Mechanism of Epithelial Lithium Transport

Evidence for Basolateral Na:Na and Na:Li Exchange

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ABSTRACT Measurement of transmural sodium fluxes across isolated, ouabain-inhibited turtle colon in the presence of a serosal-to-mucosal sodium gradient shows that in the absence of active transport the amiloride-sensitive cellular path contains at least two routes for the transmural movement of sodium and lithium, one a conductive path and the other a nonconductive, cation-exchange mechanism. The latter transport element can exchange lithium for sodium, and the countertransport of these two cations provides a mechanistic basis for the ability of tight epithelia to actively absorb lithium despite the low affinity of the basolateral Na/K-ATPase for this cation.

INTRODUCTION

The isolated turtle colon actively absorbs lithium ions by a mechanism that is pharmacologically identical to that for active sodium absorption. The absorption of either cation is blocked by mucosal amiloride or serosal ouabain (24). The sensitivity to amiloride appears to reflect a common pathway for the entry of the two cations into the cells: the amiloride-sensitive cation channels in the apical membrane. The basis for the common inhibitory effect of serosal ouabain is less clear. The glycoside is a specific inhibitor of the basolateral Na/K-ATPase, but ouabain-induced inhibition of transepithelial lithium transport cannot distinguish between two possible modes of lithium exit from the cell: (*a*) lithium transport by the basolateral Na/K-ATPase and (*b*) basolateral sodium-lithium exchange (24). In the latter mechanism the inhibitory effect of ouabain would be attributed to abolishing the transmembrane sodium gradient, which directly energizes lithium exit via countertransport.

In the present study we measured transmural fluxes of sodium and lithium

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across portions of isolated colon that were treated with ouabain to eliminate Na/K-ATPase activity so that possible exchange transport might be more readily discerned. In the presence of a transmural (S-to-M) gradient of either sodium or lithium, an amiloride-sensitive cation current from serosa to mucosa was observed. The electrical properties of this conductive pathway are discussed in a separate paper.[1] Tracer fluxes revealed that in the presence of ouabain the amiloride-sensitive path also contains a nonconductive cation-exchange mechanism that can carry out sodium-sodium or sodium-lithium exchange. Further, it was demonstrated that this transport element can produce sodium-lithium counterflow similar to that observed in the plasma membrane of the human red blood cell (10, 11, 19, 23). This basolateral cation exchanger provides a mechanistic basis for the ability of the colon (24) and other tight epithelia to actively absorb lithium ions.

METHODS

Colons were removed from turtles, stripped of musculature, and mounted as flat sheets in lucite chambers (A = 5.2 cm^2), as described elsewhere (7). Provisions for voltage-clamping were also identical to those previously described. Unless otherwise specified, portions of colon were bathed on the serosal side by a Ringer's solution that contained 114 mM Na, 114 mM Cl, 2.5 mM K, 2.5 mM HCO_3, 1.0 mM Ca, 5.0 mM D-glucose, 5.0 mM D-mannitol, and 2.5 mM pyruvate. The mucosal solution was identical except that the sodium concentration was reduced to ~3 mM by isosmotic replacement with choline-Cl to produce a transmural sodium gradient. A transmural lithium gradient was produced by bathing the tissue in solutions in which all of the sodium (3 or 114 mM) had been replaced by lithium. Both solutions were vigorously stirred with air at room temperature, and the pH of the bathing solutions was ~8.2. The sodium, potassium, and lithium content of all solutions was verified by flame photometry.

Transmural unidirectional fluxes of sodium and mannitol were measured as described previously (7) using ^{22}Na and [^{14}C]mannitol as tracers. Unidirectional lithium flows were approximated by measuring the appearance of lithium in a nominally lithium-free mucosal or serosal solution when the opposite solution contained either 114 or 5 mM lithium. 1-ml samples were taken from the mucosal bathing solution and replaced by lithium-free solution. The samples were assayed for lithium content with a flame photometer. All flux determinations were carried out under short-circuit conditions, and short-circuit current (I_{sc}) and conductance (G_T) were continuously recorded.

RESULTS

Table I summarizes the results of experiments in which the unidirectional fluxes of ^{22}Na and [^{14}C]mannitol were measured simultaneously across portions of isolated colon in which a reverse I_{sc} had been produced in the presence of ouabain and a serosal-to-mucosal sodium gradient. Shown are the short-circuit current, total tissue conductance, and unidirectional fluxes before and after the addition of 0.1 mM amiloride to the mucosal bathing solution. Before the addition of amiloride, positive current flow was from S

[1] Kirk, K. L., and D. C. Dawson. Cation permeability of sodium transporting epithelial cells in the absence of active transport. Manuscript submitted for publication.

to M, as was the net sodium flux. Mucosal amiloride reduced I_{sc} to near zero and markedly reduced the net sodium flux, whereas the unidirectional mannitol fluxes were unaffected. A comparison of the amiloride-induced reductions in I_{sc}, J_{sm}^{Na}, and J_{ms}^{Na} reveals that the amiloride-sensitive net sodium flux was equal to the amiloride-sensitive reversed short-circuit current. This result provides strong support for the notion that the reversed I_{sc} observed in the presence of ouabain and an S-to-M sodium gradient represents net sodium flow "backward" through the epithelial cells that are the normal route of active sodium absorption.

The effect of amiloride on transmural sodium fluxes is compatible with a simple model in which sodium can traverse the ouabain-inhibited colon via at least two pathways: a cellular path blocked by mucosal amiloride and a paracellular path that is unaffected by amiloride (7, 27). Fig. 1 provides

TABLE I

Transmural Fluxes of Sodium and Mannitol in the Presence of Ouabain and a Sodium Gradient

	I_{sc} (n = 12)	J_{sm}^{Na} (n = 6)	J_{ms}^{Na} (n = 6)	$J_{(\times 100)}^{man}$ (n = 12)	G_T (n = 12)
	$\mu Eq/5.2\ cm^2 \cdot h$		$\mu Eq/5.2\ cm^2 \cdot h$		$mS/5.2\ cm^2$
Ouabain	−1.08±0.12	3.95±0.25	1.03±0.14	3.29±0.43	4.80±0.27
Amiloride + ouabain	0.16±0.05	1.64±0.18	0.09±0.01	3.06±0.29	4.24±0.19
Δ	−1.24±0.14	−2.31±0.16	−0.94±0.13	−0.23±0.18	−0.56±0.10

Opposing unidirectional fluxes were measured using paired tissues. Since the values for I_{sc}, G_T, and J^{man} did not vary greatly between pairs, these have been averaged for economy. Values represent means ± SEM. The direction of positive current flow was from the mucosal to the serosal side. $[Na]_s$ = 114 mM; $[Na]_m$ = 3 mM.

additional support for this parallel path model. The figure shows J_{sm}^{Na} plotted vs. the simultaneously measured unidirectional mannitol flow, J_{sm}^{man}, in the presence and in the absence of mucosal amiloride. In a previous study (7) we showed that the transmural mannitol flow provides a marker for a paracellular shunt pathway where sodium and mannitol move as in free solution, and that in the actively transporting colon, S-to-M sodium flow behaved as a single component that was restricted to the paracellular path. Fig. 1 shows that in the presence of ouabain and a sodium gradient, J_{sm}^{Na} behaved as if the flux consisted of two components: a paracellular flow that was highly correlated with the transmural mannitol flux, and cellular flow, represented by the intercept in Fig. 1, which was abolished by amiloride. These observations are consistent with the notion that the amiloride-sensitive transmural sodium flux is a direct measure of transcellular sodium flow.

Mechanisms of Transcellular Sodium Movement: Flux-Ratio Analysis

Measurement of transmural fluxes across ouabain-treated colon confirmed the expectation that the amiloride-sensitive "reversed" I_{sc} induced by a

transmural sodium gradient was due to net sodium flow. The steady state current voltage relation for the cellular path presented in a separate paper[1] indicated that the sole driving force for this current was the sodium electrochemical potential gradient, as if the reverse sodium current might be attributable to simple diffusion. Ussing (29) showed that a simple, macroscopic test for diffusional flow is provided by the ratio of the unidirectional fluxes. If transcellular sodium flows are diffusional, the predicted flux ratio under short-circuit conditions ($V_{ms} = 0$) is:

$$\Delta J_{sm}^{Na}/\Delta J_{ms}^{Na} = [Na]_s/[Na]_m,$$

where ΔJ_{sm}^{Na} and ΔJ_{ms}^{Na} represent the cellular components of the transmural fluxes, which are operationally defined as the amiloride-sensitive fluxes. The predicted value for the flux ratio is 38 for a 114–3-mM sodium gradient.

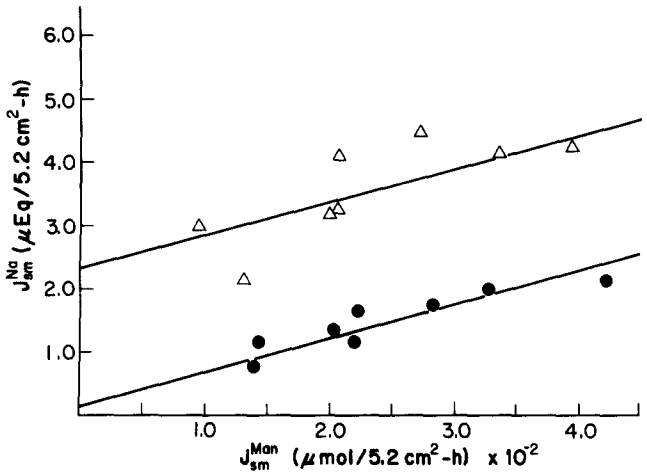

FIGURE 1. J_{sm}^{Na} plotted vs. the simultaneously measured J_{sm}^{man} for tissues exhibiting reversed currents. Fluxes were measured before and after the addition of mucosal amiloride (0.1 mM). Each point represents the mean of at least three half-hour flux periods from a single tissue. △, pre-amiloride; ●, post-amiloride. $[Na]_m$ = 3 mM; $[Na]_s$ = 114 mM; ouabain = 10^{-4} M.

The measured value, however, was 2.46 (Table I), an order of magnitude lower than that expected for simple diffusion. This result can be restated by saying that although the net flux of sodium from S to M was equal to the reversed current, the relative "backflux," ΔJ_{ms}^{Na}, was much greater than expected for simple diffusion given the relatively steep applied sodium chemical potential gradient. The simplest explanation for this behavior is that a portion of the tracer flux proceeded via an obligatory, sodium-sodium exchange mechanism. In exchange flow, acceleration of the "uphill" unidirectional flux is produced by counterflow between the abundant and tracer species of the ion (8, 9).[2]

[2] We do not attach any significance to the fact that in the presence of amiloride the ratio of the average sodium flux is 18 rather than 38. The variability in paired determinations and in the actual magnitude of the mucosal sodium concentration could easily account for this discrepancy.

Support for this hypothesis is provided by Fig. 2, in which the amiloride-sensitive sodium fluxes, ΔJ_{sm}^{Na} and ΔJ_{ms}^{Na}, are plotted vs. the amiloride-sensitive short-circuit current. The form of this plot suggests that the cellular, S-to-M sodium flux consisted of two components, one of which was highly correlated with the current and a second which was unrelated to charge movement. In contrast, ΔJ_{ms}^{Na}, the cellular blackflux, showed little or no correlation with ΔJ_{sc}. The relationship between ΔJ_{sm}^{Na} and ΔJ_{sc} can be approximated by a straight line with a unity slope and positive intercept that is equal to the average value of ΔJ_{ms}^{Na}. This result suggests that the transcellular sodium fluxes can be envisioned as consisting of two components: a conductive component that gives rise to the short-circuit current and a one-for-one exchange component that is not associated with current flow.

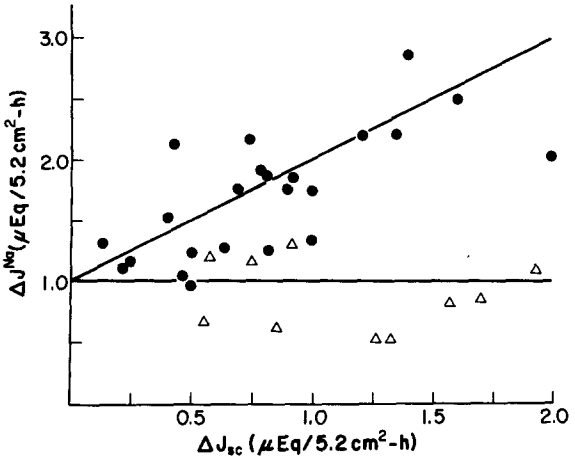

FIGURE 2. The amiloride-sensitive components of J_{sm}^{Na} and J_{ms}^{Na} (ΔJ_{sm}^{Na} and ΔJ_{ms}^{Na}) plotted vs. the amiloride-sensitive reversed current, ΔJ_{sc}. Note that the current is expressed in the same units as the opposing Na^+ fluxes. Each point represents the mean of at least three half-hour flux periods from a single tissue. ●, S to M; △, M to S. Concentrations are as in Fig. 1.

Fig. 2 suggests that in the presence of a steep transmural sodium gradient the opposing cellular fluxes can be written for practical purposes as:

$$\Delta J_{sm}^{Na} = \Delta J_{sc} + \Delta J_{ex}^{Na};$$

$$\Delta J_{ms}^{Na} = \Delta J_{ex}^{Na},$$

where ΔJ_{ex}^{Na} is the apparent "exchange component" of the amiloride-sensitive transmural flux. This simple dichotomy probably reflects the fact that the relative backflux through the conductive path is expected to be small in the presence of a steep transmural sodium gradient.

Evidence for Cation-Cation Exchange

If, in the absence of active transport, a portion of the transcellular sodium flux proceeds via sodium-sodium exchange, then the cellular component of

the S-to-M sodium flux should be highly dependent on the availability of mucosal (or cellular) sodium. Table II presents the results of an experiment in which tissues were initially bathed on the mucosal side by nominally sodium-free choline Ringer's and on the serosal side by 114 mM sodium or 114 mM lithium (Na-free) Ringer's. The table shows the amiloride-sensitive values for the short-circuit current, and the S-to-M fluxes of sodium or lithium before and after the addition of 3 mM sodium chloride to the mucosal bath. The addition of mucosal sodium did not alter ΔI_{sc} but markedly stimulated the S-to-M fluxes of both sodium and lithium. This result is consistent with the notion that the cellular path contains a cation-exchange mechanism that can operate in a sodium-sodium or a sodium-lithium exchange mode. It is of interest in this regard that a similar increment in mucosal sodium concentration produced virtually identical increments in the cellular S-to-M fluxes of

TABLE II

Effect of $[Na]_m$ on ΔJ_{sm}^{Na} and ΔJ_{sm}^{Li}

	Na gradient		Li gradient	
	ΔI_{sc}	ΔJ_{sm}^{Na}	ΔI_{sc}	ΔJ_{sm}^{Li}
	$\mu Eq/5.2\ cm^2 \cdot h$		$\mu Eq/5.2\ cm^2 \cdot h$	
$[Na]_m = 0$	0.97±0.10	1.14±0.11	1.06±0.07	1.36±0.08
$[Na]_m = 3$	0.82±0.08	1.80±0.16	1.09±0.11	2.01±0.22

Tissues were exposed to serosal ouabain and bathed initially by sodium-free choline Ringer's on the mucosal side and 114 mM sodium or lithium Ringer's on the serosal side. J_{sm}^{Na} and J_{sm}^{Li} were measured first in the absence of mucosal sodium (0.3 mM), then after the addition of 3 mM NaCl to the mucosal bath, and finally after the addition of mucosal amiloride. Each measurement was the mean of at least two successive half-hour flux periods. The amiloride-sensitive values of current and ion flux (ΔI, ΔJ) were obtained by subtracting the values measured in the presence of amiloride from those measured at each mucosal Na^+ concentration in the absence of amiloride. This procedure was justified on the basis of previous studies (24, 27) which showed that alterations in mucosal cation concentration did not alter amiloride-insensitive sodium fluxes or amiloride-insensitive currents. Data represents means ± SEM. (Na^+ gradient: $n = 10$; Li^+ gradient: $n = 8$.)

both sodium and lithium. The stimulation of cation exchange by mucosal sodium implies that cell sodium can be reduced to near zero by removing mucosal sodium. This behavior is expected if the basolateral membrane is rate-limiting for reverse sodium flow.

Fig. 3 provides additional support for the existence of an exchange component of the transcellular sodium flux. The figure shows values of ΔJ_{ms}^{Na} plotted vs. simultaneously measured values of ΔJ_{sc} before and after the addition of 3 mM NaCl to a nominally sodium-free mucosal bath. In view of the results shown in Fig. 2, both sets of points in Fig. 3 were fitted by a straight line with unity slope; however, the intercept for the fluxes measured into a nominally sodium-free mucosal bath is near zero, whereas that for 3 mM mucosal sodium is ~ 1 μEq/5.2 $cm^2 \cdot h$. The relation between ΔJ_{sm}^{Na} and ΔJ_{sc} is thus consistent with the notion that the S-to-M cellular sodium flux consists of two components: a conductive flow that gives rise to the reverse current, and a nonconductive exchange component that is dependent on the presence of mucosal sodium.

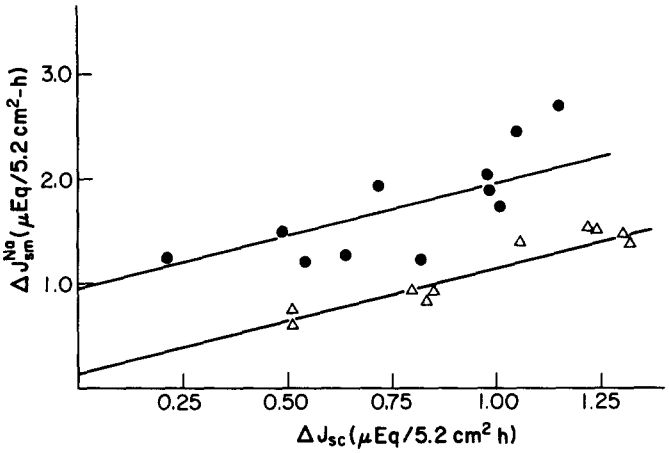

FIGURE 3. The amiloride-sensitive component of J^{Na}_{sm}, ΔJ^{Na}_{sm}, plotted vs. the amiloride-sensitive reversed current, ΔJ_{sc}, measured first in the presence of a nominally Na^+-free mucosal bath ($[Na]_m \simeq 0.3$ mM) and subsequently in the presence of 3 mM mucosal Na^+. Each point represents the mean of at least three half-hour flux periods from a single tissue. ●, $[Na]_m = 3$ mM; Δ, $[Na]_m \approx 0$ mM; $[Na]_s = 114$ mM; ouabain = 10^{-4} M.

Sodium-Lithium Counterflow

The anomalous flux ratio that is characteristic of an exchange transport mechanism is a direct result of a counterflow interaction between the tracer and abundant isotopes of the transported species (8, 9). Hence, counterflow, i.e., the driving of the net flow of one cation with the free energy in the gradient of another cation, is the most direct test for obligatory cation exchange. In the present system the data are consistent with the notion that both sodium and lithium have affinity for the exchanger so that it should be possible, in principle, to observe sodium-lithium counterflow. We measured transmural sodium fluxes in the absence of a sodium electrochemical potential gradient, with and without an imposed transmural lithium gradient, and the results are shown in Table III.

TABLE III

Sodium-Lithium Counterflow

	$[Li]_m = [Li]_s$				S-to-M Li gradient			
	I_{sc}	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{ms}/J^{Na}_{sm}	I_{sc}	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{ms}/J^{Na}_{sm}
	μEq/5.2 cm²·h				*μEq/5.2 cm²·h*			
Pre-amiloride	0.04±0.17	0.32±0.07	0.38±0.05	0.84±0.80	−0.71±0.11	1.04±0.25	0.06±0.01	17.2±3.0
Post-amiloride	0.17±0.07	0.05±0.01	0.04±0.01	1.40±0.40	0.36±0.05	0.06±0.01	0.03±0.10	2.1±0.20
Δ	0.13±0.05	−0.27±0.06	−0.34±0.05	0.80±0.10	1.05±0.09	−0.99±0.26	−0.03±0.01	−29.3±5.8

Transmural sodium fluxes were measured in the absence of cation gradients in ouabain-treated, paired tissues, both of which were bathed on both sides by Ringer's solutions containing 2 mM Na, 2 mM Li, and 112 mM choline. In a second set of tissues bidirectional fluxes were measured in the presence of a 114–2 mM, serosal-to-mucosal lithium gradient. The mucosal and serosal bathing solutions both contained 2 mM sodium. In both experiments fluxes were measured before and after the addition of mucosal amiloride (0.1 mM). All values are mean ± SEM. I_{sc}'s did not vary greatly between pairs and values were averaged for economy.

In the absence of a transmural lithium gradient net, transcellular sodium flow was near zero, although amiloride-sensitive fluxes were discernible. In the presence of an S-to-M lithium gradient, the cellular M-to-S sodium flux was nearly 30 times larger than the opposing S-to-M flux, resulting in net sodium flow in the direction opposing the transmural lithium gradient. This net flux was due to a 3-fold increase in the cellular M-to-S flux and a simultaneous, 10-fold decrease in the cellular S-to-M sodium flow. The amiloride-sensitive, reversed short-circuit current indicated that sodium absorption was accompanied by net lithium flow in the S-to-M direction. The finding that net sodium flow can be induced by a transmural lithium gradient in the absence of a sodium electrochemical potential gradient provides compelling evidence for sodium-lithium exchange.[3]

For models of active lithium absorption, the most relevant exchange mode is lithium flow driven by a sodium gradient. The results presented in Fig. 4 show that a sodium gradient can drive net lithium absorption in ouabain-treated tissues that presumably lack a functional sodium-potassium ATPase. The figure shows the total number of moles of lithium (n_{Li}) appearing on the mucosal or serosal side plotted as a function of time. Net lithium absorption was induced only in the presence of a serosal-to-mucosal sodium gradient. The addition of amiloride (0.1 mM, mucosal) abolished the accumulation of lithium on the serosal side in the presence of a sodium gradient. There was no readily discernible effect of amiloride on the other transmural flows, probably because of the small size of these cellular fluxes in the presence of only 5 mM lithium. Using the 3-h points for comparison, the M-to-S and S-to-M flows of lithium yield a net flow of $\sim$1.76 μEq/h in the presence of a serosal-to-mucosal sodium gradient, a value similar to that obtained for lithium-driven sodium absorption (Table III). It was typically observed that in the hour after amiloride addition, there was a rise in serosal lithium, followed by a decline during the next hour (Fig. 4). Lithium accumulation after mucosal amiloride addition could have been due to the exchange of serosal sodium and cellular lithium, which would be expected to continue even after lithium entry from the mucosal solution had been blocked. The decline in serosal lithium during the next hour could represent the subsequent dissipation of both sodium and lithium gradients (between serosal medium and cells) via parallel leak pathways.

The demonstration of Na-Li counterflow also serves to further clarify the results presented in Table II. Here the addition of mucosal sodium increased the S-to-M lithium flow but had virtually no effect on the current, ΔI_{sc}, which suggests that the charge carried by the additional S-to-M lithium flow was

[3] A similar experiment employing an M-to-S gradient of lithium to drive net sodium secretion is complicated by the fact that high levels of mucosal cation (sodium or lithium) lead to a reduction or loss of apical membrane conductance (27; and unpublished observations). This effect has been observed in a variety of tight epithelia and has been attributed to a "negative feedback" effect of cellular sodium on apical sodium permeability (28).

The source of the relatively small amiloride-sensitive I_{sc} measured in the presence or in the absence of a lithium gradient is unknown, but discrepancies of this magnitude could be due in part to electrode offsets.

exactly balanced by net sodium counterflow in the opposite direction. In the presence of a nominally sodium-free mucosal solution, the net sodium flow was equal to the reversed current, whereas the net lithium flow under similar conditions was greater than the reversed current. This difference probably reflects the difficulty in removing all of the sodium from the epithelial cells even by repeated replacement of the mucosal bath.

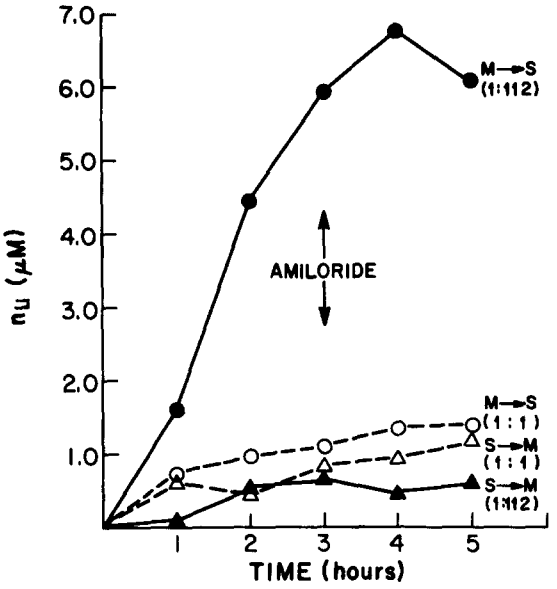

FIGURE 4. Representative experiments in which transmural lithium fluxes were measured in the presence and in the absence of a transmural sodium gradient. Reverse currents were established in all tissues as described in the text, and then the serosal solution of one pair was changed to low-sodium (1 mM) choline Ringer's. Fluxes were determined by measuring lithium appearance on one side of the tissue after the addition of 5 mM lithium to the other side in a small volume of concentrated LiCl. Numbers in parenthesis indicate the mucosal and serosal sodium concentrations, respectively. Amiloride (0.1 mM) was added to the mucosal solutions of all tissues after the third sample. The tissue conductances in this experiment averaged 3.1 mS/5.2 cm^2 (M to S, 1:112, ▲), 5.3 mS/5.2 cm^2 (S to M, 1:112), 7.8 mS/5.2 cm^2 (M to S, 1:1, ○), and 5.3 mS/5.2 cm^2 (S to M, 1:1, △); i.e., the tissue with the smallest electrical conductance exhibited the largest lithium flux.

DISCUSSION

Measurements of transmural flows of sodium and lithium across the isolated colon in the presence of ouabain and a transmural cation gradient and electrical measurements presented in a separate paper[1] are consistent with the model shown in Fig. 5. The model represents the behavior of the cells that are normally responsible for active sodium absorption after the basolateral sodium pump has been effectively removed by treatment with ouabain.

In this scheme the apical membrane contains a single conductive transport element: a channel that is permeable to both sodium and lithium and is blocked by amiloride. Electrophysiological results (16)[1] require that the basolateral membrane contain at least two conductive transport elements: a barium-sensitive potassium channel and a second channel impermeable to potassium but permeable to sodium and lithium. In addition, however, the flux measurements presented here provide evidence for a cation-exchange mechanism that operates in a one-for-one, "electrically silent" mode.

Basolateral Cation Exchange

Although the present experiments do not reveal directly the site of the cation exchange, several considerations suggest its presence in the basolateral membrane. First, previous experiments (26) indicated that the apical sodium influx measured during active sodium absorption is a purely conductive

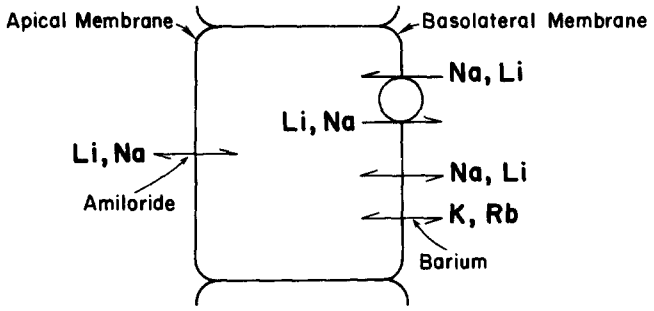

FIGURE 5. Schematic representation of the cation permeabilities of the apical and basolateral membranes of sodium-transporting cells of the turtle colon in the presence of ouabain and a serosal-to-mucosal sodium gradient.

process that is not inhibited by mucosal lithium. This behavior is not consistent with the presence of a significant component of electroneutral cation exchange at the apical membrane. In contrast, Table III indicates that raising serosal lithium markedly reduced the cellular portion of the S-to-M sodium flux and produced a simultaneous increase in the cellular portion of the mucosal-to-serosal flux. These effects are difficult to reconcile with simple inhibition by lithium of basolateral sodium permeability, but are entirely consistent with the notion that these fluxes are due to a basolateral cation exchange mechanism.

The definitive criterion for the existence of a cation exchanger is the demonstration of thermodynamic coupling: the donation of energy from the electrochemical potential gradient of one ion to the net flow of another. This coupling is conveniently measured by the flux ratio, which is a function of the total effective thermodynamic force on a transported substance (8, 9). Table III shows that the application of a transmural lithium gradient reduces the S-to-M sodium flux and increases the M-to-S sodium flux, i.e., the transcellular sodium flux ratio goes from unity in the absence of a lithium gradient to a value of ~30 in the presence of a lithium gradient.

We can use the flux ratio to compare the apparent driving force for the flow of sodium with the energy available in the transmural lithium gradient. For one-to-one stoichiometry the maximum flux ratio in the absence of a sodium gradient is given by:

$$J_{ms}^{Na}/J_{sm}^{Na} = \exp(\Delta\tilde{\mu}_{Li}/RT)$$
$$= [Li]_s/[Li]_m.$$

The observed flux ratio of 30 approaches the maximum value in the context of a 40–50-fold transmural lithium gradient. Clearly, exact quantitation is impossible since we do not know the cellular concentration of either cation. The one-to-one stoichiometry for the exchanger is suggested by the tight coupling between Na and Li fluxes and the fact that the exchange flow appeared to be "electrically silent."

Implications for Active Lithium Absorption by Epithelia

Sarracino and Dawson (24) showed that the isolated turtle colon, like the frog skin (4, 20, 30) and toad urinary bladder (14), actively absorbs lithium. Considerable evidence suggests that lithium enters the transporting cells of tight epithelia through amiloride-sensitive sodium channels in the apical membrane. In the turtle colon, the results of Thompson and Dawson (26) suggest that these apical channels are at least as permeable to lithium as they are to sodium. The nature of the exit step is less clear. Sarracino and Dawson (24) showed that, as in the frog skin (4, 20), lithium absorption by turtle colon is abolished by ouabain. This result, however, does not discriminate between models based on lithium exit directly via the basolateral sodium-potassium pump and those based on lithium exit via a basolateral sodium-lithium exchanger (10, 11, 19, 23). In an exchange model, steady state lithium transport would presumably be maintained by "recycling" (by the basolateral Na/K pump) of sodium that entered in exchange for lithium. Although metabolic and electron-microprobe studies on toad urinary bladder and frog skin have been interpreted as suggesting that there is little "recycling" of sodium across the basolateral membrane (1, 5, 21), it should be noted that a one-for-one cation exchanger will only manifest itself in these experiments if a second exchangeable cation is present. Thus, contrary to the suggestion of Sarracino and Dawson (24), the cited studies have no bearing on this issue.

Dunham and co-workers (12, 22) showed that the prototypical Na/K pump of the human red blood cell will, in fact, carry out ouabain-sensitive lithium extrusion, but that the affinity for lithium on the cytoplasmic side is so low that outward lithium transport is only detectable if the intracellular sodium concentration is <1 mM. Similarly, Siegel et al. (25) reported that a microsomal preparation of Na/K-ATPase from isolated frog skin epithelium was not activated by lithium. These observations are difficult to reconcile with lithium transport rates in turtle colon (24) and frog skin (4, 20, 30), which are comparable to those for sodium over a wide range of mucosal sodium concentrations.

The present experiments provide the first evidence for the existence of a basolateral cation exchanger that could effect that cation-cation exchange required to yield ouabain-sensitive lithium absorption. These results show clearly that in the presence of ouabain the cation exchanger can provide coupling between cation flows necessary to permit an inwardly directed sodium gradient to drive an outwardly directed lithium flow. Recent observations on the amphotericin-treated colon (16) lend additional support to a model based on basolateral cation exchange. Halm and Dawson (13) have shown that in polyene-treated colons, in the absence of mucosal and serosal sodium, mucosal lithium does not activate the sodium-potassium pump.

The present experiments do not reveal whether the cation exchanger is active during normal absorption of sodium or lithium by the colon. The simple experiment of removing serosal sodium is not definitive because of the potential effects of this maneuver on apical cation permeability (6, 28). In addition, the absence of a readily detectable cellular sodium backflux in the normally transporting tissue in the presence or in the absence of mucosal lithium appears to argue against a significant amount of basolateral cation exchange (7). The lack of cellular backflux under these conditions, however, could also reflect a steep electrochemical potential gradient at the apical membrane of transporting cells even in the presence of mucosal lithium (17).

Several investigators have, in fact, reported significant amiloride-sensitive sodium backfluxes under normal transport conditions as well as in the presence of ouabain. O'Neil and Helman (18) reported a vasopressin-induced sodium backflux in frog skin that was abolished by mucosal amiloride. In the absence of vasopressin, sodium backflux was unaffected by amiloride. Biber and Mullen (2, 3) showed that in frog skin the sodium backflux exhibited saturation kinetics, but was not affected by 6 mM serosal lithium. An amiloride-sensitive component of the sodium backflux was only observed at reduced (6 mM) mucosal sodium concentrations, however.

A model based on a one-for-one basolateral cation exchanger predicts that in the absence of mucosal sodium the short-circuit current should be equal to the net lithium flux, since the net recycling of sodium entering from the serosal side should exactly equal the lithium efflux from the cell. This is apparently the case in the frog skin (4, 20, 30) and turtle colon (24).

Physiological Role of the Cation Exchanger

The physiological significance of the basolateral cation exchanger is unclear since neither Na:Na nor Na:Li exchange is expected to play a role in normal cell functions. It must be emphasized, however, that the cation exchange behavior revealed by these experiments could arise, in principle, from a variety of cation co- or countertransport mechanisms as long as the transporter had affinity for both cations. For instance, the sodium-proton exchanger that is thought to reside in the luminal membrane of proximal tubular cells apparently has affinity for lithium (15).

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