Possible role of cytosolic calcium and Na-Ca exchange in regulation of transepithelial sodium transport

TAYLOR, ANN, AND ERICH E. WINDHAGER. Possible role of cytosolic calcium and Na-Ca exchange in regulation of transepithelial sodium transport. Am. J. Physiol. 236(6): F505-F512, 1979 or Am. J. Physiol.: Renal Fluid Electrolyte Physiol. 5(6): F505-F512, 1979.—Emerging evidence in a number of different epithelia suggests that changes in cytosolic calcium ion levels play a critical role in the regulation of transepithelial sodium transport. Maneuvers believed to raise cytosolic calcium ion activity lead to an inhibition of net sodium transport in toad urinary bladder, frog skin, and isolated perfused proximal renal tubules. Regulation of the level of ionized calcium in the cytosol of the epithelial cells appears to involve a process of coupled Na-Ca exchange across the basolateral plasma membrane, energized, at least in part, by the sodium gradient. It is suggested that changes in cytosolic calcium ion levels, secondary to changes in Na-Ca exchange, in turn depend on part on the activity of the sodium pump, constitute a link in a negative feedback mechanism. Through such a feedback mechanism, the rate of entry of sodium into the cell across the apical surface may be kept in step with its rate of extrusion across the basolateral surface.

sodium reabsorption; negative feedback model for sodium transport; toad bladder; proximal tubule; kidney

THE REGULATION OF NORMAL BODY SODIUM content and concentration—on which normal function so critically relies—is largely dependent on the control of net rates of sodium and water movement across epithelia. The basic mechanisms involved in the transepithelial movement of sodium are now reasonably well understood. Accordingly, in most sodium-transporting epithelia, the existence in the transcellular pathway of a passive, apical sodium-entry step in series with an energy dependent, ouabain-sensitive sodium pump located in the basolateral cell membrane is generally recognized. The importance of the specific morphological organization of the epithelium and of the extent of passive fluxes of ions through intercellular pathways in leaky (versus tight) epithelia is well established.

Compositional changes in both extracellular and intracellular fluid are known to influence the rate of active sodium transport. Amongst these, changes in sodium concentration have been most fully characterized. Thus, a dependence of the rate of sodium transport on the luminal (external or mucosal) (26, 32, 68), on the intracellular (16, 47), and, moreover, on the contraluminal (internal or serosal) (6, 34, 38) sodium concentration has been demonstrated in different epithelia. Whereas it is easy to understand why luminal and intracellular sodium concentration might influence the overall rate of sodium transport, it is not obvious why changes in contraluminal sodium concentration affect transcellular sodium movement. Furthermore, an unresolved issue, central to an understanding of the process of active transport across epithelia, is the means whereby the rate of entry of ions into the cell across the apical cell membrane is coupled to the rate of their exit across the basolateral cell membrane.

In certain epithelia there is evidence that the apical entry of sodium is rate limiting for the overall transport process (7, 16, 26, 34, 47); changes in the rate of passive entry of sodium into the epithelial cell are believed to lead to proportional changes in intracellular sodium activity, and, in turn, to changes in the rate of active extrusion of sodium across the basolateral cell border (16, 26, 47). According to this concept, the rate of entry of sodium limits the availability of sodium ions to the pump and directly determines the rate of active transport. In addition, however, there is mounting evidence in several epithelia for the existence of a negative feedback mechanism whereby intrinsic changes in the rate of active sodium extrusion determine the rate of apical sodium entry in such a way that entry and exit are kept in step (17, 18, 25, 36, 64). Changes in intracellular sodium ion
activity have been implicated in this feedback process (17, 18, 36). Emerging evidence in a number of different epithelia suggests that changes in cytosolic calcium ion activity, secondary to changes in Na-Ca exchange, in turn dependent (at least in part) on the rate of sodium extrusion by the pump, may constitute a link in this feedback mechanism. According to this view, cytosolic calcium ions play a decisive role in the regulation of net transport of sodium, influencing primarily the rate of passive entry of sodium ions into the cell.

Evidence That Cytosolic Calcium Influences Sodium Transport

The first indication that calcium ions may influence sodium transport was obtained in the toad urinary bladder. In the course of studies on the role of cytosolic calcium in the action of vasopressin, Taylor (48) observed that quinidine inhibits the net transport of sodium in isolated toad bladder preparations. Quinidine was tested because of its known effect on muscle contractility (30), an effect which has been attributed to its ability to raise calcium ion activity in the sarcoplasm (30). Quinidine, 10^{-4} to 10^{-3} M, has been shown to inhibit calcium uptake by isolated muscle mitochondria (5) and sarcoplasmic reticulum vesicles (3, 5, 14), and, under certain circumstances, to induce a release of bound calcium from these organelles in vivo and in vitro (3, 5, 14, 30). In the toad bladder (48; and Taylor, unpublished results), addition of quinidine to the serosal bathing medium of isolated hemibladders, at a final concentration of 4 \times 10^{-4} M, induced a progressive fall in the short-circuit current. In 19 experiments, the short-circuit current in quinidine-treated tissues averaged 43 \pm 4% of that in their paired controls 45 min after addition of the drug (P < 0.01). (Throughout the text, values are expressed as means \pm SE.) The fall in short-circuit current was associated with a somewhat lesser fall in transepithelial potential difference (PD), such that transepithelial resistance was increased by 34 \pm 9% (n = 9) (P < 0.01) relative to the controls. Addition of vasopressin, 20 mU/ml, to quinidine-treated hemibladders elicited an increase in the short-circuit current which exceeded that in their paired controls. Vasopressin, in fact, restored the inhibited short-circuit current to control levels, essentially reversing the effect of the drug. Although the mechanism of action of quinidine in the toad bladder remains to be precisely established, these observations are at least consistent with the view that changes in cytosolic calcium ion activity—perhaps in this instance mediated by drug-induced changes in the subcellular distribution of calcium—can influence the rate of transepithelial sodium transport.

Further evidence that an increase in cytosolic calcium ion activity inhibits sodium transport has been provided by studies using the calcium ionophores A-23187 and X-537A. These agents more or less specifically promote the movement of divalent, monovalent, cations across cell membranes (see Refs. 41 and 42 for reviews). Wiesmann et al. (57) first reported the effects of A-23187 on sodium transport in isolated toad bladders. These investigators observed that serosal addition of A-23187, at a final concentration of 1 \mu M, induced a progressive inhibition of the short-circuit current, which was dependent on the level of calcium in the external bathing medium. In these experiments the fall in short-circuit current was associated with a parallel fall in transepithelial PD. In separate in vitro experiments, exposure to A-23187 was shown to result in increased accumulation of \textsuperscript{45}Ca by isolated toad bladder epithelial cells. In these studies, the stimulation of the short-circuit current by 10 mU/ml vasopressin was found to be reduced in ionophore-treated tissues, although the response to cAMP was unaltered. The effect of the ionophore on the vasopressin response was attributed to a partial inhibition of vasopressin-stimulated cAMP synthesis. Ludens (37) confirmed the finding that A-23187 reduces the basal short-circuit current and transepithelial PD in parallel in the toad bladder. Moreover, he demonstrated that inhibition of the short-circuit current by the ionophore is associated with a decrease in the unidirectional mucosal-to-serosal flux of \textsuperscript{22}Na without any change in serosal-to-mucosal flux, i.e., in the passive backflux of sodium ions. Accordingly, the effect of the ionophore is evidently dependent on inhibition of the transcellular active transport pathway for sodium. Both groups of investigators (37, 57) concluded that the effects of A-23187 are mediated by an increase in calcium influx into the transporting epithelial cells of the bladder.

Taylor et al. (51, and Taylor, unpublished results), in the course of a systematic follow-up of the earlier studies with quinidine, found that both A-23187 and X-537A inhibit sodium transport in the toad bladder. Addition of A-23187 to the serosal bathing medium of isolated hemibladders at a final concentration of 10 \mu M induced a fall in short-circuit current to 46 \pm 5% (n = 12) (P < 0.01) of the control value after 60 min. As in the studies of Wiesmann et al. (57) and Ludens (37), no significant change in transepithelial resistance was observed in these experiments; however, it must be pointed out that edgadamage effects may have precluded the detection of such changes. The degree of inhibition of net sodium transport by A-23187 varied with the external calcium concentration. When hemibladders were bathed in 0.89 mM calcium the short-circuit current fell by 57 \pm 8%, whereas in their paired controls bathed in 0.1 mM calcium short-circuit current fell by only 28 \pm 11% (n = 8) (P < 0.02). In these studies the natriuretic response to 20 mU/ml vasopressin was enhanced in the ionophore-treated tissues relative to their controls. As in the earlier studies with quinidine, vasopressin completely reversed the drug-induced inhibition of sodium transport.

The effect of X-537A on the basal rate of sodium transport was essentially similar to that of A-23187 (Taylor et al., unpublished results). At a final concentration of 5 \mu M, X-537A induced a fall in short-circuit current to 52 \pm 7% (n = 7) (P < 0.01) of control values after 60 min. However, in contrast to the situation with A-23187, the effect of X-537A was not found to vary with the external calcium concentration. Vasopressin did not fully reverse the inhibition of the short-circuit current induced by X-
As with A-23187, however, the final level of sodium transport achieved in the presence of both the hormone and the ionophore varied inversely with the external calcium.

In the presence of the calcium ionophores, it is most probable that increased calcium influx across the cell membrane and/or release of calcium from intracellular stores (41, 42) lead to an increase in the level of ionized calcium in the epithelial cell cytosol. It seems reasonable to conclude, therefore, that an increase in cytosolic calcium ion activity is the common denominator in the studies with quinidine and the calcium ionophores, and that the effects on base-line short-circuit current observed in these various studies are mediated by changes in cytosolic calcium ion activity. Since vasopressin can apparently overcome the effect of an experimentally induced increase in cytosolic calcium ion activity, it is intriguing to speculate that the stimulation of sodium transport by this hormone may actually be mediated by a decrease in cytosolic calcium ion levels.

**Evidence for Na-Ca Exchange in Transporting Epithelia**

On the basis of the studies with pharmacological agents described above, it appears likely that changes in cytosolic calcium ion activity may influence the rate of transepithelial sodium transport under physiological and also pathological conditions. The question of how intracellular free calcium ion levels are regulated physiologically becomes pertinent to understanding the potential role of calcium in the regulation of sodium transport.

It is now generally recognized that cytosolic calcium ion activity may be regulated, in part, by calcium pumps in mitochondria (13, 35), endoplasmic reticulum (10, 39), and cell membranes (45), and also by a process of Na-Ca exchange across the plasma membrane. The work of Blaustein (8, 9), Baker (1, 2), and Mullins (40) has provided convincing evidence that in excitable tissue maintenance of a low cytosolic calcium ion activity depends critically on such a Na-Ca exchange process across the cell membrane. In these tissues it is well established that calcium ions enter the cells relatively freely and must be extruded against a steep electrochemical gradient in order to maintain the cytosolic calcium ion activity at its physiological level of about $10^{-7}$ M (see Ref. 44 for review). While short-term buffering of cytosolic calcium may be effected by sequestration of calcium by mitochondria, endoplasmic reticulum, and even by calcium-binding proteins, these mechanisms must ultimately have a limited capacity, and long-term regulation of cytosolic calcium must be due to calcium extrusion out of the cell. Current evidence indicates that a countertransport of sodium and calcium ions across the plasma membrane, energized at least in part by the sodium gradient, is involved in the uphill extrusion of calcium. In nerve and muscle, experimental maneuvers that influence the electrochemical gradient for sodium entry in turn influence the coupled extrusion of calcium and, hence, cytosolic calcium ion activity. Thus, when the sodium concentration in the external medium is lowered, the sodium-dependent calcium efflux is reduced and cellular ionized calcium levels increase accordingly. In sodium-free solutions, when the sodium gradient is actually reversed, sodium leaves the cell and calcium enters through reverse operation of the Na-Ca countertransport system (8, 44). Numerous early observations are consistent with the view that a similar, if not identical, Na-Ca exchange process operates also in other tissues, as first pointed out by Blaustein (8). Recently, examination of this issue has yielded evidence that Na-Ca exchange may play an important physiological role in epithelial transport.

A hypothetical model relating sodium and calcium fluxes in epithelial cells is depicted in Fig. 1. The contraluminal cell membrane of transporting epithelial cells may be compared with the plasma membrane of excitable cells. Across this membrane, sodium extrusion is brought about through the operation of a sodium pump, presumably involving the activity of Na-K-activated ATPase. A small, but finite, backflux of sodium occurs across this membrane into the cell down the electrochemical gradient established by the pump. Calcium may also enter the cell across this membrane down its electrochemical gradient, as is known to occur in excitable tissues. According to the model, at least a portion of the passive sodium entry across the contraluminal membrane is coupled to the efflux of calcium ions out of the cell. This coupled calcium extrusion process may be largely, if not exclusively, energized by the electrochemical gradient for sodium ions between extra- and intracellular fluid compartments. (In excitable tissue, it has been calculated that a 3:1 (9) or 4:1 (40) Na-Ca exchange process, powered solely by the sodium gradient, although also promoted by ATP (40), can account for the observed value of cytosolic calcium ion activity of the order of $10^{-7}$ M or less; comparable kinetic data are not available for epithelia.) In addition, calcium may be actively extruded through the operation of a calcium pump energized by ATP, i.e., through the activity of a Ca-activated ATPase (20, 31). Also depicted in Fig. 1 are sites for passive entry of sodium and calcium into the cell across the luminal plasma membrane. (A Na-Ca exchange system might

![FIG. 1. Model of sodium and calcium transport in epithelia (see text). The Na-Ca exchange ratio may be 3 or 4 to 1 (9, 40).](http://ajprenal.physiology.org/download/f507.png)
exist in the luminal plasma membrane; however, there is no experimental evidence at present concerning this matter.)

Evidence that a Na-Ca exchange process is involved in the regulation of cytosolic calcium levels in amphibian epithelia has recently been obtained in studies involving manipulation of the electrochemical gradient for sodium entry into the epithelial cells across the basolateral plasma membrane. In fact, it is a long-standing observation that removal of sodium from the medium bathing the serosal or inner surface of toad bladder (6, 34) and frog skin (38) inhibits the active net transport of sodium. As pointed out above, this phenomenon has remained unexplained.

The hypothesis that a Na-Ca exchange process might play a role in this phenomenon was first examined by Grinstein and Erlij (27). These investigators observed that the decrease in net transport of sodium induced by removal of sodium from the inner bathing medium of frog skin was prevented when calcium was removed from the inner solution. They also showed that $^{45}$Ca uptake by the isolated epithelium was increased in sodium-free solutions, nearly all the increased uptake occurring across the basal border of the cells. They concluded that a Na-Ca exchange process is normally involved in the regulation of cell calcium levels in frog skin and suggested that the inhibition of sodium transport induced by sodium-free solutions is due to entry of calcium into the epithelial cells (through reversal of the normal direction of Na-Ca exchange) and a consequent decrease in apical membrane sodium permeability.

Evidence for a functional role of Na-Ca exchange in the regulation of sodium transport has also been obtained in the toad bladder (50, 51, and Taylor and Eich, unpublished results). When the sodium concentration in the medium bathing the serosal surface of isolated hemibladders was reduced to 2 mM (substituting choline for sodium) a rapid fall in short-circuit current ensued; in 11 experiments, the short-circuit current averaged 52 ± 3% ($P < 0.01$) of its initial value 15 min after the serosal medium was changed to a low sodium solution (maintaining serosal calcium at 0.89 mM). Vasopressin again reversed the effect of the experimental maneuver, restoring the inhibited short-circuit current to the levels of the controls. In these experiments, the degree of inhibition of the short-circuit current at a given reduced serosal sodium concentration was found to be a function of the serosal calcium concentration. The fall in short-circuit current was least at the lowest serosal calcium concentrations tested (0.1 mM); inhibition of short-circuit current increased nearly linearly with increasing calcium concentrations up to 0.89 mM and remained essentially constant at serosal calcium levels between 0.89 and 5.0 mM. The inhibitory effect of low serosal calcium was reduced in the presence of lanthanum (50) and at low pH (Brem and Taylor, unpublished results), two conditions in which Na-Ca exchange is inhibited (44). (Although unidirectional sodium isotope fluxes were not measured in these experiments, Leaf (34) reported that the fall in short-circuit current induced by low serosal sodium in the toad bladder is associated with a parallel decline in mucosal-to-serosal flux of $^{22}Na$.)

The studies on amphibian epithelia discussed so far lend strong, although indirect, support to the notion that Na-Ca exchange plays an important role in the regulation of active sodium transport. This concept clearly may also apply to mammalian renal tubular epithelium. Based on the substantial evidence available in the literature for parallel reabsorption of sodium and calcium ions (56) and for sodium dependence of calcium reabsorption (55), Blaustein (8) first suggested that a Na-Ca exchange process located within the peritubular cell membrane may be responsible for “active” calcium reabsorption by the kidney. Evidence that a Na-Ca exchange process exists in renal tubular epithelium was reported by Blaustein (8). He observed in in vitro studies that the rate of $^{45}$Ca washout from isotope-loaded isolated tubules of rabbit kidneys was slower when sodium had been removed from the extracellular fluid. Direct evidence for Na-Ca exchange in renal tubular epithelium in vitro has also been reported by Kinne et al. (31) who observed sodium-dependent calcium uptake by “inside-out” vesicles prepared from contraluminal cell membranes of rat kidney cortex. The first experimental indication that a Na-Ca countertransport system may operate in the proximal convoluted tubules of rat kidneys in vivo was provided by Ullrich et al. (53). Using the stop-flow microperfusion technique in conjunction with capillary microperfusion, these authors observed that proximal tubular reabsorption of calcium was abolished when the ambient Na was replaced by choline or lithium. Calcium transport by proximal tubules of hamster kidneys was also completely inhibited when sodium transport was abolished by ouabain. These authors concluded that the active reabsorption of calcium by the proximal tubule depends on the active transport of sodium ions and on the operation of a Na-Ca countertransport system in the contraluminal cell membrane.

The evidence for a coupled Na-Ca exchange in proximal tubular epithelium made it desirable to test whether maneuvers thought to induce an increase in cytosolic calcium ion activity might decrease the rate of net reabsorption of sodium by this nephron segment. For this purpose Figueiredo et al. (24 and unpublished results) used the isolated perfused proximal tubule of rabbit kidney as originally described by Burg and Orloff (12). In these experiments, the tubular lumen was perfused with solutions containing 145 meq/liter sodium at all times. The same concentration of sodium was present in the peritubular bath during control and recovery periods. However, during experimental periods, peritubular levels of sodium were reduced to about 40 meq/liter by substitution of sodium by lithium. Fluid reabsorption ($J_V$) was measured using $[^{125}]$iodothalamate. Under control conditions, $J_V$ averaged 1.01 ± 0.1 nl-min$^{-1}$-mm$^{-1}$ length ($n = 8$); during the experimental periods when peritubular sodium had been reduced, fluid reabsorption amounted to only 0.45 ± 0.05 nl-min$^{-1}$-mm$^{-1}$ length, that is, some 40% of the control values. Fluid reabsorption recovered to values not different from the control values when sodium concentrations were restored to their physiological level in the peritubular bathing solution.

To test whether the observed reduction in fluid reabsorption was caused by decreased efflux of sodium from
been added to the luminal perfusion fluid. Radioanalysis of the collected perfusate yielded the following results. During experimental periods when NaCl was partially replaced by LiCl in the peritubular bath, sodium efflux fell significantly to 72.4% of the control value. This reduction in efflux corresponded to 51% of the simultaneously observed drop in fluid reabsorption. These results indicate that the inhibition of fluid reabsorption induced by low peritubular sodium concentrations is to a large extent caused by a decrease in unidirectional sodium transport from lumen to bath. Lithium backflux itself by low peritubular sodium concentrations is to a large extent caused by a decrease in unidirectional sodium transport from lumen to bath. Lithium backflux itself cannot explain the full magnitude of inhibition of fluid reabsorption. The decrease in sodium efflux took place despite a sodium concentration gradient of 105 meq/liter favoring sodium movement in the reabsorptive direction. Similar results were also obtained when sodium was replaced by choline or tetrachethylammonium ions.

The degree of inhibition of fluid reabsorption at a given low peritubular sodium concentration was then examined as a function of the ultrafiltrable calcium concentration of the extracellular fluid. Below 0.5 mM calcium tubular cells tend to separate from each other, so only calcium concentrations higher than 0.5 mM could be used. A nearly linear relationship, $y = 30x + 19$ (where $y$ equals percent inhibition of fluid reabsorption and $x$ is the extracellular calcium concentration in millimoles per liter), was obtained in the range 0.5–2.0 mM calcium ($n = 11$) ($P < 0.01$). Thus, low external calcium resulted in the least inhibition of fluid reabsorption at low peritubular sodium concentrations. Raising the calcium concentration resulted in increased inhibition. The degree of inhibition remained constant and maximal above a calcium level of 2.0 mM. These results are, therefore, similar to those obtained in the toad bladder. They provide indirect evidence for the existence of a Na-Ca exchange process which regulates cytosolic calcium levels and secondarily influences sodium and water reabsorption by the convoluted proximal tubules.

Need for Direct Measurements of Cytosolic [Ca$^{2+}$]

It is obvious that the interpretation of these various experiments in the toad bladder, frog skin, and the proximal tubule of the kidney is hampered by the fact that cytosolic calcium ion activity in the transporting epithelial cells has not been measured directly in these studies. Available methods for measurement of cytosolic calcium ion activity include the use of photoluminescent compounds (such as aequorin), metallochromic indicators (such as arsenazo III), and calcium-selective microelectrodes (see Ref. 44). All three methods have been applied in certain giant cells, e.g., barnacle muscle, to obtain estimates of absolute levels of ionized calcium under resting and experimental conditions. In epithelial tissues, aequorin has been used to monitor changes in calcium ion activity on microinjection of calcium into the giant salivary gland cells of Chironomus (43). Technically, the use of calcium-sensitive indicators in vertebrate epithelial cells poses as yet insurmountable problems. On the other hand, the development of calcium-selective microelectrodes has only recently been advanced to the stage at which they can be used for intracellular measurements in such cells. To our knowledge, no such measurements have as yet been published. It is obvious that direct measurements of cytosolic calcium activity in epithelial cells are needed at this point to validate the changes assumed to occur in the various studies described above.

Implications of Na-Ca Exchange and the Possible Role of Cytosolic Ca$^{2+}$

In view of the evidence, albeit indirect, that changes in cytosolic Ca$^{2+}$ can influence active sodium transport, it is clear that the existence of a sodium pump and a Na-Ca exchange system, in parallel, in the basolateral cell membranes has important implications for the overall regulation of transepithelial transport. In considering these implications, certain points should be emphasized.

First, the backflux of sodium into the cells via the Na-Ca exchange system is probably only a negligible fraction of the transepithelial unidirectional flux (lumen-to-contra-luminal compartment), and even of the net reabsorptive sodium flux. Its direct effect on cytosolic sodium activity, which is, of course, in the millimolar range, is likely to be insignificant. Second, the coupled efflux of calcium via the exchange process will, on the other hand, probably have a profound effect on cytosolic calcium ion activity, assuming that this is in fact of the order of 10$^{-7}$ M. Third, as pointed out by Blaustein (8) and Ullrich et al. (53), the sodium-dependent calcium efflux may account for a substantial fraction of net calcium reabsorption in epithelia capable of net transport of this ion. Consistent with this view is the observation of Ullrich et al. (53) that calcium reabsorption in proximal tubules is abolished when active sodium transport is blocked by high concentrations of ouabain. According to this concept, ouabain induces a primary reduction in the rate of sodium pumping, with a consequent rise in cytosolic sodium activity and, hence, a decrease in the gradient favoring sodium entry across the contraluminal cell membrane. This, in turn, leads to reduced Na-Ca exchange across the membrane and, thereby, reduced calcium extrusion out of the cell. The net effect of ouabain administration, therefore, is to reduce both sodium and calcium net reabsorption across the epithelium (53).

With regard to the mechanism whereby an increase in cytosolic calcium activity inhibits the rate of sodium transport, it is presumed that changes in free calcium ion levels influence one or more rate-limiting steps in the overall transport process. In general, changes in active sodium transport rates have been attributed to discrete effects on either 1) intrinsic pump activity, 2) the energy supply to the pump, or 3) luminal membrane permeability to sodium ions.

Concerning the first possibility, it is known that calcium is a competitive inhibitor of Na-K-ATPase (22, 52); under in vitro conditions the effect of calcium is reported to be half-maximal at concentrations of approximately 0.5 mM (16). It is conceivable, therefore, that calcium

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1 Preliminary data obtained by C. O. Lee and L. Costanzo indicate an intracellular Ca ion activity of 10$^{-7}$ M or less in proximal tubular cells of Necturus kidney.
may inhibit sodium pump activity directly in vivo, though probably only at very high (unphysiological) cytosolic Ca\(^{2+}\) levels.

Concerning the question of energy supply to the pump, it is well established that active accumulation of calcium by mitochondria is driven by the electrical potential across the mitochondrial membrane and that this process occurs as an alternative to, and at the expense of, ATP synthesis (35). Interference with mitochondrial ATP synthesis at high cytosolic calcium levels, therefore, might limit the availability of energy to the sodium pump. However, it has recently become clear that the mitochondrial calcium uptake mechanism, although it has a large capacity, has a relatively low affinity (\(K_{m} \sim 10 \mu M\)) (13). This means that calcium uptake by mitochondria is half-maximal at concentrations approximately 100 times higher than the levels of cytosolic calcium ion activity assumed to obtain under physiological conditions. Therefore, again, it seems likely that only increases in cytosolic calcium far above the physiological range could significantly impair ATP synthesis.

The third possibility, that calcium influences membrane permeability to sodium, is an old concept. Curran and Gill (15) first demonstrated that high concentrations of calcium in the external medium inhibit transepithelial sodium transport in frog skin. They attributed the effect of high external calcium to a decrease in sodium permeability of the luminal membrane of the transporting epithelial cells. More recently, it has been shown that both elevated extracellular and elevated intracellular calcium levels reduce the sodium permeability of the plasma membrane of vertebrate photoreceptors (11, 28). There is a precedent, therefore, for postulating that changes in cytosolic calcium ion activity can modify cell membrane permeability to sodium.

The possibility that changes in cytosolic calcium primarily influence luminal membrane permeability to sodium in transporting epithelial cells is attractive, because this concept can be incorporated into a model which could account for the negative feedback mechanism whereby the rate of luminal entry of sodium is coupled to the rate of basolateral sodium pump activity (17, 18, 36). According to this concept, a primary change in pump activity would alter the intracellular sodium ion concentration (and also the basolateral membrane potential, if the pump were electrogenic), and, hence, the electrochemical gradient for passive sodium backflux into the cell across the basolateral plasma membrane. If this passive entry of sodium is coupled, either entirely or in part, to an efflux of calcium out of the cell through the operation of a Na-Ca countertransport system, changes in intracellular sodium concentration will be accompanied by parallel changes in cytosolic calcium ion activity (assuming that these are not immediately buffered by other cellular calcium uptake systems). The change in cytosolic calcium ion activity could then, in turn, modify (directly or indirectly) luminal membrane permeability to sodium in such a manner that the rate of entry of sodium into the cell across its apical surface is kept in step with its rate of extrusion across the basolateral surface. Accordingly, a primary decrease in pump rate would be reflected in an increase in cytosolic calcium ion activity, and this, in turn, would induce a decrease in the rate of luminal entry of sodium (Fig. 2). Conversely, an increase in the rate of sodium extrusion by the pump would stimulate Na-Ca exchange, thus diminishing cytosolic calcium, and thereby result in an increased rate of luminal entry of sodium ions. A similar negative feedback, mediated by free calcium ions, may also underlie the saturation phenomenon (7, 26, 47) which characterizes the luminal entry of sodium ions at high luminal sodium concentrations.

It seems probable that such a negative feedback, whether mediated by cytosolic calcium, as postulated here, or by a direct effect of cytosolic sodium on luminal sodium permeability, as proposed by Cuthbert and Shum (17, 18) and others (36, 54), would play an important role in the regulation of cell volume in sodium transporting epithelial cells. Thus, in the event of a decrease in sodium pump rate or of a large increase in luminal sodium concentration, a decrease in the sodium conductance of the luminal cell membrane in response to high cytosolic calcium (or sodium) levels would prevent overload of the cell with sodium and water and consequent cell swelling and lysis (17, 18, 36).

That such a negative feedback mechanism may operate in any situation in which the sodium pump rate is experimentally altered has been pointed out by Lewis et al. (36). These authors obtained direct evidence in rabbit urinary bladder that decreases in pump rate induced by anoxia, by the addition of ouabain or metabolic inhibitors, or by removal of serosal bicarbonate, elicit increases in apical membrane resistance that account for essentially all of the observed increase in total transepithelial resistance. Similarly, in the toad bladder removal of potassium from the serosal bathing medium has been shown to inhibit active sodium transport and at the same time decrease luminal permeability to \(^{22}\)Na (29). In frog

![FIG. 2. Model of epithelial cell indicating potential role of calcium ions in negative feedback mechanism. It is predicted that a primary decrease in Na pump rate will lead to a diminished rate of calcium efflux across the contraluminal cell membrane and, therefore, an increase in both cytosolic sodium and calcium ion activity. The increase in the latter, in turn, may induce a decrease in rate of sodium entry across the luminal cell membrane (see text).](http://ajprenal.physiology.org/)

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**Diagram:**

- **Luminal Cells**
  - **Contraluminal**
  - **Na\(^+\)**
  - **K\(^+\)**
  - **Ca\(^{2+}\)**
  - **\([Na^+]_i\)**
  - **\([Ca^{2+}]_i\)**

- **Negative feedback**
  - **2(\(^{22}\)Na)**

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skin inhibition of transport by ouabain is known to be associated with a decrease in luminal sodium entry (54). We suggest that all of these effects may, in fact, be mediated by changes in the level of free calcium ions and that the latter constitute a link in the negative feedback mechanism.

Given the concept that changes in cytosolic calcium ion activity primarily influence sodium transport through an influence on luminal membrane permeability to sodium, one might argue that at least short-term changes in sodium transport rates induced by hormones might actually be mediated by changes in cellular ionized calcium levels. Thus, the stimulatory action of vasopressin on sodium transport, which is generally believed to be largely due to an increase in luminal sodium permeability (16, 26, 54), may actually be the result of a lowering of cytosolic calcium levels (see above).

In this context it should be pointed out that there is now substantial evidence that changes in cytosolic free calcium levels and the process of Na-Ca exchange play a role in the action of vasopressin on water permeability of toad and frog urinary bladder (29, 48, 50, 51). In general, maneuvers believed to increase cellular calcium ion levels (quinidine, calcium ionophores, low sodium and potassium-free serosal media) inhibit the hydroosmotic response to vasopressin and cAMP (29, 48, 50, 51). At this juncture it is not clear whether changes in cytosolic calcium influence the action of vasopressin through regulation of intracellular cAMP levels, or whether a change in cytosolic calcium levels may actually mediate the hormonal response, perhaps through a regulation of microtubule and microfilament function (49).

The experimental observations concerning the role of cytosolic calcium in the regulation of sodium transport in amphibian epithelia and mammalian renal tubules would appear to have significant implications for various clinical situations in man. Any condition in which the electrochemical gradient for sodium across the peritubular cell membrane is altered, particularly if such a condition is long lasting, must be considered in the context of changes in cytosolic calcium ion activity. Accordingly, the condition of hyponatremia in which renal tubular reabsorption of sodium is diminished (4) may, at least in part, be mediated by an increase in cytosolic calcium activity caused by reduced Na-Ca exchange across the peritubular cell membrane. Similarly, abnormalities of sodium excretion in hypokalemic and hyperkalemic states may be dependent on the operation of these same cellular mechanisms. Furthermore, the diminished renal sodium reabsorption associated with hypercalcemia (19, 21, 33), and the increased sodium reabsorption associated with hypocalcemia (46), could be mediated in part by changes in ionized calcium levels in the renal tubular epithelial cells such as might be expected to occur in the presence of long-term changes in serum calcium. The possible influence of alterations in cytosolic calcium on renal sodium reabsorption in anoxia and in acidic or alkalotic states deserves to be investigated.

It is clear that we are only beginning to understand the potential significance of Na-Ca exchange, and of the messenger function of calcium, in the regulation of transepithelial transport. The precise physiological role of this intracellular control system and its distribution in different epithelial cell types are unknown at present. Also unknown are the relative roles of cytosolic calcium buffering systems in epithelial cells, consisting of mitochondria, endoplasmic reticulum, and, perhaps, calcium-binding proteins. Nevertheless, it appears that any factor that might change cytosolic calcium activity—such as pH, inorganic phosphate, parathyroid hormone, calcitonin, or even "natriuretic hormone" and neurotransmitters—may potentially influence the overall functioning of the system.

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REFERENCES


Although there is as yet no direct evidence that changes in sodium pump rate influence luminal sodium permeability in leaky epithelia, it seems possible that such a feedback mechanism may operate, at least to some degree, in all epithelial cells.


