFR, along with PGE_2 production as compared to no effect on renal hemodynamics in sodium-replete animals (Fig. 3.25). Whether the prostanoids are produced

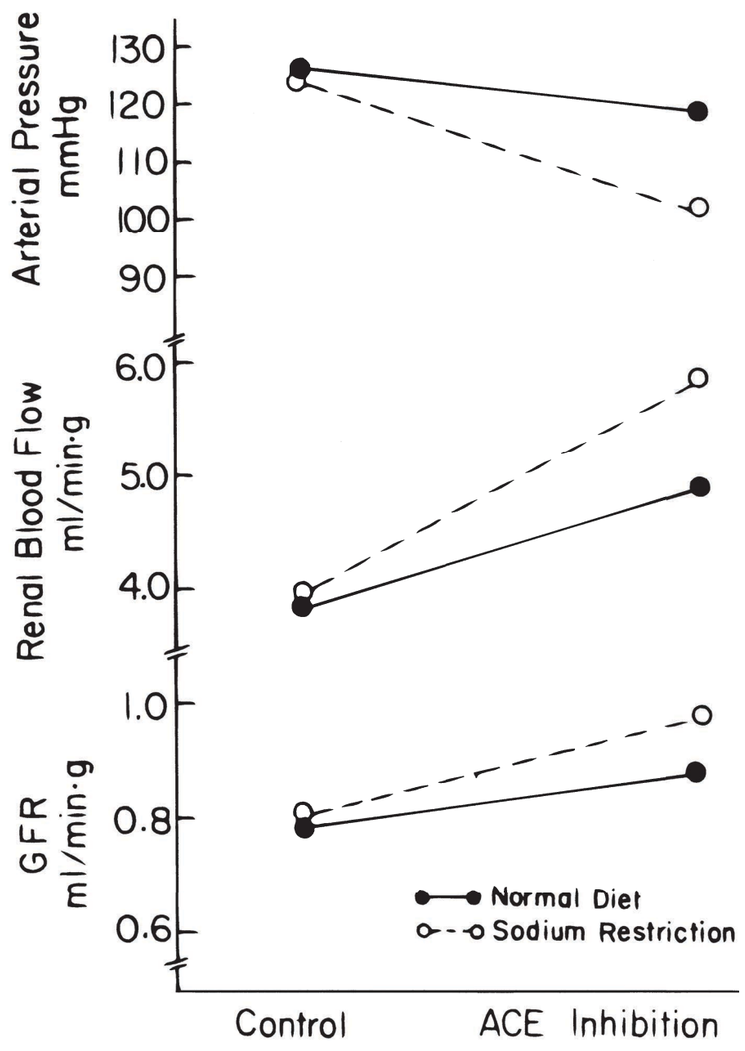

FIGURE 3.25 A comparison of renal hemodynamic and arterial pressure responses to angiotensin converting enzyme (ACE) inhibition in sodium-replete (*solid lines*) and sodium-depleted (*dashed lines*) anesthetized dogs. *GFR*, glomerular filtration rate.

by COX-1 or COX-2 is uncertain. One study finds that the renal hemodynamic changes are dependent on COX-2 activity, whereas another study reports that selective COX-2 inhibition is without effect on whole kidney hemodynamics during sodium depletion. COX-2 inhibition decreases medullary blood flow equally in animals on normal and low salt diets.[71,116,310,311]

COX activity and renal cortical production of vasodilatory prostanoids are low during volume expansion. COX inhibition in high salt animals has essentially no effect on whole kidney RBF or GFR. On the other hand, a high salt diet increases COX-2 expression in the renal medulla. Selective medullary COX-2 inhibition reduces medullary blood flow, which is associated with sodium retention and hypertension in rats consuming a high salt diet.[312–315]

Macula densa nNOS activity and NO productivity are inversely related to the sodium diet. Both endothelial eNOS and macula densa nNOS are upregulated during a low salt diet. NO generation in the kidney from L-arginine participates in adapting renal function to changes in salt intake. Dietary salt loading in animals enhances eNOS expression and activity and NO generation in the renal vasculature. Urinary nitrate + nitrite excretion, an index of NO production, increases during high sodium intake. NOS inhibition produces greater increases in arterial pressure (AP) and RVR and greater reductions in RBF in animals and humans on high versus low salt diets. NOS inhibition reduces medullary more than cortical perfusion during low and normal sodium diets. During sodium loading, NO control is similar for cortical and medullary blood flow. Macula densa nNOS is downregulated during a high salt diet. In contrast, protein expression of eNOS, iNOS, and nNOS is increased in the inner medulla. Afferent arteriolar vasodilatory responses to acetylcholine and sodium nitroprusside were unaffected by chronic high salt diets.[316–318]

Sodium restriction upregulates the kallikrein–kinin as well as the renin–Ang II system. The AT_1-receptor antagonism and ACE inhibition in dogs maintained on a low salt diet increase RBF and cortical blood flow. ACE inhibition causes larger increases in medullary flow than AT_1 receptor antagonism, an effect normalized by the blockade of bradykinin B_2 receptors. The vasodilator kallikrein–kinin system also participates in the renal adaptation to salt loading. Mice lacking vasodilator B_2 receptors respond to a high salt diet by becoming hypertensive with reduced RBF and increased RVR. In contrast, wild-type mice are normotensive with tendencies toward increased RBF and reduced RVR.[206]

Renal vascular reactivity to exogenous ET-1 is reduced by a low salt diet. ET_A receptor blockade has little effect on RBF during volume contraction. Sodium restriction upregulates pre-pro-ET-mRNA in the renal cortex, whereas ET_A and ET_B receptor density is unchanged. Low sodium intake and elevated Ang II acting on AT_1 receptors increase renal medullary ET-1 and ET_A and ET_B receptor mRNA, but not the sensitivity of renal medullary perfusion or sodium excretion to the ET_A or ET_B receptor blockade.[319,320]

Endogenous ET-1 levels are increased in the renal cortex and the medulla of animals fed a high salt diet, and renal vascular reactivity to exogenous ET-1 at the whole kidney level is enhanced. ET-1 reduces renal cortical blood flow and increases medullary perfusion in rats on a high salt diet as compared to cortical vasoconstriction, and has no effect on medullary blood flow in animals on a normal salt diet. Juxtamedullary-afferent arteriolar vasoconstriction produced by ET-1 and the ET_B receptor agonist are reduced. Arteriolar ET_B receptors, but not ET_A receptors, are upregulated presumably on endothelial cells during salt loading, and their vasodilation appears to buffer ET-1–induced vasoconstriction mediated by ET_A receptors. The ET_A-receptor blockade has no effect on whole kidney renal hemodynamics in rats placed on a high salt diet.[158,321]

Sodium loading increases renal ET-1 content and NO production that is in part mediated by ET_B receptors that are responsible for increasing medullary blood flow. A genetic deficiency of ET_B receptors leads to salt-induced hypertension. The more specific deletion of ET-1 or ET_B receptors in the collecting duct also causes salt-sensitive hypertension. In contrast, the specific mutation of ET_B receptors in vascular endothelial cells does not affect the AP sensitivity to salt.[156,322]

Normal kidneys, however, exhibit highly efficient whole kidney steady-state autoregulation of RBF whether animals are fed either a low or high salt diet and thus is independent of endogenous Ang II. Sodium depleted dogs have a normal RBF and GFR, with efficient autoregulation of RBF,

GFR, and single nephron GFR to changes in renal perfusion pressure. RBF is also effectively autoregulated during a low salt diet whether or not ACE is inhibited or AT_1 receptors are antagonized.[2] A high salt diet has little impact on dynamic RBF autoregulation, with either no change or an increased responsiveness of the myogenic mechanism in Sprague-Dawley or Long-Evans rats, respectively. Normal myogenic responses of afferent arterioles to increased luminal pressure is observed in mice fed a high salt diet for 3 months. A slight attenuation of pressure-induced myogenic responses is noted during high salt versus low salt diets for isolated interlobular arteries and afferent arterioles of Dahl salt-resistant rats. In hypertensive states, efficient autoregulatory responses to an increased arterial pressure play an important role in protecting sensitive glomerular capillaries and other renal structures from severe barotrauma. In some individuals, high salt intake may increase RBF and impair autoregulatory mechanisms such that the increased arterial pressure is transmitted to glomeruli, with the eventual development of proteinuria and glomerular sclerosis, along with interstitial inflammation.[93,323–325]

Renal cortical cytochrome P450-2 epoxygenase protein levels in renal microvessels and urinary metabolite EET excretion are increased during the consumption of a high salt diet. Epoxygenase products such as 11,12-EET exert antihypertensive effects as a result of vasodilator and natriuretic actions. Epoxygenase inhibition leads to salt-sensitive hypertension. In contrast, cytochrome P450-4A ω-hydroxylase protein levels are reduced in the renal cortex and in the renal vasculature. Nevertheless, a high salt diet increases overall renal excretion of 20-HETE in an adaptive response that contributes to increased sodium excretion. The pharmacologic inhibition of 20-HETE formation reduces sodium excretion and leads to salt-sensitive hypertension in rats.[183,318,326]

Adenosine produces more pronounced A_1 receptor–mediated renal vasoconstriction in high renin/Ang II states such as during sodium restriction. In contrast, adenosine produces dilation mediated by A_2 receptors in animals on a high salt diet, with increases observed in both the renal cortex and the outer and inner medulla. The reduction in RVR is due in part to enhanced cytochrome P450 activity and EET production during high salt intake. The pharmacologic stimulation of A_1 receptors decreases RBF and perfusion to the cortex and both the outer and inner medulla. In contrast, an A_1 receptor agonist causes reductions in RBF and cortical and outer medullary blood flow, but not inner medullar perfusion in low salt animals.[218,327,328]

Conditions that shift the balance to favor increased vasoconstrictor actions of superoxide and other reactive oxygen species over the vasodilator buffering effects of NO promote salt-sensitive hypertension that is characterized by renal vasoconstriction and enhanced salt retention. A hallmark of endothelial dysfunction is reduced NO production that can result from a reduced expression of eNOS and nNOS and impaired eNOS activation and NO production in the kidney. Salt-sensitive hypertension can be induced by pharmacologic NOS inhibition or genetic deletion of eNOS; the pressor response is reversed by the superoxide dismutase mimetic tempol. Enhanced renal vasoconstriction is associated with stronger than normal actions of Ang II and amplification by oxidative stress and NADPH oxidase–derived ROS as well as isoprostane. A chronic high salt diet leads to oxidative stress and inflammation in the kidney and vascular tissues and, eventually, hypertension, which is independent of Ang II activation of AT_1 receptors. Salt loading increases renal cortical NADPH oxidase subunit expression (NOX2 and $p47^{phox}$) and activity, increases superoxide generation, and increases urinary H_2O_2, 8-isoprostane, malondialdehyde, and thromboxane B_2 excretion, and decreases plasma NO end products. This is accompanied by the reduced expression of antioxidant superoxide dismutase isoforms. Such changes are limited more to the renal cortex than the renal medulla. It is noteworthy that the functional changes associated with oxidative stress and high salt intake are counteracted by oral L-arginine supplementation and improved NO production.[262,329–335]

Sodium restriction increases plasma renin activity and AT_1-receptor dependent oxidative stress in the kidneys as urinary excretion of 8-isoprostane is increased. Chronic activation of the renin–angiotensin system induces oxidative stress in the kidney. The administration of Ang II acting on AT_1 receptors enhances renal cortical NOX1, $p22^{phox}$ expression, superoxide production, and urinary excretion of 8-isoPGF2α (isoprostane). mRNA is decreased for NOX4 and extracellular superoxide dismutase. Although weaker, AT_2 receptors tend to blunt the actions of AT_1 receptors. The administration of the superoxide dismutase tempol increases RBF and arterial pressure, whereas GFR and sodium excretion are unchanged, suggesting predominant actions of superoxide on the renal vasculature.[262,336,337]

Changes in Protein Intake

The Western-style diet is characterized by highly processed and refined foods with a high content of sugars, salt, and fat, and high protein from meat that is chronically associated with dyslipidemia, oxidative stress, and inflammation. It is a major contributor to metabolic disturbances and the development of obesity-related diseases, including type 2 diabetes, hypertension, and cardiovascular and renal disease.

It has long been recognized that variations in the protein diet and plasma amino acid concentrations can have significant effects on the renal circulation. The consumption of protein in excess of 1 g/kg/day is usually associated with renal vasodilation in animals and humans. In dogs, the consumption of a high protein meal leads to increases in RBF and GFR, which are maximal at 3 to 6 hours and then progressively return to normal by 24 hours. The effect of protein feeding on renal function in humans is less marked than that in dogs. A short-term intravenous infusion of casein produces renal vasodilation that is sustained for up to 8 hours even though the blood amino acid concentrations

rapidly return to preexisting levels after the infusion is stopped. Various combinations of amino acids usually produce renal vasodilation and increase GFR; the changes are rapid in onset and reversible. It has been noted that only amino acids that are metabolized dilate the renal vasculature, whereas nonmetabolized amino acids do not affect RBF.[338–341]

The postprandial response to a protein-rich meal or a response to metabolizable amino acid infusion involves renal hyperemia and hyperfiltration because both RBF and GFR increase in both humans and animals. The ability of the kidneys to vasodilate and increase RBF and GFR in response to an acute protein or amino acid load is used clinically as a diagnostic index of vascular adaptability of "renal functional reserve." The greater the response, the more adaptable and thus the healthier the reserve; a weak or absent response is associated with severe nephron loss and aging. The mechanisms responsible for the renal vasodilation are multiple, involving blood-borne vasoactive agents, including pancreatic glucagon and the intrarenal release of COX-derived vasodilator prostanoids, NO, as well as reduced renin–Ang II and reduced TGF activity. Plasma renin activity and Ang II levels are unchanged and ACE inhibition does not impact on the protein-induced renal vasodilation.[154]

In healthy humans and laboratory animals, a high protein diet or the infusion of amino acids exert proportional effects on RBF, GFR, and urinary urea nitrogen excretion. Renal vascular resistance is reciprocally related to protein intake, with larger changes in RBF than in GFR. Arterial pressure remains stable and is independent of protein intake. Some reports, however, find that vegetarians have a reduced GFR relative to omnivores. The high protein diet leads to renal vasodilation and increased RBF and GFR in the face of normal arterial pressure and normal levels of plasma renin activity. Increased RBF and GFR are mediated by COX-dependent prostanoids and are largely prevented by COX inhibition. Glomerular COX and PLA_2 activities are increased, and the glomerular (but not renal papillary) production of PGE_2, $PGF_2\alpha$, and TxA_2 are increased under basal conditions and in response to Ang II. The urinary excretion of PGE_2 and PGF_2 metabolites are increased while plasma renin activity is elevated. Renal vasodilation is little affected by Ang II, as protein-induced changes in renal hemodynamics are unaffected by ACE inhibition. Nevertheless, vascular reactivity to Ang II is reduced during high protein feeding, whereas that of norepinephrine is normal. One mechanism contributing to renal vasodilation and increased GFR on a high protein diet is suppressed TGF control of preglomerular vascular resistance. Nevertheless, glomerular hyperfiltration is observed in adenosine A_1 receptor–deficient mice lacking TGF.[2,111,342,343]

Athletes and exercisers often use high-protein diets to enhance strength and muscle hypertrophy and recovery from intense exercise or injury. High protein diets combined with carbohydrate or fat restriction are also advocated for weight loss. However, it should be appreciated that chronic consumption of large amounts of protein may exacerbate or cause kidney damage. A long-term high protein intake often leads to proteinuria and increased GFR with renal hypertrophy that is accompanied with larger glomeruli and more glomerulosclerosis and tubulointerstitial fibrosis. The glomerular hypertrophy is due in part to increased vascular endothelial growth factor production.[344,345]

During a low protein diet, arterial pressure is normal, whereas plasma renin, Ang II, and aldosterone levels are reduced. Nevertheless, renal renin content is increased and exerts tonic actions on RVR. RBF and GFR are markedly reduced by protein restriction. The reduction in RBF is due to equal increases in both afferent and efferent arteriolar resistance. GFR is reduced due to reductions in plasma flow and the ultrafiltration coefficient. ACE inhibition increases RBF and GFR while reducing RVR, which is consistent with a prominent vasoconstrictor role of intrarenal Ang II. Glomerular AT_1 receptor density in the renal cortex and the medulla is increased during low protein feeding. Dietary protein restriction lowers plasma renin activity as a result of reduced PGE_2 production.[346]

In general, high protein intake aggravates renal injury, whereas restriction of dietary protein (~1 g protein per kilogram of body weight per day) while avoiding malnutrition ameliorates the progressive development of glomerular disease in various models. Based on animal studies, a low protein diet tends to be renoprotective in that it delays damage and proteinuria associated with hypertension, diabetes, obesity, or reduced renal mass and chronic renal disease, thus reducing GFR, glomerular growth, and interstitial infiltrate. A restricted protein diet reduces uremia, the progression of proteinuria, and the decline in GFR in adult patients with moderate-to-severe chronic renal disease. Dietary protein restriction reduces the glomerular permselective defect responsible for proteinuria in human renal disease. Implicated mechanisms include a hemodynamic basis, with reduced blood flow and glomerular capillary pressure, and nonhemodynamic factors related to reduced glomerular growth and less oxidative stress and inflammation. A low protein diet may reduce RBF by increasing TxA_2 production. Local Ang II levels may also participate. A protein restrictive diet lowers plasma renin activity by attenuating renin release, mediated in part by reduced PGE_2 stimulation, and increases AT_1 receptor density in the renal cortex and the medulla.[347–351]

CONCLUSION

As is evident from the previous discussion, there are many exciting issues concerning the area of renal hemodynamics and the multiple control mechanisms that are under active investigation. Modern technologic developments have allowed a more detailed and a more direct evaluation of the characteristics of specific segments of the renal microvasculature and of the various membrane and cellular mechanisms mediating differential responses. The direct assessment of

responses of individual arterioles has allowed for the clarification of long-standing controversies. In addition, developments related to interactions between endothelial cells and vascular smooth muscle cells are now receiving greater attention from investigators studying the renal circulation, as has been the case for interactions between distal tubular macula densa cells and vascular cells of the afferent arteriole and glomerulus. This has led to exciting new concepts with far-reaching implications. Additionally, many of these integrative mechanisms are now being addressed in terms of dynamic as well as steady-state characteristics. This combination of new developments has provided the impetus for renewed interest in the area of renal hemodynamics and the interactions with other intrarenal systems. These new investigations should result in a much better appreciation of the exact mechanisms that regulate renal microvascular contractility and reactivity and how disruptions of these mechanisms can lead to or predispose the kidneys to dysfunction and increased injury.

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