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Cation Selectivity in Active Transport: Properties of the Turtle Colon in the Presence of Mucosal Lithium

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Summary. If mucosal Na is completely replaced by Li the isolated turtle colon exhibits a steady-state short-circuit current (I_{sc}) consistent with the transport of Li from mucosa to serosa. I_{sc} persists in the absence of a transmural electrochemical gradient for Li and is abolished by amiloride or ouabain. In the presence of mucosal Li the amiloride-sensitive, transepithelial cation transport path can be described as a constant emf in series with a variable conductance. A comparison of equivalent circuit parameters, however, reveals that in the presence of mucosal Li the apparent emf of the cation transport path is markedly reduced, but the conductance of the active path may be greater than, equal to, or less than that observed in the presence of Na. In tissues characterized by a relatively low cellular conductance in the presence of Na, Li substitution increases the amiloride-sensitive conductance, whereas, in tissues characterized by an initially high active "Na-conductance", the amiloride-sensitive conductance is reduced in the presence of Li. Thus, the approximate identity of I_{sc} in some tissues after cation substitution is a fortuitous consequence of an increased amiloride-sensitive conductance in the presence of mucosal Li coupled with a *decreased* apparent emf for Li transport. The addition of small amounts of Li to Na-containing mucosal or serosal solutions inhibits active Na transport, suggesting that Li exerts a "toxic" effect on the transport machinery apart from its ability to serve as a substitute cation.

Transmural Na flux measurements reveal that, when the mucosal bathing solution contains comparable amounts of Na and Li, the net flow of Na through the active path is *less* than the total I_{sc}. The ratio of I_{sc} to the net Na flux approaches unity as the mucosal Li concentration approaches zero, confirming the notion that the "extra current" is carried by Li ions. The ratio of I_{sc} to the net Na flux is a linear function of the mucosal Li:Na concentration ratio and the slope of this relation can be interpreted to indicate that the ratio of the apparent rate coefficients for Li and Na movement through the active path is about 0.75.

The isolated turtle colon actively transports Na from mucosa to serosa [5]. Na which is actively transported across the epithelial cell layer must cross at least two barriers in series; the apical cell membrane, where Na entry is blocked by amiloride, and a more basal-lying barrier, presumably the basolateral membrane, where Na transport is inhibited

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by ouabain [17]. In a previous study [18] we investigated the cation selectivity of the apical membrane by measuring Na entry into epithelial cells from the mucosal bathing solution in the presence of mucosal Li. The results suggested that Li can enter Na-transporting cells through amiloride-sensitive channels in the apical membrane. This observation naturally raised the question of possible active transport of Li from the cells to the serosal bathing solution, particularly in view of previous reports of apparent active Li transport by isolated frog skin [3, 19] and toad bladder [11]. The aim of the present experiments was to investigate transmural Li transport by the turtle colon and, in particular, to obtain a quantitative comparison of transmural Na and Li movements through the active cation transport path. We compared the electrical properties of the turtle colon in the presence of mucosal Na and Li and measured transmural Na fluxes in the presence of varying concentrations of mucosal Li. The results suggest that Li is actively transported across the isolated turtle colon by a mechanism which, based on its sensitivity to amiloride and ouabain, appears to be identical to the active Na transport path. The properties of the amiloride-sensitive cation transport path, however, are markedly different in the presence of mucosal Li. These differences appear to reflect not only possible differential "selectivity" of the apical and basolateral cation transport steps, but also a pronounced toxic effect of Li on the active transport mechanism.

Materials and Methods

Colons were removed from turtles (*Psuedemys scripta elegans*) and stripped of muscular layers as previously described [5]. Portions of stripped mucosa were mounted as flat sheets in Lucite chambers so that the exposed area was approximately 1 cm^2. The chambers were equipped with two 3-M KCl-agar bridges connected to calomel electrodes for the measurement of transepithelial electrical potential differences (PD), and two Ringer's-Agar bridges connected to Ag-AgCl electrodes for passing current across the tissue. The electrodes were connected to an electronic voltage clamp which maintained the transepithelial PD at zero mV and which could be adjusted to compensate for the fluid resistance between the PD sensing bridges and the tissue surface. The total tissue conductance, G_t, was calculated from the change in current due to a brief, 10-mV change in clamping potential. During dissection, the tissue was bathed by a solution containing (in mM): Na, 114; Cl, 114; K, 2.5; HCO_3, 2.5; Ca, 1.0; D-glucose, 5.0; D-mannitol, 5.0; and pyruvate, 2.0; referred to as "Na-Ringer's." In individual experiments, as indicated in *Results*, the mucosal bathing solutions contained varying concentrations of Na and/or Li and sufficient choline to render the solution isosmotic with 114 mM Na-Ringer's. In *all* experiments the serosal bathing solution initially contained 114 mM Na-Ringer's and was altered in several instances by simply adding a small volume of concentrated LiCl directly to the 10-ml chamber volume. The compositions of these solutions were routinely

verified by osmometry, flame photometry, and atomic absorption spectrophotometry. All solutions were stirred and oxygenated with air to yield a pH of approximately 8.1 at 25 °C. Transmural fluxes of ^{22}Na and ^{3}H-mannitol were measured as previously described [5].

Results

Electrical Properties of the Colon in the Presence of Mucosal Li

The open-circuit PD (ψ_{ms}) and short-circuit current (I_{sc}) characteristic of the isolated turtle colon are, for practical purposes, entirely attributable to active Na transport [5]. I_{sc} and ψ_{ms} are reduced to near zero values by amiloride (added to the mucosal side), ouabain (added to the serosal side), or by replacing all mucosal Na by choline or K [6, and *unpublished observations*]. To determine if Li could substitute for Na in the active transport path, I_{sc} was measured after mucosal Na was *completely replaced* by Li.

Preliminary experiments (*see also* Fig. 5) suggested that substituting either choline or Li for Na in the *serosal* bathing solution markedly reduced the rate of active Na transport by the isolated colon. For this reason the composition of serosal bathing solution was maintained constant (i.e., 114 mM Na-Ringer's) in most experiments when the Na in the mucosal solution was partially or totally replaced by choline or Li. Asymmetry in the cation composition of the bathing solutions, however, results in a diffusional emf which appears across the shunt pathway. Experiments in which amiloride was added to the mucosal solution to block the active cation transport path [5, 17] yielded the values shown in Table 1 for the *amiloride-insensitive* portion of I_{sc} in the presence of

Table 1. Effect of mucosal cations on I_{sc} in the presence of amiloride when the serosal bathing solution was 114 mM Na-Ringer's

Mucosal bathing solution	$(I_{sc})_{amil}$	n
114 mM Na-Ringer's	3.0 ± 0.3	24
114 mM Li-Ringer's	5.1 ± 0.4	49
16 mM Na-Ringer's (98 mM choline)	2.9 ± 0.4	28
16 mM Li-Ringer's (98 mM choline)	4.8 ± 0.4	42

n = number of tissues. The units of I_{sc} are μA/cm^2.
$\bar{x} \pm$ SE.

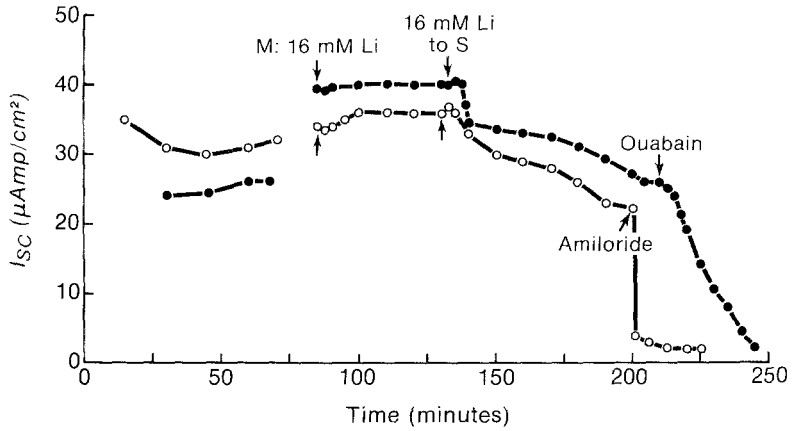

Fig. 1. Representative ion substitution experiment showing I_{sc} as a function of time. Tissues were pre-incubated in 16 mM mucosal Na (98 mM choline), 114 mM serosal Na. The mucosal bathing solution was then replaced with 16 mM Li (98 mM choline). Subsequently a small volume of a concentrated LiCl solution was added to the serosal solution to bring the final concentration to 16 mM

various mucosal cations. The average values of the amiloride-insensitive I_{sc} with asymmetric bathing solutions do not exceed 6 μA/cm^2 and differ only slightly from the value observed in the absence of transmural cation gradients. Thus, the error incurred by assuming that I_{sc} may be equated with active ion transport is negligible except in tissues characterized by a relatively low rate of active cation absorption.

Figure 1 shows a representative ion substitution experiment in which the mucosal bathing solutions of two tissues initially contained 16 mM Na. The serosal bathing solutions were initially 114 mM Na-Ringer's. After a steady-state I_{sc} was obtained, the mucosal side of both tissues was washed several times with Na-free, Li-Ringer's and the mucosal bathing solution was replaced with 16 mM Li-Ringer's. In this case the steady-state I_{sc} in the presence of mucosal Li was comparable to that observed with Na. We have previously reported that sudden exposure of the mucosal surface of the turtle colon to Li-containing solutions produces a marked transient in I_{sc} and total tissue conductance, G_t [18]. This Li-induced oscillation is highly damped after 1–2 min, however, and is not shown in this plot. After I_{sc} attained a quasi-steady-state value in the presence of mucosal Li, sufficient Li was added to the serosal bathing solution to bring the final concentration to 16 mM, thus abolishing the electrochemical potential difference for Li. This maneuver reduced but did not abolish I_{sc}. Preliminary experiments showed that the addition of

up to 16 mM Li to the serosal bathing solution (114 mM Na-Ringer's) of tissues in which active cation transport had been abolished by amiloride caused no detectable change in I_{sc} regardless of the composition of the mucosal bathing solution. Thus, the reductions in I_{sc} following exposure to serosal Li (Figs. 1, 2 and 5) cannot be attributed to changes in transepithelial diffusion emfs. As shown in Fig. 1, I_{sc} in the presence of Li was abolished by the addition of amiloride (10^{-4} M) to the mucosal bathing solution or ouabain (10^{-4} M) to the serosal bathing solution.

Figure 2 shows the results of a similar experiment in which two tissues were again pre-incubated in the presence of 16 mM mucosal Na and the mucosal solution of one tissue was replaced with 16 mM Li-Ringer's. Note that, in contrast to the result shown in Fig. 1 substitution of mucosal Na by Li resulted, in this case, in a *reduction* in the steady-state I_{sc}. The figure shows that the addition of 16 mM Li to the serosal bathing solutions (which initially contained 114 mM Na-Ringer's) reduced I_{sc} in the presence of either mucosal Li or Na. The time course of the inhibition of I_{sc} by ouabain was identical in the presence of mucosal Li or Na.

Using a protocol similar to that shown in Figs. 1 and 2, a series of ion-substitution experiments was conducted. Tissues were pre-incubated in the presence of either 114 or 16 mM mucosal Na and the mucosal bathing solution was then replaced with one in which all Na had been replaced by Li. The serosal solutions were Li-free, Na-Ringer's. After a steady-state I_{sc} was obtained in the presence of mucosal Li, 10^{-4} M amiloride

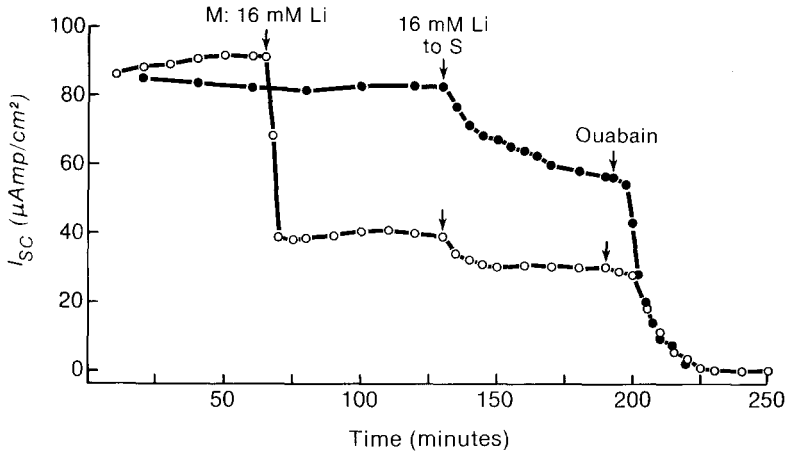

Fig. 2. Representative ion-subsitution experiment showing I_{sc} as a function of time. Tissues were pre-incubated in 16 mM mucosal Na (98 mM choline), 114 mM serosal Na, and the composition of the bathing solutions was altered as indicated

was added to the mucosal bathing solution. This procedure yielded values for I_{sc} and total tissue conductance, G_t, in the presence of mucosal Li, before and after exposure to amiloride. Previous studies [5] have shown that transmural Na movement through the active path is abolished by amiloride. Amiloride completely blocks Na entry across the apical membranes of the epithelial cells and appears to reduce the conductance of the active path to near zero values [17]. Thus, the amiloride induced *decrease* in the *total* tissue conductance provides a measure of the conductance of the cellular cation transport path.

Figure 3 shows the amiloride-sensitive, steady-state short circuit current measured in the presence of mucosal Li, ΔI_{sc}^{Li}, plotted *vs.* the steady-state current which characterized the same tissue in the presence of mucosal Na, I_{sc}^{Na}. Up to about 25 μA/cm^2, ΔI_{sc}^{Li} and I_{sc}^{Na} do not differ greatly. At the highest values of I_{sc}^{Na}, however, ΔI_{sc}^{Li} is markedly reduced from the value observed in the presence of mucosal Na. Figure 4 shows ΔI_{sc}^{Li} plotted *vs.* the amiloride-sensitive conductance, ΔG, for the same tissues shown in Fig. 3. Also shown are values for ΔI_{sc}^{Na} and ΔG obtained by adding 10^{-4} M amiloride to the mucosal surface of colons bathed by 114 mM mucosal Na. As shown in previous studies [5, 17], ΔI_{sc}^{Na} is a linear function of ΔG in the presence of mucosal Na, suggesting that variations in ΔI_{sc}^{Na} from tissue to tissue can be attributed to variation in the apparent conductance of the active path, the apparent emf, E_{Na},

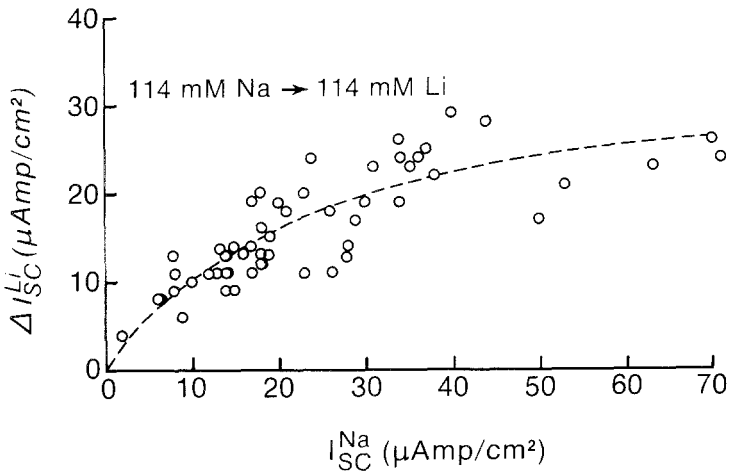

Fig. 3. Composite results of experiments in which 114 mM mucosal Na was replaced by 114 mM mucosal Li. The amiloride-sensitive I_{sc} in the presence of mucosal Li, ΔI_{sc}^{Li}, is plotted *vs.* the value of I_{sc} which characterized the same tissue prior to Li substitution. Each point represents one tissue; the line was drawn by eye

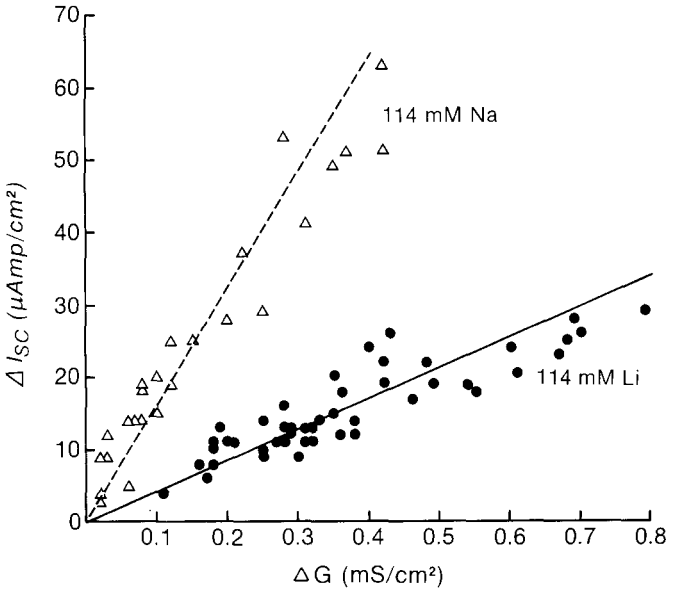

Fig. 4. The amiloride-sensitive short-circuit current, ΔI_{sc}, plotted *vs.* the amiloride-sensitive conductance, ΔG, for tissues bathed by 114 mM mucosal Na (△) and for tissues bathed by 114 mM mucosal Li (●). Each point represents one tissue. The units of conductance are milliSiemans (mS) where mS = mmho

remaining constant.[1] Likewise, Fig. 4 shows that, in the presence of mucosal Li, ΔI_{sc}^{Li} is a linear function of ΔG. This result suggests that in the presence of mucosal Li the amiloride-sensitive, cation transport path can also be described as a constant apparent emf in series with a variable dissipative element or conductance. It is clear from Fig. 4 that at similar values of ΔI_{sc} the corresponding amiloride-sensitive conductance is significantly *greater* in the presence of mucosal Li than in the presence of mucosal Na. It follows that the apparent driving force or emf of the amiloride-sensitive cation transport path is significantly less in the presence of mucosal Li. The least squares slopes of the lines shown in the figure yield values for the apparent emfs of about 160 mV in the presence of mucosal Na and 40 mV in the presence of mucosal Li. Qualitatively identical results were obtained when 16 mM mucosal Na was replaced by 16 mM Li.

The linear relation between ΔI_{sc} and ΔG in the presence of either mucosal Na or Li suggests that from tissue to tissue the variation in the

[1] The value for the overall "apparent emf" would, under the conditions of these experiments, include not only the driving force due to active transport *per se*, but also that resulting from ion concentration gradients across the active path.

rate of cation transport is referable entirely to variation in the apparent cation conductance of the transport path. Thus, the "saturable" relation between ΔI_{sc}^{Li} and I_{sc}^{Na} shown in Fig. 3 must reflect differences in the effect of cation substitution on the conductance of the cation transport path.

The most direct measure of the effect of cation substitution on the conductance of the active transport path is to compare, in the same tissue, the values of the steady-state, *amiloride-sensitive* conductance before and after substituting Li for Na. In the experiments which comprise Fig. 3, however, the amiloride-sensitive conductance was not determined in the presence of Na, before replacing with Li. The total conductance of the isolated turtle colon is for practical purposes the sum of two components; the conductance of the active path, abolished by amiloride, and the conductance of the paracellular shunt, unaffected by amiloride [5, 17]. Several experiments in which G_t was measured *in the same tissue* before and after mucosal cation substitution indicated that the average *ratio* of the amiloride-*insensitive* tissue conductance in the presence of the two cations, $(G_t^{Li}/G_t^{Na})_{amil}$, was $1.02 \pm .01$ $(n=3)$, suggesting that the conductance of the paracellular shunt path is not significant-

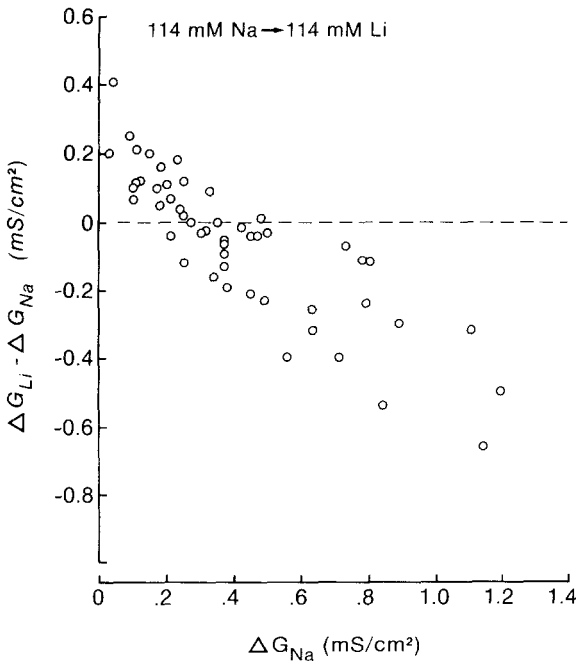

Fig. 5. The Li-induced change in the amiloride-sensitive conductance of the colon, $\Delta G_{Li} - \Delta G_{Na}$, plotted *vs.* the estimated conductance of the active path in the presence of Na. Each point represents one tissue

ly altered by substituting Li for Na in the mucosal solution. Thus, it is possible to estimate the "Na conductance" of the active path for the experiments summarized in Fig. 3 by simply correcting the total tissue conductance, measured in the presence of Na, for the value of the amiloride-*insensitive* conductance (i.e., the shunt conductance) which was measured in the presence of mucosal Li. In Fig. 5 the change in the conductance of the *active* path induced by substituting Li for Na, $\Delta G_{Li} - \Delta G_{Na}$, calculated for the experiments summarized in Fig. 3 is plotted vs. the estimated value of the conductance of the active path in the presence of mucosal Na, ΔG_{Na}. This plot reveals a negative correlation between the Li-induced change in the conductance of the active cation transport path and the initial value of the conductance in the presence of Na. At low values of ΔG_{Na} replacing Na with Li *increases* the conductance, whereas at higher values of ΔG_{Na} the conductance is actually reduced in the presence of mucosal Li.

Toxic Effects of Li on Active Na Transport

The results of Li−Na substitution experiments are consistent with the notion that Li is transported across the turtle colon via an amiloride-sensitive, ouabain-inhibitable path. The interpretation of such substitution experiments, however, is complicated by the fact that differences between the transmural movements of Li and Na may result from at least two effects. The transport steps at the apical and basolateral membranes may discriminate between the two cations to differing degrees, i.e., differences in transmural Li and Na transport by the same tissue may be the overall result of differential *cation selectivity* of the steps in the transport process. In addition, however, it is to be expected that Li, particularly in the relatively high concentrations employed in these studies, may inhibit the cation transport machinery by virtue of some "toxic" effect of Li on intracellular processes [12, 14, 16, 19]. Such effects could presumably be mediated by some additional action of Li not directly related to its ability to "substitute" for Na in the active transport path. To explore possible toxic effects of Li, we studied the effects on I_{sc} and net Na transport of adding small amounts of LiCl to the mucosal or serosal bathing solutions. Figure 6 shows two representative experiments in which the isolated colon was bathed on both sides by 114 mM Na-Ringer's and allowed to attain a steady-state value of I_{sc}. The left-hand panel of Fig. 6 shows that the addition of 15 mM

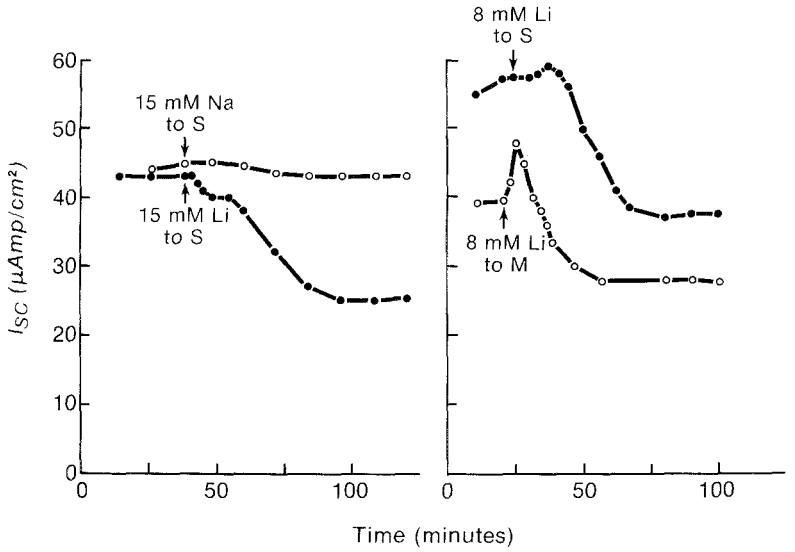

Fig. 6. *Left:* The effect on I_{sc} of adding 15 mM LiCl or 15 mM NaCl to the serosal bathing solutions. Both mucosal and serosal bathing solutions initially contained 114 mM Na-Ringer's. *Right:* The effect on I_{sc} of adding 8 mM LiCl to either the mucosal or the serosal bathing solutions. Both solutions initially contained 114 mM Na-Ringer's

LiCl to the serosal bathing solution of the colon inhibited I_{sc}, whereas the addition of a similar amount of NaCl to another tissue had no effect. The right-hand panel of Fig. 6 shows that the addition of 8 mM LiCl to *either* the mucosal or the serosal side produced an inhibition of I_{sc}. Preliminary experiments with amiloride-treated colons showed that these Li-induced reductions in I_{sc} cannot be attributed to diffusional emfs. Note that in the case of *mucosal* addition (Fig. 6) the initial response is a transient *increase* in I_{sc}, presumably representing Li entry into the transporting cells across the apical cell membrane. Under steady-state conditions, however, transmural Na flux measurements (data not shown) indicated that in the presence of these low concentrations of Li in the mucosal or serosal bathing solutions, I_{sc} is not detectably different from net Na absorption.

The Relation between Na and Li Transport

To circumvent some of the ambiguities of ion-substitution studies and to provide a direct comparison of the transmural flows of Na and Li under similar conditions, we measured transmural flows of ^{22}Na and ^{3}H

mannitol in the presence of varying amounts of mucosal Na and Li. The object of these experiments was to determine the *fraction* of I_{sc} attributable to active Na transport when the mucosal bathing solution contained comparable concentrations of Na and Li. A previous study [5] indicated that with Na-Ringer's bathing both sides of the tissue the *serosa to mucosa* flows of Na and mannitol, J_{sm}^{Na} and J_{sm}^{Man}, are confined to a paracellular shunt pathway where the flux-ratio, J_{sm}^{Na}/J_{sm}^{Man}, is characteristic of diffusion in free solution and where the flux of Na is unaffected by amiloride. Under these conditions the amiloride-sensitive, mucosa to serosa flux of Na, ΔJ_{ms}^{Na}, is equal to the *net* flow of Na through the active path. Similar experiments in the presence of mucosal Li (regardless of the Li concentration) yielded values for the ratio, J_{sm}^{Na}/J_{sm}^{Man} of 0.46 ± 0.03 in the absence of amiloride and 0.44 ± 0.04 in the presence of amiloride. These values are not discernably different from the diffusional flux ratio of 0.45 [5] and suggest that, in the presence of mucosal Li, J_{sm}^{Na} is confined to a paracellular path, i.e., that the Na backflux through the active path is undetectable. Hence, in the presence of mucosal Li we take the amiloride-sensitive portion of $J_{ms}^{Na}(\Delta J_{ms}^{Na})$ to be equal to the *net* flow of Na through the active path.[2]

Figure 7 shows the results of measurements of J_{ms}^{Na} in the presence of a mucosal bathing solution which contained 32 mM Na and 80 mM Li. The amiloride-sensitive unidirectional Na flux, ΔJ_{ms}^{Na}, is plotted vs. the amiloride-sensitive short-circuit current, ΔI_{sc}. When mucosal Na was partially replaced by choline the slope of this relation was 1.00 ± 0.03 ($n=4$) as indicated by the dashed line. The points are not shown since they are off the scale of this plot. In the presence of mucosal Li, however, the slope is significantly less than unity [0.38], indicating that in the presence of mucosal Li the net flow of Na through the active path accounts for only a fraction of the amiloride-sensitive I_{sc}.

Figure 8 summarizes the results of a series of experiments similar to that represented by Fig. 7 in which ΔJ_{ms}^{Na} and ΔI_{sc} were measured with varying concentrations of Na and Li in the mucosal solution. The values of the ratio, $\Delta I_{sc}/\Delta J_{ms}^{Na}$, are plotted *vs.* the Li to Na concentration ratios in the mucosal bathing solution. The points can be described by a straight line with an intercept of 1.0. The form of this relation is

[2] In all flux measurements with mucosal Li the serosal bathing solution contained 114 mM Na, so that a transepithelial Na gradient exists which will produce net diffusional flow of Na from serosa to mucosa through the shunt path. Thus the amiloride-sensitive portion of J_{ms}^{Na} is *not* the net flow of Na across the epithelium. Rather, ΔJ_{ms}^{Na} is the net flow of Na through the *active path*, which is the quantity of interest.

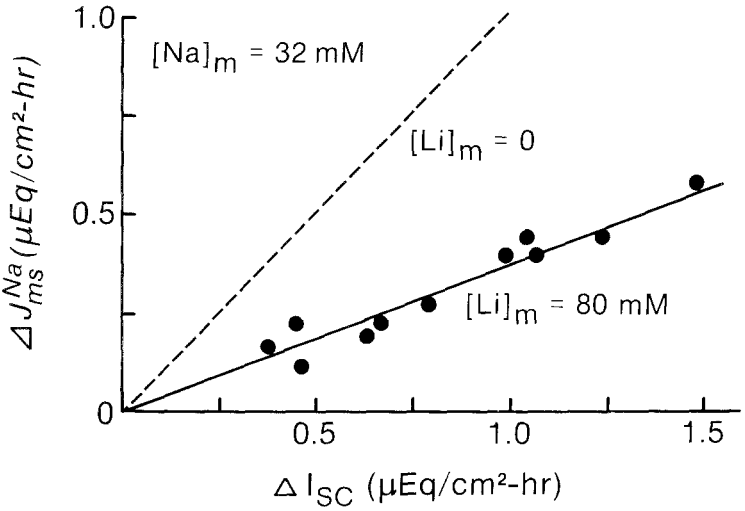

Fig. 7. Plots of the amiloride-sensitive, mucosa to serosa Na flux, ΔJ_{ms}^{Na}, *vs.* the amiloride-sensitive short-circuit current, ΔI_{sc}, for experiments in which the mucosal bathing solution contained 32 mM Na and 80 mM choline (dashed line) or 32 mM Na and 80 mM Li (solid line). Each point on the solid line represents a single tissue for which values of ΔJ_{ms}^{Na} and ΔI_{sc} were obtained by comparing the average values of these parameters over three 30-min flux periods before and three 30-min periods after the addition of 10^{-4} M amiloride to the mucosal bathing solution. The points for the dashed line are not shown because they are off the scale of this plot (*see* text). The SE for the points shown are less than the size of the filled circles

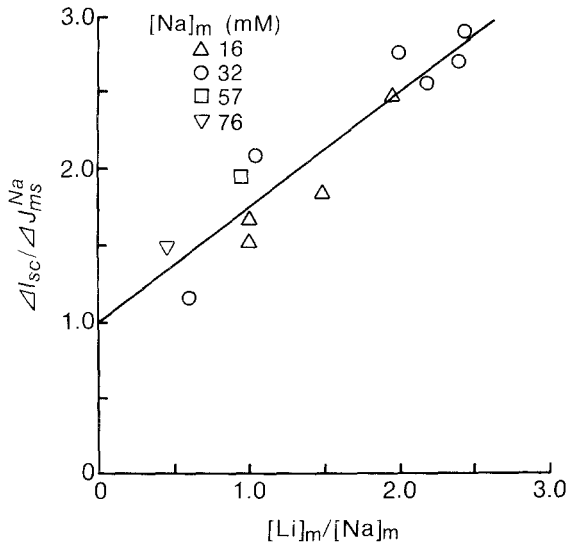

Fig. 8. Plot of $\Delta I_{sc}/\Delta J_{ms}^{Na}$ *vs.* the Li:Na concentration ratio in the mucosal bathing solution. Each point represents the average value of this ratio measured on at least 3 tissues. The SE for the points never exceeded the size of the symbols

consistent with the notion that Li ions carry the "extra current" not attributable to Na, so that the ratio, $\Delta I_{sc}/\Delta J_{ms}^{Na}$, approaches 1.0 as the mucosal Li concentration approaches zero.[3]

The data in Fig. 8 suggest that the total amiloride-sensitive I_{sc} is equal to the sum of the net flows of Na and Li through the amiloride-sensitive path, ΔJ_{net}^{Na} and ΔJ_{net}^{Li}, i.e.,

$$\Delta I_{sc} = \Delta J_{net}^{Na} + \Delta J_{net}^{Li} \tag{1}$$

and

$$\Delta I_{sc}/\Delta J_{net}^{Na} = 1 + \Delta J_{net}^{Li}/\Delta J_{net}^{Na}. \tag{2}$$

If it is assumed that the net fluxes of both ions through the amiloride-sensitive path are, for practical purposes, *unidirectional* then each may be written as the product of a rate coefficient and the mucosal ionic concentration, i.e.,

$$\Delta J_{ms}^{Na} = \lambda_{Na}[Na]_m \qquad \Delta J_{ms}^{Li} = \lambda_{Li}[Li]_m \tag{3}$$

where λ_{Na} and λ_{Li} represent the rate coefficients for *transepithelial* Na and Li movement through the amiloride-sensitive path and $[Na]_m$ and $[Li]_m$ are the concentrations of the cations in the mucosal bathing solution. In general, the values of the rate coefficients are expected to be functions of the cation concentrations in the mucosal bathing solution. In the absence of mucosal Li, I_{sc} is a saturable function of the mucosal Na concentration [17] so that λ_{Na} is, for instance, a function of the mucosal Na concentration but may also depend on the mucosal Li concentration.

Combining Eqs. (2) and (3), we obtain

$$\Delta I_{sc}/\Delta J_{ms}^{Na} = 1 + (\lambda_{Li}/\lambda_{Na})([Li]_m/[Na]_m). \tag{4}$$

The form of the observed relation $\Delta I_{sc}/\Delta J_{ms}^{Na}$ and $[Li]_m/[Na]_m$ shown in Fig. 8 is consistent with that predicted by Eq. (4) and suggests that the slope of this plot may be interpreted as the ratio of the rate coefficients for *transmural* movement of Li and Na through the amiloride-sensitive path. The least squares slope of the line yields a value for $\lambda_{Li}/\lambda_{Na}$ of 0.75. The linear relation suggests that this ratio is independent of the ratio of Li to Na in the mucosal bathing solution. In additional experiments (data not shown) we estimated the ratio, $\lambda_{Li}/\lambda_{Na}$, by measuring *tracer* Na

[3] Additional experiments (data not shown) have shown that in the presence of mucosal lithium, the *net* Cl flux is zero so that transmural Cl transport does not contribute to I_{sc} under these conditions.

movement in the presence of 114 mM mucosal Li but in the virtual absence of *abundant* Na. These experiments also yield a value of about 0.75 for $\lambda_{Li}/\lambda_{Na}$.

Discussion

Li Transport by the Colon

The results of this study suggest that Li is actively transported across the isolated turtle colon via a pathway identical to that normally responsible for active Na absorption. Previous studies have suggested that Li is actively transported by the isolated frog skin [3, 13, 19] and toad urinary bladder [11], but the available data did not permit a direct comparison of the properties of the active cation transport path(s) in the presence of Na or Li. The results presented here suggest that, although the rates of transmural transport of the two cations are similar under some circumstances, there are marked differences in the behavior of the epithelium in the presence of Li which appear to reflect not only the selectivity of the transport path but also toxic effects of Li.

The conclusion that Li is actively transported by the turtle colon is based on several observations. First, if mucosal Na is entirely replaced by Li the resulting steady-state I_{sc} is inhibited by mucosal amiloride or serosal ouabain, i.e., I_{sc} in the presence of mucosal Li exhibits responses to these agents identical to those observed when I_{sc} is due solely to active Na transport. Second, it appears that in the presence on the mucosal side of both Li and Na the portion of the amiloride-sensitive I_{sc} which is not due to active Na transport must be due to net Li transport from mucosa to serosa. This "extra" current approaches zero as the mucosal Li concentration approaches zero and the *net* transmural movement of Cl, the only other ion in significant concentration in the bathing solutions, is zero under these conditions.[4] Third, it appears that transmural Li

[4] It seems unlikely that the "extra" I_{sc} observed in the presence of mucosal Li could be the result of some nonsteady-state process whereby Li carries current across the apical membrane but does not leave the cell, the current across the basolateral membranes being carried, for instance, by net K exit. I_{sc} often varied less than 10% over a period as long as 3 hr after mucosal Na had been partially or totally replaced by Li, an observation which seems incompatible with a continuous intracellular accumulation of Li and depletion of K. If, for instance, exchangable intracellular K in the turtle colon is of the same order as that in the rabbit colon, 1 μeq/cm² [10], then a Li current of 27 μA/cm² (~ 1 μeq/cm² hr) would totally deplete intracellular K in 1 hr.

transport is indeed an "active" process. If the mucosal bathing solution contains only Li and choline as the major cations, an amiloride-sensitive I_{sc} persists in the *absence* of an electrochemical potential gradient for Li. Previous studies [18] have shown that Li can enter the epithelial cells of the colon through amiloride-sensitive channels in the apical membrane, and the simplest interpretation of the present results is that Li also exits via the ouabain-sensitive cation extrusion mechanism presumably located at the basolateral membrane of the transporting cells.

Clearly, these results cannot exclude the alternative hypothesis that Li extrusion from the cell is "indirectly coupled" to metabolic energy via a Na gradient, for instance. In the present experiments the serosal solutions uniformly contained 114 mM Na and it is to be expected that under short-circuit conditions a substantial electrochemical potential gradient for Na was present across the basolateral membranes of the Na transporting cells. Since there is ambiguity as to the identity of the ions which carry current across the basolateral membranes [14], we cannot exclude the possibility that Li exit is driven by an *electrically neutral* Na $-$Li exchange mechanism such as that which appears to be responsible in part for Li extrusion from the human red blood cell [6, 7, 9]. The exit of each Li ion would be accompanied by the entry (from the serosal bathing solution) of one Na which would then be "recycled" via the Na pump. In another Na-transporting, tight epithelium, toad urinary bladder, however, there appears to be little "recycling" of Na across the basolateral membranes under normal conditions [1, 4].

Zerhan [18] obtained the first definitive evidence for active, transepithelial Li transport. He showed that when the isolated frog skin was bathed by solutions containing Na and Li there was an "extra (short circuit) current which was attributable to net Li absorption. Net Li transport occurred in the presence of an unfavorable electrochemical potential gradient for Li. More recently Candia and coworkers [3, 13] showed that active Li transport by the frog skin was inhibited by amiloride or ouabain and was enhanced by vasopressin. These investigators suggested, however, that although Li transport is ouabain sensitive in the frog skin, Li does not interact directly with the Na$-$K ATPase presumably responsible for Na extrusion from the cell. This conclusion was based on the observation that a microsomal ATPase fraction obtained from frog skin was not activated by Li [13]. Dunham and Senyk [8], however, have recently obtained evidence that in the human red cell it is possible to demonstrate ouabain-sensitive Li efflux via the Na$-$K pump.

The Effect of Li on the Active Cation Transport Path

The results presented here confirm studies from other laboratories showing that Li inhibits active Na transport [2, 3, 19]. In the turtle colon we observed significant reduction in I_{sc} when small amounts of Li were added to either the mucosal or the serosal bathing solution (Fig. 6). In a previous study [18] we found that sudden exposure of the apical surface of the colon to Li caused a marked increase in I_{sc}, but during the first 60 sec there was no detectable effect on the rate of Na entry across the apical membrane. Figure 6 shows that the initial effect of the addition of Li to the mucosal bathing solution was a transient increase in I_{sc} followed by a sustained inhibition. Taken together, these observations suggest that Li does not inhibit active Na transport by virtue of a Li effect on the *mucosal side* of the apical membrane, but instead that inhibition is produced only after Li enters Na transporting cells. The mechanism of the Li toxicity is unknown and could presumably result from a generally deleterious effect of Li on intracellular metabolic processes [12, 14] or a more direct effect on the cation transport system. The observation that Li inhibits Na transport when added to the bathing solutions in amounts so small that Li is not carrying an appreciable current suggests that the inhibitory effects of Li cannot be attributed solely to simple competition of Li and Na for the exit step. One question raised by these results is whether mucosal and serosal Li inhibit transport by the same mechanism, i.e., by entering the Na transporting cells. Figure 5 indicates that mucosal and serosal Li produced a similar inhibition of I_{sc} except that the effect of serosal Li was slower in onset. This difference could simply reflect the delay in reaching the epithelial cells due to the submucosal connective tissue layer which remains in the "stripped" colon preparation. In additional experiments, however, we have found that small amounts of serosal Li produce similar inhibition of I_{sc} when the mucosal solution contains 114 mM Li. In this circumstance the amount of Li added to the serosal bathing solution would presumably have little effect on the total Li accumulated by the transporting cells. This result suggests, therefore, that serosal Li inhibits ouabain-sensitive cation transport by a separate-mechanism, perhaps by virtue of some interaction with the active extrusion mechanism on the *serosal* side of the basolateral membrane.

Cation Selectivity of the Active Transport Path

If it is assumed, provisionally, that Li and Na are transported across the colonic epithelium via the same path, then a comparison of the relative rates of transport of the two cations could presumably yield information as to the cation "selectivity" of the transport mechanism. Even if the assumption of identical transport paths is acceptable, however, it is clear that caution must be exercised in the interpretation of ion substitution studies for at least two reasons. First, an actively transported cation must cross at least two membranes in series, the apical and basolateral membranes of the cell layer, and it is to be expected, *a priori*, that the physical principles which govern the interaction of cations with these two membranes will be distinctly different. Second, the results of this and other studies [2, 11, 16, 19] suggest that the changes in the properties of the cation transport system which result from substituting Li for Na are, at least in part, attributable to "toxic" effects of Li on the transport machinery.

The amiloride-sensitive cation transport path can be described by an electrical equivalent circuit consisting of a constant emf in series with a variable conductance when either Na or Li is the transported cation. The results shown in Figs. 1 and 3 could give the impression that in some tissues the active transport path does not discriminate between the two cations. It is apparent from Figs. 3–5, however, that in electrophysiological terms the approximate identity of I_{sc} in some tissues before and after mucosal cation substitution is a fortuitous consequence of the fact that the presence of mucosal Li increases the conductance of the active path, whereas the emf, or apparent driving force for Li transport, is reduced as compared to that observed with mucosal Na.

The differential effects of mucosal Li on the apparent conductance and emf of the transport path may be attributable, in part, to the expected differential selectivities of the cation transport steps at the apical and basolateral membranes of the epithelial cell layer. A previous study [18] showed that sudden exposure of the apical membranes of the colon to Li produces a transient increase in the amiloride-sensitive conductance which is specific for Li. The rapid onset of this conductance change and the fact that the "Li-response" was unaltered by inhibiting active transport with ouabain led us to speculate that the increased conductance was referable to the interaction of Li with the apical membranes. The present experiments show that under steady-state conditions, in tissues with a relatively low active Na conductance, Li

substitution increases the amiloride-sensitive conductance, a result consistent with a Li-induced increase in *apical* membrane conductance. In tissues characterized by a relatively high active "Na conductance", however, Li substitution reduces the amiloride-sensitive conductance of the epithelium. Thus, if Li substitution does, in fact, result in increased apical membrane conductance then there is an additional inhibitory effect of Li, perhaps on the apical or basolateral membranes, which is only detectable when the *total* conductance of the transcellular path is relatively high. The decreased apparent emf of the active transport path in the presence of mucosal Li could reflect, in part, a reduced affinity of Li for the ouabain-sensitive active transport step at the basolateral membranes as well as the deleterious effects of Li on the active transport machinery.

The most direct comparison of transmural Li and Na transport emerges from experiments in which Na flow through the active path was measured in the presence of varying amounts of mucosal Li (Figs. 7 and 8). As indicated in *Results*, the relationship between the net Na flow through the active path and the total amiloride-sensitive I_{sc} (Fig. 8) can be interpreted so as to yield the ratio of the rate coefficients for the transmural flows of Li and Na. This ratio, $\lambda_{Li}/\lambda_{Na}$, appears to be about 0.75, independent of the amounts of Li or Na in the mucosal bathing solution. This result shows that under similar conditions, i.e., when the transport of either cation would presumably be subject to the toxic effects of intracellular Li, Na is transported more readily than Li through the active path. If it is assumed that the permeability of the apical membrane to Li is at least as great, if not greater, than that to Na (*see above*), then we may infer that the reduced rate coefficient for *transmural* Li transport, compared to Na, must result from a reduced affinity of Li for the ouabain-sensitive exit step. Zehran [19] did not present a quantitative analysis of the relative rates of net Na and Li transport across the frog skin, but he concluded that since the percent of I_{sc} attributable to transepithelial Li movement appeared to be similar to the percent of the total transportable cation (Na+Li) which was Li, "lithium and sodium compete on an equal basis in the transport process". If the data of Zehran are analyzed according to the method illustrated by Fig. 7, it is possible to derive a value of the ratio $\lambda_{Li}/\lambda_{Na}$ for frog skin which lies between 0.5 and 1.0.

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