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Ion Selectivity of the Apical Membrane Na Channel in the Toad Urinary Bladder

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Summary. The ion selectivity of the apical membrane Na channel in the toad urinary bladder was investigated. The electrical potential difference and resistance across the basal-lateral membrane were reduced using high concentrations of KCl in the serosal bathing medium, and gradients for various ions were imposed across the apical membrane by altering the composition of the mucosal bathing medium. Ion fluxes through the channel were measured as the transepithelial current inhibited by amiloride, a specific blocker of the channel's Na conductance. The selectivity sequence for alkali metal cations was $H > Li > Na \gg K$. K permeability was barely detectable; the selectivity for Na over K was about 1000:1. Ammonium, hydroxyl ammonium and hydrazinium ions were, like K, virtually impermeant. The results suggest that the size of the unhydrated ion is an important factor in determining permeability in this channel.

Key words epithelial Na transport · toad urinary bladder · apical membrane · ion selectivity · Na channels

Introduction

High resistance epithelia such as amphibian skin and bladder can absorb NaCl from dilute external or luminal fluids. According to the model of Koefoed-Johnsen and Ussing (1958) this process is accomplished by the concerted actions of the apical and basal-lateral plasma membranes of the epithelial cells, which respectively allow Na to diffuse into the cell, and extrude the ions into the serosal compartment against an electrochemical activity gradient. In this model of transepithelial transport, the apical membrane is selectively permeable to Na ions. In fact, *in vitro* measurements of the transepithelial electrical potential as a function of the mucosal Na and K concentrations implied a selectivity for Na over K of about 20:1 (Lindley & Hoshiko, 1964). It has more recently become apparent, however, that the mucosal surface of epithelium does not constitute a homogeneous barrier to ion movement. Rather, there exists a small number of discrete sites (channels) in the membrane which have a high conductance specific for Na ions (Lindemann & Van Driessche, 1977). In parallel with these transmembrane elements are paracellular pathways which have relatively little ion selectivity (Ussing & Windhager, 1964; DiBona & Civan, 1973). The purpose of this investigation was to examine the selectivity properties of the Na channels using the diuretic drug amiloride as a specific inhibitor of this pathway (Bentley, 1968; Ehrlich & Crabbé, 1968). The results indicate a very high selectivity ($\sim 1000:1$) for Na over K. H and Li were more permeant than Na, while NH_4, NH_3OH and NH_3NH_2 were not significantly more permeant than K.

Materials and Methods

Female toads (*Bufo marinus*, Dominican Republic origin) were obtained from National Reagents (Bridgeport, Conn.). Animals were double pithed, the urinary bladders excised and mounted as everted sacs (mucosal side out) on plastic cannulae, or as flat sheets in Lucite chambers, and electrical connections made as described previously (Rossier, Wilce & Edelman, 1974; Palmer, Edelman & Lindemann, 1980).

To reduce the electrical potential and resistance across the basal-lateral membranes, the serosal solution consisted of KCl 85, sucrose 50, $CaCl_2$ 1, $MgSO_4$ 0.5, glucose 5 and K-phosphate 3.5 buffered to pH 7.5 (all in mM). This solution was previously shown to substantially depolarize the serosal surface of the epithelium, without altering cell volume, amiloride-insensitive conductance or basal or hormone-stimulated Na transport (Palmer et al., 1980). Mucosal solutions contained the chloride salts of K, Na, NH_4, choline, NH_3OH, NH_3NH_2 or Li 85 mM; $CaCl_2$ 1 mM; $MgSO_4$ 0.5 mM and K phosphate 3.5 mM buffered to pH 7.5 except where indicated. Amiloride (a gift of Merck, Sharp and Dohme) was dissolved in deionized H_2O at 10 mM and added to the mucosal solution to a final concentration of 5 to 100 μM. Antidiuretic hormone (ADH) was added as aqueous pitressin (Parke Davis, Detroit, Mich.) to the serosal solution to a final concentration of 10 mU/ml.

Transepithelial potential differences (PD) and short-circuit current (I_{sc}) were measured in sac preparations as described previously (Rossier et al., 1974). Transepithelial slope conductance was measured as the current required to change the PD from 0 to −10 mV, or, when the open circuit PD was negative, as the current required to reduce the PD by 10 mV.

In Na-free experiments, hemibladders were preincubated for 2–3 hr in 3 to 4 washes of Na-free mucosal solutions, the final

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wash being the first test solution. After reaching a steady state in the first test solution, the tissues were challenged with amiloride, and changes in PD and resistance recorded. The amiloride was then washed off, the hemibladders preincubated in the second test solution, and challenged again with amiloride. Normally the paired hemibladder from the same animal was treated identically, but the order of the two test solutions was reversed to cancel any time-dependent effects.

To measure Li permeability, hemibladders were mounted as flat sheets in Lucite chambers as described by Palmer et al. (1980). Since transient, rather than steady-state data were used, and Li produced large changes in both PD and resistance, continuously short-circuited preparations were studied. The transepithelial PD was clamped to zero and the short-circuit current monitored continuously on a strip chart recorder. The mucosal solution (volume 3 ml) was continuously renewed by gravity feed. Ouabain (Sigma Chemical Co.) was added to the serosal solution to a final concentration of 5 mM. As shown previously, this drug inhibits transepithelial transport by increasing intracellular Na activity, thereby reducing the driving force for Na influx across the apical membrane without substantially changing the Na permeability under these conditions (Palmer et al., 1980).

When the short-circuit current had fallen to near zero, the mucosal solution containing 25 mM NaCl was rapidly replaced by solutions containing no Na, but various concentrations of LiCl, with KCl added to maintain constant ionic strength. After exposure to each Li-containing solution for 1–3 min, the mucosal solution was returned to 25 mM NaCl. When the short-circuit current had stabilized the procedure was repeated with another LiCl solution. In each experiment at least one LiCl solution gave a positive current transient, and one a negative current transient, bracketing the hypothetical Li concentration producing no transient.

To assess NH_3OH and NH_3NH_2 permeability, mucosal solutions containing these ions were prepared from stock solutions containing $NH_2OH \cdot HCl$ (Sigma) or $NH_2NH_2 \cdot 2\,HCl$ (Sigma) which were titrated with KOH to give a pH of 5.75 for NH_3OH and 6.2 for NH_3NH_2. These stock solutions were used to prepared Ringer's solutions with the same Cl concentration as the standard mucosal solutions. Since NH_3OH and NH_3NH_2 have pK's of 5.95 and 7.97, respectively (Hille, 1971), these solutions contained 52 mM NH_3OH and 41 mM NH_3NH_2.

Hemibladders were equilibrated with KCl solution (pH 6.0) on the mucosal side. The mucosal solution was then replaced with the NH_3OH or the NH_3NH_2 solution. After a new steady state was attained, the solution was replaced with one of identical composition except for the addition of amiloride (10 μM) to block any flux of the cations through the Na channels.

Theory

Previous work has indicated that with high K-sucrose solution on the serosal side, the PD and resistance across the basal-lateral membrane of the toad bladder are largely eliminated, and the transepithelial electrical properties are dominated by those of the apical membrane (Palmer et al., 1980). We therefore assume that under these conditions, the epithelium contains two electrical pathways: an amiloride-sensitive pathway with a resistance that is largely confined to the apical membrane, in parallel with an amiloride-insensitive conductance that can be either transcellular, paracellular or both. The consequences of neglecting the electrical properties of the basal-lateral membrane are considered below.

Initially the results were analyzed in terms of conductances. Amiloride-sensitive Na conductance was measured directly as the difference in transepithelial conductance in the absence and presence of amiloride in the mucosal bath. K conductance through the amiloride-sensitive pathway was estimated using the electrical circuit model; the measured change in PD with amiloride, the measured resistance in the presence of amiloride, and the estimated emf for K across the apical membrane were used to calculate the K conductance. Permeabilities for Na and K were then estimated from conductances using the constant field equation. Subsequently, a slightly different approach was used. Amiloride-sensitive currents were computed from the measured values of PD and resistance, and permeability coefficients calculated from the constant field equation and the assumed concentration differences (*see below*). The two methods gave similar results, but the latter precedure gave somewhat more consistent selectivity ratios, and was therefore used. This method has the additional convenience that amiloride-sensitive current could be measured directly with continuously short-circuited preparations. This was not done in the present study, as the data was collected before the method of analysis was adopted.

In the presence of 20 mM Na in the mucosal solution, the *I–V* relationship of the amiloride-sensitive pathway of the depolarized toad bladder can be described by the constant field equation, with a Na permeability coefficient of about 1×10^{-5} cm/sec and an intracellular Na activity of about 2 mM (Palmer et al., 1980). Under these conditions, with the transepithelial PD and, by inference, the apical PD clamped to zero, the permeability coefficient can be estimated from the short-circuit current using the relationship:

$$I_{Na} = FP_{Na}(Na_o - Na_c) \tag{1}$$

where I_{Na} is the amiloride-sensitive short-circuit current, F the Faraday constant, and Na_o and Na_c the Na activities of the mucosal solution and the cytoplasm, respectively.

To calculate the K permeability of the channel, the analogous relationship

$$I_K = FP_K(K_o - K_c) \tag{2}$$

was used. I_K was calculated under open-circuit conditions, but in the absence of Na, so that the transepithelial PD and, by inference, the apical PD are within a few millvolts of zero. The change in transepithelial PD in response to amiloride is measured directly, and converted to amiloride-sensitive current by dividing by the transepithelial resistance in the presence

of amiloride. For the voltage range and the small voltage changes observed, the resistance of the tissue in the absence of Na is essentially ohmic (Palmer et al. 1980).

Since amiloride sometimes had small effects on the PD even in the absence of a K gradient, which were presumably not due to specific effects on the channel, the apparent I_K in the absence of a K gradient was subtracted from that in the presence of a gradient for each hemibladder:

$$I_K = (\Delta V/R)_{Ch/K} - (\Delta V/R)_{K/K} \quad (3)$$

where ΔV and R are the amiloride-induced PD change and the resistance, respectively, and the subscripts refer to the major cations on either side of the apical membrane.

Similarly, the current carried by NH_4, when that ion replaced K in the mucosal solution, was estimated from:

$$I_{NH_4} = (\Delta V/R)_{NH_4/K} - (\Delta V/R)_{K/K} - I_K. \quad (4)$$

Here I_K had to be subtracted, as an outward K gradient was present. I_K was assumed to be independent of mucosal NH_4.

The current carried by H was calculated from:

$$I_H = (\Delta V/R)_{pH\,5r1} - (\Delta V/R)_{pH\,7r5} \quad (5)$$

where both values of ΔV were measured in the absence of a K gradient. Here the subscripts refer to the pH of the mucosal solution. The same background current, measured in the absence of a transepithelial pH gradient, was subtracted to compute the specific H current.

Results

Figure 1 shows a typical response of the transepithelial PD of a toad bladder to amiloride in the absence of mucosal Na. In the presence of a K gradient (88.5 mM serosal, 3.5 mM), the PD changes abruptly, usually reaching a new steady-state within 30 sec. Subsequent addition of a higher concentration of amiloride did not produce further effects. The direction of the change in PD is that expected for blockade of an outward K current by amiloride. When the K gradient was abolished by addition of K to the mucosal solution, the effect of amiloride was much smaller.

In the absence of Na, amiloride did not measurably change the transepithelial resistance. With Na-free solution on the mucosal side, the ratio of resistance after amiloride to that before amiloride was 1.01 ± 0.02 ($n=46$). This finding is consistent with amiloride's blocking a very small K conductance in the apical membrane. Estimates of K conductance (G_K) of the Na channels were made from the equation:

$$G_K = I_K/E_K$$

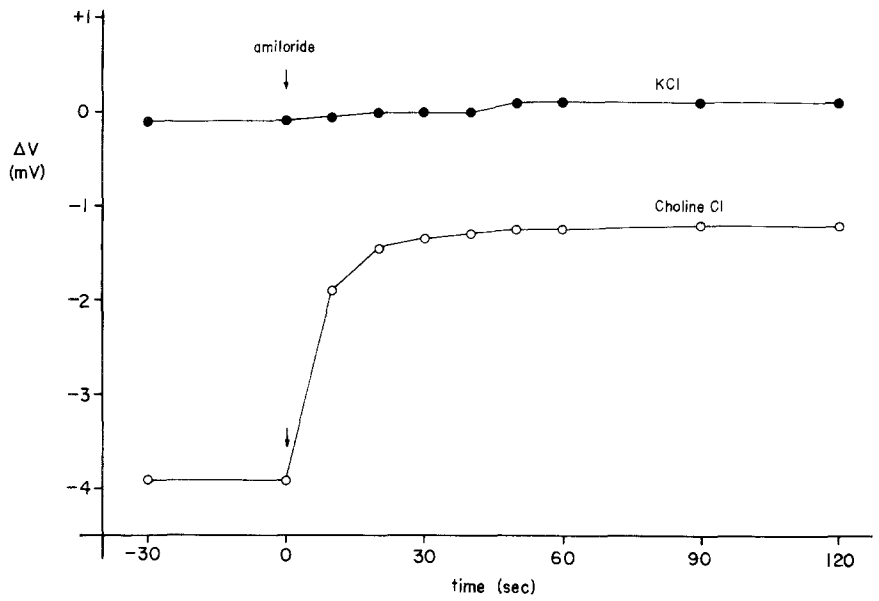

Fig. 1. Time course of the effect of amiloride on open-circuit potential in Na-free solution. A single hemibladder was mounted as a sac and equilibrated with high K sucrose on the serosal side, and Na-free KCl solution on the mucosal side. At time 0, amiloride (50 μM) was added to the mucosal solution and the potential recorded at 10-sec intervals (filled circles). The amiloride was then washed off, and the hemibladder was reequilibrated in a mucosal solution in which KCl was replaced by choline Cl. The response to amiloride was then remeasured (open circles). ΔV is defined as mucosal minus serosal potential. Resistance was 294 ohms (KCl) and 870 ohms (choline Cl). I_K was calculated to be 0.7 μA (KCl) and 3.1 μA (choline Cl)

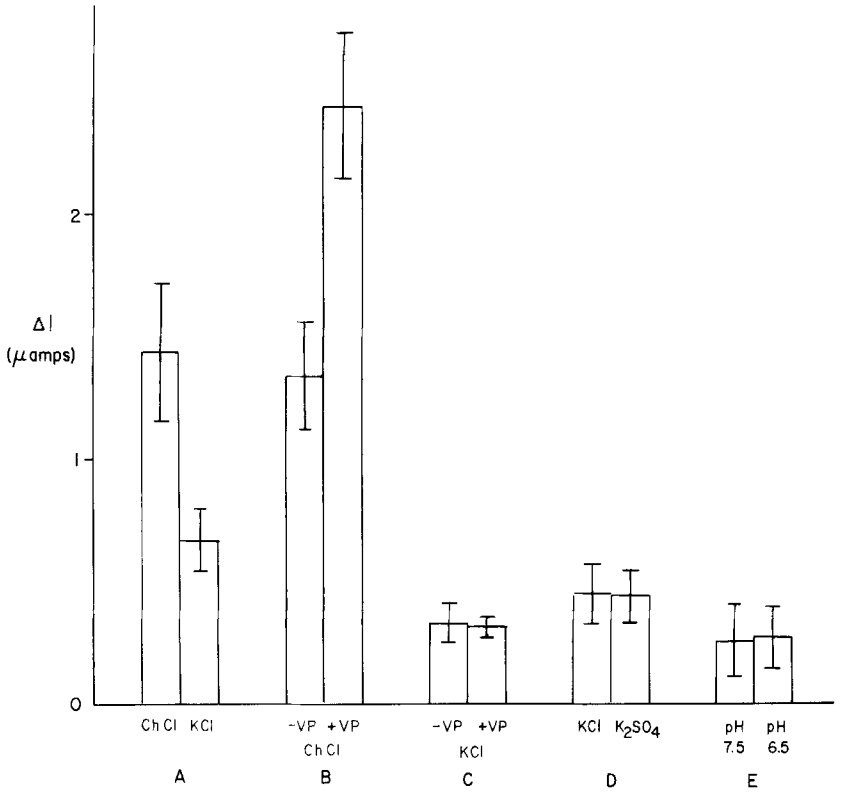

Fig. 2. Changes in transepithelial current after addition of amiloride. Each pair of bars represents measurements made on the same tissues, each hemibladder serving as its own control. The mucosal solutions contained (concentrations in mM): (*A*) choline Cl 85, KCl 85, $n=12$; (*B*) choline Cl 85 with and without ADH, 20 mU/ml on the serosal side, $n=12$; (*C*) KCl 85, with and without ADH, 20 mU/ml on the serosal side, $n=7$; (*D*) KCl 85, K_2SO_4 68, $n=11$; (*E*) KCl 85 at pH 7.5 or 6.5, $n=4$. Means ± SEM are illustrated. A positive ΔI indicates a reduction in outward (serosa-to-mucosa) positive current.
Resistance values were (in ohms): (*A*) ChCl: 750±70; KCl: 660±90; (*B*) +ADH: 560±50; −ADH: 800±80; (*C*) +ADH: 590±90; −ADH: 630±70; (*D*) KCl: 830±160; K_2SO_4: 740±150; (*E*) pH 7.5: 610±140; pH 6.5: 600±90

Table 1. Relative permeabilities of the amiloride-sensitive channel to Na, K and H[a]

	n	$I_i(\mu A)$	Δa_i (mM)	P_{Na}/P_i
Na	12	106 ±20	17	1
K	12	0.83±0.20	65	670 ±170
Na	6[b]	142 ±24	17	1
	6[c]	48 ±14	17	1
H	6[b]	0.70±0.18	0.008	0.15±0.02[d]
H	6[c]	0.48±0.09	0.025	

[a] The amiloride-sensitive short-circuit current (I_i) and the activity gradient across the apical membrane (Δa_i) were estimated for each ion as explained in the text. The permeability ratio P_{Na}/P_i was obtained by averaging individual permeability ratios obtained from each experiment. Data are given as means ±SEM. n is the number of observations.
[b] Mucosal pH 5.1.
[c] Mucosal pH 4.6.
[d] Data from pH 5.1 and pH 4.6 lumped; $n=12$.

where E_K is the driving force for K across the apical membrane, calculated from the Nernst equation. G_K was calculated to be 0.005±0.001 of the shunt conductance. Thus the expected change in resistance with amiloride is less than 1% in Na-free solutions.

Mean values of outward current in the presence of a K gradient, a Cl gradient and a H gradient of 1 pH unit are shown in Fig. 2. Only the K gradient produced a detectable current above the background current, measured in the absence of any transepithelial ion gradient. This is consistent with the idea that K, but not choline, Cl, or SO_4 has a finite permeability through the amiloride blockable channels.

To further examine the possibility that this outward K current was mediated by the Na channels, I_K was measured before and 20 min after addition of ADH to the serosal solution. This hormone increases the permeability of the apical membrane of the toad bladder by increasing the number of conducting Na channels by roughly twofold (Li, Palmer, Edelman & Lindemann, 1979). As illustrated in Fig. 2, ADH doubled the amiloride-sensitive K current in the presence of a K gradient, but had no effect in the absence of a gradient. I_K, therefore, increased in response to the hormone, as expected for a Na channel-mediated permeability.

In another series of experiments, I_K was determined either before or after measurement of I_{Na}, the amiloride-sensitive short-circuit current in the presence of 25 mM Na in the mucosal solution. P_{Na} and P_K were calculated from Eqs. (1) and (2) using ion activity values of 19 for Na_o, 2 for Na_c (Palmer et al., 1980), 2.7 for K_o and 68 for K_c (assumed to be equal to the K activity in the serosal solution, *see below*). Activity coefficients in the bathing solutions were assumed to be 0.77. The results for 12 hemibladders are summarized in Table 1. P_{Na}/P_K was 670±170

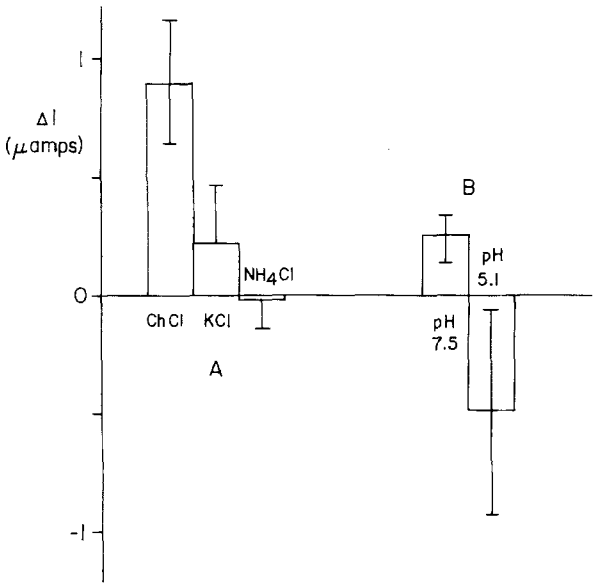

Fig. 3. Changes in transepithelial current after addition of amiloride. Each set of bars represents measurements made on the same tissues, each hemibladder serving as its own control. The mucosal solutions contained (concentrations in mM): (*A*) choline Cl 85, KCl 85, NH_4Cl 85, $n=12$; (*B*) KCl 85 at pH 7.5 and 5.1 ($n=6$). Means ± SEM are represented. A positive ΔI indicates a reduction in outward (serosa-to-mucosa) positive current. Resistance values were (in ohms): (*A*) ChCl: 690±90; KCl: 490±70; NH_4Cl: 430±60. (*B*) pH 7.5: 970±150; pH5.1: 1,050±230

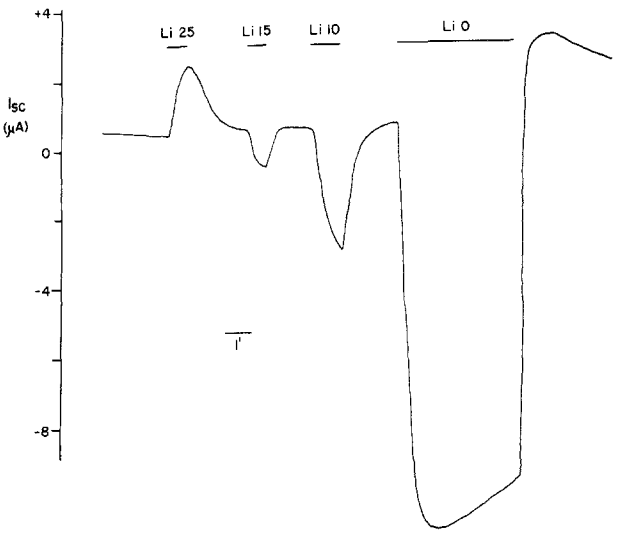

Fig. 4. A hemibladder was mounted as a flat sheet, and preincubated with KCl-sucrose solution containing 5 mM ouabain on the serosal side. The mucosal solution contained 25 mM NaCl. The tissue was continuously short-circuited, and the Figure is a tracing of the original short-circuit current recording. When the current had declined to a low, steady-state value, the mucosal solution was rapidly exchanged with one containing 25 mM LiCl and no Na (Li 25), inducing an inward (mucosa-to-serosa) positive current. The mucosal solution was replaced with 25 mM NaCl, and the current returned to the original level. Replacement with 20 or 15 mM LiCl (KCl added to maintain ionic strength constant) resulted in small outward (serosa-to-mucosa) currents. When the mucosal solution was both Na-free and Li-free (Li 0) a large outward transient was observed

(mean ± SEM) which, as discussed below, is an underestimate of the true permeability ratio.

With NH_4 replacing K in the mucosal solution, the amiloride-sensitive current was small and in the inward direction, relative to the background current. This suggests that NH_4 has a permeability coefficient that is only slightly higher than that of K. P_{NH_4}/P_K, calculated from Eq. (2) and an analogous equation for NH_4, was 1.8±0.6 (6 hemibladders).

When mucosal pH was lowered to 5.1 or 4.6, the amiloride-sensitive current was inward, presumably as a result of a H flux from mucosa to serosa through the Na channels (Fig. 3). In a series of 12 hemibladders, in which the mucosal pH was either 5.1 or 4.6, I_H was calculated from Eq. (5), and P_H estimated from I_H and the H gradient. The intracellular pH was assumed to remain close to 7 (Leaf, Keller & Dempsey, 1964), so that intracellular H activity could be neglected in computing the H gradient. P_{Na}/P_H was found to be 0.15±0.02, indicating that the channel is more permeable to H than to Na (Table 1).

Figure 4 shows a recording of short-circuit current from a hemibladder which had been exposed to 5 mM ouabain on the serosal side for 1 hr, reducing the current to 0.4 μA/3 cm^2 tissue area. When Na was rapidly removed from the mucosal solution, a large outward current transient developed, indicating that Na was moving from the cells into the mucosal solution. When Li was used to replace Na in the mucosal solution, the outward transient was reduced, indicating the simultaneous inward movement of Li and outward movement of Na. When the Li concentration was 25 mM or more, a net inward current transient was observed.

The direction of the current transient changed between 15 and 25 mM Li in every experiment. Since in the ouabain-blocked state the steady-state current was very small, but the apical Na permeability was still significant, the intracellular Na concentration was assumed to be close to 25 mM. Under biionic conditions, with Na in the cell at concentration Na_c, and Li in the mucosal solution at concentration Li_o and zero transapical potential, the Li solution for which no current transient will occur will be determined by $P_{Na} \cdot Na_c = P_{Li} \cdot Li_o$. Thus, P_{Li}/P_{Na} was between 1.0 and 1.6. When the size of the current transients was measured and linear interpolation used to determine Li_o for zero current flow, P_{Li}/P_{Na} was calculated to

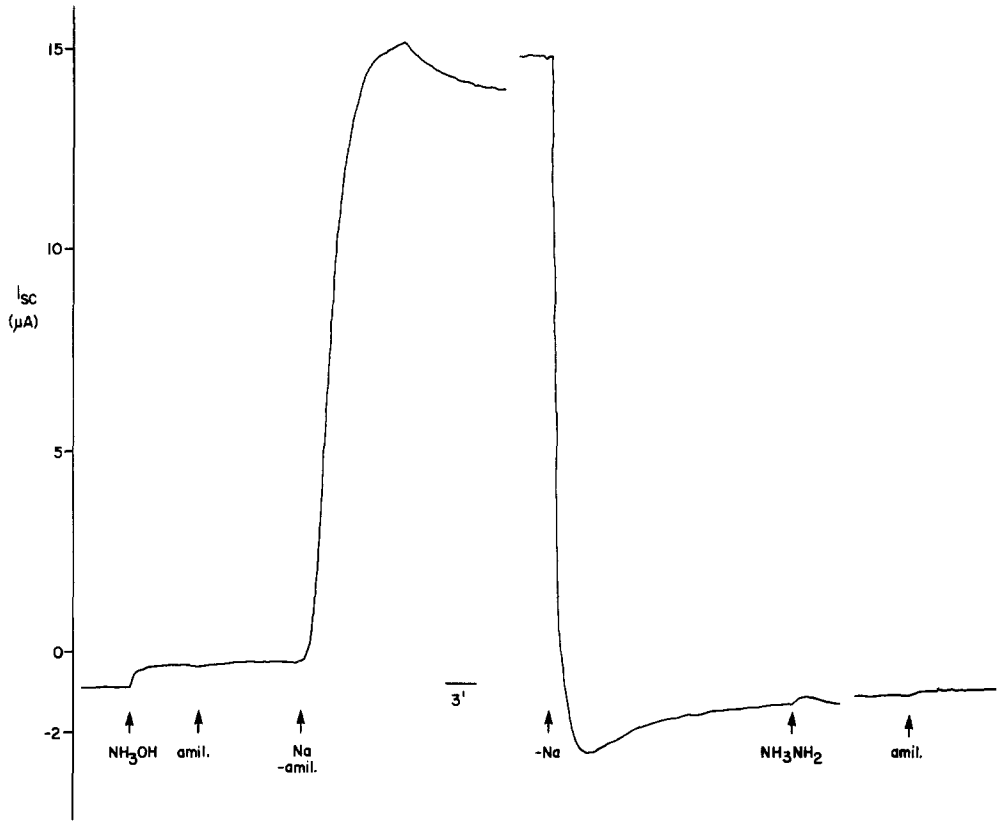

Fig. 5. A hemibladder was mounted as a flat sheet and preincubated with KCl-sucrose solution on the serosal side. The mucosal solution contained 85 mM KCl at pH 6.0. The tissue was continuously short-circuited and the Figure is a tracing of the original short-circuit current recording. Initially the current was small and negative. When a NH_3OH-containing solution was applied (arrow) the current became slightly less negative, indicating a flow of positive charge from mucosa to serosa. This current was not affected by amiloride (10^{-4} M). When the amiloride and NH_3OH were washed off and the mucosal solution contained 25 mM NaCl, a short-circuit current in the usual direction (positive) developed. Subsequently, the Na was removed, and the experiment was repeated using NH_3NH_2 as the test cation. This ion also failed to support a measurable amiloride-sensitive inward current

be 1.3 ± 0.1 (mean $\pm$ SEM for experiments). In the presence of 50 μM amiloride, no current transients were observed, indicating that the Li permeability was mediated by the Na channels.

When bladders equilibrated with KCl on the mucosal side were subjected to step changes in the concentrations of NH_3OH or NH_3NH_2, only minimal changes in short-circuit current were seen (Fig. 5). Furthermore, when amiloride was added to the mucosal solution, under these conditions, no changes in current were observed. No quantitative permeability ratios were computed for these ions. It is clear, however, that neither ion was significantly more permeant than K.

Discussion

As pointed out in the theory section, the quantitative analysis of these results depends on the assumptions that the basal-lateral membrane PD and resistance are negligible, and that K is distributed at electrochemical equilibrium across this membrane. Evidence supporting these assumptions has been previously presented (Palmer et al., 1980). In addition, recent experiments using a–c impedance analysis have shown that depolarization with high K-sucrose reduces the basal-lateral impedance to undetectable levels, so that the epithelium appears electrically as a single membrane in parallel with a shunt resistance (Warncke & Lindemann, 1981). Nevertheless, the basal-lateral membrane may retain a small residual resistance, PD and emf for K, which should be recognized in considering the calculations made here.

1. A resistance in series with the apical membrane would imply that the apical membrane permeability would be underestimated by fitting the transepithelial I–V relationships with the constant field equation (Fuchs, Hviid Larsen & Lindemann, 1977). This effect will be larger on the estimate of P_{Na}, since the relative apical/basal-lateral resistances are expected to be higher in the absence than in the presence of Na. P_{Na}/P_K would, therefore, be underestimated.

2. In the presence of a cell-negative PD the permeability would be overestimated from the constant field equation: a 10-mV cell potential would lead to a

21% overestimate of both P_{Na} and P_K[1]. Since the cell potential at short-circuit is likely to be more negative, if anything, in the absence of Na transport (Higgins, Gebler & Frömter, 1977; Narvarte & Finn, 1980), the effect of the potential would also be to underestimate P_{Na}/P_K.

3. If K is not at electrochemical equilibrium across the basal-lateral membrane, but is accumulated within the cell by an active transport process (DeLong & Civan, 1978), this mechanism would serve as an additional driving force for outward K movement through the channels. Thus P_K would be overestimated by the above treatment, which assumed only electrodiffusional driving forces, again leading to an underestimate of P_{Na}/P_K.

In addition to these considerations, there are two other obvious sources of error in the calculation of the permeability ratio:

4. Amiloride was assumed to affect only the transcellular transport pathway. Balaban, Mandel and Benos (1979) reported, however, that the drug can also inhibit ion movement to some extent through the paracellular pathway; at least in low resistance epithelia. This would imply that measurements of I_K could reflect, in part, amiloride-sensitive movement through the paracellular shunt. P_K of the apical membrane would, therefore, be overestimated.

5. Ion permeabilities in this system are concentration dependent. Fuchs et al. (1977) have shown that in the frog skin apical Na permeability decreases when mucosal Na is increased between 0 and 20 mM. In the frog skin, it has recently been shown that mucosal K can also apparently reduce Na permeability, although not as effectively as Na itself (Benos, Mandel & Simon, 1980; Biber & Mullen, 1980). Using apparent inhibition constants of 10 mM for Na and 39 mM for K (Benos et al., 1980), the expected reduction in P_{Na} when the Na-containing solution used here (25 mM NaCl = 60 mM KCl) is substituted for 85 mM KCl is calculated to be 35%. Taking this figure into account, P_{Na}/P_K would be 1030, if P_{Na} and P_K were measured under identical conditions (85 mM KCl; Na concentration approaching zero). Although information on the variation of P_{Na} with Na and K concentrations and with voltage is available, nothing is known about the possible concentration and voltage dependence of P_K in this system. Thus even if the value for the permeability ratio were precise, it would be valid for only a single set of experimental conditions, and is not necessarily expected to be an invariant property of the channel.

Because of these uncertainties, and the indirect method of measurement, the value of the permeability ratio should be considered as an order of magnitude estimate.

Clearly a more direct technique, such as measurement of amiloride-sensitive conductance or tracer fluxes would be preferable, but, in the absence of Na, no effect of amiloride on transepithelial conductance could be measured. In retrospect, this is not surprising, since the calculated K conductance through the Na channels was always less than 1% of the total tissue conductance.

As discussed above, the value of P_{Na}/P_K may be an underestimate. This is nevertheless a very high selectivity ratio, and is considerably larger than the value of 20 found in frog skin by Lindley and Hoshiko (1964). As mentioned in the Introduction, the value reported by these authors reflects the properties of the entire apical membrane, including the paracellular shunt pathway, while the selectivity found in the present study represents that of the amiloride-sensitive channels themselves.

A high selectivity for Na over K was also reported in the frog skin by Benos et al., (1980), who found no detectable amiloride-sensitive K influx across the apical membrane. From the limits of detection of their method, they concluded that K permeability was less than 1/100 that of Na. In contrast, Lewis and Wills (1980) found Na/K selectivity ratios of 2:1 and 10:1 for the amiloride-sensitive transporter in the rabbit urinary bladder, a mammalian tight epithelium. Here the selectivity depended on the Na content of the animals' diet.

The experiments performed at low mucosal pH indicate that H can readily traverse the Na channel, suggesting that this transport pathway may be of physiological significance in the process of urinary acidification. However, the small absolute H ion gradients developed by Na-transporting epithelia make this unlikely. In the turtle bladder, mucosal pH can reach values as low as 5.0 as a result of acid secretion (Steinmetz, 1974). The maximal concentration gradient across the apical membrane of this tissue is, therefore, about 10^{-5} M, four orders of magnitude lower than that of Na, which in the toad bladder with Ringer's solution on both sides is about 10^{-1} M (Rick, Dörge, Macknight, Leaf & Thurau, 1978). The maximal flux of H into the cell through the Na channels is thus about 1/1000 that of Na. The rate of H secretion by the turtle bladder under these conditions, however, is of the same order of magnitude as that of Na absorption (Al-Awqati, Norby, Mueller & Steinmetz, 1976). Thus, the backflux of secreted H into the cell through the Na channels should not contribute significantly to limiting the ability of this tissue to acidify the urine.

The finding that the toad bladder Na channel is permeable to Li is in accord with other studies indicat-

[1] This calculation is based on varying the intracellular PD at constant intracellular electrochemical ion activities, and estimating the permeability from the slope conductance at $V=0$

ing that Li can substitute for Na in amiloride-sensitive transport systems in the frog skin, turtle colon and toad bladder (Herrera, Egea & Herrera, 1971; Sarracino & Dawson, 1979; Benos et al., 1980; Macknight & Hughes, 1981).

Thus the Na channel is highly permeable to H and Li ions, which in the unhydrated state are smaller than Na, and virtually impermeable to K, NH_4, NH_3OH and NH_3NH_2 ions, which are larger than the unhydrated Na ion. Since NH_3OH and NH_3NH_2 are comparable in size to a monohydrated Na ion (Hille, 1971), it is conceivable that Na ions must be completely stripped of H_2O in order to traverse the channel. This situation contrasts with that in the frog node of *Ranvier*, where NH_3OH and NH_3NH_2 were found to be high permeant to the excitable Na channel (Hille, 1971). In addition, the Na/K selectivity in this preparation was about 10:1 (Hille, 1972), much lower than in the toad bladder.

The selectivity sequence for permeant ions: H > Li > Na is different from the sequence of aqueous ion mobilities: H > Na > Li. This indicates that the channel environment is not that of a bulk aqueous solution. Rather, the sequence suggests the existence within the channel of a negative fixed-charge site with a high field strength. Such a site would preferentially bind smaller ions (Eisenman, 1962).

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