# Conductance and Gating of Epithelial Na Channels from Rat Cortical Collecting Tubule

## *Effects of Luminal Na and Li*

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ABSTRACT The behavior of individual Na channels in the apical membrane of the rat cortical collecting tubule (CCT) was studied at different concentrations of the permeant ions Na and Li. Tubules were opened to expose their luminal surfaces and bathed in K-gluconate medium to minimize tubule-to-tubule variation in cell membrane potential and intracellular Na concentration. The patch-clamp technique was used to resolve currents through individual channels. The patch-clamp pipette was filled with solutions containing variable concentrations of either NaCl or LiCl. In one series of experiments, the concentrations were changed without substitutions. In another series, the ionic strength and Cl concentration were maintained constant by partial substitution of Li with *N*-methyl-D-glucamine (NMDG). In cell-attached patches, both the single-channel conductance ($g$) and the single-channel current ($i$) saturated as functions of the Na or Li activity in the pipette. Without NMDG, the saturation of $i$ was well described by Michaelis-Menten kinetics with an apparent $K_m$ of ~20 mM activity for Na and ~50 mM activity for Li. $K_m$ was independent of voltage for both ions. With substitution for Li by NMDG, the apparent $K_m$ value for Li transport through the channels increased. The values of the probability of a channel's being open ($P_o$) varied from patch to patch, but no effect of pipette ion activity on $P_o$ could be demonstrated. A weak dependence of $P_o$ on membrane voltage was observed, with hyperpolarization increasing $P_o$ by an average of 2.3%/mV.

## INTRODUCTION

Na channels in tight epithelia are responsible for mediating the influx of Na from mucosal fluids into the cell across the apical membrane. The activity of these channels is regulated, and changes in channel activity are important in the control of transepithelial transport (Palmer, 1986; Garty and Benos, 1988; Eaton and Hamilton, 1988).

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One of the major factors determining the flux of Na through the channels is the mucosal or luminal Na concentration. In most tight epithelia, including the frog skin, toad and *Necturus* urinary bladder, rabbit colon, cortical collecting tubule (CCT), and urinary bladder, and A6 cells in culture, active Na transport saturates as the mucosal Na concentration is increased (Kirschner, 1955; Frazier et al., 1962; Lewis and Diamond, 1976; Turnheim et al., 1978; Benos et al., 1980; Stokes, 1981; Thomas et al., 1983; Sariban-Sohraby et al., 1983). Since this saturation can occur without a large change in the driving force for Na entry, it implies that the permeability of the apical membrane is reduced at high mucosal Na concentrations (Fuchs et al., 1977; Li et al., 1982; Thomas et al., 1983).

The mechanism underlying this phenomenon is controversial. Olans et al. (1984) found that the conductance of single Na channels reconstituted from A6 cells into lipid bilayers is a hyperbolic function of the medium Na concentration, with 18–44 mM Na required for half-maximal conductance. This suggests that the saturation of transepithelial transport reflects the saturation of currents through individual Na channels. However, using noise analysis, Van Driessche and Lindemann (1979) found little saturation of single-channel currents in frog skin up to 60 mM Na activity. They emphasized the role of a decreased number of conducting channels in the apical membrane in the saturation of transport by that tissue.

We have recently identified Na channels in the apical membrane at the rat cortical collecting tubule using the patch-clamp technique (Palmer and Frindt, 1986). In this article, we report the behavior of these channels in the presence of varying concentrations of the permeant ions Na and Li.

## MATERIALS AND METHODS

Sprague-Dawley rats of either sex (100–150 g), raised free of viral infections (Charles River Laboratories, Kingston, NY), were maintained on a low-Na diet (ICN, Cleveland, OH) for 1-3 wk before use. Animals were killed and single CCTs were prepared for patch-clamp experiments as described previously (Palmer and Frindt, 1986).

In order to better control the electrical and chemical driving forces for Na across the luminal membrane, we used a high-K solution to bathe the tubules. This solution contained (millimolar): 140 K-gluconate, 2 $CaCl_2$, 1 $MgCl_2$, 2 glucose, and 10 HEPES, buffered to pH 7.4 with KOH. Pipette solutions contained 14–280 mM NaCl or LiCl and 5 mM HEPES, buffered to pH 7.2 with NaOH. In some experiments, *N*-methyl-D-glucamine (NMDG) HCl was added to the LiCl solutions to match the osmolarity, ionic strength, and Cl concentration of the 140 mM LiCl solution.

Cell-attached and inside-out patches were obtained and currents across the patches were recorded at room temperature as described previously (Palmer and Frindt, 1986, 1987). The sizes of current transitions were measured from digitized records with the aid of a digital oscilloscope (Nicolet Corp., Madison, WI) equipped with an electronic cursor. 10–20 transitions were generally measured for each pipette voltage in each patch and the current sizes were averaged. To measure the mean number of open channels ($NP_o$), current records of 1–10 min duration from patches containing 1–10 channels were analyzed as described previously (Palmer and Frindt, 1987). Briefly, the record was broken into time intervals between transitions delineating the opening or closing of a channel. The probability of finding $i$ channels open ($P_i$) was computed from all the intervals in the record. $NP_o$ was then calculated as $\Sigma_i i P_i$ for all observed values of $i$.

To obtain estimates of $P_o$, the probability of a channel's being open, the number of conducting channels ($N$) in the patch was first assumed to be equal to the number of observed current levels minus one. A first guess for $P_o$ was then obtained by dividing $NP_o$ by $N$, and the values for each $P_i$ predicted by the binomial distribution were compared with the observed values. The value of $N$ was then adjusted to optimize the fit to the binomial distribution by least squares. The adjustment of $N$ involved the assumption that the state with all the channels open and/or all the channels closed was not observed. In 80% of the records studied in this way, the best fit was obtained using the original guess for $N$. This implies that in most experiments, the states in which all channels were open and all channels were closed were both observed.

To fit current vs. ion activity ($i$-$a$) relationships with the Michaelis-Menten kinetic scheme, the data were linearized using Eadie-Hofstee plots of $i$ vs. $i/a$. The best fit to the data assuming the relationship

$$i = i_{max}/(1 + K_m/a) \quad (1)$$

was then obtained by linear regression. An identical procedure was used to fit conductance-activity ($g$-$a$) relationships with Michaelis-Menten kinetics. The Na and Li concentrations in the pipette-filling solutions were measured using a flame photometer (943, Instrumentation Laboratories, Lexington, MA) and the ion activities calculated from standard tables in Robinson and Stokes (1959).

## RESULTS

With high K and no Na in the bathing medium, and NaCl or LiCl in the pipette, Na channel activity in cell-attached patches could be readily recognized by the inward currents at zero pipette potential ($V_p$). These currents increased in magnitude when $V_p$ was made positive, and decreased, but did not reverse, when $V_p$ was made negative (Fig. 1). The other channel whose activity was frequently observed under these conditions was one with an 8-pS unitary conductance that carried only outward current and appeared to be selective for K. Other channel types seen included high-conductance, Ca-activated K channels (Frindt and Palmer, 1987) and nonselective cation channels with an intermediate conductance (23 pS). Both of these channel types were observed relatively rarely and were easily distinguishable from the Na channels.

Na channel activities in cell-attached patches with high (280 mM) and low (14 mM) concentrations of Li in the pipette are shown in Fig. 1. The currents are similar in their kinetic pattern to those observed previously in which Na is the conducted ion (Palmer and Frindt, 1986). The $i$-$V$ relationships for conduction of Li and Na are shown in Fig. 2. Because these data were obtained from cell-attached patches, the abscissa is, strictly speaking, the voltage difference between the interior of the pipette and the bath, and the true potential across the patch will include the resting potential of the cell. We assume, however, that with high-K solution in the bath, the cell membrane is to a large extent depolarized, and the membrane potential contributes relatively little to the trans-patch voltage. Support for this assumption was obtained by comparing currents in cell-attached patches with those measured after excising the patches into the Na-free, high-K medium. As shown in Fig. 3, the $i$-$V$ relationships in the cell-attached and excised, inside-out configurations are nearly identical, which implies that the cell potential is small, probably <5 mV. Alterna-

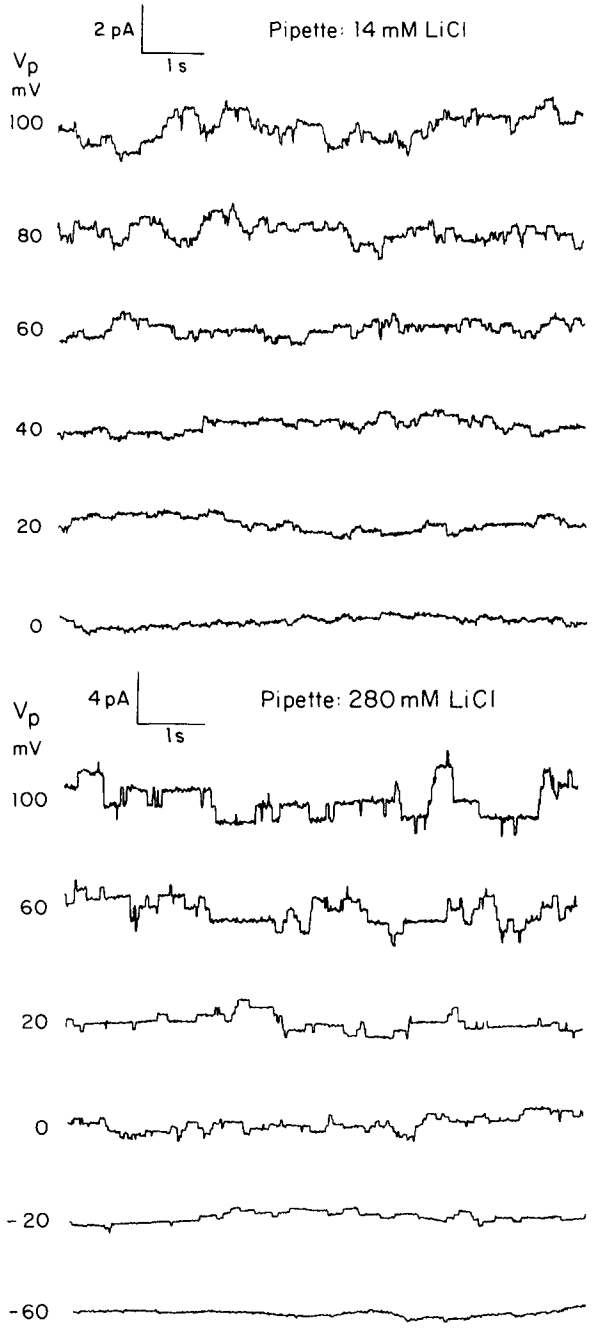


FIGURE 1. Na channel currents in the presence of high and low Li concentrations in the pipette. Recordings are from two cell-attached patches, one with 280 mM LiCl, and the other with hypotonic 14 mM LiCl. All other conditions were identical. With high Li, single-channel currents could be resolved with pipette voltages ($V_p$) from −60 to +100 mV. With low Li, currents could be resolved between 0 and +100 mV. The sampling rate was 500 Hz. The low-pass filter was at 50 Hz.

tively, it is possible that the additional driving force owing to a negative cell potential in the cell-attached patch is offset by a finite intracellular Na concentration. We feel, however, that the intracellular Na concentration was probably quite low, since there was no Na in the bath. In most cases, the Na channel activity in excised, inside-out

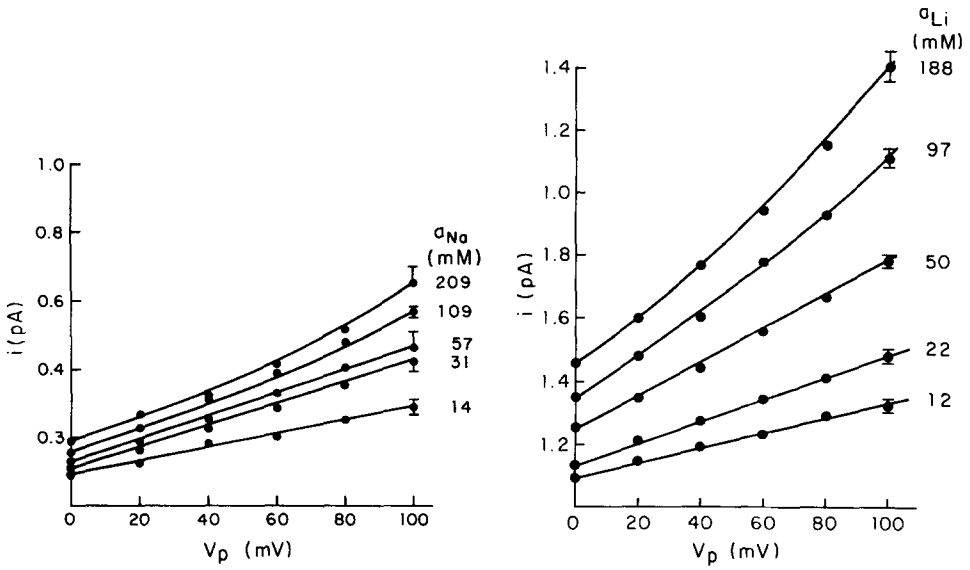


FIGURE 2. Current-voltage relationships for Na channels as a function of the activities of Na and Li in the pipette. Values of $i$ were determined from records like those shown in Fig. 1 by averaging the amplitudes of current transitions from individual patches and then averaging these mean values from 5–10 different patches. The solid lines are drawn by eye through the points. Standard error bars are shown only at $V_p$ = 100 mV for clarity.

patches diminished too quickly to permit a satisfactory measurement of the $i$-$V$ relationship or of the $P_o$ of the channels. Therefore, most of the results we present and analyze below were obtained from cell-attached patches.

The $i$-$V$ relationships are linear over the voltage range 0–100 mV for low concentrations of either Na or Li (Fig. 2). At high concentrations, they become slightly concave upward. Given the findings in a number of epithelia that the macroscopic amiloride-sensitive $i$-$V$ relationship can be described by the constant-field equation,

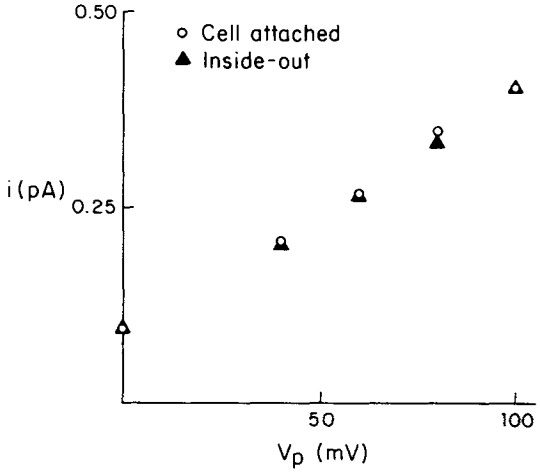


FIGURE 3. Current-voltage relationships for Na channels in a single patch in two configurations. The $i$-$V$ relationship was first obtained from a cell-attached patch with 28 mM NaCl in the pipette. The patch was then excised, presumably in the inside-out configuration, from the cell into K-gluconate solution and the $i$-$V$ relationship was measured again. The currents are plotted as the means of 15–25 transitions for each voltage. Standard errors were <5% of the means.

it was of interest to investigate how closely the single-channel $i$-$V$ curve followed this theoretical formulation. Fig. 4 shows the $i$-$V$ relationship in a cell-attached patch that was unusually stable over a wide range of $V_p$ with a high Li concentration in the pipette. As in Fig. 2 for the case of high salt, the curve was concave upward, as predicted from the constant-field equation. We were not able to fit the data exactly over the entire voltage range with this equation, although restricted ranges could be described well. The fits were made assuming that the intracellular Na concentration was zero. Introducing a finite intracellular Na concentration made the fits worse. At

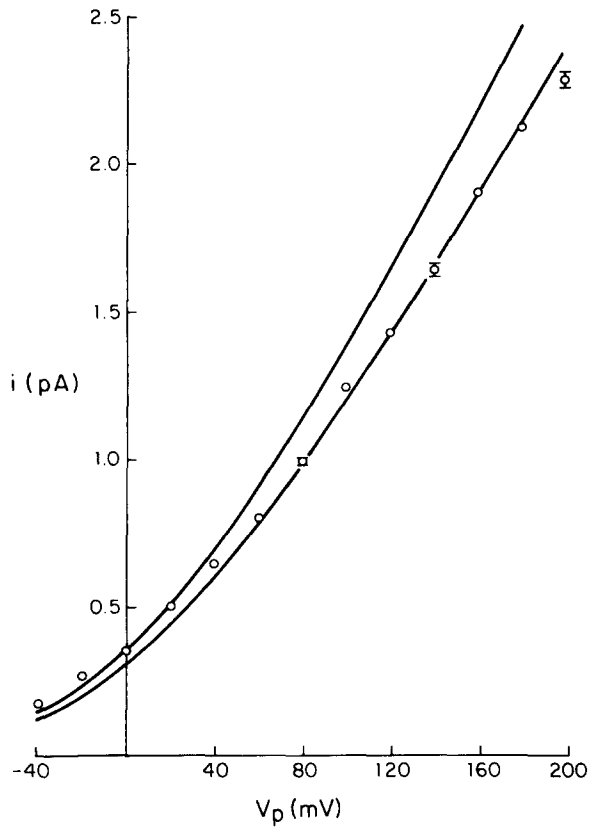


FIGURE 4. Comparison of the current-voltage relationship of Na channels with the constant-field equation. The $i$-$V$ relationship was measured in a single cell-attached patch with 280 mM LiCl in the pipette. The patch was unusually stable, enabling a wider-than-normal voltage range to be studied. Each point represents the mean of at least 20 current transitions. Standard errors were <5% of the means. The solid lines represent the prediction of the constant-field equation. These were obtained by assuming that the intracellular Na concentration was zero and that the channels were perfectly selective for Na. In the upper curve, the permeability coefficient ($P_{Na}$) was adjusted so that the line would pass through the data point at $V_p = 0$. In the lower curve, the value of $P_{Na}$ was chosen so that the line would pass through the data point at $V_p = 160$ mV.

lower salt concentrations, the measured $i$-$V$ curves were more linear and were less well described by the constant-field equation.

The Na or Li concentration dependence of conduction was assessed in two ways. First, $g$ was measured in each individual experiment, using the range 0–100 mV (0–60 mV at 140–280 mM). The average values of $g$ were then plotted as $g$ vs. $g/a$, as shown in Fig. 5. For both Na and Li, $g$ could be fairly well described by Eq. 1 with $g_{max} = 5.0$ pS for Na and 11.1 pS for Li, and $K_m = 25$ mM activity for Na and 47 mM activity for Li. These data were obtained by varying the salt concentration in the pipette solution. Thus, in addition to the concentration of permeant cations, the

ionic strength and Cl concentration of the pipette solution were also varied at the same time. To test for possible effects of these ancillary changes, another series of conductance measurements was made by varying the Li concentration at constant ionic strength. Li was partially replaced with NMDG-Cl at a constant salt concentration of 140 mM. With 28 mM LiCl in the pipette, the addition of 112 mM NMDG-Cl reduced $g$ by 27%, from 3.50 ± 0.08 to 2.75 ± 0.06 pS, with little change in the calculated Li ion activity. This led to increases in the calculated values of $g_{max}$ from 11.1 to 15.0 pS and of $K_m$ from 47 to 92 mM (Fig. 5).

In a second assessment of the concentration dependence of conduction, currents for a given Na or Li activity were averaged at each voltage to create a set of plots of $i$ vs. activity plots, as shown in Fig. 6. While $i_{max}$ values increased monotonically with increasing $V_p$, values of $K_m$ were, to a first approximation, voltage independent. The

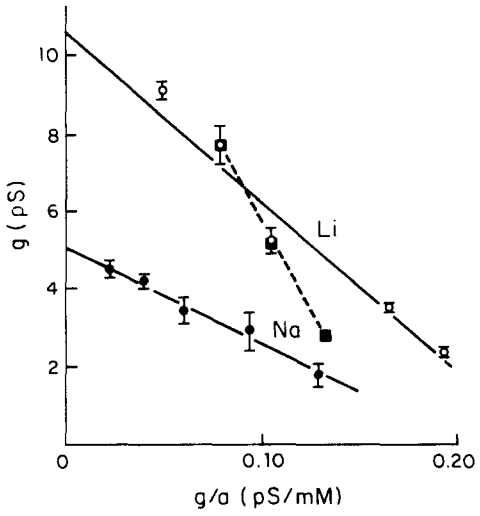


FIGURE 5. Conductance-activity relationships for Na and Li transport through the Na channels. Values of slope conductance were obtained for 4–10 individual patches at each ion activity. The data are represented in an Eadie-Hofstee plot of $g$ vs. $g/a$ for Na (filled circles), Li (open circles), and Li with NMDG substitution (squares). The points were fitted with straight lines by linear regression, $g_{max}$ was estimated from the $g$-intercept, and $K_m$ was estimated from the negative of the slope. Values of $g_{max}$ were 5.0 pS (Na), 10.6 pS (Li), and 15.0 pS (Li with NMDG substitution). Values of $K_m$ were 25 mM (Na), 47 mM (Li), and 92 mM (Li with NMDG substitution), all expressed in activity.

apparent $K_m$ values for saturation of $i$ were similar to those obtained for saturation of $g$. In both cases, $K_m$ values were higher for Li transport than for Na transport.

These data indicate that Li is conducted more rapidly through the channels than Na, particularly at high salt concentrations. To assess the permeability ratio between Na and Li, currents through the channels were studied in excised, inside-out patches under biionic conditions with 140 mM LiCl in the pipette and 140 mM NaCl in the bath. Results of a representative experiment are shown in Fig. 7. The $i$-$V$ relationship under these conditions showed an interpolated reversal potential of ~12 mV, which indicates a permeability ratio $P_{Na}/P_{Li}$ of ~0.6. This is a rough estimate owing to the uncertainty involved in the interpolation. The slope conductance was higher for Li transport ($V_p > 0$) than for Na transport ($V_p < 0$).

In light of the findings of Van Driessche and Lindemann (1979) that the number of conducting channels in frog skin measured with noise analysis decreases with increasing mucosal Na, we estimated values of $P_o$ for the Na channel in the rat CCT

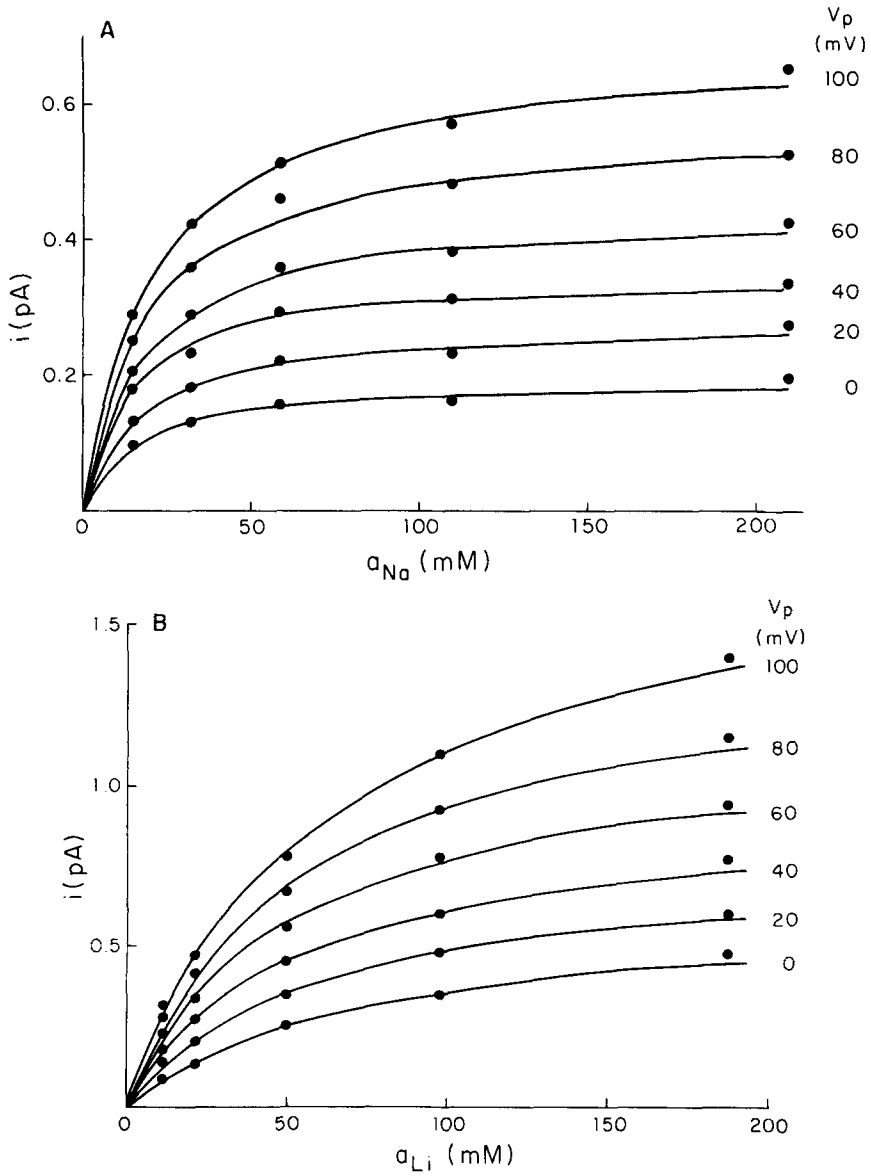


FIGURE 6.

at various concentrations of Na and Li in the pipette. These measurements were made from records where $V_p$ was between +60 and +80 mV. The values of $P_o$ were estimated from multichannel patches using the binomial distribution to estimate the number of channels in the membrane, as outlined in the Materials and Methods. In most cases, the fit of the distribution of open-state probabilities was well described by the binomial distribution, as shown in the top panel of Fig. 8, which confirms our earlier results (Palmer and Frindt, 1986). In a few cases, however, the channels did

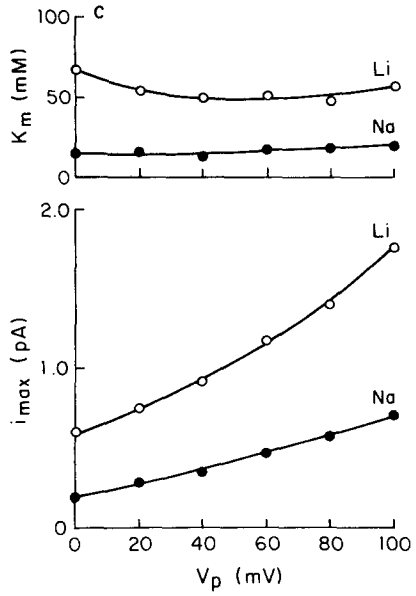


FIGURE 6. Current–ion activity relationships for transport of Na and Li through Na channels. Data from Fig. 2 are replotted to show single-channel current as a function of Na (*A*) or Li (*B*) activity for different voltages. The lines in *A* and *B* (opposite) represent best fits of the data to Eq. 1 using Eadie-Hofstee plots as in Fig. 5. From these plots, values of $i_{max}$ and $K_m$ were calculated and are shown as a function of $V_p$ in *C*.

not conform to the expectations of the binomial distribution, as illustrated in the bottom panel of Fig. 8. In these cases, the probability of finding exactly one channel open was much higher than that of finding either zero channels open or two channels open. These data could be explained if two channels were present, one with a very high $P_o$ and the other with a very low $P_o$. This would violate the assumption made in applying the binomial distribution that all channels in a given patch are identical and independent. In these cases, which amounted to <10% of the patches

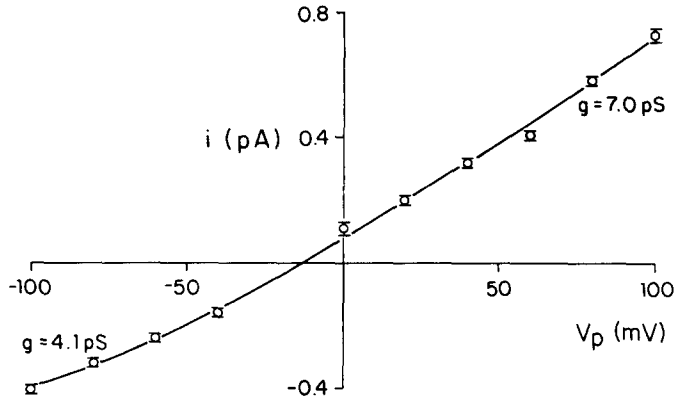


FIGURE 7. Current-voltage relationship for Na channels in an excised, inside-out patch with 140 mM LiCl in the pipette and 140 mM NaCl in the bath. Currents are the mean values of the amplitudes of 5–20 transitions at each voltage. Error bars represent standard deviations. The line was drawn by eye through the points. The slope conductance was 4.1 pS at negative pipette potentials (driving Na into the pipette) and 7.0 pS at positive pipette voltages (driving Li out of the pipette).

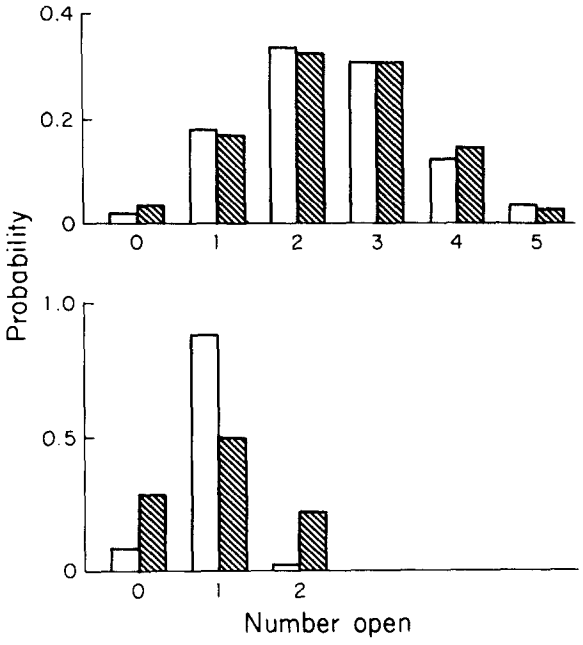


FIGURE 8. Distribution of the number of open Na channels in multichannel patches. The probability of finding zero, one, two, etc., channels open is shown for two cell-attached patches. The top panel illustrates a patch in which there was evidence for five channels with identical conductances. The measured probability of finding $n$ channels open for $n = 0$–5 is shown by the open bars. The shaded bars represent the predicted probabilities for finding $n$ channels open from the binomial distribution given five independent channels, each with an open probability equal to 0.49, which was the mean for all five channels. The bottom panel illustrates a patch that apparently contained two channels, one with high open probability and one with a low open probability. The open bars correspond to the observed data and the filled bars give values predicted from the binomial distribution assuming that both channels had an open probability of 0.47, which was the mean for the two channels.

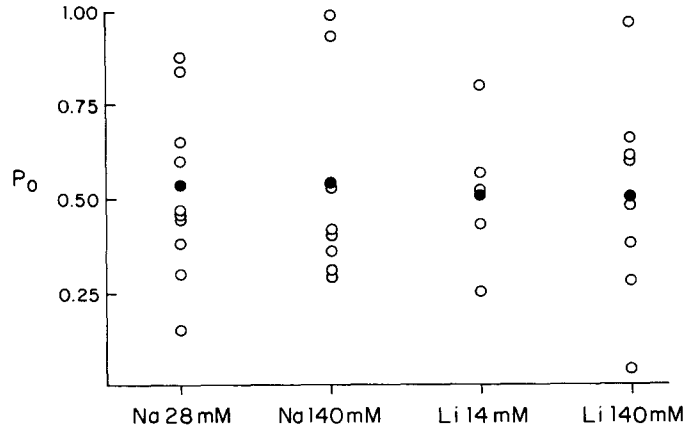


FIGURE 9. $P_o$ values for Na channels in cell-attached patches with high and low concentrations of Na and Li in the pipette solutions. The open circles represent values from individual patches obtained at pipette potentials of +60 to +80 mV. The filled circles represent the mean values for each pipette solution.


FIGURE 10. Na channel current records from a patch showing a significant voltage dependence of $P_o$. The pipette contained 140 mM LiCl. The pipette voltage was changed from +60 mV to 0 and then to +100 mV. Values of $P_o$ were 0.015 at $V_p$ = 0, 0.038 at $V_p$ = +60 mV, and 0.157 at $V_p$ = +100 mV. The sampling rate was 500 Hz. The low-pass filter was 50 Hz.

studied, the number of channels was estimated from the number of current levels observed minus one.

As seen in Fig. 9, there was considerable variation in the calculated $P_o$ values under each ionic condition. For the entire set of experiments, $P_o$ ranged from 0.05 to 0.98. There was no effect of the nature or concentration of the permeant cation on the average value of $P_o$ that was discernible from these measurements.

In some experiments, $P_o$ was calculated at two or three voltages. In most of these cases, there was a modest activation of the channels as $V_p$ was made more positive, i.e., as the membrane was hyperpolarized. An example of a patch that showed a relatively vigorous activation in response to voltage is shown in Fig. 10. This effect of voltage appeared to be rapid, although the slow kinetics and the usually small activation prevented an accurate measurement of the time course of the effect. The activation was also readily reversible. However, not all patches showed the degree of

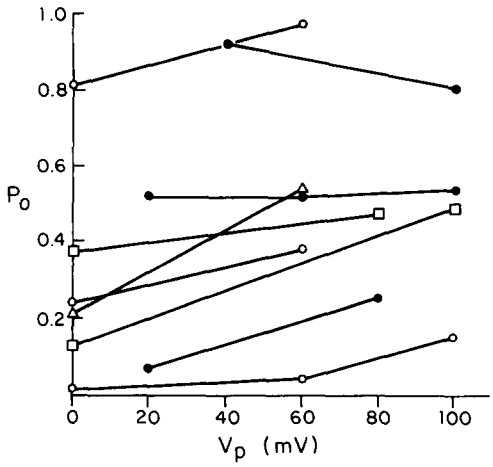


FIGURE 11. Effect of pipette voltage of $P_o$ on Na channels in nine cell-attached patches. $P_o$ was computed at two or three different voltages in each patch. Values from the same patch are connected by straight lines.

voltage dependence seen in Fig. 10. A composite record showing the response to voltage changes in nine patches is shown in Fig. 11. Some patches had almost no response, while in others the response was substantial. The average behavior of the channels was assessed as the percent change in $P_o$ per millivolt change in $V_p$, or

$$fP_o = (P'_o - P''_o)/[P''_o(V'_p - V''_p)], \quad (2)$$

where the primes refer to the different voltages tested. Values of $fP_o$ averaged 2.3 ± 0.8%/mV for the experiments shown in Fig. 11, significantly different from zero ($P < 0.01$).

## DISCUSSION

### *Shape of the Single-Channel i-V Relationship for Na Channels*

The macroscopic *i-V* relationship of the amiloride-sensitive pathway for Na of a number of tight epithelia was found to obey the constant-field equation, assuming a perfect selectivity of the channels for Na (Fuchs et al., 1977; Palmer et al., 1980; Thomas et al., 1983; DeLong and Civan, 1984; Schoen and Erlij, 1985). In this study, the single-channel *i-V* relationship was found to behave qualitatively as predicted by the constant-field equation with high concentrations of permeant ions in the pipette, although systematic deviations from the theoretical curve were evident (Fig. 4). At lower concentrations of permeant ions, the *i-V* relationship was linear over the range of voltages studied. Similar results were obtained in a microelectrode study of the apical membrane of *Necturus* urinary bladder by Thomas et al. (1983). These authors also reported that the *i-V* relationship of the amiloride-sensitive pathway was well described by the constant-field equation at high mucosal Na concentrations, but had less curvature at large electrical driving forces than predicted when the mucosal Na concentration was low. We did not investigate deviations from constant-field equation in more detail, since this equation is not expected a priori to apply to channels in which there are significant interactions between the channel and the conducted ions, as we propose below.

### *Selectivity between Na and Li*

The Na channels had a mild selectivity for Li over Na on the basis of both relative conductance and reversal potential under biionic conditions. Previous results also indicated a selectivity for Li over Na in amphibian epithelia. Benos et al. (1980) found that Li carried a slightly higher short-circuit current than did Na in the frog skin. Sarracino and Dawson (1979) observed an increase in the amiloride-sensitive short-circuit current in the turtle colon when Li replaced Na in the mucosal solution. Kirk and Dawson (1985) showed that the passive retrograde current through the amiloride-sensitive pathway in the same tissue in the presence of a serosal-to-mucosal ion gradient was greater for an Li gradient than for an Na gradient. Palmer (1982) reported a $P_{Li}/P_{Na}$ ratio of ~1.2 under approximately biionic conditions across the apical membrane of the toad urinary bladder.

The finding of different apparent $K_m$ values for Na and Li transport implies that the two ions interact differently with the channel. One simple interpretation is to assume the presence of a presumably negatively charged site within the lumen of the

pore, which binds Na more tightly than Li. A simple kinetic scheme for such a model can be written as:

$$B^{o} + Na_{o} \underset{k_{-1}}{\overset{k_{1}}{\rightleftharpoons}} NaB \xrightarrow{k_{2}} Na_{c} + B^{o},$$

where $Na_o$ represents the mucosal Na concentration, $B^o$ is the density of unoccupied channels, $NaB$ is the density of occupied channels, and $Na_c$ is the intracellular Na concentration. $Na_c$ is presumed to be sufficiently low that backflux into the channel is negligible. Translocation through the channel is then proportional to the occupancy of the channel:

$$i = k_2 NaB,$$

where $i$ is the single-channel current. The occupancy in turn is given by

$$NaB = B^{T}/(1 + K_m/Na_o),$$

where $B^T$ is the total density of channels, or $B^T = B^o + NaB$, and the apparent Michaelis-Menten constant is given by

$$K_m = (k_{-1} + k_2)/k_1.$$

Since the maximal single-channel conductance is higher for Li than for Na, the rate of exit from the channel, $k_2$, is apparently larger for Li. An increase in $k_2$ will also tend to increase $K_m$, as was observed. This could be the result of a deeper energy well within the channel for Na, in which case $k_{-1}$ would also be higher for Li than for Na. Alternatively, it is possible that the energy barrier for Li exit from the channel into the cell is selectively reduced, in which case the other rate constants could be similar for Na and Li. We do not have data to distinguish these possibilities.

### *Saturation of Single-Channel Currents*

Saturation of the overall Na transport rate with increasing mucosal Na concentration has been observed in a number of tight epithelia (Kirschner, 1955; Frazier et al., 1962; Turnheim et al., 1978; Benos et al., 1980; Thomas et al., 1983; Sariban-Sohraby et al., 1983). In most of these instances, the epithelia were short-circuited, so that the saturation was observed without a change in the electrical driving force for Na across the tissues. Saturation of net Na fluxes with increasing luminal Na in the range of 0–140 mM has also been observed in the rabbit cortical collecting tubule (Stokes, 1981; Frindt, G., and E. E. Windhager, manuscript in preparation). In this case, however, the tubules were open-circuited, so the contribution of an increased lumen-negative potential to the saturation of net fluxes is difficult to evaluate.

Olans et al. (1984) studied amiloride-sensitive Na channels from A6 cells reconstituted into planar lipid bilayers. They found that the single-channel conductance saturated as a function of the Na concentration in the medium, which was varied between 10 and 200 mM. They described two populations of channels. One population had a relatively low maximal conductance of 4 pS and an apparent $K_m$ of 17 mM. The other population had a maximal conductance of 44 pS and an apparent $K_m$ of 47 mM. The channels we have studied in the rat CCT more closely resemble

the low-conductance, low-$K_m$ channels described by Olans et al., although the rat channels seem to have a higher selectivity for Na over K (>10:1; Palmer and Frindt, 1986) than those reconstituted from A6 cells (2:1; Olans et al., 1984).

The value of apparent $K_m$ of 25 mM activity for Na transport through the channels found here is considerably lower than that of 75 mM in concentration or 55 mM activity reported previously by us (Palmer and Frindt, 1986). The discrepancy may be related to the different conditions used. In our previous studies, we varied the Na concentration by substitution with NMDG to maintain constant ionic strength, while in the present study, no ion substitutions were made. This idea is supported by the finding that addition of NMDG to Li solutions to preserve ionic strength increased the apparent $K_m$ value for Li transport from 47 to 92 mM in activity.

The effect of NMDG-Cl on conductance could have at least two explanations. First, the cation could interact with specific sites in the conduction pathway, acting as a weak antagonist of Li transport. This would diminish the conductance in the presence of high NMDG and increase the apparent $K_m$ for transport. Second, the external mouth of the Na channel could have fixed negative charges in its surrounding environment. Decreasing the ionic strength by diluting the NaCl concentration in the pipette would then decrease the amount of screening of this surface charge, thereby increasing the concentration of Na at the outer mouth of the channel. This will elevate the conductance at low NaCl concentrations and thereby decrease the apparent $K_m$. Such a screening mechanism is thought to influence the *g-a* relationship in excitable Na channels (Green et al., 1987). On the other hand, Benos et al. (1981) reported evidence that surface charge at the apical surface of frog skin does not affect the conductance through the Na channels in that membrane.

With 28 mM Li in the pipette, the *i-V* relationship was linear to a good approximation over the range of 0–100 mV in the presence and absence of NMDG-Cl. There was no evidence for a voltage-dependent block by NMDG, as might be anticipated if the site of the block were deep within the conduction pathway. Thus, NMDG probably exerts its effects on the channel at or near its outer mouth. Whether this involves a specific interaction with a site near the beginning of the conduction pathway or a more general interaction with nearby surface charges cannot be discerned from these data.

Using noise analysis to measure single-channel currents in the frog skin outer membrane, Van Driessche and Lindemann (1979) did not observe any saturation of $i$ as the mucosal Na ion activity was increased up to 60 mM. In these experiments, Na activity was reduced at constant ion strength by substitution with K. This ion is known to block Na conduction through the amiloride-sensitive channels in toad bladder (Palmer, 1984). Thus, as discussed above, in this protocol currents at low Na (high K) will be decreased and the apparent $K_m$ value will be increased, which perhaps explains the lack of saturation found in that study.

### *Independence of $P_o$ on Na and Li Concentrations*

The number of conducting Na channels in the frog skin as measured with noise analysis decreases as the mucosal Na concentration is raised (Van Driessche and Lindemann, 1979). Similar observations have been reported in hen copradeum (Chris-

tensen and Bindslev, 1982) and in rabbit urinary bladder (Lewis et al., 1984). Although the physical mechanism underlying the phenomenon termed "self-inhibition" (Lindemann, 1984) is unclear, the down-regulation can be described formally as a blocked state of the channel that is promoted by high mucosal Na. This blocked state must be long-lived compared with the kinetics of amiloride block in order to show up in the analysis of amiloride-induced noise as a decrease in channel number, rather than in single-channel current. Since the kinetics of the spontaneous open/closed transitions of the rat Na channel are indeed slow relative to the kinetics of amiloride block (Palmer and Frindt, 1986), we tested the hypothesis that rates of the spontaneous transitions could be affected by changes in the luminal (pipette) Na concentration. Although we did not estimate the individual rate constants for opening and closing in these experiments, we did measure $P_o$, which is determined by the ratio of the rate constants for opening and closing. There was no systematic effect on $P_o$ of a 5-fold increase in Na or a 10-fold increase in Li concentration in the pipette.

Several factors could account for our failure to observe the change in the apparent number of conducting channels as found by noise analysis. First, there could be tissue differences such that the self-inhibition of Na channels by Na might not exist in the rat CCT. Second, the large variation in $P_o$ values found in our studies might have masked a systematic effect of ion concentration on channel kinetics. Finally, in the experiments in which noise analysis was applied, the entire mucosal surface was exposed to changes in Na concentration, while in the patch-clamp studies, only the patch under study was affected. This raises the possibility that some effects of increasing mucosal Na may be indirect, perhaps involving an increase in Na influx into the cell and subsequent effects on membrane voltage, cell Ca, or cell pH (see below). Since under our experimental conditions, Na could enter the cell only through a small patch of the luminal membrane, such indirect effects of Na may not be observable in this preparation. It is also conceivable that the high-K bath employed in our studies somehow altered the response of the channels to external Na. It should be pointed out, however, that the hypothesis of regulation of apical Na channels by external Na was based on observations of K-depolarized frog skin (Fuchs et al., 1977). The average value of $P_o$ observed in the present studies with high K was ~0.5 (Fig. 9). This is similar to the mean value of $P_o$ of 0.41 reported previously for tubules bathed in NaCl Ringer's solution (Palmer and Frindt, 1986). Thus, the high bath K did not appear to have any marked systematic effects on $P_o$.

*Variations in $P_o$*

The variability in the estimated values of $P_o$ measured under identical conditions, at least outside the cell, is striking (Fig. 9). Two factors that might be involved in this variability are the intracellular pH and the intracellular Ca ion activity. Both pH and Ca were shown to influence Na channel activity in the rat CCT, the former by a direct effect on the channels and the latter by an indirect one (Palmer and Frindt, 1987). However, there are almost certainly other factors involved. We observed the activities in a few patches of two channels that apparently had very different $P_o$ values despite the fact that they must have experienced the same intracellular ionic environment (Fig. 8). In such a case, differences in the gating patterns may arise

from two different populations of channel types with the same conductance or from alterations in the channels that are slow with respect to the time of measurement, such as a phosphorylation or methylation of the channel protein.

*Voltage Dependence of $P_o$*

The voltage dependence of the Na channels in the rat CCT is such that hyperpolarization of the apical cell membrane activates the channels and is therefore opposite to the voltage-gating of Na channels from excitable tissues. The voltage effects on the epithelial channels are also weaker and do not appear to have any associated inactivation. This voltage dependence will tend to stabilize both the activity of the channels and the membrane potential. Thus, any increase in the current through the Na channels, whether mediated by an effect on single-channel current, gating, or channel number, will tend to depolarize the apical membrane and hence to decrease $P_o$. This negative feedback loop is similar in nature to that proposed by Taylor and Windhager (1979), in which the cytoplasmic Ca ion activity rather than the membrane potential is the factor mediating the down-regulation of the channels. Still other negative feedback systems could involve cell pH and metabolic stress (Palmer, 1986; Palmer and Frindt, 1987). Although the relative importance of these factors in regulating Na permeability under physiological conditions is unknown, their common actions will be to stabilize the transepithelial transport rate, to reduce Na permeability as luminal Na is raised, and to blunt the effects of hormones and other agents that might directly affect the Na channels.

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