7.6 Regulation of Fluid and Electrolyte Balance

Learning Objectives

- Describe how afferent and efferent arteriolar contractions affect the GFR
- Define the autoregulation of RBF and GFR
- Describe the myogenic mechanism and tubuloglomerular feedback as mechanisms of autoregulation
- Describe what is meant by glomerulotubular balance and distinguish it from tubuloglomerular feedback
- Describe the chemical nature of ADH
- Describe the glandular origin of ADH
- Describe the mechanism by which ADH alters distal tubule water permeability
- List the signals leading to increased ADH secretion
- Describe the chemical nature and origin of renin
- List the signals leading to increased renin release
- Describe the cascade of events leading to angiotensin II
- List the actions of angiotensin II
- Recognize the chemical structure of aldosterone and identify its glandular origin
- List the signals leading to increased aldosterone release
- List the actions of aldosterone

REGULATION OF GLOMERULAR FILTRATION RATE AFFECTS URINE OUTPUT

Pressures produce the glomerular ultrafiltrate according to

$$\text{GFR} = K_f[P_{GC} - P_{BS} - \pi_{GC}] \quad [7.6.1]$$

Theoretically, the regulation of the glomerular filtration rate (GFR) can be accomplished by regulating K_f, the filtration coefficient; P_{GC}, the hydrostatic pressure within the glomerular capillary; P_{BS}, the hydrostatic pressure within Bowman's space; or π_{GC}, the oncotic pressure of the glomerular capillary blood.

P_{BS} IS NORMALLY CONSTANT AND NOT SUBJECT TO PHYSIOLOGICAL REGULATION

The hydrostatic pressure within Bowman's space is a consequence of the volume within that structure and its compliance. Typically the pressure in Bowman's space is a modest 20 mmHg, and it is not subject to large variation. If the kidney's urinary path becomes blocked, pressure within the urinary conduits will rise until filtration equilibration is reached. This is a pathological condition. Although there is no evidence that P_{BS} is physiologically regulated, it seems that increased GFR ought to increase P_{BS} as a direct effect of compliance. Increased P_{BS} should then increase flow down the tubule because the driving force is increased. Similarly, reductions in GFR ought to decrease P_{BS} because there would be less fluid distending the cavity. Thus the P_{BS} may not be independently regulated, but it may vary directly with the GFR. The consequence is that the resistance of the tubule to flow naturally opposes changes in GFR. There is little information about the relative importance of this resistance.

PLASMA ONCOTIC PRESSURE INCREASES WITH DEHYDRATION AND REDUCES GFR

The plasma oncotic pressure is that part of the total osmotic pressure of the plasma that is due to impermeant proteins. During prolonged water restriction or after water loss due to sweat, the blood becomes more concentrated and its oncotic pressure increases. Since this pressure opposes the GFR, dehydration also reduces the GFR due to reduction in the net driving force. This produces a negative feedback loop: dehydration increases π_{GC} which decreases the GFR, thereby lessening further loss of fluid through the urine. However, the kidneys are merely conservatory organs. They cannot alone set aright the dehydration. Drinking water is necessary. Dehydration engages the mechanism of **thirst** to motivate us to seek water.

CONTRACTION OF MESANGIAL CELLS ALTERS K_F

Mesangial cells surround the glomerular capillaries and have contractile properties. In response to a variety of vasoactive agents, these cells can alter the effective surface area for filtration, perhaps also altering the dimensions of glomerular capillary fenestrae and podocyte slit pore size. The result is that K_f is reduced by vasoconstrictors and increased by vasodilators. The physiological importance of this mechanism is unknown.

THE AFFERENT ARTERIOLE AND EFFERENT ARTERIOLE EXERT SEPARATE EFFECTS ON P_{GC}

The glomerular capillary hydrostatic pressure is the driving force for formation of the glomerular ultrafiltrate.

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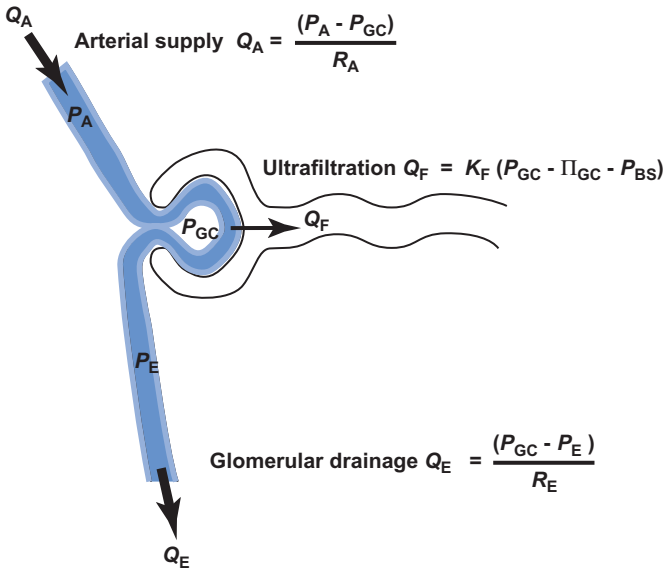


FIGURE 7.6.1 Flows into and out of the glomerulus. Input is the flow through the afferent arteriole, which is determined by the resistance of the afferent arteriole and the pressure difference that drives flow. Output consists of the ultrafiltrate and flow through the efferent arteriole. The afferent and efferent arteriolar resistances largely determine the glomerular capillary hydrostatic pressure.

Consider the arrangement of flows shown in Figure 7.6.1. The conservation of fluid volume at steady state requires that the flow into the glomerular capillaries through the afferent arterioles equals the flow out of the glomerular capillaries through the efferent arterioles and the glomerular ultrafiltrate. We write this as

$$Q_A = Q_E + Q_F \quad [7.6.2]$$

where Q_A is the blood flow through the afferent arteriole, Q_E is the flow through the efferent arteriole, and Q_F is the flow of the ultrafiltrate, or the GFR. The flow through each is determined by the pressure difference and the resistance. We write these as

$$\frac{(P_A - P_{GC})}{R_A} = \frac{(P_{GC} - P_E)}{R_E} + K_f[P_{GC} - P_{BS} - \pi_{GC}] \quad [7.6.3]$$

where P is the pressure in the respective spaces, R_A and R_E are the resistances of the afferent and efferent arterioles, respectively, and K_f is the filtration coefficient for the glomerular filtration, as written in Eqn (7.6.1). We can manipulate Eqn (7.6.3) algebraically to solve for P_{GC}, the pressure in the glomerular capillary. We find

$$P_{GC} = P_A \frac{R_E}{R_E + R_A + K_f R_E R_A} + P_E \frac{R_A}{R_E + R_A + K_f R_E R_A} + (\pi_{GC} + P_{BS}) \frac{K_f R_E R_A}{R_E + R_A + K_f R_E R_A} \quad [7.6.4]$$

The utility of this equation is reduced by the fact that π_{GC} and, to a lesser extent, P_{BS} are not independent of P_{GC}. Because P_{GC} drives the ultrafiltration, the filtration fraction generally increases with increases in P_{GC}—but not always—and so π_{GC} also increases with P_{GC} and glomerular filtration is opposed. Therefore, the last term on the right-hand side of the equation does not have a clear interpretation. Nevertheless, the relative normal values of R_E, R_A, and K_f can illuminate the relative importance of the three terms in determining P_{GC}. If we use $P_A \approx 90$ mmHg and $P_{GC} \approx 60$ mmHg, and the normal value for $Q_A \approx 1.1$ L min^{-1}, we can calculate $R_A \approx 27.3$ mmHg L^{-1} min. Similarly, we can estimate R_E for the normal condition as (60 mmHg − 10 mmHg)/0.98 L min^{-1} = 51.0 mmHg L^{-1} min. K_f under normal conditions can be estimated from the average values of the GFR and driving forces as K_f = 0.12 L min^{-1}/10 mmHg = 0.012 L min^{-1} mmHg^{-1}. Inserting these values into Eqn (7.6.4) gives

$$P_{GC} = 0.537P_A + 0.287\,P_E + 0.176(\pi_{GC} + P_{BS}) \quad [7.6.5]$$

This equation is NOT causative, but it is descriptive: although true for the given state of the glomerulus and afferent and efferent arterioles, the coefficients will change with other states. If we double R_E without changing any other variable, e.g., the three coefficients become 0.627, 0.167, and 0.205 for P_A, P_E, and $(\pi_{GC} + P_{BS})$, respectively, and P_{GC} increases from 60 to 67.3 mmHg. The equation allows us to see that the factors multiplying the afferent arteriolar pressure dominate the calculation. Because P_A is very much greater than P_E, and the coefficient multiplying $(\pi_{GC} + P_{BS})$ is so small, the coefficient multiplying P_A has the greatest importance. This coefficient is given as

$$\frac{R_E}{R_E + R_A + K_f R_E R_A} \quad [7.6.6]$$

so the resistance of the efferent arteriole is the most important factor in determining P_{GC} and, therefore, the GFR. Thus the constriction of the efferent arteriole causing an increase in R_E results in an increase in P_{GC} and an increase in GFR. This conclusion has its limits. If R_E increases too much, the flow through the glomerular capillaries is reduced and GFR similarly falls because pressure equilibrium will be reached and there will be less blood flow to support the GFR. On the other hand, increasing R_A by vasoconstriction should increase the denominator in the P_A term, reducing the pressure in the glomerular capillaries and reducing the GFR. The effect of dilation or constriction of afferent and efferent arterioles on GFR and filtration fraction is shown in Figure 7.6.2.

RBF AND GFR EXHIBIT AUTOREGULATION

AUTOREGULATION MAINTAINS A RELATIVELY CONSTANT RBF AND GFR

According to Eqn (7.6.1), we should expect that decreased arterial blood pressure would decrease RBF and decrease the GFR and that increased arterial blood pressure would increase both RBF and GFR. The experimental observation in dogs is that RBF and GFR remain

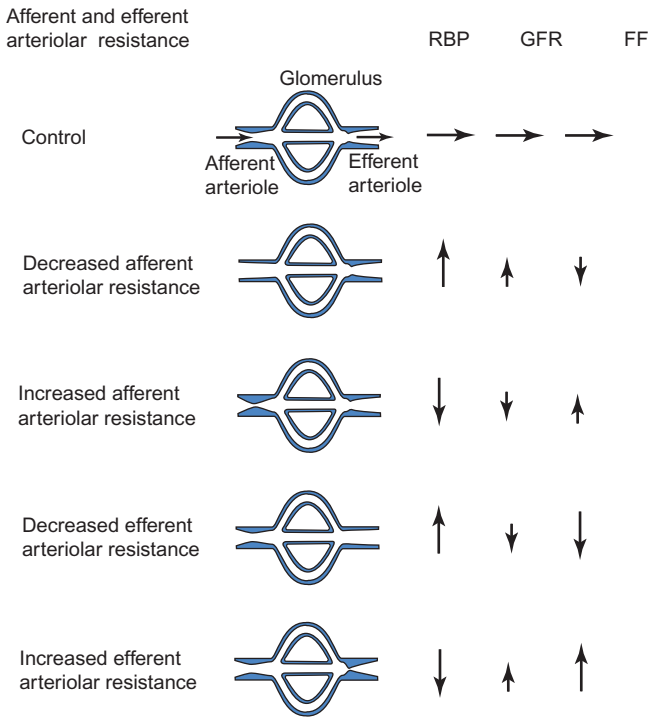


FIGURE 7.6.2 Effect of afferent arteriolar and efferent arteriolar constrictions and dilation on renal blood flow (RBF), glomerular filtration rate (GFR), and filtration fraction (FF). Dilating the afferent arteriole increases RBP because the overall resistance to flow is decreased. The GFR is also increased because of the increase in glomerular capillary pressure, but this increase is slight. The result is a decrease in the filtration fraction. Constricting the afferent arteriole reduces RBP because the vascular resistance is increased. This constriction also reduces the pressure downstream from the constriction, which reduces the GFR approximately proportionately. However, reducing RBF gives more time for filtration to reach equilibrium, so that the filtration fraction will rise slightly upon afferent arteriolar constriction. Constriction of the efferent arteriole alone also reduces RBF but with an increase in glomerular capillary pressure. This favors a relative increase in the GFR over the RBF, so that the filtration fraction is increased.

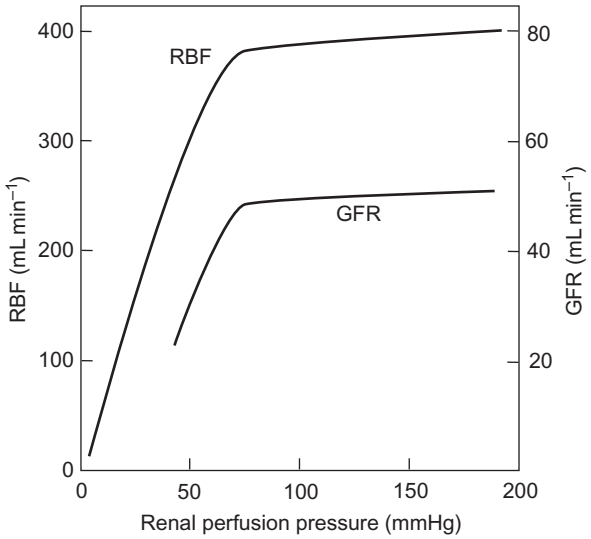


FIGURE 7.6.3 Autoregulation of renal blood flow (RBF) and glomerular filtration rate (GFR) as a function of renal perfusion pressure in dog kidneys. Assuming that the hematocrit was 0.45 in this animal, the filtration fraction would be approximately 50 mL min^{-1}/(400 mL min^{-1} × 0.55) = 0.23. *Adapted from L.G. Navar, Renal autoregulation: perspectives from whole kidney and single nephron studies.* Am. J. Physiol. ***234***:*F357, 1978.*

nearly constant over a wide range of arterial blood pressure, as shown in Figure 7.6.3. This phenomenon is called **autoregulation**. Because both RBF and GFR are maintained fairly constant between 80 and 180 mmHg perfusion pressure, we conclude that afferent arteriolar constriction causes autoregulation. If the efferent arteriole was involved, we would expect changes in the GFR.

THE MYOGENIC MECHANISM AND TUBULOGLOMERULAR FEEDBACK ACCOUNTS FOR AUTOREGULATION

The dynamic response of autoregulation reveals a fast component and a slow component. The fast component is due to **myogenic vasoconstriction**, whereas the slower component is mediated by feedback from the early distal tubule, called **tubuloglomerular feedback**. Tubuloglomerular feedback requires changes in GFR to affect the composition or volume of fluid delivered to the distal tubule, and this takes time to work its way down from the glomerulus to the distal tubule. Alterations of blood pressure that stretch the afferent arteriole, however, occur rapidly.

THE MYOGENIC MECHANISM AUTOREGULATES GLOMERULAR BLOOD FLOW AND PROTECTS AGAINST RENAL DAMAGE FROM OVERPRESSURE

The myogenic response is the reflex response of the afferent arterioles to changes in blood pressure. Increased blood pressure increases the tension in the vascular wall, and the vascular smooth muscle contracts. Similarly, decreased blood pressure decreases the tension and the smooth muscle relaxes. These responses originate in the smooth muscle cells themselves, triggered by stretch. Although the myogenic mechanism provides a nearly constant P_{GC}, thereby regulating the GFR, the myogenic mechanism also protects the glomerulus from damage by high pressure. Studies of the frequency response of autoregulation show that oscillating pressure causes sustained contractions. The myogenic mechanism responds to the systolic pressure and not the mean pressure. The mean pressure drives the GFR, whereas the myogenic mechanism protects the glomerulus from the systolic pressure. Since systolic and mean pressure generally change in concert, protection against systolic pressure autoregulates the GFR as a by-product.

The mechanism of the myogenic response connects mechanical stretch of the renal vasculature with the contractile machinery of vascular smooth muscle cells. The general mechanism of myogenic responses has been discussed in Chapter 5.11. The myogenic response involves three stages: a membrane sensor for mechanical stretch, transduction of that stretch response to a cytosolic signal, and then, lastly, the contractile

response to that signal. The membrane response involves detection of stretch by integrins, membrane-bound dimers of one α and one β subunit, whose deformation activates tyrosine protein kinases, mitogen-activated protein kinases (MAPKs), or extracellular receptor kinases (ERKs). These modulate surface membrane voltage-operated calcium channels (VOCC) and ryanodine receptors (RyR) on the sarcoplasmic reticulum to alter cytosolic [Ca^{2+}], thereby altering the activity of myosin light chain kinase (MLCK) and the phosphorylation state of the myosin light chains, altering the contractile state of the vascular smooth muscle. A variety of other mechanosensitive sensors and pathways have been proposed to explain the myogenic response.

TUBULOGLOMERULAR FEEDBACK REGULATES THE SINGLE NEPHRON GFR

Tubuloglomerular feedback refers to the feedback regulation of the GFR in a single nephron based on sensory information about the distal tubule fluid. This feedback regulation involves the **juxtaglomerular apparatus (JGA)**, a collection of specialized cells where the thick ascending limb contacts the afferent and efferent arterioles. The JGA includes the **macula densa** cells that line the thick ascending limb at its junction with the distal tubule, **granule cells** in the afferent arteriole wall that release the enzyme **renin** into the circulation, and **mesangial cells** that lie between these structures and which may relay signals between them (see Figure 7.6.4). Increased distal tubular Na^+ concentration causes the macula densa cells to swell by activation of the NKCC transporter. These cells release ATP that either directly activates arteriolar cells through purinergic receptors or is converted to adenosine by extracellular nucleotidases. The resulting adenosine can activate adenosine A1 receptors that activate afferent arteriolar contraction. Swelling of the macula densa cells signals constriction of the afferent arteriole of its own nephron so that the glomerular filtration of the same nephron is decreased. This forms a negative feedback loop in which the increased distal tubular load of Na^+ is decreased by reducing the GFR and subsequently allowing more time for Na^+ reabsorption because flow is slower. This regulation of the GFR occurs locally in a single nephron. The thick ascending limb of the nephron regulates its own single nephron GFR (SNGFR).

THE NEPHRON ADJUSTS REABSORPTION OF WATER AND SALT TO MATCH CHANGES IN THE GFR

Autoregulation of the GFR by either the myogenic mechanism or tubuloglomerular feedback does not mean that the GFR does not change. It can change drastically due to sympathetic nervous stimulation of the renal arterioles, for example. When the GFR changes as a primary event, the amount of salt and water reabsorbed by the proximal tubule also changes, so that a constant fraction of the filtered load, about 0.67, is reabsorbed. This phenomenon is called **glomerulotubular balance**. It should not be confused with tubuloglomerular feedback. Glomerulotubular balance is partially explained by two mechanisms: peritubular oncotic pressure and Na^+-cotransport mechanisms. If the GFR increases without a concomitant change in RBF, as might occur if the efferent arterioles constrict while the afferent arterioles relax, then the filtration fraction will increase. The blood perfusing the peritubular capillaries then would have increased oncotic pressure because a greater fraction of protein-free ultrafiltrate would concentrate the remaining proteins more. This effect is not linear, so that the oncotic pressure would increase more than the concentration. The increased oncotic pressure would favor reabsorption of fluid from the tubule lumen. In addition, an increased GFR without a change in the RBF implies that a greater pressure drop occurs across

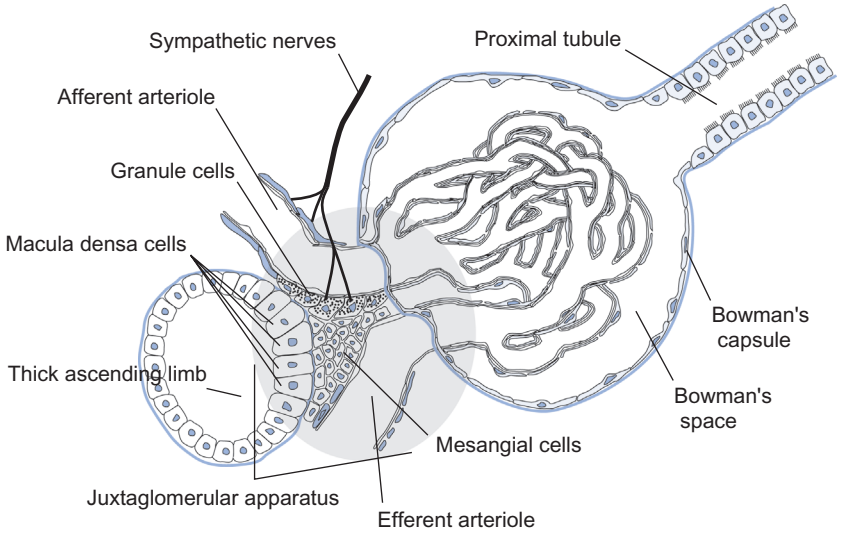


FIGURE 7.6.4 Structures of the juxtaglomerular apparatus. The macula densa cells of the thick ascending limb of the loop of Henle sense the distal tubular load of Na^+ by swelling in response to high Na^+ loads. Signals from the macula densa cells are believed to be relayed by mesangial cells to smooth muscle and granule cells in the wall of the afferent arteriole.

the efferent arteriole than before, so that the hydrostatic pressure within the peritubular capillaries will also be less. Thus both the decrease in hydrostatic pressure and the increased oncotic pressure of the peritubular blood will favor fluid reabsorption along the peritubular capillaries.

Increasing the GFR increases the filtered load of all solutes, including glucose and amino acids and other solutes that are absorbed by Na^+ cotransport. Since these solutes are reabsorbed completely in the proximal tubule, increasing their load increases the amount of Na^+ reabsorption proportionately.

WATER BALANCE IN THE BODY IS MEDIATED BY ANTIDIURETIC HORMONE

The typical water exchanges in moderate climates are shown in Figure 7.6.5. Water inputs include the water content of drink and food plus water made in the body during oxidation. Water outputs include the **insensible loss** through the skin and lungs, even in the absence of sweating, plus losses in urine and feces. These numbers are highly variable. High ambient temperatures or elevated body temperature due to exercise can cause losses by sweating as much as 4 L of water per hour. Sweat is a hypoosmotic fluid that contains NaCl and KCl, and excessive sweating also leads to excessive electrolyte loss. Large losses of water can occur through vomiting and diarrhea. Urinary water excretion is a major daily loss of water. After prolonged and strenuous exercise, urine volume is small and its color is deep, indicating concentration of its solutes. After drinking excessively, the urine volume is large and it is nearly colorless because it is dilute. This everyday experience shows us that the urinary volume varies inversely with the amount of sweat and directly with fluid intake, whereas urinary concentration typically varies inversely with urinary volume. Thus the regulation of urinary volume and concentration is a major component in the regulation of total body water. However, the kidneys must always excrete an obligatory solute load of about 600 mOsmol per day, which requires at least 500 mL of urine per day. The kidneys cannot make water. Loss of body water can be corrected only by drinking water. Thus the behavioral mechanism of thirst is also crucial for maintenance of total body water. The regulation of urinary water excretion is mediated by **ADH**, **antidiuretic hormone**.

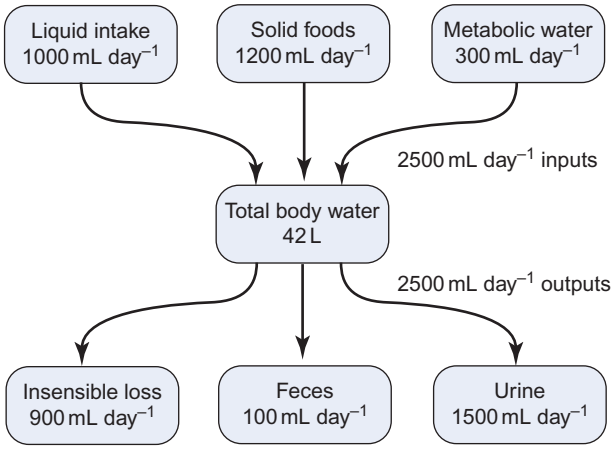


FIGURE 7.6.5 Typical water exchanges at steady state in a "typical" 70-kg adult male. Total body water is about 42 L. Daily loss is about 2.5 L day^{-1}, with most through the urine (1.5 L day^{-1}) and through insensible loss through the skin and lungs (0.9 L day^{-1}). Almost all of this loss is replenished through food (1.2 L day^{-1}) and drink (1 L day^{-1}) with a relative small component through metabolic water (0.3 L day^{-1}). These numbers vary greatly depending on environmental conditions and activity level.

ADH SECRETION BY THE BRAIN IS INCREASED BY HYPOVOLEMIA AND HYPEROSMOLARITY

As its name implies, ADH has an "antidiuresis" effect; it inhibits urine flow. It is also called vasopressin because it also causes vasoconstriction. This hormone is a peptide consisting of nine amino acids whose chemical structure is shown in Figure 7.6.6. The hormone is synthesized in the brain in cells in the **supraoptic nucleus** and **paraventricular nucleus** as a precursor protein called propressophysin. It is carried by axoplasmic transport down to the posterior pituitary gland where the precursor is cleaved by proteolysis to its final products: ADH, neurophysin II, and a glycoprotein. Upon stimulation of the cell bodies in the supraoptic nucleus and paraventricular nucleus, ADH and its carrier neurophysin are released from their nerve terminals by exocytosis. ADH dissociates from neurophysin and circulates to its target tissues (see Chapter 9.2).

Specialized cells in the anterior hypothalamus, close to the supraoptic nucleus, sense the osmolarity of blood. Increases in the concentration of impermeant solutes cause these **osmoreceptor cells** to shrink, and this activates them to signal cells in the supraoptic nucleus and paraventricular nucleus to release ADH. Permeable solutes like urea and glucose do not activate the osmoreceptors, so these solutes could increase plasma osmolarity without evoking an increased ADH release. **Changes in blood osmolarity are the strongest factor in regulating ADH release** (see Figure 7.6.7).

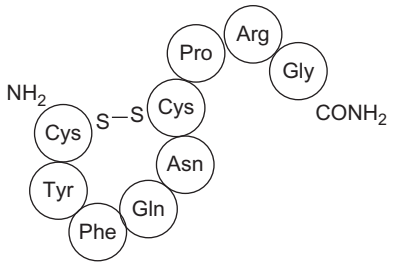


FIGURE 7.6.6 Chemical structure of antidiuretic hormone. The cyclic peptide is released from nerve cell terminals in the posterior pituitary to circulate to target tissues. The cell bodies, located in the hypothalamus, make the hormone as a precursor that is carried by axoplasmic transport along axons that terminate in the posterior pituitary. ADH is released in response to low blood pressure, low circulating blood volume, and blood hyperosmolarity.

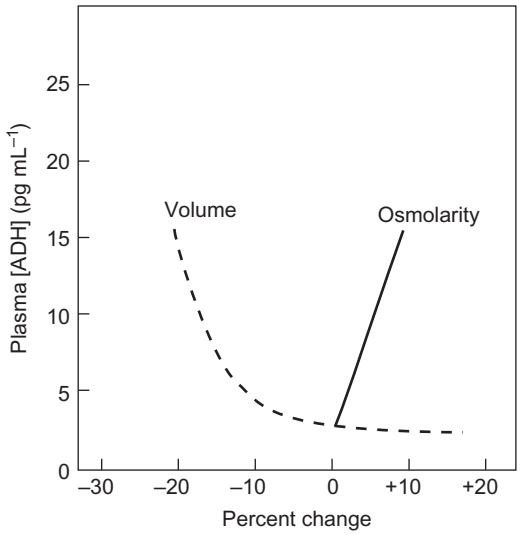


FIGURE 7.6.7 Relative sensitivity of ADH secretion to changes in plasma osmolarity and blood volume. Hyperosmolarity markedly increases ADH secretion as evidenced by higher circulatory [ADH]. Hypovolemia also increases circulating levels of ADH, but larger changes are required compared to changes in plasma osmolarity. The steep curve for osmolarity indicates that ADH secretion is most sensitive to changes in plasma osmolarity.

Stretch receptors in the low-pressure side of the circulation increase their action potential frequency with stretch of the walls of the left atria and intrathoracic veins. The afferent input of these stretch receptors tonically inhibits ADH release. Thus increased stretch caused by excess venous volume reduces ADH release, and decreased stretch increases ADH release. Similarly, immersion in water or exposure to cold tends to send blood back to the central veins, causing a reflex diuresis mediated through reduced ADH release by increased activity of the central stretch receptors (see Figure 7.6.7).

Other factors that influence ADH release include pain, trauma, emotional distress, nausea, ethanol ingestion, angiotensin II, catecholamines, and atrial natriuretic factor. Stimulation of α adrenergic sympathetics is diuretic, whereas β adrenergic stimulation is antidiuretic. Alcohol inhibits ADH release, causing a diuresis.

ADH INCREASES WATER PERMEABILITY OF THE DISTAL NEPHRON

Like most peptide hormones, ADH exerts its effect on cells through receptors on the surface of its target cells. The vasopressin receptors include V1a, V1b, and V2. The V1 receptor subtype is linked to a G_q mechanism involving the activation of phospholipase C and the liberation of inositol trisphosphate (IP3) and diacylglycerol (DAG). (See Chapter 2.8 for a description of G_q-linked receptors.) The V1 receptor subtypes are responsible for the vasoconstrictor effects of ADH. The kidney possesses V2 receptors, which are linked to a G_s mechanism. Occupancy of the V2 receptor liberates the α subunit of the heterotrimeic G_s protein, and this subunit then activates adenylyl cyclase to produce cAMP from ATP. The increased cytosolic concentration of cAMP then activates protein kinase A (PKA). PKA phosphorylates subunits of **aquaporin**, the water channel protein. In the kidney, **AQP2** is phosphorylated at multiple amino acids. Phosphorylation of AQP2 signals the insertion of AQP2 into the apical membrane of the cell, thereby rendering the entire cell permeable to water. The AQP2 channels continuously cycle between the apical membrane and endocytotic vesicles. Channels that are insufficiently phosphorylated are removed from the membrane and phosphorylated channels are inserted into the membrane. **Protein phosphatase** assures that in the unstimulated state the channels become endocytosed and the water permeability of the cell is low. Endocytosis is facilitated by ubiquitinylation of AQP2. Thus the continued presence of ADH is necessary to maintain high water permeability. This mechanism of ADH action is shown in Figure 7.6.8. Although many parts of the nephron possess aquaporins, only the late distal tubule and collecting duct have AQP2 on the apical membrane. AQP1 is located in the proximal tubule and descending thin limb of the loop of Henle, and AQP3 and AQP4 are on the basolateral membrane of the collecting duct principal cells. AQP1, AQP3, and AQP4 are not sensitive to ADH. ADH also increases the total AQP2 content of the tubule cells by increasing transcription of the gene coding for AQP2 (see Figure 7.6.8).

ADH also increases the urea permeability of the inner medullary collecting duct. The urea transporter on the apical membrane has been identified as UT-A1, and ADH increases the insertion of these transporters in the apical membrane, increasing the urea permeability, and producing a higher osmotic gradient along the cortical–medullary axis due to urea recycling (see Chapter 7.5). The action of ADH of increasing the urea permeability of the inner medullary collecting duct occurs through similar mechanisms as the insertion of AQP2 into the apical membranes (see Figure 7.6.9).

THE ADH-RENAL SYSTEM FORMS NEGATIVE FEEDBACK LOOPS

Increasing the osmolarity of blood increases ADH release, which increases the water permeability of the terminal nephron. This causes the excretion of a small volume of concentrated urine. Since solutes are excreted in excess of water in a concentrated urine, this has the effect of removing salt from the extracellular fluid. Thus this system would correct the hyperosmolarity and return body fluids to normal.

Increased blood volume increases stretch that in turn increases inhibition of ADH release. The lower circulating [ADH] results in low water and urea permeability of the terminal nephron and excretion of a large volume of dilute urine. This tends to deplete the blood volume, correcting the perturbation in blood volume. Similarly, lowering the blood volume engages the system so that water is lost less rapidly and any additional fluids

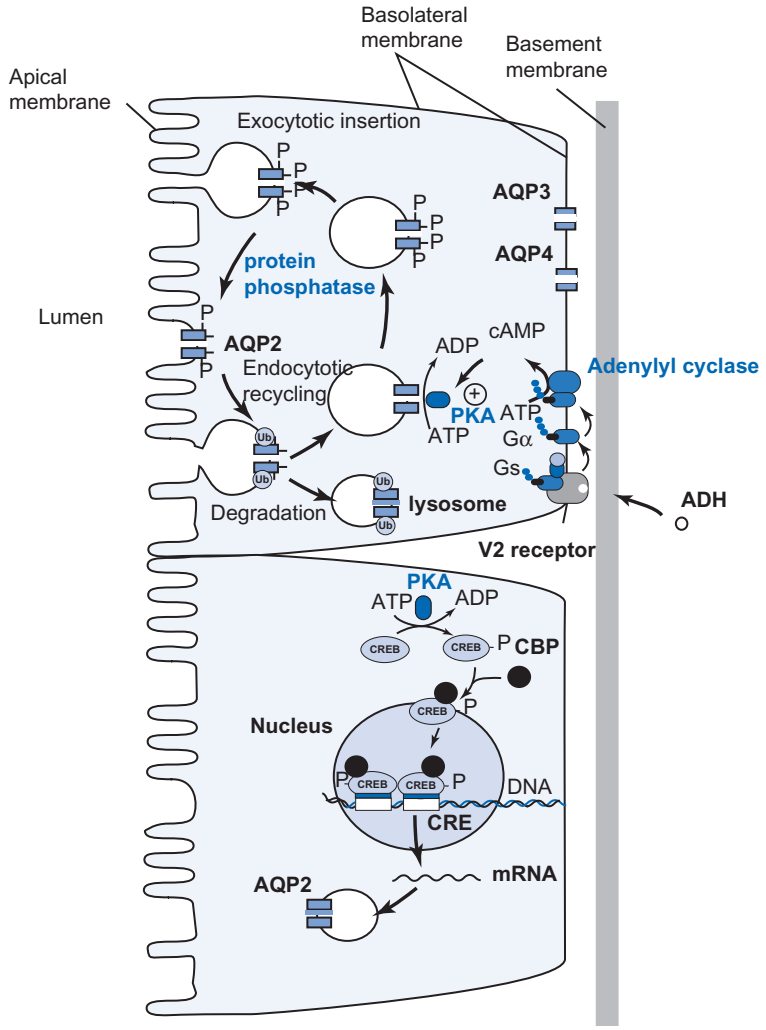


FIGURE 7.6.8 Simplified mechanism of ADH action on water permeability of distal tubule and collecting duct principal cells. AQP2 channels are sequestered in subapical vesicles in the nonstimulated state. ADH binds to V2 receptors on the basolateral membrane, activating a G_s mechanism. The α subunit of a heterotrimeric G-protein exchanges GTP for GDP and dissociates from the $\beta\gamma$ subunits. The $G\alpha$ subunit activates adenylyl cyclase, converting ATP to 3′,5′-cAMP. The cytosolic cAMP then activates protein kinase A (PKA), which phosphorylates AQP2 channels in vesicles. The AQP2 channels are then inserted into the apical membrane, thereby increasing the cell's permeability to water. AQP2 is dephosphorylated by protein phosphatases and is also ubiquitinylated. These signal for endocytosis of the AQP2, which may then be recycled or degraded by lysosomal enzymes. ADH also increases the total AQP2 in the cell over a longer time by PKA-mediated phosphorylation of the cyclic AMP response element binding protein (CREB), which then binds the CREB-binding protein (CBP) and then activates the AQP2 promoter to increase expression of AQP2. The long-term effect of ADH is mediated by another protein, EPAC (exchange protein activated directly by cAMP).

that are consumed are retained by the kidneys. These feedback loops are illustrated in Figure 7.6.10.

THE *FREE WATER CLEARANCE* QUANTIFIES THE OVERALL CONCENTRATION OR DILUTION OF URINE

The free water clearance is given by

$$C_{H_2O} = Q_U\left(1 - \frac{U_{osm}}{P_{osm}}\right) \quad [7.6.7]$$

where C_{H2O} is the free water clearance, Q_U is the urine flow, U_{osm} is the total osmolarity of urine, and P_{osm} is the total osmolarity of plasma. Note that this equation is not the same as the typical clearance, $C_x = Q_U U_x / P_x$, because the concentration of water in a solution is inversely related to the osmolarity: higher osmolarity implies less water per unit volume of urine. You can see that this equation makes intuitive sense. If the urine osmolarity is the same as plasma, the urine is isosmolar and there is no gain or loss of free water. There is still loss of water, equal to Q_U, but the body fluids are not being concentrated or diluted. If the free water clearance is positive, it means that $U_{osm} < P_{osm}$ and free water is being excreted or cleared. In this case the urine is hyposmolar and body fluids are being concentrated by removal of water. If the free water clearance is negative, it means $U_{osm} > P_{osm}$ and the urine is hyperosmolar: free water is being retained in the body while excess salt

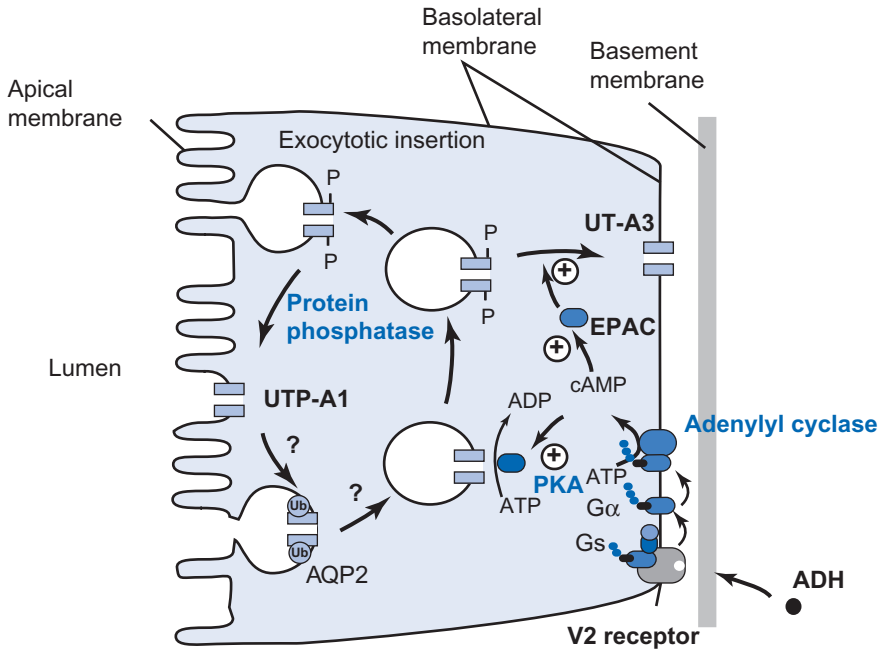


FIGURE 7.6.9 Simplified mechanism of action of ADH on urea permeability of the inner medullary collecting duct cells. ADH (=vasopressin) binds to a GPCR (G-protein-coupled receptor) on the basolateral membrane of the cells, causing the exchange of GTP for GDP on the α subunit of the heterotrimeric G-protein. The G_α subunit activates adenylyl cyclase to increase cytosolic concentrations of 3',5' cyclic AMP. This cAMP activates PKA (protein kinase A) and EPAC (exchange protein activated by cAMP) that phosphorylates UT-A1 channels, signaling their incorporation into the apical membrane. cAMP also activates EPAC that also mediates UT-A1 and UT-A3 function.

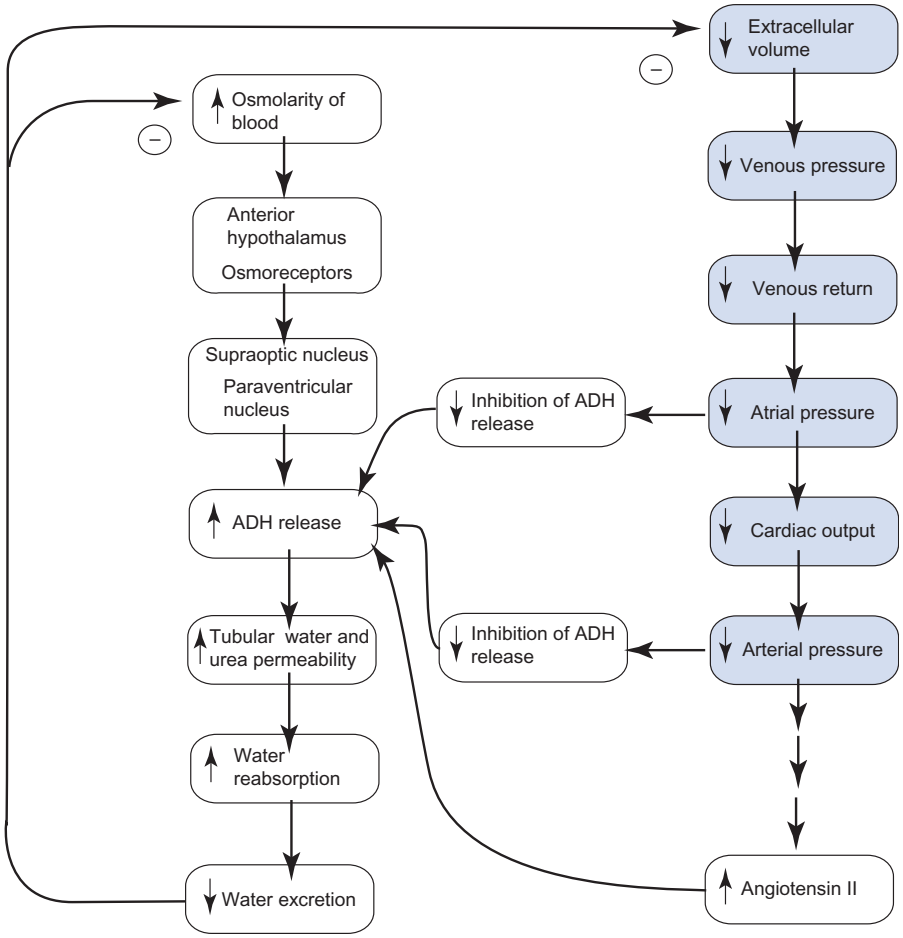


FIGURE 7.6.10 Negative feedback loops involving ADH. The main determinants of ADH release are increased plasma osmolarity and decreased blood volume. ADH released in response to these perturbations increases the water and urea permeability of the distal nephron resulting in more water reabsorption and excretion of a small volume of concentrated urine. This can correct the hyperosmolarity but cannot correct the reduced blood volume. However, it sets the stage for retention of water when we drink.

and other osmolytes are being excreted. The free water clearance thus measures the volume of plasma that is being completed cleared of water. Unlike other clearances, the free water clearance can be positive, zero, or negative, and reflects the relative treatment of osmolytes—primarily NaCl—and water.

REGULATION OF Na^+ BALANCE INVOLVES THE *RENIN–ANGIOTENSIN–ALDOSTERONE* SYSTEM

RENIN *BEGINS A CASCADE OF EVENTS LEADING TO THE PRODUCTION OF CIRCULATING* ANGIOTENSIN II

Specialized granule cells called juxtaglomerular cells or JG cells in the afferent arteriole release **renin** into the circulation. Renin is a proteolytic enzyme that converts an inactive plasma protein, an α_2 globulin, called **angiotensinogen**, into **angiotensin I**. Angiotensin I is a polypeptide 10 amino acids in length. **Angiotensin converting enzyme (ACE)** cleaves off two additional amino acids to produce **angiotensin II**. ACE is located predominantly in the lung, but the kidney also has some ACE activity. Angiotensin II exerts multiple effects that are discussed below. A third enzyme, **ACE2**, converts angiotensin II to **angiotensin (1–7)** that binds to a GPCR called Mas whose activation opposes many of the functions of angiotensin II.

JG cells synthesize prorenin which is converted to renin by proteases. JG cells release prorenin at a constant and slow rate that is not affected by stimuli for renin release. Prorenin is not converted to renin in the circulation.

MULTIPLE SIGNALS CAUSE RENIN RELEASE FROM THE GRANULE CELLS OF THE AFFERENT ARTERIOLE

Three main signals stimulate renin release:

1. Decreased afferent arteriolar pressure increases renin release.
2. Renal sympathetic nerve stimulation increases renin release.
3. Decreased distal tubule [NaCl] increases renin release.

Decreased afferent arteriolar pressure is sensed locally within the arteriole and a signal passes to the granule cells directly. The renal sensor for arteriolar pressure is called the "intrarenal baroreceptor." The response of the system shows a threshold arterial pressure of about 85 mmHg that separates an almost constant level of renin secretion at higher pressures from a steep increase at lower pressures (see Figure 7.6.11). Renal sympathetic nervous stimulation can occur when there is a drop in systemic arterial pressure or during strenuous exercise or in the "fight or flight" reaction in which the renal arteries are vasoconstricted. Sympathetic stimulation of renin release occurs at low levels of sympathetic renal nerve activity that precedes the renal vasoconstriction that occurs at higher sympathetic output to the kidneys. This mechanism involves β_2 adrenergic receptors

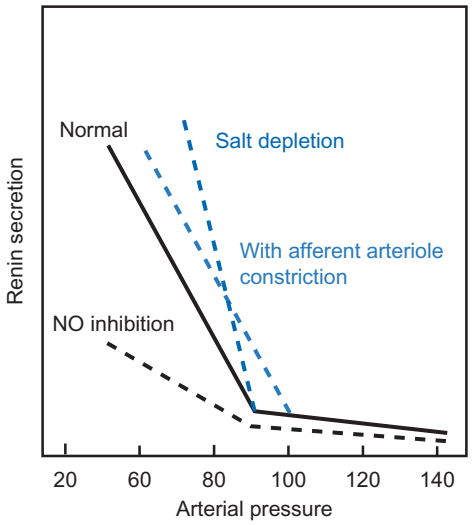


FIGURE 7.6.11 Control of renin secretion. Renin secretion has a low and nearly constant level at high arterial pressure. At a threshold of about 85 mmHg, renin secretion rises steeply with decrease in pressure. This effect is blocked by the inhibition of NO production. The threshold is increased by afferent arteriolar constriction caused by high levels of renal sympathetic nerve stimulation or by pathological conditions such as renal stenosis. Salt depletion increases renin release at any given pressure, indicating the multifactorial nature of renin secretion.

on the granule cells. However, the vasoconstriction of the afferent arteriole through α-adrenergic receptors also lowers the pressure in the glomerulus and sensitizes the intrarenal baroreceptor, causing increased renin secretion for a given reduction in arterial pressure.

Decreased distal tubule [NaCl] is sensed by cells in the **macula densa**, which swell in response to increased [NaCl] in the distal tubule. A diffusible paracrine signal passes from the macula densa cells to the granule cells, probably being relayed by the intervening mesangial cells. Three candidates for this paracrine signal include adenosine, nitric oxide (NO), and prostaglandins. Isolated juxtaglomerular apparatus (JGA) produces PGE_3 that can stimulate renin secretion through EP2 or EP4 receptors. In isolated perfused JGA, stimulation of renin secretion by low [NaCl] in the lumen is blocked by COX-2 (cyclooxygenase, an enzyme necessary for prostaglandin synthesis) blockers. Adenosine inhibits renin release and may be involved in the suppression of renin release by high [NaCl] or increased arterial pressure, but it appears to be unimportant in the increased secretion caused by low [NaCl] in the tubule fluid at the macula densa. Macula densa cells expresses the neuronal nitric oxide synthase (NOS-I) that produces NO that stimulates renin release in response to low luminal [NaCl].

Renin release is also feed back inhibited by angiotensin II.

ANGIOTENSIN II EXERTS MULTIPLE EFFECTS

Angiotensin II exerts five major effects:

1. AII causes vasoconstriction.
2. AII promptly releases **aldosterone** from the **zona glomerulosa** of the adrenal gland.
3. AII induces the sensation of thirst.

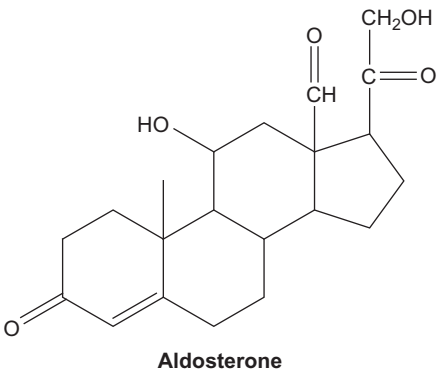
4. AII releases ADH.
5. AII increases reabsorption of Na^+ and HCO_3^- in the proximal tubule.

All of these effects work to increase the extracellular fluid volume and to maintain the integrity of the circulation. The vasoconstriction helps to bring the systemic circulatory pressure back up by increasing total peripheral resistance, TPR. Aldosterone helps reabsorb filtered Na^+. This helps expand, or prevents further loss of, extracellular fluid volume. The sensation of thirst drives us to drink fluids to replenish the ECF volume. Release of ADH helps recapture filtered water, which prevents further loss of ECF. Increasing reabsorption of Na^+ reduces Na^+ loss in the urine, further helping to preserve the ECF volume. These effects of ANG II are mediated by angiotensin receptors (AT_1 and AT_2) that are G-protein-coupled receptors (GPCR) residing on the cell membrane of target cells.

ALDOSTERONE IS A MINERALOCORTICOID THAT INCREASES Na^+ RETENTION AND K^+ EXCRETION

Aldosterone is a steroid hormone produced in the outermost layer of the adrenal gland, the **zona glomerulosa**. Its secretion is controlled predominantly by angiotensin II and plasma $[K^+]$. Increasing plasma $[K^+]$ increases aldosterone secretion. Other stimuli include decreased plasma $[Na^+]$ and **adrenocorticotrophic hormone (ACTH)**. Aldosterone's chemical structure is shown in Figure 7.6.12.

Aldosterone acts according to the classical steroid hormone model in which the hormone binds to cytosolic receptors and is transferred to the nucleus where it binds to nuclear receptors that stimulate the transcription of specific genes. Aldosterone affects Na^+ and water absorption and K^+ secretion in the gut, salivary glands, sweat glands, and kidneys. In the kidneys, aldosterone increases Na^+ reabsorption and increases K^+ secretion in cells in the cortical collecting duct. It increases the activity of the basolateral Na^+,K^+-ATPase, ENaC, and the apical K^+ channel, ROMK (see Figure 7.5.7). Its major effect is to increase ENaC. The mechanism of action of aldosterone is discussed in Chapter 9.5.



Aldosterone

FIGURE 7.6.12 Chemical structure of aldosterone.

ATRIAL NATRIURETIC PEPTIDE AND ENDOGENOUS DIGITALIS-LIKE SUBSTANCE INCREASE Na^+ EXCRETION IN HYPERVOLEMIA

Atrial natriuretic peptide (ANP) originates in the atria and B-type or brain natriuretic peptide (BNP) originates in the ventricles. A third form, CNP, is made in many organs including the brain, kidney, bone, and vasculature. Both ANP and BNP are made as pro-hormones that are converted to their active forms by a membrane-bound protease called corin. Stretch of the atrial wall and other stimuli such as endothelins cause release of atrial natriuretic peptide (ANP) into the blood. Pressure or volume overload that stretches the ventricular wall causes BNP release. Both ANP and BNP bind to natriuretic peptide receptor type A (NPR-A) which is a quanylyl cyclase. ANP decreases TPR and increases renal Na^+ and water excretion. ANP appears to increase Na^+ excretion by inhibiting apical ENaC and basolateral Na, K-ATPase in the medullary collecting duct.

The adrenal cortex synthesizes a steroid hormone that appears to inhibit the Na^+,K^+-ATPase. This substance may be structurally similar to a class of compounds called cardiac glycosides because they increase the contractile force of the heart. The compound **digitalis** was first isolated from the Foxglove plant. Related drugs are **ouabain** and **digoxin**. These compounds all inhibit the Na^+,K^+-ATPase. For this reason, the compound is called endogenous digitalis-like substance or EDLS. The role of EDLS in Na^+ balance is not established.

THE INTEGRATED RESPONSE TO DECREASED BLOOD VOLUME

Figure 7.6.13 illustrates the main events in the response to decreased blood volume. The kidney engages the renin–angiotensin–aldosterone (RAA) system in response to decreased afferent arteriolar pressure, increased renal sympathetic nervous stimulation, and decreased NaCl delivery to the macula densa. Angiotensin II that results from increased renin release causes vasoconstriction, increased aldosterone release, increased ADH release, increased Na^+ reabsorption, and increased thirst. All of these actions tend to raise the ECF volume or to prevent its further loss. The kidneys cannot make new volume. Loss of water or salt through sweat or diarrhea or vomiting or hemorrhage can be reversed only by the consumption of water and electrolytes.

INTEGRATED RESPONSE TO INCREASED Na^+ LOAD OR VOLUME EXPANSION

Excess ingestion of NaCl, followed by its absorption from the gastrointestinal tract, leads to an increased osmolarity of the blood that stimulates thirst and ADH release. Thus there is an increase in water intake and

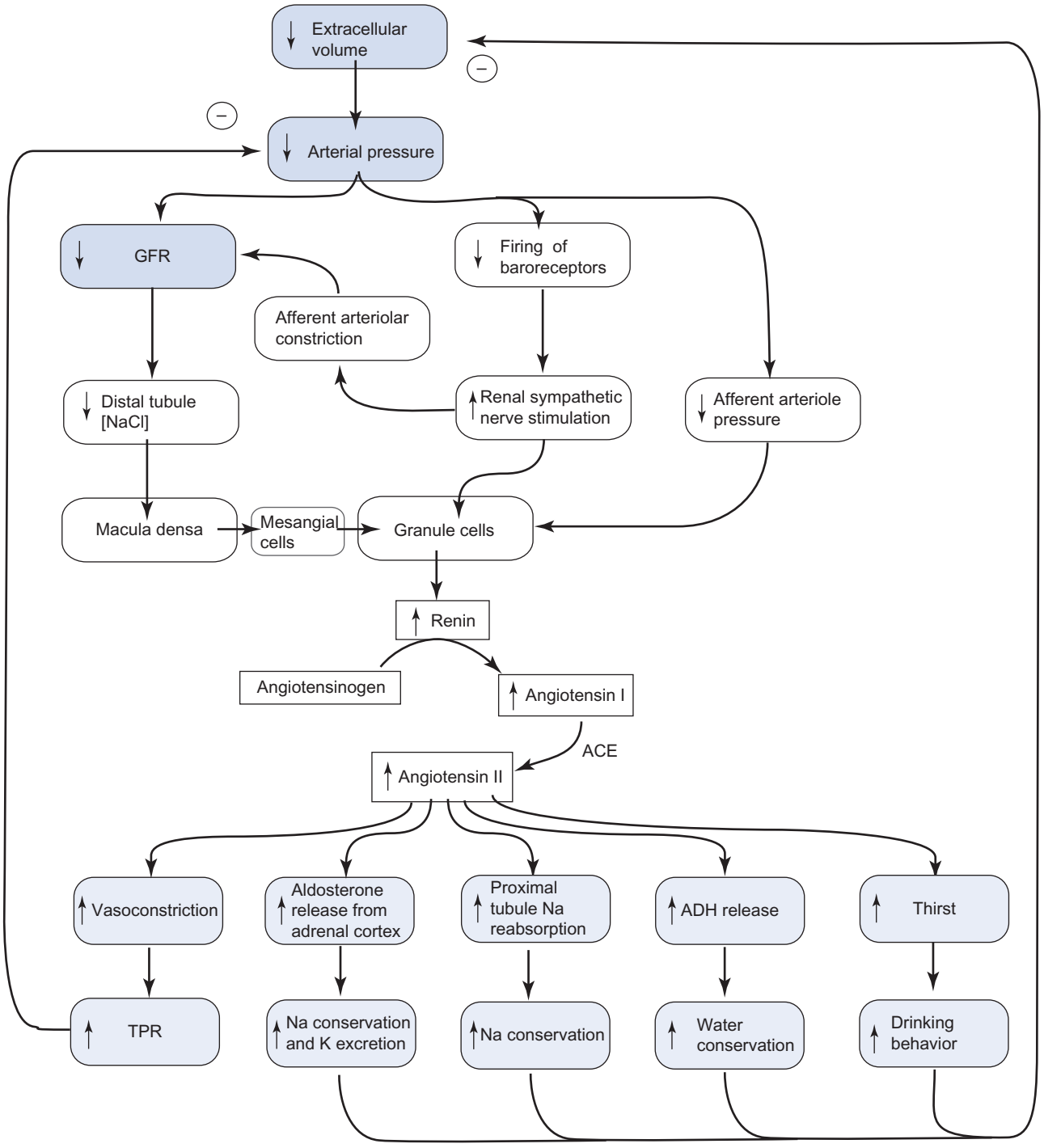


FIGURE 7.6.13 Feedback mechanisms for the response to decrease in blood volume.

conservation until the osmolarity of the blood returns to normal. This can be achieved only by an expansion of the ECF volume, as described in Chapter 7.1. These events occur rapidly and then the body is presented with a normal blood osmolarity but an expanded blood volume. All of the systems shown in Figures 7.6.10 and 7.6.13 are then brought to bear to correct the situation, but this time they all work in reverse. Thus there is a reduction in ADH levels and in aldosterone levels, with the resulting excretion of a more dilute urine, approximately isoosmolar with plasma, so that the ECF volume returns to normal while maintaining normal osmolarity.

SUMMARY

The renal system regulates the volume and osmolarity of the plasma and, by extension, of the ISF and ICF, by excreting either a concentrated urine or a dilute urine. This is controlled principally by ADH, but other factors

are involved including the RAA system, stretch receptors in the left atria and intrathoracic veins, and the thirst mechanism. Increased osmolarity increases ADH release, which results in excretion of a highly concentrated urine. This eliminates salt in excess of water, so that the blood osmolarity returns toward normal. Stretch of the left atria inhibits ADH release, and less stretch during plasma depletion relieves the inhibition, causing increased ADH release. This in itself cannot restore plasma volume, but it helps retain water when it is ingested.

ADH exerts its effects on the kidney through V2 receptors linked to a G_s mechanism. Binding of ADH to V2 receptors increases cytosolic [cAMP] that activates PKA to phosphorylate aquaporin channels (AQP2) that in turn signal the cell to transport the channels to the apical membrane. This increases the water permeability of the late distal tubule and the collecting duct. In the absence of ADH, protein phosphatases gradually dephosphorylate the AQP2 channels and they are removed from the apical membrane and stored in endocytotic vesicles.

The RAA system is activated by: (1) decreased afferent arteriolar pressure; (2) increased renal sympathetic nervous stimulation; and (3) decreased distal tubule [NaCl]. These all signal the granule cells in the afferent arteriole to release renin, an enzyme that breaks down plasma angiotensinogen to angiotensin I, which is further converted to the active angiotensin II by ACE. Angiotensin II has multiple effects, including: (1) vasoconstriction; (2) release of aldosterone from the adrenal cortex; (3) release of ADH; (4) increased thirst; and (5) increased absorption of Na^+ and HCO_3^- from the proximal tubule. All of these actions defend against reduced blood pressure and blood volume. The vasoconstriction helps return blood pressure to normal, aldosterone retains Na^+ by preventing renal loss, ADH minimizes renal water loss, increased thirst motivates replacement of lost fluids, increasing Na^+ reabsorption in the proximal tubule complements actions of aldosterone in the distal nephron.

REVIEW QUESTIONS

1. What effect would increased blood pressure have on the GFR? On the filtration fraction? What effect does autoregulation have on this relationship?
2. What mechanisms are responsible for autoregulation of renal blood flow and GFR? Which operates more quickly?
3. What effect does dehydration have on GFR?
4. What is ADH? Where does it originate? What are the stimuli for its release? What are its renal actions? What are its actions on the vasculature?
5. What is renin? What cells secrete it? What are the stimuli for its release? What does it do?
6. What is angiotensin? How is it produced? What does it do?
7. What is aldosterone? What stimuli release it? What does it do?
8. What are the sensors for hypovolemia? How does hypovolemia get corrected?
9. What are the sensors for hyperosmolarity? How does hyperosmolarity get corrected?