CHAPTER 66

Mechanisms of Diuretic Action

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The term diuretic derives from the Greek diouretikos, which means, "to promote urine."* Even though many substances promote urine flow, the term diuretic is usually taken to indicate a substance that can reduce the extracellular fluid volume by increasing urinary solute and water excretion. In 1553, Paracelsus recorded the first truly effective medical treatment for dropsy (edema), namely inorganic mercury (calomel). Inorganic mercury remained the mainstay of diuretic treatment until the first part of the 20th century. In 1919, the ability of organic mercurial antisyphilitics to affect diuresis was discovered by Vogl, then a medical student. This observation led to the development of effective organic mercurial diuretics that continued to be used through the 1960s. In 1937, the antimicrobial sulfanilamide was found to cause metabolic acidosis. This drug was soon thereafter shown to inhibit the enzyme carbonic anhydrase, which had been discovered in 1932. Pitts then showed that sulfanilamide inhibited sodium (Na) bicarbonate reabsorption in dogs and Schwartz showed that sulfanilamide could induce diuresis when administered to patients with congestive heart failure. Soon, more potent sulfonamide-based carbonic anhydrase inhibitors were developed, but these drugs suffered from side effects and limited potency. Nevertheless, a group at Sharp & Dohme Inc. was stimulated by these developments to explore the possibility that modification of sulfonamide-based drugs could lead to small molecules that enhanced Na excretion with chloride rather than bicarbonate, thus better enhancing depletion of the extracellular fluid, which is composed primarily of sodium chloride (NaCl) and water. The result of this program was the synthesis of chlorothiazide and its marketing in 1957. This drug ushered in the modern era of diuretic therapy and revolutionized the clinical treatment of edema.

The search for more potent classes of diuretics led to the development of ethacrynic acid and furosemide in the United States and Germany, respectively. The safety and efficacy of these drugs led them to replace the organic mercurials as drugs of choice for severe and resistant edema. Spironolactone, marketed in 1961, was developed after the properties and structure of aldosterone had been discovered and steroidal analogues of aldosterone were found to have aldosterone-blocking activity. Triamterene was initially synthesized as a folic acid antagonist, but was found to have diuretic and potassium (K)-sparing activity. The identification of the arginine vasopressin (AVP) receptor subtypes led to the more recent development of vasopressin antagonists, which have recently entered clinical practice. The identification and cloning of natriuretic peptides led to the development of drugs with similar effects.

The availability of safe, effective, and relatively inexpensive diuretic drugs has made it possible to treat edematous disorders and hypertension effectively. Incidentally, driven by clinical drug development, specific ligands that interact with discrete Na and Cl transport proteins in the kidney were developed, permitting these transport proteins to be identified. Subsequently, these ligands were used to clone the Na and Cl transport proteins that mediate the bulk of renal Na and Cl reabsorption. The diuretic-sensitive transport proteins that have been cloned include the sodium hydrogen exchanger (NHE3; gene symbol SLC9A3), the bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2; gene symbol SLC12A1), the thiazide-sensitive Na-Cl cotransporter (NCC; gene symbol SLC12A3), and the epithelial Na channel (ENaC; gene symbols SCNN1A, SCNN1B, SCNN1G). The information derived from molecular cloning has also permitted identification of inherited human diseases that are caused by mutations in these diuretic-sensitive transport proteins. The phenotypes of several of these disorders resemble the manifestations of chronic diuretic administration. Thus, the development of clinically useful diuretics permitted identification and later cloning of specific ion transport pathways. The molecular cloning is now helping to define mechanisms of diuretic action and diuretic side effects. The use of animals in which diuretic-sensitive transport pathways have been "knocked out" permits a clearer understanding of which diuretic effects result directly or secondarily from actions of the drugs on specific ion transport pathways and which effects result from actions on other pathways or other organ systems.

*Many older and historical references have been omitted for brevity. These references can be found in prior editions of this text.

NORMAL RENAL NaCl HANDLING

The normal human kidneys filter approximately 23 moles of NaCl in 150 liters of fluid each day. According to data from NHANES III, typical dietary sodium consumption in the United States is 4.3 g daily for men and 2.9 g daily for women.[1] The sex difference reflects differences in caloric intake, not differential food choices. As 17 mEq of Na is 1 g of table salt and 43 mEq of Na is in 1 g of sodium, men typically consume 100 mEq of Na and women typically consume 67 mEq on a daily basis. To maintain balance, renal NaCl excretion must equal this, ignoring the modest losses in feces and sweat. Under normal circumstances, approximately 99.2% of the filtered NaCl is reabsorbed by kidney tubules generating a normal fractional sodium excretion of <1% (100 mEq in urine per 23,000 mEq filtered = 0.4%). Sodium, chloride, and water reabsorption by the nephron is driven by the metabolic energy provided by ATP. The ouabain-sensitive Na/K ATPase is expressed at the basolateral cell membrane of all Na transporting epithelial cells along the nephron. This pump maintains large ion gradients across the plasma membrane, with the intracellular Na concentration maintained low and the intracellular K concentration maintained high. Because the pump is electrogenic and associates with a K channel in the same membrane, renal epithelial cells have a voltage across the plasma membrane oriented with the inside negative relative to the outside.

The combination of the low intracellular Na concentration and the plasma membrane voltage generates a large electrochemical gradient favoring Na entry from lumen or interstitium. Specific diuretic-sensitive Na transport pathways are expressed at the apical (luminal) surface of cells along the nephron, permitting vectorial transport of Na from lumen to blood. Along the proximal tubule, where approximately 50% to 60% of filtered Na is reabsorbed, an isoform of the Na/H exchanger is expressed at the apical membrane. Along the thick ascending limb, where approximately 20% to 25% of filtered Na is reabsorbed, an isoform of the Na-K-2Cl cotransporter is expressed at the apical membrane. Along the distal convoluted tubule, where approximately 5% of filtered Na is reabsorbed, the thiazide-sensitive Na-Cl cotransporter is expressed. Along the connecting tubule (CNT) and cortical collecting duct (CCD), where approximately 3% of filtered Na is reabsorbed, the amiloride-sensitive epithelial Na channel is expressed. These apical Na transport pathways are the primary targets for diuretic drug action.

This chapter discusses the molecular and physiologic bases for diuretic action in the kidney. Although some aspects of clinical diuretic usage are discussed, physiologic principles and mechanisms of action are emphasized. Several recent texts provide detailed discussions of diuretic treatment of clinical conditions.[2] Extensive discussions of diuretic pharmacokinetics are also available.[3,4]

A rational classification of diuretic drugs (Table 66.1) is based on the primary nephron site of action. Such a scheme emphasizes that different chemical classes of drugs can affect the same ion transport mechanism and exhibit many of the same clinical effects and side effects. Furthermore, although

66.1 Effects of Diuretics on Electrolyte Excretion

	Na	Cl	K	Pi	Ca	Mg
Osmotic diuretics[6,205,388–390]	□(10%–25%)	□(15%–30%)	□(6%)	□(5%–10%)	□(10%–20%)	□(>20%)
Carbonic anhydrase inhibitors[57,98,205]	□(6%)	□(4%)	□(60%)	□(>20%)	□ or □ (<5%)	□(<5%)
Loop diuretics[98,177,205,344,391,392]	□(30%)	□(40%)	□(60%–100%)	□(>20%)	□(>20%)	□(>20%)
DCT diuretics[177,205,391,393]	□(6%–11%)	□(10%)	□(200%)	□(>20%)	□	□(5–10%)
Collecting duct diuretics[177,205,344]	□(1%–5%)	□(6%)	□(8%)	□	□	□

Figures indicate approximate maximal fractional excretions of ions following acute diuretic administration in maximally effective doses. □ indicates that the drug increases excretion; □ indicates that the drug decreases excretion; □ indicates that the drug has little or no direct effect on excretion. During chronic treatment, effects often wane (Na excretion), may increase (K excretion during distal convoluted tubule diuretic treatment), or may reverse as with uric acid (not shown).
Na, sodium; Cl, chloride; K, potassium; Pi, phosphate; Ca, calcium; Mg, magnesium.

most diuretic drugs affect transport processes along several nephron segments, most owe their clinical effects primarily to their ability to inhibit Na transport by one particular nephron segment. An exception is the osmotic diuretics. Although these drugs were initially believed to inhibit solute and water flux primarily along the proximal tubule, subsequent studies have revealed effects in multiple segments. Other diuretics, however, are classified according to their primary site of action.

OSMOTIC DIURETICS

Osmotic diuretics are substances that are freely filtered at the glomerulus, but are poorly reabsorbed (Fig. 66.1). The pharmacologic activity of drugs in this group depends entirely on the osmotic pressure exerted by the drug molecules in solution. It does not depend on interaction with specific transport proteins or enzymes. Mannitol is the prototypical osmotic diuretic.[5] Because the relationship between the magnitude of diuretic effect and concentration of osmotic diuretic in solution is linear, all osmotic diuretics are small molecules. Other agents considered in this class include urea, sorbitol, and glycerol.

Urinary Electrolyte Excretion

Although osmotic agents do not act directly on transport pathways, ion transport is affected. Following mannitol infusion, sodium, potassium, calcium, magnesium, bicarbonate, and chloride excretion rates increase (see Table 66.1). Rates of sodium and water fractional reabsorption are reduced by 27% and 12%, respectively, following the infusion of mannitol.[6] Reabsorption of magnesium and calcium is also reduced in the proximal tubule and loop of Henle. In contrast, phosphate reabsorption is only inhibited slightly by mannitol in the presence of parathyroid hormone.[7]

Mechanism of Action

The functional consequences that result from intravenous infusion of mannitol include an increase in cortical and medullary blood flow; a variable effect on glomerular filtration rate; an increase in sodium, water, calcium, magnesium, phosphorus, and bicarbonate excretion; and a decrease in medullary concentration gradient. The most pronounced effect observed with mannitol is a brisk diuresis and natriuresis. The mechanisms by which mannitol produces a diuresis include: (1) an increase in osmotic pressure in the lumens of the proximal tubule and loop of Henle, thereby retarding the passive reabsorption of water and (2) an increase in renal blood flow and washout of medullary tonicity.

Mannitol is freely filtered at the glomerulus and its presence in tubule fluid minimizes passive water reabsorption. Normally, within the proximal tubule, sodium reabsorption creates an osmotic gradient for water reabsorption. When an osmotic diuretic is administered, however, the osmotic force of the nonreabsorbable solute in the lumen opposes the osmotic force produced by sodium reabsorption. Isosmolality of tubule fluid is preserved because molecules of mannitol replace sodium ions reabsorbed. However, sodium reabsorption eventually stops because the luminal sodium concentration is reduced to a point where a limiting gradient is reached and net transport of sodium and water ceases. The validity of this mechanistic explanation has been confirmed by stationary micropuncture studies. Quantitatively, mannitol has a greater effect on inhibiting Na and water reabsorption in the loop of Henle than in the proximal tubule. Free-flow micropuncture studies following mannitol infusion in dogs demonstrated a modest decrease in fractional reabsorption of sodium and water by the proximal tubule, but a much larger effect by the loop of Henle.[7] Within the loop of Henle, the site of action of mannitol appears to be restricted to the thin descending limb, decreasing water reabsorption.[8] In the thick ascending limb, Na reabsorption continues, in proportion to its delivery to this segment. The sum of net transport in the thin and thick limbs determines the net effect of mannitol in the loop of Henle. Further downstream in the collecting duct, mannitol reduces sodium and water reabsorption.[9]

Renal Hemodynamics

During the administration of mannitol, its molecules diffuse from the bloodstream into the interstitial space. In the interstitial space, the increased osmotic pressure draws water from the cells to increase extracellular fluid volume. This effect increases total renal plasma flow.[9] Cortical and medullary blood flow rates both increase following mannitol infusion.[9] Single nephron glomerular filtration rate (GFR), on the other hand, increases in cortex but decreases in medulla.[8] The mechanisms by which mannitol reduces the GFR of deep nephrons are not known, but it has been postulated that mannitol reduces efferent arteriolar pressure. Micropuncture studies examining the determinants of GFR in superficial nephrons have demonstrated that the increase in single nephron GFR results from an increase in single nephron plasma flow and a decrease in oncotic pressure.[10] The net effect of mannitol on total kidney GFR has been variable, but most studies indicate that the overall effect is to increase GFR.[10]

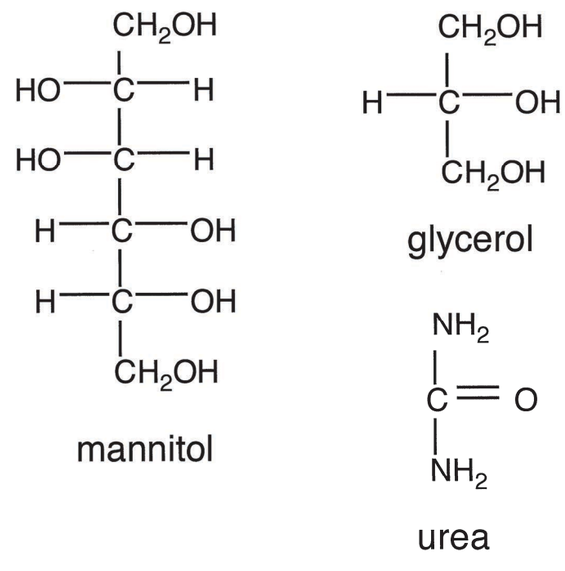


FIGURE 66.1 Structures of osmotic diuretics.

The combination of enhanced renal plasma flow and reduced medullary GFR washes out the medullary osmotic gradient by reducing papillary sodium and urea content. Experimental studies indicate that the osmotic effect of mannitol to increase water movement from intracellular to extracellular space leads to a decrease in hematocrit and in blood viscosity. This fact contributes to a decrease in renal vascular resistance and increase in renal blood flow. In addition, secretion of vasodilatory substances is stimulated by mannitol infusion. Both prostacyclin (PGI_2)[11] and atrial natriuretic peptide[12] could mediate the effect of mannitol on renal blood flow. The vasodilatory effect of mannitol is reduced when the recipient is pretreated with indomethacin or meclofenamate, suggesting that PGI_2 is involved in the vasodilatory effect. Alterations in renal hemodynamics contribute to the diuresis observed following administration of mannitol. An increase in medullary blood flow rate reduces medullary tonicity primarily by decreasing papillary sodium and urea content[13] and increasing urine flow rate.[14]

Pharmacokinetics

Mannitol is not readily absorbed from the intestine[5]; therefore, it is routinely administered intravenously. Following infusion, mannitol distributes in extracellular fluid with a volume of distribution of approximately 16 liters[15]; its excretion is almost entirely by glomerular filtration.[16] Of the filtered load, less than 10% is reabsorbed by the renal tubule, and a similar quantity is metabolized, probably in the liver. With normal glomerular filtration rate, plasma half-life is approximately 2.2 hours.

Clinical Use

Mannitol is used prophylactically to prevent acute kidney injury.[17] In the past, it was administered to patients with established acute kidney injury, but it has proven ineffective in this situation. Mannitol improves renal hemodynamics in a variety of situations of impending or incipient acute kidney injury. Mannitol (along with hydration and sodium bicarbonate) has been recommended by some,[18,19] but not all[20] investigators for the early treatment in myoglobinuric acute kidney injury and to prevent posttransplant acute kidney injury.[21] Mannitol is frequently used perioperatively to treat patients undergoing cardiopulmonary bypass surgery. The beneficial effects may relate to its osmotic activity thereby reducing intravenous fluid requirement[22] and its ability to act as a free radical antioxidant.[23] Although some studies have shown a beneficial effect when used prophylactically to treat patients at risk for contrast nephropathy,[24] most prospective controlled studies have not found mannitol beneficial in preventing acute kidney injury and it is not currently recommended.[25,26]

Mannitol is used for short-term reduction of intraocular pressure.[27] By increasing the osmotic pressure, mannitol reduces the volume of aqueous humor and the intraocular pressure by extracting water. Mannitol also decreases cerebral edema and the increase in intracranial pressure associated with trauma, tumors, and neurosurgical procedures,[22,23,28] although hypertonic saline appears more effective for this purpose.[29]

Mannitol and other osmotic agents have been used to treat dialysis disequilibrium.[30,31] This syndrome is characterized by acute symptoms during or immediately following hemodialysis, and is especially common when dialysis is first initiated. Most significant symptoms are attributable to disorders of the central nervous system such as headache, nausea, blurred vision, confusion, seizure, coma, and death. Rapid removal of small solutes such as urea during dialysis of patients who are markedly azotemic is associated with the development of an osmotic gradient for water movement into brain cells producing cerebral edema and neurologic dysfunction. Dialysis disequilibrium syndrome can be minimized by slow solute removal, using low blood flow and short treatment times; raising plasma osmolality with saline or mannitol can also be employed.

Adverse Effects

Patients who have a reduced cardiac output may develop pulmonary edema when mannitol is infused. Intravenous mannitol administration increases cardiac output and pulmonary capillary wedge pressures.[15] Acute and prolonged administration of mannitol leads to different electrolyte disturbances. Acute overzealous use or the accumulation of mannitol leads to dilutional metabolic acidosis and hypertonic hyponatremia (as mannitol shifts sodium-free water from cells to the extracellular space). Accumulation of mannitol also produces hyperkalemia[32] as a result of the same osmotic forces. An increase in plasma osmolality increases potassium movement from intracellular to extracellular fluid from bulk solute flow and increase in the electrochemical gradient for potassium secretion. Prolonged administration of mannitol generates urinary losses of sodium and potassium potentially leading to volume depletion, hypernatremia (as urinary loss of sodium is invariably less than water), and hypokalemia.[33] Although mannitol is sometimes used to induce hypernatremia to reduce intracranial pressure, studies have shown that the adverse effects of hypernatremia outweigh the benefits of reduced intracranial pressure, especially when serum sodium exceeds 160 mmol/l.[34]

Marked accumulation of mannitol in patients can lead to reversible acute kidney injury that appears to be caused by vasoconstriction and tubular vacuolization.[35,36] Mannitol-induced acute kidney injury usually occurs when large cumulative doses of ~295 g are given to patients with previously compromised renal function.[35]

PROXIMAL TUBULE DIURETICS (CARBONIC ANHYDRASE INHIBITORS)

Through the development of carbonic anhydrase inhibitors, important compounds were discovered that have utility as therapeutic agents and as research tools. Carbonic anhydrase

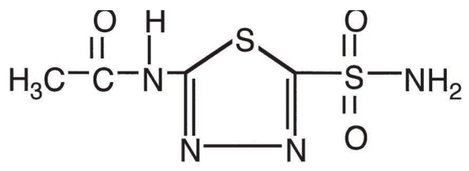


FIGURE 66.2 Structure of a carbonic anhydrase inhibitor.

inhibitors have a limited therapeutic role as diuretic agents, however, because they are only weakly natriuretic when employed chronically. They are used primarily to reduce intraocular pressure in glaucoma, to enhance bicarbonate excretion in metabolic alkalosis or chronic hypercapnia, and to prevent mountain sickness. Structures of carbonic anhydrase inhibitors are shown in Figure 66.2.

Urinary Electrolyte Excretion

Through their effects on carbonic anhydrase in the proximal tubule, carbonic anhydrase inhibitors increase bicarbonate excretion by 25% to 30% (see Table 66.1). The increase in sodium and chloride excretion is smaller than might be expected, because these ions are reabsorbed by more distal segments of the nephron.[37] However, a residual small but variable amount of sodium is excreted along with bicarbonate (Table 66.1). Calcium and phosphate reabsorption are also inhibited along the proximal tubule by carbonic anhydrase inhibitors. Because distal calcium reabsorption is stimulated by increased distal delivery, fractional calcium excretion does not increase. In contrast, phosphate appears to escape distal reabsorption resulting in an increase in fractional excretion of phosphate by ~3%. Although proximal tubule magnesium transport is inhibited by carbonic anhydrase inhibitors, fractional excretion of magnesium is either unchanged or increased as a result of variable distal reabsorption.[38]

Carbonic anhydrase inhibitors increase potassium excretion. It is likely that several indirect effects contribute to the observed kaliuresis. Carbonic anhydrase inhibition could block proximal tubule potassium reabsorption and increase delivery to the distal tubule, but this has not been established clearly. Whereas carbonic anhydrase inhibitors decrease proximal tubule sodium, bicarbonate, and water absorption during both free flow micropuncture and microperfusion, the effects of carbonic anhydrase inhibitors on proximal tubule potassium transport have been less consistent. In free flow micropuncture studies, carbonic anhydrase inhibition did not affect proximal tubule potassium reabsorption,[39] whereas it did reduce net reabsorption by microperfused proximal tubules.[40] The effect of carbonic anhydrase inhibitors on the proximal tubule ion transport does, however, facilitate an increase in tubular fluid flow rate and sodium and bicarbonate but not chloride delivery to the distal nephron. This effect is thought to increase the concentration of bicarbonate in the distal tubule lumen, which increases lumen negative voltage[41] and increases flow rate,[42] factors known to increase potassium secretion by the distal tubule. Carbonic anhydrase inhibitors can also produce a luminal composition that is low in chloride and high in bicarbonate. This luminal fluid composition has been demonstrated to stimulate potassium secretion by the distal nephron independent of a change in lumen negative voltage.[43]

Mechanism of Action

In the kidney, carbonic anhydrase inhibitors act primarily on proximal tubule cells to inhibit bicarbonate absorption.[44] Carbonic anhydrase, a metalloenzyme containing one zinc atom per molecule, is important in sodium bicarbonate reabsorption and hydrogen ion secretion by renal epithelial cells. The biochemical, morphologic, and functional properties of carbonic anhydrase have been reviewed.[45] Carbonic anhydrase isoforms (CA) can be categorized into four groups: (1) cytosolic, I, II, III, VII; (2) mitochondrial, V; (3) membrane associated IV, IX, XII, XIV; and (4) secreted, VI.[45] Carbonic anhydrases regulate cellular H ion secretion through catalyzing the formation of HCO_3 from OH and CO_2 and by binding to transporters and directly regulating activity. There are three major renal carbonic anhydrases. Type II carbonic anhydrase (CAII) is distributed widely comprising more than 95% of the overall activity in kidney and is sensitive to inhibition by sulfonamides. CAII is expressed in the cytoplasm and facilitates the secretion of H ions by catalyzing the formation of HCO_3 from OH and CO_2 (see equation 66.3). In addition, CAII binds to the C-terminal region of NHE1 and likely regulates the transport efficiency of Na/H exchange. CAIV is bound to renal cortical membranes, comprising up to 5% of the remaining overall activity in rodent kidney, and is also sensitive to sulfonamides. Carbonic anhydrase activity at basolateral and luminal plasma membranes of proximal tubule cells and luminal membrane of intercalated cells catalyzes the dehydration of intraluminal carbonic acid generated from secreted protons. The carbonic anhydrase activity at the basolateral and luminal plasma membranes of proximal tubule cells is thought to be due in part to CAIV.[46] CAIV has been shown to also bind to the extracellular loop of $NaHCO_3$ transporter 1 (NBC1) regulating its transport activity.[47] Evidence for the physiologic importance for carbonic anhydrase is apparent as a deficiency of CAII leads to a renal acidification defect resulting in renal tubular acidosis. Furthermore, metabolic acidosis leads to an adaptive increase in both CAII and IV carbonic anhydrase mRNA expression in kidney[48] suggesting the importance of both carbonic anhydrase isoforms in this disorder. CAXII is also expressed in proximal tubules and collecting ducts and may contribute to the carbonic anhydrase activity in these segments.[49–51]

Normally the proximal tubule reabsorbs 80% of the filtered load of sodium bicarbonate and 60% of the filtered load of sodium chloride. Early studies by Pitts and micropuncture studies by DuBose and others indicated that hydrogen ion secretion is responsible for bicarbonate absorption and renal acidification. The cellular mechanism by which proximal

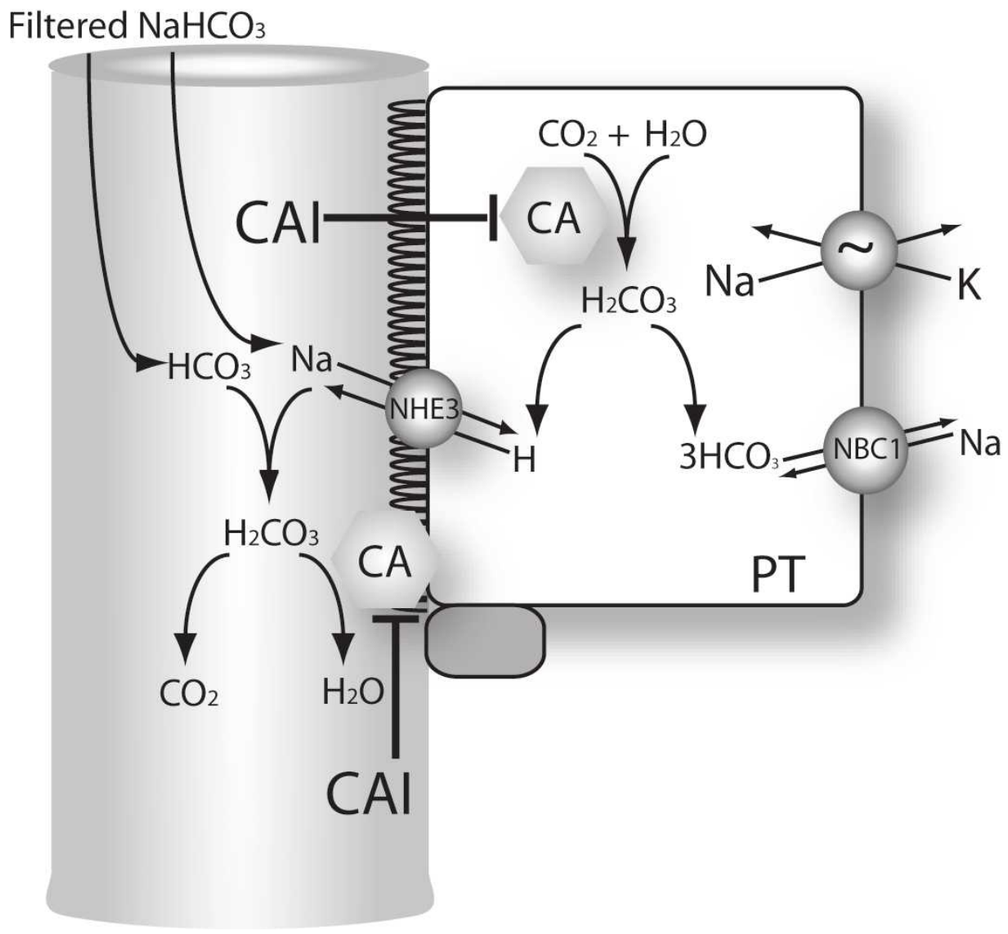


FIGURE 66.3 Mechanisms of diuretic action in the proximal tubule. The figure shows functional model of proximal tubule (PT) cells. Many transport proteins are omitted from the model, for clarity. Carbonic anhydrase (CA) catalyzes inside the cell the formation of HCO_3 from H_2O and CO_2. This is the result of the two-step process (please see equations in the text for additional details). Bicarbonate leaves the cell via the Na, HCO_3, cotransporter.[186,187] A second pool of carbonic anhydrase is located in the brush border (CA). This participates in disposing of carbonic acid, formed from filtered bicarbonate and secreted H^+. Both pools of carbonic anhydrase are inhibited by acetazolamide and other carbonic anhydrase inhibitors (see text for details).

tubules reabsorb bicarbonate is depicted in Figure 66.3. The effect of carbonic anhydrase to accelerate bicarbonate is a result of the reactions that occur in both luminal fluid and in the cell. The mechanism of carbonic anhydrase action in luminal fluid,[52] is shown here, where E represents the carbonic anhydrase enzyme:

Luminal Fluid

$$EH_2O + HCO_3^- \rightleftharpoons H_2O + CO_2 + EOH \quad (1)$$

$$EOH + H^+ \rightleftharpoons EH_2O \quad (2)$$

$$HCO_3^- + H^+ \rightleftharpoons CO_2 + H_2O \quad (3)$$

Note that the addition of reactions 1 and 2 leads to the classic reaction 3. In this scheme, the enzyme is viewed as a superhydroxylator.

Luminal carbonic anhydrase prevents H from accumulating in tubule fluid, which would eventually stop all Na/H exchange. Once formed, carbon dioxide diffuses rapidly from the lumen into the cell across the apical membrane.

The mechanism by which intracellular carbonic anhydrase participates in net H^+ secretion is functionally the reverse of the reactions shown previously.

Intracellular Fluid

$$EH_2O \rightleftharpoons EOH + H^+ \quad (2R)$$

$$EOH + H_2O + CO_2 \rightleftharpoons EH_2O + HCO_3^- \quad (1R)$$

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \quad (3R)$$

In this case, the enzyme splits water, thereby providing an hydroxyl ion to form bicarbonate. The bicarbonate ions then exit the basolateral membrane via $Na(HCO_3)_3$ cotransport.[53] Thus, in the early proximal tubule, the net effect of the process described results in the isosmotic reabsorption of $NaHCO_3$. The lumen chloride concentration increases because water continues to be reabsorbed, thereby producing a lumen positive potential. These axial changes provide an electrochemical gradient for transport of chloride, via paracellular and transcellular pathways. The latter pathway for chloride likely involves an exchange of Cl with anions, including oxalate and formate, operating in parallel with a Na/H proton exchanger. The dual operation of these parallel exchangers results in net NaCl absorption.[54]

Carbonic anhydrase inhibitors act primarily on proximal tubule cells, where approximately 60% of the filtered load of sodium chloride is reabsorbed. Despite the magnitude of sodium chloride reabsorption in the proximal tubule segment, the natriuretic potency of carbonic anhydrase inhibitors is relatively weak. Several factors explain this observation. First, proximal sodium reabsorption is mediated by carbonic anhydrase-independent as well as carbonic anhydrase-dependent pathways. Second, the increased sodium delivery to distal nephron segments is largely reabsorbed by these distal nephron segments. Third, carbonic anhydrase inhibitors generate a hyperchloremic metabolic acidosis further reducing the effects of subsequent doses of carbonic anhydrase inhibitor. Metabolic acidosis also produces resistance to carbonic anhydrase action. Following the induction of metabolic acidosis, the K_i for bicarbonate absorption by membrane impermeant carbonic anhydrase inhibitors was increased by a factor of 100 to 500, suggesting that metabolic acidosis is associated with changes in the physical properties of the carbonic anhydrase protein.[55] For these reasons, carbonic anhydrase inhibitors alone are rarely used as diuretic agents.

Following carbonic anhydrase inhibitor administration, proximal tubule bicarbonate reabsorption declines between 35% and 85%. Additional sites of action of carbonic anhydrase inhibitors include proximal straight tubule or loop of Henle, distal tubule, and the collecting and papillary collecting ducts. Yet, despite the effect of carbonic anhydrase inhibitors on proximal tubules as well as other nephron segments, compensatory reabsorption of bicarbonate at other downstream

tubular sites limits net fractional excretion of bicarbonate to ~25% to 30%, even during acute administration.[56,57]

The relative contributions of membrane-bound and intracellular components of cellular carbonic anhydrase have been examined. Both species contribute to bicarbonate absorption. The role of membrane-bound carbonic anhydrase was addressed in studies that employed carbonic anhydrase inhibitors with different abilities to penetrate proximal tubule cell membranes. Benzolamide is charged at normal pH and does not penetrate cell membranes well, whereas acetazolamide enters the cell relatively easily.[58] Proximal tubular perfusion of benzolamide inhibits bicarbonate reabsorption by 90%[59] indicating that luminal carbonic anhydrase inhibition contributes importantly to bicarbonate absorption. Inhibition of luminal carbonic anhydrase causes lumen pH to decrease because of the continued secretion of hydrogen ions into the tubule lumen.[59] The conclusion that tubular fluid is in direct contact with membrane carbonic anhydrase was substantiated by the use of dextran-bound carbonic anhydrase inhibitors.[60,61] In proximal tubules perfused in vivo, Lucci et al. determined that dextran-bound inhibitors, which inhibit only luminal carbonic anhydrase, decreased proximal tubule bicarbonate absorption by approximately 80% and reduced lumen pH.[61]

Although these studies establish the importance of luminal carbonic anhydrase, they also support an important role for intracellular and basolateral carbonic anhydrase. Both acetazolamide and benzolamide inhibit proximal tubule bicarbonate reabsorption to a similar degree yet they produce opposite effects on tubule fluid pH, suggesting that intracellular carbonic anhydrase contributes to proximal tubule luminal acidification. Furthermore, inherited deficiency of the predominant renal carbonic anhydrase, CAII, causes proximal renal tubular acidosis.[62]

The expression of carbonic anhydrase in the basolateral membrane of proximal tubule cells suggests that this membrane-bound enzyme also has an important role in basolateral bicarbonate transport. Although it is well known that carbonic anhydrase inhibitors inhibit intracellular generation of substrate for the transporter,[63,64] the direct interaction between CAIV and NBC1, the sodium/bicarbonate cotransporter,[47] suggests the possibility that carbonic anhydrase inhibitors may also directly regulate anion transport activity. Functional studies using an impermeant carbonic anhydrase inhibitor, p-fluorobenzyl-aminobenzamide, that is 1% as permeable as acetazolamide, demonstrated the importance of basolateral membrane-bound carbonic anhydrase. p-Fluorobenzyl-aminobenzamide reduced fluid and bicarbonate absorption when applied to the basolateral membrane of rabbit proximal tubules perfused in vitro.[50]

In the collecting duct, carbonic anhydrase facilitates acid secretion that is mediated by a vacuolar H adenosine triphosphatase (H-ATPase)[65] and a P-type gastric H-K-ATPase.[66–68] Luminal administration of acetazolamide produced an acid disequilibrium pH in the outer medullary collecting duct suggesting the contribution of luminal carbonic anhydrase.[69] Using a membrane-impermeant carbonic anhydrase inhibitor (F-3500; aminobenzamide coupled to a nontoxic polymer polyoxyethylene), bicarbonate absorption was reduced confirming the presence of carbonic anhydrase in the luminal membrane of the outer medullary collecting duct.[55] The K_i for inhibition of bicarbonate absorption was 5 μM, consistent with the inhibition of CAIV.

Renal Hemodynamics

Inhibition of carbonic anhydrase decreases GFR acutely. Systemic acetazolamide infusion decreased GFR by 30%. Single nephron glomerular filtration rate (SNGFR) was 23% lower during acetazolamide infusion partly because increased solute delivery to the macula densa activates the tubuloglomerular feedback (TGF) mechanism, which reduces GFR. Similar results were observed following infusion of benzolamide.[70] Nevertheless, the effects of carbonic anhydrase inhibitors to reduce GFR are not simply the result of TGF activation. Sarala[8-] angiotensin I, an angiotensin II antagonist, prevented the decrease in SGNFR suggesting the involvement of local angiotensin II in response to benzolamide.[70] Further, infusion of benzolamide into targeted adenosine-1 receptor knockout mice (i.e., mice that lack a TGF response) reduced GFR by 21%.[71] Taken together, these results suggest complex mechanisms by which carbonic anhydrase inhibitors reduce GFR.

Pharmacokinetics

Acetazolamide is well absorbed from the gastrointestinal (GI) tract. More than 90% of the drug is plasma protein bound. The highest concentrations are found in tissues that contain large amounts of carbonic anhydrase (e.g., renal cortex, red blood cells). Renal effects are noticeable within 30 minutes and are usually maximal at 2 hours. Acetazolamide is not metabolized but is excreted rapidly by glomerular filtration and proximal tubular secretion. The half-life is approximately 5 hours and renal excretion is essentially complete in 24 hours.[16] In comparison, methazolamide is absorbed more slowly from the GI tract, and its duration of action is long, with a half-life of approximately 14 hours.

Adverse Effects

Generally, carbonic anhydrase inhibitors are well tolerated with infrequent serious adverse effects. Side effects of carbonic anhydrase inhibitors may arise from the continued excretion of electrolytes. Significant hypokalemia and metabolic acidosis may develop. In elderly patients with glaucoma treated with acetazolamide (250 to 1,000 mg per day), metabolic acidosis was a frequent finding, in comparison to a control group.[72] Acetazolamide is also associated with nephrocalcinosis and nephrolithiasis due to its effects on urine pH, facilitating stone formation. Premature infants treated with furosemide and acetazolamide are particularly susceptible to nephrocalcinosis, presumably due to the combined effect of

an alkaline urine and hypercalciuria.[73] Other adverse effects include drowsiness, fatigue, central nervous system depression, and paresthesias. Bone marrow suppression has been reported.[74,75]

Clinical Use

As noted, these drugs are almost never used as first-line diuretics because of the availability of much more potent drugs. Daily use produces systemic acidemia from an increase in urinary excretion of bicarbonate. Nevertheless, acetazolamide can be administered for short-term therapy, usually in combination with other diuretics, to patients who are resistant or who do not respond adequately to other agents.[76] The rationale for using a combination of diuretic agents is based on summation of their effect at different sites along the nephron.

The major indication for the use of acetazolamide as a diuretic agent is in the treatment of patients with metabolic alkalosis accompanying edema[77,78] or the treatment of chronic respiratory acidosis in chronic obstructive lung disease.[79,80] In patients with cirrhosis, congestive heart failure, or nephrotic syndrome, aggressive diuresis with loop diuretics promotes intravascular volume depletion and secondary hyperaldosteronism, conditions that promote metabolic alkalosis. Administration of sodium chloride to correct the metabolic alkalosis simply exacerbates the edema. Acetazolamide can improve metabolic alkalosis by decreasing proximal tubule bicarbonate reabsorption thereby increasing the fractional excretion of bicarbonate. An increase in urinary pH (>7.0) indicates enhanced bicarbonaturia. However, it should be noted that potassium depletion should be corrected prior to acetazolamide use because acetazolamide will also increase potassium excretion. The time course of the acetazolamide effect is rapid. In critically ill patients on ventilators, following the correction of fluid and electrolyte disturbances, intravenous acetazolamide produced an initial effect within 2 hours and a maximum effect in 15 hours.[81]

Acetazolamide is used effectively to treat chronic open-angle glaucoma. The high bicarbonate concentration in aqueous humor is carbonic anhydrase dependent and oral carbonic anhydrase inhibition can be used to reduce aqueous humor formation. Topical formulations of carbonic anhydrase inhibitors were 82, and these drugs are now available to treat glaucoma.

Acute mountain sickness usually occurs in climbers within the 12 to 72 hours of ascending to high altitudes. Symptoms include headache, nausea, dizziness, and breathlessness. Carbonic anhydrase inhibitors improve symptoms and arterial oxygenation.[83]

The administration of acetazolamide has been used in the treatment of familial hypokalemic periodic paralysis,[84,85] a disorder characterized by intermittent episodes of muscle weakness and flaccid paralysis. Its efficacy may be related to a decrease in influx of potassium as a result of a decrease in plasma insulin and glucose[86] or to metabolic acidosis. Carbonic anhydrase inhibitors can also be used as an adjunct treatment of epilepsy,[87] pseudotumor cerebri,[88] and central sleep apnea.[89]

By increasing urinary pH, acetazolamide has been used effectively in certain clinical conditions. Acetazolamide is used to treat cystine and uric acid stones by increasing their solubility in urine, although urinary alkalinization is no longer recommended for prevention of tumor lysis syndrome.[90] Acetazolamide in combination with sodium bicarbonate infusion has been used to treat salicylate toxicity, but acetazolamide is now considered to be contraindicated in this situation. Other indications for CAIs are experimental but emerging and include possible application of CAIs in conditions as diverse as obesity, cancer, and infection.[91]

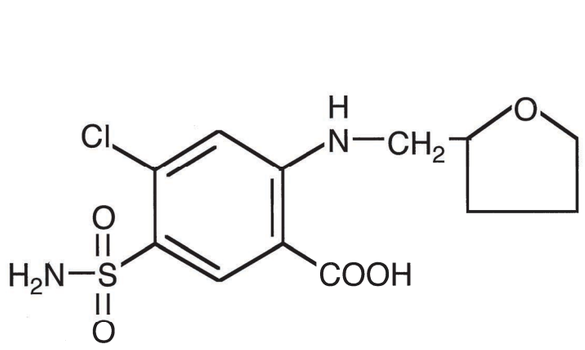
LOOP DIURETICS

The loop diuretics inhibit sodium and chloride transport along the loop of Henle and macula densa. Although these drugs also impair ion transport by proximal and distal tubules under some conditions, these effects probably contribute little to their action clinically. The loop diuretics available in the United States include furosemide, bumetanide, torsemide, and ethacrynic acid (Fig. 66.4).

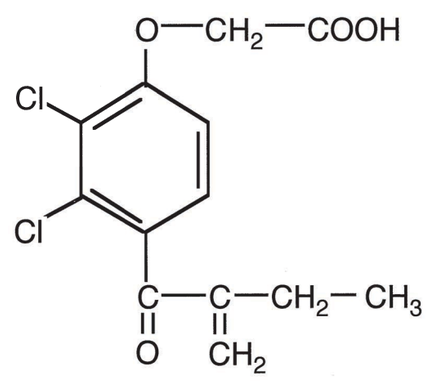
Loop diuretics are organic anions. Studies that utilized radiolabeled bumetanide suggest that loop diuretics bind to one of the chloride (anion) sites on the transporter.[92] According to this model, the loop diuretic would bind because of its negative charge (and its shape) and then inhibit the transport of ions because it is not transported. Studies utilizing chimeric cloned proteins, comprising portions of different members of the cation chloride cotransporters, however, have indicated that diuretic binding and ion affinities are properties of the central hydrophobic domain of the proteins.[93] Isenring and colleagues[94,95] found that transmembrane domains 2 to 6 and 10 to 12 play roles in defining loop diuretic affinity, whereas chloride affinity is regulated by transmembrane domains 4 and 7.[95] This suggests that loop diuretics do not simply bind to one of the chloride sites on the transporter. The results of the chimeric studies, however, have been complex and it would appear that interactions between various transmembrane domains might reconcile the apparent differences in results. Recent models, based on crystallization of related proteins, may provide more definitive information about these results.[96]

Urinary Electrolyte and Water Excretion

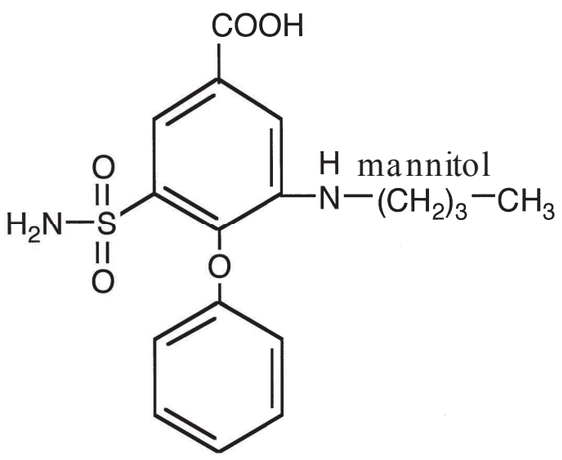
Loop diuretics increase the excretion of water, Na, K, Cl, phosphate, magnesium, and calcium (see Table 66.1). The dose-response relationship between loop diuretic and urinary Na and Cl excretion is sigmoidal (Fig. 66.5). The steep dose response relation has led many to refer to loop diuretics as "threshold" drugs.[3] Loop diuretics have the highest natriuretic and chloruretic potency of any class of diuretics; they are sometimes called "high ceiling" diuretics for this reason. Loop diuretics can increase Na and Cl excretion up to 25% of the filtered load. If administered during water loading, solute-free water clearance (C_{H_2O}) decreases and osmolar clearance increases, although the urine always remains dilute. This effect



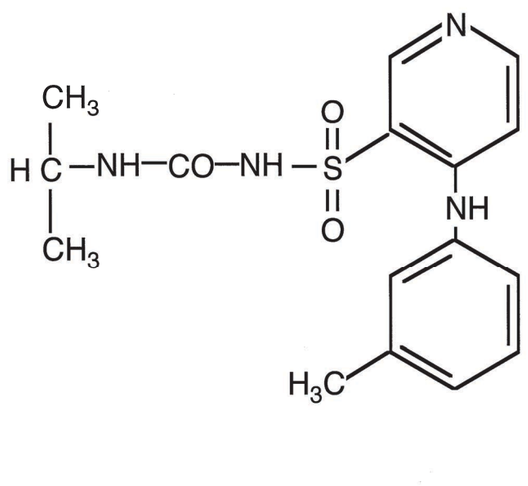
furosemide



ethacrynic acid



bumetanide



torsemide

FIGURE 66.4 Structures of loop diuretics.

contrasts with that of osmotic diuretics which increase osmolar clearance and C_{H_2O}.[97] During hydropenia, loop diuretics impair the reabsorption of solute-free water ($T^C_{H_2O}$). During maximal loop diuretic action, the urinary Na concentration is usually between 75 to 100 mM.[98] Because urinary K concentrations during furosemide-induced natriuresis remain low, this means that the clearance of electrolyte free water (C_{H_2Oe}) is increased when loop diuretics are administered during conditions of water diuresis or hydropenia.[98] This effect of loop diuretics has been exploited to treat hyponatremia, when combined with normal or hypertonic saline.[99,100]

Mechanisms of Action

Sodium and Chloride Transport

The predominant effect of loop diuretic drugs is to inhibit the electroneutral Na-K-2Cl cotransporter at the apical surface of thick ascending limb cells. The loop of Henle, defined as the region between the last surface proximal segment and the first surface distal segment, reabsorbs from 20% to 50% of the filtered Na and Cl load[101]; approximately 10% to 20% is reabsorbed by thick ascending limb cells. The model in Figure 66.6 shows key components of Na, K, and Cl transport pathways in a thick ascending limb cell. As in other nephron segments, the Na/K ATPase at the basolateral cell membrane maintains the intracellular Na concentration low (approximately 10-fold lower than interstitial) and the K concentration high (approximately 20-fold higher than interstitial). Potassium channel(s)[102] in the basolateral cell membrane permit K to diffuse out of the cell, rendering the cell membrane voltage oriented with the intracellular surface negative, relative to extracellular fluid. A chloride channel in the basolateral cell membrane permits Cl to exit the cell.[102] Together with the apical K channel, described below, this chloride channel generates a transepithelial voltage, oriented in the lumen-positive direction.

The transporter inhibited by loop diuretics is a member of the cation chloride cotransporter family.[93,103] This protein—referred to as the bumetanide-sensitive cotransporter, first isoform (BSC-1), or as the Na-K-2Cl cotransporter, second isoform (NKCC2)—is encoded by the gene SLC12A1. It apparently comprises 12 membrane-spanning domains, exists as a dimer,[96] and is expressed at the apical membrane of the thick ascending limb[104] and macula densa (MD) cells.[105,106] A K channel (ROMK) is also present in the same membrane, permitting potassium to recycle from the cell to the lumen.[107] Greger et al. showed that the asymmetrical orientation of channels (apical versus basolateral) and the action of the Na/K ATPase and Na-K-2Cl cotransporter combine to create a transepithelial voltage that is oriented with the lumen positive, with respect to the interstitium.[108] This lumen-positive potential drives absorption of Na^+, Ca^{2+}, and Mg^{2+} via the paracellular pathway. The paracellular component of Na reabsorption comprises 50% of the total transepithelial Na transport by thick ascending limb cells.[109] It should be noted, however, that both the transcellular and the paracellular components of Na transport are inhibited by loop diuretics, the former directly and the latter indirectly. The thick ascending limb is virtually impermeable to water. The combination of solute absorption and water impermeability determines the role of the thick ascending limb as the primary diluting segment of the kidney.

Although direct inhibition of ion transport is the most important natriuretic action of loop diuretics, other actions may contribute. Thick ascending limb cells have been shown to produce prostaglandin E_2 following stimulation with furosemide,[110]

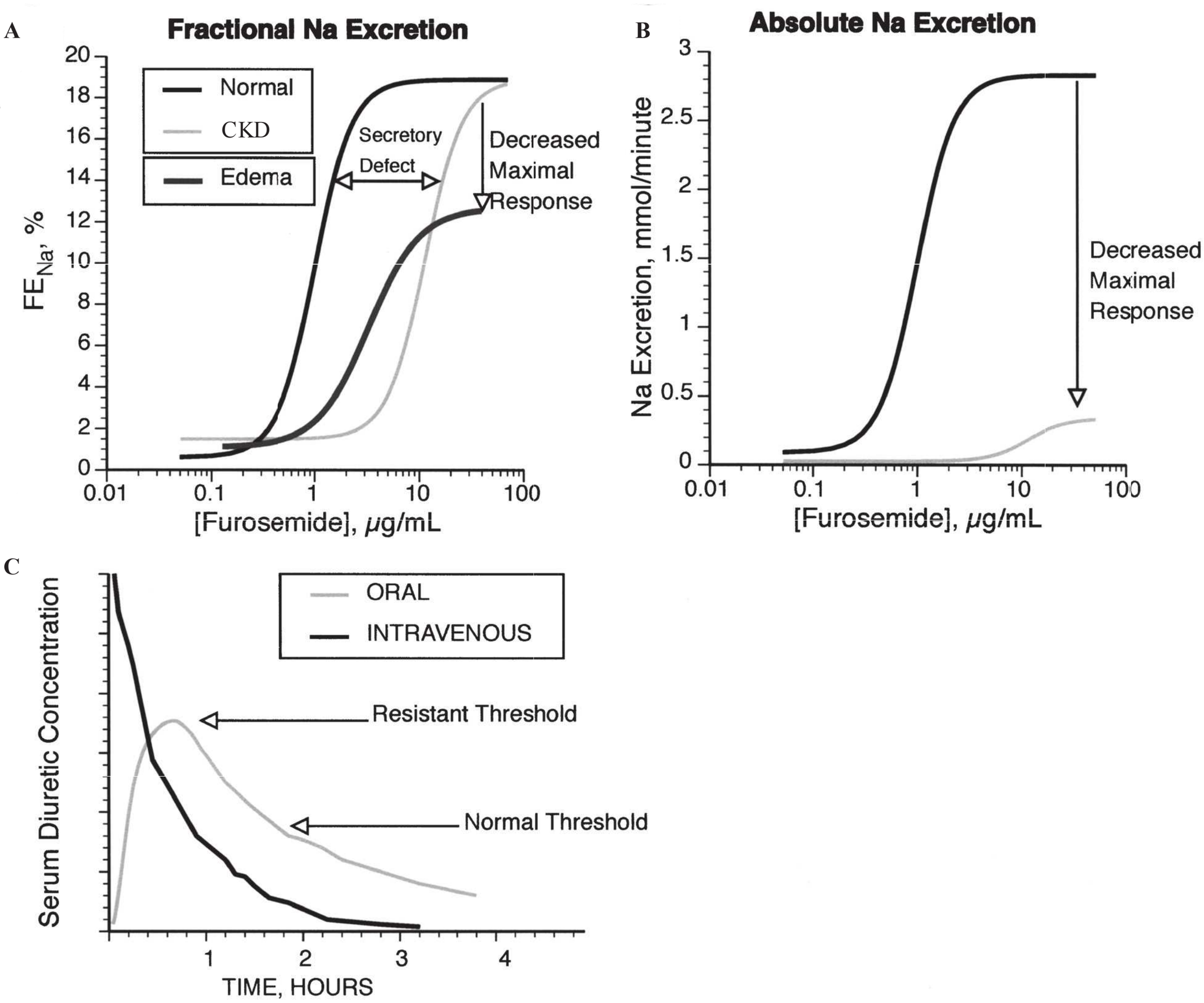


FIGURE 66.5 Dose response curve for loop diuretics. **A:** The fractional Na excretion (FE_{Na}) as a function of loop diuretic concentration. Compared with normal patients, patients with chronic renal failure (CKD) show a rightward shift in the curve, owing to impaired diuretic secretion. The maximal response is preserved when expressed as FE_{Na}, but when expressed as absolute Na excretion **(B)**, maximal natriuresis is reduced in patients with CKD. Patients with edema demonstrate a rightward and downward shift, even when expressed as FE_{Na} **(A)**. **C:** Compares the response to intravenous and oral doses of loop diuretics. In a normal individual (Normal), an oral dose may be as effective as an intravenous dose because the time above the natriuretic threshold (indicated by the *normal* line) is approximately equal. If the natriuretic threshold increases (as indicated by the *dashed line*, from an edematous patient), then the oral dose may not provide a high enough serum level to elicit natriuresis.

perhaps via inhibition of prostaglandin dehydrogenase.[111,112] Blockade of cyclooxygenase reduces the effects of furosemide to inhibit loop segment chloride transport in rats,[113,114] and this effect appears to be important clinically because nonsteroidal anti-inflammatory drugs (NSAIDs) are common causes of diuretic resistance (see below). Increases in renal prostaglandins may also contribute to the hemodynamic effects of loop diuretics, described later.

Calcium and Magnesium Transport

Loop diuretics increase the excretion of the divalent cations calcium (Ca) and magnesium (Mg). Although a component of magnesium and calcium absorption by thick ascending limbs may be active (especially when circulating parathyroid hormone levels are high[115]), a large component of their absorption is passive and paracellular, driven by the transepithelial voltage. As described above, active NaCl transport by thick ascending limb cells leads to a transepithelial voltage, oriented in the lumen positive direction. The paracellular pathway in the thick ascending limb expresses claudin-16 (paracellin-1) and claudin-19, which interact to form a tight junction that mediates both magnesium and calcium movement.[116,117] Mutations in these genes lead to the clinical syndrome familial hypomagnesemia and hypercalciuria (FHHNC), an autosomal recessive tubular disorder that is frequently associated with renal failure.[118] The positive voltage in the lumen, relative to

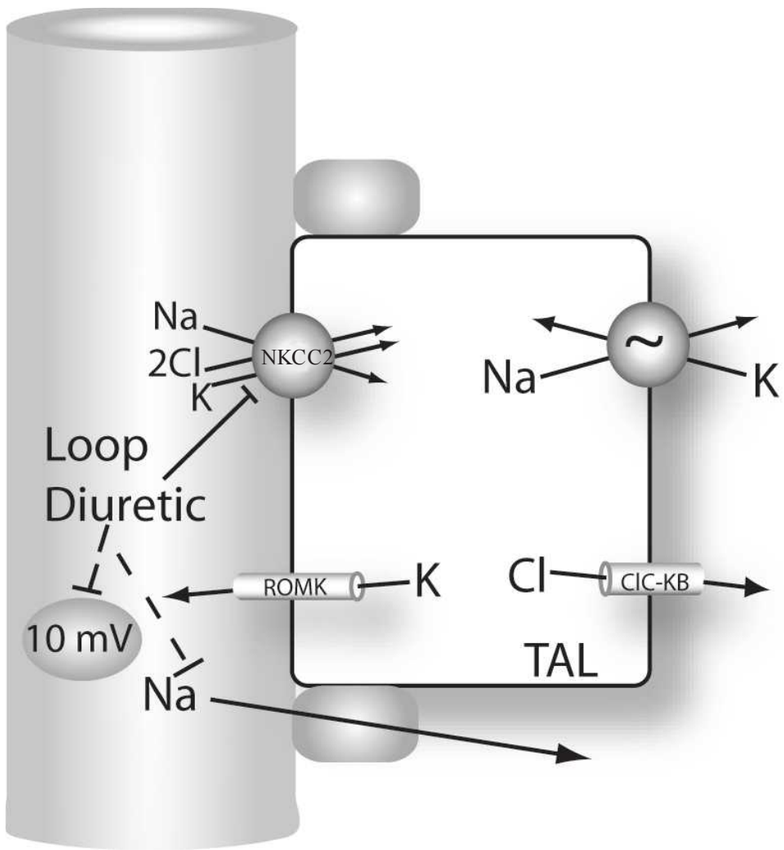


FIGURE 66.6 Mechanisms of diuretic action along the loop of Henle. Figure shows model of thick ascending limb (TAL) cells. Na and Cl are reabsorbed across the apical membrane via the loop diuretic-sensitive Na-K-2Cl cotransporter, NKCC2. Loop diuretics bind to and block this pathway directly. Note that the transepithelial voltage along the TAL is oriented with the lumen positive relative to blood (circled value, given in millivolts, mV). This transepithelial voltage drives a component of Na (and calcium and magnesium, see Fig. 66.9) reabsorption via the paracellular pathway. This component of Na absorption is also reduced by loop diuretics because they reduce the transepithelial voltage.

the interstitium, drives calcium and magnesium absorption through the paracellular pathway. Loop diuretics, by blocking the activity of the Na-K-2Cl cotransporter at the apical membrane of thick ascending limb cells, reduce the transepithelial voltage toward or to 0 mV. This stops passive paracellular calcium and magnesium absorption.

Renin Secretion

In addition to enhancing Na and Cl excretion, effects that result directly from inhibiting Na and Cl transport, loop diuretics also stimulate renin secretion. Although a component of this effect is frequently related to contraction of the extracellular fluid volume (see later), loop diuretics also stimulate renin secretion by inhibiting Na-K-2Cl cotransport directly. Macula densa cells, which control renin secretion, sense the NaCl concentration in the lumen of the thick ascending limb.[119] High luminal NaCl concentrations in the region of the macula densa lead to two distinct but related effects. First, they activate the tubuloglomerular feedback (TGF) response, which suppresses GFR. Second, they inhibit renin secretion. The relation between these two effects is complex and has been reviewed,[120] but both effects appear to result largely from NaCl movement across the apical membrane.[121] Most of the ion transport pathways of macula densa cells are expressed by thick ascending limb cells. This includes the loop diuretic-sensitive Na-K-2Cl cotransporter (NKCC2) at the apical surface.[105,106] Under normal conditions, an increase in luminal NaCl concentration in the thick ascending limb raises the NaCl concentration inside macula densa cells.[121] Because the activity of the basolateral Na/K ATPase is lower in macula densa cells than in surrounding thick ascending limb cells,[120] the cell NaCl concentration is much more dependent on luminal NaCl concentration in macula densa than in thick ascending limb cells.[122] When luminal and macula densa cell NaCl concentrations decline, production rates of nitric oxide and prostaglandin E_2 are stimulated. Although the mechanisms by which Na and Cl transport regulate nitric oxide and prostaglandin production rates are not known, both mediators appear to participate importantly in effecting renin secretion. Interestingly, loop diuretics also may stimulate renin secretion by inhibiting NKCC1, the secretory form of the three ion cotransport mechanism. Genetic deletion of NKCCC1 leads to an increase in plasma renin activity and a failure of renin exocytosis in response to furosemide,[119] suggesting a role for alternative pathways.

The constitutive (neuronal) isoform of nitric oxide synthase (nNOS) is expressed at high levels by macula densa cells, but not by other cells in the kidney.[123] Nitric oxide produced by macula densa cells has a paracrine effect to increase cellular cAMP in adjacent juxtaglomerular cells. Cyclic AMP through protein kinase A helps to stimulate renin secretion. In juxtaglomerular cells, nitric oxide may act by increasing cellular cGMP which inhibits phosphodiesterase 3,[124] leading to phosphodiesterase 3 inhibition and cAMP accumulation. Several laboratories reported that furosemide-induced stimulation of renin secretion is dependent on an intact nitric oxide system because nonspecific nitric oxide inhibition interferes with this phenomenon.[125–127] More recent studies, however, utilized knockout models to examine the role of nitric oxide in diuretic-induced renin secretion. Using this approach, it appears that neither neuronal nor endothelial nitric oxide synthases are required for loop diuretic–induced renin secretion. Instead, nitric oxide appears to play a permissive, rather than necessary, role in facilitating diuretic-induced renin secretion.[128]

Prostaglandin production also participates in regulating renin secretion. Cyclooxygenase, COX-2, is expressed by macula densa cells and by interstitial cells in the kidney.[129–132] This isoform is often found only after induction by inflammatory cytokines. Blockade of prostaglandin synthesis either by nonspecific cyclooxygenase inhibitors[133] or by specific COX-2 blockers[134,135] reduces both the natriuresis induced by loop diuretics and dramatically inhibits the renin secretory response. These results have been corroborated in humans.[136]

Renal Hemodynamics

GFR and renal blood flow (RBF) tend to be preserved during loop diuretic administration,[137] although GFR and RPF can decline if extracellular fluid volume contraction is severe. Loop diuretics reduce renal vascular resistance and increase

RBF under experimental conditions.[138,139] This effect is believed related to the diuretic-induced production of vasodilatory prostaglandins (discussed previously).

Another factor that may contribute to the tendency of loop diuretics to maintain GFR and RBF despite volume contraction is their effect on the TGF system. The sensing mechanism that activates the TGF system involves NaCl transport across the apical membrane of macula densa cells by the loop diuretic sensitive Na-K-2Cl cotransporter.[140] Under normal conditions, when the luminal concentration of NaCl reaching the macula densa rises, GFR decreases via TGF. To a large degree, the TGF-mediated decrease in GFR is believed to be due to afferent arteriole constriction. In response to changes in NaCl transport across the apical membrane of macula densa cells, ATP is released across the basolateral membranes through a NaCl sensitive ATP-permeable large-conductance (380 pS) anion channel.[141] ATP appears to be degraded to adenosine which activates A1 adenosine receptor (P1 purinergic receptor class) expressed on afferent arteriole,[142,143] as reviewed.[140] Loop diuretic drugs block TGF by blocking the sensing step of TGF.[144] In the absence of effects on the macula densa, loop diuretics would be expected to suppress GFR and RPF by increasing distal NaCl delivery and activating the TGF system. Instead, blockade of the TGF permits GFR and RPF to be maintained.

Systemic Hemodynamics

Acute intravenous administration of loop diuretics increases venous capacitance.[145] Some studies suggest that this effect results from stimulation of prostaglandin synthesis by the kidney.[146,147] Other studies suggest that loop diuretics have effects in peripheral vascular beds as well.[148] Pickkers and coworkers examined the local effects of furosemide in the human forearm. Furosemide had no effect on arterial vessels, but did cause dilation of veins, an effect that was dependent on local prostaglandin production.[149] More recently, loop diuretic-induced vasodilation was shown to depend on increased nitric oxide production.[150] Although venodilation and improvements in cardiac hemodynamics frequently result from intravenous therapy with loop diuretics, the hemodynamic response to intravenous loop diuretics may be more complex.[151] Johnston et al. reported that low dose furosemide increased venous capacitance, but that higher doses did not.[152] It was suggested that furosemide-induced renin secretion leads to angiotensin II-induced vasoconstriction. This vasoconstrictor might overwhelm the prostaglandin-mediated vasodilatory effects in some patients. In two series, 1 to 1.5 mg per kg furosemide boluses, administered to patients with chronic heart failure, resulted in transient deteriorations in hemodynamics (during the first hour), with declines in stroke volume index, increases in left ventricular filling pressure,[153] and exacerbation of heart failure symptoms. These changes may be related to activation of both the sympathetic nervous system and the renin/angiotensin system by the diuretic drug. Evidence for a role of the renin/angiotensin system in the furosemide-induced deterioration in systemic hemodynamics includes the temporal association between its activation and hemodynamic deterioration,[153] and the ability of angiotensin-converting enzyme (ACE) inhibitors to prevent much of the pressor effect.[154] The effects of renal denervation on sympathetic responses to furosemide were studied. These results confirm that the effects are mediated by both direct renal nerve traffic and indirectly, by activation of the renin/angiotensin axis.[155,156] Many other studies have shown that acute loop diuretic administration frequently produces a transient decline in cardiac output; whether diuretic administration increases or decreases left atrial pressure acutely may depend primarily on the state of underlying sympathetic nervous system and renin/angiotensin axis activation.

Pharmacokinetics

The three loop diuretics that are used most commonly—furosemide, bumetanide, and torsemide—are absorbed quickly after oral administration, reaching peak concentrations within 30 minutes to 2 hours. Furosemide absorption is slower than its elimination in normal subjects; thus, the time to reach peak serum level is slower for furosemide than for bumetanide and torsemide. This phenomenon is called "absorption-limited kinetics," as the rate of absorption is often slower than the rate of elimination.[3] The bioavailability of loop diuretics varies from 50% to 90% (Table 66.2); furosemide bioavailability is approximately 50%[4]; when furosemide dosing is switched from intravenous to oral, the dose may need to be increased to compensate for its poor bioavailability.[3] The half-lives of the loop diuretics available in the United States vary, but all are relatively short (ranging from approximately 1 hour for bumetanide to 3 to 4 hours for torsemide). The half-lives of muzolimine, xipamide, and ozolinone, none of which are available in the United States, are longer (6 to 15 hours).

Loop diuretics are organic anions that circulate tightly bound to albumin (>95%), thus their volume of distribution is small except during extreme hypoproteinemia.[157] Approximately 50% of an administered dose of furosemide is excreted unchanged into the urine. The remainder appears to be eliminated by glucuronidation, probably by the kidney. Torsemide and bumetanide are eliminated both by hepatic processes and through renal excretion. The differences in metabolic fate mean that the half-life of furosemide is altered by renal failure, whereas this is not true for torsemide and bumetanide. Similar to CAIs and thiazides, loop diuretics gain access to the tubular fluid almost exclusively by proximal secretion. The peritubular uptake is mediated by the organic anion transporters OAT1 and OAT3, whereas the apically located multidrug resistance–associated protein 4 (Mrp-4) mediates secretion into the tubular fluid. Mice lacking OAT1, OAT3, or Mrp-4 are remarkably resistant to both loop and thiazide diuretics, illustrating the functional importance of these proteins.[158–160] In humans, polymorphisms in the gene encoding the organic anion transporter OATP1B1 resulted in a slower elimination of torsemide.[161]

66.2 Pharmacokinetics of Loop Diuretics

		Elimination Half-Life (hours)			
	Oral Bioavailability (%)	Healthy	Renal Disease	Liver Disease	Heart Failure
Furosemide	10–100	1.5–2	2.8	2.5	2.7
Bumetanide	80–100	1	1.6	2.3	1.3
Torsemide	80–100	3–4	4–5	8	6

Adapted from the data in Brater DC. Diuretic therapy. N Engl J Med. 1998;339:387–395.

Interestingly, the response to loop diuretics is also associated with polymorphisms in the genes encoding the more distal sodium transporters NCC and ENaC.[162]

Clinical Use

Loop diuretics are used commonly to treat the edematous conditions, congestive heart failure, cirrhosis of the liver, and nephrotic syndrome.[163] In addition, a variety of other electrolyte, fluid, and acid base disorders can respond to loop diuretic therapy. Details of loop diuretic use for the treatment of edematous conditions are beyond the scope of this chapter.

Adverse Effects

There are at least three types of adverse effects of loop diuretics. The first and most common side effects are those that result directly from the effects of these drugs on renal electrolyte and water excretion. The second class of side effects is toxic, effects that are dose-related and predictable. The third class includes idiosyncratic allergic drug reactions.

Loop diuretics are frequently administered to treat edematous expansion of the extracellular fluid volume. Edema usually results from a decrease in the "effective" arterial blood volume. Overzealous diuretic usage or intercurrent complicating illnesses can lead to excessive contraction of the intravascular volume leading to orthostatic hypotension, renal dysfunction, and sympathetic overactivity. Patients suffering from heart failure are typically treated with both diuretics and ACE inhibitors or angiotensin receptor blockers (ARBs); this combination is especially likely to worsen renal function, under certain circumstances. Functional renal failure in such patients often responds to reduced diuretic doses and liberalization of dietary NaCl intake, permitting continued administration of the ACE inhibitor/ARBs.[164,165]

Other patients at increased risk for renal dysfunction during diuretic therapy include the elderly,[166] patients with preexisting renal insufficiency,[167] patients with right-sided heart failure or pericardial disease, and patients taking NSAIDs. In a case control study of NSAID use and renal failure, diuretic users had a 2.77 relative risk of acute kidney injury, compared with nonusers.[168]

Disorders of Na and K concentration are among the most frequent adverse effects of loop diuretics. Hyponatremia is less common with loop diuretics than with distal convoluted tubule diuretics (see later), but can occur. Its pathogenesis is usually multifactorial, but involves the effect of loop diuretics to impair the clearance of solute free water. Additional factors that may contribute include the nonosmotic release of arginine vasopressin,[169] hypokalemia, and hypomagnesemia.[170] Conversely, loop diuretics have been used to treat hyponatremia when combined with hypertonic saline, in the setting of the syndrome of inappropriate ADH secretion.[100,171] The combination of loop diuretics and ACE inhibitors has been reported to ameliorate hyponatremia in the setting of congestive heart failure.[172] The value of adding a loop diuretic to treatment with a vasopressin V2 receptor antagonist for hyponatremic syndrome of inappropriate ADH secretion has been suggested.[173,174] Hypokalemia occurs commonly during therapy with loop diuretics, although the magnitude is smaller than that induced by distal convoluted tubule diuretics (loop diuretics, 0.3 mM, vs. distal convoluted tubule [DCT] diuretics, 0.5–0.9 mM).[175,176] Loop diuretics increase the delivery of potassium to the distal tubule because they block potassium reabsorption via the Na-K-2Cl cotransporter. In rats, under control conditions, approximately half the excreted potassium was delivered to the "early" distal tubule. During furosemide infusion, the delivery of potassium to the early distal tubule rose to 28% of the filtered load.[177] Thus, it appears that a large component of the effect of loop diuretics to increase potassium excretion acutely reflects their ability to block potassium reabsorption by the thick ascending limb. Nevertheless, during chronic diuretic therapy, the degree of potassium wasting correlates best with volume contraction and serum aldosterone levels.[178] These data suggest that, under chronic conditions, the predominant effect of loop diuretics to stimulate potassium excretion results from their tendency to increase mineralocorticoid hormones while simultaneously increasing distal Na and water delivery.

Metabolic alkalosis is very common during chronic treatment with loop diuretics. Loop diuretics cause metabolic alkalosis via several mechanisms. First, they increase the excretion of urine that is bicarbonate free but contains Na and Cl. This leads to contraction of the extracellular fluid around a fixed amount of bicarbonate buffer; a phenomenon known as "contraction alkalosis." This probably contributes only slightly to the metabolic alkalosis that commonly accompanies chronic loop diuretic treatment. Loop diuretics directly inhibit transport of Na and Cl into thick ascending limb cells. In some species, these cells also express an isoform of the Na/H exchanger at the apical surface. When Na entry via the Na-K-2Cl cotransporter is blocked by a loop diuretic, the decline in intracellular Na activity will stimulate H secretion via the Na/H exchanger.[179–181] Loop diuretics stimulate the renin/angiotensin/aldosterone pathway, both directly and indirectly, as discussed previously. Aldosterone stimulates Na reabsorption by principal cells of the CNT and CCT, which renders the tubule lumen more negative and increases H-ATPase activity.[182] Aldosterone also directly activates the vacuolar H^+-ATPase in the outer medullary collecting tubule.[183,184] Thus, through different mechanisms, aldosterone stimulates H^+ secretion via H^+ ATPase present at the apical membrane of α intercalated cells. Hypokalemia itself also contributes to metabolic alkalosis by increasing ammonium production,[185] stimulating bicarbonate reabsorption by proximal tubules,[186,187] and increasing the activity of the H/K ATPase in the distal nephron.[67,188] Finally, contraction of the extracellular fluid volume stimulates Na/H exchange in the proximal tubule and may reduce the filtered load of bicarbonate. All of these factors may contribute to the metabolic alkalosis observed during chronic loop diuretic treatment.

Ototoxicity is the most common toxic effect of loop diuretics that is unrelated to their effects on the kidney. Deafness, which is usually temporary but can be permanent, was reported shortly after the introduction of loop diuretics. It appears likely that all loop diuretics cause ototoxicity, because ototoxicity can occur during use of chemically dissimilar drugs such as furosemide and ethacrynic acid.[189,190] The mechanism of ototoxicity remains unclear, although the stria vascularis, which is responsible for maintaining endolymphatic potential and ion balance, appears to be a primary target for toxicity.[191] Loop diuretics reduce the striatal voltage from +80 mV to −10 to −20 mV within minutes of application.[192] A characteristic finding in loop diuretic ototoxicity is strial edema. This suggests that toxicity involves inhibition of ion fluxes.[191] Ikeda and Morizono detected functional evidence for the presence of a Na-K-2Cl cotransporter in the basolateral membrane of marginal cells in the inner ear.[193] According to the model proposed by these investigators, marginal cells resemble secretory cells in other organ systems, with a Na-K-2Cl cotransporter and Na/K ATPase at the basolateral cell membrane and channels for K and Cl at the apical surface. According to this model, loop diuretic induced shrinkage of marginal cells results from inhibition of cell Na, K, and Cl uptake across the basolateral cell membrane. This model received molecular confirmation when the secretory isoform of the Na-K-2Cl cotransporter, NKCC1, was localized in the lateral wall of the cochlea, using specific antibodies[194] and real-time polymerase chain reaction (RT-PCR).[195] Furthermore, disruption of the ubiquitous form of the Na-K-Cl cotransporter, NKCC1, leads to deafness in mice.[196–198] Loop diuretics cause loss of outer hair cells in the basal turn of the cochlea, rupture of endothelial layers, cystic formation in the stria vascularis, and marginal cell edema in the stria vascularis.[199]

Ototoxicity appears to be related to the peak serum concentration of loop diuretic and therefore tends to occur during rapid drug infusion of high doses. For this reason, this complication is most common in patients with uremia.[200] It has been recommended that furosemide infusion be no more rapid than 4 mg per minute.[201] In addition to renal failure, infants, patients with cirrhosis, and patients receiving aminoglycosides or cis-platinum may be at increased risk for ototoxicity.[200]

Myalgias have been reported with bolus infusion of bumetanide.[202]

DISTAL CONVOLUTED TUBULE DIURETICS

The first orally active drug to be developed that inhibited Na and Cl transport along the distal convoluted tubule (DCT) was chlorothiazide. Chlorothiazide was developed as sulfonamide-based carbonic anhydrase inhibitors were modified in pursuit of substances that increased Cl excretion rather than bicarbonate. The identification of a substance that increased Na and Cl excretion rates was immediately recognized as clinically significant, because extracellular fluid contains predominantly NaCl rather than $NaHCO_3$, and because acidosis limits the effectiveness of carbonic anhydrase inhibitors. Subsequent development led to a wide variety of benzothiadiazide (thiazide) diuretics (Fig. 66.7); all are analogs of 1,2,4-benzothiadiazine-1,1-dioxide. Other structurally related diuretics include the quinazolines (such as metolazone) and substituted benzophenone sulfonamide (such as chlorthalidone). Although the term "thiazide diuretics" is frequently used to describe this class of drugs, a more accurate descriptor is the term distal convoluted tubule diuretics.

Urinary Electrolyte and Water Excretion

Acute administration of these drugs increases the excretion of Na, K, Cl, HCO_3, phosphate, and urate (see Table 66.1). The increases in HCO_3, phosphate, and urate excretion are probably related primarily to carbonic anhydrase inhibition, and not to inhibition of the Na-Cl cotransporter (see later). As such, the effects of DCT diuretics to increase HCO_3, phosphate, and urate excretion may

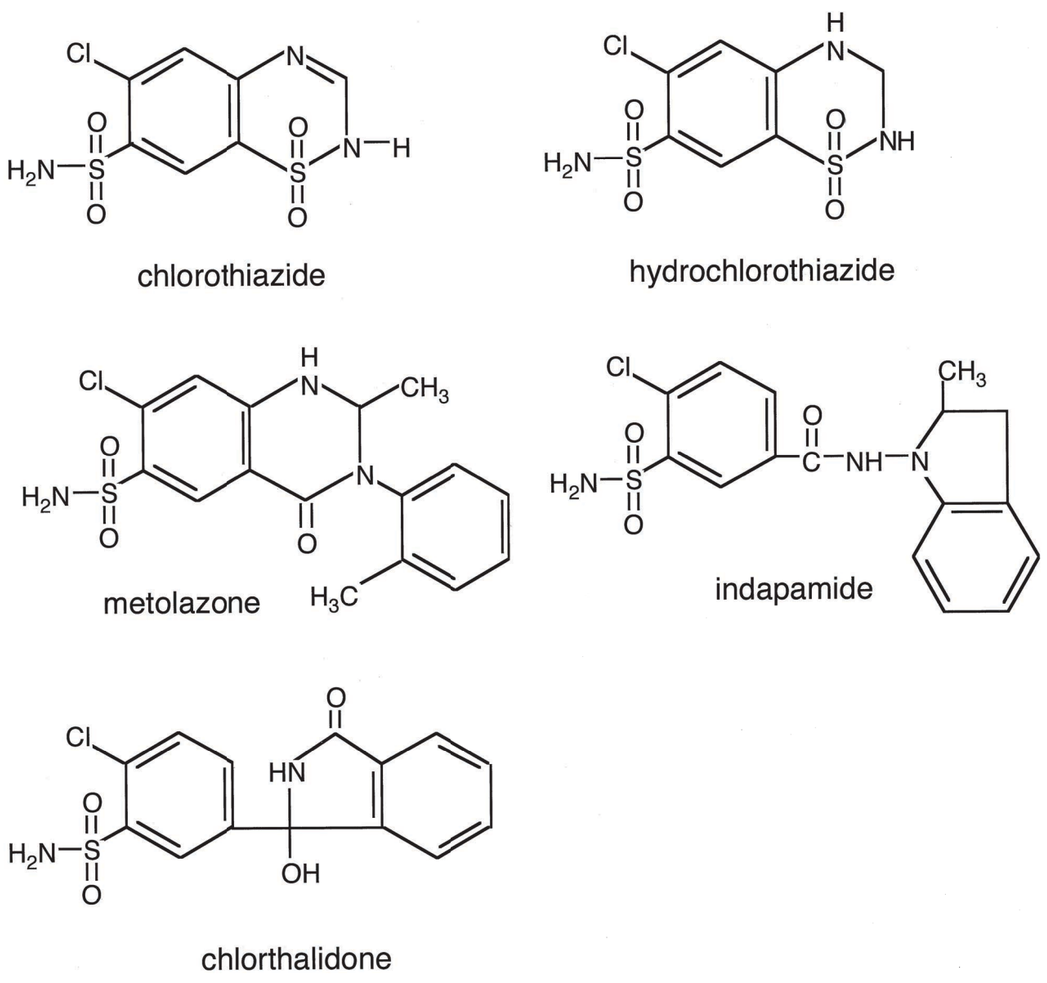


FIGURE 66.7 Structures of distal convoluted tubule diuretics.

vary, depending on the carbonic anhydrase inhibiting potency of a particular drug. Chronically, as contraction of the extracellular fluid volume occurs, uric acid excretion declines, and hyperuricemia can occur.[203] Further, bicarbonate excretion ceases, and continuing losses of chloride without bicarbonate coupled with extracellular fluid volume contraction may lead to metabolic alkalosis. In contrast to loop and proximally acting diuretics, DCT diuretics strongly reduce urinary calcium excretion.[204] Although the effects on urinary calcium excretion can be variable during acute administration,[205–207] these drugs uniformly lead to calcium retention when administered chronically.

DCT diuretics inhibit the clearance of solute free water when administered during water diuresis. This effect is similar to that of loop diuretics and originally led to the mistaken inference that they act along the thick ascending limb. In contrast to loop diuretics, however, DCT diuretics do not limit water retention during antidiuresis.

Mechanism of Action

Sodium and Water Transport in the Proximal Tubule

As discussed previously, DCT diuretics are related chemically to carbonic anhydrase inhibitors and most DCT diuretics retain some carbonic anhydrase inhibiting activity.[208] Carbonic anhydrase inhibitors interfere with the activity of the apical Na/H exchanger expressed at the luminal membrane of proximal tubule cells by indirect mechanisms described above. Although this effect of DCT diuretics may occur when these drugs are administered acutely (as during intravenous chlorothiazide administration), it probably contributes little to the overall natriuresis during chronic use.[209,210] Yet this effect may play a role in the tendency for DCT diuretics to reduce the GFR and activate the TGF mechanism.[40] The relative carbonic anhydrase inhibiting potency (shown in parentheses) of some commonly used DCT diuretics is chlorthalidone (67) > benzthiazide (50) > polythiazide (40) > chlorothiazide (14) > hydrochlorothiazide (1) > bendroflumethiazide (0.07).[211]

NaCl Absorption in the Distal Nephron

As the name indicates, the predominant site at which DCT diuretics inhibit ion transport is the DCT. This region of the nephron, between the macula densa and the confluence with another nephron to form the CCD, is cytologically heterogeneous.[212,213] It comprises a short stretch of post–macula densa thick ascending limb, the DCT, the CNT, and the initial portion of the CCD. In previous editions, the controversies surrounding the predominant sites of expression of the thiazide-sensitive transporter were described. The interested reader is referred to those editions for more details. These controversies were resolved, when the thiazide-sensitive Na-Cl cotransporter (now called NCC) was expression-cloned from the flounder bladder,[214] and later cloned from rat, mouse, rabbit, and human kidney[215–218]; the gene is SLC12A3 and is closely related to NKCC2, and is a member of the cation chloride cotransporter gene family. In rat, human, and mouse, NCC message and protein are expressed by DCT cells. A model of NaCl transport by DCT cells is shown in Figure 66.8. In human, rat, and mouse, expression of NCC extends into a transitional segment, referred to as the DCT2, which shares properties of DCT and CNTs.[219,220] In the rabbit, the thiazide-sensitive Na-Cl cotransporter was also shown to be expressed exclusively by DCT cells[221]; CNT cells do not express the transporter. Thus, from a molecular standpoint, the NCC is expressed by distal convoluted tubule cells in all mammalian species examined to date.

DCT diuretics are organic anions that bind to and inhibit the transporter. Based on studies performed before NCC was identified at the molecular level, [^{3}H] metolazone binding to kidney cortical membranes was studied. DCT diuretics were shown to bind to the cotransporter at an anion site.[222] This conclusion derives from the observation that chloride inhibits the binding of [^{3}H] metolazone in a competitive manner. Unlike loop diuretic binding to the Na-K-2Cl cotransporter, [^{3}H] metolazone binding to the Na-Cl cotransporter does not require the presence of Na, suggesting either that chloride binds first to the transporter or that binding of ions to the transporter is not "ordered." Monroy and colleagues[223] studied thiazide inhibition of the cloned transporter and reported that the inhibitory activity of DCT diuretics could be inhibited by increasing concentration of either Cl^- or Na^+. These workers suggested a model that incorporates Na and Cl binding to the transporter in random order, with alterations in diuretic affinity occurring secondarily. Morena and colleagues used a chimeric approach to study sites of diuretic binding to NCC. They reported that

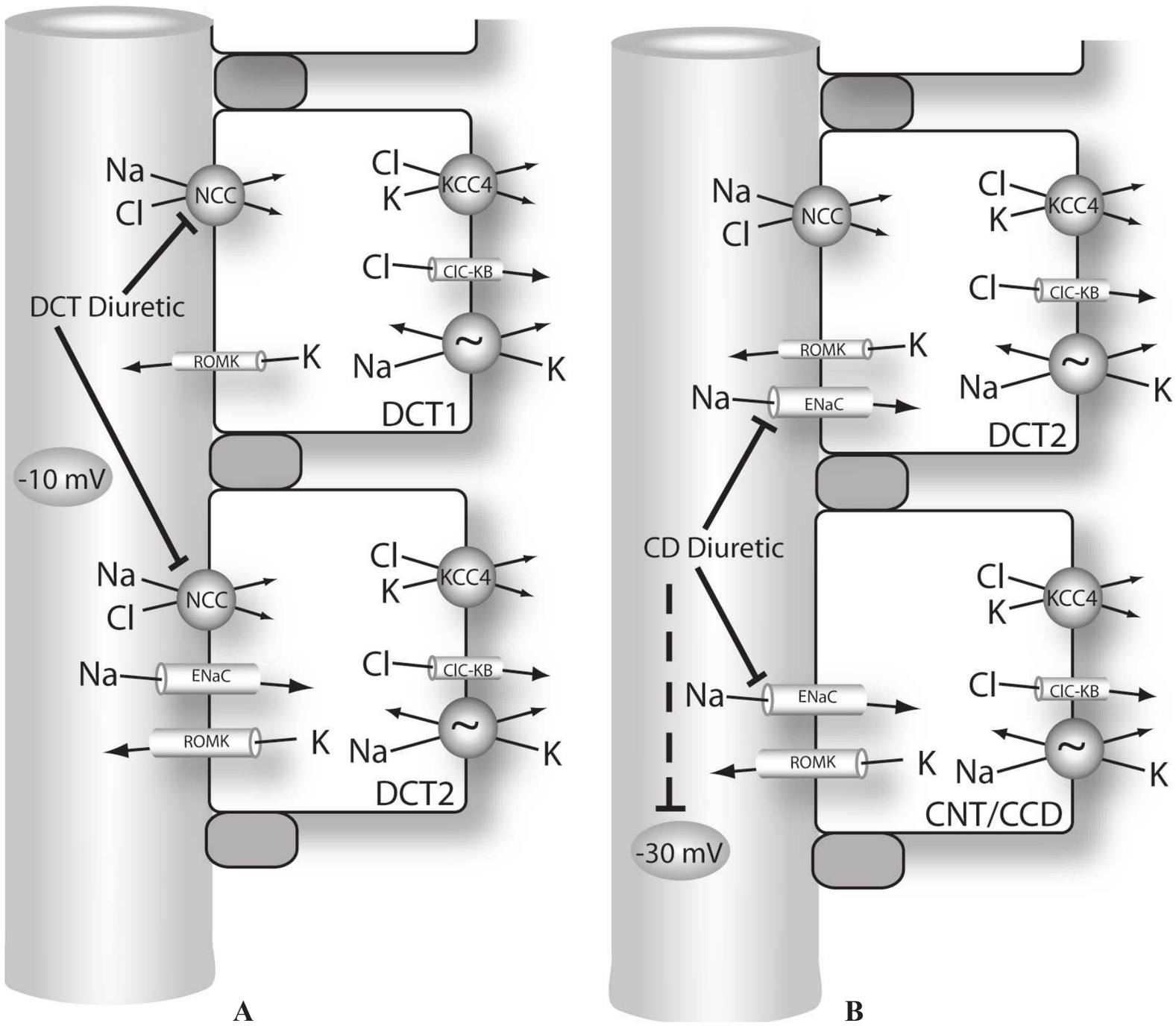


FIGURE 66.8 Mechanisms of distal convoluted tubule (DCT) and collecting duct (CD) diuretics. **A:** Mechanism of action of DCT diuretics. In rat, mouse, and human, two types of distal convoluted tubule cells have been identified, referred to here as DCT-1 and DCT-2. Na and Cl are reabsorbed across the apical membrane of DCT-1 cells only via the thiazide-sensitive Na-Cl cotransporter. This transport protein is also expressed by DCT-2 cells where Na can also cross through the epithelial Na channel, ENaC.[219,220,395] Thus, the transepithelial voltage along the DCT-1 is near to 0 mV, whereas it is finite and lumen-negative along the DCT-2. **B:** Mechanism of action of CD diuretics. The late distal convoluted tubule cells (DCT2 cells) and connecting (CNT) or CD cells are shown. Na is reabsorbed via the epithelial Na channel (ENaC), which lies in parallel with a K channel (ROMK). The transepithelial voltage is oriented with the lumen negative, relative to interstitium (shown by the *circled value*), generating a favorable gradient for transepithelial K secretion. Drugs that block the epithelial Na value reduce the voltage toward 0 mV (effect indicated by *dashed line*), thereby inhibiting K secretion.

the site determining metolazone affinity was near the last transmembrane domain, whereas the region defining chloride affinity was near the 5 to 6 transmembrane domains. Although these conclusions appear to be contradictory, it should be mentioned that in one study diuretic binding was determined, whereas in the others changes in diuretic affinity were determined. As is the case for the Na-K-2Cl cotransporter, described previously, firm conclusions about sites of diuretic binding await crystallization of the transport protein.

Evidence for thiazide action in other nephron segments has also been obtained. In vivo catheterization experiments demonstrated a component of thiazide-sensitive Na transport in medullary collecting tubules of rats.[224] Terada and Knepper detected thiazide-sensitive Na-Cl transport in rat CCDs perfused in vitro,[225] a finding recently confirmed when it was shown that thiazides can inhibit a sodium-dependent chloride-bicarbonate exchanger in the collecting duct.[226] The role of this effect in the clinical response to DCT diuretics remains to be established.

Calcium and Magnesium Transport

When administered chronically, DCT diuretics reduce calcium excretion. This effect has been utilized clinically to treat calcium nephrolithiasis (see later). Much progress in understanding mechanisms of the hypocalciuric effect of DCT diuretics has been made during the past 10 years, but the mechanisms involved remain controversial. Acute administration of DCT diuretics has a variable effect on calcium excretion, sometimes leading to increases in calcium excretion.[205,227] This probably reflects the carbonic anhydrase inhibiting capacity of these drugs, because carbonic anhydrase inhibitors increase urinary calcium excretion acutely. Calcium reabsorption by proximal tubules is coupled functionally

to sodium reabsorption; drugs that inhibit proximal Na reabsorption also inhibit proximal calcium reabsorption.[228] Thus, during chronic treatment, the filtered calcium load may decrease (owing to ECF volume depletion) and proximal calcium reabsorption may increase (owing to the ECF volume contraction), which stimulates proximal Na^+ and water reabsorption.

DCT diuretics also increase renal calcium reabsorption in the distal nephron. Although rat distal nephrons reabsorb both Na and Ca, Constanzo showed[229] that thiazide diuretics dissociate the two; Na reabsorption is inhibited whereas calcium reabsorption is stimulated. Several factors are now believed to contribute to the effect of DCT diuretics to stimulate calcium reabsorption (Fig. 66.9). As in most other cells, the intracellular calcium concentration of DCT cells is low, compared with extracellular fluid calcium.[230] Calcium enters DCT cells passively, down its electrochemical gradient via specific calcium channels, primarily Trpv5.[231,232] DCT diuretics increase the intracellular calcium activity, suggesting that a primary effect is to increase apical calcium entry.[230]

Bindels and colleagues showed that a member of the transient receptor potential family, TrpV5, is expressed at the apical membranes of cells along the second part of the distal convoluted tubule (the DCT2) and along the CNT. It is regulated by vitamin D and appears to possess several characteristics suggesting that it is one of the primary apical calcium entry pathways. Lee and colleagues confirmed an acute effect of DCT diuretics on distal calcium uptake, but found that a large portion of the chronic effects of DCT diuretics results from extracellular fluid volume depletion. They speculated that acute exposure to DCT diuretics hyperpolarizes DCT cells, as suggested by Friedman and colleagues,[230] activating TRPV5 channels and also increasing their expression at the mRNA level.[233] During chronic exposure, however, ECF

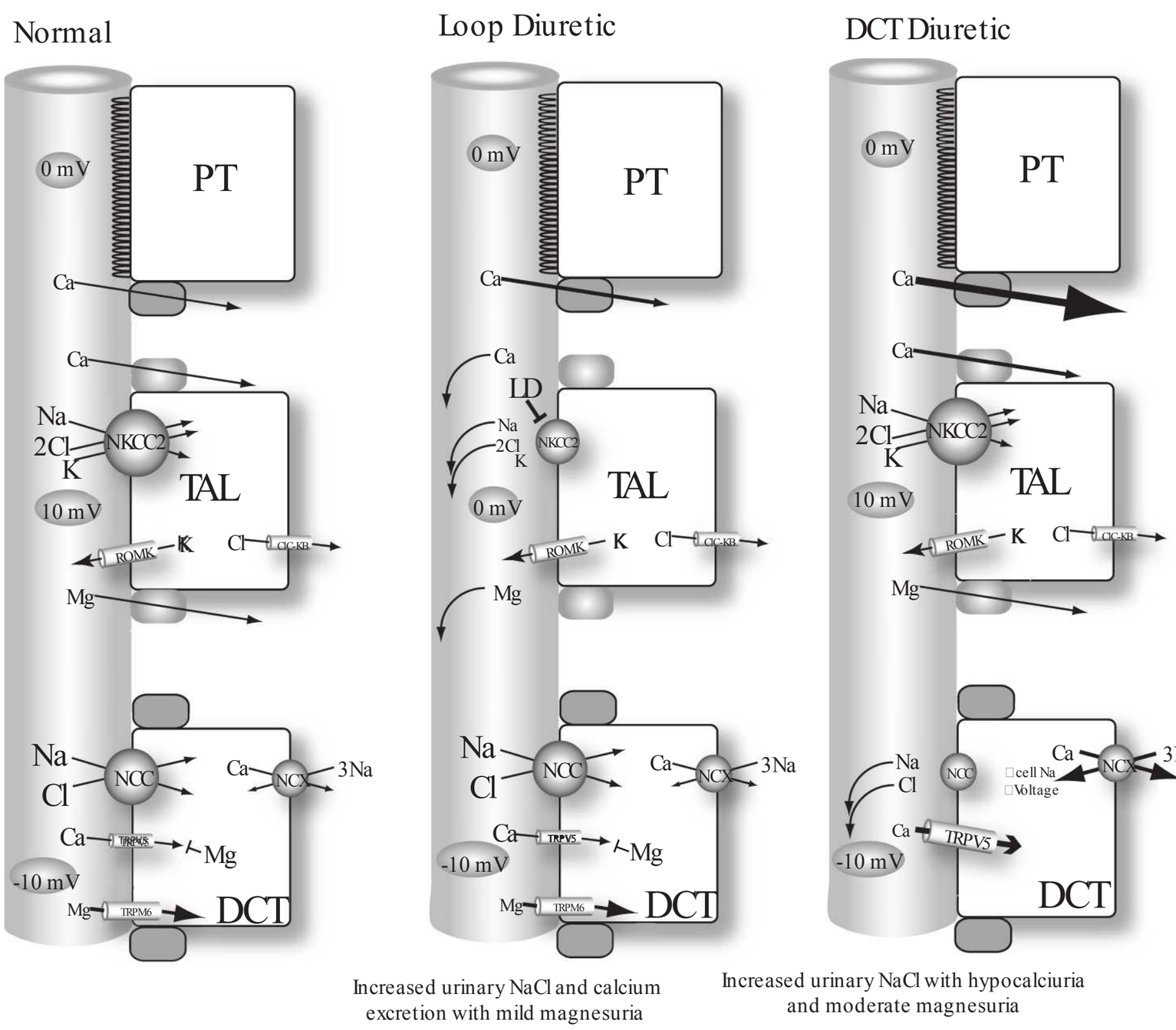


FIGURE 66.9 Possible mechanisms of diuretic effects on calcium and magnesium excretion. Typical cells from the proximal tubule (PT), thick ascending limb (TAL), distal convoluted tubule (DCT) are shown. Calcium reabsorption occurs along the DCT largely via a transient receptor potential channel (TRPV5). Magnesium reabsorption occurs along the DCT largely via a transient receptor potential channel (TRPM6). Transepithelial voltages (representative but arbitrary values, given in millivolts, mV) are shown. Net effects on electrolyte excretion are shown at the bottom. Normal conditions are at the left. Treatment with loop diuretics (LDs) is shown in the middle; treatment with DCT diuretics is shown on the right. LDs reduce the magnitude of the lumen-positive transepithelial voltage, thereby retarding passive calcium and magnesium reabsorption. Passive calcium and magnesium reabsorption appears to traverse the paracellular pathway. Chronic treatment, especially with DCT diuretics, increases proximal Na and Ca reabsorption; thus, less calcium is delivered distally. Enhanced distal calcium absorption, driven by DCT diuretics, also occurs.[204] Effects of DCT diuretics to increase magnesium excretion remain incompletely understood, but likely include decreased TRPM6 abundance.

volume contraction reduces distal NaCl delivery limiting the DCT diuretic-induced hyperpolarization.[233]

DCT diuretics not only stimulate entry of calcium across the apical membrane, but also stimulate calcium transport across the basolateral cell membrane into the interstitium. DCT cells, at least in rat, mouse, and human, express the Na/Ca exchanger and a calcium ATPase. The Na/Ca exchanger is electrogenic and is believed to carry three Na ions into the cell in exchange for one calcium ion out. When DCT diuretics block the luminal entry pathway for Na and Cl, the intracellular Na^+ concentration declines, and the cells hyperpolarize. Both the hyperpolarization and the decline in cell Na^+ concentration increase the electrochemical driving force favoring calcium movement from cell to interstitium. Although the data describing effects on apical and basolateral calcium transport were obtained in different model systems and from different species, taken together, they suggest that DCT diuretics stimulate both the apical entry pathway and the basolateral exit pathway that permit calcium reabsorption. The calcium reabsorptive pathway of the distal tubule is quite potent and the passive calcium permeability of this tubule segment is low; in stationary microperfusion experiments, the distal tubule was able to reduce the luminal calcium concentration below 0.1 mM.[229]

The net effect of DCT diuretics to reduce calcium excretion appears most likely to involve both effects along the proximal and the distal tubule. The interested reader is referred to the review by Reilly and Huang for a more detailed description of the controversy and the data supporting effects along different segments.[204]

DCT diuretics enhance magnesium excretion, but the effects are generally much less profound than their effects on calcium excretion. This is in contrast to the effects of genetic NCC deletion or inactivity, as occurs in Gitelman syndrome, where hypomagnesemia is a cardinal feature. Acute infusions have been reported to have little effect on magnesium excretion.[205,234,235] In contrast, DCT diuretics increase urinary magnesium excretion and can cause hypomagnesemia when administered chronically.[236–238] One important pathway for magnesium reabsorption across the apical membrane of DCT cells is the transient receptor potential, TRPM6.[239] This protein localizes to DCT cells (and intestinal cells) at the apical membrane and has functional characteristics suggesting it is, or is part of, the magnesium entry step along the distal nephron.[240]

The mechanisms by which chronic DCT diuretic administration lead to hypomagnesemia remain controversial. Quamme proposed that DCT diuretics, by hyperpolarizing DCT cells, enhance magnesium uptake, thereby downregulating the expression of magnesium channels, leading to magnesemia.[241] Ellison suggested that magnesium wasting consequent to DCT diuretic treatment resulted from the actions of aldosterone to increase the lumen-negative transepithelial voltage of the DCT.[242] This would be expected to increase the electrochemical gradient favoring magnesium secretion (or inhibiting its reabsorption). Loffing and colleagues suggested that magnesium wasting results from the destruction of DCT cells, resulting from apoptosis induced by DCT diuretics.[243,244] Several groups have reported that inactivation of NCC reduces the abundance of Trpm6, which would be expected to impair magnesium reabsorption.[245,246]

Renal Hemodynamics

DCT diuretics increase renal vascular resistance and decrease the GFR when given acutely. Okusa et al.[40] showed that intravenous chlorothiazide reduced the GFF by 16% when measured as whole kidney clearance or by micropuncture of a superficial distal tubule. In contrast, however, when flow to the macula densa was blocked and the single nephron GFR was measured by micropuncture of a proximal tubule, intravenous chlorothiazide had no effect on GFR. These data suggest that diuretic-induced stimulation of the TGF system mediates the effect of DCT diuretics on GFR; yet, the recent data described previously (in which carbonic anhydrase inhibition was shown to reduce the GFR of adenosine-1 receptor–deficient, TGF-deficient, animals) suggest that other mechanisms may be involved as well.[71]

During chronic treatment with DCT diuretics, mild contraction of the ECF volume develops, thereby increasing solute and water reabsorption by the proximal tubule. This effect reduces Na delivery to the macula densa. This would be expected to return GFR toward baseline values during chronic treatment with DCT diuretics.[210,247] Thus, when used chronically, DCT diuretics lead to a state of mild ECF volume contraction, increased fractional proximal reabsorption, and relatively preserved glomerular filtration.[210,247]

When administered acutely, the effect of DCT diuretics on renin secretion is variable.[248] If urinary NaCl losses are replaced, these drugs tend to suppress renin secretion,[249] probably by increasing NaCl delivery to the macula densa.[40] In contrast, during chronic administration, renin secretion increases both because solute delivery to the macula densa declines[210] and because volume depletion activates the vascular mechanism for renin secretion.

Pharmacokinetics

DCT diuretics are organic anions that circulate in a highly protein-bound state. As with loop diuretics, the amount reaching the tubule fluid by filtration across the glomerular basement membrane is small; the predominant route of entry into tubule fluid is by secretion via the organic anion secretory pathway in the proximal tubule.[3] DCT diuretics are rapidly absorbed across the gut, reaching peak concentrations within 1.5 to 4 hours.[3] The amount of administered drug that reaches the urine varies greatly,[3] as does the half-life. Short-acting DCT diuretics include bendroflumethiazide, hydrochlorothiazide, tizolemide, and trichlormethiazide. Medium-acting DCT diuretics include chlorothiazide, hydroflumethiazide, indapamide, and mefruside. Long-acting DCT diuretics include chlorthalidone, metolazone, and polythiazide.[3] The clinical effects of the differences in half-life are unclear, except in the

incidence of hypokalemia, which is more common in patients taking the longer acting drugs such as chlorthalidone.[175,250] The longer half life may also contribute to their increased efficacy in essential hypertension (see following). The individual response to DCT diuretics is also associated with polymorphisms in genes encoding proteins that directly or indirectly regulate NCC, including with-no-lysine kinase 1 (WNK1)[251] and α-adducin.[252]

Clinical Use

DCT diuretics are used most commonly to treat essential hypertension.[253] Despite a great deal of debate about the potential complications of DCT diuretics, these drugs continue to be recommended as first-line therapy for hypertension because they are at least as effective as more expensive agents at reducing mortality.[254] Although hydrochlorothiazide has become most commonly used, at least to treat hypertension, there is increasing evidence that chlorthalidone and indapamide may be more effective than hydrochlorothiazide.[255]

DCT diuretics are also used occasionally to treat edematous conditions, although they may be less effective than loop diuretics. Although the maximal effect of loop diuretics to increase urinary Na, Cl, and water excretion is greater than that of DCT diuretics, Reyes and colleagues have shown that the cumulative effects of DCT diuretics on urinary Na and Cl excretion are often greater than those of once daily furosemide.[256] Although these studies were conducted in normal volunteers, they may extend to patients with mild cases of edema. In addition, DCT diuretics have proved useful to treat edematous patients who have become resistant to loop diuretics. In this case, the addition of a DCT diuretic to a regimen that includes a loop diuretic frequently increases urinary Na and Cl excretion dramatically. The interested reader is referred elsewhere for a discussion of combination diuretic therapy and diuretic synergism.[76,151]

DCT diuretics have become drugs of choice to prevent the recurrence of kidney stones in patients with idiopathic hypercalciuria. In several controlled and many uncontrolled studies, the recurrence rate for calcium stones has been reduced by up to 80%.[257–259] Relatively high doses of DCT diuretics are often employed for the treatment of nephrolithiasis.[260] Some studies suggest that the hypocalciuric effect of DCT diuretics wanes during chronic use, in the setting of absorptive hypercalciuria.[261] The observation that Gitelman syndrome, an inherited disorder of NCC inactivity, may present during adulthood with hypocalciuria suggests that compensatory mechanisms may not exist for the effects of DCT diuretics on calcium transport.[242] The ability of DCT diuretics to reduce urinary calcium excretion suggests that these drugs may prevent bone loss. Some,[262,263] but not all,[264,265] epidemiologic studies suggest that DCT diuretics reduce the risk of hip fracture and osteoporosis. A randomized controlled study confirmed that DCT diuretics reduce bone loss in women.[266] A case series suggests that DCT diuretics also provide rapid recovery of bone mass in osteoporotic men.[267] Another study confirmed an effect of DCT diuretics to reduce hip fracture, but noted that the protective effect wanes within 4 months of discontinuance.[268] Others have indicated that DCT diuretics can be effective in patients with primary hypoparathyroidism, when combined with a low salt diet.[269] Concomitant potassium bicarbonate administration may increase renal calcium retention induced by DCT diuretics.[270]

DCT diuretics are also employed to treat nephrogenic diabetes insipidus, causing a paradoxical decrease in urinary volume flow rate. This action of DCT diuretics results from the combination of mild extracellular fluid volume contraction (owing to diuretic-induced natriuresis), suppression of glomerular filtration (owing largely to diuretic-induced activation of the TGF mechanism), and impaired solute reabsorption along the DCT. The DCT, like the thick ascending limb, is nearly impermeable to water.[271] Solute reabsorption by the thiazide-sensitive Na-Cl cotransporter therefore contributes directly to urinary dilution. The central role of extracellular fluid volume contraction in the efficacy of DCT diuretics in diabetes insipidus was highlighted by the observation that dietary salt restriction is necessary to reduce urinary volume effectively.[247,272] DCT diuretics may also increase the antidiuretic hormone-independent water permeability of the medullary collecting tubule.[273] When administered to rats lacking antidiuretic hormone (similar to patients with central diabetes insipidus), DCT diuretics did not alter the abundance of the apical water channel of the collecting duct (aquaporin-2),[274] even though they reduced urine volume. In contrast, DCT diuretic treatment increased the abundance of aquaporin-2, NCC, and the alpha subunit of the epithelial Na channel[275] when administered to rats with lithium-induced nephrogenic diabetes insipidus. It was suggested that the upregulation of the abundance of the renal Na and water transporters might explain the antidiuretic effectiveness of DCT diuretics.

Adverse Effects

Electrolyte disorders, such as hypokalemia, hyponatremia, and hypomagnesemia, are common side effects of DCT diuretics. A measurable decline in serum K concentration is nearly universal in patients given DCT diuretics, but most patients do not become frankly hypokalemic. In the ALLHAT trial, mean serum potassium concentrations declined from 4.3 to 4.0 and 4.1 mM at 2 and 4 years of treatment, respectively.[276] The clinical significance of diuretic-induced hypokalemia continues to be debated. Unlike the loop diuretics, DCT diuretics do not influence K transport directly.[277] Instead, they increase K excretion indirectly. DCT diuretics increase tubule fluid flow in the CNT and collecting duct, the predominant sites of K secretion along the nephron. Increased flow stimulates K secretion along the distal nephron, which enhances K secretion by large calcium activated K channels.[278] In addition, DCT diuretic-induced extracellular fluid volume contraction activates the renin/angiotensin/aldosterone system, further stimulating K secretion via the renal outer medullarly potassium channel (ROMK). Evidence for the central role of aldosterone in diuretic-induced hypo-

kalemia includes the observation that hypokalemia is more common during treatment with long-acting DCT diuretics, such as chlorthalidone, than with shorter-acting DCT diuretics, such as hydrochlorothiazide, or with the very short-acting loop diuretics.[175] Another reason that DCT diuretics may produce more potassium wasting than loop diuretics is the differences in effects on calcium transport. As discussed previously, loop diuretics inhibit calcium transport by the thick ascending limb, increasing distal calcium delivery. In contrast, DCT diuretics stimulate calcium transport, reducing calcium delivery to sites of potassium secretion. Okusa and colleagues[279] showed that high luminal concentrations of calcium inhibit the functional activity of ENaC in the distal nephron, thereby inhibiting potassium secretion. DCT diuretics also increase urinary magnesium excretion and can lead to hypomagnesemia, as discussed previously. Hypomagnesemia may cause or contribute to the hypokalemia observed under these conditions.[280–282] Some studies suggest that maintenance magnesium therapy can prevent or attenuate the development of hypokalemia,[280] but this has not been supported universally.

Diuretics have been reported to contribute to more than one half of all hospitalizations for serious hyponatremia. Hyponatremia is especially common during treatment with DCT diuretics, compared with other classes of diuretics, and the disorder is potentially life-threatening.[283] A recent case control study suggested that hyponatremia during thiazide treatment is more common than generally appreciated, but that in most cases, it does not prove morbid.[284] Several factors contribute to DCT diuretic-induced hyponatremia. First, as discussed previously, DCT diuretics inhibit solute transport in the terminal portion of the "diluting segment," the DCT. This impairs the ability to excrete solute-free water. Second, DCT diuretics can reduce the GFR, primarily by activating the TGF system. This limits solute delivery to the diluting segment and impairs solute-free water clearance. Third, DCT diuretics lead to volume contraction, which increases proximal tubule solute and water reabsorption, further restricting delivery to the "diluting segment." Fourth, hyponatremia has been correlated with the development of hypokalemia in patients receiving DCT diuretics.[285] Finally, susceptible patients may be stimulated to consume water during therapy with DCT diuretics; although the mechanisms are unclear, this may contribute importantly to the sudden appearance of hyponatremia that can occur during DCT diuretic therapy. Of note, one report suggests that patients who are predisposed to develop hyponatremia during treatment with DCT diuretics will demonstrate an acute decline in serum sodium concentration in response to a single dose of the drug.[286] Other studies suggest that risk factors for DCT diuretic-induced hyponatremia include older age, lower body mass, and concomitant administration of selective serotonin reuptake inhibitors.[287,288]

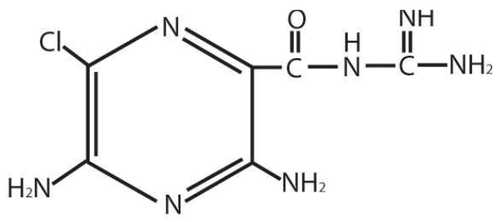
DCT diuretics frequently cause mild metabolic alkalosis. The mechanisms are similar to those described above for loop diuretics, except that DCT diuretics do not stimulate Na/H exchange in the thick ascending limb.

DCT diuretics cause several disturbances of endocrine glands. Glucose intolerance has been a recognized complication of DCT diuretic use since the 1950s. This complication appears to be dose-related.[289,290] In the ALLHAT trial, patients experienced a 1.8% increase in new onset diabetes at 4 years of treatment, compared with patients treated with calcium channel blockers.[291] This difference did not translate into adverse clinical outcomes in the diuretic group, but has generated a great deal of discussion. The pathogenesis of DCT diuretic–induced glucose intolerance remains unclear, but several factors have been suggested to contribute. First, diuretic-induced hypokalemia may decrease insulin secretion by the pancreas, via effects on the membrane voltage of pancreatic β cells. When hypokalemia was prevented by oral potassium supplementation, the insulin response to hyperglycemia normalized, suggesting an important role for hypokalemia.[292] Hypokalemia may also interfere with insulin mediated glucose uptake by muscle, but most patients demonstrate relatively normal insulin sensitivity.[203] Volume depletion may stimulate catecholamine secretion, but volume depletion during therapy with DCT diuretics is usually very mild. It has also been suggested that DCT diuretics directly activate calcium-activated potassium channels that are expressed by pancreatic β cells.[293] Activation of these channels is known to inhibit insulin secretion. Inhibiting the renin/angiotensin/aldosterone axis appears to reduce the development of new diabetes.[294] Drugs that inhibit this pathway might attenuate the effects of diuretics to impair glucose homeostasis, but this has not been tested directly. Other factors may contribute to glucose intolerance as well, including drug-specific factors.[295]

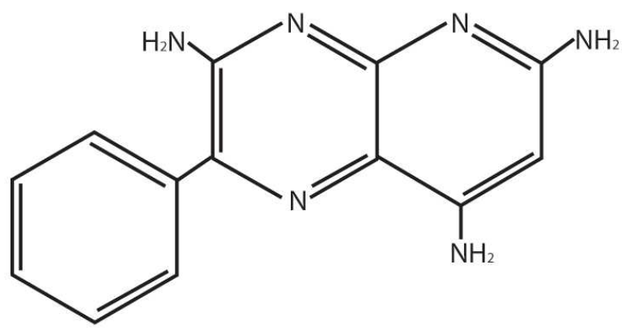
DCT diuretics increase levels of total cholesterol, total triglyceride, and LDL cholesterol and reduce the HDL.[203,296] Definitive information about the mechanisms by which DCT diuretics alter lipid metabolism is not available, but many of the mechanisms that affect glucose homeostasis have been suggested to contribute. Hyperlipidemia, like hyperglycemia, is a dose-related side effect, and one that wanes with chronic diuretic use. In the ALLHAT study, treatment with chlorthalidone resulted in a total cholesterol 2.2 mg per dL higher than did treatment with ACE inhibitors.[291] In several large clinical studies, the effect of low dose DCT diuretic treatment on serum LDL was not significantly different from placebo.[297,298] Further, treatment of hypertension with DCT diuretics reduces the risk of stroke, coronary heart disease, congestive heart failure, and cardiovascular mortality.

CORTICAL COLLECTING TUBULE DIURETICS

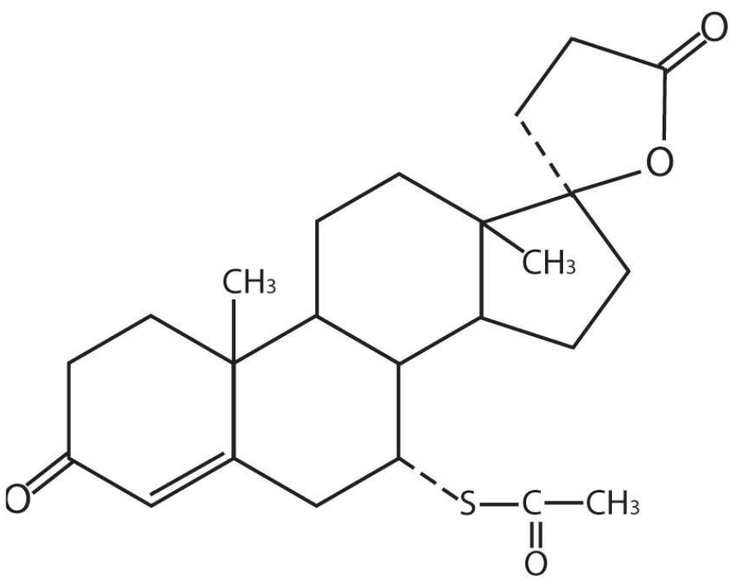
Diuretic drugs that act primarily in the cortical collecting tubule or the CNT and CCD (potassium-sparing diuretics) comprise three pharmacologically distinct groups: aldosterone antagonists (spironolactone and eplerenone), pteridines (triamterene), and pyrazinoylguanidines (amiloride; Fig. 66.10). The site of action for all diuretics of this class



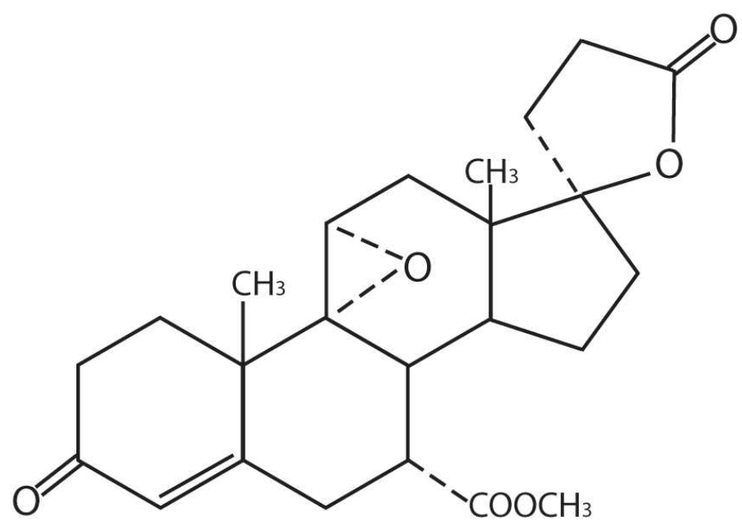
amiloride



triamterene



spironolactone



eplerenone

FIGURE 66.10 Structure of collecting duct diuretics.

is the last part of the distal convoluted tubule (the DCT2), the CNT and the CCD, where they interfere with sodium reabsorption and indirectly potassium secretion (the CNT may be especially important in this regard[299]). Because of the ability to minimize the normal tendency of diuretic drugs to increase potassium excretion, amiloride[300] and triamterene[301,302] are considered potassium sparing. The recently introduced vasopressin V2-receptor antagonists (tolvaptan, mozavaptan, and lixivaptan) also act in the collecting duct and could be categorized as diuretics.[303] These drugs selectively inhibit water reabsorption in the collecting duct and are therefore also sometimes referred to as "aquaretics." Because vasopressin-receptor antagonists are primarily used for the treatment of hyponatremia secondary to the syndrome of inappropriate antidiuretic hormone secretion, heart failure, or liver cirrhosis, these compounds are discussed in more detail in the chapter on hyponatremia.

The diuretic activity of amiloride, triamterene, and aldosterone antagonists is weak partly because fractional sodium reabsorption in the collecting tubule usually does not exceed 3% of the filtered load. Another reason, however, may relate to the tendency for these drugs to produce only partial blockade of Na channels. In support of this hypothesis, knockout or disruption of sodium channel (ENaC) function leads to profound renal salt wasting.[304] Because potassium-sparing drugs are relatively weak natriuretic agents, they are used most commonly in combination with thiazides or loop diuretics, often in a single preparation, to restrict potassium losses and sometimes augment diuretic action. However, in certain conditions potassium sparing diuretics are used as first line agents (Table 66.3). For example spironolactone is used in the treatment of edema in patients with cirrhosis[305] and amiloride or triamterene is used as a first-line treatment of Liddle syndrome.[306,307] These drugs are also used for Bartter syndrome,[308] although this indication is controversial.[309] Mineralocorticoid blocking drugs have become standard parts of the treatment of patients with systolic dysfunction heart failure. Spironolactone reduces mortality of patients with congestive heart failure.[310] Eplerenone was shown to reduce mortality in patients with left ventricular dysfunction following myocardial infarction.[311]

Urinary Electrolyte Excretion

Amiloride, triamterene, and spironolactone are weak natriuretic agents when given acutely (see Table 66.1), although some studies suggest that these drugs are as effective as furosemide in some clinical settings.[305] Additionally, these three diuretic agents decrease hydrogen ion secretion by the late distal tubule and collecting ducts. Evidence that spironolactone decreases hydrogen ion excretion comes from the finding of metabolic acidosis associated with mineralocorticoid deficiency,[312,313] and the finding that spironolactone produces metabolic acidosis in patients with cirrhosis who have mineralocorticoid excess.[314] In rats, the administration of amiloride and triamterene has been shown to inhibit urinary acidification.[300,302] A common mechanism is likely to be

66.3 Indications for Diuretic Drugs

I. Indications for osmotic diuretics
- A. Acute or incipient renal failure, especially owing to heme pigment
- B. To reduce intraocular or intracranial pressure

II. Indications for carbonic anhydrase inhibitors
- A. Glaucoma (*generally outmoded)
- B. Acute mountain sickness
- C. Metabolic alkalosis
- D. Cystinuria
- E. Resistant edema (used in combination with other diuretics)

III. Indications for loop diuretics
- A. Edematous conditions
  1. Congestive heart failure
  2. Cirrhotic ascites
  3. Nephrotic syndrome
- B. Hypercalcemia (*controversial)
- C. Hyperkalemia
- D. Hyponatremia (with hypertonic saline)
- E. Hyperkalemic, hyperchloremic metabolic acidosis (type 4 RTA)
- F. Hypermagnesemia
- G. Intoxications
- H. Hypertension
- I. Acute kidney injury

IV. Indications for distal convoluted tubule diuretics
- A. Hypertension
- B. Edematous conditions
  1. Congestive heart failure
  2. Cirrhotic ascites
  3. Nephrotic syndrome
- C. Nephrolithiasis
- D. Nephrogenic diabetes insipidus
- E. Osteoporosis
- F. Hypoparathyroidism
- G. Diuretic resistance (used in combination with other diuretics)

V. Indications for collecting duct diuretics
- A. Cirrhotic ascites
- B. Lithium-induced diabetes insipidus
- C. Prevention of hypokalemia (owing to potassium-wasting diuretics)
- D. Prevention of hypomagnesemia (owing to potassium-wasting diuretics)
- E. Diuretic resistance (used in combination with other diuretics)

involved in mediating the effects of all three diuretic agents on hydrogen ion secretion. These drugs reduce the lumen-negative potential difference (voltage) and thus decrease the electrochemical gradient favoring hydrogen ion secretion.

Clearance studies in rats have demonstrated that amiloride decreases calcium excretion.[315] In these studies, amiloride produced both a decrease in the Ca clearance-to-Na clearance ratio (C_{Ca}/C_{Na}), as well as a decrease in the fractional excretion of calcium. The effect of triamterene on clearance of calcium was less clear, although it did decrease the C_{Ca}/C_{Na} ratio. In vivo microperfusion of rat distal tubules demonstrated that the effect of chlorothiazide on calcium absorption was enhanced with amiloride, but that amiloride's action was along the "late" distal tubule (probably the CNT) rather than in the true DCT.[316] Furthermore, in vitro perfusion of CNTs has shown that amiloride stimulates calcium absorption.[317] Amiloride is believed to stimulate calcium absorption through its ability to block sodium channels, thereby hyperpolarizing the apical membrane.[318] Hyperpolarization of the apical membrane stimulates calcium entry through hyperpolarization-activated calcium channels, as discussed previously. Amiloride has also been reported to reduce magnesium excretion[236,319] and to prevent the development of hypomagnesemia during therapy with a DCT diuretic.[320]

Mechanism of Action

The site of action of potassium-sparing diuretics is the DCT2, CNT, and collecting duct. Although a great deal of interest has centered on control of Na and K transport by the collecting duct, recent evidence has reemphasized the central role played by the CNT.[212,299] Molecular studies have indicated that sites of DCT diuretic action overlap with sites of CCD diuretic action. Thus, in rat, mouse and human, a transitional segment, with characteristics of both DCT and CNT, is present along the distal tubule. This segment, which may comprise the bulk of the distal tubule in humans, expresses both NCC and the amiloride-sensitive epithelial Na channel.

Although the connecting and cortical collecting tubules reabsorb only a small percentage of the filtered Na load, two characteristics render this segment important in the physiology of diuretic action. First, this nephron segment is the primary site of action of the mineralocorticoid aldosterone, a hormone that controls sodium reabsorption and potassium secretion. Second, virtually all of the potassium that is excreted is due to the secretion of potassium by the connecting and collecting tubules. Thus, this segment contributes to the hypokalemia seen as a consequence of diuretic action.

The collecting tubule is composed of two cell types that have entirely separate functions. Principal cells (collecting duct cells) are responsible for the transport of sodium, potassium, and water, whereas intercalated cells are primarily responsible for the secretion of hydrogen or bicarbonate ions. The apical membrane of principal cells expresses separate channels that permit selective conductive transport of sodium and potassium (Fig. 66.8). Connecting tubule

cells also express apical Na and K channels, permitting electrogenic Na reabsorption and K secretion. The mechanism by which sodium reabsorption occurs is through conductive sodium channels. The low intracellular sodium concentration as a result of the basolateral Na, K-ATPase generates a favorable electrochemical gradient for sodium entry through sodium channels. Because sodium channels are present only in the apical membrane of principal and CNT cells, sodium conductance depolarizes the apical membrane resulting in an asymmetric voltage profile across the cell. This effect produces a lumen-negative transepithelial potential difference. The lumen-negative potential difference together with a high intracellular to lumen potassium concentration gradient provides the driving force for potassium secretion.

Amiloride-sensitive sodium conductance is a function of the epithelial sodium channel (ENaC). The functional channel comprises three homologous subunits, α, β, and γ ENaC[321]; the channel subunit structure has been debated, but the recent crystal structure of a homologous channel, and other work, suggest that it may comprise heterotrimers.[322,323] A number of factors regulate this channel, including hormones such as aldosterone, vasopressin, oxytocin; intracellular signaling elements such as G-proteins and cAMP; protein kinase C; intracellular ions sodium, hydrogen, and calcium[321]; and the cystic fibrosis transmembrane conductance regulator.[324,325] Alterations in systemic acid-base balance[326] and sodium intake[327] have also been shown to regulate ENaC function. Studies that used selective subunit antisera have demonstrated specific regulation of subunit abundance or pattern of expression. In mice adapted to a high sodium diet, the α-subunit was undetectable, and the β- and γ-subunits were expressed in the cytoplasm.[327] In contrast, mice on a low sodium diet displayed subapical or apical expression of all three subunits.[327] Administration of dDAVP to Brattleboro rats increased expression of all three subunits to varying degrees.[328] Long term acid-loading decreases and base loading increases β- and γ-subunits.[326]

The amount of sodium and potassium present in the final urine is tightly controlled by aldosterone action on connecting and collecting duct cells. Extensive studies have demonstrated that in epithelia, aldosterone produces an early increase in sodium conductance[329] followed by a sustained increase in transepithelial sodium transport. As a result, transepithelial sodium transport is increased, an effect that depolarizes the apical membrane. An increase in the lumen negative-potential in turn enhances potassium secretion through conductive potassium channels located in the apical membrane. The cellular mechanisms that are responsible for these events have been extensively studied and reviewed.[330] Aldosterone has been shown to have heterogeneous effects on ENaC subunits. In mammals, aldosterone has been shown to increase the abundance of the α subunit of ENaC,[329] to redistribute all three subunits to the apical region of principal cells,[331] and to induce a shift in the molecular weight of the γ-subunit from 85 kDa to 70 kDa. In contrast, however, a mineralocorticoid knockout mouse demonstrated essentially normal expression of ENaC subunits in the kidney, suggesting aldosterone regulation ENaC function in a posttranscriptional manner.[332] Additional nongenomic activation of ENaC function by aldosterone has been reported.[333]

Mineralocorticoid Receptor Blockers

Spironolactone (Fig. 66.10) is an analog of aldosterone that is extensively metabolized,[334,335] having the principal effect of blocking aldosterone action.[336,337] Spironolactone is converted by deacylation to 7α-thiospironolactone or by diethioacetylation to canrenone.[336] In the kidney, spironolactone and its metabolites enter target cells from the peritubular side, bind to cytosolic mineralocorticoid receptors, and act as competitive inhibitors of the endogenous hormone. In studies using radiolabelled spironolactone or aldosterone, [^{3}H]-spironolactone-receptor complexes were excluded from the nucleus. In contrast, [^{3}H]-aldosterone-receptor complexes were detected in the nucleus.[338] These results are consistent with the proposal that aldosterone antagonists block the translocation of mineralocorticoid receptors to the nucleus. The mechanism by which aldosterone antagonists block nuclear localization of antagonist-receptor complexes is not known; however, it has been suggested that they destabilize mineralocorticoid receptors facilitating proteolysis.[339] Mineralocorticoid receptors, like other steroid receptors, contain a steroid-binding unit associated with other cellular components including HSP90, in its inactive state. Steroid binding produces dissociation of HSP90 from the steroid binding unit uncapping the DNA-binding sites. Spironolactone facilitates the release of HSP90 and in combination with rapid dissociation of ligand could lead to degradation of the receptor.[339]

Spironolactone induces a mild increase in sodium excretion (1%–2%) and a decrease in potassium and hydrogen ion excretion.[340,341] Its effect depends on the presence of aldosterone. Spironolactone is ineffective in experimental adrenalectomized animals[306] and in patients with Addison disease[306] or humans on a high salt diet. In cortical collecting tubules perfused in vitro, spironolactone added to the bath solution reduced the aldosterone-induced lumen-negative transepithelial voltage.[301] By blocking sodium absorption in the collecting tubule, a decrease in lumen negative potential reduces the driving force for passive sodium and hydrogen ion secretion.[301]

Spironolactone causes troubling estrogenic side effects commonly, a fact that constrains its use (see later). Renewed interest in the utility of aldosterone blockers, especially in the setting of congestive heart failure, led to the development of antialdosterone agents that are more specific inhibitors of the mineralocorticoid receptor. Eplerenone is a second competitive aldosterone antagonist, currently in clinical use. Eplerenone (see Fig. 66.10) is Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo, γ-lactone, methyl ester (7α, 11α, 17α), and was derived from spironolactone by the

introduction of a 9α,11α-epoxy bridge and substitution of the 17α-thioacetal group of spironolactone with a carbomethoxy group. In vitro, eplerenone exhibits 10- to 20-fold lower affinity for the mineralocorticoid receptor than spironolactone, but in humans, it appears to be 50% to 75% as potent.[342,343] This change significantly enhances the relative affinity of the drug for mineralocorticoid receptors over other steroid receptors.

Amiloride and Triamterene

Amiloride and triamterene (see Fig. 66.10) are structurally different but are organic cations that use the same primary site of action (see Fig. 66.8). Triamterene is an aminopteridine chemically related to folic acid and amiloride is a pyrazinoylguanidine. Systemically administered amiloride results in an increase in sodium excretion and decrease in potassium excretion.[38] Their actions on sodium and potassium transport, unlike spironolactone, are not dependent on aldosterone. Systemically administered amiloride produced a small increase in sodium excretion and a much larger decrease in potassium excretion.[344,345] Sampling of tubule fluid from the distal tubule demonstrated an inhibition of the normal rise in the tubule fluid to plasma potassium ratio. These results indicated that amiloride decreased distal tubule potassium secretion. Experiments employing in vivo microperfusion of distal tubules[277,346] and in vitro perfusion of isolated cortical collecting tubules[347,348] demonstrated that luminally administered amiloride reduced sodium absorption and potassium secretion. Similar results were obtained following in vivo microperfusion with benazamil,[349] a more potent amiloride analog. Amiloride decreases potassium secretion by blocking ENaC in the apical membrane of CNT and collecting tubule cells[350,351] thereby decreasing the electrochemical gradient for potassium secretion.

In high concentrations (>100 μM), amiloride interacts with different transporters, enzymes, and receptors. At concentrations of 0.05 to 0.5 mM, however, amiloride interacts specifically with ENaC.[352,353] The molecular mechanism by which amiloride blocks ENaC remains incompletely defined. It is likely, however, that the positive charge on the guanidinium moiety plays an important role in occluding the sodium channel (see Fig. 66.8).[353,354]

Several groups, using mutational analysis[355,356] and anti-amiloride antibodies[357] have demonstrated contributions to amiloride binding by all three subunits in close proximity to the channel pore.[358] A putative amiloride binding domain, WYRFHY, of the a-subunit of ENaC has been identified.[357,359]

Clearance and free-flow micropuncture studies using triamterene demonstrated results similar to studies with amiloride[177] although the mechanism of action is not as clearly defined. In earlier studies of rabbit cortical collecting tubules perfused in vitro, triamterene produced a gradual, reversible inhibition of the potential difference after a latent period of 10 minutes. More recent studies, however, suggest that triamterene binds to the epithelial sodium channel and thus has a mechanism of action similar to amiloride.[360]

Pharmacokinetics

Spironolactone is poorly soluble in aqueous fluids. Bioavailability of an oral dose is approximately 90% in some but not all commercial preparations. The drug is rapidly metabolized in the liver into a number of metabolites. Canrenone is one metabolite of spironolactone.[334,335] This conclusion was based on fluorometric assays. Assays of spironolactone and its metabolites by the use of high performance liquid chromatography (HPLC) demonstrated that fluorometrically measured levels of canrenone overestimated true canrenone levels.[361] Using HPLC, the predominant metabolite, 7α-methylspironolactone,[362] appears to be responsible for roughly 80% of the potassium-sparing effect. Spironolactone and its metabolites are extensively bound to plasma protein (98%). In normal volunteers, taking spironolactone (100 mg per day) for 15 days, the mean half-lives for spironolactone, canrenone, 7α-thiomethylspironolactone, and 6β-hydroxy-7α-thiomethylspironolactone were 1.4, 16.5, 13.8, and 15 hours, respectively. Thus, although unmetabolized spironolactone is present in serum, it has a rapid elimination time. The onset of physiologic action is extremely slow for spironolactone, with peak response sometimes occurring 48 hours or more after the first dose; effects gradually wane over a period of 48 to 72 hours. Spironolactone is used in cirrhotic patients to induce a natriuresis. In these patients, pharmacokinetic studies indicate that the half-lives of spironolactone and its metabolites are increased. The half-lives for spironolactone, canrenone, 7α-thiomethylspironolactone, and 6β-hydroxy-7α-thiomethylspironolactone are 9, 58, 24, and 126 hours, respectively.[363]

Eplerenone is rapidly absorbed, with peak serum levels at 1.5 hours.[343] Its volume of distribution is 43 to 90 liters, with approximately 50% protein bound. It is cleared primarily via the CYP4503A4 system to inactive metabolites with an elimination half-life of 4 to 6 hours.[343] This is in contrast to spironolactone, where the half-life of the parent compound is short, but the half-life of metabolites is very long. The maximal plasma concentration and area under the curve are increased in people >65 years of age and with kidney failure; eplerenone is not removed by hemodialysis.[343]

Clinical Use

CCT diuretics can be used for the treatment of hypertension, primary aldosteronism, and secondary aldosteronism; they are also used to limit the kaliuretic effects of loop or DCT diuretics, and sometimes primarily to treat hypokalemia due to renal potassium loss of various causes.

Spironolactone (or eplerenone) plays an important role in four clinical situations. First, it is the treatment of choice in patients with primary aldosteronism (due to bilateral adrenal hyperplasia).[364,365] Second, the drug is especially appropriate for the treatment of cirrhosis with ascites, a condition invariably associated with secondary hyperaldosteronism.[305] In comparison to loop or thiazide diuretics, spironolactone is equivalent or more effective.[366] A combination of loop

diuretic in addition to spironolactone can be used to boost natriuresis when the diuretic effect of spironolactone alone is inadequate, and it has been reported that a ratio of 100 mg spironolactone per 40 mg furosemide carries the best ratio of efficacy/safety.[367] A recent animal study, however, showed that amiloride may also be effective in lowering portal pressure in liver cirrhosis by inhibiting intrahepatic vasoconstriction.[368] A third use of spironolactone is in systolic heart failure, where mineralocorticoid antagonists have been shown to reduce morbidity and mortality.[369] A subsequent study showed that eplerenone reduced morbidity and mortality of patients with left ventricular dysfunction following a myocardial infarction.[370] Most recently, eplerenone, as compared with placebo, reduced both the risk of death and the risk of hospitalization among patients with systolic heart failure and even mild symptoms.[371] Finally, there is growing interest in using spironolactone to treat resistant hypertension, even when demonstrable hyperaldosteronism is not present.[372]

Triamterene or amiloride is generally used in combination with potassium-wasting diuretics (thiazide or loop diuretics), especially when maintenance of normal serum potassium concentrations is clinically important. In addition, amiloride (or triamterene) has also been used as initial therapy in potassium wasting states such as primary hyperaldosteronism,[373,374] Liddle,[307] Bartter, or Gitelman syndrome,[308] although, as noted previously, use in the latter situation has been disputed.[309] Amiloride is recommended to treat lithium-induced nephrogenic diabetes insipidus.[375] The efficacy of amiloride in this disorder relates to the ability of amiloride to block collecting duct sodium channels, a pathway which lithium uses to gain entry into cells. Recently, a small placebo-controlled cross-over trial[376] and an animal study[377] confirmed these effects.

Adverse Effects

The most serious adverse reaction encountered during therapy with spironolactone is hyperkalemia. Serum potassium should be monitored periodically even when the drug is administered with a potassium-wasting diuretic. Patients at highest risk are those with low GFRs, patients with concurrent medication predisposing to hyperkalemia, and individuals who take potassium supplements concurrently. This problem has become more important because of the wide use of aldosterone blocking drugs, together with ACE inhibitors, ARBs, and beta-blockers in patients with congestive heart failure.[378] Risk factors include kidney failure, older age, coexistent diabetes mellitus, and concomitant treatment with beta-blockers.[379] It is important to note that the original RALES study specifically excluded patients with several of these comorbidities. Renal failure appears to be another complication in this group.[379] Another group at risk for hyperkalemia are elderly patients receiving chronic treatment with spironolactone who are intermittently treated with trimethoprim-sulfamethoxazole for a urinary tract infection.[380] Surprisingly, however, another recent population-based study showed that despite a marked increase in the use of spironolactone, no increase was seen in hospital admissions for hyperkalemia and that outpatient hyperkalemia actually fell; the authors ascribed these findings to more careful monitoring.[381]

In patients with cirrhosis and ascites treated with spironolactone, hyperchloremic metabolic acidosis can develop independent of changes in renal function.[314] Gynecomastia may occur in men, especially as the dose is increased[382] but even at low doses[369]; decreased libido and impotence have also been reported. Women may develop menstrual irregularities, hirsutism, or swelling and tenderness of the breast. Spironolactone-induced agranulocytosis has also been reported.[383]

Triamterene and amiloride may also cause hyperkalemia. The risk of hyperkalemia is highest in patients with limited renal function (e.g., renal insufficiency, diabetes mellitus, and elderly patients). Additional complications included elevated serum blood urea nitrogen and uric acid, glucose intolerance, and gastrointestinal disturbances. Triamterene induces crystalluria or cylindruria[384] and may contribute to or initiate formation of renal stones[385] and acute kidney injury when combined with NSAIDs.[386,387] The drugs are contraindicated in patients with hyperkalemia, individuals taking potassium supplements in any form, and in patients with severe renal failure with progressive oliguria.

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