TUBULAR SITES AND MECHANISMS OF DIURETIC ACTION[1]

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In its strictest sense diuresis is a term that implies increase in urine flow, and diuretics those agents that effect this change. In the present review, however, diuretics will be defined as clinically useful pharmacological agents which induce a *net loss* of water and sodium in the urine. In bringing about this change a number of renal and extrarenal systems and functions may be altered. Within the kidney itself a number of complex vascular, humoral, and biochemical events may ensue. Although virtually all diuretics in common use alter the GFR their actions are generally independent of it. Inhibition by diuretics of tubular reabsorption of sodium and water, therefore, may be inferred. This review pertains to the current concepts of the tubular actions of diuretics and the evidence on which they rest.

TYPES AND POTENCY OF DIURETICS

The past two decades have witnessed the emergence of a large number of agents that alter the reabsorption of electrolytes by the renal tubules. These agents vary widely in their chemical structure, potency, and the pattern of electrolyte excretion they induce. Consequently, a number of classifications have been proposed based on structure, potency, or mechanism of action. No one classification is entirely satisfactory. For convenience we propose to group these agents in classes that take all of these variables into account. Thus, the diuretics in common use may be classified for the purposes of discussion as follows: (*a*) organomercurials, (*b*) carbonic anhydrase inhibitors, (*c*) sulfonamides, also called thiazides although not all members of this group are thiazides e.g. chlorthalidone, (*d*) potassium-sparing diuretics, and (*e*) high-ceiling diuretics, a term that describes a group of highly potent drugs with relatively steep dose-response relationship and includes agents such as furosemide and ethacrynic acid.

[1] The following abbreviations shall be used throughout this review: GFR = glomerular filtration rate; ADH = antidiuretic hormone; ECF = extracellular fluid; $T^c_{H_2O}$ = tubular reabsorption of solute-free water; C_{H_2O} = clearance of solute-free water; V = urine flow rate; C_{Na} = clearance of sodium; C_{osm} = osmolal clearance; TF/P = the ratio of concentration in the tubular fluid to that in plasma; AMP, ADP, and ATP = adenosine mono-, di-, and tri-phosphate; ATPase = adenosine triphosphatase; Na^+, K^+-ATPase = sodium, potassium-activated adenosine triphosphatase.

To understand the basis for the different characteristics and potency of the diuresis induced by these agents it is necessary to determine the site or sites in the nephron where individual agents act and the mechanisms of such action. The stop-flow technique introduced in 1958 by Malvin, Wilde & Sullivan (1) was utilized extensively for this purpose. It was subsequently shown, however, that this technique suffered from major interpretational pitfalls (2). The discovery by Wirz, Hargitay & Kuhn (3) of the countercurrent mechanism for urine dilution and concentration provided another rational basis for the study of the pattern of water and electrolyte excretion for the localization of diuretic action in the nephron. Finally, the revival by Gottschalk & Mylle (4) of the technique of micropuncture provided investigators with yet another tool to study the tubular mechanisms of diuretic action.

Studies of Water and Sodium Excretion

A brief review of the events taking place in the normal nephron and the theoretical alterations that occur should be of value in understanding the data on diuretics to be reviewed subsequently. Depending on the species and the state of hydration, about 50–70% of the glomerular filtrate is reabsorbed isosmotically in the proximal tubule (5–11); the remaining fluid enters the loop of Henle. In the descending limb, water is lost to the hypertonic medullary interstitium and the fluid at the tip of the loop is hypertonic (4, 6, 12–15). At or near the bend of the loop the tubular cells probably begin to reabsorb sodium; this process continues throughout the length of the ascending limb (about 25% of the filtered load) to the practical exclusion of water resulting in the emergence of dilute fluid (4, 12–16). The reabsorption of sodium in this segment provides the single effect that sets into motion the countercurrent multiplier system responsible for the establishment of the osmotic gradient in the renal medulla from the corticomedullary junction to the papillary tip. Sodium reabsorption continues in the late ascending limb and early distal convoluted tubule, resulting in the further generation of water unobligated by solute. In most of the remainder of the distal convoluted tubule, however, sodium reabsorption provides the driving force for hydrogen ion and potassium secretion into the tubular lumen, and in the dog relatively little solute-free water is generated (17) or lost (18). In dog (18), monkey (19), and other species (20–22) the tubular fluid leaving the distal convoluted tubules remains hypotonic. At low flow rates, the fluid in the collecting ducts equilibrates in the presence of ADH with the surrounding interstitium and the urine emerges concentrated (4). In the absence of ADH water continues to be lost to the medullary interstitium because of the high osmolar gradient between lumen and interstitial space (23, 24). The fact that this obligatory water loss is small relative to the volume presented, and that solute is reabsorbed in the collecting duct contributes to the final excretion of a dilute urine (14, 24, 25).

Reabsorption in the proximal tubule has been well studied and shown to vary in response to changes in the ECF volume (7–9, 26–28). Regulation of absorption in the loop of Henle has not been as well studied; it is known from clearance and micropuncture studies, however, to possess a high reabsorptive capacity for

sodium when the delivery of filtrate to it is increased (8, 9, 26, 29, 30). Increased absorption of sodium in the loop of Henle increases $T^c_{H_2O}$ in the hydropenic animal (31) and C_{H_2O} in the hydrated animal (32). Because of the dependence of these functions of the loop on the rate of delivery of filtrate to it, an estimate of delivery is crucial in assessing these changes. Urine flow rate has been used as an index of delivery during water diuresis because of the small and relatively constant volume of water back-diffusion from the descending limb and the collecting duct. In the presence of unreabsorbed solute such as glucose or mannitol, V becomes an overestimate of the rate of delivery of sodium to the ascending limb of Henle's loop. A better index of delivery under these circumstances is the sum of C_{Na} and C_{H_2O} based on the assumption that sodium leaving the proximal tubule is either excreted, or reabsorbed forming solute-free water (33). It is worth emphasizing that these indices underestimate delivery by a volume equivalent to the obligatory water back-diffusion. Unfortunately, no truly good index of delivery exists in hydropenia and C_{osm}; a measure of the solute escaping reabsorption in the loop of Henle is employed instead. To recapitulate, inhibition of sodium reabsorption in the proximal tubule would be expected to increase V and C_{H_2O} in the hydrated animal, and C_{osm} and $T^c_{H_2O}$ in the hydropenic animal, in a normal proportion. Inhibition of the reabsorption of sodium in the ascending limb of Henle's loop during hydration should lower C_{H_2O} at any level of V. Because only the portion of the ascending limb in the renal medulla is involved in the concentrating mechanism, inhibition of this segment (medullary diluting segment) impairs $T^c_{H_2O}$ at any level of C_{osm}. Inhibition of the cortical portion of the ascending limb (cortical diluting segment) would not be expected to affect adversely the urinary concentrating process but should lower C_{H_2O} at any V (34).

Action in the proximal tubule.—An increase in V during water diuresis has been reported with the use of the organomercurials (34–38), the carbonic anhydrase inhibitors (39–41), and the high-ceiling diuretics furosemide (34, 40, 42–45), ethacrynic acid (34, 40, 43–48), and triflocin (49, 50). The potassium-sparing agents produce no effect on V (44) and the effects of the thiazides are equivocal, some studies reporting an increase (39, 42, 51, 52) and others no change (44, 53, 54).

Changes in V may be difficult to interpret. First, the diuretic may contain additives, which may affect this parameter. This is the case with preparations of meralluride, which contain aminophylline. This xanthine may exert a proximal effect that could be mistakenly attributed to the mercurial (35, 38). Other organomercurial compounds also increase V, however, and it appears reasonably certain that this effect is specifically that of this group of compounds (36–38). Second, in most experiments reported the urinary losses of water and salt induced by the diuretic are not carefully replaced. The shrinkage of ECF volume that may ensue could influence the data in one of several ways: (*a*) ADH may be stimulated, resulting in increased water back-diffusion in the distal nephron and obscuring any proximal effect of the diuretic agent, (*b*) fractional reabsorption in the proximal tubule may be increased (9, 10) overcoming any effect of the

diuretic agent, and (*c*) GFR may fall, thereby obliterating any effect of the diuretic; when V is expressed as a fraction of the filtered load, however, an increase may then become apparent (39). When these factors are taken into consideration, thiazides may be shown also to increase V (39). The final problem in the interpretation of changes in V concerns the ability of V to reflect changes truly in proximal tubular reabsorption. Since water is lost both from the descending limb of Henle's loop and from the collecting duct during water diuresis changes in the osmotic driving forces may alter the volume of water backdiffused. Thus agents that inhibit sodium reabsorption in the ascending limb of Henle's loop (vide infra) diminish the medullary osmotic gradient and decrease the loss of water out of the descending limb. Inhibition of sodium reabsorption in the ascending limb (even in the cortical diluting segment), also raises tubular fluid osmolality in the distal convoluted tubule and diminishes water loss out of the collecting duct. Urine flow rate may increase, therefore, even though the proximal tubule may not have been inhibited. An action in the proximal tubule, therefore, may be safely inferred only for agents that produce a considerable increase in V such as the high-ceiling diuretics, or for agents that have little effect on the loop of Henle such as the carbonic anhydrase inhibitors.

Action in the loop of Henle.—A decrease in $T^c_{H_2O}$ and C_{H_2O} following the administration of organomercurials has been reported by some (55–60). Others report no change (35–38, 61). The decrease in $T^c_{H_2O}$ is particularly marked in acidotic animals (55, 56). The reason for the discrepancy in these results is not readily apparent but may be due to the combined effect of these agents on the proximal tubule and on the loop of Henle. Increasing delivery of filtrate out of the proximal tubule may increase, while inhibition of sodium reabsorption in the ascending limb may decrease C_{H_2O} and $T^c_{H_2O}$, the net result being no change. When examined over a wide range of V and C_{osm}, however, C_{H_2O} and $T^c_{H_2O}$ appear to be uniformly reduced in comparison to the normal relationship established with hypotonic or hypertonic saline or mannitol (34, 35, 55). Thus it seems reasonable to conclude that organomercurials do exert an effect on the ascending limb of Henle's loop.

The actions of carbonic anhydrase inhibitors on the loop of Henle are somewhat unique. Although C_{H_2O} has been shown to increase following the administration of these agents (40), it is lower than normal especially at high rates of V or of C_{osm} (39, 41). $T^c_{H_2O}$ has also been noted to decrease (55). This does not appear to be the consequence of inhibition of sodium reabsorption in the loop of Henle but the result instead of the delivery to this segment of $NaHCO_3$, a relatively nonreabsorbable solute (41). This conclusion is supported by the fact that the infusion of $NaHCO_3$, like mannitol, produces a degree of reduction of C_{H_2O} relative to V similar to that produced by acetazolamide (41).

The sulfonamide diuretics produce either no change or a decrease in C_{H_2O} (39, 42, 44, 51–54). When compared to V, however, C_{H_2O} is uniformly but modestly reduced (34, 42, 52). No change is seen in $T^c_{H_2O}$ at low levels of C_{osm} (39, 42, 52, 54). At high C_{osm}, however, T_{H_2O} is increased (39, 42, 52). This is a

unique effect of this group of agents and may be due to the delivery of less hypotonic fluid to the collecting duct and therefore the earlier achievement of isotonicity. Since $T^c_{H_2O}$ measures the reabsorption of solute-free water beyond the point of isotonicity, this results in a greater calculated value for $T^c_{H_2O}$ even though the total amount of water reabsorbed may be unchanged or reduced (because of lower osmotic gradient). Thus, the partial inhibition of C_{H^2O} with an unchanged concentrating mechanism suggests that sulfonamides exert their effect in the cortical diluting segment.

High-ceiling diuretics severely impair the concentrating mechanism and virtually abolish $T^c_{H_2O}$ (34, 42, 43, 46–50, 62). Ethacrynic acid and triflocin also produce a consistent reduction of C_{H_2O} (34, 40, 43–50). Furosemide, on the other hand, may increase or decrease C_{H_2O} (34, 40, 42–45). When related to V, however, there is a clear-cut and marked reduction in C_{H_2O} (34, 42). This effect of furosemide, like the mercurials, appears to be due to a proximal effect that tends to increase C_{H_2O} and an effect in the loop of Henle that decreases it. An increase in C_{H_2O} may be observed, therefore, when the rate of delivery and C_{H_2O} are low, and a decrease when both are high (42). The inhibition by high-ceiling diuretics of both dilution and concentration indicates a site of action in the ascending limb of Henle's loop. The observation that $T^c_{H_2O}$ may be abolished while some residual C_{H_2O} remains suggests that the action of these agents is restricted to the medullary diluting segment.

The conclusion that organomercurials, furosemide, ethacrynic acid, and triflocin exert an effect in the medullary segment of the ascending limb of Henle's loop is supported by the observation that these agents lower the gradient for sodium from cortex to medulla or to papilla (48, 56, 63, 64).

The above conclusions are concerned only with the major sites of action of the diuretics discussed and do not exclude additional action in the distal convoluted tubule and collecting duct. For instance, organomercurials seem to work in the distal convoluted tubule to inhibit potassium secretion (65, 66) and so do the potassium-sparing diuretics (67, 68). Furthermore, it is important to recognize that an action in the proximal tubule does not necessarily imply a high diuretic potency since the loop of Henle possesses a high capacity for sodium absorption and can blunt the action of such diuretics as the sulfonamides unless it is inhibited too.

Studies of Other Electrolytes

Parallel changes in the renal handling of sodium, phosphate, calcium, and magnesium have been demonstrated under a variety of experimental and clinical conditions (69–73). Diuretics increase the excretion of these ions and alter their relationship to sodium excretion. These changes are not a simple function of the intrinsic capacity of diuretics to inhibit sodium transport, but depend on a number of other factors. First is the nephronal site of action of the agent relative to the site of reabsorption of phosphate, calcium, and magnesium. A second factor is the shrinkage of ECF volume induced by these agents and its effect on the renal handling of these ions. Acute or chronic shrinkage of ECF volume may

lead to enhanced proximal tubular reabsorption and reduce the excretion of these ions to the extent that they may be absorbed in the proximal tubule (74–76). A third factor is change in the blood levels of phosphate, calcium, and magnesium resulting from urinary losses and the alteration in circulating levels of parathormone that may follow (77, 78).

Studies on phosphate.—Micropuncture study of inorganic phosphate handling by rat and dog kidney indicates that its reabsorption takes place largely in the proximal tubule (78, 79) although a small fraction may be reabsorbed distally (80). Recently it has been shown in the dog that the infusion of a moderate saline load results in a 35–40% inhibition of proximal tubular fractional sodium and phosphate reabsorption (78). The bulk of the phosphate rejected proximally appears in the urine while sodium excretion rises minimally, consistent with the presence of reabsorptive sites in the distal nephron for sodium but not phosphate (78). Changes in phosphate excretion induced by diuretic agents, therefore, may be used to infer changes in proximal tubular sodium reabsorption (40, 77).

Intravenous ethacrynic acid and furosemide result in an increase in the clearance of both phosphate and sodium in dog (77) and man (40, 75). In man, when urinary losses are not replaced, ethacrynic acid-induced phosphaturia is abolished while that of furosemide, although still present, becomes blunted (75). This observation may explain the failure of others to demonstrate the phosphaturic action of these agents when urinary losses were not replaced (40, 45, 81).

The suggestion has been made that alkalinization of the urine increases phosphate excretion (82, 83). Presumably this is the result of an increase in alkaline tubular fluid of the concentration of dibasic phosphate ion which is more polar and, therefore, less well absorbed (83). This mechanism has been invoked to explain the phosphaturic effect of bicarbonate infusion and acetazolamide administration (82, 83). Sodium bicarbonate and acetazolamide inhibit proximal tubular reabsorption[2] and increase sodium excretion which in itself could result in phosphaturia (10). Furthermore, ethacrynic acid and mercurials that have no carbonic anhydrase-inhibitory activity and do not alkalinize the urine also increase phosphate excretion. The greater increase of phosphate excretion relative to that of sodium induced by chlorothiazide and acetazolamide may be the consequence of the reabsorption of sodium in the loop of Henle where these agents have little or no effect.

Studies of calcium and magnesium.—The bulk of calcium and magnesium reabsorption occurs in the proximal tubule (86–88). Calcium is also absorbed in the loop of Henle (86) while magnesium is reabsorbed in the distal convoluted tubule (89). Micropuncture studies have shown that the ratio of tubular fluid to plasma concentration of sodium, calcium, and magnesium is close to unity at the end of the accessible portion of the proximal tubule (86–89). Since the

[2] The first by volume expansion and the second by carbonic anhydrase inhibition (26, 84, 85).

major fraction of the filtered load is reabsorbed at this site, inhibition of proximal reabsorption of these ions should result in their excretion in similar proportion unless there is distal dissociation of their transport. Dissociation of calcium and sodium reabsorption in the distal nephron has been shown by several investigators (76, 78, 90, 91). Adrenalectomy, mineralocorticoids, and parathyroid hormone alter the relationship of calcium to sodium excretion (76, 78, 90, 91). The effect of diuretics on the excretion of these ions, therefore, will depend on whether they act at a site where these ions are reabsorbed in association with sodium, or at a site where their reabsorption is dissociated.

Increased calcium excretion during the acute administration of ethacrynic acid, furosemide, mercurials, chlorothiazide, and acetazolamide has been shown (40, 45, 74, 77, 92–95). The absolute increase in calcium excretion induced by chlorothiazide and acetazolamide, however, is at best minimal despite a significant increase in the excretion of sodium (74, 77, 81, 94–98). Should these agents inhibit proximal reabsorption but have no effect on the medullary part of the loop of Henle, the calcium delivered out of the proximal tubule will be reabsorbed at this site and little or no calciuresis would result. Chlorothiazide also acts on a distal reabsorptive site for sodium, which may not be shared by calcium. Recently, direct evidence for a differential effect on distal tubular calcium and sodium transport was presented by micropuncture studies (88). A highly significant correlation between early distal TF/P sodium and calcium was shown during hydropenia indicating parallel sodium and calcium reabsorption in the loop of Henle. Saline infusion did not alter this relationship. After thiazides, however, mean fractional rejection of sodium increased by 6% while that of calcium was not significantly altered. This differential effect on reabsorption of sodium forms the basis of the therapeutic usefulness of thiazides in patients with hypercalciuria (76, 99, 100). The reduction in calcium excretion in these patients may result from increased excretion of sodium without calcium, shrinkage of the ECF volume, and increased proximal tubular reabsorption of sodium and calcium (76, 95). In contrast to thiazides, the mercurials, furosemide and ethacrynic acid, which in addition to a proximal action exert an effect in the loop of Henle, result in a significant increase in the excretion of calcium. This may explain why furosemide does not correct hypercalciuria (100).

The effect of diuretics on magnesium has been less well studied. Diuretics have been shown to increase magnesium excretion. The increase in fractional clearance of magnesium is similar to that of calcium (77, 81, 92–95). Hypomagnesemia complicating diuretic administration has been reported (101).

Uric acid.—Uric acid is freely filtered, reabsorbed, and secreted (102, 103). While no definite proof exists that secretion is involved in the excretion of uric acid, several lines of evidence support this hypothesis. Experiments using stop flow in the dog show that pyrazinamide inhibits a secretory mechanism that seems to be located in the distal tubule in the mongrel dog (104). The effect of pyrazinamide, however, is complex because it may also stimulate the active tubular reabsorption of urate (105). Most diuretics are organic acids and may

exert a competitive action on other organic acids, such as uric acid, in the proximal tubule. It is apparent then that interpretation of the effect of any diuretic on uric acid excretion is fraught with interpretational difficulties.

Hyperuricemia is a frequent complication of thiazide therapy. This has been attributed to competition for secretory sites by thiazides, which are organic acids (106). An alternate mechanism has been proposed by Suki, et al (76), who demonstrated that thiazide-induced hyperuricemia is corrected by replenishment of salt deficits despite continued administration of thiazides, and that hyperuricemia may be induced in normal subjects by low salt diets alone. The hyperuricemia may be attributed, therefore, to ECF volume contraction with secondary enhancement of urate reabsorption rather than inhibition of secretion (76, 107). This hypothesis is supported by the observation that furosemide and ethacrynic acid given intravenously are uricosuric when diuretic-induced salt and water losses are replaced continuously; if fluid losses are not replaced, uric acid excretion decreases sharply within two hours (108).

Localization by Micropuncture

To determine more directly the sites and mechanisms of diuretic action in the nephron, investigators have utilized micropuncture techniques. While general agreement exists with respect to the effects of diuretics on the distal nephron, the presence or absence of a net reduction of sodium reabsorption in the proximal tubule remains highly debated.

Action in the proximal tubule.—Studies designed to demonstrate inhibition of sodium reabsorption in the proximal tubule suffer from several major difficulties: (*a*) when the urinary losses of sodium and water go unreplaced, ECF volume shrinks and fractional reabsorption increases (9, 10) tending to mask any depression that may be produced by diuretics, (*b*) significant reduction of GFR, whether occurring spontaneously due to increased intratubular pressure or brought about by decreased ECF, itself increases fractional reabsorption (109, 110), and (*c*) the increased intratubular pressure may result in the inadvertent retrograde collection of tubular fluid with high concentration of inulin, thereby spuriously elevating fractional reabsorption (111).

In a study in which urinary losses were not replaced and GFR fell, Dirks, Cirksena & Berliner reported an increase in proximal reabsorption after chlorothiazide (84). In the same study acetazolamide produced no change (84) while in another conducted under comparable conditions it increased proximal reabsorption (112). On the basis of these studies, therefore, an effect of thiazides or carbonic anhydrase inhibitors on the proximal tubule cannot be excluded.

The earliest studies on furosemide revealed a small depression of proximal tubular fractional reabsorption in experiments in which GFR was significantly reduced (113, 114) but not when the GFR was relatively well maintained (113). Given without replacement of urinary losses, furosemide resulted in an increase in proximal fractional reabsorption (84), and given against the background of a

saline load it was without effect (115). In more careful studies in which volume was replaced and retrograde contamination of collected fluid was avoided, furosemide was found to inhibit sodium and water reabsorption (111) in the rat but not in the dog (19, 116). Like furosemide, ethacrynic acid had no effect when GFR fell moderately (84, 117) or when it was given against the background of a salt load (84); it increased proximal reabsorption when urinary losses were not replaced (84) or GFR fell markedly (117). Only when GFR is stable can an effect be demonstrated in the proximal tubule of both dog (117) and rat (118). Thus, under the proper conditions both furosemide and ethacrynic acid appear to inhibit sodium reabsorption in the proximal tubule.

The organomercurials also raise proximal reabsorption when fluid and electrolyte losses are not replaced (84), or leave it unchanged (119). Proximal reabsorption is not changed when mercurials are given against a salt load (84). Whether these agents exert an effect on the proximal tubule surely cannot be ascertained from the present studies.

Action in the loop of Henle.—Although incomplete, studies on the effects of diuretics on the loop of Henle are in general agreement.

Chlorothiazide has been reported to increase the tubular fluid sodium concentration in the early distal convoluted tubule (120) and also to increase osmolality (121), suggesting an action on the loop of Henle. Acetazolamide produced only a slight increase in tubular fluid osmolality (121). Thus thiazides do exert an effect on the loop of Henle while carbonic anhydrase inhibitors have a very small effect.

Furosemide has been reported to increase markedly sodium (120) and chloride (114) concentration and the osmolality (121) of early distal tubular fluid. It also decreases the reabsorption of sodium and water in the loop of Henle (17, 19, 113–115, 122). Thus furosemide exerts a definite and major effect on the loop of Henle. No such studies have been done with ethacrynic acid.

Organomercurials also increase the osmolality (121) and the sodium concentration of distal tubular fluid and the delivery of sodium and water (119) into it. Although an effect on the loop of Henle may be concluded, this effect appears intermediate in its potency between the thiazides and furosemide.

Action in the distal convoluted tubule and collecting duct.—Furosemide appears to have small or no effect on the distal convoluted tubule. As a matter of fact, sodium reabsorption in this segment appears to be increased in the rate because of the large delivery of filtrate to it (122). Furosemide also greatly reduces water absorption from the collecting duct, most likely because of the decreased osmotic gradient in the medulla (114, 115); in high doses it also seems to reduce sodium reabsorption in the collecting duct (115).

Organomercurials reduce potassium delivery to the distal convoluted tubule and reduce the high concentration of potassium relative to plasma in potassium-loaded rats (119). Thus mercurials appear to inhibit potassium secretion in the distal tubule.

Amiloride also decreases potassium secretion in the distal convoluted tubule but has no perceptible effect on sodium absorption in this segment (122).

It appears from the foregoing discussion that wherever a definite conclusion can be drawn, the results of micropuncture studies appear consonant with the conclusions derived from studies utilizing the pattern of water, sodium, and electrolyte excretion to localize the sites of action of the diuretics in the nephron.

Cellular Metabolic Alterations

Alterations in cellular metabolism seem to be a likely mechanism by which diuretics interfere with sodium reabsorption across renal epithelial membranes. The bulk of renal sodium reabsorption is principally dependent on oxidative metabolism, although there is some evidence that anaerobic processes also play a role, particularly in outer and inner medulla (123–126). Production of ATP from either of these two forms of metabolism is important for the proper function of membrane ATPase, which appears to be intimately related to the sodium transport process in the kidney (127, 128). This enzyme system is mainly associated with sodium reabsorptive processes in the outer medulla and its specific in vivo inhibition by cardiac glycosides in experimental animals leads to profound depression of C_{H_2O} and $T^c_{H_2O}$ and marked natriuresis (129–132). Since the natriuretic effect of the cardiac glycosides is exerted principally in the ascending limb of the loop of Henle, diuretics such as ethacrynic acid, furosemide, and the thiazides having their principal action at this site, may be candidates for inhibition of Na^+, K^+-ATPase.

Ethacrynic acid inhibits renal Na^+, K^+-ATPase of a variety of species in vitro (133–135). The drug blocks phosphorylation of the enzyme, prevents the ADP-ATP exchange reaction, and leads to stabilization of the spontaneous disappearance of the phosphorylated intermediate. This last effect of ethacrynic acid in the steps of enzyme turnover is different from that of cardiac glycosides and has been suggested to account for its diuretic action in vivo (136). The recent reports by Duggan & Noll (137) and by Nechay & Contreras (138) that in vivo administration of the diuretic to the dog is associated with enzyme inhibition, give credence to the possible involvement of membrane ATPase in the mechanism of action of ethacrynic acid. Whether in vivo inhibition of the Na^+, K^+-ATPase is responsible for the diuretic effect of ethacrynic acid, however, is still unclear. First, binding of radioactive ethacrynic acid to membrane fractions in the dog is 1000–2000 fold less than the binding required for 50% inhibition of the enzyme (139). Second, high doses of ethacrynic acid lead to natriuresis in rabbits without demonstrable inhibition of the enzyme (140). Third, in extensive studies in the dog, Inagaki, Martinez-Maldonado & Schwartz (unpublished observations) have been unable to reproduce the results of Duggan & Noll (137) and Nechay & Contreras (138). The reasons for the different results are not apparent, because enzyme isolation and assay, and dose of drug utilized, were comparable in these studies. Despite this conflict, it appears clear that the diuretic may interact in vivo with the enzyme in an as yet unidentified manner. Nechay et al (139) and Martinez-Maldonado et al (141) have demonstrated that prior

administration of cardiac glycosides blunts or prevents the natriuretic effect of ethacrynic acid; conversely, ethacrynic acid reduces or abolishes the natriuretic effect of the glycoside. Furthermore, ethacrynic acid reduces the metabolic rate of the medulla in vivo, an effect that is abolished by prior ouabain administration (142). At present, these results are the best evidence that Na^+, K^+-ATPase may be involved, although in a manner different from cardiac glycosides, in the mechanism of action of ethacrynic acid.

Other aspects of cellular function may be inhibited by ethacrynic acid: alterations in membrane permeability and/or a direct effect on cellular energy metabolism could result from its administration. An effect of ethacrynic acid on energy metabolism has been demonstrated in vitro (143–157). Concentrations of ethacrynic acid ranging between 0.1–1.0 mM depress respiration and decrease intracellular K^+ concentration of kidney slices from rat, rabbit, and dog. Furthermore, ethacrynic acid inhibits the respiration of isolated kidney mitochondria, the major site of ATP formation in cells. These effects of ethacrynic acid could result in reduced substrate for Na^+, K^+-ATPase and interference with its normal activity. This suggestion is strengthened by the observation that membrane ATPase-stimulated mitochondrial respiration is inhibited by doses of ethacrynic acid (0.05 mM) which do not inhibit ATPase in vitro (145). Furthermore, increased levels of ADP in the incubation medium reverse the inhibition of mitochondrial respiration in the presence but not in the absence of membrane fractions (145). On the other hand, inhibition of respiration of mitochondria isolated from cortical tissue could not be demonstrated following the in vivo administration of ethacrynic acid (10 mg/kg) to rabbits (140). Moreover, glycolysis of a cell cytoplasmic fraction of rabbit kidney cortex and medulla was not altered by 0.2 mM ethacrynic acid. These results have been interpreted by Landon & Fitzpatrick (140) as evidence that, in vivo, ethacrynic acid does not have a primary action on cellular metabolism. Alternative explanations for these results may be advanced. First, ethacrynic acid may be lost from some critical site in the cell during isolation of cell fractions. Charnock & Almeida (148) have shown that guinea pig kidney cortical slices can accumulate ethacrynic acid against a concentration gradient and that this process is blunted by reducing temperature of incubation, omission of glucose from the incubation medium, anoxia, metabolic inhibitors, and ouabain. Although these studies did not provide information about the specific cellular or intracellular site at which the drug was concentrated, this could have been any intracellular structure. Thus, one cannot rule out the possibility that in the experiments of Landon & Fitzpatrick (140) in vivo alterations of mitochondrial respiration took place which were not demonstrable in vitro. Second, most of Landon & Fitzpatrick's experiments were done on cortical slices, while the major site of action of ethacrynic acid is the medulla (34). Moreover, since two distinct mitochondrial populations appear to exist in both cortex and medulla (149) the possibility of a different response to ethacrynic acid by each population needs to be explored. In fact, preliminary data of Martinez-Maldonado & Schwartz indicate that medullary mitochondria may be more sensitive to ethacrynic acid than cortical mitochondria (unpub-

lished observations). Third, although the glycolysis of a cortical cell cytoplasm fraction was not perturbed, this tissue in vivo does not depend primarily on glycolysis for its function (123). In addition, although glycolysis was also not inhibited in medullary[3] cell cytoplasm fractions the incubation under 100% O_2 does not simulate the conditions that exist in this portion of the kidney in vivo (150). Evidence for an inhibitory effect of ethacrynic acid on glycolysis in kidney slices has been advanced by Levin & Cortes (151) who also showed that medulla is more sensitive in this respect than cortex. Furthermore, lactate formation is decreased by 1 mM ethacrynic acid in cell-free medullary preparations (147).

Experiments conducted in dogs also suggest that ethacrynic acid may have an effect on metabolism in vivo. Washington & Holland (153) have shown that during saline or osmotic diuresis urinary P_{O_2} is reduced. Since both saline and osmotic diuresis result in increased sodium delivery to and enhanced sodium reabsorption in the ascending limb, these authors proposed that the drop in urine P_{O_2} results from enhanced oxygen consumption for the reabsorptive process. The reduced urine P_{O_2} was returned to normal by ethacrynic acid, which also raised urine P_{O_2} during hydropenia. Since ethacrynic acid inhibits sodium reabsorption principally in the medullary segment of the ascending limb of Henle's loop, the rise in urine P_{O_2} was attributed to inhibition of active sodium transport and, therefore, oxygen consumption at this site. More direct evidence for an in vivo effect of ethacrynic acid on metabolism has been obtained by Aukland, Johannesen & Kiil (154). Utilizing fine thermocouples inserted into the outer medulla they demonstrated that ethacrynic acid markedly reduced local metabolic rate as estimated from the changes in local temperature. Wolf, Bieg & Fülgraff (155), on the other hand, have reported that during massive natriuresis induced by ethacrynic acid whole-kidney oxygen consumption remains unchanged, and advanced this as evidence that this agent does not directly inhibit sodium reabsorption in vivo but that the natriuresis is due to changes in membrane permeability. Measurements of whole-kidney oxygen consumption, however, are difficult to interpret because: (*a*) these studies have major technical pitfalls, (*b*) total renal O_2 consumption is the sum of basal and transport-associated O_2 consumption each of which may vary independently, and (*c*) the O_2 cost of reabsorption may vary from one segment of the nephron to another.

Another possible cellular mechanism of action of ethacrynic acid is its inhibition of protein-bound sulfhydryl groups (156). Although a critical discussion of this interesting problem is beyond the scope of this review, it should be pointed out that inhibition of sulfhydryl groups by other compounds may occur without an increase in sodium excretion (157).

Finally, changes in the permeability of tubular cell membranes may occur after ethacrynic acid administration allowing back leak of reabsorbed fluid and

[3] No mention is made whether outer or inner medullary tissue or both was utilized in these experiments. Since these two tissues have different metabolic profiles and capacities (123–126) and different content of mitochondria (152), this factor may have been critical.

solute into the tubular lumen. Although this possibility has been suggested (155, 158), direct evidence for it is lacking. In isolated anuran membranes, ethacrynic acid inhibits the natriferic and hydro-osmotic effects of vasopressin but not those of caffein and cyclic AMP (159). This effect is independent of SH-group inhibition: ethacrynic acid analogs with little or no SH-combining activity may still act as antagonists of naturiferic and hydro-osmotic effect. Furthermore, it must take place early in the sequence of events following receptor-hormone interaction, because cyclic AMP effects are not blocked (159).

Evidence also exists that furosemide produces its natriuresis through its effects on Na^+, K^+-ATPase and cellular metabolism (160, 161). Studies in vivo (154) and in vitro (147, 151) indicate that furosemide also depresses renal sodium reabsorption principally in the medulla and affects cellular metabolism in that region of the kidney.

The principal effect of chlorothiazide is on the cortical portion of the ascending limb of Henle's loop (34). There is no evidence known to us that suggests that the effects of chlorothiazide on cellular metabolism are similar to those of ethacrynic acid or furosemide (146). On the other hand, it has been shown that chlorothiazide depresses net renal uptake of nonesterified fatty acid (NEFA) (162). Since NEFA oxidation may in part provide energy for Na^+ transport in the cortex, it appears possible that alterations in energy metabolism are also part of the mechanism of action of this group of diuretics.

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