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Sodium Uptake across the Apical Border of the Isolated Turtle Colon: Confirmation of the Two-Barrier Model

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Summary. The initial rate of Na uptake by the turtle colon from the mucosal bathing solution consists of two operationally distinct components. One component is a linear function of mucosal Na concentration, is unaffected by amiloride, and appears to represent Na uptake into the paracellular shunt path. The major component of Na uptake is abolished by amiloride and is virtually equal to the short-circuit current over a wide range of mucosal Na concentrations, suggesting that this portion of Na uptake represents Na movement into Na-transporting cells of the colon. The amiloride-sensitive component of Na uptake, at low mucosal Na concentrations, was unaffected if net Na transport was abolished by ouabain. Similarly, at low mucosal Na concentrations the amiloride-sensitive conductance of the colon was identical in the presence and in the absence of net Na transport.

These results show that the isolated turtle colon behaves as two distinct barriers to transmural Na transport, an apical barrier blocked by amiloride and a more basal-lying barrier where active, transmural Na transport is blocked by ouabain. In addition, these experiments appear to provide the first unambiguous demonstration that the initial-rate isotope uptake technique can provide a *direct* measure of the properties of the amiloride-sensitive barrier to transmural Na movement, presumably the apical membranes of the Na-transporting cells. The results are consistent with the notion that the rate of transmural active Na transport and the conductance of the active Na-transport path are determined by the properties of the apical membrane.

The two-barrier model for transepithelial Na transport was formulated by Koefoed-Johnsen and Ussing [14] to account for their observations on the electrical behavior of the isolated frog skin, and this model has served as a starting point for subsequent studies of active Na transport in a variety of epithelia. The two-barrier model suggests that active, transepithelial Na movement consists of two distinct, sequential steps. Na first enters the epithelial cells by crossing the apical cell membrane, driven by the Na-electrochemical potential difference across the apical

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membrane. Subsequently, Na is extruded from the epithelial cells across the basolateral membranes at the expense of metabolic energy.

Schultz *et al.* [23] introduced the technique of determining the properties of the apical membranes of epithelia by measuring the *initial rate* of isotope uptake from the apical side. Attempts to use this technique to measure directly the properties of the apical barrier to Na entry in Na-transporting, "tight" epithelia, however, have produced results not entirely consistent with a simple, two-barrier model. In the frog skin, for instance, Na entry under some conditions is inhibited by ouabain [1, 20] leading one group of investigators to suggest that the isolated frog skin may behave as a single barrier to transmural Na transport [20]. In addition, it has been suggested that Na entry into the frog skin [1] and the toad bladder [9] may be in part an active process.

We have previously shown that the isolated colon of the freshwater turtle actively transports Na and that changes in the net transport of Na across the tissue are identical to changes in the short-circuit current [5]. Furthermore, variations in Na transport and I_{sc} appear to be attributable to variations in the apparent conductance of the active transport path. In the present experiments, we have attempted to estimate the initial rate of Na entry from the mucosal bathing solution into the cells of the turtle colon in order to answer two questions: Does the initial rate of Na uptake provide a direct measure of the properties of the apical membrane and, if so, what is the contribution of the apical membrane to the transport properties of the tissue?

The results of these studies indicate that Na which traverses the active path across the isolated turtle colon must cross at least two barriers in series, an apical barrier where Na entry is blocked by amiloride and a second barrier which is the site of action of ouabain. The short-term uptake of Na from the mucosal bathing solution appears to provide a direct measure of the properties of the apical barrier, presumably the apical cell membranes of the Na transporting cells. In addition, these results indicate that the properties of the apical cell membrane determine the apparent conductance of the active Na transport path and, hence, the rate of transmural Na transport.

Materials and Methods

Colons were removed from freshwater turtles, *Pseudemys scripta elegans* (Mogul-Ed, Oshkosh, Wisc.) and stripped of circular and longitudinal musculature as previously de-

scribed [5]. In order to reduce edge-damage effects [13], portions of stripped colon were initially mounted by gluing the serosal side of the tissues to rubber washers (~1 cm OD) with a cyano-acrylate tissue adhesive (Eastman 910). The rubber washers were fabricated from 1/32″ silicon rubber sheeting (McMaster-Carr, Chicago, Ill.). Because the adhesive bonds virtually instantaneously, the degree of stretch of the tissue could be relatively easily controlled. The circular piece of mucosa with the serosal washer was then cut out using a tissue punch. The resulting disc was sealed on the washer side using silicone vacuum grease to the inner portion of an influx chamber which was essentially identical in design to that described in detail by Biber and Curran [2]. The disc of tissue was held in place by a cap which was also gently applied to the mucosal side. Typically 18 to 24 1-cm^2 portions of tissue were mounted in this manner on individual "inner chambers" and placed mucosal side down in an aerated incubation bath with approximately 0.75 ml of an appropriate Ringer's solution on the serosal side. Preliminary experiments suggested that there is no marked variation in the transport properties of the colon along its length.

Unidirectional Sodium Influx

The unidirectional influx of Na from the mucosal bathing solution into the mucosal cell layer was estimated by measuring the uptake of ^{22}Na according to the technique employed by Biber and Curran [2] and subsequently by Dawson and Curran [6]. Portions of stripped mucosa, mounted as described above, were placed in the influx chamber mucosal side down. The chamber was, in principle, identical to that described by Biber and Curran [2] except that the mucosal surface of the colon 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 due to a slight hydrostatic head on the serosal side. This arrangement provided 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 which could be adjusted to compensate for the fluid resistance between the tissue surface and the PD-sensing electrodes. The short-circuit current 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.

Unless otherwise indicated, the serosal surface of the tissue was bathed by 0.75 ml of a Ringer's solution which contained (in mM) Na, 114; Cl, 114; K, 2.5; HCO_3, 2.5; Ca, 1; D-glucose, 5; D-mannitol, 5; and pyruvate, 2.5. The mucosal surface was bathed by 5 ml of an identical solution, except where the Na concentration was varied by isosmotic replacement with choline-Cl. Na concentrations were routinely verified by flame photometry. 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 approximately 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 5–8 μCi of ^{22}Na and 60 μCi of ^{3}H-mannitol in the appropriate Ringer's solution. After 30 to 60 sec, the inner chamber was rapidly removed and the influx was terminated in one of two ways. One method consisted of blotting the tissue on several layers of filter paper and subsequently rapidly removing the tissue with a punch. The alternate method involved first "washing" the mucosal surface of the tissue by briefly (2–3 sec) immersing the inner chamber in a large volume of "cold" Ringer's, followed by blotting and punching. Differences in results using the two procedures will be discussed in the

text. The tissue was extracted for at least 2 hr in distilled water, and an aliquot of the extract was assayed for ^3H and ^{22}Na. The uptake of ^3H-mannitol served to estimate the volume of fluid adhering to the tissue after blotting. ^{22}Na in excess of that in the mannitol space was defined as Na uptake by the tissue.

Results

Sodium Uptake as a Function of Time

The aim of these short-term uptake experiments was to obtain a measure of the *initial rate* of Na entry from the mucosal bathing solution into the colon. In order to obtain an exposure time appropriate for an initial rate determination, we measured Na uptake by the colon as a function of time at Na concentrations ranging from 2 to 114 mM. In all cases Na uptake was a linear function of time over a 60-sec period, but when the influx was terminated by blotting only, the intercepts of the uptake *vs.* time plots were nonzero, as indicated in Fig. 1 by the dashed line. The mannitol space in these experiments was virtually constant over the range of exposure times. As shown in Fig. 1 by the solid line, the nonzero intercept is eliminated by briefly washing the mucosal surface in cold Ringer's solution prior to blotting. The efficacy of washing is identical whether the washing solution contains high or low Na concentrations or amiloride. In addition, sampling of the serosal bathing solution indicated that over a 60-sec period virtually no tracer crossed the tissue. These results indicate that Na uptake measured using

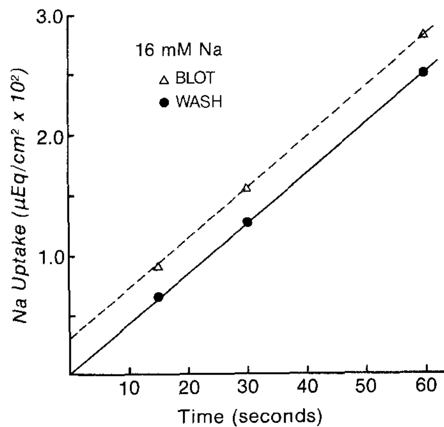

Fig. 1. Na uptake by colon as a function of time of exposure to the test solution for two conditions: (△) uptake terminated by blotting only; (●) uptake terminated by washing and then blotting. Each point is the mean of at least six determinations

a 30 to 60-sec exposure time followed by a brief wash provides an adequate estimate of the initial rate of Na uptake, i.e., the unidirectional flux of Na from the mucosal bathing solution into the tissue.

Sodium Influx and I_{sc}

Na influx was measured under short-circuit conditions at mucosal Na concentrations of 2, 8, 16, 25, 80 and 114 mM. In most experiments the serosal solution contained 114 mM Na, but in some experiments the serosal Na concentration was also reduced. The Na concentration in the serosal bathing solution appeared to have no effect on the relation between Na influx and I_c. Experiments in which active Na transport was abolished by amiloride indicated that the small diffusion potential expected in the presence of ion concentration gradients across the shunt path had a negligible effect on I_{sc}. Exposure of the serosal side to 114 mM Na-Ringer's, however, appeared to promote better long-term survival of the tissue so that this serosal solution was employed most frequently.

Figure 2 shows the unidirectional Na influx, J_i^{Na}, plotted *vs.* the simultaneously measured I_{sc} at mucosal Na concentrations of 2 and 114 mM. Each point represents an influx and current determination on a single tissue. Each set of points can be described by a straight line with a unity slope and a positive intercept. The relation between J_i^{Na} and I_{sc} is identical at 2 and 114 mM Na except that the intercept, $(J_i^{Na})_{I_{sc}=0}$, is higher at the higher Na concentration. Also shown in Fig. 2 are the mean values of J_i^{Na} and I_{sc} in the presence of 10^{-4} M amiloride. Previous studies [5] have shown that the addition of amiloride to the mucosal side of the isolated turtle colon reduces I_{sc} to near zero in seconds. In the presence of amiloride active Na transport is abolished and transmural Na fluxes appear to be confined to paracellular shunt pathways. It can be seen in Fig. 2 that amiloride markedly reduces I_{sc} and J_i^{Na}. The action of amiloride at the mucosal surface is sufficiently rapid that the results are identical whether amiloride is added to the tissue prior to the measurement of Na influx or is only present during the uptake period.

Examination of similar plots of J_i^{Na} *vs.* I_{sc} at a variety of mucosal Na concentrations suggested that the slopes did not differ from unity, while the intercepts (and the amiloride-insensitive portion of J_i^{Na}) increased monotonically with increasing mucosal Na concentration. Figure 3 shows the average values of J_i^{Na} measured in the presence of amilo-

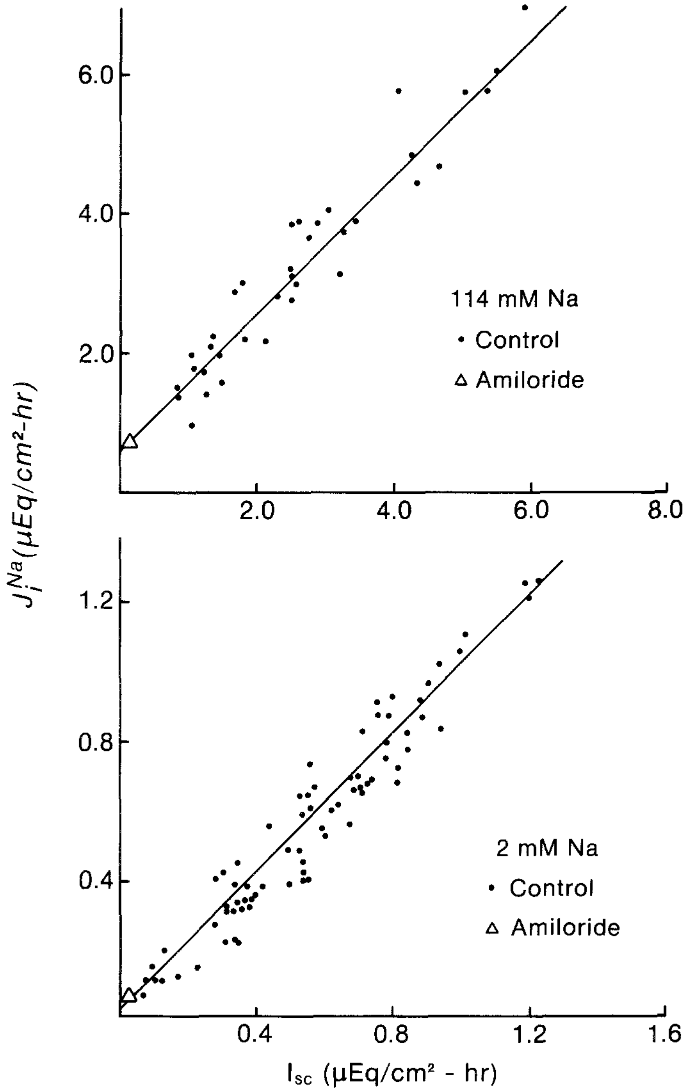

Fig. 2. J_i^{Na} plotted *vs.* I_{sc}. Each dot represents a single tissue in the absence of amiloride. Also shown (△) are the *average* values of J_i^{Na} and I_{sc} in the presence of amiloride

ride plotted as a function of mucosal Na concentration. The maximum SE for these values is approximately twice the size of the filled circles. The least squares line describing these points has an intercept not different from zero and a slope of 0.006 ± 0.003 cm/hr. Also shown are the average values of the intercepts of the J_i^{Na} *vs.* I_{sc} plots. These points fall very close to the line which describes the amiloride-insensitive Na influx as a function of Na concentration, suggesting that the amiloride-insensitive

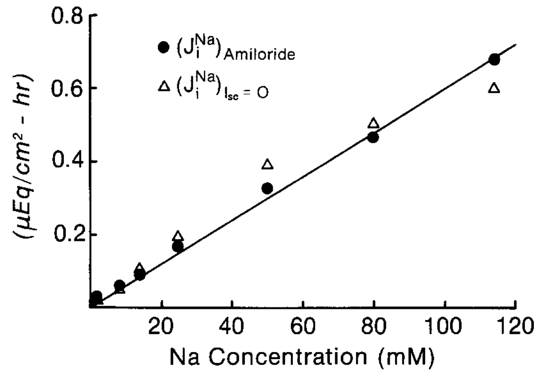

Fig. 3. Na influx in the presence of amiloride (●) and the intercepts of J_i^{Na} *vs.* I_{sc} plots (△), plotted as a function of mucosal Na concentration

component of J_i^{Na} represents Na uptake into some compartment or pathway not involved in net Na transport across the tissue.

Na influx from the mucosal bathing solution into the epithelial cell layer thus appears to consist of at least two operationally distinguishable components. The major component of Na influx is identical to the short-circuit current at all mucosal Na concentrations and is abolished by mucosal amiloride. The second and smaller component is a linear function of mucosal Na concentration, is unaffected by amiloride, and is apparently unrelated to net Na transport across the tissue. The total influx, J_i^{Na}, can be written as,

$$J_i^{\mathrm{Na}} = I_{sc} + \beta[\mathrm{Na}]_m$$

where β represents the apparent permeability of the amiloride-insensitive component, 0.006 cm/hr.

The Linear Component of J_i^{Na}

The amiloride-insensitive portion of J_i^{Na} presumably represents Na which does not enter the active transport path. This component of Na uptake could represent Na entry into cells not involved in transmural Na transport, nonspecific Na "binding" to the apical surface of the epithelium, or Na uptake into the paracellular shunt path. We have attempted to discriminate among these alternatives by determining the effect of "opening" the epithelial tight junctions on the amiloride-insensi-

Table 1. Effect of mucosal hyperosmolarity on Na uptake in the presence of amiloride ($[Na]_m = 114$ mM)

	G_t (mmho/cm^2)	J_i^{Na} (μeq/cm^2-hr)	J_i^{Na}/G_t	n
Control	1.33 ± 0.10	0.65 ± 0.11	0.52 ± 0.10	15
Hyperosmolar	2.52 ± 0.36	1.17 ± 0.19	0.49 ± 0.06	13

$\bar{x} \pm$ SE, n = number of tissues

Control tissues were exposed to 114 mM Na-Ringer's on the mucosal side. The experimental group was pre-incubated in an identical solution which was made approximately 300 mosmol hyperosmolar by the addition of mannitol to the mucosal bathing solutions only. Each tissue was exposed to the hyperosmolar solution for from 6–10 min and the increase in G_t was monitored. After G_t had increased by from 50 to 150%, Na uptake was measured as previously described, except with a hypertonic test solution.

tive portion of J_i^{Na}. DiBona and Civan [7] showed that it was possible to modify the permeability of the paracellular shunt pathway in the toad urinary bladder by raising the osmolarity of the mucosal solution. In the turtle colon this treatment caused "blisters" in the tight junctions and a resultant increase in the tissue conductance and transmural Na diffusion through the shunt [5]. Table 1 shows the results of an experiment in which Na uptake was measured after treatment with amiloride (10^{-4}M) in the presence and in the absence of a hyperosmotic mucosal bathing solution. In accord with previous observations [5] mucosal hyperosmolarity results in a marked increase in total tissue conductance. In addition, the amiloride-insensitive Na uptake in the treated tissues is significantly higher than in the controls. Despite the marked increase in G_t and J_i^{Na}, however, the ratios, J_i^{Na}/G_t, in the presence of amiloride are similar for the two groups.

The Amiloride-Sensitive Component of J_i^{Na}

The major portion of J_i^{Na} is, within the error of our measurements, identical to I_{sc} and is abolished by amiloride. One interpretation of this behavior is that the initial rate of Na uptake is, in fact, a measure of the initial step in transmural Na transport, which according to a two-barrier hypothesis, would correspond to Na entry across the apical cell membrane into the so-called "transport pool". According to this

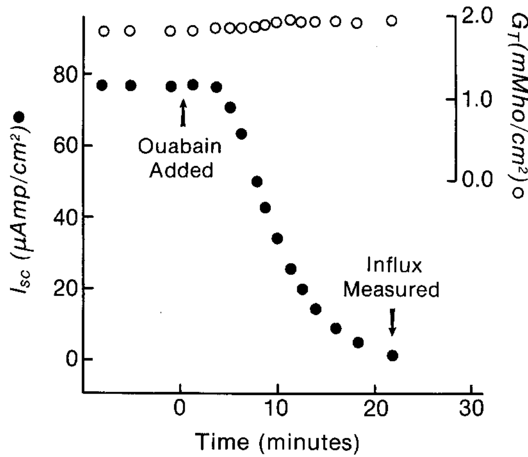

Fig. 4. Protocol for measurement of J_i^{Na} after I_{sc} has been reduced to zero by ouabain

hypothesis, Na uptake over short time periods has not yet traversed the Na-pump, presumably located at the serosal membrane, and thus this portion of J_i^{Na} would provide a direct measure of the properties of the apical cell membranes of Na transporting cells. An alternative interpretation, however, is that even at times as early as 30–60 sec Na taken up by the epithelium has already been actively transported, i.e., Na uptake is "past" the pump. This behavior would be expected if the intracellular "Na transport pool" were vanishingly small or if a ouabain-sensitive active Na transport step resides at the apical membrane. In order to interpret measurements of Na uptake unambiguously, it is necessary to dissociate the entry step from ouabain-sensitive, transmural Na transport.

The action of ouabain is thought to be localized at the basolateral Na pump [19] and according to the simplest version of the two-barrier model should not directly affect Na entry. Figure 4 indicates the protocol for experiments designed to measure Na uptake by tissues in which I_{sc} has been reduced to zero by ouabain. I_{sc} (solid circles) and total tissue conductance (open circles) are plotted *vs.* time for a representative experiment at a mucosal Na concentration of 16 mM. The steady-state value of I_{sc} was recorded at $t=0$ and ouabain was added to the serosal bathing solution (10^{-4}M). Typically, I_{sc} declined to zero over about 25 min while the tissue conductance remained virtually unchanged. When I_{sc} reached $0 \pm 10\%$, Na uptake was measured as previously described. Using this protocol it was possible to compare J_i^{Na} (measured at zero

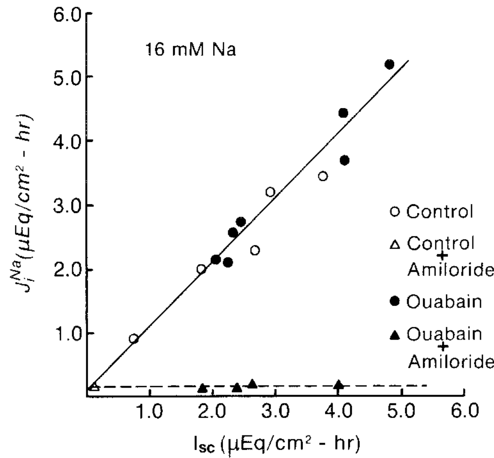

Fig. 5. J_i^{Na} plotted *vs.* I_{sc} for four conditions. J_i^{Na} measured in the absence of ouabain (○, △) is plotted *vs.* simultaneously measured I_{sc}. J_i^{Na} measured in the presence of ouabain ($I_{sc}=0$, ●, ▲) is plotted *vs.* *pre*-ouabain steady-state I_{sc}

I_{sc}) with the steady-state I_{sc} which characterized the tissue *prior* to treatment with ouabain. Our previous results indicate near identity between the amiloride-inhibitable portions of J_i^{Na} and I_{sc} under control conditions. If Na uptake is a measure of Na entry and if ouabain has no effect on the entry step, then J_i^{Na} measured in the presence of ouabain should be identical to the *pre-ouabain* value of I_{sc}.

Figure 5 shows J_i^{Na} plotted *vs.* I_{sc} at 16 mM mucosal Na for 4 conditions. The open circles are control influxes measured in the absence of ouabain and plotted *vs.* the simultaneously measured I_{sc}, as before. As expected from previous results, these points are described by a line with a unity slope and an intercept in agreement with the average value of J_i^{Na} measured in the presence of mucosal amiloride (open triangle). The solid circles represent values of J_i^{Na} measured in the *presence* of ouabain after I_{sc} had been reduced to zero. These values, however, are plotted *vs.* the *pre-ouabain,* steady-state I_{sc}. Note that J_i^{Na} measured in the presence of ouabain ($I_{sc}=0$) exhibits a relation to the *pre-ouabain* I_{sc} which is identical to that obtained for the controls, despite the fact that Na uptake was measured under conditions of zero net Na transport. Furthermore, as shown by the solid triangles, even though J_i^{Na} is unaffected by ouabain, it is abolished if amiloride is added to the mucosal solution only during the 30 to 60-sec uptake period. Similar experiments indicated that the amiloride-sensitive portion of Na uptake is unaffected by ouabain at mucosal Na concentrations of 2, 8, 16, and 25 mM. Subsequent studies,

however, have shown that after ouabain inhibition of I_{sc} amiloride-sensitive Na uptake is decreased by as much as 40% at a mucosal Na concentration of 114 mM (*unpublished observations*).

The Effect of Ouabain on the Sodium-Conductance of the Active Path

Experiments such as that shown in Fig. 4 suggested that at low mucosal Na concentrations ouabain reduced I_{sc} to zero but did not affect the *total* tissue conductance. This observation suggests that the conductance of the *active* transport path, ${}_aG_{Na}$, is not affected by ouabain. To document this point, we examined the relation between active Na transport and the conductance of the active path in control tissues and in tissues in which I_{sc} had been reduced to zero by ouabain. The Na current, I_{sc}^{Na}, is defined as the amiloride-sensitive portion of I_{sc}. Likewise, ${}_aG_{Na}$ is defined as the amiloride-sensitive portion of G_t. Figure 6 shows ${}_aG_{Na}$ plotted as a function of I_{sc}^{Na} at 8 mM mucosal Na for two conditions. The open circles represent control tissues in which ${}_aG_{Na}$ and I_{sc}^{Na} were obtained by adding amiloride. As expected from our previous study [5], ${}_aG_{Na}$ is highly correlated with I_{sc}^{Na}. Using a protocol similar to that illustrated in Fig. 4, the total conductance, G_t, was also measured *after* I_{sc} had been reduced to zero by ouabain. Amiloride (10^{-4}M) was then added to the mucosal solution and the amiloride-induced reduction

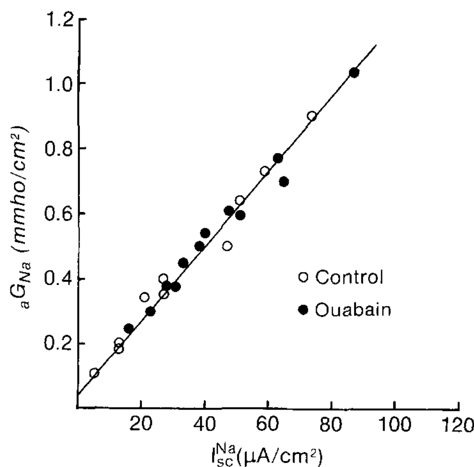

Fig. 6. Na conductance of the active path, ${}_aG_{Na}$, plotted *vs.* the Na current though the active path, I_{sc}^{Na}. For controls (○) ${}_aG_{Na}$ is plotted *vs.* the simultaneously determined I_{sc}^{Na}. For the ouabain points (●) ${}_aG_{Na}$ determined in the presence of ouabain ($I_{sc}=0$) is plotted *vs.* the *pre*-ouabain steady-state I_{sc}. ($[Na]_m = 8$ mM)

in G_t determined. In this manner it was possible to compare ${}_aG_{Na}$ measured in the *absence* of net Na transport with the value of I_{sc} which characterized the tissue *prior* to ouabain inhibition. (I_{sc} in the presence of ouabain and amiloride was not significantly different from zero.) The filled circles in Fig. 6 represent values of ${}_aG_{Na}$ determined at $I_{sc}=0$ and plotted *vs.* the *pre*-ouabain steady-state I_{sc}. Clearly, ${}_aG_{Na}$ measured in the absence of net Na transport exhibits a relation to the *pre*-ouabain I_{sc} which is identical to that for the control tissues, indicating that abolishing *net* Na transport with ouabain has no detectable effect on the conductance of the active path. Additional experiments indicate that when the mucosal Na concentration is increased to 114 mM, inhibiting I_{sc} with ouabain *reduces* the value of ${}_aG_{Na}$ (*unpublished observations*).

Sodium Concentration Dependence of J_i^{Na} and I_{sc}

Since the amiloride-sensitive portion of J_i^{Na} appeared to be equal to I_{sc} over a wide range of mucosal Na concentrations, it was of interest to determine the concentration dependence of both parameters. Tissues were initially incubated with 114 mM Na on both sides. After a steady value of I_{sc} was obtained, the mucosal solution was exchanged for one in which the Na concentration had been reduced by substitution with choline-Cl, and a new steady-state value of I_{sc} was obtained. This process was repeated with at least 5 Na concentrations on a single tissue. Figure 7 shows I_{sc} normalized to 114 mM plotted *vs.* mucosal Na concentration. This figure represents data from 4 tissues which varied in their initial currents from 40 to 80 μA/cm². Despite this variability, the normalized

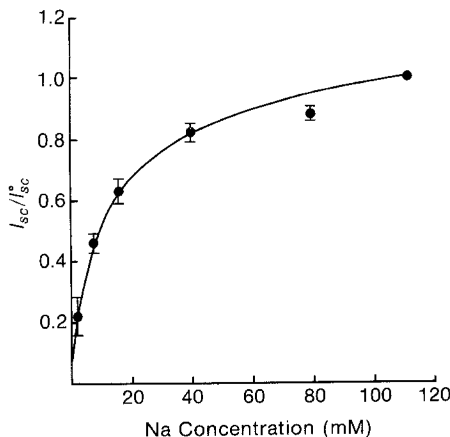

Fig. 7. Values of I_{sc} normalized to the value at $[Na]_m = 114$ mM (I_{sc}^0) plotted as a function of mucosal Na concentration. Each point is the average of four tissues

values of I_s indicate that individual segments of colon behave similarly. Two features of this plot are of interest. First, as expected from studies on other isolated epithelia [2, 6], I_{sc} appears to be a saturable function of mucosal Na concentration. If the values are appropriately plotted, an apparent "K_T" of about 10 mM can be calculated. The correspondence of the normalized values of I_{sc} for different tissues suggests that, in Michaelis-Menten terminology, tissue-to-tissue variability lies primarily in the values of I_{sc}^{max} rather than K_T.

Discussion

Barriers to Sodium Transport through the Active Path

Previous attempts to use initial-rate, isotope uptake techniques to identify the properties of the apical barrier to transmural Na transport in "tight" epithelia have produced ambiguous results, because it has not been possible to completely dissociate the initial rate of Na entry into the epithelium from the process of *transmural* Na transport. In the frog skin, Na uptake from the outside bathing solution is either partially [1] or totally [8, 20, 22] inhibited by ouabain, an agent which is presumed to exert its *direct* effect by interfering with the active Na extrusion step. Although several explanations for the inhibitory effect of ouabain on isotope uptake have been advanced [1, 8, 20], it has not been possible to eliminate the alternative that tracer Na which is taken up from the apical side during very short exposure times has already traversed the ouabain-sensitive "Na-pump". In this experimental setting an epithelium with a vanishingly small "Na transport pool" might well behave as a single barrier [20 and S.G. Schultz, *personal communication*].

The results presented here appear to provide an unambiguous demonstration that in the isolated turtle colon the initial-rate, isotope uptake technique provides a direct measure of the properties of the amiloride-sensitive barrier to transepithelial Na transport, presumably the apical cell membranes of the Na transporting cells. The major component of Na uptake by the turtle colon is highly correlated with I_{sc}, is blocked by mucosal amiloride, and thus appears to represent Na uptake by cells which participate in transmural Na transport across the epithelial cell layer. Furthermore, at low mucosal Na concentrations, the unidirectional influx of Na from the mucosal solution *into the cells*, J_{mc}^{Na}, is unaffected when *net*

Na transport is abolished by ouabain. This result demonstrates that the amiloride-sensitive entry mechanism is proximal to the ouabain-inhibitable transport step. In this experimental setting the turtle colon clearly behaves as two series barriers to transmural Na transport.

At low mucosal Na concentrations ouabain abolishes I_{sc} but does not cause a discernable change in either the rate coefficient for Na entry or the conductance of the active path, ${}_aG_{Na}$. Taken together, these observations suggest that J_{mc}^{Na} and ${}_aG_{Na}$ are both measures of the properties of the same barrier, the apical cell membrane of the Na transporting cells, and that the properties of this membrane determine the rate of transmural Na transport. This conclusion is consistent with microelectrode studies on a variety of Na-transporting epithelia which indicate that the conductance of the active Na transport path represents essentially the conductance of the apical membranes [4, 12, 15, 16, 21, 24].

Inhibitory effects of ouabain on Na entry in frog skin [1, 20] and toad bladder [9] have raised the possibility that Na entry in these tissues may have an active component. In contrast, the present results are consistent with the notion that in the turtle colon Na entry is driven solely by the Na electrochemical potential difference across the apical cell membrane, $\Delta\tilde{\mu}_{Na}$. These experiments show that over a wide range of mucosal Na concentrations the unidirectional rate of Na entry, J_{mc}^{Na}, is within the error of its determination identical to I_{sc}. Thus for practical purposes, J_{mc}^{Na} is equal to the *net* flux of Na into the cells. Similar relations between J_{mc}^{Na} and I_{sc} have been found for the frog skin [1], the toad colon [6], and the rabbit colon [11]. The relation between J_{mc}^{Na}, J_{cm}^{Na} (the unidirectional flux from cell to mucosal solution) and I_{sc} in the steady-state can be written as

$$J_{mc}^{Na}/I_{sc} = (1 - J_{cm}^{Na}/J_{mc}^{Na})^{-1}.$$

The lack of any systematic variation of J_{mc}^{Na}/I_{sc} with $[Na]_m$ indicates that over a wide range of mucosal Na concentrations the Na flux ratio, J_{cm}^{Na}/J_{mc}^{Na}, across the apical membrane, is much less than unity. For passive Na entry this is consistent with a significant electrochemical potential difference favoring Na entry at all mucosal Na concentrations.

If the ionic permeability of the apical cell membranes of the colon can be attributed solely to a highly selective "Na-channel" through which Na ions move in response to their electrochemical potential gradient, then the electrical PD across the apical membrane under short-circuit conditions, ${}_0\Delta\psi_m$, is given by:

$${}_0\Delta\psi_m = E_{Na}^m - I_{sc} R_{Na}^m.$$

Where ${}_0\Delta\psi_m$ is defined as $\psi_c - \psi_m$, the potential of the cell with respect to the mucosal solution when the transepithelial PD is zero; E_{Na}^m is the Naemf across the apical membrane given by (RT/zF) $\ln\{\gamma_m[Na]_m/\gamma_c[Na]_c\}$; and R_{Na}^m is the Na resistance of the apical cell membrane (γ_m and γ_c are the activity coefficients for Na in the mucosal solution and the cell and RT/zF has its usual significance). The lack of an effect of ouabain on the rate coefficient for Na entry indicates that, at low mucosal Na concentrations, ${}_0\Delta\psi_m$ is not detectably changed when I_{sc} is reduced to zero. Thus the condition for zero *net* Na movement across the apical membrane ($\Delta\tilde{\mu}_{Na}=0$) must be attained through a change in intracellular Na concentration. If the apical membrane PD in the absence of ouabain were of the order of 50 mV, cell negative, as found in the rabbit descending colon [24]; constancy of ${}_0\Delta\psi_m$ would require that cellular Na concentration rise to a level greater than that in the mucosal bathing solution. Alternatively, if, in the absence of ouabain, ${}_0\Delta\psi_m$ does not differ greatly from zero, as reported for the toad urinary bladder [10], then a constant ${}_0\Delta\psi_m$ would require only that cellular Na rise to the level of that in the mucosal solution when net Na transport is abolished. In the absence of information as to the magnitude of ${}_0\Delta\psi_m$ in the turtle colon, we cannot discriminate between these alternatives.

The Effect of Mucosal Sodium on J_{mc}^{Na}

J_{mc}^{Na} and I_{sc} conform to an identical saturable function of mucosal Na concentration. This behavior indicates that raising mucosal Na concentration *reduces* the rate coefficient for Na movement from the mucosal bathing solution into the cells. Similar behavior of J_{mc}^{Na} has been observed in the isolated frog skin [2] and the toad colon [6]. Presently, at least three possible explanations for this behavior must be considered which are not mutually exclusive. The saturation of J_{mc}^{Na} could reflect an inhibitory interaction of *mucosal* Na ions with the entry mechanism as in competitive inhibition, for instance, or via a "modifier" site as suggested by Lindemann [17]. Alternatively, increasing mucosal Na concentration could result in a value of ${}_0\Delta\psi_m$ which is more positive inside the cell. A third possibility which has been considered, however, is "trans-inhibition" of Na entry, i.e., an inhibitory interaction of *cellular* Na ions with the entry mechanism [16, 25, 26]. As discussed above, the ouabain-induced reduction of *net* Na entry can probably be attributed to an expected rise in the apparent intracellular Na concentration [18]. Thus

at low mucosal Na concentrations there is no evidence for trans-inhibition of J_{mc}^{Na}. We have found, however, that at 114 mM mucosal Na, both J_{mc}^{Na} and ${}_aG_{Na}$ are reduced in the presence of ouabain *(unpublished observations)*. Although a reduction of J_{mc}^{Na} by ouabain in the colon could be attributed to the development of an unfavorable electrical PD across the apical cell membrane, this would not account for the reduction of ${}_aG_{Na}$. Similar reductions in amiloride-sensitive conductance by agents such as ouabain or amphotericin B, which presumably lead to elevated cellular Na content, have been interpreted as evidence for a negative-feedback, trans-inhibition of Na entry in the rabbit urinary bladder [16] and descending colon [25, 26].

The Linear Component of Sodium Uptake

A previous study [5] suggested that in the presence of mucosal amiloride transmural Na fluxes could be attributed to diffusional movement of Na through a paracellular shunt path. The results of the present experiments suggest that the amiloride-insensitive component of J_i^{Na} represents Na uptake into this same shunt pathway. The amiloride-insensitive portion of J_i^{Na} increases linearly with mucosal Na concentration and can be characterized by an apparent permeability of 0.006 cm/hr, a value in good agreement with the average Na permeability of the shunt measured previously by transmural fluxes, 0.005 cm/hr [5]. In addition, the amiloride-insensitive component of J_i^{Na} is increased in the presence of a hyperosmotic mucosal solution. We showed previously that this maneuver disrupts tight junctions in the turtle colon and increases the permeability of the shunt path to Na. The behavior of the amiloride-*insensitive* portions of J_i^{Na} and J_{ms}^{Na} (the *transmural* unidirectional Na flux) in the presence of hyperosmotic mucosal solutions is consistent with the notion that the rate limiting barrier for Na movement is in both cases the epithelial tight junction, near the apical side of the epithelium. Previous experiments [5] indicated that *in the presence of amiloride* approximately 90% of the conductance of isolated turtle colon is attributable to a paracellular shunt path and that approximately 40% of this total shunt conductance is attributable to Na. If Na uptake in the presence of amiloride represents predominantly Na diffusion into a paracellular shunt, then under short-circuit conditions the ratio, $(J_i^{Na}/G_t)_{amil}$, is a measure of the fraction of the total shunt conductance attributable to Na. This ratio is nearly identical for control and treated

tissues (Table 1), and the value of about 0.50 is in reasonable agreement with the estimate obtained from transmural fluxes.[1]

It is possible, therefore, to account for the *total* Na uptake by the colon in terms of Na entry into two pathways: one, the apical cell membranes of the Na transporting cells, and the other, the paracellular shunt path. This suggests that the cellular component of Na influx behaves as if the apical surface of the epithelium represents a *homogeneous* population of Na-transporting cells, i.e., Na entry into cells not involved in active transmural Na transport is negligible. This observation is consistent with our study of transmural Na movements [5], in which we showed that the only appreciable *transcellular* movement of Na is via the active Na transport path. In contrast, uptake studies on the frog skin [2] and toad colon [6] indicated that the portion of Na uptake unrelated to net Na transport was in excess of that attributable to the shunt path. Our comparison of blotting and washing techniques (Fig. 1) suggests that at least a portion of this discrepancy may be eliminated by washing the mucosal surface.

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1 In a previous study we showed that ^{3}H-mannitol can traverse the paracellular shunt path in the turtle colon. In short-term uptake experiments, however, the amount of ^{3}H-mannitol entering the shunt path can be estimated to be less than 10% of the total ^{3}H-mannitol "space". In addition, the ^{3}H-mannitol space in the presence of a hyperosmotic mucosal solution was not significantly different from controls.

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