Kidney International, Vol. 36 (1989), pp. 427–433

Electroneutral NaCl transport in the distal tubule

JOHN B. STOKES

Laboratory of Epithelial Transport, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa, USA

The process of electrically silent NaCl translocation across cell membranes can take one of three general forms. a) The process can involve a single "carrier" that translocates 1Na, 1K, and 2Cl ions together. This quarternary transporter is inhibited by loop diuretics. b) There can be synchronous operation of Na-H and Cl-base exchangers. The parallel operation of these two exchangers effects net NaCl translocation linked indirectly to cell pH and is not directly coupled. c) The third and least well characterized system is that of simple NaCl cotransport.

For over a decade, it has been recognized that interdependent Na and Cl transport exists in a wide variety of cells [1]. Prominent among cells exhibiting such systems are epithelial cells responsible for fluid secretion and absorption. A major advance in our understanding of the operational differences among different NaCl cotransport processes occurred when Geck et al [2] provided evidence that the movement of Na and Cl in Ehrlich ascites tumor cells was tightly coupled to the transport of K. They determined that the stoichiometry of the transporter was 1Na:1K:2Cl, that the translocation was sensitive to furosemide, and that the process was not influenced by membrane voltage. Since their discovery, it has been found in a wide variety of cells, the most important of which in the kidney is the thick ascending limb of Henle's loop (as described in another manuscript in this Symposium). Armed with this insight, investigators have carefully examined electrically silent NaCl cotransport processes and have discovered that the 1Na:1K:2Cl transporter or the synchronous operation of the Na-H and Cl-base exchangers are the most common mechanisms of electrically silent NaCl cotransport across cell membranes.

The distal tubule, however, appears to possess a distinct NaCl cotransport process that is independent of K and is not a synchronous operation of Na-H Cl-base exchangers. The available evidence indicates that the early portion of the rat distal tubule has a "simple" NaCl cotransporter that is relatively insensitive to loop diuretics, amiloride, and anion exchange inhibitors. Rather, this NaCl cotransporter is sensitive to the thiazide-type diuretics. This review identifies what is known about the operation of this system as deduced by various models, describes the operation as it pertains to the distal tubule, and discusses the relevance of this type of NaCl transport as it pertains to K secretion and Ca^{++} balance.

Characteristics of NaCl cotransport

The understanding of neutral NaCl cotransport has been greatly facilitated by the discovery of such a system in the urinary bladder of *Pseudopleuronectes americanus*, the winter flounder. It has been known for some time that Na and Cl transport in this tissue is not influenced by perturbations of transepithelial voltage [3] and that it absorbs Na and Cl in equal amounts [4]. Furthermore, the transporter exists in a high resistance epithelium, an unusual combination of features [5]. Most epithelial cells expressing electrically silent cotransport occur in electrically "leaky" cells such as the proximal convoluted tubule or the thick ascending limb of Henle's loop, both of which have transepithelial resistances of 50 ohm · cm^2 or less. The discovery of electrically-silent NaCl cotransport in a high resistance epithelium (1000 to 2000 ohm · cm^2) provided the opportunity to examine the function of the cotransporter in an intact epithelium without a large paracellular ion flux. In this setting ion transport through the cell is relatively large.

The flounder bladder also has the ability to secrete K via an apical K channel. K secretion under short circuited conditions can be completely blocked by application of 4 mM Ba^{++} to the mucosal (lumen) solution [6]. Thus, by applying mucosal Ba^{++} and preventing K secretion, one can test the K dependence of NaCl translocation. Figure 1 shows the lack of stimulation of Na transport by raising mucosal K concentration. Similarly mucosal K concentration has no effect on Cl transport [5]. Because of the low rate of backleak of K from the serosal solution and the inhibition of K secretion with Ba^{++}, one can be reasonably certain that the K concentrations in the microenvironment of the apical membrane approximate those measured in bulk solution. The equality of the net fluxes for Na and Cl, the demonstration of complete interdependence, and the lack of influence of mucosal K concentration on either Na or Cl flux provide strong evidence for NaCl cotransport not requiring K [5].

Further experiments demonstrated that NaCl cotransport in the flounder bladder was not owing to synchronous operation of Na-H and Cl-base exchangers. Na transport was not inhibited by high concentrations of amiloride even when mucosal Na concentration was 15 mM. Likewise, Cl absorption was not reduced by the disulfonic stilbene, DIDS, indicating that Cl-base exchange was an unlikely mode of operation. The failure of carbonic anhydrase inhibition to alter the rate of Na or Cl transport also argued against synchronous operation of parallel antiporters [5].

The uniqueness of the flounder bladder NaCl cotransporter became even more apparent when it was discovered that 0.1

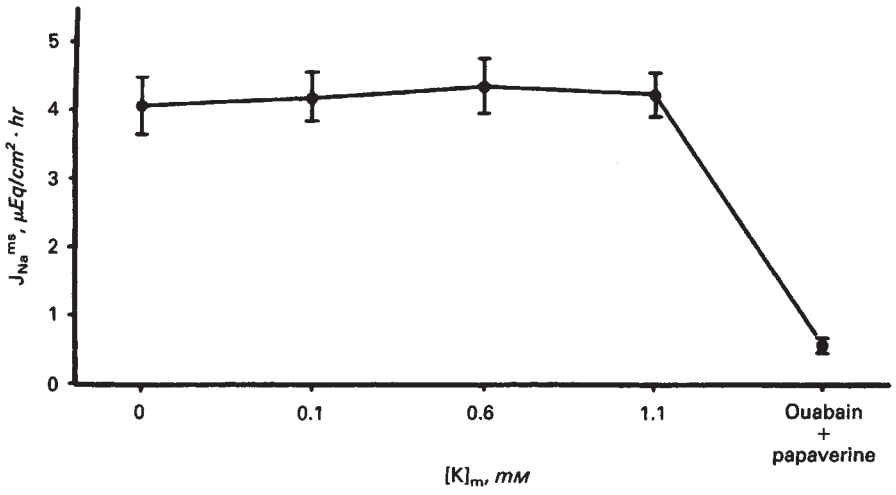

Fig. 1. *Effect of increasing mucosal (luminal) K concentration ($[K]_m$) on Na absorption (mucosal to serosal flux, J_{Na}^{ms}).* K concentration was measured during each period. Ouabain and papaverine were added to inhibit active transport. Similar results can be demonstrated with Cl transport. (Drawn from data reported in ref. 5).

mM bumetanide had no effect on the rate of Na or Cl transport, that 1 mM furosemide had only a partial effect on some tissues, and that hydrochlorothiazide and metolazone both inhibited NaCl transport almost completely [5]. The distinction between the efficacy of thiazide-type diuretics and loop diuretics has been underscored by the fact that thiazides have no effect on the Na:K:2Cl cotransport system [7, 8]. Thus, the diuretic "fingerprint" helps to define the system as we know it today.

NaCl cotransport by the early distal tubule

Before beginning a discussion of NaCl cotransport in the distal tubule, it is important to recognize that different investigators have used different terms for this segment. As defined in classic micropuncture physiology, the term distal tubule applies to the nephron segment between the macula densa and the first branch of the collecting system. However, the distal tubule actually comprises at least three functionally distinct regions: the early distal tubule (often called the distal convoluted tubule), the connecting tubule, and the initial collecting tubule. In the rat, where most of the in vivo data on distal tubule function has been collected, the precise delineation between these segments is not possible because the transitions are gradual [9]. A few investigators have been able to identify early and late segments of sufficient length to permit assessment of transport. In these cases early distal tubule seems to represent, for the most part, histologically homogeneous distal convoluted tubule while the late distal tubule may represent a combination of connecting and initial collecting tubules. In this review, I will use the term distal tubule to indicate the nephron segment between the macula densa and the first branch of the collecting system and the term "early distal tubule" to indicate the nephron segment distal to the macula densa representing the first loop available for micropuncture. The term "early distal tubule" implies that an element of uncertainty exists regarding its homogeneity but that there is a good probability that most or all of the epithelium is histologically "distal convoluted tubule".

The deduction that the neutral NaCl cotransporter described in the flounder urinary bladder also operates in the early distal tubule can be made from a variety of results from several different laboratories. Kunau, Weller and Webb [10] were the first to document that the site of action of chlorothiazide was the distal tubule. Subsequently, Costanzo [11] provided evidence that the thiazide effect was on the early portion of the distal tubule. Most recently, Ellison, Velasquez and Wright [12] have demonstrated that the thiazide-sensitive portion of the distal tubule is the early portion and that the distal tubule late portion is not sensitive to thiazide diuretics. Rather, the late portion has a Na absorptive system that is sensitive to amiloride. That 1 mM chlorothiazide can completely inhibit NaCl absorption, even when absorption is maximal, is good evidence that the major, if not the only, salt absorbing process is NaCl cotransport. The evidence for identity between this NaCl transport system and that of the flounder urinary bladder extends throughout the pharmacological fingerprint. Both systems are insensitive to bumetanide and partially sensitive to high concentrations of furosemide [5, 8]. The data for the distal tubule are presented in Figure 2. That furosemide does not produce further inhibition of NaCl absorption when chlorothiazide is present [8] provides additional evidence that the 1Na:1K:2Cl cotransporter and the NaCl cotransporter do not coexist in the early distal tubule. Experiments from both in vitro perfused thick ascending limbs of Henle's loop [7] and in vivo perfused loops [8] demonstrate no effect of thiazide-type diuretics on loop function. Thus, the separate actions of bumetanide and thiazide diuretics are clean and convincing, and the modest effect of furosemide on NaCl cotransport is evident both in the early distal tubule and in the flounder urinary bladder.

The evidence for an interdependence of Na and Cl transport in the distal tubule is also convincing. Velazquez, Good and Wright [13] have shown that, when lumen Na concentration is near 0, Cl absorption is 0. Conversely, when lumen Cl concentration is near 0, Na absorption is 0. Figure 3 demonstrates these interactions in distal tubules perfused in vivo. They further demonstrate the apparent kinetic constants; when lumen Na concentration is ~9 mM, Cl absorption is half-maximal, and, when Cl concentration is ~12 mM, Na absorption is half-maximal. These kinetics are in reasonable agreement with those observed in the flounder bladder [5], although such a

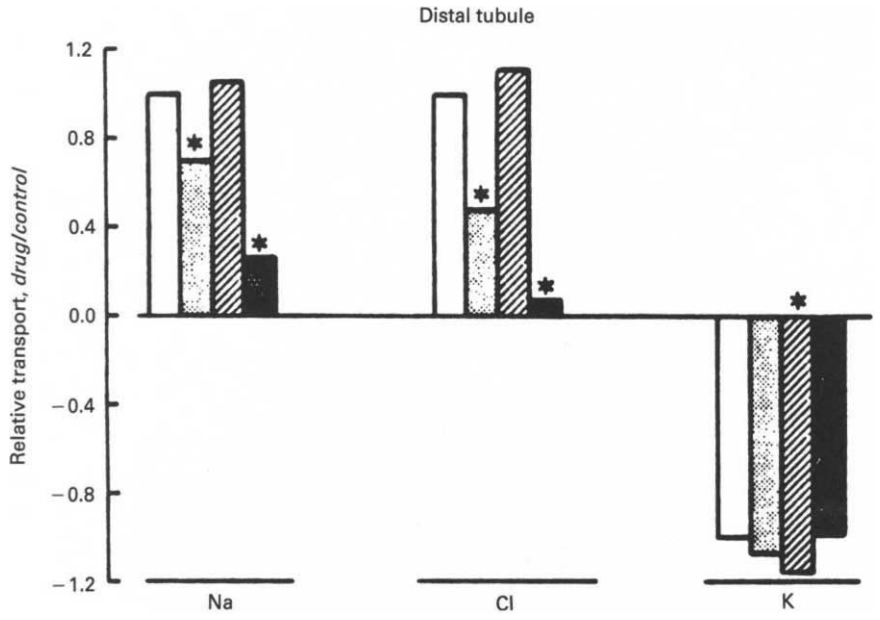

Fig. 2. *Effect of 0.1 mM furosemide, 0.1 mM bumetanide, and 1 mM chlorothiazide on Na, Cl, and K transport by the distal tubule.* Negative values indicate secretion, positive values absorption. Reproduced from ref. 8 with permission. Symbols are: (□) control; (▨) FUR; (▨) BUM; (■) CTZ.

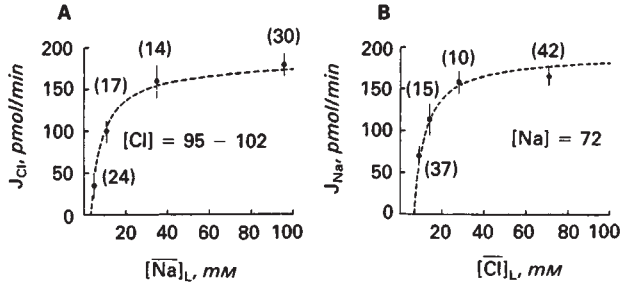

Fig. 3. *Interdependence of Na and Cl absorption in the distal tubule.* The magnitude of (**A**) Na and (**B**) Cl absorption is dependent on the concentration of the other ion. Reproduced from ref. 13 with permission.

detailed study has yet to be conducted in the latter tissue. Additional experiments supporting electroneutral NaCl transport in the distal tubule include those demonstrating small to absent changes in transepithelial voltage following reductions in lumen Cl concentration [14, 15]. Experiments where voltage has been measured after lumen Na removal show depolarization [16]. However, it is probable that this voltage change was influenced by active transport and diffusion voltages in the late distal tubule.

Special aspects of NaCl cotransport in distal tubule

The location of the NaCl cotransporter in the early portion of the distal tubule may have special importance to the overall functioning of the distal nephron that transcends simple NaCl absorption and regulation of the extracellular volume. There is evidence to support the idea that this cotransporter may be involved in: a) the regulation of Ca^{++} reabsorption, b) an element of NaCl secretion, and c) the regulation of K secretion.

Ca^{++} reabsorption

It has long been recognized that diuretics acting on the distal nephron increase Ca^{++} reabsorption and thus reduce Ca excretion [17]. More recently, Costanzo [18] has demonstrated that the thiazide diuretics enhance Ca^{++} absorption by the early portion of the distal tubule. The interaction between thiazide diuretics and enhanced Ca^{++} transport is not confined to the mammalian kidney but is also evident in the flounder bladder [19]. This interaction, occurring in the portion of the nephron where calcitonin produces its major stimulation of adenylate cyclase [20] may indicate a physiologically important (inverse) linkage between the magnitude of NaCl absorption and Ca^{++} absorption by this segment.

The mechanism of this linkage is not clear. Inhibition of NaCl absorption in other nephron segments does not necessarily enhance Ca^{++} absorption. For example, in the thick ascending limb of Henle's loop furosemide inhibits Ca^{++} absorption [21]. On the other hand, the stimulation of Ca^{++} absorption is not unique to the cells displaying thiazide-sensitive NaCl cotransport. Amiloride also stimulates Ca absorption by the late distal tubule [11]. This segment does not display thiazide-sensitive Na absorption [12] so it is possible that the thiazide effect on Ca^{++} transport is not intimately linked to the NaCl cotransporter. Rather the diuretic effects on Ca^{++} absorption could be an indirect one mediated by membrane voltage. Mucosal thiazides probably hyperpolarize cells of the (early) distal nephron [22]. They also hyperpolarize flounder bladder cell membranes [23]. If Ca^{++} channels are present in the apical membrane of DCT cells, the hyperpolarization could accelerate the rate of absorption. As intriguing as these possibilities are, the exact nature of the interaction between NaCl and Ca^{++} absorption and the effects of inhibitors on Ca^{++} transport remains to be fully elucidated.

NaCl secretion by early distal tubule

The kidney expends a substantial amount of energy reabsorbing filtered NaCl and water. In general, the distal nephron is

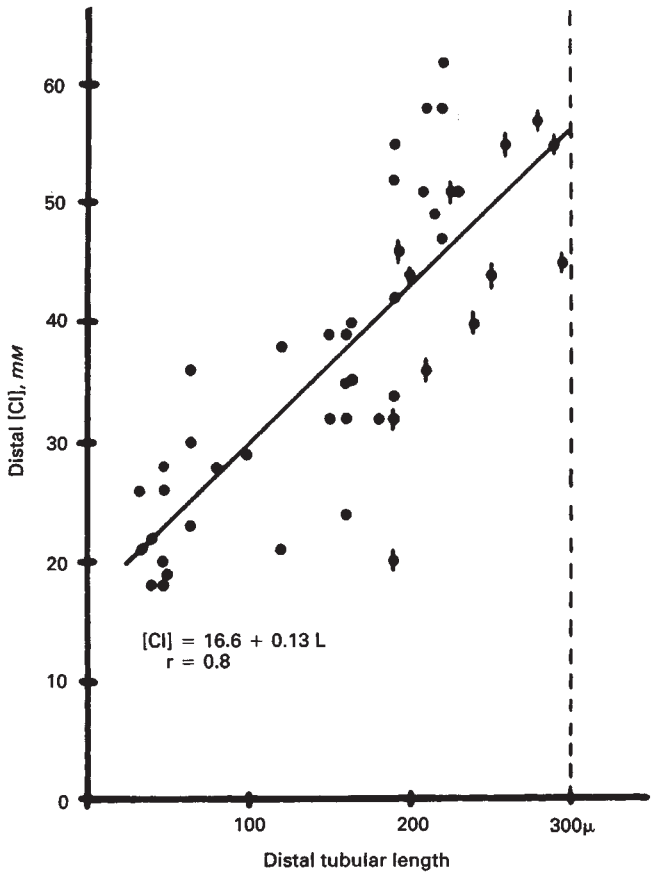

Fig. 4. *Cl concentration as a function of length along the first 300 μm of the early DCT of Munich-Wistar rats.* No Cl addition was detected after 300 μm. The increase in [Cl] was not secondary to water absorption. Reproduced from ref. 24 with permission. Symbols are: (●) surface glomeruli; (●) no surface glomeruli.

responsible for establishing and maintaining concentration gradients for Na, K, Cl, and H^+. It, therefore, came as some surprise when Schnermann, Briggs and Schubert [24] reported NaCl secretion by the very early (post macula densa) portion of the distal tubule. The data for Cl are reproduced in Figure 4. The possibility that NaCl secretion could occur in this segment had previously been demonstrated [25, 26] for tubules perfused with extremely low concentrations of NaCl. That NaCl secretion occurs normally in the very early distal tubule raises the important question of what is the mechanism of this secretion.

Although the pathway(s) for NaCl secretion by the early distal tubule have not yet been addressed experimentally, there are two general possibilities. First, it is possible that the majority of the NaCl entering the lumen (and raising the NaCl concentration from 20 to 50 mM) does so via the paracellular pathway. If so, the paracellular resistance of the epithelium would be rather low—substantially lower than that of the cortical thick ascending limb of Henle's loop. A second possibility is that a portion of the NaCl secretion occurs by a transcellular pathway. A transcellular pathway might permit a greater degree of regulation of a secretory process. One common mechanism of NaCl secretion is exemplified by the shark rectal gland [27] and the tracheal epithelium [28]. In these tissues, Cl is secreted via a Cl conductive pathway in the apical membrane. There is not enough known about the cell membranes of the early distal tubule to say with certainty if such a pathway exists. However, it is also possible that NaCl secretion could occur via the NaCl cotransporter on the apical membrane. In the flounder bladder, a large transcellular backflux (serosa to mucosa) of Na and Cl exists [5, 29]. The extent to which this backflux can occur in the presence of an appropriately low lumen NaCl concentration is not known. However, the potential for backflux of NaCl through the cell clearly exists. The regulation of the overall process might only require regulation of the basolateral membrane Na permeability since basolateral Cl permeability is probably already high enough to allow for Cl exit during active NaCl absorption.

Linkage of apical membrane NaCl and KCl cotransporters

The mechanism of K secretion by the early distal tubule is probably different from that of the late distal tubule. The cellular heterogeneity of the distal tubule has made clear conclusions regarding structure and function difficult. However, recent experiments in which separate early and late segments have been perfused in vivo have added considerably to our understanding. Given the presently available information, it is reasonable to conclude that a portion of the K secretion across the apical membrane of distal tubule cells occurs via a KCl cotransporter. Velazquez, Ellison and Wright [30] have recently provided evidence that both early and late distal tubule cells may have such a transporter.

The data supporting the conclusion that the luminal membrane of distal tubule cells contain a KCl cotransporter are beyond the scope of the present review. The most important series of observations are those demonstrating that reducing lumen Cl concentration enhances K secretion in the absence of consistent changes in transepithelial voltage [14, 30]. The KCl cotransporter may exist in both the early and late portions of the distal tubule. It is the (putative) KCl cotransporter on the apical membrane of the early distal tubule on which we now focus our attention.

The presence of a KCl cotransporter on the same apical membrane as the NaCl cotransporter as postulated by Velazquez, Ellison and Wright [31] creates a situation whereby lumen Cl concentration influences both Na absorption and K secretion. Such an arrangement predicts that at any given lumen Cl concentration, increasing lumen Na concentration will increase Na (and Cl) absorption. The increase in Na entry into the cell will increase the turnover rate of the basolateral Na-K pump and thereby increase K entry into the cell. Because lumen Cl concentration is low, a more favorable gradient exists for KCl exit across the apical membrane and more K will be secreted. According to this model, Cl cycles across the apical membrane entering the cell with Na and exiting with K. The overall operation is one of Cl dependent Na-K exchange. The kinetic details of such a system will need to be examined in greater detail, but it is not difficult to envision a situation where the magnitude of K secretion would be inversely proportional to the lumen Cl concentration over a physiologically relevant range. Preliminary evidence that such a process might operate in this fashion has been presented by Velazquez et al [31].

The parallel arrangement of NaCl and KCl cotransporters on the apical membrane of the early distal tubule may provide a

partial explanation for the accelerated K loss in metabolic alkalosis. In metabolic alkalosis when HCO_3 is delivered to the DCT in substantial quantities, there is a concomitant increase in Na concentration or a decrease in Cl concentration or both. By creating a difference in the lumen Na and Cl concentrations of the early distal tubule, K secretion would be favored. The recent experiments of Velazquez et al [30] suggest that such a situation could transform the early DCT into a K-secreting epithelium when under normal circumstances it is not.

The understanding of the mechanisms of K secretion by the DCT may help explain why NaCl is secreted by the very early distal tubule. NaCl secretion may exist to ensure an adequate lumen Na concentration in the late distal tubule and collecting tubule. The rate of K secretion is limited when lumen Na concentration falls below 30 mM and is little affected by raising lumen Na concentration over 40 mM [16, 26, 32]. The NaCl secretory system in the very early DCT raises Na concentration to this range [24]. If Cl concentration in the early distal tubule is similar to that of Na, the balance of the relevant chemical gradients may not favor K secretion by this segment. Rather there might be more influence on the late distal tubule K secretory system where the mechanisms of Na absorption are different.

NaCl cotransport in other tissues

Many investigators have identified NaCl cotransport processes in other tissues. Often the unambiguous distinction between a NaCl cotransporter, a 1Na:1K:2Cl cotransporter and parallel Na-H and Cl-base exchangers is not possible from the information available. At a minimum, the demonstration of a NaCl cotransporter similar to that found in the early distal tubule and the flounder urinary bladder requires evidence of: a) interdependence of Na and Cl transport, b) NaCl transport independent of K, c) nearly complete inhibition by thiazide diuretics, and d) insensitivity to 0.1 mM bumetanide. The failure to demonstrate appropriate sensitivities to these pharmacologic agents does not preclude the presence of NaCl cotransport. However, if such a system(s) exists, it must be different from that of the distal tubule and flounder bladder.

Several investigators have provided good evidence for NaCl cotransporters in other cells or tissues but, to date, none meet all the above criteria. Ericson and Spring [33] noted a NaCl transport mechanism in the apical membrane of the *Necturus* gallbladder, a process further determined to be independent of K and inhibited by bumetanide [34]. Although this process seemed to represent NaCl cotransport, Reuss [35] was able to demonstrate independent pathways for Na and Cl entry across the apical membrane. The reason(s) for the different results is not clear. In the Ehrlich ascites tumor cell, Hoffman, Sjoholm and Simonsen [36] have demonstrated that cell swelling and subsequent shrinkage induces a bumetanide-sensitive NaCl cotransport. Eveloff and Calamia [37] have demonstrated a similar process in isolated cells from the medullary thick ascending limb of Henle's loop. These processes are clearly different from the distal tubule in their sensitivity to bumetanide. They seem to be able to operate independently of K. However, further experiments will be needed to characterize these transport processes fully.

There are two other tissues where a NaCl cotransport process exists that may be similar to that of the distal tubule. Benos and Biggers [38] have described NaCl cotransport in preimplantation rabbit blastocysts. This transport process operates independently of K, does not have characteristics of parallel Na-H and Cl-HCO_3 exchangers, and is only partially sensitive to furosemide. Other inhibitors were not tested. A second possible candidate exists in the rabbit gallbladder. Cremaschi et al [39, 40] have described a K-independent, bumetanide-insensitive, NaCl cotransporter that appears to be separate from parallel Na-H and Cl-HCO_3 exchangers in the same tissues. At the present time, it is not known if this cotransporter is sensitive to thiazide diuretics.

NaCl cotransport might also be identified by examining the effect of thiazide diuretics on transport. Wilson, Honrath and Sonnenberg [41] have reported that hydrochlorothiazide inhibits Na and Cl absorption by the rat inner medullary collecting duct. In an unrelated tissue, Duncan and Baumgarten [42] have demonstrated that a portion of the NaCl influx into rabbit ventricle is thiazide sensitive. The growing awareness of the thiazide sensitivity of NaCl cotransport may lead to the discovery of other cells possessing this cotransporter. It seems likely that since this transport molecule has been conserved throughout evolution, more cells than just early distal tubule cells will be found to express it.

Therapeutic implications of the NaCl cotransporter

That the site and mechanism of action of the thiazide diuretics are separate from the loop diuretics provides a rationale for their combined use in selected patients. Loop diuretics such as bumetanide or furosemide are potent inhibitors of NaCl absorption in the thick ascending limb of Henle's loop. The thiazides (and metolazone) inhibit NaCl absorption in the early distal tubule. By acting at different sites along the nephron their actions are additive. Under some circumstances they may be synergistic [43, 44]. Such a situation may exist in a patient who has been treated for weeks with a loop diuretic and who then has a thiazide added. Furosemide, by simultaneously delivering larger NaCl loads to the early distal tubule and contracting volume, causes that segment to hypertrophy [45, 46]. The addition of a thiazide diuretic under these conditions can produce a substantial increment in saliuresis. However, the physician electing to use this combination of diuretics must exercise extreme caution. The inhibition of NaCl absorption along such an extensive length of the nephron proximal to the K secretory site can produce large increments in urinary K loss. Severe hypokalemia can be produced in hours. Other patients with severe refractory edema and/or renal insufficiency may not respond at all. Predicting which patients will or will not respond is difficult and therefore special measures must be taken to ensure that the adverse reactions of severe K loss, should they occur, can be dealt with emergently.

Conclusion

There is good evidence supporting the idea that the early distal tubule has a NaCl cotransporter on the apical membrane. This transport process does not consist of separate parallel Na and Cl transporters. Neither is it the more commonly found 1Na:1K:2Cl cotransporter. Its sensitivity to thiazide diuretics and relative insensitivity to "loop" diuretics are important pharmacologic features. Its location in the early distal tubule may be strategically important for the regulation of NaCl

balance, K excretion and the renal response to metabolic alkalosis.

Acknowledgments

The author appreciates the secretarial assistance of Ms. Syd Harned. Dr. Stokes is an Established Investigator of the American Heart Association.

Reprint requests to John B. Stokes, M.D., Division of Nephrology, Department of Internal Medicine, University of Iowa Hospitals, Iowa City, Iowa 52242, USA.

References

1. FRIZZELL RA, FIELD M, SCHULTZ SG: Sodium-coupled chloride transport by epithelial tissues. *Am J Physiol* 236(Renal Fluid Electrolyte Physiol 5):F1–F8, 1979
2. GECK P, PIETRZYK C, BURCKHARDT B-C, PFEIFFER B, HEINZ E: Electrically silent cotransport of Na, K and Cl in Ehrlich cells. *Biochim Biophys Acta* 600:432–447, 1980
3. RENFRO JL: Water and ion transport by the urinary bladder of the teleost *Pseudopleuronectes americanus*. *Am J Physiol* 228:52–61, 1975
4. RENFRO JL: Interdependence of active Na^+ and Cl^- transport by the isolated urinary bladder of the teleost, pseudopleuronectes americanus. *J Exp Zool* 199:383–390, 1978
5. STOKES JB: Sodium chloride absorption by the urinary bladder of the winter flounder. *J Clin Invest* 74:7–16, 1984
6. DAWSON DC, ANDREW D: Differential inhibition of NaCl absorption and short circuit current in the urinary bladder of the winter flounder, *Pseudopleuronectes americanus*. *Bull Mt Desert Isl Biol Lab* 19:46–49, 1979
7. SCHLATTER E, GREGER R, WEIDTKE C: Effect of "high ceiling" diuretics on active salt transport in the cortical thick ascending limb of Henle's loop of rabbit kidney. Correlation of chemical structure and inhibitory potency. *Pflügers Arch* 396:210–217, 1983
8. VELAZQUEZ H, WRIGHT FS: Effects of diuretic drugs on Na, Cl, and K transport by rat renal distal tubule. *Am J Physiol* 250(Renal Fluid Electrol Physiol 19):F1013–F1023, 1986
9. KAISSLING B: Structural aspects of adaptive changes in renal electrolyte excretion. *Am J Physiol* 243(Renal Fluid Electrol Physiol 12):F211–F226, 1982
10. KUNAU RT, WELLER DR, WEBB HL: Clarification of the site of action of chlorothiazide in the rat nephron. *J Clin Invest* 56: 401–407, 1975
11. COSTANZO LS: Comparison of calcium and sodium transport in early and late rat distal tubules: effect of amiloride. *Am J Physiol* 246(Renal Fluid Electrol Physiol 15):F937–F945, 1984
12. ELLISON DH, VELAZQUEZ H, WRIGHT FS: Thiazide-sensitive sodium chloride cotransport in early distal tubule. *Am J Physiol* 253(Renal Fluid Electrol Physiol 22):F546–F554, 1987
13. VELAZQUEZ H, GOOD DW, WRIGHT FS: Mutual dependence of sodium and chloride absorption by renal distal tubule. *Am J Physiol* 247(Renal Fluid Electrol Physiol 16):F904–F911, 1984
14. VELAZQUEZ H, WRIGHT FS, GOOD DW: Luminal influences on potassium secretion: Chloride replacement with sulfate. *Am J Physiol* 242(Renal Fluid Electrol Physiol 11):F46–F55, 1982
15. ELLISON DH, VELAZQUEZ H, WRIGHT FS: Stimulation of distal potassium secretion by low lumen chloride in the presence of barium. *Am J Physiol* 248:(Renal Fluid Electrol Physiol 17):F638–F649, 1985
16. GOOD DW, VELAZQUEZ H, WRIGHT FS: Luminal influence on potassium secretion: Low lumen sodium concentration. *Am J Physiol* 246(Renal Fluid Electrol Physiol 15):F609–F619, 1984
17. COSTANZO LS, WEINER IM: Relationship between clearances of Ca and Na: Effect of distal diuretics and PTH. *Am J Physiol* 230:67–73, 1976
18. COSTANZO LS: Localization of diuretic action in microperfused rat distal tubules: Ca and Na transport. *Am J Physiol* 248(Renal Fluid Electrol Physiol 17):F527–F535, 1985
19. ZIYADEH F, KELEPOURIS E, AGUS Z: Thiazides stimulate calcium absorption in urinary bladder of winter flounder. *Biochim Biophys Acta* 897:52–56, 1987
20. MOREL F: Sites of hormone action in the mammalian nephron. *Am J Physiol* 240(Renal Fluid Electrol Physiol 9):F159–F164, 1981
21. BOURDEAU JE, BUSS SL, VUREK GG: Inhibition of calcium absorption in the cortical thick ascending limb of Henle's loop by furosemide. *J Pharmacol Exp Ther* 221:815–819, 1982
22. HANSEN LL, SCHILLING AR, WIEDERHOLT M: Effect of calcium, furosemide and chlorothiazide on net volume reabsorption and basolateral membrane potential of the distal tubule. *Pflügers Arch* 389:121–126, 1981
23. DUFFEY ME, FRIZZEL RA: Flounder urinary bladder: Mechanism of inhibition by hydrochlorothiazide (HCT). (abstract) *Fed Proc* 43:444, 1984
24. SCHNERMANN J, BRIGGS J, SCHUBERT G: In situ studies of the distal convoluted tubule in the rat. I. Evidence for NaCl secretion. *Am J Physiol* 243(Renal Fluid Electrol Physiol 12):F160–F166, 1982
25. COSTANZO LS, WINDHAGER EE: Calcium and sodium transport by the distal convoluted tubule of the rat. *Am J Physiol* 235(Renal Fluid Electrol Physiol 5):F492–F506, 1978
26. GOOD DW, WRIGHT FS: Luminal influences on potassium secretion: Sodium concentration and fluid flow rate. *Am J Physiol* 236(Renal Fluid Electrol Physiol 5):F192–F205, 1979
27. GREGER R, SCHLATTER E, WANG F, FORREST JN: Mechanism of NaCl secretion in rectal gland tubules of spiny dogfish *(Squalus acanthias)*. III. Effects of stimulation of secretion by cyclic AMP. *Pflügers Arch* 402:376–384, 1984
28. WELSH MJ: Electrolyte transport by airway epithelia. *Physiol Rev* 67:1143–1184, 1987
29. STOKES JB, BLACKMORE M, LEE IB: Pathways for passive Na and Cl transport across the urinary bladder of the winter flounder *Pseudopleuronectes americanus*. *Bull Mt Desert Island Biol Lab* 24:40–41, 1984
30. VELAZQUEZ H, ELLISON DH, WRIGHT FS: Chloride-dependent potassium secretion in early and late renal distal tubules. *Am J Physiol* 253(Renal Fluid Electrol Physiol 22):F555–F562, 1987
31. VELAZQUEZ H, ELLISON DH, WRIGHT FS: Na Dependent K secretion by distal tubule inhibited by thiazide diuretics. (abstract) *Fed Proc* 46(4):1393, 1987
32. STOKES JB: Potassium secretion by cortical collecting tubule: Relation to sodium absorption, luminal sodium concentration and transepithelial voltage. *Am J Physiol* 241(Renal Fluid Electrol Physiol 10):F395–F402, 1981
33. ERICSON AC, SPRING KR: Coupled NaCl entry into necturus gallbladder epithelial cells. *Am J Physiology* 243:(Cell Physiol 12):C140–C145, 1982
34. LARSON M, SPRING KR: Bumetanide inhibition of NaCl transport by necturus gallbladder. *J Membr Biol* 74:123–129, 1983
35. REUSS L: Independence of apical membrane Na^+ and Cl^- entry in necturus gallbladder epithelium. *J Gen Physiol* 84:423–445, 1984
36. HOFFMANN EK, SJOHOLM C, SIMONSEN LO: Na^+, Cl^- cotransport in Ehrlich ascites tumor cells activated during volume regulation (regulatory volume increase). *J Membr Biol* 76:269–280, 1983
37. EVELOFF JL, CALAMIA J: Effect of osmolarity on cation fluxes in medullary thick ascending limb cells. *Am J Physiol* 250(Renal Fluid Electrol Physiol 19):F176–F180, 1986
38. BENOS DJ, BIGGERS JD: Sodium and chloride co-transport by preimplantation rabbit blastocysts. *J Physiol* 342:23–33, 1983
39. CREMASCHI D, MEYER G, BERMANO S, MARCATI M: Different sodium chloride cotransport systems in the apical membrane of rabbit gallbladder epithelial cells. *J Membr Biol* 73:227–235, 1983
40. CREMASCHI D, MEYER G, BOTTA G, ROSSETTI C: The nature of the neutral Na^+-Cl^--coupled entry at the apical membrane of rabbit gallbladder epithelium: II. Na^+-Cl^- symport is independent of K^+. *J Membr Biol* 95:219–228, 1987
41. WILSON DR, HONRATH U, SONNENBERG H: Thiazide diuretic effect on medullary collecting duct function in the rat. *Kidney Int* 23:711–716, 1983
42. DUNCAN SWN, BAUMGARTEN CM: Chlorothiazide-sensitive, Na-dependent Cl cotransport in rabbit ventricle. (abstract) *Circulation* 74:(suppl II)II–264, 1986

43. GHOSE RR, GUPTA SK: Synergistic action of metolazone with "loop" diuretics. *Br Med J* 282:1432–1433, 1981
44. WOLLAM GL, TARAZI RC, BRAVO EL, DUSTAN HP: Diuretic potency of combined hydrochlorothiazide and furosemide therapy in patients with azotemia. *Am J Med* 72:929–938, 1982
45. KAISSLING B, STANTON B: Adaptation of distal tubule to increased fluid delivery. (abstract) *Kidney Int* 31:437, 1987
46. ELLISON DH, VELAZQUEZ H, WRIGHT FS: Dissociation of cell hypertrophy and NaCl transport capacity in distal tubule. (abstract) *Kidney Int* 31:432, 1987