J. Membrane Biol. 87, 55–65 (1985)

The Journal of
Membrane Biology

Kinetics of the Effect of Amiloride on the Permeability of the Apical Membrane of Rabbit Descending Colon to Sodium

William M. Moran, Randall L. Hudson, and Stanley G. Schultz
Department of Physiology and Cell Biology, University of Texas Medical School, Houston, Texas 77225

Summary. The effects of the addition of graded concentrations of amiloride, $(A)_m$, to the mucosal bathing solution on the permeability of the apical membrane of rabbit descending colon to Na (P^m_{Na}) were determined when the Na activity in the mucosal bathing solution, $(Na)_m$, was 18, 32 or 100 mM. P^m_{Na} was obtained from current-voltage relations determined on tissues bathed with a high-K serosal solution before and after the addition of a maximally inhibitory concentration of amiloride to the mucosal solution as described by Turnheim et al. (Turnheim, K., Thompson, S.M., Schultz, S.G. 1983. *J. Membrane Biol.* **76**:299–309).

The results indicate that: (1) As demonstrated previously (Turnheim et al., 1983), P^m_{Na} decreases with increasing $(Na)_m$. (2) P^m_{Na} also decreases hyperbolically with increasing $(A)_m$. Kinetic analyses of the effect of amiloride on P^m_{Na} are consistent with the conclusions that: (i) the stoichiometry between the interaction of amiloride with apical membrane receptors that results in a decrease in P^m_{Na} is one-for-one; (ii) there is no evidence for cooperativity between amiloride and these binding sites; (iii) the value of $(A)_m$ needed to halve P^m_{Na} at a fixed $(Na)_m$ is 0.6–1.0 μM; and, (iv) this value is independent of $(Na)_m$ over the fivefold range studied.

These findings are consistent with the notion that the sites with which amiloride interacts to bring about closure of the channels through which Na crosses the apical membrane are *kinetically* distinct from the sites with which $(Na)_m$ interacts to bring about closure (i.e., "self-inhibition"). In short, the effects of $(Na)_m$ and $(A)_m$ on P^m_{Na} in this tissue appear to be independent and additive.

Key Words rabbit colon · amiloride · sodium permeability · "self-inhibition"

Introduction

Since the discovery by Bentley (1968) that the pyrazine diuretic, amiloride, rapidly and reversibly inhibits Na absorption by toad urinary bladder, this agent has become a valuable tool in the study of Na transport by a wide variety of "tight epithelia." In an effort to gain insight into the possible mechanism of action of this diuretic, numerous attempts have been made to determine the kinetics of its inhibitory effect with strikingly variable results. Thus, in some instances the inhibitory action appears to be strictly noncompetitive (Bentley, 1968; Benos, Mandel & Balaban, 1979; Biber, 1971; Benos, 1982); in other instances it appears to be competitive (Zeiske & Lindemann, 1974; Benos et al., 1979; Cuthbert, 1981; Benos, 1982); and, in some instances varying degrees of "mixed" competition have been observed (Salako & Smith, 1970; Turnheim, Luger & Grasl, 1981; Benos, 1982; Bindslev et al., 1982). Benos (1982) has suggested that these different kinetic patterns may reflect species differences. While this is certainly possible, there may be another explanation for these discrepant findings. In the large majority of the studies cited above the approach has been to determine the effect of graded concentrations of amiloride on the short-circuit current (I_{sc}) in the presence of different Na concentrations. However, this approach is incorrect for the following reasons:

First, microelectrophysiologic studies have clearly established that amiloride, by blocking the Na conductance of the apical membrane, results in a hyperpolarization of that barrier (i.e., the cell interior becomes increasingly more negative with respect to the mucosal solution) (Helman & Fisher, 1977; Schultz, Frizzell & Nellans, 1977; Sudou & Hoshi, 1977; Thomas et al., 1983). In addition, there is compelling evidence that Na entry across the apical membrane of these "tight epithelia" conforms to the Goldman-Hodgkin-Katz (GHK) "constant field" flux equation (Goldman, 1943; Hodgkin & Katz, 1949) for simple electrodiffusion over a reasonably wide range of electrical potential differences (Fuchs, Larson & Lindemann, 1977; Palmer et al., 1980; Thompson, Suzuki & Schultz, 1982*a*; Thomas et al., 1983; DeLong & Civan, 1984) and almost certainly takes place through channels (Lindemann & Van Driessche, 1977). Further, evidence has been presented that Na transport through single channels also conforms to this relation (Henrich &

Lindemann, 1984). It follows that when the Na activity in the mucosal solution, $(Na)_m$, is constant, the rate of entry of Na across the apical membrane will be influenced by the electrical potential difference across that barrier, (ψ^{mc}), the chord conductance of that barrier to Na, (G^m_{Na}), and the intracellular Na activity, $(Na)_c$.

Thus, when a submaximal inhibitory dose of amiloride is added to the mucosal solution, a certain number of channels will be inhibited. But, at the same time, the electrical driving force, ψ^{mc} (with reference to the mucosal solution), and perhaps the chemical driving force, for Na entry across the remaining, noninhibited channels will increase as will the Na conductances of these remaining channels[1]. Further, this effect will be more marked in the presence of high $(Na)_m$ than in the presence of low $(Na)_m$ since in the latter instance ψ^{mc} already approaches the value observed in the presence of a maximum inhibitory concentration of amiloride (*cf.* Thomas et al., 1983). The overall effect will be to *decrease the apparent effectiveness* of a given dose of amiloride, and this effect will be greater in the presence of high $(Na)_m$ than in the presence of low $(Na)_m$.

In addition, evidence has been presented (Palmer, 1984*a*) that the binding of amiloride to its receptor sites on the apical membrane is affected by ψ^{mc} such that binding increases as ψ^{mc} becomes more negative. If so, the inhibitory effectiveness of a given concentration of amiloride would be lower in the presence of a high $(Na)_m$ than in the presence of a low $(Na)_m$, thereby adding to the effects cited above.

Thus, even if the actual molecular mechanism of the inhibitory effect of amiloride were strictly noncompetitive (i.e., uninfluenced by the Na concentration), the kinetics of the effects of graded doses of this agent on the I_{sc} will, in general, be influenced by $(Na)_m$ suggesting, at the very least, mixed-competition.

Second, in some studies on the kinetics of amiloride inhibition of the I_{sc} the results are interpreted using a simple enzyme-substrate-inhibitor model (e.g., Benos et al., 1979) in which Na is viewed as the substrate or activator of transport and amiloride as the inhibitor. However, this may be an oversimplified view of a much more complex system in which Na is not only an activator but is also an inhibitor of the entry step. The results reported by Van Driessche and Lindemann (1979) for frog skin strongly suggest that the saturable relation between $(Na)_m$ and I_{sc} is not due to saturation of a *fixed* number of entry sites or channels; instead, the single channel conductance appears to increase linearly with increasing $(Na)_m$, whereas, at the same time, the number of open channels decreases hyperbolically. Thus, the mechanism of "self-inhibition" may not be analogous to the mechanism responsible for classic Michaelis-Menten behavior of a simple, saturating enzyme-substrate interaction where the amount of enzyme is assumed to remain constant.

It follows that one correct way to examine the kinetics of the interaction between amiloride and the Na entry mechanism is to determine the effects of graded doses of this agent on the Na permeability of the apical membrane, P^m_{Na}, when $(Na)_m$ and the intracellular Na activity, $(Na)_c$, are constant. This parameter is voltage-independent and is the best macroscopic measure of the number of open Na channels.

The present studies were designed to determine the effects of graded doses of amiloride on P^m_{Na} of rabbit descending colon in the presence of different mucosal Na activities. The results indicate that the inhibitory effectiveness of amiloride on P^m_{Na} is not affected by $(Na)_m$ or $(Na)_c$ over the ranges studied.

Materials and Methods

Segments of descending colon were obtained from white rabbits (3–4 kg), which were killed by a blow to the head. The colon was opened along its mesenteric border, rinsed free of intestinal contents and the outer muscle layers were stripped off. The resulting "partial mucosal strips" were mounted in Ussing-type chambers (Frizzell, Koch & Schultz, 1976).

The solution bathing the mucosal surface of the tissue contained (in mM): 140 Na, 114.6 gluconate, 4.8 Cl, 2.4 HPO_4, 0.6 H_2PO_4, 20 HCO_3, 1.2 Ca, and 1.2 Mg. Solutions with lower Na concentrations were prepared by isosmotic replacement of Na with tetramethylammonium (TMA). The serosal solution contained a high K concentration in order to depolarize, and reduce the resistance of, the basolateral membrane (Fuchs et al., 1977; Thompson et al., 1982*a*; Turnheim et al., 1983; Palmer, 1984*b*) and contained (in mM): 100 K, 50 gluconate, 29.4 Cl, 20 HCO_3, 2.4 HPO_4, 0.6 H_2PO_4, 1.2 Ca, 1.2 Mg, and 83 mannitol. Both solutions contained 10 mM glucose and had osmolarities of 280 mOsm determined by vapor-pressure osmometry. All solutions were gassed with a mixture of 95% O_2–5% CO_2 and had a pH of 7.4 at 37°C.

Gluconate was employed as the major anion in these experiments for two reasons. First replacement of Cl in the mucosal solution with gluconate stimulates the I_{sc}; a similar effect of replacement of Cl with a variety of other anions has been reported for this epithelium by Turnheim, Frizzell and Schultz (1977). Second, Thompson has found (*personal communication*) that a high-K, gluconate serosal bathing solution is as effective in depolarizing the basolateral membrane and reducing its effective resistance as high-K solutions where the dominant anion is SO_4; this observation is consistent with the findings of Wills, Lewis and Eaton (1979) that the basolateral membrane of rabbit descending colon is predominantly permeable to K.

[1] A decline in $(Na)_c$ in the presence of a constant $(Na)_m$ would lead to an increase in the driving force for entry. Further, since Na entry conforms to the GHK equation, G^m_{Na} will increase as ψ^{mc} becomes more negative.

In the series of experiments designed to determine the effects of graded submaximal doses of amiloride on the current-voltage (*I-V*) relations of the apical membrane in the presence of a fixed mucosal Na activity, $(Na)_m$, the tissues were short-circuited using an automatic voltage-clamp which compensated for fluid resistance. The clamp was controlled by a minicomputer (LSI 11/03) so that a train of alternating polarity current pulses could be passed across the tissue of sufficient magnitudes to clamp the transepithelial electrical potential difference, ψ^{ms}, over the range of 0 to ±120 mV in steps of 10 mV (i.e., ψ^{ms} = 0, +10, 0, −10, 0, +20, 0, −20, 0 . . . . 0, +120, 0, −120). Each pulse had a duration of 100 msec, and the interval between pulses was 500 msec. The transepithelial current (I^{ms}) and the corresponding ψ^{ms} were recorded by a chart recorder and the values of I^{ms} and ψ^{ms} determined 20 and 90 msec after the onset of each pulse were relayed to the computer via an analog-to-digital converter and stored for later processing. This approach has been described in detail previously (Thompson et al., 1982*a*). We have previously reported (Thompson et al., 1982*a*) that the *I-V* relations of the apical membrane determined at 16 and 90 msec are essentially identical so that in the present study the data obtained at 20 msec were employed.

The experimental protocol was as follows:

When the short-circuit current (I_{sc}) and tissue resistance (r_t) had stabilized (generally 30 min after mounting) a control *I-V* relation was obtained. Then, graded submaximal concentrations of amiloride, $(A)_m$, (i.e., 3.3×10^{-8} M, 10^{-7} M, 3.3×10^{-7} M, 10^{-6} M and 3.3×10^{-6} M) were added to the mucosal solution, in turn. An *I-V* relation was obtained after each addition of amiloride once the I_{sc} had stabilized (usually 2–4 min). Finally, a maximum inhibitory concentration of amiloride (10^{-4} M) was added to the mucosal solution and a final *I-V* relation was obtained. Separate series of experiments were carried out when $(Na)_m$ was 100, 32, and 18 mM. Because accurate determinations of the effects of graded concentrations of amiloride on the I_{sc} could only be made when the initial I_{sc} exceeded 15 $\mu A/cm^2$, experiments were not carried out when the initial I_{sc} was below that value; in all, 2 tissues out of 10 were rejected when $(Na)_m$ = 32 mM and 4 tissues out of 10 were rejected when $(Na)_m$ = 18 mM.

As discussed previously (Thompson et al., 1982*a*; Turnheim et al., 1983), when rabbit colon is bathed by a high-K serosal solution, ψ^{ms} is a good measure of the electrical potential difference across the apical membrane (ψ^{mc}) and the difference between the value of I^{ms} at a given ψ^{ms} before and after exposing the tissue to a maximum inhibitory concentration of amiloride is a measure of the Na current across the apical membrane (I^m_{Na}) at that value of ψ^{ms} (or "ψ^{mc}")[2]. In this way, the relations between I^m_{Na} and "ψ^{mc}" were obtained in the presence of graded submaximal concentrations of amiloride when $(Na)_m$ was 100, 32, or 18 mM. The values of the permeability of the apical membrane to Na, (P^m_{Na}), and the intracellular Na activity, $(Na)_c$, were obtained by fitting the experimental *I-V* relations to the Goldman-Hodgkin-Katz equation over the range "ψ^{mc}" = −20 mV to I^m_{Na} = 0 using nonlinear regression analysis as described previously (Turnheim et al., 1983). In essence, two data points within this range (i.e., two values of I^m_{Na} at two values of "ψ^{mc}") were substituted into the GHK equation yielding two equations with two unknowns. The simultaneous solution of these two equations provided "initial estimates" of P^m_{Na} and $(Na)_c$. These values were then employed to obtain the "best fit" and the final values of P^m_{Na} and $(Na)_c$. In approximately 15% of the experiments the data could not be fit by the GHK equation regardless of the initial estimates. In almost every other instance the initial estimates did not differ from the final "best fit" values. In all, the data reported represent the results of five experiments when $(Na)_m$ = 100 mM, six experiments when $(Na)_m$ = 32 mM and five experiments when $(Na)_m$ = 18 mM.

A second series of experiments was carried out in order to examine the effect of a fixed submaximal concentration of amiloride on the kinetics of Na entry across the apical membrane in the presence of varying $(Na)_m$. The protocol was as follows:

After mounting, the tissue was bathed by a mucosal solution having a Na activity of 100 mM; the serosal surface of the tissue was bathed with the high-K solution. When the I_{sc} and r_t had stabilized, 3.3×10^{-7} M amiloride was added to the mucosal solution and the resulting I_{sc} was recorded after it had stabilized. Then the mucosal reservoir and chamber were rinsed three times with an amiloride-free solution containing *either* 32, 18 or 12 mM Na in random order; a series of control studies indicated that three rinses is sufficient to remove all amiloride from the system and restore the I_{sc} to the pre-amiloride (control) value. When the I_{sc} stabilized in the presence of the new Na concentration, 3.3×10^{-7} M amiloride was added to the mucosal solution and the resulting I_{sc} was recorded. This rinsing and replacement procedure was repeated until the I_{sc} was obtained *on the same tissue* before and after the addition of 3.3×10^{-7} M amiloride when $(Na)_m$ was 100, 32, 18, and 12 mM. Then 10^{-4} M amiloride was added to the mucosal solution and the amiloride-insensitive I_{sc} was recorded; this value was subtracted from all other values of I_{sc} to obtain the rate of transepithelial Na transport when ψ^{ms} = 0, ${}_0I_{Na}$[3].

All results are reported as the arithmetic mean ± the standard error of the mean (SEM). Statistical analyses were performed using the paired or unpaired Student *t*-test and/or an analysis of variance with a value $P < 0.05$ chosen as the level of statistical significance.

Results and Discussion

P^m_{Na} as a Function of Time after Addition of a Maximum Inhibitory Concentration of Amiloride to the Mucosal Solution

Because the experiments designed to examine the effects of graded doses of amiloride on the *I-V* relations in the presence of a fixed $(Na)_m$ last approximately 15 min, it was important to determine whether prolonged exposure to amiloride affects any of the conductive pathways in parallel with the Na-entry step[4]. To this end, a series of five experiments was performed in which an *I-V* relation was

[2] The quotation marks around ψ^{mc} simply designate that this is not a directly measured value but is presumed to be reasonably close to ψ^{ms}. The validity of this approximation is examined in the Appendix.

[3] As discussed below, the amiloride-insensitive I_{sc} can be attributed to small diffusion potentials arising from the ionic asymmetries in the two bathing solutions.

[4] If the ionic conductances of any pathway in parallel with the amiloride-sensitive Na entry step change with time, the differences between I^{ms} before and after amiloride will not be a valid measure of I^m_{Na} (Thompson et al., 1982*a*).

determined when $(Na)_m$ = 100 mM in the absence of amiloride, then 10^{-4} M amiloride was added to the mucosal solution and *I-V* relations were determined 2, 5, 8, 11 and 14 min thereafter. The results are shown in Fig. 1. The finding that the calculated P^m_{Na} of the tissue is independent of time after exposure to a maximum inhibitory dose of amiloride indicates that this agent does not affect any of the conductive pathways in parallel with the Na-entry step over the time range employed in these studies.

I-V Relations in the Presence of Graded Concentrations of Amiloride

The time course of a typical experiment in which *I-V* relations were determined under control conditions and after the addition of increasing concentrations of amiloride to the mucosal solution is shown

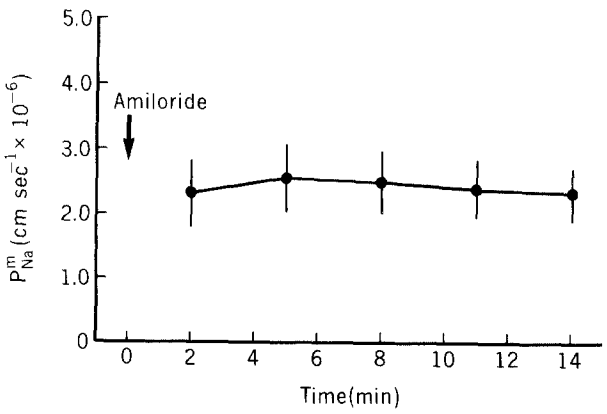

Fig. 1. The results of a series of studies in which an *I-V* relation was determined when $(Na)_m$ = 100 mM and then at 2–3 min intervals after the addition of 10^{-4} M amiloride to the mucosal bathing solution. P^m_{Na} was determined as described in the text. The bars represent SEM of 5 experiments

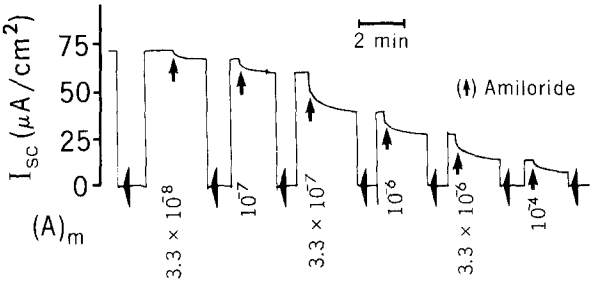

Fig. 2. The effect of graded concentrations of amiloride on the I_{sc}. The small triangles indicate the deflections in I^{ms} during the *I-V* determinations before and after the addition of graded concentrations of amiloride to the mucosal solution (arrows). [*Note*: As indicated in the Methods section, the tissues were "short-circuited" throughout these experiments except during brief displacements by transepithelial current pulses over the range ψ^{ms} = 0 to ψ^{ms} = ±120 mV. During these current pulses the response of the chart recorder was attenuated 25-fold]

in Fig. 2. As expected, the addition of amiloride resulted in a concentration-dependent decrease in the I_{sc}. In the presence of 10^{-4} M amiloride, which completely abolishes active Na transport, there is a small residual I_{sc} that can be attributed to a small diffusion potential due to the different ionic compositions of the mucosal and serosal bathing solutions. The computer program automatically corrects for this amiloride-insensitive current yielding the amiloride-sensitive I^m_{Na}.

An example of the relations between the amiloride-sensitive I^m_{Na} and "ψ^{mc}" in the presence of graded concentrations of amiloride is shown in Fig. 3. The curves represent the best fits of the experimental points to the GHK equation. In every experiment in which the data obtained under control conditions and in the presence of submaximal concentrations of amiloride complied with the GHK equation (~85%) there was an excellent "fit" over the range −20 mV < "ψ^{mc}" < 60 mV. The departures from the predicted curves at larger negative values of "ψ^{mc}" was a consistent finding. The direction of these departures is consistent with the recent finding by Palmer (1984*a*) that the effect of amiloride on toad urinary bladder is voltage-dependent; the implication of this finding will be discussed below.

The values of P^m_{Na}, $(Na)_c$ and ${}_0I_{Na}$ under control conditions and in the presence of graded concentrations of amiloride are given in the Table.

Relation Between P^m_{Na} and $(Na)_m$

The values of P^m_{Na}, in the absence of amiloride, are plotted as a function of $(Na)_m$ in Fig. 4. As demonstrated previously using this preparation (Turnheim et al., 1983), P^m_{Na} decreases with increasing $(Na)_m$. The interpretation of this finding is complicated by the fact that $(Na)_c$ also increases with increasing

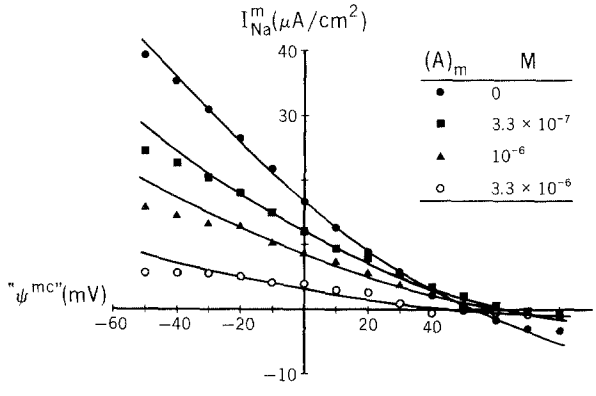

Fig. 3. Relations between I^m_{Na} and "ψ^{mc}" in the absence and presence of graded concentrations of amiloride. The curves correspond to the best fit of the experimental data to the GHK equation over the range "ψ^{mc}" = −20 mV to I^m_{Na} = 0

$(Na)_m$ (Table) so that the observed effect on P^m_{Na} could be the combined results of "self-inhibition" by $(Na)_m$ as well as "negative feedback" by $(Na)_c$. However, for the reasons discussed by Turnheim et al. (1983), it is likely that "self-inhibition" by $(Na)_m$ is the dominant factor underlying the relation shown in Fig. 4. Garty and Lindemann (1984) and Palmer (1985) have reported that in K-depolarized toad urinary bladder G^m_{Na} and P^m_{Na} are minimally affected by changes in $(Na)_c$ below 20–40 mM.

Effect of Graded Concentrations of Amiloride on $(Na)_c$ and $_0I_{Na}$

As shown in the Table, in the presence of fixed $(Na)_m$ there was an overall tendency of $(Na)_c$ to decrease with increasing $(A)_m$, but the effect is not large and is of borderline statistical significance. Nagel, Garcia-Diaz and Armstrong (1981) have reported that $(Na)_c$ is not affected following a short-term (5 min) exposure of frog skin to maximum inhibitory concentrations of amiloride in spite of the fact that I_{sc} decreased within that period to only 10–20% of the control value.

The values of $_0I_{Na}$ under control conditions and in the presence of graded concentrations of amiloride are also given in the Table. Inasmuch as experiments were not carried out on tissues displaying an initial I_{sc} less than 15 $\mu A/cm^2$, which resulted in the rejection of a number of tissues when $(Na)_m$ = 32 or 18 mM, these data cannot be overinterpreted. Nonetheless, it is of interest to compare these results with those reported from this laboratory by Turnheim et al. (1983) who found, using essentially the same approach employed in this study, that reduction of the Na activity of the solution bathing the mucosal surface of tissues from the *same* animal resulted in hyperbolic decreases in $(Na)_c$ and $_0I_{Na}$; the relation between $(Na)_c$ and $_0I_{Na}$ was sigmoidal and consistent with the notion that 2–3 Na interact with the basolateral pump per cycle. The present results indicate that among single, selected tissues from different animals there is no clear-cut relation between $(Na)_c$ and $_0I_{Na}$.

Effect of Graded Concentrations of Amiloride on P^m_{Na}

The relations between $(1/P^m_{Na})$ and $(A)_m$ when $(Na)_m$ = 100, 32 and 18 mM are given in Fig. 5; the lines drawn through the experimental points were determined by least-squares linear regression analysis and all have correlation coefficients greater than 0.99. These linear relations indicate that when $(Na)_m$ is constant the permeability of the apical membrane to Na in the presence of amiloride, $P^{m'}_{Na}$ is given by the relation

$$P^{m'}_{Na} = P^m_{Na} K_A / [(A)_m + K_A] \quad (1)$$

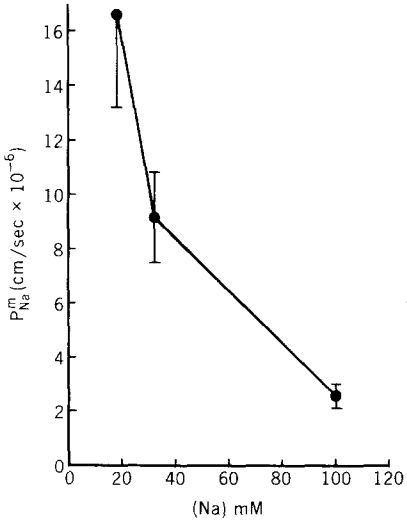

Fig. 4. Relation between P^m_{Na} and $(Na)_m$

Table. Effects of Amiloride on the Sodium Permeability of the Apical Membrane Cell Sodium Activities and Sodium Transport

$(Na)_m$ (mM)		$(A)_m$ (M) 0	3.3×10^{-8}	1×10^{-7}	3.3×10^{-7}	1×10^{-6}	3.3×10^{-6}
18 (n = 5)	P^m_{Na}	16.6 ± 3.4	15.2 ± 3.3	12.8 ± 2.9	9.0 ± 1.8	5.6 ± 1.3	3.0 ± 0.1
	$(Na)_c$	5 ± 1	5 ± 1	4 ± 1	4 ± 1	4 ± 1	7 ± 5
	$_0I_{Na}$	22 ± 1	21 ± 1	19 ± 1	14 ± 1	9 ± 1	4 ± 1
32 (n = 6)	P^m_{Na}	9.2 ± 1.6	8.3 ± 1.7	7.9 ± 1.6	5.7 ± 1.1	4.2 ± 1.7	2.2 ± 0.3
	$(Na)_c$	9 ± 2	9 ± 2	8 ± 2	6 ± 1	6 ± 1	6 ± 1
	$_0I_{Na}$	22 ± 2	21 ± 2	20 ± 2	15 ± 2	11 ± 2	5 ± 2
100 (n = 5)	P^m_{Na}	2.6 ± 0.4	2.5 ± 0.4	2.3 ± 0.4	1.6 ± 0.2	1.1 ± 0.2	0.5 ± 0.1
	$(Na)_c$	22 ± 4	18 ± 4	15 ± 4	16 ± 4	16 ± 6	16 ± 3
	$_0I_{Na}$	23 ± 2	22 ± 2	19 ± 2	14 ± 2	8 ± 2	4 ± 2

P^m_{Na} in cm · sec^{-1} × 10^{-6}; $(Na)_c$ in mM; $_0I_{Na}$ in $\mu A/cm^2$; n = number of tissues.

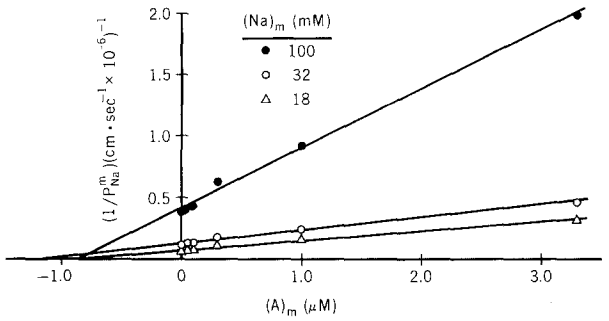

Fig. 5. Relations between $(1/P^m_{Na})$ and $(A)_m$

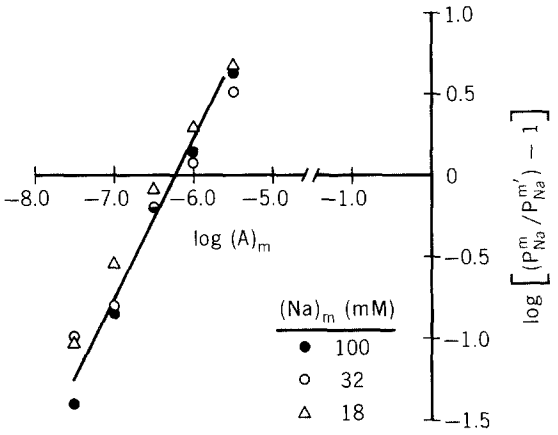

Fig. 6. Hill plot of the effect of $(A)_m$ on P^m_{Na} as described by Eq. (2)

where P^m_{Na} is the permeability of the apical membrane to Na in the absence of amiloride and K_A is that value of $(A)_m$ at which $P^{m'}_{Na} = (P^m_{Na}/2)$.

The values of K_A when $(Na)_m$ was 100, 32 and 18 mM are 0.85, 1.1 and 0.95 μM, respectively, and do not differ significantly.

An alternate and somewhat more sensitive (Segel, 1975) way of linearizing Eq. (1) is analogous to the logarithmic form of the Hill equation, i.e.

$$\log[(P^m_{Na} - P^{m'}_{Na})/P^{m'}_{Na}] = \log(A)_m - \log K_A. \quad (2)$$

The relations between $\log[(P^m_{Na} - P^{m'}_{Na})/P^{m'}_{Na}]$ and $\log(A)_m$ at the three different values of $(Na)_m$ are shown in Fig. 6. The relations at each value of $(Na)_m$ are linear ($r > 0.99$). When $(Na)_m$ = 100, 32 and 18 mM, the slopes are 1.0, 0.8 and 0.8, respectively, and are not statistically different ($P > 0.05$). The values of K_A are 0.7 ± 0.1, 0.7 ± 0.1 and 0.5 ± 0.1 μM, respectively; an analysis of variance of the individual values of K_A at the three values of $(Na)_m$ yielded a value of F that was not significant at the $P > 0.05$ level. The single line shown in Fig. 6 describes the composite data and has a slope of 0.9 which does not differ significantly from unity; the value of K_A derived from the intercept on the abscissa is 0.6 μM.

Thus, these data are consistent with the conclusions that (i) the stoichiometry of the interactions between amiloride and the apical membrane receptors that led to a decrease in P^m_{Na} is one-for-one; (ii) there is no evidence for cooperative interactions between amiloride and these binding sites; (iii) the value of $(A)_m$ required to halve P^m_{Na} at a fixed $(Na)_m$ is 0.6–1.0 μM; and, (iv) this value is independent of $(Na)_m$ over the fivefold range examined within the limits of experimental error. Further, K_A does not appear to be influenced by $(Na)_c$ over the range 5–22 mM.

The Effect of Amiloride on the Relation Between $(Na)_m$ and I^m_{Na}

The relations between I^m_{Na} and $(Na)_m$ in the absence of amiloride and in the presence of 3.3×10^{-7} M amiloride are shown in Fig. 7. The curves correspond to the relation

$$I^m_{Na} = I^m_{Na(m)}\,(Na)_m/[(Na)_m + K_{Na}] \quad (3)$$

where in the absence of amiloride $I^m_{Na(m)}$ = 40 μA/cm^2 and K_{Na} = 19 mM, while in the presence of 0.33 μM amiloride $I^m_{Na(m)}$ = 24 μA/cm^2 and K_{Na} = 19 mM. Thus, the value of $(Na)_m$ at which $I^m_{Na} = (I^m_{Na(m)}/2)$ (i.e., K_{Na}) is not affected by amiloride at a concentration close to that required to halve the values of P^m_{Na}. While it would have been desirable to examine these relations at higher values of $(A)_m$ it proved to be very difficult to reverse completely the effects of amiloride by repeated rinsing when $(A)_m$ was significantly greater than 3.3×10^{-7} M.

Conclusions

The primary purpose of the present study was to determine the effects of graded doses of amiloride on the permeability of the apical membrane of rabbit descending colon to Na in the presence of a fivefold range of $(Na)_m$. P^m_{Na} was obtained from I-V relations determined on tissues bathed with a high-K serosal solution before and after the addition of a maximally inhibitory dose of amiloride to the mucosal solution. Before discussing the implications of the results we will consider possible problems arising from the methods employed.

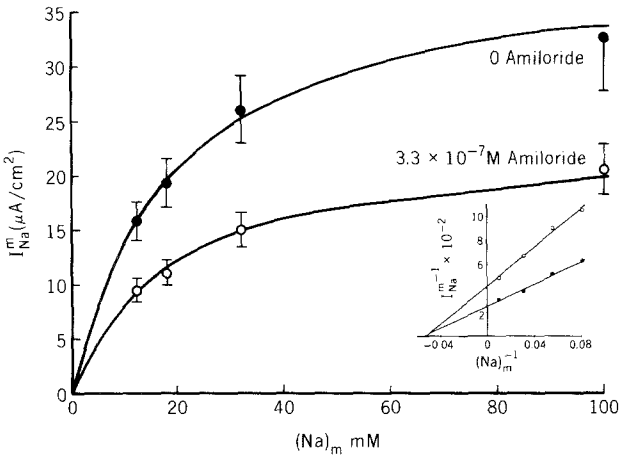

Fig. 7. Relations between I^m_{Na} and $(Na)_m$ under control conditions and in the presence of 3.3×10^{-7} M amiloride. The values of K_{Na} and $I^m_{Na(m)}$ were determined from the double-reciprocal plot (inset) and used to construct the curves shown. The data shown are the means ± SEM of 10 experiments

First, we have previously demonstrated (Thompson et al., 1982*a*) that when rabbit descending colon is bathed with a serosal solution containing a high-K concentration, the electrical potential difference across the basolateral membrane, ψ^{cs}, is essentially abolished and the resistance of that barrier (r^s) is markedly reduced; similar findings have been reported for frog skin (Klemperer, Garcia-Diaz & Essig, 1984) and toad urinary bladder (Palmer, 1984*b*). However, although r^s is reduced it is not abolished. Thus, when current is passed across the cell $\psi^{cs} \neq 0$ so that $\psi^{mc} \neq \psi^{ms}$. However, as discussed in the Appendix it seems highly unlikely that any "residual" r^s significantly distorts our findings.

Second, Palmer (1984*a*) has presented evidence that the effect of amiloride on Na entry across the apical membrane of toad urinary bladder is voltage-dependent and concluded that this agent interacts with a site that "senses" approximately 12–15% of ψ^{mc}. Similar observations have been reported by Warncke and Lindemann for frog skin (J. Warncke & B. Lindemann, *submitted*). If the same is true for rabbit colon, this might be the explanation for the systematic departures of the *I-V* relations observed when $\psi^{mc} < -20$ mV (e.g., Fig. 3). Nonetheless, while this may distort the shape of the *I-V* relation, the excellent agreement between the intercepts of the GHK curves on the ordinate ("ψ^{mc}" = 0) and abscissa ($I^m_{Na} = 0$) with the experimental data lends credibility to our calculated values of P^m_{Na} and $(Na)_c$.

The Effects of Amiloride and Sodium on the Entry Step

As discussed above, both $(Na)_m$ and amiloride are *inhibitors* of Na channels in the apical membranes of descending colon, frog skin, toad urinary bladder and *Necturus* urinary bladder.

The inhibition by $(Na)_m$, referred to as "self-inhibition," was first reported by Lindemann and his co-workers (Lindemann & Gebhardt, 1973; Lindemann, 1977) and, like the effect of amiloride, appears to be the result of a decrease in the number of open Na channels (Van Driessche & Lindemann, 1979). Results obtained on frog skin (Fuchs et al., 1977), toad urinary bladder (Palmer et al., 1982) and rabbit colon (Turnheim et al., 1983) further indicate that P^m_{Na} decreases hyperbolically with increasing $(Na)_m$ so that

$$P^m_{Na} = (P^m_{Na})_0 \, K_{Na}/[(Na)_m + K_{Na}] \quad (4)$$

where $(P^m_{Na})_0$ is the value of P^m_{Na} when $(Na)_m = 0$ and K_{Na} is the value of $(Na)_m$ when $P^m_{Na} = (P^m_{Na})_0/2$.

As discussed above, $(A)_m$ also brings about a hyperpolic decline in P^m_{Na} given by Eq. (1) which is isomorphic with Eq. (4). *But, the finding that K_A is not affected by $(Na)_m$ indicates that the sites with which amiloride interacts to bring about closure of Na channels differ from the sites with which $(Na)_m$ interacts to bring about closure of these channels.*

The findings reported in Fig. 7 corroborate this conclusion. Thus, inasmuch as the saturable relation between I^m_{Na} (or I_{sc}) and $(Na)_m$ appears to be primarily due to "self-inhibition," the finding that K_{Na} is not affected by $(A)_m$ indicates that the "self-inhibition" site and the amiloride-binding site differ.

Before proceeding, it seems important to clarify an issue that is a source of confusion in the literature dealing with this subject. This confusion seems to stem from the fact that $(Na)_m$ is often viewed solely as an *activator* of Na entry rather than as *both an activator and an inhibitor* of Na entry. Thus, a number of authors have inferred that results such as these have a bearing on the nature of the interaction between amiloride and Na *for the Na entry site or channel*[5]. This clearly need not be the

[5] It is important to define clearly our use of the word "channel." We restrict this word to designate the *pathway* for electrodiffusion of Na through an integral protein that spans the apical membrane. In other words, when we refer to "interaction with the channel" we refer to an interaction within the "hole" that permits Na entry into and passage through the diffusional pathway and not with the proteinaceous structure that surrounds this "hole."

case. All that can be concluded from the data available to date is that the apical membrane possesses channels that are highly selective for Na (and Li) and that the number of open channels can be reduced (not necessarily "blocked" or "plugged") by amiloride and $(Na)_m$. The findings of Palmer (1984*a*) and of Warncke and Lindemann (*submitted*) that the inhibitory effect of amiloride is voltage-dependent are certainly consistent with a "plugging" action within the mouth of the channel. However, it is also conceivable that amiloride binds to a site outside of the channel and brings about a conformational change within the channel that is voltage-dependent. Thus, the present data are equally consistent with two extreme models; i.e., (i) amiloride interacts with a receptor that is distinct from the Na channel, and $(Na)_m$ interacts with a different receptor that is also distinct from the Na channel, and both interactions independently and additively bring about closure; or (ii) both amiloride and $(Na)_m$ interact within the Na channel but at different sites and independently and additively bring about closure. In short, all that can be concluded from these data is that the amiloride binding sites and the Na self-inhibition sites differ.[6]

Comparison with Previous Results

Turnheim et al. (1981) have examined the kinetics of the inhibitory effect of amiloride on the I_{sc} across rabbit descending colon in the presence of varying $(Na)_m$. These investigators concluded that the kinetics of inhibition is "mixed" *but predominantly noncompetitive* and that the stoichiometry between amiloride and the receptor that results in the inhibition of I_{sc} is one-for-one; the values for K_A found in those studies are in reasonable agreement with those reported in this paper. Thus the major differences between the results of Turnheim et al. (1981) and the present results is the finding of a small effect of $(Na)_m$ on K_A and a small effect of $(A)_m$ on K_{Na}; it is quite possible that these differences can be attributed to the effect of amiloride on ψ^{mc} as discussed in the Introduction. The effect of amiloride on ψ^{mc} *may also be the explanation for the "shallow dependence" of* K_A on $(Na)_m$ reported by Bindslev et al. (1982) for hen coprodaeum.

For the case of toad urinary bladder, Cuthbert and Shum (1975) reported a marked dependence of K_A on $(Na)_m$. More recently, Palmer (1984*a*) examined the *I-V* relations across toad urinary bladder, bathed with a high-K serosal solution, in the presence of graded doses of amiloride and concluded that (i) Na and other cations compete with amiloride for its binding site but the affinity for Na is quite low (i.e., K_A is doubled in the presence of 100 mM Na); and (ii) the site with which Na interacts to bring about self-inhibition differs from the amiloride binding site. Except for the fact that we were unable to detect a statistically significant effect of $(Na)_m$ on K_A over the range $(Na)_m$ = 18 mM to $(Na)_m$ = 100 mM our conclusions are in agreement with those of Palmer (1984*a*).

The results in the case of frog skin seem to vary among species, and noncompetitive, mixed and competitive inhibition have been reported (*cf.* Benos, 1982; Li & Lindemann, 1983*b*). All of these studies examined the effect of amiloride on the I_{sc} across intact skins so that, as discussed in the Introduction, these results could be misleading.

The important point, however, is not whether $(Na)_m$ affects K_A but *whether the amiloride binding site and the Na self-inhibition site are the same or different.* If the kinetics of inhibition are strictly noncompetive, they *must* differ. But they may still differ even if the kinetics are of the mixed or competitive type. Inasmuch as amiloride is only effective in its cationic form (Benos et al., 1976; Cuthbert, 1981; Benos, 1982), it is quite possible that Na and other cations might interact with the amiloride binding site and that the affinities of these interactions could very well be dependent upon a variety of factors, including species. Thus, as reported by Palmer (1984*a*), $(Na)_m$ may inhibit the effect of amiloride but with an affinity constant that differs markedly from the "self-inhibition" affinity; i.e., K_{Na} of Eq. (3).

This issue was recently addressed by Li and Lindemann (1983*a*) for the case of the skin of *Rana esculenta* (*ridibunda*) where earlier studies indicated that the effect of amiloride is purely competitive (Zeiske & Lindemann, 1974). These investigators found that the macroscopic K_A's of amiloride, and a number of other related organic compounds, are much greater than the microscopic inhibition constants determined from fluctuation analysis. They concluded that these differences could be explained by competition between the organic channel blocker and the Na self-inhibition effect. On the other hand, Lewis et al. (1984) have reported excellent agreement between the macroscopic inhibitor constant of amiloride (K_A) and the microscopic constant determined from fluctuation analysis of the Na-entry step across rabbit urinary bladder.

[6] There are at least two findings that are difficult to reconcile with the simple notion that the *sole* action of amiloride is to "plug" Na channels. First, at very low concentrations amiloride stimulates Na transport (*cf.* Li & Lindemann, 1983*b*). Second, the biguanide phenformin, whose structure somewhat resembles that of amiloride, stimulates Na transport across frog skin, and this action can be inhibited by amiloride (Saito & Yoshida, 1984).

The Effects of Sulfhydryl-Reactive Agents

The Na-entry steps across the apical membrane of rabbit descending colon and the outer membrane of frog skin (*R. ridibunda*) share a number of common features. Both conform to the GHK equation for simple electrodiffusion (Fuchs et al., 1977; Thompson et al., 1982*a*); both appear to be mediated by channels having similar single channel conductances (Lindemann, 1984; Zeiske, Wills & Van Driessche, 1982); both are inhibited by amiloride with K_A's in the submicromolar range (i.e., Turnheim et al., 1981; Li & Lindemann, 1983*a*; present data); and, both are subject to self-inhibition by $(Na)_m$ as well as "negative feedback" by $(Na)_c$ (Turnheim, Frizzell & Schutz, 1978; Lindemann, 1984).

But there are two substantive differences revolving about the actions of sulfhydryl reactive agents such as *p*-chloromercuribenzene sulfonate (PCMBS).

In the case of rabbit descending colon, Gottlieb et al. (1978) demonstrated that PCMBS prevents and reverses the inhibitory action of amiloride on the I_{sc}. Subsequently, Luger and Turnheim (1981) reported that the organic mercurial, mersalyl, (i) noncompetitively blocks the inhibitory action of amiloride on the I_{sc}; (ii) stimulates the I_{sc} when it is below a mean value of approximately 2.5 μEq/cm^2 hr but inhibits the I_{sc} when it is above that value; and, (iii) blunts or abolishes the effects of $(Na)_m$ ("self-inhibition") and perhaps $(Na)_c$ ("negative feedback") on P_{Na}^m. These investigators also demonstrated that *p*-chloromercuribenzoate conjugated with dextran has the same effects as mersalyl, suggesting that sulfhydryl groups located superficially in the apical membrane are involved.

On the other hand, Lindemann and his coworkers (*cf.* Li & Lindemann, 1983*b*) have found that SH-reactive agents such as *p*-chloromercuribenzoate and *p*-chloromercuriphenyl sulfonate consistently stimulate P_{Na}^m of *R. ridibunda* skin by inhibiting Na self-inhibition and thereby increasing the number of open channels; and, at the same time the macroscopic affinity of amiloride for inhibition of these channels is *increased*. Benos, Mandel and Simon (1980) have also found that PCMB conjugated with dextran stimulates the I_{sc} of *R. catesbeiana* skin but, in this tissue, the affinity for amiloride inhibition is unchanged.

Li and Lindemann (1983*b*) have suggested that the large increase in the macroscopic affinity for amiloride elicited by PCMPS in *R. ridibunda* skin, is consistent with the finding that amiloride and Na appear to compete for the self-inhibition site and that the observations of Benos et al. (1980) are consistent with the finding that in the skin of *R. catesbeiana* the interactions between amiloride and Na are noncompetitive. But, this argument does not appear to hold for rabbit descending colon where: (i) the interactions of amiloride and Na are noncompetitive; (ii) PCMBS relieves Na self-inhibition and at the same time prevents the inhibitory action of amiloride. In other words, in rabbit descending colon, PCMBS (and other organic mercurials) appears to simultaneously affect two sites that are, at least, kinetically distinct.

Clearly, further speculation on the possible mechanisms responsible for the regulation of P_{Na}^m at this time is unwarranted. Suffice it to say that, given the many similarities between the behavior of the Na channels in frog skin, rabbit descending colon, and other tight epithelia, it is tempting to suggest that these differences do not stem from fundamentally different modes of regulation and that unravelling these differences may provide considerable insight into these important regulatory mechanisms.

This research was supported by a grant from the NIH-NIADDK (AM-26690). Dr. Moran was supported by a NIH Training Grant (AM-07408), and Dr. Hudson was the recipient of a NIH Postdoctoral Research Fellowship (AM-06526).

Amiloride was a generous gift from Merck, Sharp and Dohme, Rahway, New Jersey.

The authors gratefully acknowledge fruitful discussions with Drs. J. Lapointe and Klaus Turnheim.

References

Benos, D.J. 1982. Amiloride: A molecular probe of sodium transport in tissues and cells. *Am. J. Physiol.* **242:***C*131–*C*145

Benos, D.J., Mandel, L.J., Balaban, R.S. 1979. On the mechanism of the amiloride-sodium entry site interaction in anuran skin epithelia. *J. Gen. Physiol.* **73:**307–326

Benos, D.J., Mandel, L.J., Simon, S.A. 1980. Effects of chemical group specific reagents on sodium entry and the amiloride binding site in frog skin: Evidence for separate sites. *J. Membrane Biol.* **56:**149–158

Benos, D.J., Simon, S.A., Mandel, L.J., Cala, P.M. 1976. Effect of amiloride and some of its analogues on cation transport in isolated frog skin and thin lipid membranes. *J. Gen. Physiol.* **68:**43–63

Bentley, P.J. 1968. Amiloride: A potent inhibitor of sodium transport across the toad bladder. *J. Physiol. (London)* **195:**317–330

Biber, T.U.L. 1971. Effect of changes in transepithelial transport on the uptake of sodium across the outer surface of the frog skin. *J. Gen. Physiol.* **58:**131–144

Bindslev, N., Cuthbert, A.W., Edwardson, J.M., Skadhauge, E. 1982. Kinetics of amiloride action in the hen copradaeum in vitro. *Pfluegers Arch.* **392:**340–346

Cuthbert, A.W. 1981. Sodium entry step in transporting epithelia: Results of ligand-binding studies. *In*: Ion Transport by

Epithelia. S.G. Schultz, editor. pp. 181–195. Raven Press, New York

Cuthbert, A.W., Shum, W.K. 1975. Effects of vasopressin and aldosterone on amiloride binding in toad bladder epithelial cells. *Proc. R. Soc. London B* **189:**543–575

DeLong, J., Civan, M.M. 1984. Apical sodium entry in split frog skin: Current-voltage relationship. *J. Membrane Biol.* **82:**25–40

Frizzell, R.A., Koch, M.J., Schultz, S.G. 1976. Ion transport by rabbit colon. I. Active and passive components. *J. Membrane Biol.* **27:**297–316

Fuchs, W., Larsen, E.H., Lindemann, B. 1977. Current-voltage curve of sodium channels and concentration dependence of sodium permeability in frog skin. *J. Physiol. (London)* **267:**137–166

Garty, H., Lindemann, B. 1984. Feedback inhibition of sodium uptake in K-depolarized toad urinary bladders. *Biochim. Biophys. Acta* **771:**89–98

Goldman, D.E. 1943. Potential, impedance and rectification in membranes. *J. Gen. Physiol.* **27:**37–60

Gottlieb, G.P., Turnheim, K., Frizzell, R.A., Schultz, S.G. 1978. *p*-Chloromercuribenzene sulfonate blocks and reverses the effect of amiloride on sodium transport across rabbit colon in vitro. *Biophys. J.* **22:**125–129

Helman, S.I., Fisher, R.S. 1977. Microelectrode studies of the active Na transport pathway of frog skin. *J. Gen. Physiol.* **69:**571–604

Henrich, M., Lindemann, B. 1983. Voltage dependence of channel current and channel densities in the apical membrane of toad urinary bladder. *In*: Intestinal Absorption and Secretion. E. Skadhauge and K. Heintze, editors. pp. 209–220. MTP Press, Lancaster

Hodgkin, A.L., Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (London)* **108:**37–77

Klemperer, G., Garcia-Diaz, J.F., Essig, A. 1984. Effects of high serosal K concentration on electrophysiological parameters of frog skin. *J. Gen. Physiol.* **84:**29*a*

Lewis, S.A., Ifskin, M.S., Loo, D.D.F., Diamond, J.M. 1984. Studies of sodium channels in rabbit urinary bladder by noise analysis. *J. Membrane Biol.* **80:**135–151

Li, J.H-Y., Lindemann, B. 1983*a*. Chemical stimulation of Na transport through amiloride-blockable channels of frog skin epithelium. *J. Membrane Biol.* **75:**179–192

Li, J.H-Y., Lindemann, B. 1983*b*. Competitive blocking of epithelial sodium channels by organic cations: The relationship between macroscopic and microscopic inhibition constants. *J. Membrane Biol.* **76:**235–251

Lindemann, B. 1977. A modifier-site model for passive Na^+ transport into frog skin epithelium. *In*: Intestinal permeation. M. Kramer and F. Lauterbach, editors. pp. 217–288. Excerpta Medica, Amsterdam-Oxford

Lindemann, B. 1984. Fluctuation analysis of sodium channels in epithelia. *Annu. Rev. Physiol.* **46:**497–515

Lindemann, B., Gebhardt, U. 1973. Delayed changes of Na-permeability in response to steps of (Na) at the outer surface of frog skin and frog bladder. *In*: Transport Mechanisms in Epithelia. H.H. Ussing and N.A. Thorn, editors. pp. 115–130. Munksgaard, Copenhagen

Lindemann, B., Van Driessche, W. 1977. Sodium-specific membrane channels of frog skin are pores: Current fluctuations reveal high turnover. *Science* **195:**292–294

Luger, A., Turnheim, K. 1981. Modification of cation permeability of rabbit descending colon by sulphydryl reagents. *J. Physiol. (London)* **317:**49–66

Nagel, W., García-Díaz, J.F., Armstrong, W. McD. 1981. Intracellular ionic activities in frog skin. *J. Membrane Biol.* **61:**127–134

Palmer, L.G., 1984*a*. Voltage-dependent block by amiloride and other monovalent cations of apical Na channels in the toad urinary bladder. *J. Membrane Biol.* **80:**153–165

Palmer, L.W. 1984*b*. Use of potassium depolarization to study apical transport properties in epithelia. *Curr. Topics Membr. Transp.* **20:**105–121

Palmer, L.G. 1985. Modulation of apical Na permeability of the toad urinary bladder by intracellular Na, Ca and H. *J. Membrane Biol.* **83:**57–69

Palmer, L.G., Edelman, I.S., Lindemann, B. 1980. Current-voltage analysis of apical sodium transport in toad urinary bladder: Effects of inhibitors of transport and metabolism. *J. Membrane Biol.* **57:**59–71

Saito, T., Yoshida, S. 1984. Effects of biguanides on the short-circuit current in frog skin. *Am. J. Physiol.* **247:***F*277–*F*281

Salako, L.A., Smith, A.J. 1970. Changes in sodium pool and kinetics of sodium transport in frog skin produced by amiloride. *Br. J. Pharmacol.* **39:**99–109

Schultz, S.G., Frizzell, R.A., Nellans, H.N. 1977. Active sodium transport and the electrophysiology of rabbit colon. *J. Membrane Biol.* **33:**351–384

Segel, I.H. 1975. Enzyme Kinetics: Behaviour and Analysis of Rapid Equilibrium and Steady-State Systems. J. Wiley and Sons, Inc., New York

Sudou, K., Hoshi, T. 1977. Mode of action of amiloride in toad urinary bladder: An electrophysiological study of drug action on sodium permeability of the mucosal border. *J. Membrane Biol.* **32:**115–132

Thomas, S.R., Suzuki, Y., Thompson, S.M., Schultz, S.G. 1983. Electrophysiology of *Necturus* urinary bladder: I. "Instantaneous" current-voltage relations in the presence of varying mucosal sodium concentrations. *J. Membrane Biol.* **73:**157–175

Thompson, S.M., Suzuki, Y., Schultz, S.G. 1982*a*. The electrophysiology of rabbit descending colon: I. Instantaneous transepithelial current-voltage relations and the current-voltage relationship of the Na-entry mechanism. *J. Membrane Biol.* **66:**41–54

Thompson, S.M., Suzuki, Y., Schultz, S.G. 1982*b*. The electrophysiology of rabbit descending colon: II. Current-voltage relations of the apical membrane, the basolateral membrane, and the parallel pathways. *J. Membrane Biol.* **66:**55–61

Turnheim, K., Frizzell, R.A., Schultz, S.G. 1977. Effect of anions on amiloride-sensitive, active sodium transport across rabbit colon, *in vitro*. *J. Membrane Biol.* **37:**63–84

Turnheim, K., Frizzell, R.A., Schultz, S.G. 1978. Interaction between cell sodium and the amiloride-sensitive sodium entry step in rabbit colon. *J. Membrane Biol.* **39:**233–256

Turnheim, K., Luger, A., Grasl, M. 1981. Kinetic analysis of the amiloride-sodium entry site interaction in rabbit colon. *Molec. Pharmacol.* **20:**543–550

Turnheim, K., Thompson, S.M., Schultz, S.G. 1983. Relation between intracellular sodium and active sodium transport in rabbit colon. *J. Membrane Biol.* **76:**299–309

Van Driessche, W., Lindemann, B. 1979. Concentration dependence of currents through single sodium-selective pores in frog skin. *Nature (London)* **282:**519–520

Wills, N.K., Lewis, S.A., Eaton, D.C. 1979. Active and passive properties of rabbit descending colon: A microelectrode and nystatin study. *J. Membrane Biol.* **45**:81–108

Zeiske, W., Lindemann, B. 1974. Chemical stimulation of Na current through the outer surface of frog skin epithelium. *Biochim. Biophys. Acta* **352**:323–326

Zeiske, W., Wills, N., Van Driessche, W. 1982. Na^+ channels and amiloride-induced noise in the mammalian colon. *Biochim. Biophys. Acta* **688**:201–210

Received 9 January 1985; revised 23 April 1985

Appendix

The results of the studies by Thompson et al. (1982*b*) are consistent with a general model of the resistive elements of rabbit descending colon illustrated in Fig. A1; r^m_{Na} is the slope resistance of the apical membrane to Na, r^m_i is the lumped slope resistance of conductive pathways to ions i across the apical membrane in parallel with the Na entry pathway, r^s is the lumped resistance of the basolateral membrane and r^p is the lumped resistance of all pathways, cellular or paracellular, that are electrically isolated from and in parallel with the amiloride-sensitive absorptive cells. A similar model has been proposed by Wills et al. (1979).

Now if $r^m_i \gg r^m_{Na}$, then the transcellular resistance, r^c, is given by

$$r^c = (r^m_{Na} + r^s) = [r_t r'_t/(r'_t - r_t)] \tag{A1}$$

where r_t is the total transepithelial resistance in the absence of amiloride and r'_t is the total transepithelial resistance in the presence of a maximally effective concentration of amiloride; the latter is generally assumed to provide a good approximation of r^p [*cf.* Thomas et al., 1983].

However, if r^m_{Na} and r^m_i do not differ markedly (as is the case for rabbit descending colon), the situation becomes considerably more complex and, in general, (assuming that r^m_i, r^s and r^p are not affected by amiloride)

$$[r_t r'_t/(r'_t - r_t)] = (r^m_{Na} r^m_i + r^s(r^m_{Na} + r^m_i))(r^m_i + r^s)/r^{m^2}_i. \tag{A2}$$

When r^s is very small compared to r^m_{Na} and r^m_i (i.e., approaches zero), Eq. (A2) reduces to

$$r_t r'_t/(r'_t - r_t) = r^m_{Na} = 1/g^m_{Na}. \tag{A3}$$

In the present experiments, the values of r_t in the absence of amiloride and in the presence of submaximal inhibitory concentrations of amiloride were obtained from the value of I^{ms} needed to displace ψ^{ms} by 10 mV; r'_t was obtained from the value of I^{ms} necessary to displace ψ^{ms} by 10 mV in the presence of 10^{-4} M amiloride. In Fig. A2 we have plotted the calculated values of $[(r'_t - r_t)/r_t r'_t]$ designated by g, the computed values of P^m_{Na} (assuming that ψ^{cs} and r^s are zero) and the observed values of ${}_0J_{Na}$ *vs.* $-\log$ [A] in the absence of amiloride and in the presence of increasing concentrations of amiloride when $(Na)_m = 100$ mM. Clearly there is a close parallelism between the effects of graded concentrations of amiloride on all of these parameters; the concentrations of amiloride needed to halve each of these parameters are given in the inset and are virtually identical.

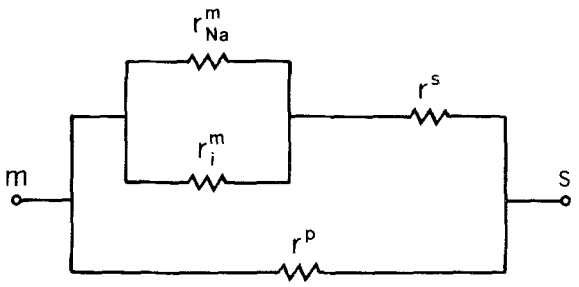

Fig. A1. Model of resistive elements of rabbit descending colon (Thompson et al., 1982*b*)

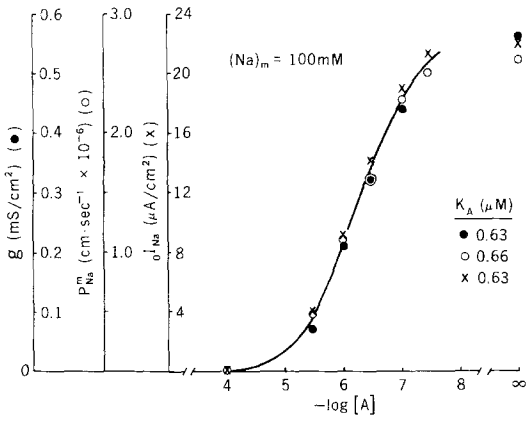

Fig. A2. Relations among g, P^m_{Na} and ${}_0J_{Na}$ and $-\log(A)_m$ when $(Na)_m = 100$ mM

Identical relations were observed when $(Na)_m$ was 32 and 18 mM.

The close parallelism between the effects of graded concentrations of amiloride on g and ${}_0J_{Na}$ strongly suggests that $g \approx g^m_{Na}$ and therefore that r^s, under these experimental conditions (*compare* Eqs. (A2) and (A3)), is negligible. The same conclusion can be drawn from the parallelism between the effects of submaximal doses of amiloride on the relation between g and P^m_{Na}.

In short, these findings strongly support the contention that in the presence of a high-K serosal solution, r^s is negligible so that the behavior of the cell approaches that of a single resistive barrier.