

Cation Selectivity of the Apical Membrane of the Turtle Colon

Sodium Entry in the Presence of Lithium

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ABSTRACT Exposure of the apical surface of the isolated turtle colon to Li produced a marked transient in short-circuit current (I_{sc}) and total tissue conductance (G_T) which was abolished by amiloride but was unaffected by ouabain or by removing Na or Cl from the mucosal bathing solution. Despite marked changes in I_{sc} , Na uptake across the apical membrane was a linear function of time during exposure to Li-containing solutions, and except at very high Li concentrations, the initial rate of Na uptake, J_i^{Na} , was identical to its pre-Li value. In the presence of Li, however, J_i^{Na} was significantly less than the total I_{sc} . The apparent "transference number" for Na in the apical membranes was a function of the Li:Na concentration ratio in the mucosal bathing solution. These results suggest that Li can carry substantial amounts of current through amiloride-sensitive channels in the apical membrane of the colon without having any effect on the rate coefficient for Na entry. This behavior is not consistent with "competition" of Na and Li for a membrane "carrier" but rather suggests that the Na entry mechanism may be a population of pores or channels through which Na and Li may pass with negligible interaction.

INTRODUCTION

In a previous paper (Thompson and Dawson, 1978), we presented evidence that the initial rate of Na uptake by the turtle colon from the mucosal bathing solution provides a direct measure of the amiloride-sensitive Na entry mechanism located in the apical membrane of the epithelial cells. When the mucosal bathing solution contained principally Na, choline, and Cl, it appeared that all of the amiloride-sensitive short-circuit current (I_{sc}) across the apical membrane was carried by Na. Furthermore, it was not necessary to invoke any driving force other than the Na-electrochemical potential gradient to account for Na entry. These observations are consistent with the existence of an Na-selective channel in the apical cell membrane through which Na might move by diffusion. We also observed, however, that Na entry is a saturable function of mucosal Na concentration, suggesting the possibility of some type of Na-Na interaction at the apical cell membrane. In this paper, we present experiments designed to test directly for possible cation-cation interactions in the apical membrane and to evaluate the cation selectivity of the Na entry mechanism. We have examined

the effect of mucosal Li on the Na entry step and on the relation of Na entry to the amiloride-inhibitable I_{sc} . A study of the effects of Li was prompted by observations in the literature suggesting that Li can carry current through the Na-selective pores in axon membranes (Hille, 1975) and by the observation of Biber and Curran (1970) that Na and Li exhibit a competitive interaction for the entry step in frog skin. The principal aim of these experiments, therefore, was to determine (a) whether Li ions carry current through the amiloride-sensitive Na channel in the apical membrane of the turtle colon and (b) whether mucosal Li has any effect on the rate coefficient for Na entry. The results indicate that Li can carry substantial amounts of current across the apical membranes without having any detectable effect on the rate of Na entry.

METHODS

Colons were removed from freshwater turtles, *Pseudemys scripta elegans* (The Mogul Corp., Chagrin Falls, Ohio) and stripped of circular and longitudinal musculature as previously described (Dawson, 1977). To reduce edge-damage effects (Helman and Miller, 1971), portions of stripped colon were initially mounted by gluing the serosal side of the tissues to rubber washers (≈ 1 cm OD) with a cyanoacrylate tissue adhesive (Eastman 910, Eastman Kodak Co., Rochester, N. Y.). The rubber washers were fabricated from $1/32$ -inch silicon rubber sheeting (McMaster-Carr Supply Co., Chicago, Ill.). Because the adhesive bonds virtually instantaneously, the degree of stretch could be controlled with relative ease. The circular piece of mucosa with the serosal washer was then cut out with a tissue punch. The resulting disk was sealed on the washer side with silicone vacuum grease to the inner portion of an influx chamber essentially identical in design to that described in detail by Biber and Curran (1970). The disk of tissue was held in place by a cap that was also gently sealed on the mucosal side with silicone vacuum grease. Typically, 18–24 1-cm² portions of tissue were mounted in this manner on individual "inner chambers" and placed, mucosal side down, in an aerated incubation bath with ≈ 0.75 ml of an appropriate Ringer's solution on the serosal side.

Unidirectional Na Influx

The unidirectional influx of Na from the mucosal bathing solution into the mucosal cell layer was estimated by measuring the uptake of ²²Na according to the technique employed by Biber and Curran (1970) and subsequently by Dawson and Curran (1976). 1-cm² portions of stripped mucosa, mounted as described above, were placed in the influx chamber, mucosal side down. The chamber was, in principle, identical to those described by Biber and Curran (1970) except that the mucosal surface of the tissue was held at an angle of about 30° with respect to the horizontal. This allowed a stream of air bubbles, injected through the base, to wipe across the mucosal surface which was mildly distended because of a slight hydrostatic head on the serosal side. This arrangement provides vigorous stirring at the mucosal surface.

The chamber was equipped with four agar bridges; two 3 M KCl bridges for the measurement of the transepithelial potential difference (PD) and two Ringer's agar bridges for passing current across the tissue. The bridges were connected through appropriate electrodes to an electronic voltage clamp that could be adjusted to compensate for the fluid resistance between the tissue surface and the PD sensing electrodes. The I_{sc} was continuously monitored on a strip chart recorder. Tissue conductance was calculated from the change in transepithelial current due to a brief, 10-mV change in clamping potential.

In all experiments the serosal surface of the tissue was bathed by ≈ 0.75 ml of a

Ringer's solution that contained 114 mM Na, 114 mM Cl, 2.5 mM K, 2.5 mM HCO₃, 1.0 mM Ca, 5.0 mM D-glucose, 5 mM D-mannitol, and 2.5 mM pyruvate. All tissues were preincubated in a mucosal solution identical to that on the serosal side except that the Na concentration was reduced to 8 or 16 mM by isosmotic replacement with choline-Cl. During the brief period (30–60 s) over which Na uptake was measured, the mucosal solution contained Na at a concentration identical to the preincubation solution and, in addition, variable amounts of Li which were added by isosmotic replacement of choline-Cl. Na concentrations were routinely verified by flame photometry and Li concentrations by atomic absorption spectrophotometry. Preliminary experiments indicated that the isolated tissue could be adequately maintained by aerating the mucosal solution only. This solution was stirred and oxygenated by a stream of air to yield a pH of $\cong 8.1$ at 25°C.

Tissues were incubated in the influx chamber until a stable I_{sc} was obtained. The mucosal bathing solution was then rapidly aspirated and replaced by 5 ml of a test solution containing 3–6 μ Ci of ²²Na and 60 μ Ci of [³H]mannitol in the appropriate Ringer's solution. After 30–45 s, the inner chamber was rapidly removed and the influx was terminated by first "washing" the mucosal surface of the tissue by briefly immersing (2–3 s) the inner chamber in a large volume of "cold" Ringer's, and then blotting and punching out the tissue. The tissue was extracted for at least 2 h in distilled water, and an aliquot of the extract was assayed for ³H and ²²Na. [³H]Mannitol served to estimate the amount of test solution adhering to the apical surface after blotting. The amount of ²²Na in excess of that in the mannitol space is defined as the Na uptake by the tissue.

I_{sc} was monitored continuously during preincubation and during the influx period on a strip chart recorder. In addition, due to transients in I_{sc} caused by exposure of the apical surface to Li, the average current during the influx period was calculated by digitally integrating $I_{sc}(t)$ to obtain the total charge and then dividing by the elapsed time.

RESULTS

Effect of Li on the Electrical Behavior of the Isolated Colon

When the solution bathing the apical surface of the colon is exchanged for a new solution containing a higher Na concentration, the response of I_{sc} is typically an initial peak and a rapid decline to a quasi-steady value. An example of the change in I_{sc} that resulted from raising mucosal Na concentration from 2 to 30 mM is shown by the dashed line in Fig. 1. In marked contrast is the response of I_{sc} when a mucosal solution containing 2 mM Na is replaced with a solution containing 2 mM Na plus 30 mM Li, shown by the solid line in Fig. 1. The current peaks and begins to decline but then rises and proceeds through a second peak before declining to a new quasi-steady-state value. This transient response is abolished if the apical surface is treated with amiloride (10^{-4} M) and the transient I_{sc} can be aborted by adding amiloride to the mucosal bathing solution during the response. I_{sc} in the presence of amiloride rarely exceeded about 5 μ A/cm² regardless of the ionic composition of the mucosal bathing solution. The response of I_{sc} to Li is qualitatively identical in the absence of mucosal Na or Cl.

The marked rise and fall in I_{sc} caused by Li is accompanied by a corresponding change in the total tissue conductance, G_T . As shown by the closed circles in Fig. 1, G_T rises and falls approximately in phase with I_{sc} . Thus the marked transient in I_{sc} is due at least in part to an Li-induced change in the conductance of an amiloride-sensitive path. Identical Li-induced changes in I_{sc} and G_T are obtained

if the pre-Li I_{sc} is reduced to zero by adding 10^{-4} M ouabain to the serosal side of the tissue.¹

The objective of these experiments was to compare the unidirectional influx of Na across the apical cell membrane with the total I_{sc} in the presence of mucosal Li. For at least two reasons, it was desirable to conduct these experiments by exposing the apical surface to Li only for the brief period during which Na uptake is measured. First, brief exposure to Li on the mucosal side may reduce the possibilities of "secondary" effects due to the intracellular accumulation of Li (Sarracino and Dawson, 1978). Second, at early times after Li exposure, it may be reasonable to assume that the intracellular Li concentration is negligible in any attempt to model the behavior of the apical cell membrane.

Na Uptake during the Li-Induced Transient in I_{sc}

We measured the time-course of Na uptake during the Li-induced transient in I_{sc} for two reasons. First, it was necessary to establish a time period over which

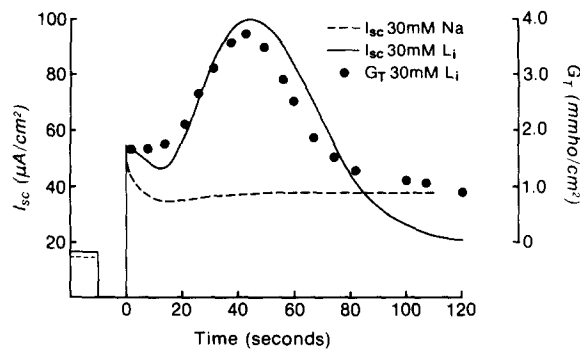


FIGURE 1. The response of I_{sc} and G_T to a sudden exposure of the mucosal surface of the colon to Li or an increased Na concentration. Traces to the left of zero time represent the initial currents in 2 mM Na Ringers. The break in the traces represents the time required to change the mucosal solution to either 2 mM Na + 30 mM Na (---) or 2 mM Na + 30 mM Li (—).

Na uptake may be used to calculate the initial rate of Na entry, J_i^{Na} . Previous experiments (Thompson and Dawson, 1978) showed that, in the absence of Li, Na uptake was a linear function of time over a period of 60 s. In addition, given the substantial changes in I_{sc} during the uptake period in the presence of Li, it was necessary to determine whether the apparent rate coefficient for Na influx varied during the uptake period.

Fig. 2 shows Na uptake as a function of time, when the test solution injected

¹ If I_{sc} is reduced to zero by ouabain, a sudden elevation of the mucosal Na concentration will also evoke a transient I_{sc} that rises within seconds to a peak and then declines in a quasi-exponential fashion to zero over a period of a few minutes. This transient I_{sc} , as well as that observed with Li under similar conditions, presumably represents cation entry across the apical membrane and accumulation within the cell. The identities of the ions carrying current across the basolateral membrane during the transient are unknown.

at $t = 0$ contained 2 mM Na and 30 mM Li. The dashed line represents the average value of $I_{sc}(t)$ during this time. Clearly, despite the marked changes in I_{sc} , Na uptake is a linear function of time and the intercept of the plot is not distinguishable from zero. This behavior indicates that Na uptake over a 45-s period provides a reasonable estimate of the initial rate of Na entry in the presence of Li and, in addition, suggests that the marked variation of I_{sc} during this time has little or no effect on Na uptake. Similar results were obtained at 16 mM mucosal Na with Li concentrations as high as 98 mM, over exposure times as long as 60 s.

We have previously shown (Thompson and Dawson, 1978) that the initial rate of Na uptake across the apical border of the colon consists of two components. The major component of Na uptake represents the unidirectional Na influx across the apical membranes of Na transporting cells. This component of Na uptake is highly correlated with I_{sc} and is abolished by amiloride. In addition,

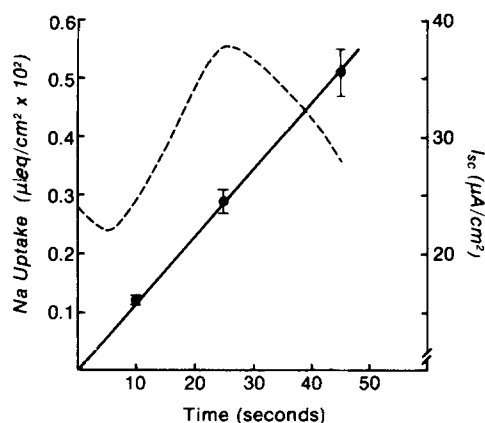


FIGURE 2. Na uptake as a function of time during Li-induced transient in I_{sc} (---). Each point is the mean of six determinations. All 18 tissues were obtained from a single colon. The test solution contained 2 mM Na and 30 mM Li.

there is a small, amiloride-insensitive component of Na uptake that appears to represent Na movement into the paracellular shunt path. This portion of Na uptake is a linear function of mucosal Na concentration and is characterized by an apparent permeability of 0.006 cm/h. In the present experiments, Na uptake was measured at mucosal concentrations of 8 and 16 mM so that the linear component of Na uptake is negligible. In addition, preliminary experiments showed that Na uptake in the presence of amiloride is unaffected by Li.

In each experiment, tissues were preincubated in either 8 or 16 mM Na, and the Na uptake was measured at an identical Na concentration but in the presence of Li. By virtue of this protocol three parameters are available for comparison. The initial rate of Na entry, J_i^{Na} , may be compared with the average current, \bar{I}_{sc} , during the influx period in the presence of Li. This average current was computed by digitally integrating the current during the Li-induced transient in I_{sc} and dividing by the elapsed time. In addition, the Na

influx may be compared with the pre-Li current, i.e., the steady-state value of I_{sc} which characterized the tissue in the presence of Na only, before exposing the mucosal surface to the Li-containing solution. Previous experiments (Thompson and Dawson, 1978) indicated that Na influx is identical to I_{sc} over a wide range of mucosal Na concentrations when Na is replaced by choline. Thus the value of the pre-Li I_{sc} affords for one tissue a direct comparison of Na influx in the presence and in the absence of Li.

Fig. 3 shows the results from a representative experiment in which Na influx was measured in the presence of 8 mM Na and 13 mM Li. Values of J_1^{Na} are plotted vs. both values of I_{sc} , the pre-Li current (Δ) and the average current in the presence of Li (\bullet). Both sets of points can be described by straight lines with identical intercepts that do not differ significantly from zero. As discussed

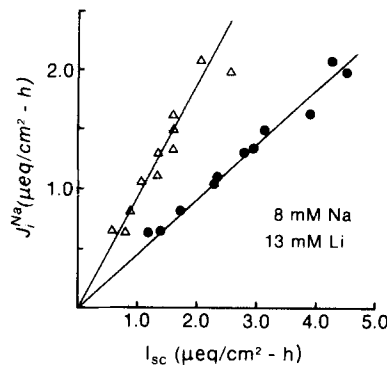


FIGURE 3. J_1^{Na} , measured in the presence of 8 mM Na and 13 mM Li, plotted vs. the pre-Li I_{sc} (Δ) and the average I_{sc} in the presence of Li (\bullet). All determinations on tissues from a single colon.

previously, this behavior is consistent with the notion that under these conditions J_1^{Na} is, for practical purposes, equal to the initial rate of Na entry into the Na transporting cells of the colon. The slope of the relation between J_1^{Na} and the pre-Li I_{sc} , 0.92 ± 0.03 , does not differ greatly from unity. In contrast, the relation between J_1^{Na} and the average I_{sc} in the presence of Li is characterized by a slope of 0.45 ± 0.004 . We will refer to this slope as the apparent "transference number" for Na in the presence of Li because we will argue in the discussion that this slope represents a measure of the fraction of the total I_{sc} carried by Na. This definition is clearly somewhat arbitrary because the current carried by Li is changing with time and the average "Li current" will depend on the time of integration. Table I lists the slopes for similar plots of J_1^{Na} vs. I_{sc} measured before and during exposure to Li. Inasmuch as the mucosal Na concentration never exceeded 16 mM, the slopes listed in Table I were calculated for the best fit to a line through the origin. The values for the slope of J_1^{Na} on the pre-Li I_{sc} cluster around unity for Li concentrations below about 30 mM, suggesting that these concentrations of Li have no detectable effect on the rate coefficient for Na entry. At higher Li concentrations, the results suggest that mucosal Li inhibits Na influx. In contrast, the slope relating J_1^{Na} to the average I_{sc} in the

presence of Li, t^{Na} , appears to decrease monotonically as a function of mucosal Li. At 16 mM Na and 31 mM Li, for instance, Li has no detectable effect on J_1^{Na} but J_1^{Na} accounts for $<1/3$ of the total I_{sc} in the presence of Li.

Fig. 4 shows the reciprocal of t^{Na} plotted vs. the Li:Na concentration ratio in the mucosal bathing solution. The quantity $1/t^{\text{Na}}$ is the ratio of the average I_{sc} in the presence of Li to the unidirectional Na influx. The points can be described by a straight line with an intercept not significantly different from 1.0 and a slope of 0.98 ± 0.02 . Qualitatively, this plot indicates that the ratio of the total current to the Na influx approaches 1.0 as the mucosal Li concentration approaches zero. This behavior is consistent with our previous study (Thompson and Dawson, 1978) which showed that in the absence of Li the cellular portion of J_1^{Na} is equal to I_{sc} over a wide range of mucosal Na concentrations. On the

TABLE I
VALUES OF $\partial J_1^{\text{Na}}/\partial I_{\text{sc}}$ (\pm SE) IN THE PRESENCE OF LITHIUM

n^*	[Na] _m mM	[Li] _m mM	$\partial J_1^{\text{Na}}/\partial I_{\text{sc}}^{\text{Na-Li}}$	t^{Na} ($\partial J_1^{\text{Na}}/\partial I_{\text{sc}}^{\text{Na}}$)
12	8.0	5.8	0.98±0.019	0.58±0.013
12	7.9	6.9	1.14±0.114	0.57±0.012
14	8.0	11.9	0.98±0.028	0.43±0.007
14	8.0	13.0	0.92±0.03	0.46±0.004
13	8.9	25.2	1.00±0.05	0.25±0.002
14	8.1	32.0	0.60±0.04	0.20±0.001
14	15.7	4.6	0.87±0.005	0.72±0.004
12	15.5	5.7	1.05±0.009	0.93±0.009
19	15.5	10.8	0.80±0.007	0.54±0.003
8	15.9	13.5	1.05±0.023	0.45±0.009
9	16.0	16.0	1.19±0.018	0.50±0.005
7	16.0	19.5	1.13±0.04	0.47±0.005
8	16.0	27.0	1.06±0.013	0.44±0.006
8	16.0	31.0	1.06±0.018	0.32±0.005
8	15.5	40.3	0.77±0.012	0.32±0.020
8	16.0	80.0	0.64±0.015	0.17±0.002
7	16.0	98.0	0.52±0.006	0.13±0.001

* Number of tissues.

basis of several simplifying assumptions (detailed in the Discussion), the slope of the plot shown in Fig. 4 can be interpreted to be equal to the ratio of the apparent rate coefficients for the movement of Li and Na across the apical membranes of the colon.

DISCUSSION

When Li is added to the solution bathing the apical membranes of the isolated turtle colon, a transient increase in I_{sc} occurs, accompanied by a transient increase in tissue conductance. These Li-induced changes in I_{sc} and G_T are abolished by amiloride but are qualitatively unaffected by reducing I_{sc} to zero with ouabain or by eliminating Na or Cl from the mucosal bathing solution. This behavior strongly suggests that the transients in I_{sc} and G_T result from the

movement of Li ions through an amiloride-sensitive channel, across the apical cell membranes of the colon. The mechanism underlying the Li-induced transient is unknown but these results suggest that the time-dependent changes in I_{sc} and G_T are specific for Li; the rate coefficient for Na entry appears to be constant throughout most of the transient. The relatively slow time-course of the Li-induced increase in G_T may indicate that Li must enter the cells to produce the effect, and that cellular accumulation of Li may in some way increase the Li conductance of the apical membranes. Finkelstein (1961) reported that exposing the apical surface of isolated frog skin to 100 mM LiCl caused sustained oscillations in open-circuit potential and conductance having a period of 3–5 min. We have noted similar oscillations in I_{sc} after partial or complete replacement of mucosal Na with Li, but these fluctuations are highly damped after the initial transient has subsided, i.e., after 2–3 min.

A previous study (Thompson and Dawson, 1978) showed that over a wide range of mucosal Na concentrations, the amiloride-inhibitable portion of J_i^{Na} is

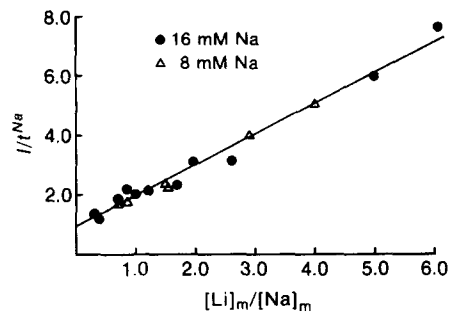


FIGURE 4. The inverse of the apparent transference number for Na in the apical membrane plotted vs. the Li:Na concentration ratio in the mucosal bathing solution.

equal to I_{sc} when NaCl is replaced by choline-Cl. In the presence of Li, however, J_i^{Na} is significantly less than the total I_{sc} . As previously discussed, this extra current appears to be carried by Li ions moving through amiloride-sensitive channels in the apical cell membrane. In addition, it can be demonstrated (Sarracino and Dawson, 1978) that in the presence of mucosal Li, the net, transmural flow of Na through the active path is significantly less than the total, ouabain-sensitive I_{sc} . This observation suggests that at least a portion of the Li that enters epithelial cells through amiloride-sensitive channels may enter the Na "transport pool" and be extruded from the cell by an ouabain-sensitive mechanism. Thus at least a portion of the Li current appears to be passing through the same population of amiloride-sensitive channels that are the route of Na entry across the apical membrane. Nevertheless, the presence of Li has no detectable effect on the rate coefficient for Na entry at Li concentrations where Li appears to be carrying a significant amount of current across the apical membranes. At higher Li concentrations, i.e. >30 mM, Na entry appears to be inhibited somewhat by mucosal Li.

Quantitative Treatment of Na Entry in the Presence of Li

To obtain a quantitative measure of the relative rates of Na and Li movement across the apical membrane we must make several simplifying assumptions. The results suggest that the amiloride-inhibitable I_{sc} is carried exclusively by Na and Li. To facilitate a dissection of this total current into its two components we assume that in the presence of Li the unidirectional influx of Na, J_i^{Na} , is equal to the net flux of Na across the apical membranes. This equality has been demonstrated in the absence of Li (Thompson and Dawson, 1978) over a wide range of mucosal Na concentrations. Likewise, we assume that during the brief exposure of the apical surface of the colon to Li the intracellular concentration of Li is negligibly small and hence that Li entry is unidirectional. Thus the influxes of Na and Li may be expressed as

$$J_i^{Na} = \lambda_{Na} [Na]_m,$$

and

$$J_i^{Li} = \bar{\lambda}_{Li} [Li]_m, \quad (1)$$

where λ_{Na} and $\bar{\lambda}_{Li}$ are the unidirectional rate coefficients for Na and Li entry, respectively. These relations are written as though both J_i^{Na} and J_i^{Li} are constant over the period of the Na uptake measurement, yet the total current exhibits significant changes during this time. The measurement of Na uptake as a function of time during the Li-induced transient in I_{sc} (Fig. 2) indicates that J_i^{Na} does not vary significantly during exposure to Li. In addition, the fact that the Li-induced transients appear to be identical in the presence or absence of Na or Cl, suggests that the time-varying component of I_{sc} is due exclusively to changes in J_i^{Li} . Thus, the rate coefficient defined by Eq. 1 for Li represents an average value over the uptake period.

According to the assumptions introduced above, the total average current in the presence of Li is given by

$$I_{sc} = J_i^{Na} + J_i^{Li} = \lambda_{Na} [Na]_m + \bar{\lambda}_{Li} [Li]_m, \quad (2)$$

where I_{sc} is expressed in units of ion flux, microequivalents per square centimeter per hour. The transference number for Na in the apical membranes is simply J_i^{Na}/I_{sc} so that the inverse of the transference number is given by

$$1/t^{Na} = 1 + J_i^{Li}/J_i^{Na} = 1 + (\bar{\lambda}_{Li}/\lambda_{Na})([Li]_m/[Na]_m). \quad (3)$$

Interpreted in this manner, Fig. 4 represents essentially a plot of J_i^{Li}/J_i^{Na} vs. $[Li]_m/[Na]_m$. The form of this plot suggests that the ratio of the rate coefficients for Li and Na entry is virtually constant over the range of mucosal Li concentrations studied.

If it is assumed that Li and Na enter the cells of the colon through a single, homogeneous population of amiloride-sensitive channels, then at least two classes of models for the entry process can predict the behavior seen in Fig. 4. Starting with the model of Koefoed-Johnsen and Ussing (1958), we may assume that the amiloride-sensitive entry step is an electrodiffusion process. The simplest approach is to propose that Li and Na do not interact in the channel,

i.e., that these ions obey the so-called "independence principle" (Hodgkin and Huxley, 1952), and that the movements of Na and Li can be described by the constant field equation (Goldman, 1943; Hodgkin and Katz, 1949). Because both fluxes are assumed to be unidirectional, the backflux terms are negligible, and because the Li and Na fluxes appear in a ratio, the terms in the apical membrane PD drop out. The resulting expression for $1/t^{\text{Na}}$ is simply

$$1/t^{\text{Na}} = 1 + (\bar{P}_{\text{Li}}/P_{\text{Na}})([\text{Li}]_m/[\text{Na}]_m), \quad (4)$$

where \bar{P}_{Li} and P_{Na} are the apical membrane permeabilities for Li and Na, i.e., they are functions of physical parameters that describe the ion-membrane interaction such as ion-channel partition coefficients, diffusion coefficients, and channel length. Viewed within this framework, the unity slope relation of Fig. 4 would be consistent with a ratio for $\bar{P}_{\text{Li}}/P_{\text{Na}}$ of unity. This ratio, however, must be regarded as a somewhat arbitrary, operational comparison of Li and Na entry because the value for $\bar{P}_{\text{Li}}/P_{\text{Na}}$ would clearly depend on the time over which the I_{sc} is integrated.

Another starting point is to assume, as did Biber and Curran (1970), that both J_1^{Na} and J_1^{Li} may be described by simple Michaelis-Menten functions, i.e.,

$$J_1^{\text{Na}} = \frac{J_{\text{Na}}^{\text{M}}[\text{Na}]_m}{K_{\text{Na}} + [\text{Na}]_m},$$

(5)

and

$$J_1^{\text{Li}} = \frac{J_{\text{Li}}^{\text{M}}[\text{Li}]_m}{K_{\text{Li}} + [\text{Li}]_m},$$

where J_{Na}^{M} and J_{Li}^{M} represent the maximal fluxes of Na and Li and K_{Na} and K_{Li} are the apparent Michaelis constants for the two cations. If we assume, in addition, that both ions "compete" for a common "site" or "carrier" in the apical membrane and that the interaction can be described by classical competitive inhibition kinetics then Eqs. 5 become:

$$J_1^{\text{Na}} = \frac{J_{\text{Na}}^{\text{M}}[\text{Na}]_m}{K_{\text{Na}} \left(1 + \frac{[\text{Li}]_m}{K_{\text{Li}}} \right) + [\text{Na}]_m},$$

(6)

and

$$J_1^{\text{Li}} = \frac{J_{\text{Li}}^{\text{M}}[\text{Li}]_m}{K_{\text{Li}} \left(1 + \frac{[\text{Na}]_m}{K_{\text{Na}}} \right) + [\text{Li}]_m}.$$

The expression for $1/t^{\text{Na}}$ again assumes a simple form, given by

$$1/t^{\text{Na}} = 1 + (K_{\text{Na}}/K_{\text{Li}})([\text{Li}]_m/[\text{Na}]_m). \quad (7)$$

Viewed from this perspective, the unity slope of Fig. 4 is also consistent with equal apparent Michaelis constants or apparent "affinities" for a saturable entry mechanism. Again we must emphasize that this ratio rests on the somewhat

arbitrary choice of the 60-s integration period for the time-varying Li current. Our purpose here, however, is only to compare the predictions of two simple models for the entry process on an equal basis.

Clearly, on the basis of Fig. 4 alone these two classes of models can not be distinguished. A model based on simple, competitive inhibition, however, would also predict that J_1^{Na} in the presence of Li should be significantly less than in the absence of Li. A previous study (Thompson and Dawson, 1978) indicated that I_{sc} in the turtle colon was a saturable function of mucosal Na concentration consistent with an apparent Michaelis constant (K_{Na}) of about 10 mM. Assuming that $K_{\text{Li}} = K_{\text{Na}} = 10$ mM, a simple competition model predicts nearly a 40% inhibition of J_1^{Na} at 16 mM Na in the presence of 16 mM Li, well within the sensitivity of these measurements. The data in Table I indicates, however, that at mucosal Li concentrations below about 30 mM Li has no discernable effect on the rate of Na entry. These experiments cannot, of course, exclude some combination of inhibitory and stimulatory effects which fortuitously cancel.

The predictions of even these oversimplified models are subject to a number of qualifications because of the assumptions involved. The population of cation-selective channels in the apical membranes is homogeneous only in that all of the channels appear to be blocked by amiloride. These results cannot exclude the possibility that there are two populations of amiloride-sensitive channels in the apical cell membrane, one specific for Na and a second specific for Li. A complete dichotomy of Na and Li entry seems unlikely, however. In addition, we cannot be certain that the unidirectional influx of Na through the amiloride-sensitive channels is equal to the net influx of Na in the presence of Li. If J_1^{Na} in the presence of Li is an overestimate of the net flux of Na, then the ratio, $\bar{P}_{\text{Li}}/P_{\text{Na}}$, is underestimated. Likewise, if a portion of the amiloride-inhibitable Li current enters a compartment separated from the "Na transport pool" (as suggested by Morel and Leblanc [1975] for frog skin) then the ratio $\bar{P}_{\text{Li}}/P_{\text{Na}}$ is overestimated to this extent. It must also be stressed that due to the time-variant nature of the Li current the value of $\bar{P}_{\text{Li}}/P_{\text{Na}}$ is not uniquely defined and is dependent on a somewhat arbitrarily chosen integration time for I_{sc} . The value of the apparent rate coefficient for Na, however, does not appear to vary significantly during the Li-induced transient in I_{sc} (Fig. 2).

The Mechanism of Na and Li Entry

The unidirectional influx of Na across the apical membrane has been measured directly in the frog skin (Biber and Curran, 1970), the toad colon (Dawson and Curran, 1976), the rabbit colon (Frizzell and Turnheim, 1978) and the turtle colon (Thompson and Dawson, 1978). Uniformly, these studies indicate that the initial rate of Na entry is a saturable function of mucosal Na concentration. This observation can be restated more directly by saying that the rate coefficient for Na entry is decreased as Na concentration in the solution bathing the apical membranes is raised. This behavior invites the suggestion that Na ions cross the apical membrane via some Na-specific "site" or "carrier" for which tracer Na and unlabeled Na ions compete. In addition, Biber and Curran (1970) found that increasing Li concentrations on the apical surface of frog skin inhibited Na entry and that this inhibition conformed to classic competitive inhibition

kinetics. Their results do not indicate, however, whether Na entry in the presence of Li could account for the total I_{sc} . Dawson and Curran (1976) found in the colon of the toad (*Bufo marinus*) that the ratio of Na entry to the total I_{sc} was decreased in the presence of Li, and they also interpreted these results as an inhibition of Na entry rather than as a contribution of Li to the current. They did not consider the relation between Na entry and the pre-Li values of I_{sc} , the most sensitive test for "inhibition" of Na entry. In fact, the data presented in Fig. 8 of Dawson and Curran (1976) can be interpreted within the analysis presented here to indicate a P_{Li} to P_{Na} ratio of about 0.75 which, within the error of those measurements, would not be distinguishable from unity. Nagel (1977) recently studied the effect of Li on intracellular potentials in the frog skin. The results were consistent with passive Li entry from the outside solution through an amiloride-sensitive "channel" and suggested that the inhibitory effects of Li on Na uptake by the frog skin previously reported by Biber and Curran (1970) may be attributable to Li-induced changes in the apical membrane PD.

Our results show clearly that under conditions where Li ions carry as much as $1/2$ - $2/3$ of the total amiloride-sensitive I_{sc} across the apical membranes of the turtle colon, Na entry through amiloride-sensitive channels in the apical membrane is not affected. This result is not compatible with an entry mechanism that involves the competition of Na and Li for a specific "site" or "carrier" in the membrane. As discussed above, the simplest model that accounts for our observations is a cation-selective "pore" through which Na and Li ions may move with negligible interaction. Recently, Lindemann and Van Driessche (1977) analyzed the current fluctuations in the apical membrane of the frog skin caused by amiloride and concluded that single Na-transport sites translocate more than 10^6 Na ions per second, suggesting that an Na-selective pore is a physically realistic model for the Na entry mechanism.

In the absence of additional information as to the nature of the time-variant I_{sc} in the presence of mucosal Li, we cannot attach great quantitative significance to the value for \bar{P}_{Li}/P_{Na} of unity obtained over a 60-s uptake period. Additional experiments, however, have shown that if the apical surface of the turtle colon is bathed by a solution containing 114 mM Li (in the absence of Na) the amiloride-sensitive conductance in the steady state (after all transients have vanished) is significantly greater than that seen with 114 mM Na (Sarracino and Dawson, 1978). If, under these conditions, the amiloride-sensitive conductance is attributable primarily to the conductance of the apical membranes, this result suggests that the ratio P_{Li}/P_{Na} may be significantly greater than unity in the steady state.

The behavior of Na and Li in the apical membrane of the turtle colon is also reminiscent of the results obtained with Li:Na substitution in axon membranes. Several studies have suggested that the ratio P_{Li}/P_{Na} is approximately unity for the Na channel in the squid axon membrane (Moore, 1958; Chandler and Meves, 1965). Hille (1972, 1975) reported that P_{Li}/P_{Na} averaged about 0.93 for the Na channel in myelinated nerve and, in addition, that Li also had a blocking effect on the channel such that peak inward currents were 28% lower in Li Ringer than in Na Ringer. In the apical membrane of the turtle colon the

behavior of I_{sc} and J_{Na}^i in the presence of Li are consistent with a constant ratio, P_{Li}/P_{Na} , which may be unity or greater, but it is clear that Li also "blocks" Na entry to a certain extent at higher Li concentrations. In the present experiments, however, the inhibitory effects of high Li concentrations may be attributable to changes in the apical membrane PD.

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