CHAPTER

4 Regulation of Water Balance: Urine Concentration and Dilution

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Water is by far the largest component of the body and accounts for approximately 50% to 65% of body weight. Maintenance of body fluid homeostasis, including fluid volume and solute concentration, is essential for cell function and whole-organism survival. The osmolality of body fluid, a concentration of all of the solute in water, is kept within a remarkably narrow range (280 to 295 mOsm per kilogram of water), in spite of large fluctuations of solute and water intakes and losses. Although this constancy is made by a variety of regulatory mechanisms in the body, the most critical regulatory capacities are provided by the kidney's urine concentration and dilution mechanisms.

Body water homeostasis is maintained by the balance between the input and output of water. Each side has regulated and unregulated components. The regulated component of water input is oral intake of fluids in response to a perceived sensation of thirst. The unregulated components of water input are oral intake of liquids and water in foods, and metabolic water of oxidation. Oral intake of water usually varies enormously in excess of homeostatic need because of social, cultural, or psychological influences. Solute-free water excretion by the kidney is the only route of regulated water output. The unregulated component of water excretion occurs via various kinds of insensible water losses including sweat, evaporative loss, gastrointestinal loss, and the obligate amount of water that is required to excrete the solutes in the urine. Sweat volume is determined by the requirements of temperature regulation. Evaporative loss is determined by body temperature and surface area, ventilation, and environmental temperature and humidity. Gastrointestinal water loss is affected by disturbance of its function. Both the input and output of water have very substantial unregulated components, and these can vary tremendously as a result of factors that are unrelated to the maintenance of body water homeostasis. Therefore, the regulated components, which are urine excretion and water intake caused by thirst, must compensate for whatever perturbations result from the unregulated water gains and losses. The daily urine excretion range is as low as 0.5 L to as high as 25 L depending on the requirements for water balance. When the kidney's capacity to conserve water is maximized to the limit due to dehydration, a sensation of thirst is activated, causing oral water intake to be increased.

Because the solute concentrations in water in the body must be kept nearly constant, water loss must be regulated by a mechanism that decouples water and the solutes. The kidney can excrete the appropriate amount of water without marked perturbations in solute excretion. When water intake is too large and dilutes blood plasma, urine diluted more than plasma is excreted to concentrate the plasma. When water intake is too small to concentrate plasma, urine concentrated more than plasma is excreted to dilute the plasma. In both cases, the solute excretion varies little. Renal solute-free water excretion is mainly regulated by the antidiuretic hormone vasopressin. Vasopressin is secreted from the posterior pituitary gland into systemic circulation in response to increases in the tonicity, which is an effective osmotic pressure in the plasma, to decreases in the effective circulating volume or pressure, or to several other stimuli. In response to changing levels of vasopressin in the plasma, the kidney is capable of wide variations in free water excretion. The molecular entity of a major effector of vasopressin in the kidney is aquaporin-2 (AQP2), which is a member of the aquaporin (AQP) water channel family. Molecular identification of the AQP family has revolutionized the understanding of water transport in the body, including urine concentration and dilution.[1,2] AQP2 is abundant in the collecting duct, which is the chief site of regulation of water reabsorption.[3] Acute stimulation of vasopressin promotes AQP2 translocation from an intracellular reservoir to the luminal cell surface, and its chronic stimulation increases the cellular abundance of AQP2, both of which elevate the water permeability of the collecting duct cells, resulting in the promotion of water reabsorption from the urinary tubule. Its impairments result in various water balance disorders including nephrogenic diabetes insipidus (NDI). In addition to AQP2, six other AQP isoforms are expressed in the kidney and play key roles in water transport activities specific for their localization in the nephron segments without

affecting solute transport. In addition to AQPs, the localizations of urea transporters and ion transporters are highly specific through the renal tubule segments. Together with a three-dimensional configuration of the nephron, the specific transport activities of water, urea, and ion all through the renal tubule segments enable the mechanism of urine concentration and dilution.

ANATOMIC CONSIDERATIONS FOR URINE CONCENTRATION AND DILUTION MECHANISMS

General Features of the Nephron Structure

The kidney consists of two populations of nephrons: short-looped nephrons and long-looped nephrons (Fig. 4.1). Both types of loops have a hairpin or a U-shape configuration, and are defined by the length of their Henle loop. Short-looped nephrons originate from superficial glomeruli and have a Henle loop, which turns near the inner-outer medullary border and consists of a proximal straight tubule, a thin descending limb, and a thick ascending limb. Long-looped nephrons originate from the juxtamedullary glomeruli, extend into the inner medulla, bend at various levels of the inner medulla, and contain a thin ascending limb in addition to segments found in short-looped nephrons. Thin ascending limbs are found only in the inner medulla and their transition to thick ascending limbs defines the inner-outer medullary border. Thus, only thick ascending limbs are found in the outer medulla.

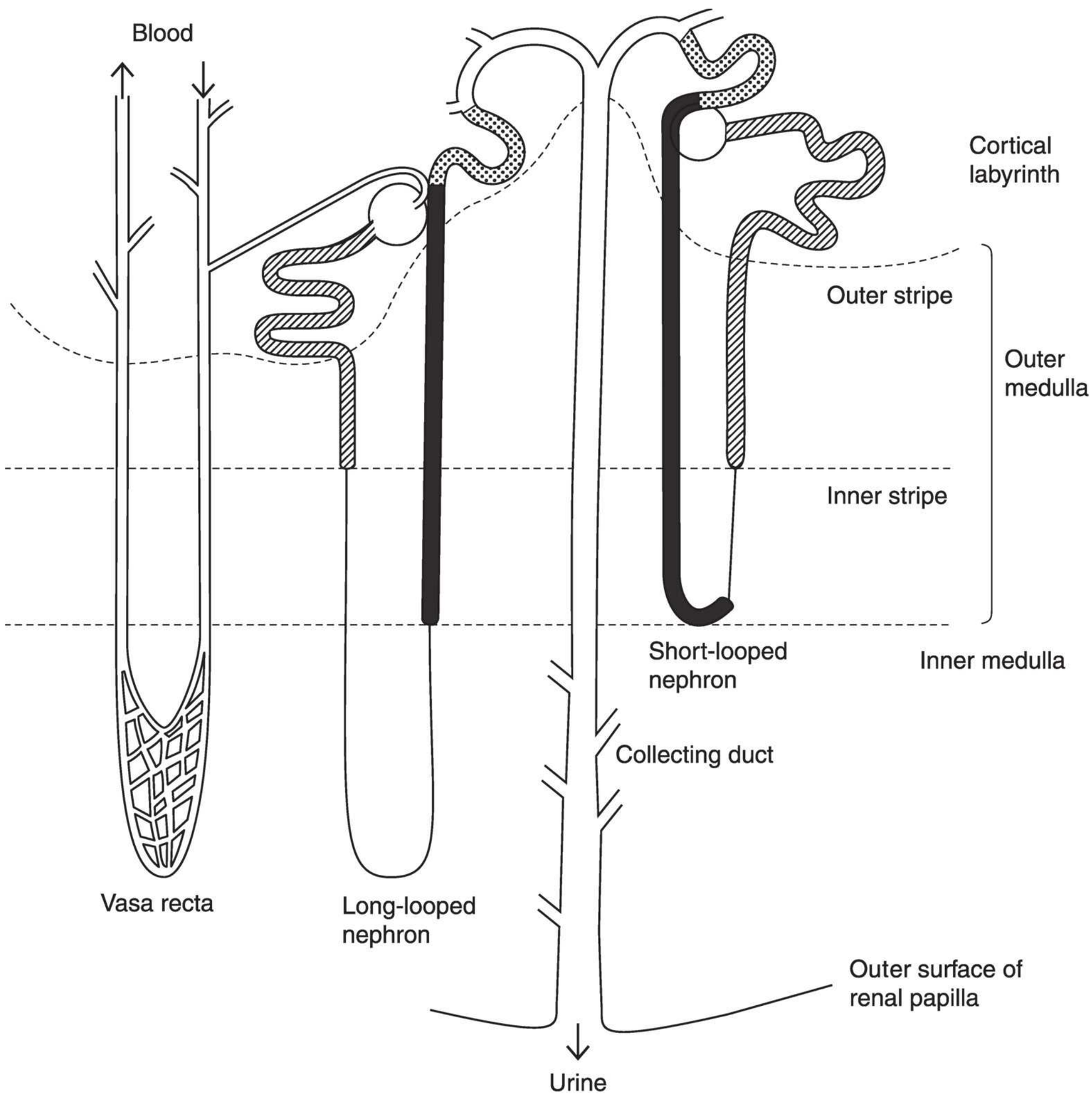


FIGURE 4.1 The kidney structure. The configurations of both a long-looped and a short-looped nephron are shown. The vasa recta is shown in the left. The major portions of the nephron are glomeruli (*circles*), proximal tubules (*hatched*), the thin limbs of the Henle loop (*single lines*), thick ascending limbs of the Henle loop (*solid*), distal convoluted tubules (*stippled*), and the collecting duct system (*open*). (Modified from Knepper MA, Stephenson JL. Urinary concentrating and diluting processes. In: Andreoli TE, Fanestil DD, Hoffman JF, Schultz SG, eds. *Physiology of Membrane Disorders*, 2nd ed. New York: Plenum, 1986:713, with permission.)

The Descending Part of the Henle Loop

The descending part of the Henle loop consists of the S2 proximal straight tubule in the medullary ray, the S3 proximal

straight tubule in the outer stripe of the outer medulla, and the thin descending limbs in the inner stripe of the outer and inner medulla. AQP1 is abundant in both the apical membrane and the basolateral plasma membrane in the S2 and S3 proximal tubules and the descending thin limbs.[4–6] AQP1 is constitutively active and is not regulated by vasopressin. AQP1 is present in sufficient abundance to account for the observed water permeability of these tubule segments.[7] The osmotic water permeability of the thin descending limb is extremely high due to the presence of the AQP1 water channel.[6] However, there is an axial heterogeneity in the expression levels of AQP1 in the thin descending limb; AQP1 is present in entirety through the thin descending limb of the short-looped nephron, whereas it is located in the upper half of the thin descending limb of long-looped nephron.[8–10] A comparison of water permeability and the abundance of AQP1 in these tubule segments indicates that AQP1 is the main route for water movement across the tubules.[8] The high water permeability of these segments is critical for the countercurrent multiplication system that is described in the section Countercurrent Multiplication in the Outer Medulla and Other Mechanisms in the Inner Medulla, which follows. As expected, a severely impaired urine concentrating ability is observed in AQP1 knockout mice.[11]

In addition to AQP1, AQP7 is expressed in the apical membrane of the S3 proximal tubule. AQP7 transports glycerol as well as water, thus AQP7 is an aquaglyceroporin.[12] Studies in AQP7 knockout mice suggest that water absorption mediated by AQP7 is small compared to that of by AQP1 and minimally contributes to urine concentration; whereas glycerol absorption by AQP7 may be important in glycerol metabolism in the body.[13]

A urea transporter UT-A2 is present in the thin limb of the long-looped nephron in the inner medulla and the thin descending limb of the short-looped nephron in the outer medulla. Thus, these segments have a high urea permeability[14] and play an important role for urea-recycling pathways that serve to maintain high urea concentrations within the inner medullary interstitium, which is critical for the urinary concentration that is described in the section Urea Accumulation in the Inner Medulla, which follows.

The Ascending Part of the Henle Loop

The ascending part of the Henle loop consists of the thin ascending limb in the inner medulla, the medullary thick ascending limb in the inner stripe of the outer medulla, and the cortical thick ascending limb in the medullary rays. The thin ascending limb is located exclusively in the inner medulla, is present only in the long-looped nephron, and becomes a thick ascending limb at the inner-outer medullary border. The thin ascending limb has extremely low water permeability and AQPs are not detected. By contrast, the ClC-K1 chloride channel is expressed in the apical and basolateral membrane of the entire length of the thin ascending limb.[9,15,16] Studies with immunohistochemical labeling and computer-assisted reconstruction in rats' inner medullas show that the thin ascending limb marked by ClC-K1 expression occurs about 160 μm before the loop bend.[9]

The thick ascending limb has essentially no water permeability and does not express AQPs. On the other hand, Na-K-2Cl cotransporter (NKCC2) is present in the apical membrane,[17] and Na-K-ATPase, a Na pump, is present in the basolateral membrane in the thick ascending limb. NaCl is actively absorbed by these transporters, resulting in the dilution of the luminal fluid in this segment, which works as the single effect in the countercurrent multiplication (described in the following sections). Urea permeability in the cortical thick ascending limb is higher than in the medullary thick ascending limb.[18] This may also contribute to the dilution of the luminal fluid as it flows up to the cortex by passive urea absorption.

The Distal Convoluted Tubule and Connecting Tubule

After exiting the Henle loop, the luminal fluid enters the distal convoluted tubule that expresses the thiazide-sensitive Na-Cl cotransporter NCC.[17,19] NCC is important for NaCl reabsorption that reduces fluid delivery to the collecting duct, leading to increases in urinary concentrating ability. Water permeability in the distal convoluted tubule is low and the expression of AQP2 or vasopressin V2 receptor is not observed.

Several distal tubules merge to form a connecting tubule arcade. The connecting tubule cells express both AQP2 and vasopressin V2 receptor, suggesting that, like the collecting ducts, the connecting tubules are sites of regulated water absorption. Moreover, vasopressin-regulated water absorption in the connecting tubule can result in a considerable amount of free-water absorption during antidiuresis because the large aggregate epithelial surface area of the connecting arcades is estimated to be roughly equivalent to that of the cortical collecting ducts.[20]

An AQP2 knockin mice model of nephrogenic diabetes insipidus, a rare disease manifested by an inability to respond to vasopressin, shows a severe urinary concentrating defect that results in neonatal death.[21] Meanwhile, AQP2 conditional selective knockout in the collecting duct but not in the connecting tubule rescues mice from the lethal phenotype that is observed in mice lacking AQP2 globally.[22] Although all types of mice show a severe urinary concentrating defect, these findings confirm an important role of the connecting duct for urinary concentration.

The Collecting Duct

The collecting duct is the final structure in the nephron. The luminal fluid from the connecting tubules then enters the collecting tubules in the superficial cortex. The collecting duct spans all the regions of the kidney. From the superficial cortex, the collecting duct descends through the cortex and the outer medulla. In the inner medulla, these collecting ducts

continually merge, finally forming the ducts of Bellini that open into the renal pelvis at the papillary tip. The renal pelvis is continuous with the ureter. The collecting ducts are arrayed parallel to the Henle loop. The collecting ducts have several morphologically discrete tubule segments: the cortical collecting duct, the outer stripe portion of the outer medullary collecting duct, the inner stripe portion of the outer medullary collecting duct, the initial part of the inner medullary collecting duct (IMCD), and the terminal part of the IMCD.

The collecting duct, under the control of vasopressin and other factors, is the nephron segment responsible for the final control of water excretion. The collecting duct expresses the vasopressin V2 receptor and AQP2, which are the primary targets for vasopressin regulation. In the absence of vasopressin, AQP2 resides in the vesicles below the apical cell surface and the entire collecting duct is very water impermeable. When the body is dehydrated, plasma osmolality is increased, which is sensed by the hypothalamic osmoreceptors and results in vasopressin secretion by the posterior pituitary. Vasopressin binds to V2 receptors in the basolateral membrane of the principal cells of the collecting duct, stimulates adenylate cyclase to produce cAMP, activates protein kinase A, phosphorylates AQP2, and inserts this water channel into the apical cell surface, which results in a significant increase in the collecting duct water permeability and water reabsorption.[23–25] This process is described in detail in the section Aquaporin-2, which follows. Not only are AQP2 water channels present, but also are AQP3 and AQP4 water channels in the collecting duct principal cells. There is axial heterogeneity in the abundance of AQP3 and AQP4; AQP3 is abundant in the connecting tubule, the cortical collecting duct, and the outer medullary collecting duct; whereas AQP4 is most abundant in the inner medullary collecting duct.[26] AQP3 and AQP4 are located in the basolateral membrane of these cells and represent a potential exit pathway from the cell to the interstitium for water entering via AQP2.

Water reabsorption mainly occurs in the cortex and the outer medulla. The inner medulla has the highest osmolality and is important for the reabsorption of the remaining water when maximal water reabsorption is required. Rather, water reabsorption by the terminal part of IMCD is greater during diuresis than during antidiuresis[27] because of its large transepithelial osmolality difference and relatively high basal water permeability compared to other portions of the collecting duct.

The connecting tubule and the cortical collecting duct express the amiloride-sensitive sodium channel ENaC.[28,29] Sodium is actively absorbed via ENaC in the apical membrane of the principal cells and exits the cells via Na-K-ATPase in the basolateral membrane. Sodium reabsorption via ENaC is increased by vasopressin or aldosterone and plays an important role for reducing fluid delivery to the medullary collecting duct, thus leading to increases in urinary concentrating ability.[30] On the other hand, the sodium permeability is low in the medullary collecting duct.

The urea permeability is extremely high only in the terminal IMCD and low in other portions of the collecting duct.[31] UT-A1 and UT-A3 urea transporters are present in the terminal IMCD cells.[32] Urea reabsorption in this segment is important for the preservation of high urea concentrations in the inner medullary interstitium, which are essential for its urine concentrating ability and are also important for minimizing the urinary loss of urea that is a major part of the urea recycling pathway. This process is described in detail in the section Urea Accumulation in the Inner Medulla, which follows.

The Vasculature

The blood vessels named the vasa recta carry blood into and out of the renal medulla. Like the Henle loop, there are the descending and ascending parts in the vasa recta, both of which are arranged in parallel and in mutual proximity. Blood from efferent arterioles of juxtamedullary nephrons enters into the descending vasa recta in the medulla, passes through the capillary plexus located at various depths within the medulla, then merges to form the ascending vasa recta. Different from the Henle loop, the descending and ascending vasa recta are separated by the capillary plexus (see Fig. 4.1).

The AQP1 water channel and the UT-B urea transporter are present in the descending vasa recta and enhance the countercurrent exchange of water and urea between the descending and ascending vasa recta in the medulla.[6,33] This countercurrent exchange reduces the effective blood flow, contributing to the conservation of osmolality gradients in the medullary interstitium.

The Medullary Interstitium

The interstitium of the outer medulla and the outer portion of the inner medulla is a narrow space that is important for limiting solute diffusion upward along the medullary axis.[34] To the contrary, the interstitium of the inner half of the inner medulla is much larger. In this region, the interstitial cells are interspersed in a gelatinous matrix of highly polymerized hyaluronic acid,[35] which is largely devoid of capillary plexuses or lymphatics. This structure slows the diffusion of solute and water in the inner medulla.

The Pelvis

The collecting duct finally opens at the papillary surface and urine enters the pelvic space. In the kidney with only one papilla, such as in hamsters and rats, the renal pelvis is an intrarenal urinary space surrounding the papilla. In the kidney with many papillae, such as in humans, each papilla is surrounded by a funnel-shaped calyx. In the human kidney, it is the compartment between the calyces and the ureter that is called the pelvis.[36] This compartment is not present in kidneys with one papilla, where the pelvis is a direct extension of the ureter. The renal pelvic (calyceal) wall contains smooth muscle layers. Contractions of these muscles induce regular peristaltic contractions of the wall that strongly

compress the medullary tissue, resulting in intermittent flow in the collecting ducts and the Henle loop.[37]

URINE CONCENTRATION MECHANISMS

General Features of Urine Concentration

In an adult human, glomerular filtrate is about 180 L per day, most of which is reabsorbed by the high water permeable proximal tubule and descending limb of the Henle loop. However, regulation of water excretion mainly occurs after the luminal fluid reaches the distal tubule. The osmolalities of luminal fluid along the rat nephron are shown in Figure 4.2. The luminal fluid in the proximal tubule is isosmotic to plasma, regardless of antidiuresis or diuresis. This fluid then enters the thin descending limb of Henle and is concentrated as it flows down to the bend of the Henle loop because of water reabsorption. As the fluid flows up the ascending limb of the Henle loop, the luminal fluid becomes diluted because this segment is impermeable to water, and the thick ascending limb actively absorbs NaCl. This diluted fluid finally enters the collecting duct system. In diuresis (low AVP), the fluid remains hypotonic. On the other hand, in antidiuresis (high AVP), the fluid is concentrated to a level far greater than plasma by water reabsorption as it flows down the collecting duct. This is due to a vasopressin-induced significant increase in water permeability of this segment and the existence of an axial osmolality gradient in the medulla. This osmolality gradient with the highest degree of hyperosmolality at the papillary tip is maintained by several mechanisms, including countercurrent multiplication, countercurrent exchange, and urea accumulation in the inner medulla, as described subsequently.

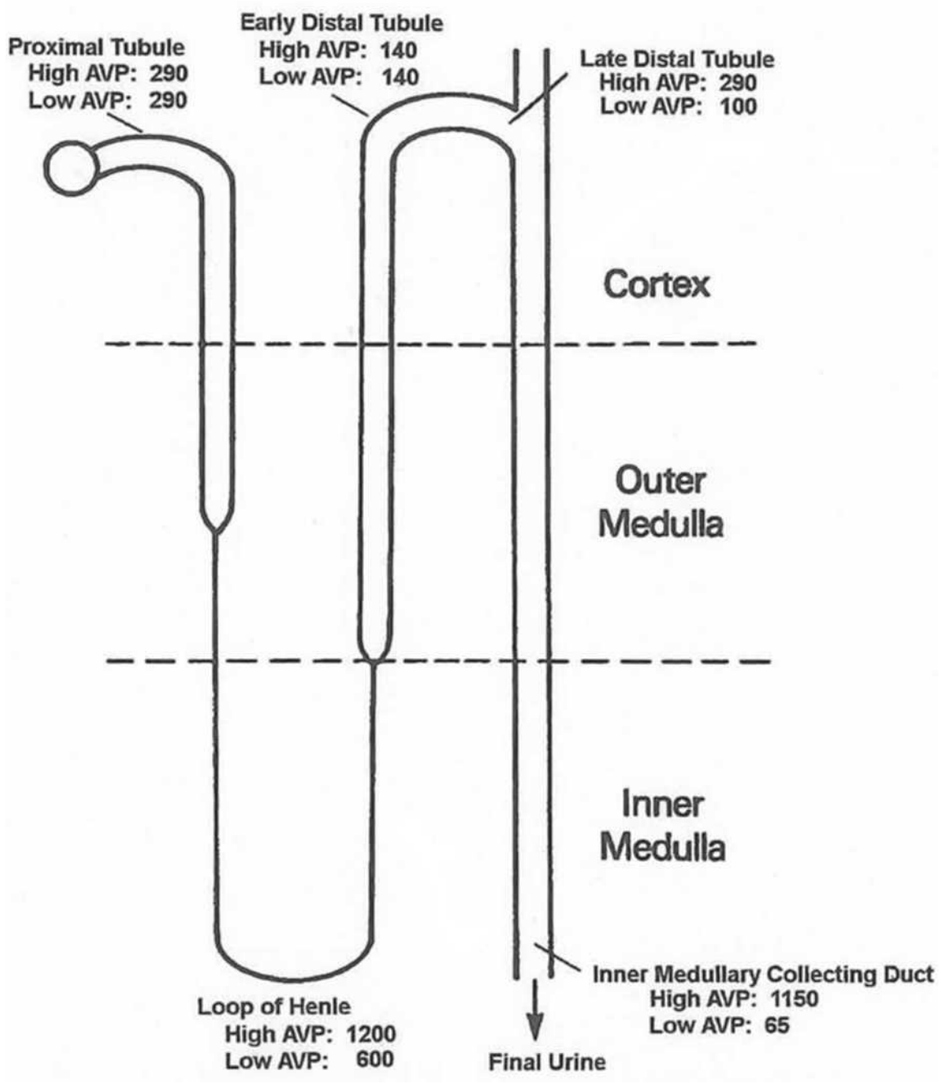


FIGURE 4.2 The typical tubular fluid osmolalities (in milliosmoles per kilogram of water) found along the nephron segments of the rat kidney. Fluid osmolality in the proximal tubule is isotonic, and it increases to 600 to about 1,200 mOsm per kilogram of water at the bend of the loop. Fluid emerging from the thick ascending limb is hypotonic, and final urine osmolalities are determined by urine concentration in the collecting duct depending on circulating vasopressin levels (65 to about 1,150 mOsm per kilogram of water). *AVP*, arginine vasopressin. (Based on micropuncture studies by Wirz H. Der osmotische Druck in den corticalin Tubuli der Ratten niere. *Helv Physiol Pharmacol Acta*. 1956;14:353; Gottschalk CW, Mylle M. Micropuncture study of the mammalian urinary concentrating mechanism: evidence for the countercurrent hypothesis. *Am J Physiol*. 1959;196:927; Jamison RL, Buerkert J, Lacy F. A micropuncture study of collecting tubule function in rats with hereditary diabetes insipidus. *J Clin Invest*. 1971;50:2444, with permission.)

Countercurrent Multiplication in the Outer Medulla and Other Mechanisms in the Inner Medulla

Countercurrent multiplication is an essential process for generating a medullary osmotic gradient along the corticomedullary axis and occurs in the Henle loop.[38] The counterflow arrangement of this loop makes the osmolality difference between the ascending and descending limbs to be multiplied, resulting in an enormous increase in osmolality toward the bend of this loop. Figure 4.3 illustrates the basic components of this mechanism in the short Henle loop. In the thick ascending limb of Henle, which corresponds to the right limb in Figure 4.3, NaCl is actively reabsorbed at any level of this ascending limb. Because this segment is impermeable to water, the luminal fluid is diluted and NaCl concentrations of the surrounding interstitium become higher than the luminal fluid. On the other hand, the descending limb of the Henle loop, which corresponds to the left channel in Figure 4.3, is highly permeable to water due to the presence of an AQP1 water channel, but is impermeable to NaCl. Water is passively reabsorbed from the descending limb lumen to the interstitium, which has a high osmolality due to NaCl accumulation. The luminal fluid in the descending limb is concentrated by water reabsorption and is continually concentrated downward to the bend of the loop, and then enters to the ascending limb. High NaCl concentrations of this fluid further promote NaCl reabsorption in the ascending limb. This fluid is then diluted by this NaCl absorption while flowing toward the top of the loop. Because the transverse concentration difference between the two limbs is always maintained by active NaCl absorption by the ascending limb (this is called the single effect) and water permeability of the descending limb at each level of the longitudinal axis is high, the bend of the loop achieves a progressively higher osmolality. This results in an enormous increase in osmolality toward the bend of the Henle loop.

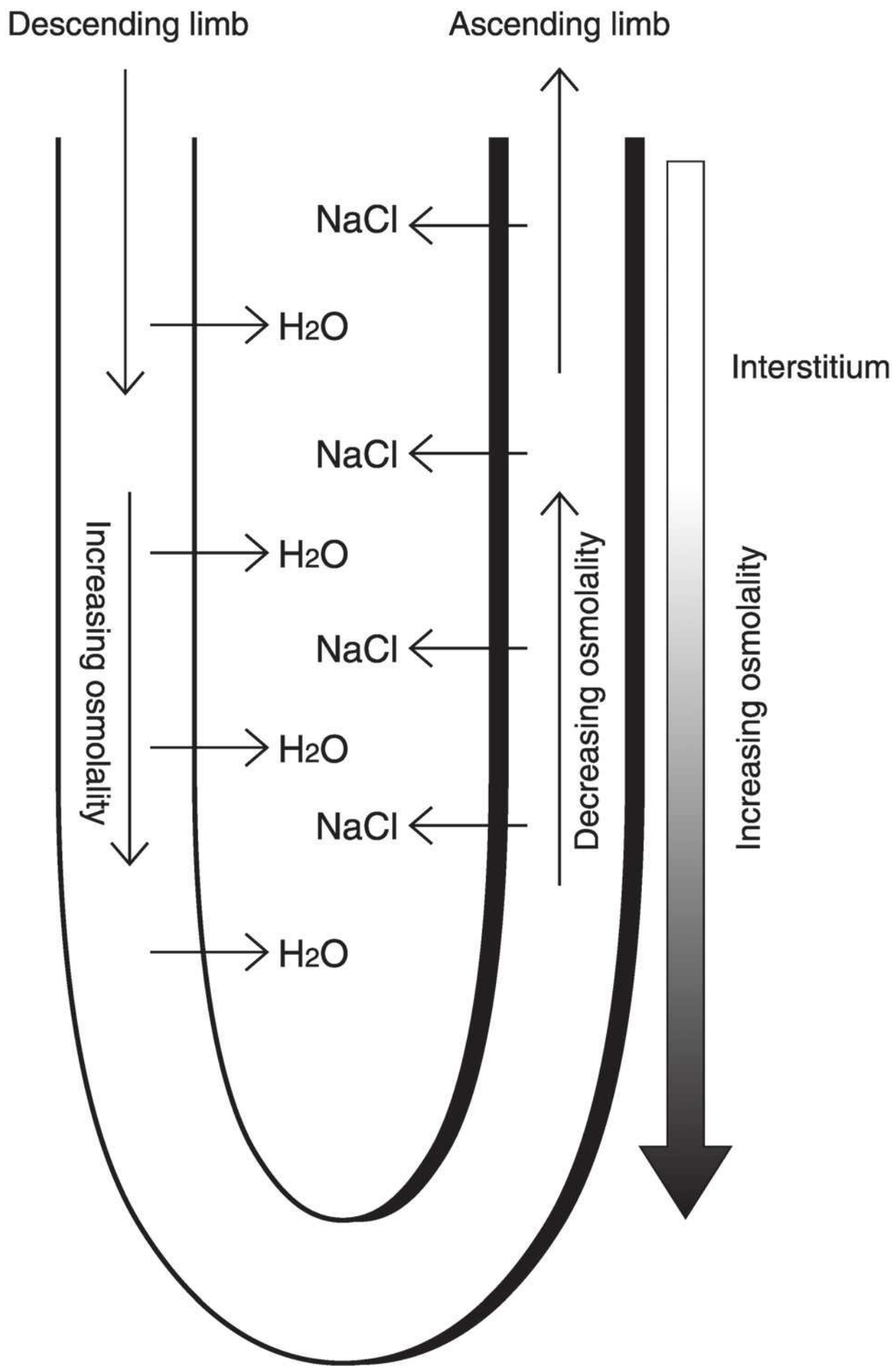


FIGURE 4.3 A countercurrent multiplication in the short Henle loop. In the thick ascending Henle limb, NaCl is actively reabsorbed. Because this segment is impermeable to water (indicated by *bold line*), the luminal fluid is diluted and the interstitium around this segment has high NaCl concentrations. On the other hand, the descending limb of the Henle loop is highly permeable to water, and water reabsorption occurs from the descending limb lumen to the interstitium with high NaCl concentrations. This results in an enormous increase in osmolality toward the bend of the Henle loop.

A countercurrent mechanism is accepted for generating the axial osmolality gradient in the outer medulla, where active NaCl reabsorption occurs in the thick ascending limb. However, in the inner medulla, there is little activity of NaCl transport despite the fact that osmolality continues to increase toward the tip of the papilla. The thin ascending limb of the Henle loop in the inner medulla cannot actively reabsorb NaCl. The high inner medullary interstitial osmolality provides a critical driving force for osmotic water flow across the collecting ducts, where the water permeability is regulated by vasopressin and AQP2 water channel, as described later. For explaining this concentrating effect in the inner medulla, a passive mechanism is widely accepted.[39,40] In this model, urea efflux from the terminal IMCD, which is mentioned previously, results in high urea concentrations in the inner medullary interstitium. This causes osmotic withdrawal of water from the thin descending limb and increases NaCl concentrations of the luminal fluid. This highly concentrated NaCl then exits passively from the thin ascending limb (works as single effect), and the luminal fluid is progressively diluted as it flows up. This passive transport process may produce an axial NaCl concentration gradient in the inner medulla. This model requires that the thin descending limb is highly permeable to water and but not to NaCl or urea, whereas the thin ascending limb is permeable to NaCl but not to water or urea. A microperfusion study of the thin ascending limb shows that the permeability of chloride and sodium are higher than urea, and that luminal dilution takes place when tubule segments are perfused in a condition simulating an in vivo situation.[41] Consistent with these physiologic studies, the ClC-K1 chloride channel is exclusively expressed in the thin ascending limb. ClC-K1 knockout mice show a large urinary concentrating defect with impaired urea as well as NaCl accumulation in the inner medulla.[42,43] This finding confirms the importance of a rapid chloride exit in the thin ascending limb and supports the passive mechanism.

Simulations based on many mathematical models incorporating physiologic parameters have been examined to show the validity of the passive model, but they usually failed to show satisfactory results. However, this does not necessary neglect the passive model because solute permeability and water permeability are sometimes different in species, in nephrons (long looped versus short looped), and within the segment (axial heterogeneity). Moreover, based on extensive studies of the rat inner medulla by immunohistochemical labeling and computer-assisted reconstruction, Pannabecker et al.[10] show that three-dimensional tubular and vascular relationships are more complex than thought before and a more comprehensive understanding of three-dimensional functional architecture is critically important for modeling urine concentration mechanisms of the inner medulla.

Countercurrent Exchange

Although the hypertonic medulla is essential for the ability to concentrate urine, blood flow may decrease the high solute concentrations in the medulla. The osmolality of blood entering to the medulla from the general circulation is far lower than the medullary interstitium and may decrease the osmolality of the interstitium as they come to equilibrium. Meanwhile, blood going out the medulla to the general circulation can carry out the solutes from the interstitium. To minimize this dissipation of the high solute concentrations in the medulla, there is a process called countercurrent exchange. Blood supply to the medulla is done by the vasa recta with the descending and ascending limbs arranged in a counterflow configuration. The vasa recta are permeable to water, sodium, and urea. Therefore, in the descending vasa recta, blood loses water and gains solutes as it flows down because

of the increasing osmolality in the medullary interstitium. After entering the ascending vasa recta, blood gains water and loses solutes as it flows up because of the decreasing osmolality of the surrounding interstitium. This exchange of water and solute between the descending and ascending vasa recta is called countercurrent exchange. This mechanism minimizes the solute washout from the inner medulla to the systemic circulation. Thus, this exchange at each level in the medulla preserves the axial solute concentration gradients in the medullary interstitium. If the blood flow is decreased, for instance in the conditions of volume depletion in the body, the efficiency of countercurrent exchange is further increased by getting enough time for achieving osmotic equilibration, leading to an increase in urinary concentrating ability.

The AQP1 water channel and the UT-B urea transporter are located in the descending vasa recta, which helps the rapid countercurrent exchange of water and urea.[6,33] Indeed, an impaired urine concentration ability is observed in AQP1 and UT-B knockout mice.[11,44]

Urea Accumulation in the Inner Medulla

The major solute responsible for the inner medullary osmolality gradient is urea and NaCl; although, in the outer medulla, it is NaCl.[45] High osmolality in the inner medulla is maintained by urea accumulation in this region in addition to NaCl. There are several mechanisms to maintain the high urea accumulation in the medulla that are called urea recycling (Fig. 4.4).[46,47]

Among the collecting duct, only the terminal IMCD has a high urea permeability due to the presence of UT-A1 and UT-A3 urea transporters in this segment.[31,32] During antidiuresis, water is absorbed in the collecting duct segments in the cortex and the outer medulla, which are impermeable to urea. Thus, the urea concentrations of the luminal fluid are progressively increased as it flows down through the connecting tubule, the cortical collecting duct, and the outer medullary collecting duct. When the luminal fluid reaches the terminal IMCD, which is highly permeable to urea, urea is rapidly absorbed from the lumen to the surrounding interstitium. During antidiuresis, the urea permeability of the inner collecting duct is increased by vasopressin, and this accounts for further rapid reabsorption of urea.[48,49]

Once reabsorbed, urea is not rapidly washed out to the general circulation because of countercurrent exchange and the low effective blood flow of the vasa recta, resulting in the high urea accumulation in the inner medullary interstitium. In addition, during antidiuresis, urea reabsorption by the terminal IMCD has another advantage for decreasing the luminal osmolality and preventing the osmotic diuresis when luminal fluid is very concentrated by enhanced water reabsorption in the upper portions of the collecting duct.

After being reabsorbed by the terminal IMCD, some urea in the inner medullary interstitium enters into the thin limbs of the long Henle loop where urea permeability is high due to the presence of UT-A2.[50] Urea entered in the thin limbs is then carried upward to the cortex through the thick ascending limb, the distal convoluted tubule, the connecting tubule, and the cortical collecting duct, and again enters into the medulla through the outer and inner medullary collecting ducts. Because these segments before the terminal IMCD have low urea permeability, urea can be returned to this region with little loss. Urea is again reabsorbed by the terminal IMCD and recycled to the inner medullary interstitium (Fig. 4.4).

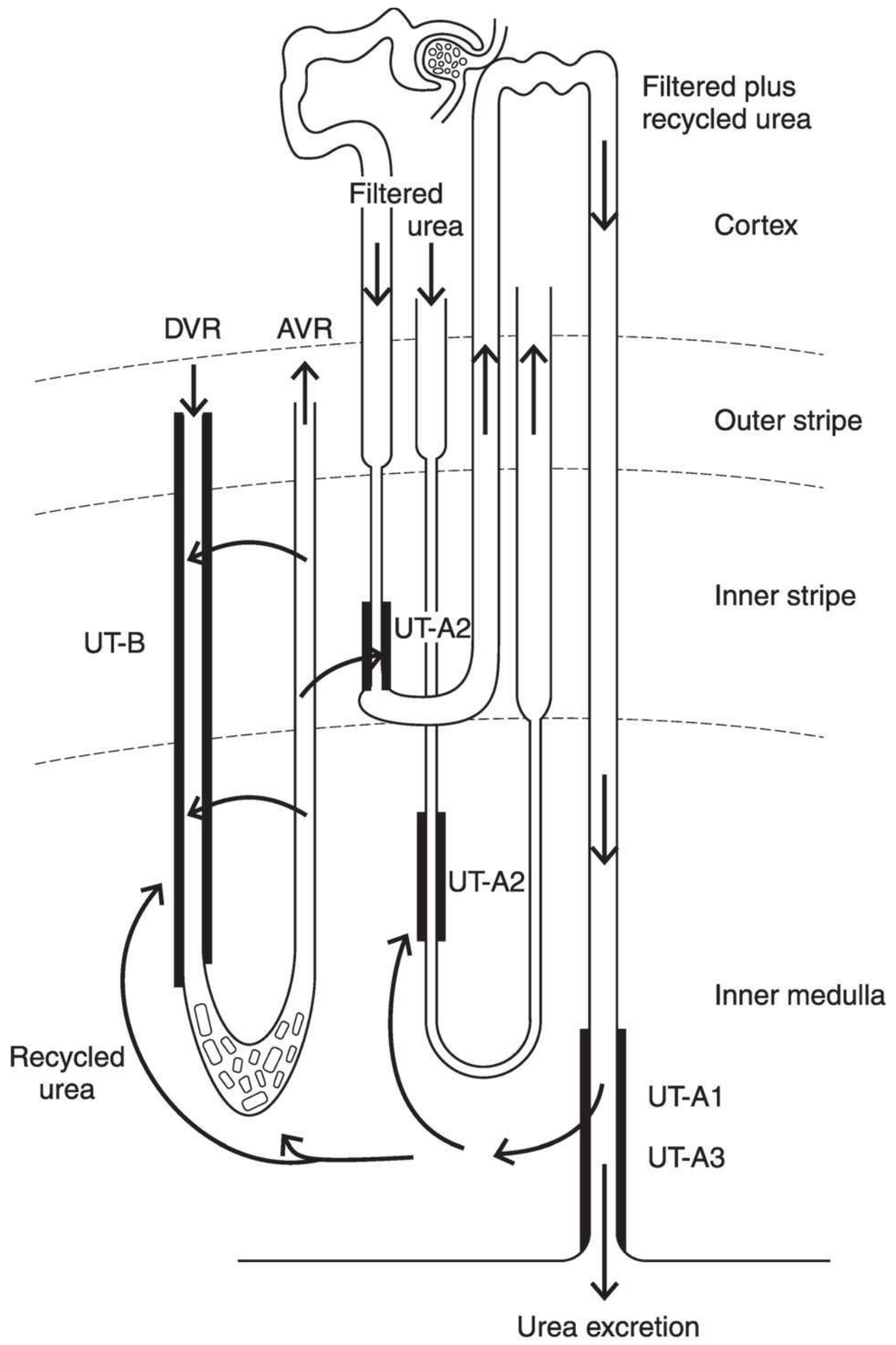


FIGURE 4.4 The urea recycling pathways within the kidney. The terminal portion of the inner medullary collecting duct (IMCD) efficiently reabsorbs urea through urea transporters UT-A1 and UT-A3, and accumulates urea in the inner medullary interstitium. Some of the urea is recycled to the IMCD by being reintroduced to the thin descending limb where UT-A2 is located. A high urea concentration in the medulla is maintained by countercurrent exchange (recycling) of urea between the descending vasa recta (*DVR*) and the ascending vasa recta (*AVR*), which is helped by UT-B in DVR. Some of the urea in the AVR is reintroduced to the thin descending limb and fed again to the IMCD. (Redrawn from Yang B and Bankir L. Urea and concentrating ability: new insights from studies in mice. *Am J Physiol Renal Physiol.* 2005;288:F881, with permission.)

In addition to the recycling pathway via the long loop in the inner medulla, there is another recycling pathway using the short loop in the outer medulla. Some parts of the urea reabsorbed by the terminal IMCD exit the inner medulla via the vasa recta. The vasa recta and the short Henle loop are arranged in parallel and in mutual proximity, and the descending limb of the short loop expresses the UT-A2 urea transporter and has a high urea permeability. Therefore, urea carried by the vasa recta is able to enter the short loop, then is convected through the distal convoluted tubule, the connecting tubule, and the collecting duct. When the luminal fluid finally reaches the terminal IMCD, urea is again reabsorbed to the surrounding inner medullary interstitium (Fig. 4.4).

The reabsorption sites among the collecting duct system are different between water and urea. Water is reabsorbed mainly in the cortex and the outer medulla, where blood flow is so high that water can be rapidly supplied to the systemic circulation. Furthermore, this reabsorption does not dilute the inner medulla. On the other hand, urea is reabsorbed in the inner medulla. Owing to low blood flow in this region and urea recycling pathways, urea can be trapped in the inner medulla, which is required for the passive mechanism, as described previously.

Regulated Water Reabsorption in the Collecting Duct

A countercurrent multiplication in the Henle loop generates a hypertonic medulla. A countercurrent exchange in the vasa recta minimizes the dissipation of this osmotic gradient. However, either of these processes has the ability to regulate water reabsorption in response to the body water balance. This function is performed by the collecting duct. The collecting duct is responsible for the final control of urine concentration, and its water permeability is regulated by vasopressin and other factors. In the absence of vasopressin, the collecting duct has an extremely low water permeability. Because the luminal fluid exiting the Henle loop is diluted, the fluid remains diluted after passing through the collecting duct, yielding a large volume of hypotonic urine. In the presence of vasopressin, the water permeability of the collecting duct is dramatically increased. Based on the hypertonic medullary interstitium generated by countercurrent multiplication and other processes, as described previously, an increase in water permeability of the collecting duct results in significant water reabsorption by the osmotic gradient between the lumen and the surrounding interstitium (Fig. 4.2). The molecular entity of this regulation of water permeability of the collecting duct is vasopressin V2 receptors and the AQP2 water channel expressed in the principal cells of the collecting duct.[26,51–55] V2 receptors and AQP2 are present in the connecting tubule and all segments of the collecting duct. When vasopressin binds to V2 receptors in the basolateral membrane of these cells, it stimulates adenylate cyclase to produce cAMP, activates protein kinase A, phosphorylates AQP2, and inserts this water channel into the apical cell surface, which results in a significant increase in the collecting duct water permeability and water reabsorption. In addition, vasopressin stimulation increases AQP2 protein abundance in the cell (Fig. 4.5). These processes are described in detail in the section Aquaporin-2, which follows. On the basolateral cell surface, AQP3 and AQP4 are located and represent a potential exit pathway from the cell to the interstitium for water entering via AQP2.

Water reabsorption mainly occurs in the connecting tubule, the cortical collecting duct, and the outer medullary collecting duct. In the cortex and the outer medulla, blood flow is sufficiently high so that absorbed water can be carried out to the general circulation without diluting the interstitium. On the other hand, the inner medulla has the highest osmolality and is important for reabsorption of the remaining water when maximal water reabsorption is required.

URINE DILUTION MECHANISMS

Approximately 50 mOsm per kilogram of water is the limit of the urinary diluting ability of humans. The mechanisms responsible for urinary dilution nearly overlap with that for urinary concentration. These two conditions between diuresis and antidiuresis are switched mainly by the collecting duct water permeability, which is regulated by vasopressin stimulation and the AQP2 water channel.

In the thick ascending limb, the luminal fluid is diluted by active NaCl absorption through NKCC2[56] and Na-H exchanger NHE3,[57] regardless of antidiuresis or diuresis. In addition, water impermeability of this segment preserves the luminal low osmolality by preventing water fluxes. The distal convoluted tubule also has the ability to actively absorb NaCl due to the presence of Na-Cl cotransporter NCC[19] and is impermeable to water, resulting in the dilution of the luminal fluid to an osmolality of about 100 mOsm per kilogram of water. In diuresis, this diluted fluid passes through the collecting duct because its water permeability is very low. Active Na reabsorption through Na channel ENaC in the collecting duct[29] can further dilute the luminal fluid. [58]

In addition to diuresis, there is another mechanism that further promotes urinary dilution. The terminal IMCD has a higher basal water permeability than other portions of the collecting duct and is surrounded by a high inner medullary interstitium. Because the osmolality of the luminal fluid reaching the terminal IMCD in diuresis is lower than that in antidiuresis, the transepithelial osmolality gradient in diuresis is larger than that in antidiuresis, resulting in a higher water reabsorption in this segment.[27] This reduces the inner medullary interstitial osmolality, leading to a further decrease in urinary concentrating ability.

VASOPRESSIN

The primary determinant of solute-free water excretion is the regulation of urinary water excretion by circulating levels of vasopressin in plasma. This section describes the regulation of vasopressin secretion from the neurohypophysis.

Structure and Synthesis

Arginine vasopressin is the antidiuretic hormone of most mammals, although members of the pig family have lysine vasopressin, in which a lysine replaces the arginine in position 8 of arginine vasopressin. Vasopressin is produced by the hypothalamic neurohypophyseal tract. This tract is composed of magnocellular neurons that arise bilaterally in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus and project medially to merge in the pituitary stalk and form the posterior pituitary gland in the sella tunica. Vasopressin is synthesized as part of a protein precursor of approximately 21,000 Da of molecular mass that incorporates a signal peptide at its amino terminus and vasopressin, neurophysin, and copeptin at its carboxyl terminus. In the endoplasmic reticulum, the signal peptide is removed. The prohormone then moves through the Golgi apparatus and into the neurosecretory granules that travel down the axon. In the neurosecretory granules, the prohormone is processed to yield amidated vasopressin, neurophysin, and copeptin.[59] Vasopressin and neurophysin form an insoluble complex in the nerve terminal and dissociate from each other after its release into the general circulation.

Regulation of Vasopressin Secretion by the Tonicity of Body Fluid

Under physiologic conditions, the most important determinant of vasopressin secretion is the tonicity of body fluid. Tonicity defines the forces that determine the net flux of water between two solutions separated by a membrane permeable to water but impermeable to certain solutes. On the other hand, osmolality refers to the forces generated by solutes that reduce the random movement of water molecules. The osmolality is a concentration of all of the solute in water, whereas the tonicity is an effective osmotic pressure and a concentration of all of the osmotically effective solutes. There are two types of solutes: osmotically effective and noneffective. Osmotically effective solutes, such as Na and Cl, are characterized by their inability to move across the cell membrane, resulting in a difference in their concentrations between the intracellular and extracellular compartments. On the other hand, osmotically noneffective solutes, such as urea and ethanol, are characterized by their ability to diffuse freely across the cell membrane and their concentrations between these two compartments are similar. Glucose is confined in the extracellular compartment and is osmotically effective in the absence of insulin; whereas in the presence of insulin, glucose is able to enter the cell by insulin-activated transporters, thereby becoming osmotically noneffective. Vasopressin secretion is finely regulated by the tonicity of body fluid.

The changes in the tonicity of body fluid are sensed by a group of cells called osmoreceptor neurons. These neurons are located in several brain areas including the organum vasculosum laminae terminalis (OVLT), the SON, and the PVN nuclei of the hypothalamus.[60] It is postulated that an increase in the tonicity of the extracellular compartment causes water withdrawal and shrinkage of the osmoreceptor neurons. Owing to the presence of mechanosensitive cation channels in these cells, cell shrinkage induces a positive charge influx and depolarizes the cell membrane, resulting in the triggering of neuronal action potentials. Such neurons send axonal projections to the SON, where they release the excitatory transmitter glutamate to synaptically excite the magnocellular neurons that release vasopressin in their distal axons.[60,61]

Recent studies show that the transient receptor potential vanilloid (TRPV) channels are likely to be the major component of the osmoreceptor. The N-terminal truncated TRPV1 channel is selectively expressed in osmosensory neurons in OVLT. The responses of these neurons to hypertonic stimuli, including reactive increases in cation channel conductance, membrane depolarization, and increased frequency of neuronal action potentials, were greatly inhibited in TRPV1 knockout mice. Furthermore, TRPV1 knockout mice showed a higher serum osmolality than wild-type mice, indicating a decreased sensitivity of osmosensation.[62] However, these results were not reproduced by another study.[63] In addition to TRPV1, TRPV2 and TRPV4 are present in osmoreceptor neurons and functional characteristics of these channels show responsiveness to the tonicity. Further studies, such as examining a possible hetero-oligomerization of these TRPV channels, are necessary for a molecular understanding of osomosensation.[60,61]

The functional properties of osmoregulatory mechanisms have a discrete threshold for vasopressin secretion, above which a linear relationship between plasma osmolality and vasopressin levels occurs.[64] When the plasma osmolality is below a threshold level, vasopressin secretion is suppressed to low or undetectable levels (0.5 pg per milliliter). Above this threshold, vasopressin secretion increases linearly in direct proportion to plasma osmolality. Both the threshold level and the slope of the regression line relating vasopressin secretion to plasma osmolality vary between persons due to unknown genetic factors and between the different conditions in the same individual. In general, the threshold is approximately 280 mOsm per kilogram of water, and above this threshold, a rise in plasma osmolality of 1% increases plasma vasopressin by approximately 0.4 to 1.0 pg per milliliter. The renal response to circulating vasopressin is also linear, with urinary concentration that is proportional to vasopressin levels from 0.5 to 5 pg per milliliter, above which urinary osmolality is maximal and cannot increase further. Therefore, changes of as little as 1% in plasma osmolality are sufficient to cause a significant increase in urinary concentration, and maximal antidiuresis is achieved by an increase in plasma osmolality of only about 10 to 15 mOsm per kilogram of water above the threshold. Furthermore, vasopressin secretion occurs within minutes in response to changes in plasma osmolality. After release into the systemic circulation, vasopressin distributes quickly because of its small size, and the equilibration between the vascular and extravascular compartments is almost complete within 10 minutes. The half-life of vasopressin in plasma is within

30 minutes. Therefore, the changes in plasma osmolality are rapidly transferred to changes in urinary concentration.

There are several factors that can alter the threshold and the sensitivity, which is the slope of the regression line relating vasopressin secretion to plasma osmolality. The most important factor is hemodynamic changes, including blood pressure and effective arterial blood volume, which are described in the next section. Aging enhances the sensitivity of vasopressin secretion.[65] In pregnancy, relaxin, an ovarian hormone produced by the corpora, reduces the threshold and increases vasopressin secretion, contributing partly to the increase in blood volume.[66]

Hemodynamic Regulation of Vasopressin Secretion

Vasopressin secretion is also affected by changes in blood volume and pressure.[67] A decrease in blood volume, such as with bleeding, sequestration or redistribution of blood, or fluid loss by sweating, diarrhea, or vomiting, can increase vasopressin secretion. The vasopressin release mechanism is much less sensitive to small changes in blood volume than to comparable changes in osmolality. Small reductions under 8% in blood volume usually have little effect on plasma vasopressin concentration. On the other hand, further acute reduction in blood volume significantly stimulates vasopressin secretion. Usually, 20% to 30% reductions in blood volume increase vasopressin secretion to the levels of 20 to 30 times normal. The stimulus-response relationship follows an exponential pattern. The vasopressin response to an acute reduction in blood pressure is similar to the response to blood volume. Reductions in blood pressure under 10% have little effect on vasopressin secretion, whereas blood pressure decreases of 20% to 30% result in significant increase in plasma vasopressin. The effects of reductions in blood volume and pressure are exerted through shifting the threshold and sensitivity of vasopressin secretion to osmotic stimuli.[68]

These hemodynamic effects on vasopressin secretion are mediated at least in part by neural pathways that originate in stretch-sensitive receptors called baroreceptors in the cardiac atria, the aorta, and the carotid sinus. From these receptors, afferent nerve fibers ascend in the vagus and glossopharyngeal nerves to the nuclei of the tractus solitarus (NTS) in the brainstem.[69] A variety of postsynaptic pathways from the NTS then project, both directly and indirectly, to the SON and the PVN nuclei of the hypothalamus.

Vasopressin secretion through the baroreceptor mechanism is promoted not only by a blood volume decrease but also by the reduction in effective arterial circulating blood volume. For example, upright posture, congestive heart failure, and cirrhosis stimulate vasopressin secretion.[70,71]

Other Influences for Vasopressin Secretion

The sensation of nausea, with or without vomiting, is a powerful stimulus to vasopressin secretion.[72] Nausea increases plasma vasopressin in excess of 200 to 400 pg per milliliter. The pathways responsible for this effect are located in the chemoreceptor zone in the postrema area of the brainstem. Water loading blunts, but does not abolish, the effect of nausea on vasopressin secretion, suggesting that the mechanism of emetic effect has a possible common pathway with that of an osmotic effect.

The renin-angiotensin system also influences the osmotic regulation of vasopressin secretion.[73] Neurons in the subfornical organ (SFO) contain angiotensin II and project axons onto hypothalamic magnocellular neurosecretory cells (MNCs) located in the SON and PVN nuclei. It is suggested that the release of angiotensin II may contribute to the potentiation of osmotically evoked action potential firing and vasopressin release.

Acute hypoglycemia induces a modest increase in plasma vasopressin,[65] although the pathways responsible for this effect are unknown.

The effects of pregnancy on the osmotic regulation of vasopressin secretion are complex. The systemic hemodynamic profile of pregnancy is characterized by a decrease in mean arterial pressure, a rise in cardiac output and plasma volume, and a decrease in body tonicity. This is due to a decrease in the osmotic threshold for vasopressin secretion because of arterial underfilling secondary to systemic arterial vasodilatation.[74,75] In addition, relaxin, an ovarian hormone produced by the corpora, reduces the threshold and increases vasopressin secretion, which contributes partly to the increase in blood volume.[66] On the other hand, the metabolic clearance of vasopressin is increased, owing to circulating cystine aminopeptidase (vasopressinase) produced by the placenta. This mechanism rarely causes transient diabetes insipidus in late pregnancy[76] because of the increased release of vasopressin.

MOLECULAR MECHANISMS OF VASOPRESSIN-REGULATED WATER PERMEABILITY OF THE COLLECTING DUCT

Vasopressin-regulated water permeability of the collecting duct is responsible for the final control of urine concentration. Upon vasopressin stimulation, the water permeability of the collecting duct is dramatically increased. Based on the hypertonic medullary interstitium, the increased water permeability results in significant water reabsorption and urine concentration. The vasopressin V2 receptor and AQP2 in the collecting duct are the primary targets for vasopressin. Vasopressin-regulated AQP2 translocation and abundance change the water permeability of the collecting duct principal cells. The essential roles of the vasopressin V2 receptor and AQP2 in urine concentrations are demonstrated by mouse models. Male mice that were introduced with a nonsense mutation into the AVPR2 gene, which codes the vasopressin V2 receptor and is located in the X chromosome, died within 7 days after birth due to hypernatremic

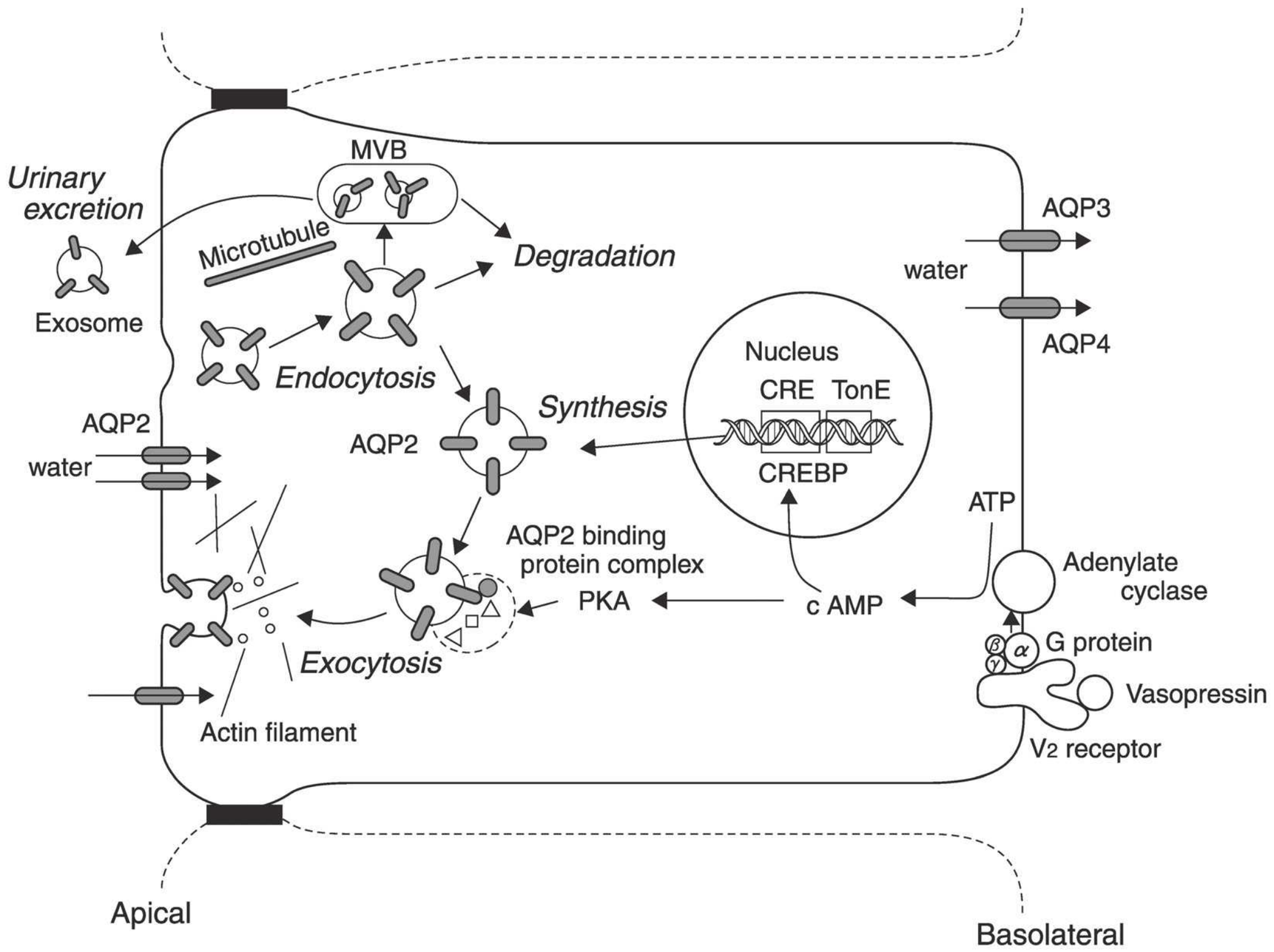


FIGURE 4.5 A schematic representation of the AQP2 protein dynamics within the cell. Vasopressin binds to the V2 receptor and this stimulates adenylate cyclase via G protein, resulting in the production of cAMP. cAMP stimulates the synthesis of the AQP2 protein through the interaction of the cAMP responsive element (*CRE*) of the AQP2 gene and the CRE binding protein (*CREBP*). Synthesized AQP2 are stored in subapical vesicles. Protein kinase A (*PKA*) directly phosphorylates AQP2, and this alters the binding dynamics of AQP2 and its binding proteins (AQP2 binding protein complex), which induces a reorganization of actin and stimulates the exocytosis of AQP2 containing vesicles to the apical membrane. Upon withdrawal of vasopressin stimuli, AQP2 is internalized by endocytosis and degraded. Some of the internalized AQP2 is transferred to the multivesicular body (*MVB*) and is excreted to the urine as an exosome. Thus, water transport—crossing the apical membrane through AQP2 and exiting the basolateral membrane through AQP3 and AQP4—is regulated by vasopressin.

dehydration.[77] Mice models with AQP2 protein deletion in the collecting duct also showed a severe defect in urinary concentration.[22,78] This section describes the molecular mechanisms for the regulation of water permeability of the collecting duct principal cells. Figure 4.5 illustrates several major mechanisms.

Vasopressin V2 Receptor

When circulating vasopressin reaches the kidney, vasopressin binds to the vasopressin V2 receptor expressed on the basolateral plasma membrane of the collecting duct principal cells and initiates the signal transduction in the cell. The V2 receptor is a 371 amino acid protein with 7 membrane-spanning domains and couples to heterotrimeric G-proteins.[79,80] The binding of the V2 receptor to vasopressin promotes the disassembly of the bound heterotrimeric G-protein (Gs) into Gα and Gβγ subunits. GDP-GTP exchange occurs in the Gα subunit and this activated Gsα then stimulates adenylate cyclase, resulting in an increase in intracellular cAMP levels (Fig. 4.5). Increased cAMP activates protein kinase A, which phosphorylates AQP2, and this phosphorylation event is required to increase the water permeability and water reabsorption of renal principal cells, which is described in the next section.

Aquaporin-2

AQP2 is a 271 amino acid protein with 6 membrane-spanning domains and 2 conserved, membrane-embedded asparagine–proline–alanine (NPA) motifs, and selectively permeates water.[3] AQP2 is predominantly expressed in the collecting duct principal cells (Fig. 4.6).[81,82] In response to vasopressin, AQP2 recycles between the luminal cell surface membrane (also called the apical membrane) and the intracellular subapical storage vesicles of the collecting duct principal cells. In the absence of vasopressin, AQP2 predominantly resides in the subapical vesicles. Because water molecules slowly diffuse through the lipid bilayer, the water permeability of the cell membrane without AQPs is low. Therefore, basal water permeability of the principal cells is also low. In the presence of vasopressin, AQP2-containing vesicles fuse with the apical membrane via exocytosis, thus inducing an exceedingly high

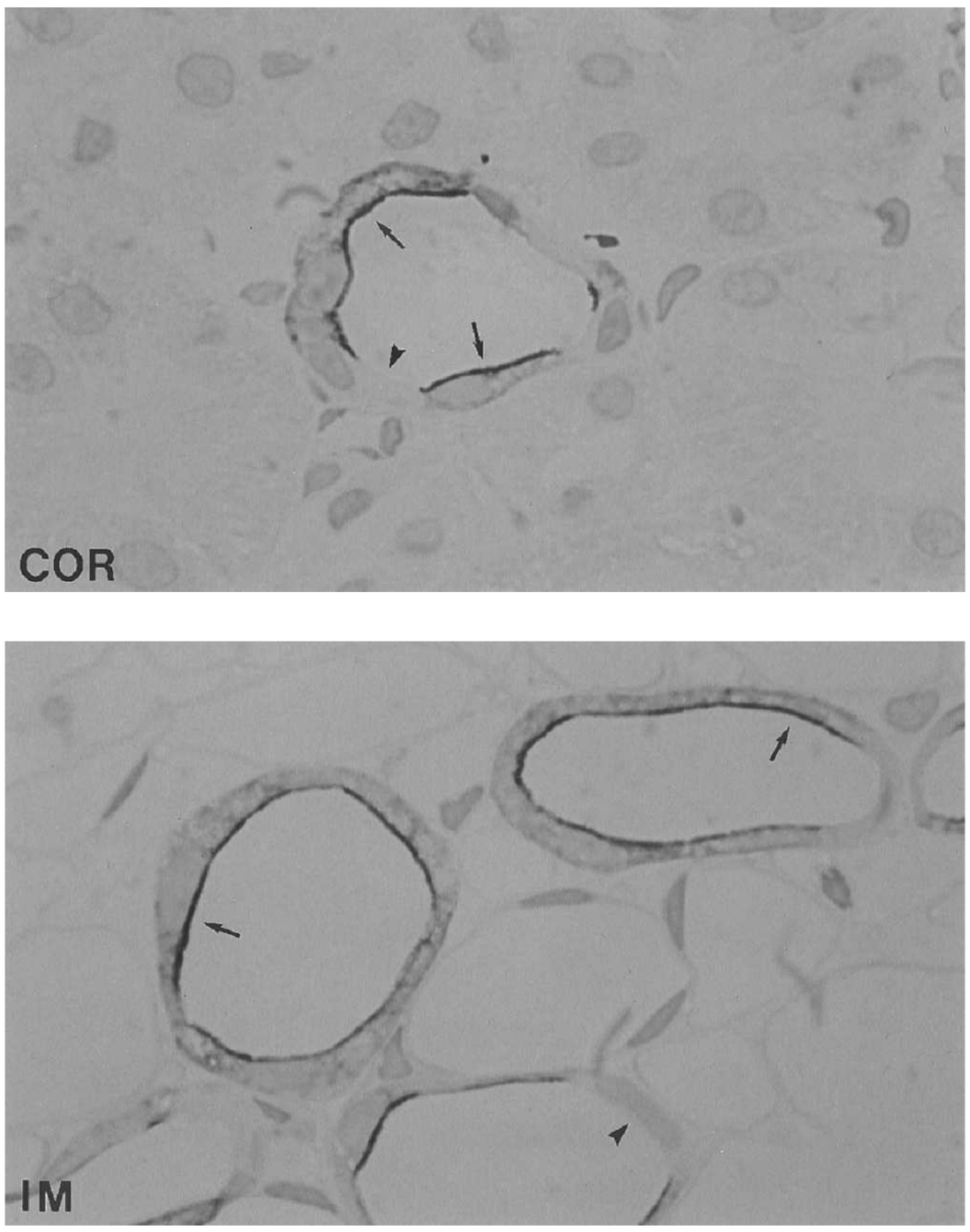


FIGURE 4.6 The immunoperoxidase labeling of AQP2 in the cortical (*COR*), the outer medullary (*OM*), and the inner medullary (*IM*) collecting duct. AQP2 is very abundant in the apical plasma membrane domains, as well as in the subapical domains (*arrows*), whereas intercalated cells are unlabeled (*arrowheads*). In the inner medullary collecting duct, AQP2 is also present in the basolateral part of the cell. (Magnification × 1,100.) (Reprinted from Nielsen S, Knepper MA, Kwon TH, Frøkiaer J. Urinary concentration and dilution. In: Schrier RW, ed. *Diseases of the Kidney & Urinary Tract*, 8th ed. Philadelphia: Lippincott Williams & Wilkins 2005:98, with permission.)

water permeability of this apical membrane (Fig. 4.7).[23–25] Vasopressin increases the water permeability by a factor of 10 to 100 in the cortical collecting duct,[49] 20 to 30 in the outer medullary collecting duct,[83] and 10 to 30 in the initial IMCD.[49] The terminal IMCD has a higher basal water permeability and vasopressin increases the water permeability by a factor of 10 in the terminal IMCD.[49]

The water permeability of the basolateral membrane of the principal cells is always high because of the presence of AQP3 and AQP4, and is not rate limiting for water transport. Thus, water that enters into the cell from the lumen via AQP2 can exit to the hypertonic medullary interstitium by the osmotic gradient. Upon the binding of vasopressin to its receptors, AQP2 is phosphorylated and this phosphorylation event is a requisite for AQP2 translocation to the apical membrane (Fig. 4.5).

Aquaporin-2 Phosphorylation and Trafficking

AQP2 forms homotetramers,[84] and at least three of four monomers in AQP2 tetramers must be phosphorylated for successful apical membrane localization.[85] Phosphorylation of serine 256 is required for the trafficking of AQP2 to the cell surface in cultured cells.[86,87] Protein kinase A and its substrates are present throughout the cell; therefore, localization of protein kinase A in specific sites is necessary for PKA to effectively phosphorylate its target. The phosphorylation process is assisted by protein kinase A anchoring proteins

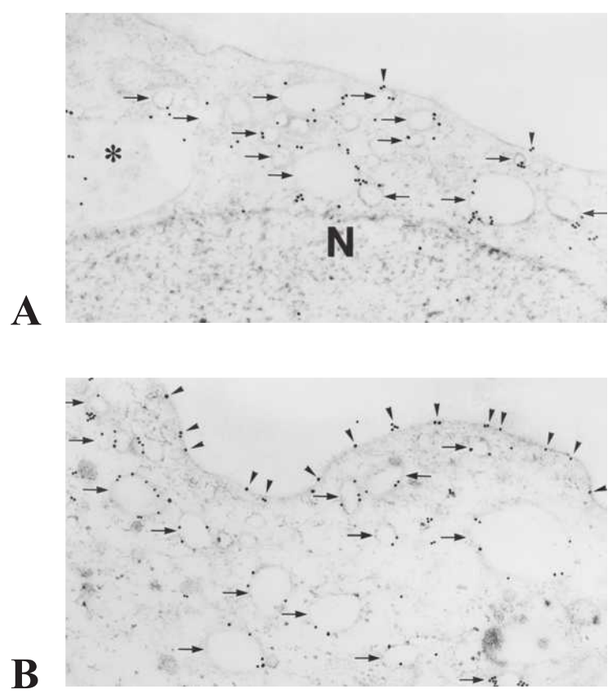


FIGURE 4.7 AQP2 immunogold labeling in the inner medullary collecting duct cells of control **(A)** and vasopressin-treated **(B)** Brattleboro rats. Immunogold labeling is obvious in the subapical cytoplasm in close association with cytoplasmic vesicles (*arrows*) or in the multivesicular body (*) but is sparse on the apical membrane (*arrowheads*). In contrast, heavy AQP2 labeling is observed on the apical membrane after vasopressin treatment (*arrowheads*). *N*, nucleus. (Magnification × 64,000.) (Reprinted from Yamamoto T, Sasaki S, Fushimi K, et al. Vasopressin increases AQP-CD water channel in apical membrane of collecting duct cells in Brattleboro rats. *Am J Physiol.* 1995;268:C1546, with permission.)

(AKAPs). The tethering of PKA to AKAPs is required for AQP2 shuttling to the cell surface.[88] A splice variant of AKAP-18, AKAP-18δ, is specifically involved in AQP2 shuttling[89] and the involvement of AKAP-220 has also been reported.[90]

AQP2 phosphorylation by kinases other than PKA might also be involved in the regulation of AQP2 trafficking. Serine 256 in AQP2 is also a substrate for Golgi casein kinase. AQP2 transition through the Golgi apparatus is associated with a PKA-independent increase in AQP2 phosphorylation at serine 256, suggesting that phosphorylation by Golgi casein kinase may be required for Golgi transition.[91] van Balkom et al.[92] showed that the activation of protein kinase C mediates AQP2 endocytosis, which is independent of the phosphorylation state of serine 256. In addition, a cyclic-GMP-dependent pathway is shown to be involved in AQP2 exocytosis,[93] and an inhibitor of cyclic GMP phosphodiesterase is able to induce AQP2 translocation to the cell surface.[94]

In addition to serine 256, there are three additional phosphorylation sites near the AQP2 C-terminus. These modifiable residues are serine 261, serine 264, and serine 269. Vasopressin induces the phosphorylation of AQP2 also at serine 264, and serine 264-phosphorylated AQP2 is translocated to the plasma membrane similarly to serine 256-phosphorylated AQP2.[95] On the other hand, vasopressin decreases the phosphorylation levels at serine 261, and the localization of serine-261-phosphorylated AQP2 is different from that of serine 256-phosphorylated AQP2, which suggests distinct roles for these residues in AQP2 trafficking.[96] Lu et al.[97] reported that the phosphorylation state of serine 261 does not affect AQP2 trafficking. Serine 269 is shown to be involved in the plasma membrane retention of AQP2.[98] Moeller et al.[99] showed that the phosphorylation of serine 264 and serine 269 depends on the prior phosphorylation of serine 256, and that the phosphorylation of serine 261 partially depends on the phosphorylation of serine 264 and serine 269. In contrast, serine 256 phosphorylation is not dependent on the state of any of the other phosphorylation sites, suggesting that serine 256 is the most important phosphorylation site of AQP2.

Role of Calcium in Aquaporin-2 Regulation

Intracellular Ca^{2+} mobilization is also involved in vasopressin-mediated AQP2 trafficking,[100] although its precise role remains unclear. In addition to increasing cAMP levels in the cytoplasm of the principal cells of the collecting duct, vasopressin binding to V2 receptor triggers a rapid increase of intracellular Ca^{2+}, which is followed by sustained temporal oscillations of the level of this ion. This process seems to be involved in AQP2 exocytosis. Balasubramanian et al.[100] suggest several plausible candidates as downstream effectors of this signaling cascade, such as calmodulin and MLCK. MLCK is a calmodulin-dependent kinase that regulates actin filament organization by phosphorylating the regulatory light chain of myosin II, and thus also activates myosin motor activity. Myosin II and its regulatory light chain are found in the AQP2-binding protein complex,[101] supporting their involvement in AQP2 trafficking. Nevertheless, Lorenz et al.[102] demonstrated that cyclic AMP alone is sufficient to induce AQP2 translocation without the need for an increase in cytosolic Ca^{2+} levels in the inner medullary collecting duct cells.

Involvement of extracellular Ca^{2+} in AQP2 regulation has also been indicated by several findings.[103–106] Urinary AQP2 excretion correlates with the severity of enuresis, a disease characterized by nocturnal polyuria and hypercalciuria.[103] Clinical amelioration demonstrated by a low calcium diet is accompanied by the regulation of urine output through the remodulation of AQP2 expression/trafficking.[104] Drug-induced hypercalcemia/hypercalciuria causes polyuria and reduces AQP2 expression in rats.[105] AQP2 translocation to the apical membrane prompted by forskolin-induced increases in cyclic AMP levels is inhibited by increased levels of extracellular Ca^{2+}.[106] This process is probably mediated by the endogenous calcium-sensing receptor and is associated with an increase in F-actin levels.

Other Influences for Aquaporin-2 Trafficking

Several other factors have recently been reported to affect AQP2 trafficking. Nejsum et al.[107] used Madin–Darby canine kidney epithelial cells transfected with AQP2 and showed that prostaglandin E2 and dopamine induce the internalization of AQP2, regardless of AQP2 dephosphorylation. de Seigneux et al.[108] reported that aldosterone induces a basolateral expression of AQP2, suggesting a role for aldosterone in water metabolism in conditions of increased sodium reabsorption in the collecting ducts.

Role of the Cytoskeleton in Aquaporin-2 Trafficking

The actin cytoskeleton is reported to function as a barrier for AQP2 exocytosis. Actin depolymerization is necessary for the cAMP-dependent translocation of AQP2. [109] In fact, the stimulation of prostaglandin E3 receptors has been shown to inhibit vasopressin-induced inactivation of Rho GTPase, vasopressin-induced F-actin depolymerization, and AQP2 translocation induced by vasopressin, cAMP, or forskolin.[110] Rho GTPase activation by bradykinin stabilizes cortical F-actin and inhibits AQP2 trafficking.[111]

GTPase-activating protein Spa-1 (SPA-1) binds to the C-terminus of AQP2, and this binding is required for AQP2 trafficking.[112] SPA-1 may inhibit Rap1 GTPase activating protein, which triggers F-actin disassembly and may maintain the basal mobility of AQP2.[55,113] SPA-1-deficient mice show impaired AQP2 trafficking and hydronephrosis.[112] In humans, mutations in the C-terminus of AQP2, which is the binding region of SPA-1, causes nephrogenic diabetes insipidus (NDI), a disease characterized by a massive loss of water through the kidney.[114,115] Furthermore, AQP2 binds to a multiprotein complex that includes the actin cytoskeleton, and a pattern of competing interactions between AQP2 and G-actin or tropomyosin directs AQP2 trafficking to the apical membrane (Fig. 4.5).[101,116,117]

F-actin assembly might have both inhibitory and facilitatory effects on an AQP2 transport to the cell surface.[118] Drug-induced actin depolymerization inhibits AQP2 translocation from the early endosomes that express early endosome antigen 1 to the subapical storage vesicles that express Rab-11.[119] Myosin II and its regulatory light chain are found in an AQP2-binding protein complex,[101] and vasopressin induces myosin light chain phosphorylation, which enhances the myosin–actin filament interaction and the formation of actin fibers.[120] Myosin has also been shown to be critical for AQP2 recycling.[121] In addition to acting as a barrier to prevent AQP2 trafficking, actin fibers may function as "cables" that promote and direct AQP2 transport. Dynamic actin reorganization may be responsible for the transformation of the actin barrier into actin cables. Further studies are necessary for understanding these sequential events.

Fusion of Aquaporin-2 Vesicles with the Apical Membrane

The docking and fusion of AQP2-containing vesicles with the apical membrane involves the action of N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins, including VAMP-2, SNAP-23, syntaxin-3, and syntaxin-4.[52] Syntaxin-binding protein 2 (also called Munc18b) is reported to function as a negative regulator of SNARE complex formation and AQP2-vesicle fusion to the apical membrane.[122]

Aquaporin-2 Recycling and Endocytosis

AQP2 is a recycling membrane protein. Upon vasopressin stimulation, AQP2 is transported to the apical membrane, rendering the cell water permeable, as described previously. After vasopressin stimulation is terminated, AQP2 is shuttled back to the cell cytoplasm, a process that restores the water-impermeability of the cell (Fig. 4.5). This internalization process consists of AQP2 retrieval into early endosomes that express early endosome antigen 1, and subsequent transferal of this water channel to storage vesicles that express Rab-11.[123] From Rab-11-positive vesicles, AQP2 is able to go again to the apical membrane. Actually, this recycling process occurs constitutively, and many signaling pathways are involved for the regulation of each part of this recycling itinerary. Vasopressin signaling is the most potent and most important factor that enhances the exocytotic process among the recycling itinerary.

During the endocytotic process of the AQP2 recycling pathway, AQP2 accumulates in clathrin-coated pits and is internalized via a clathrin-mediated process.[124,125] Dynamin is a GTPase that is involved in the formation and pinching off of clathrin-coated pits to form clathrin-coated vesicles, and its dominant-negative mutant K44A renders the protein GTPase deficient and arrests clathrin-mediated endocytosis. This GTPase-deficient dynamin mutant K44A is shown to accumulate AQP2 in the plasma membrane even without vasopressin stimulation.[125] Furthermore, this dynamin mutant K44A or methyl-β-cyclodextrin is able to accumulate the phosphorylation-deficient mutant in the cell surface despite the fact that AQP2-S256A accumulation in the cell surface is not induced by vasopressin. Methyl-β-cyclodextrin depletes membranes of cholesterol and results in a rapid inhibition of endocytosis.[126] These data also support the constitutive recycling of AQP2 and the presence of the processes that are not dependent on phosphorylation of AQP2 at serine 256.

A heat shock protein hsc70, which is important for uncoating clathrin-coated vesicles, binds to the C-terminus of nonphosphorylated AQP2 and is required for AQP2 endocytosis.[127)] Kamsteeg et al.[128] reported that the myelin and lymphocyte protein (also known as MAL), which is involved in the organization of the glycosphingolipid-enriched membrane, interacts with AQP2 and enhances accumulation of AQP2 in the apical membrane by decreasing the level of internalization of the protein. Ubiquitination at lysine 270 of AQP2 is important for AQP2 endocytosis and degradation.[129] Furthermore, LIP5, which is involved in multivesicular body formation, interacts with AQP2 and facilitates its lysosomal degradation.[130]

Regulation of Water Transport Activity of Individual Aquaporin-2s

As described previously, AQP2 phosphorylation induces its apical membrane insertion, rendering the collecting duct cells water permeable. However, whether this phosphorylation regulates the water permeability of individual AQP2 water channels remained unclear until recently. Several groups had examined the role of phosphorylation on osmotic water permeability (P_f) of individual AQP2s. In the Xenopus oocytes expression system, one study showed that PKA phosphorylated AQP2 at serine 256, which increased the P_f of oocytes without a significant increase in the amount of AQP2 on the oocyte surface.[131] However, another study using a similar method showed that the P_f values that corrected for the plasma membrane abundance of AQP proteins were not different between WT-AQP2 and the nonphosphorylation-mimicking mutant S256A-AQP2 expressing oocytes, indicating a lack of an effect of phosphorylation at S256 on the P_f of individual AQP2 proteins.[99] Lande et al.[132] purified endosomes derived from the apical membrane of rat IMCD cells that were highly enriched for AQP2. Then, these endosomes were phosphorylated by an exogenous PKA catalytic subunit or were dephosphorylated by an exogenous alkaline phosphatase. There was no significant difference in P_f between these two samples, suggesting that the P_f of AQP2 is not changed by its phosphorylation. However, involvement of other proteins, such as endogenous phosphatases, cannot be neglected.[132]

To clarify whether the P_f of AQP2 is regulated by its phosphorylation event alone, the experimental system that does not contain any other regulatory proteins is required. Eto et al.[133] performed a large-scale expression of full-length recombinant human AQP2, purification and reconstitution in proteoliposomes, and examined the protein function.

The results showed that the P_f of proteoliposomes was enhanced approximately twofold by phosphorylation at serine 256. This observation indicates that AQP2 water channel activity is directly regulated by its phosphorylation, and the mechanism involved may be the channel gating that is well studied in plant AQPs by X-ray crystal structure analysis.[134] Thus, in addition to AQP2 translocation to the luminal membrane, the water transport activity of individual AQP2 proteins may be involved in the regulation of water reabsorption in kidney collecting ducts.

Actually, vasopressin increases the water permeability of the collecting ducts more than tenfold.[49,83] Thus, vasopressin-induced short-term regulation of P_f of the collecting ducts still seems to be mainly due to AQP2 translocation, and the altered water transport activity of individual AQP2s may have a role of doubling the effect.

Long-Term Regulation of Water Permeability of the Collecting Duct by Altered Aquaporin-2 Abundance

In addition to short-term regulation of collecting duct water permeability described previously, long-term regulation also plays an important role in body water balance. Long-term regulation of collecting duct water permeability is seen when water intake is restricted for 24 hr or more, resulting in an increased maximum concentrating ability. This response is mainly induced by an increase in AQP2 abundance due to increased transcription of the AQP2 gene.[135,136] An increased AQP2 expression level during water restriction is a downstream of vasopressin signaling.[137,138] It is known that the cAMP responsive element (CRE) is present in the 5′-flanking region of the AQP2 gene and regulates the transcription of the gene.[139,140] Hasler et al.[141] examined the AQP2 gene transcription in a cultured cell line, which endogenously expresses AQP2 and showed that the vasopressin-stimulated transcription of AQP2 was mediated through CRE.

Medullary collecting ducts are surrounded by interstitial hypertonicity and the hypertonicity may be another determinant of AQP2 transcription.[142,143] Hypertonicity affects transcription of many genes through the interaction between the tonicity-responsive enhancer (TonE) and its transcription factor TonEBP. TonE is observed in the AQP2 gene, suggesting that TonE/TonEBP contributes to AQP2 transcription. This possibility was examined in TonEBP gene knockout mice, which had mostly embryonic lethality or died within a few weeks after birth. However, the surviving mice showed atrophy of the kidney medulla, and the protein expression of AQP2 but not of AQP3 was clearly downregulated in the knockout mice, confirming the role of TonE/ToneEBP in AQP2 transcription.[144] Taken together, AQP2 protein abundance in long-term regulation is likely to be mediated by stimulated transcription of the AQP2 gene through CRE and TonE motifs. In addition, the presence of AP1 and GATA elements are known in the promoter region of the AQP2 gene, but their roles remain speculative (Fig. 4.5).

CHANNELS AND TRANSPORTERS CONTRIBUTING TO URINE CONCENTRATION AND DILUTION

Urine concentration and dilution are enabled by the specialized organization of the renal tubule and vasculature, and the presence of channels and transporters with their specific localizations. This section describes water channel aquaporin family members other than AQP2 and other channels and transporters involved in urine concentration and dilution. Table 4.1 summarizes the basic characteristics of AQP members in the kidney, and tissue localization and phenotypes of mice and humans caused by gene knockout or mutations. Figure 4.8 shows the localization of these channels and transporters along the nephron. Figure 4.9 shows the maximal urine osmolalities of the knockout mice in which channels and transporters involved in urinary concentration are deleted.

Aquaporin-1

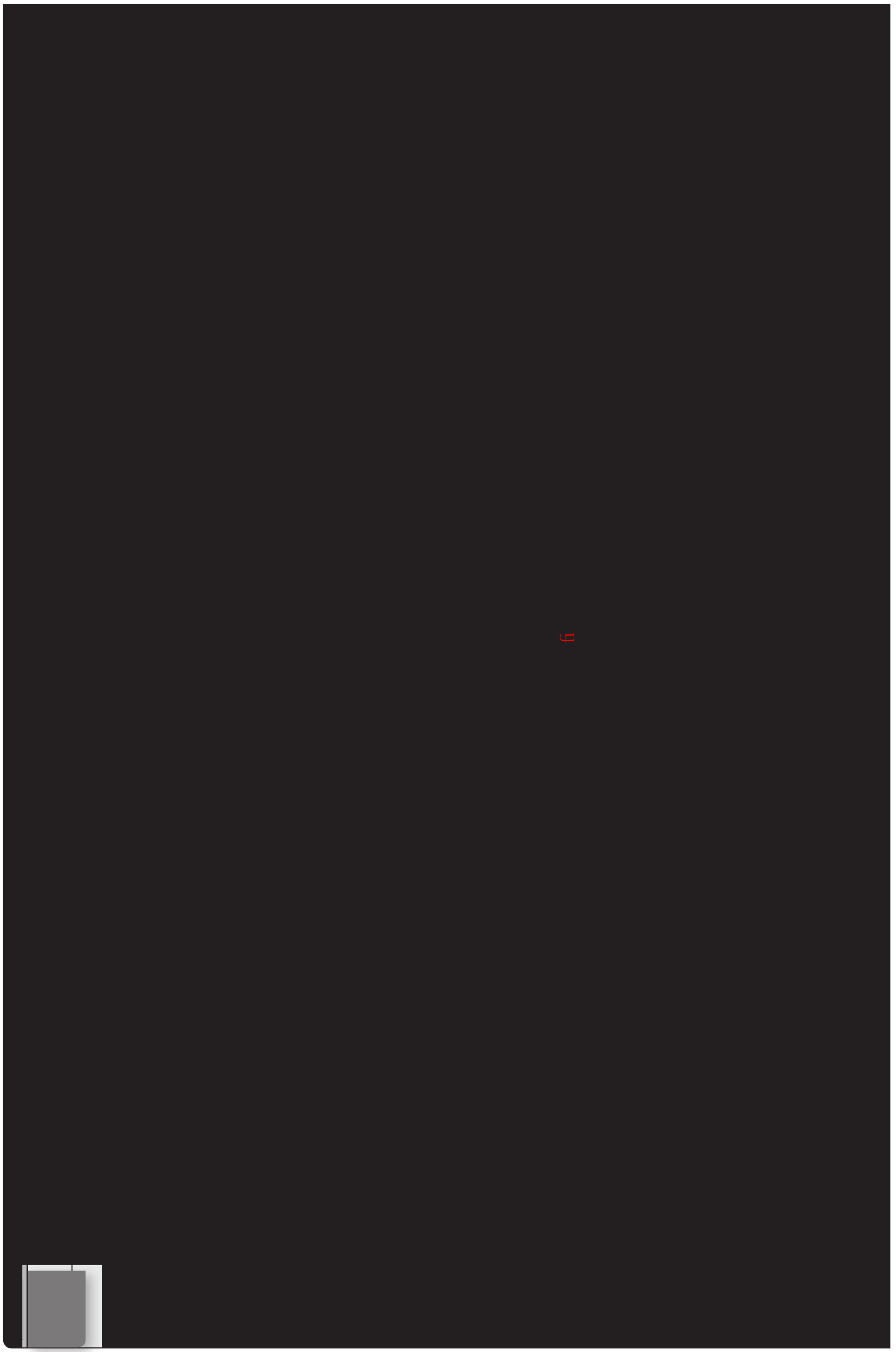
AQP1 is localized on the apical and basolateral membrane of the proximal tubules, the descending thin Henle limbs, and the outer medullary descending vasa recta.

AQP1 is constitutively active as a water-selective pore in these segments.[1,26]

AQP1-null mice show marked polyuria. Urinary osmolality in AQP1-null mice is low and unresponsive to vasopressin secretion or water deprivation.[11,145] As shown in Figure 4.9, maximal urine osmolalities of AQP1 knockout mice is 23% of that of wild-type mice under the same stimulated condition (i.e., 36 hr water deprivation). This phenotype was explained by two distinct mechanisms: impaired near iso-osmolar water reabsorption by the proximal tubule and reduced medullary hypertonicity resulting from impaired countercurrent exchange.[146] Analysis of AQP1-null mice clarified that AQP1 is the principal water channel in the proximal tubules and the descending thin Henle limbs and provides the major route for transepithelial water permeability in these segments.[146] In 2001, two unrelated individuals with a deficiency in AQP1 expression were reported.[147] One of these individuals was homozygous for a deletion of exon 1 of AQP1. The second individual was homozygous for a frame-shift mutation in exon 1 of AQP1. Although both patients seemed asymptomatic under normal conditions, they showed an impaired urinary concentrating ability following 24 hr of water restriction as compared with healthy controls (450 versus 1,000 mOsm per kilogram of water). Such a defect can become clinically meaningful in circumstances that require maximal urinary concentration; for instance, during vomiting and diarrhea.[148]

Aquaporin-3

The AQP3 water channel is present in the principal cells of the connecting tubule and the collecting duct, and enables water entry into the interstitium in these segments.[149] AQP3 is constitutively localized in the basolateral plasma



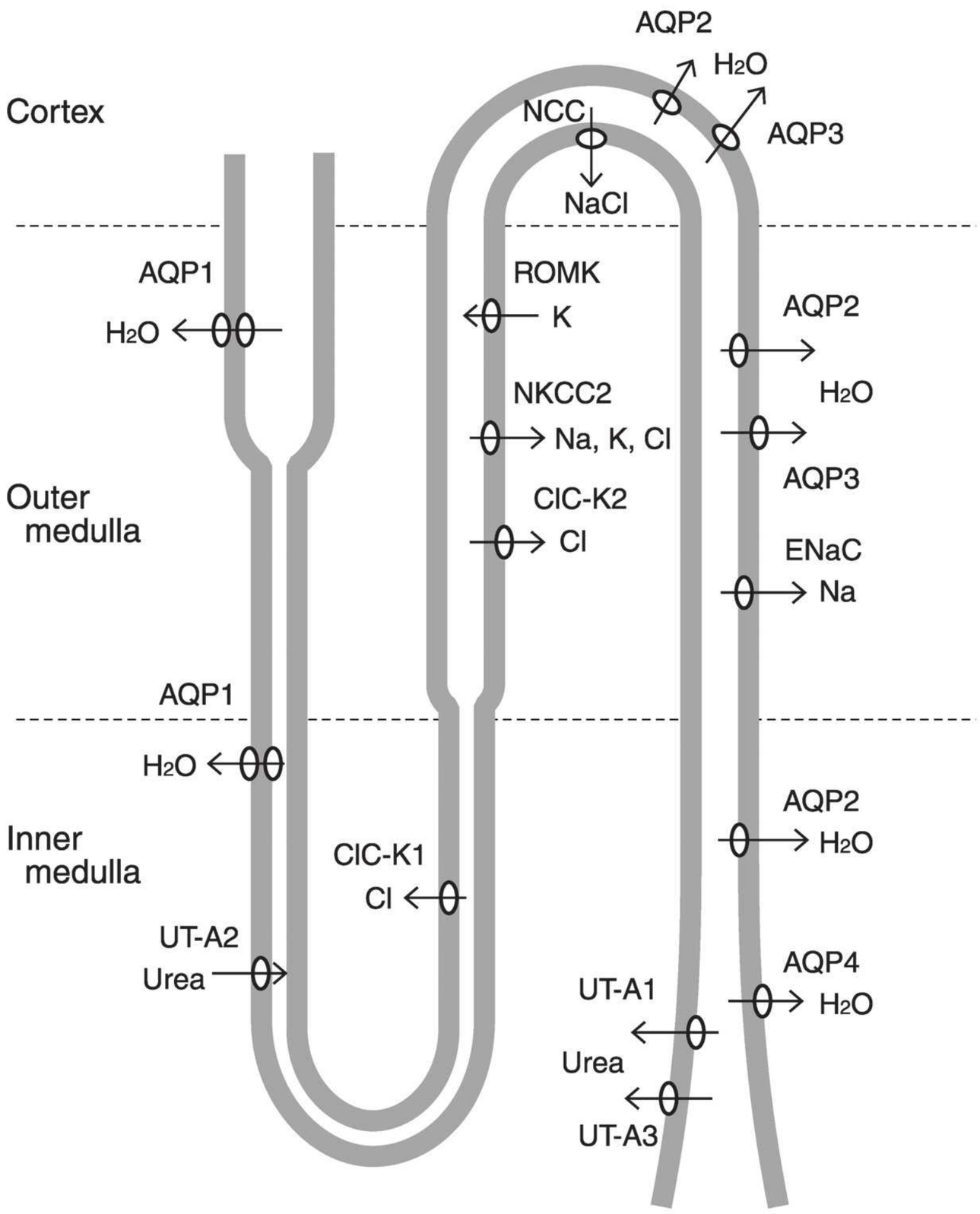


FIGURE 4.8 Transporters and channels involved in urine concentration along the nephron. Water is reabsorbed in the proximal tubule and the thin descending limb by AQP1. Chloride with sodium is passively reabsorbed in the thin ascending limb by the ClC-K1 chloride channel. NaCl is actively reabsorbed across the thick ascending limb by the apical plasma membrane Na-K-2Cl cotransporter (NKCC2). Potassium is recycled through an apical plasma membrane potassium channel, ROMK, and chloride is transported across the basolateral membrane by ClC-K2. Water is reabsorbed across the apical membrane of the collecting duct by AQP2 water channels. Water is reabsorbed across the basolateral membrane by AQP3 in the cortical and outer medullary collecting ducts and by both AQP3 and AQP4 in the inner medullary collecting duct. Urea is reabsorbed in the terminal inner medullary collecting duct by the urea transporters UT-A1 and UT-A3.

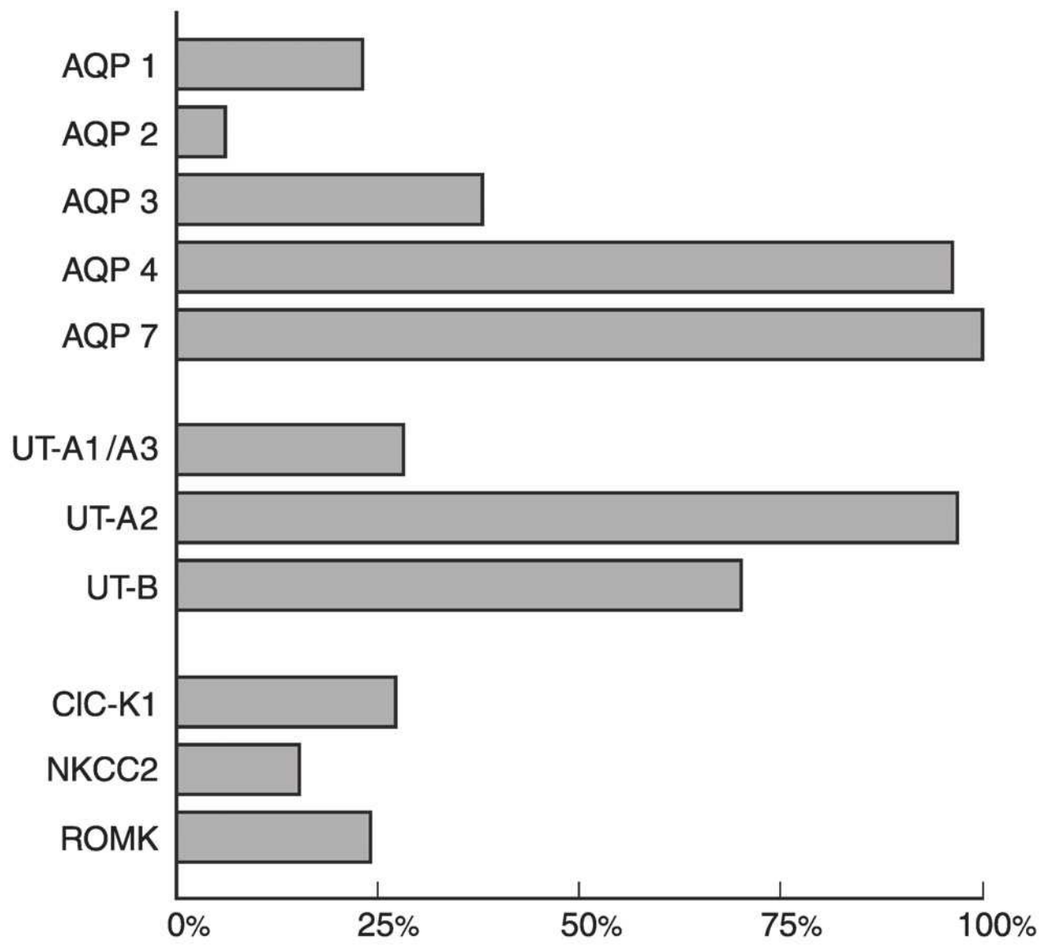


FIGURE 4.9 Maximal urine osmolalities of knockout mice in which transporters or channels involved in urine concentration are deleted. Data are expressed as a percentage of the maximal urine osmolalities of the wild-type mice in the same study. A maximally stimulated urine concentration was induced by water deprivation for 6 to 36 hr or by vasopressin administration. Data are taken from References 11, 13, 32, 42, 44, 78, 152, 158, 172, 180, and 186.

membrane. AQP3, together with AQP4, represents potential exit pathways from these cells for water entering the cell via AQP2. The AQP3 expression is shown to be increased by thirst and by vasopressin or aldosterone secretion.[150,151] AQP3 knockout mice exhibit an NDI-like phenotype, indicating that AQP3 has an important role in urine concentration (Fig. 4.9).[152,153] AQP3 deficiency was reported in humans.[154] This defect is caused by homozygous mutation affecting the 5′ donor splice site of intron 5 of AQP3. This mutation causes the skipping of exon 5 and generates a frameshift change and premature stop codon. However, phenotypes associated with this defect were not reported, possibly because the patients seemed normal.

Aquaporin-4

AQP4 is present in the principal cells of the collecting duct.[155] AQP4 is more abundant in the inner medullary collecting duct cells than is AQP3. The organization of AQP4 into orthogonal arrays of particles might enhance AQP4 water permeability,[156] and this process might be regulated by vasopressin.[157] Similarly to AQP3, AQP4 is constitutively localized in the basolateral plasma membrane. AQP4 knockout mice have a very little urinary concentrating defect (Fig. 4.9).[153,158] In contrast to AQP3, AQP4 expression is not regulated by thirst or by vasopressin secretion. AQP4 water permeability is decreased by high levels of protein kinase C and dopamine. This effect is mediated by phosphorylation at serine 180 of AQP4.[159]

Aquaporin-6

The water channel family member AQP6 is localized in the intracellular vesicles in intercalated cells in the collecting duct.[160–162] AQP6 has low water permeability and acts primarily as an anion transporter.[163] AQP6 is expressed in acid-secreting type-A intercalated cells and colocalizes with V-type H^+-ATPase; therefore, AQP6 is suggested to function to promote urinary acid secretion.

Aquaporin-7

AQP7 is localized in the brush border of proximal straight tubules (S3 segment)[12,164,165] and is able to transport glycerol as well as water.[12] AQP7 knockout mice showed marked glyceroluria,[13] indicating that glycerol can be reabsorbed through AQP7 in the proximal straight tubule, and that there might be no other glycerol reabsorbing system to compensate for this defect in the distal nephron segments.

In AQP7 knockout mice, water permeability of the brush border membrane vesicles was slightly but significantly lower than that in wild-type mice,[13] which suggests that AQP7 makes a small contribution to the water permeability of the proximal straight tubules. This contribution has been estimated to be one-eighth that of AQP1.[166] As expected from the small decrease in water permeability, AQP7 knockout mice did not show a urine concentrating defect (Fig. 4.9).[13]

The case of a man with a point mutation in AQP7 has been reported. However, no renal symptoms were mentioned, although he did have a defective glycerol metabolism.[167]

Aquaporin-11

AQP family members have two widely conserved, membrane-embedded NPA motifs that are essential for forming the water-permeable pore structure. However, AQP11, together with AQP12, have atypical NPA boxes, suggesting that their structure and function are unique.[168] In the kidney, AQP11 is present in the proximal tubular cells. Within the cells, AQP11 is localized in the endoplasmic reticulum (ER) membrane, but not localized in the cell surface plasma membrane. AQP11 knockout mice are born healthy but grow poorly and die before weaning because of uremia caused by enlarged multiple cysts in the kidney.[169] The cysts originate from kidney proximal tubule cells, inside which swollen ER are observed. The role of AQP11 is still unclear, although it seems to have an important role in ER membrane function in proximal tubular cells. Kidney cyst cells in AQP11 knockout mice showed an abnormal gene expression pattern, which is similar to that observed in polycystic kidney disease (PKD) model animals, suggesting that AQP11 knockout mice and PKD animals share common pathogenic mechanisms for cyst formation.[170] Yakata et al.[171] measured the Pf of the vesicles from the membrane fraction of Sf9 cells expressing AQP11 and showed that AQP11 has slow but constant water transport activities. Further studies are required for clarifying the role of AQP11.

Urea Transporters

In the kidney, there are four urea transporters: UT-A1, UT-A2, UT-A3, and UT-B (Figs. 4.4 and 4.8). UT-A1 and UT-A3 are expressed in the terminal IMCD,[32] and UT-A2 is present in the thin descending limb of the long-looped nephron in the inner medulla and the thin descending limb of the short-looped nephron in the outer medulla.[14] Their localization is essential for the urea recycling pathway as described in the section Urea Accumulation in the Inner Medulla, discussed previously. Vasopressin stimulation promotes the phosphorylation of UT-A1 and UT-A3 and the accumulation of UT-A1 and UT-A3 in the plasma membrane of the IMCD cells. This is responsible for the increased urea permeability of the terminal IMCD that results in greater urea reabsorption during antidiuresis.[50] Vasopressin stimulation also increases UT-A2 protein abundance.[14]

UT-A1/UT-A3 knockout mice show a large urinary concentrating defect (Fig. 4.9). This concentrating defect is ameliorated by a low protein diet, indicating that this defect is caused by a urea-dependent osmotic diuresis.[32] Another possibility may be that a low protein diet reduces urea concentrations in the medulla in both UT-A1/UT-A3 knockout mice and wild-type mice, and the difference of urine concentrating abilities of these two groups becomes small. Nevertheless, UT-A1/UT-A3 greatly contributes to urine

concentration by accumulating urea in the medulla when protein intake is normal.

On the other hand, in UT-A2 knockout mice, a small urinary concentrating defect and a reduction in medullary urea content are observed only on a low protein diet, but not on a normal diet (Fig. 4.9; a value on a normal diet is shown).[172] This finding indicates a relative role of recycling urea through the "tubular route"; urea leaves from IMCD to the interstitium, diffuses around, then enters into the tubular lumen at the thin descending limb (mediated by UT-A2), and again comes back the IMCD (Fig. 4.4), is small, and becomes significant when urea delivery to the IMCD is low.

UT-B is localized in the descending vasa recta endothelial cells.[46] UT-B knockout mice show a decrease in urea accumulation in the medulla and a urinary concentrating defect, indicating that the recycling of urea by a countercurrent exchange via the vasa recta is important for urine concentration abilities (Fig. 4.9).[44] Humans genetically lacking UT-B (Kidd blood group antigen) are identified and their maximal urinary concentrating ability is reduced (UOSM, max = 819 mOsm per kilogram of water), which is consistent with the results of the knockout mice.[173]

ClC-K1 and ClC-K2

The chloride channel ClC-K1 is mainly expressed in the thin ascending Henle limb and its expression is upregulated by water deprivation.[10,15,16] ClC-K2 is present from the thick ascending limb through to the collecting duct.[174,175] ClC-K1 knockout mice show a significantly reduced osmolality of the papilla and a severe defect in urinary concentrating ability (Fig. 4.9).[42] This finding indicates that the rapid chloride exit to the medullary interstitium is important for the maintenance of the high inner medullary interstitial osmolality and supports the passive mechanism as described in the section Countercurrent Multiplication in the Outer Medulla and Other Mechanisms in the Inner Medulla, discussed previously. Severities of impaired urine concentrating abilities are comparable in ClC-K1 and UT-A1/UT-A3 knockout mice (Fig. 4.9), implying a functional linkage between the chloride transport by ClC-K1 and the urea transport by UT-A1/UT-A3, which is again consistent with the operation of the passive model in the inner medulla.

The human orthologue of the ClC-K2 gene is one of the genes that, when mutated, causes Bartter syndrome in humans, which is a disease manifested by salt wastage, hypokalemia, metabolic alkalosis, and hyperaldosteronism.[176,177] Polyuria and polydipsia are commonly observed in Bartter syndrome and these symptoms are not relieved by vasopressin, thus showing the presence of NDI.[178] Thus, NaCl reabsorption in the thick ascending limb mediated by a basolateral chloride channel, ClC-K2, importantly contributes to urine concentration through the operation of the countercurrent multiplication system.

NKCC2 and ROMK

NKCC2 is expressed in the thick ascending limb and contributes to NaCl reabsorption in this segment.[56] The gene encoding NKCC2 is another gene, the mutations of which cause Bartter syndrome.[179] Different from NHE3, NKCC2 is also present in the macula densa.[56] NKCC2 knockout mice cannot survive to weaning because of severe dehydration and polyuria. This may be because the compensatory decrease in glomerular filtration does not occur in response to increased distal fluid delivery.[180] This finding indicates that NKCC2 plays an important role in the macula densa in the mediation of tubuloglomerular feedback and is critical for distal fluid delivery. Treatment with indomethacin rescues the knockout mice, and the surviving mice show a large vasopressin-resistant defect in urine concentrating ability (Fig. 4.9).[180] Thus, in the same mechanism as in ClC-K2, NKCC2 contributes to urine concentration.

Another Na-K-Cl cotransporter isoform, NKCC1, is expressed in the collecting duct and contributes to NaCl secretion.[181,182] NKCC1 knockout mice show a reduced capacity to excrete free water.[183] NKCC1 is present also in the juxtaglomerular afferent arteriole and the glomerular and extraglomerular mesangium, where it is thought to participate in the process of tubuloglomerular feedback and the regulation of blood pressure.

The ATP-sensitive, inwardly rectifying potassium channel, ROMK, is expressed in the apical membrane of the thick ascending limb and throughout the distal nephron segments and is involved in NaCl reabsorption in the thick ascending limb.[176,184] Mutations of the gene encoding ROMK are also found in patients with Bartter syndrome.[185] Knockout mice with ROMK have hydronephrosis, are severely dehydrated, and 95% die before 3 weeks of age. The urine concentrating ability of the surviving mice is severely impaired, as expected (Fig. 4.9).[186]

A Na-H exchanger isoform NHE3 is expressed in the thin descending Henle limb and the thick ascending Henle limb and contributes to sodium reabsorption in these segments.[57] NHE3 knockout mice show a marked reduction in proximal tubule fluid absorption. Nevertheless, these mice maintain a relatively normal distal delivery, and its urinary concentrating defect is mild.[187,188] This is due to a compensatory decrease in the glomerular filtration rate owing to an intact tubulo-glomerular feedback.[188] Furthermore, the concentrating defect of NHE3 knockout mice is explained by the decreased expression of NKCC2, a Na-K-Cl cotransporter isoform.[189]

NCC and ENaC

The Na-Cl cotransporter NCC is present in the distal convoluted tubule,[19] and the Na channel ENaC is present in the connecting tubule and the cortical collecting tubule (Fig. 4.8).[28,29] Both the proteins mediate sodium reabsorption beyond the macula densa and contribute to urine concentration by reducing fluid delivery to the medullary

collecting duct. Long-term vasopressin stimulation increases the abundance of NCC and ENaC.[190] Short-term vasopressin stimulation promotes ENaC accumulation in the apical membrane, resulting in increased sodium reabsorption.[191]

URINE CONCENTRATING DEFECTS

Central Diabetes Insipidus

Central diabetes insipidus is caused by inadequate secretion of vasopressin from the posterior pituitary in response to osmotic stimulation. In most cases, this is due to the destruction of the neurohypophysis by a variety of lesions, including granulomas (e.g., histiocytosis, sarcoidosis), neoplasms (e.g., craniopharyngioma, germinoma, lymphoma, leukemia, meningioma, pituitary tumor, metastasis), infections (e.g., meningitis, tuberculosis, encephalitis), trauma, and cerebrovascular diseases (e.g., cerebral hemorrhage, infarction). Several cases with inherited central diabetes insipidus are also reported, although it is a rare disorder.[192–194] Most of these cases show an autosomal dominant mode of inheritance and are caused by mutations in the gene coding for the vasopressin-neurophysin precursor. It is postulated that these mutations cause the production of an abnormal precursor protein that accumulates and damages the neurons.

Osmoreceptor Dysfunction

The osmoreceptors that control vasopressin secretion and thirst are located in the anterior hypothalamus. Osmoreceptor dysfunction is caused by their damage by a variety of brain lesions including granulomas, neoplasms, and cerebrovascular diseases. In contrast to central diabetes insipidus, osmoreceptor dysfunction-causing lesions usually occur more rostrally in the hypothalamus because of the location of the osmoreceptors. One lesion unique to this disorder is an anterior communicating cerebral artery aneurysm. In this disorder, osmoregulation of both vasopressin secretion and thirst is impaired.[195] Patients with osmoreceptor dysfunction typically have osmolalities in the range of 300 to 340 mOsm per kilogram of water, whereas patients with central diabetes insipidus maintain their plasma osmolality within the normal range by polydipsia. This underscores the importance of a normal thirst mechanism. On the other hand, in patients with osmoreceptor dysfunction, the baroreceptor mediated pathway[196] and responses to other nonosmotic stimuli such as nausea are intact. These patients have normal vasopressin and renal concentrating responses to hypovolemia and hypotension.

Nephrogenic Diabetes Insipidus

NDI is caused by the inability of the kidney to respond to vasopressin stimulation. Thus, plasma vasopressin levels in NDI patients are increased or normal depending on their plasma osmolality. Hyperosmolar patients with NDI have elevated vasopressin levels, whereas those with central NDI have an absence of blunted vasopressin responses relative to their plasma osmolality. There are congenital and acquired forms of NDI.

Congenital Nephrogenic Diabetes Insipidus

In more than 90% of cases of congenital NDI, the condition results from a loss of function mutations in the AVPR2 gene encoding the vasopressin V2 receptor, which makes the collecting duct cells insensitive to vasopressin. The AVPR2 gene is located in chromosome region Xq28, and its mutations cause the X-linked inheritable form of NDI. To date, over 200 mutations have been reported in the AVPR2 gene, which are categorized into five classes according to the cellular fate.[197–199] Class I mutations result in premature stop codons or unstable mRNA, and include promoter alterations, exon skipping, aberrant splicing, frameshift, and non-sense mutations that result in truncated proteins. On the other hand, mutations of class II, III, IV, and V result in fully translated proteins. Class II comprises the missense or insertion/deletion mutations that lead to the misfolding of this protein. Misfolded proteins are retained in the ER and, subsequently, are mostly targeted for proteasome degradation. Class II is most prevalent, comprising approximately 45% of all mutations in the AVPR2 gene. Class III and IV mutations do not interrupt the translocation to the plasma membrane. Class III mutations interfere with binding to the stimulatory Gs protein, leading to the reduced activation of adenylate cyclase, whereas Class IV mutations interfere with binding to vasopressin. Class V mutations result in trafficking defects beyond the early secretory pathway and misrouting to different organelles in the cell. Although most female carriers of the X-linked V2 receptor defect have no clinical disease, some females were reported with symptomatic NDI.[200] Rare female patients manifest severe NDI due to the inactivation of the normal X chromosome.[201,202] Furthermore, a significant number of de novo mutations have also been reported.[203]

Congenital NDI can also result from mutations of the autosomal gene that codes for AQP2. AQP2, the gene that encodes AQP2, is located in 12q13 and is comprised of four exons.[139,204,205] To date, over 43 mutations in the AQP2 gene have been reported (Fig. 4.10).[51,206,207] Two inheritance types are possible for the disease: Autosomal-recessive NDI is associated with 35 mutations, and autosomal-dominant NDI is associated with 8 mutations. Almost all of the mutations in recessive NDI are located in the core region of the protein, and they lead to misfolded proteins that become trapped in the ER and then are targeted for rapid degradation by the proteasome. On the other hand, AQP2 homotetramers composed only of wild-type proteins are properly translocated to the apical membrane. This effect explains the healthy phenotype of patients' parents.[206]

All mutations in autosomal-dominant NDI locate in the cytosolic C-terminus of AQP2 (Fig. 4.10). This region is important for AQP2 trafficking and these mutations impair trafficking to the apical membrane, although the water channel function of these mutants is preserved. Arg254Leu and

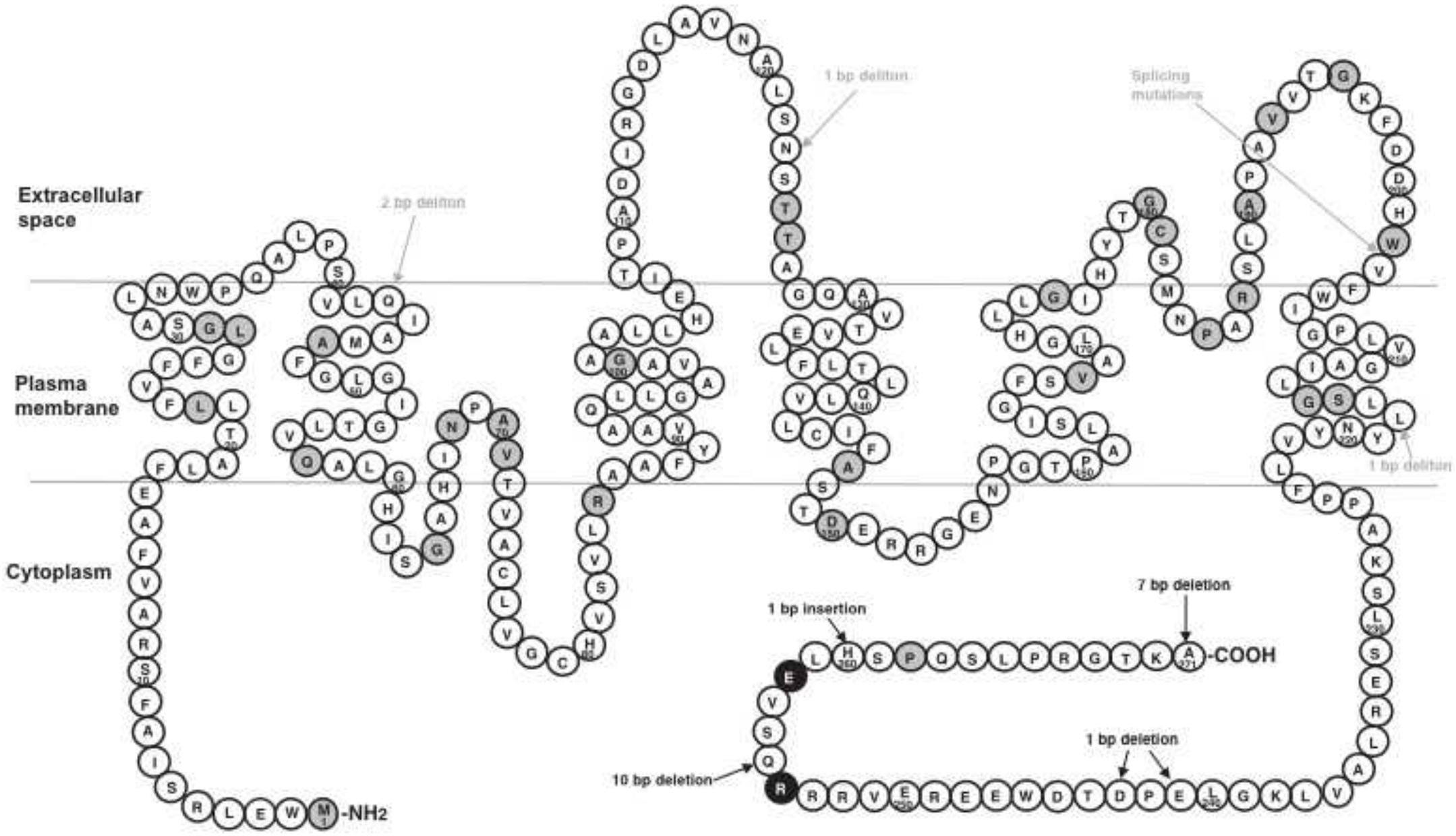


FIGURE 4.10 The AQP2 membrane topology and mutations causing nephrogenic diabetes insipidus. Amino acids that, when mutated, cause the autosomal-recessive form of nephrogenic diabetes insipidus are shown in grey. Also, *grey arrows* show AQP2 deletions and the location of the first amino acid of the protein affected by these nucleotide deletions, which causes the autosomal-recessive form of nephrogenic diabetes insipidus. Amino acids, when mutated, that cause the autosomal-dominant form of nephrogenic diabetes insipidus are shown in *black*. Similarly, in *black* are also reported AQP2 deletions or insertions, and the location of the first amino acid of the protein affected by these nucleotide changes, which cause the autosomal-dominant form of nephrogenic diabetes insipidus. *bp*, base pair.

Arg254Gly mutations destroy the site for PKA phosphorylation, so that forskolin-induced trafficking to the plasma membrane is impaired.[207,208] The Glu258Lys mutant of AQP2 is missorted to multivesicular bodies and/or lysosomes.[209] AQP2 mutants resulting from three gene deletions, 721delG, 763–772del, and 812–818del, have similar extended C-terminal tails, which contain the basolateral-membrane-sorting dileucine (LeuLeu) motif, so these mutated proteins are wrongly translocated to the basolateral membrane.[114,210] In contrast to the AQP2 mutants associated with the recessive form of the disease, AQP2 mutants associated with the dominant form of the disease are not misfolded, so they are able to form heterotetramers with wild-type AQP2. Because of the dominance of the missorting motif in the mutant proteins, tetramers composed of the mutant and wild-type forms are missorted, which leads to severely decreased amounts of AQP2 on the apical membrane. This effect explains the dominant mode of NDI inheritance in patients with these mutations. Sohara et al.[211] generated gene knockin mice with the heterozygous mutant AQP2 resulting from a gene deletion (763–772del) that produces a mouse model of dominant NDI. The mutant AQP2 is wrongly translocated to the basolateral membrane; it forms a heterotetramer with wild-type AQP2 and shows a dominant-negative effect on the normal apical sorting of wild-type AQP2. The urine concentrating ability of these gene knockin mice is severely reduced.

Several other mouse models of NDI caused by AQP2 mutations have also been generated. Yang et al.[21] created mice with a T126M knockin mutation in the AQP2 gene. These homozygous mutant mice died within 6 days after birth, suggesting that the mice may be highly sensitive organisms with regard to water homeostasis, and are unable to survive with polyuria. Lloyd et al.[212] generated mice with a F204V mutation in the AQP2 gene that survived beyond the neonatal period and had a much milder form of NDI.

Acquired Nephrogenic Diabetes Insipidus

Acquired NDI is more common than congenital NDI and is caused by various conditions, including drug treatments, electrolyte disturbances, and urinary tract obstruction. Dysregulation of AQP2 plays a fundamental role in many cases of acquired NDI.

Lithium is widely used for treating bipolar disorder and 20% to 30% of patients treated with lithium develop NDI.[213] In lithium-induced NDI, both AQP2 expression and its trafficking to the apical membrane are inhibited. Lithium enters cells expressing AQP2 via the epithelial sodium channel (ENaC) in the apical membrane and accumulates

intracellularly. This accumulation leads to the inhibition of signaling pathways that involve glycogen synthase kinase-3β (GSK3β). Although the mechanism by which AQP2 is dysregulated in this context is not established, the involvement of GSK3β is speculated. Inhibition of GSK3β by lithium increases the expression of cyclo-oxygenase 2 and the local excretion of prostaglandin E2.[214] Prostaglandin E2 is suggested to counteract vasopressin activity by causing an endocytic retrieval of AQP2 from the plasma membrane, thus impairing the urinary concentrating ability of the cell. Furthermore, lithium increases the intracellular accumulation of β-catenin,[215] which can serve as an activator of T-cell factor-dependent transcription. AQP2 downregulation may be achieved via this transcription mechanism. In addition, AQP3 expression is also decreased. Moreover, lithium treatment caused a marked reduction in the fraction of the principal cells in the collecting duct with a parallel increase in the population of intercalated cells.[216] This restructuring of the collecting duct, together with the downregulation of collecting duct AQPs, may be important in lithium-induced NDI.

Hypokalemia and hypercalcemia cause downregulation of AQP2 expression, which results in a vasopressin-resistant urinary concentrating defect. With regard to hypercalcemia, in addition to AQP2, the expression levels of AQP1 and AQP3 are also decreased.[217] In addition, hypercalcemia reduces the expression of NKCC2 and ROMK,[218] resulting in sodium absorption defects in the thick ascending limb, which would affect the countercurrent multiplication.

Ureteral obstruction decreases AQP2 expression and impairs urine concentrating capacity.[219] In addition to AQP2, AQP1, AQP3, and AQP4 are also decreased by ureteral obstruction.[219]

A urinary concentrating defect is also observed in patients with nephrotic syndrome.[220,221] AQP2 expression is decreased in nephrotic rats.[220,222] However, changes in AQP2 expression levels have not yet been confirmed in patients with nephrotic syndrome.

Mouri et al.[223] reported that AQP2 translocation to the apical membrane is inhibited by metabolic acidosis, a mechanism that might be responsible for the diuresis in patients with chronic renal failure.

Water Retention by Urine Diluting Defects

AQP2 has a critical role in the pathophysiology of many diseases associated with water balance disorders. The best known example is congestive heart failure (CHF). Water retention and hyponatremia are common and clinically important complications of CHF. Plasma vasopressin levels are suppressed by hyponatremia in healthy individuals; however, these levels are not suppressed in patients with hyponatremia who have CHF.[224,225] In CHF, effective blood volume and arterial filling are decreased, which is sensed by aortic and carotid baroreceptors, thus resulting in stimulation of vasopressin secretion. Upregulation of AQP2 expression and increased AQP2 trafficking to the apical membrane of principal cells of the collecting duct have been shown in rat models of cardiac failure.[226,227] Furthermore, water retention and hyponatremia in these rats are reversed by a V2 receptor antagonist.[227] These findings indicate that hyponatremia is caused by nonosmotic stimulation of vasopressin, which promotes the expression and trafficking of AQP2. In patients with heart failure, V2 receptor antagonists promote electrolyte-free water excretion and elevate serum sodium concentration.[228–230] In these patients, vasopressin antagonists also have been shown to improve several symptoms of heart failure, such as dyspnea.[231]

Water retention and hyponatremia are observed in patients with hepatic cirrhosis. In these patients, the nonosmotic secretion of vasopressin occurs secondary to splanchnic arterial vasodilatation and relative arterial underfilling.[225] In cirrhotic rats, AQP2 expression was increased and correlated with the volume of ascites.[232] In patients with hyponatremic cirrhosis, V2 receptor antagonists are effective at inducing free water diuresis and raising plasma sodium levels.[229,233]

During pregnancy, arterial underfilling secondary to systemic arterial vasodilatation with nonosmotic vasopressin secretion and upregulation of AQP2 is observed.[75,234] The administration of a V2 receptor antagonist increases electrolyte-free water excretion in pregnant rats.[234]

The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is a condition in which plasma vasopressin levels are not appropriately suppressed despite hypoosmolality. SIADH is the predominant cause of euvolemic hyponatremia and is a commonly encountered disorder.[235] SIADH occurs frequently in association with vascular, infectious, or neoplastic abnormalities in the lung or central nervous system. In patients with SIADH, a V2 receptor antagonist is shown to be effective in increasing urine volume and plasma sodium levels.[236] However, the long-term effects of its administration are limited in rats with SIADH.[237] Although the AQP2 protein expression is reduced shortly after the administration of the V2 receptor antagonist to rats with SIADH, it increases again in parallel with the decline of the therapeutic effects.

The nephrogenic syndrome of inappropriate antidiuresis (NSIAD) is a rare hereditary disease caused by gain of function mutations of the V2 receptor gene. Two causative mutations at the arginine residue at the position 137, Arg137Cys and Arg137Leu, have been reported.[238] In patients with this disease, endogenous vasopressin is completely suppressed, whereas antidiuresis persists due to the constitutive activation of the receptor, showing the same clinical pictures as SIADH.[238] The clinical presentation of the disease starts from childhood, but the same mutations seem to explain some sporadic episodes of euvolemic hyponatremia in adults. To differentiate NSIAD from SIADH, it may be helpful to observe the responses to V2 receptor antagonists; the former does not respond to the antagonist, whereas the latter does.[239]

The urinary AQP2 excretion level is associated with vasopressin activity in the kidney and is, therefore, a clinically useful biomarker.[240] AQP2 is excreted into the urine through the secretion of exosomes originating from the internal vesicles of

multivesicular bodies (Fig. 4.5).[241] During this process, the outer membrane of multivesicular bodies fuses with the apical plasma membrane. Urinary AQP2 excretion is increased by dehydration or vasopressin and is decreased by hydration. Urinary AQP2 excretion is also increased in patients with CHF and hepatic cirrhosis and in pregnant women.[242–244] In patients with CHF, the administration of a V2 receptor antagonist produced a significant increase in urine flow and solute-free water excretion, and was accompanied with a dramatic decrease in urinary AQP2 excretion.[242] An increased urinary excretion of AQP2 is also found in SIADH and NSIAD.[245,246] Thus, the urinary excretion of AQP2 is a useful marker of the antidiuretic activity of the collecting duct.

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