Sodium Transport Effects on the Basolateral Membrane in Toad Urinary Bladder

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ABSTRACT In toad urinary bladder epithelium, inhibition of Na transport with amiloride causes a decrease in the apical (V_{mc}) and basolateral (V_{cs}) membrane potentials. In addition to increasing apical membrane resistance (R_a), amiloride also causes an increase in basolateral membrane resistance (R_b), with a time course such that R_a/R_b does not change for 1–2 min. At longer times after amiloride (3–4 min), R_a/R_b rises from its control values to its amiloride steady state values through a secondary decrease in R_b. Analysis of an equivalent electrical circuit of the epithelium shows that the depolarization of V_{cs} is due to a decrease in basolateral electromotive force (V_b). To see if the changes in V_{cs} and R_b are correlated with a decrease in Na transport, external current (I_e) was used to clamp V_{mc} to zero, and the effects of amiloride on the portion of I_e that takes the transcellular pathway were determined. In these studies, V_{cs} also depolarized, which suggests that the decrease in V_b was due to a decrease in the current output of a rheogenic Na pump. Thus, the basolateral membrane does not behave like an ohmic resistor. In contrast, when transport is inhibited during basolateral membrane voltage clamping, the apical membrane voltage changes are those predicted for a simple, passive (i.e., ohmic) element.

INTRODUCTION

In toad urinary bladder epithelium, both the apical membrane potential (V_{mc}) and the basolateral membrane potential (V_{cs}) depolarize after mucosal addition of amiloride and hyperpolarize after the introduction of Na to a Na-free mucosal bath (Reuss and Finn, 1975*b*; Sudou and Hoshi, 1977). Because the change in V_{cs} is in a direction opposite to that expected of a decrease in internal current dropping across a passive resistor, Finn and Reuss (1978) ascribed the changes in V_{cs} to the deactivation or activation of a rheogenic Na pump during Na transport inhibition or stimulation, respectively. Not noted, however, was the fact that the ratio of apical (R_a) to basolateral (R_b) membrane resistances is initially unchanged after Na transport inhibition or stimulation even though total tissue resistance is increased or decreased, respectively (see

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Figs. 1 and 2, Reuss and Finn, 1975*b*; Fig 1, Finn and Reuss, 1978). Since amiloride has no effect on shunt resistance, the rapid increase in transepithelial resistance indicates an equally rapid increase in cell resistance. This increase, coupled with the initial constancy of R_a/R_b can only mean that R_a and R_b increase simultaneously. Since R_b increases during transport inhibition, it is possible that all or part of the change in V_{cs} during this time is related in some way to the resistance change.

Observations in this and other tissues have indicated that R_b may change in concert with Na transport. Frömter and Gebler (1977) observed that R_b was high in *Necturus* urinary bladder when the short-circuit current was spontaneously low; Nagel and Crabbé (1980) observed a close correlation between basolateral membrane conductance and short-circuit current in aldosterone-stimulated toad skin; Narvarte and Finn (1980*a*) showed that in toad urinary bladder R_b increases after mucosal [Na] reductions; and Warncke and Lindemann (1981) found in the same tissue that R_b decreases after Na transport stimulation by antidiuretic hormone. In this paper, we examine in toad urinary bladder the time course of the changes in cell current and R_b during Na transport inhibition in both the open-circuit state and when the apical membrane is voltage clamped. The results indicate that both the basolateral membrane emf and R_b change during Na transport inhibition, but these changes are temporally separate in their effects on V_{cs}.

Preliminary reports from this study have been presented elsewhere (Davis and Finn, 1980*a*, *b*, 1982).

METHODS

Urinary bladders were removed from doubly pithed toads (*Bufo marinus*) of Dominican or Mexican origin (Jacques Weil Co., Rayne, LA, or National Reagents, Bridgeport, CT). The tissues were mounted on a nylon mesh with the mucosal side upward and placed in a Lucite chamber (exposed area = 4.9 cm^2) as previously described (Reuss and Finn, 1974). The mucosal side was continuously perfused with Ringer's solution through paired pipettes radially arranged 120° to one another and to a vacuum pickup pipette. A second pair of pipettes, placed side by side with the first, was used to deliver experimental solutions: the choice of solutions was controlled by an electronically actuated pinch valve (Angar Scientific, Cedar Knolls, NJ).

Solutions

The Ringer's solution had the following composition (mM): 109 NaCl, 2.5 KCl, 2.4 $NaHCO_3$, 0.9 $CaCl_2$; it was gassed with room air and had a pH of ~8.4. Amiloride (a gift from Merck, Sharp, and Dohme, West Point, PA) was added to the mucosal solution to yield a final concentration of 10^{-4} M.

Electrical Measurements

POTENTIALS The transepithelial potential (V_{ms}) was measured with silver-silver chloride electrodes that contacted the serosal and mucosal solutions via Ringer-filled bridges. Current was passed across the epithelium through ring-shaped platinum-iridium electrodes placed directly in the serosal and mucosal solutions.

Apical (V_{mc}) and basolateral (V_{cs}) membrane potentials were measured with glass microelectrodes prepared by pulling glass tubing (1 mm OD, 0.58 mm ID; WP Instruments, Inc., Hamden, CT) containing an internal glass fiber. The electrodes

were filled with 4 M potassium acetate and bevelled (Brown and Flaming, 1974) to a final resistance of 10–20 MΩ; tip potentials were <8 mV. Impalements were performed with a motor-driven micromanipulator after prepositioning the electrode with the aid of an inverted phase-contrast microscope (Leitz Wetzlar, Federal Republic of Germany). Criteria for successful impalements were as previously described (Reuss and Finn, 1974). The mucosal solution was taken as ground.

All electrodes were connected to a voltage-current clamp device (input impedances $>10^{12}$ Ω) that had the capability of current-clamping transepithelially or clamping the potential between any two of the three potential-sensing electrodes. Regardless of the particular potential being clamped, the voltage clamp was achieved by the passage of current across the entire epithelium. The desired current and potential outputs of the clamp device were displayed on a storage oscilloscope (R5103N; Tektronix, Inc., Beaverton, OR) and sampled by a computer (Med-80; Nicolet Instruments, Madison, WI; or MINC-11; Digital Equipment Corp., Maynard, MA). The command input to the voltage-current clamp was achieved with a stimulator (model 302-T; WP Instruments Inc., Hamden,CT) or the MINC-11.

Total tissue resistance (R_t) was calculated from the deflection in V_{ms} caused by the passage of a transepithelial constant current pulse (2.5–5.0 $\mu A \cdot cm^{-2}$) or from the change in current and V_{ms} resulting from a brief (<500-ms) change in clamp command voltage.[1] The ratio of apical to basolateral membrane resistances was calculated as the ratio $\Delta V_{mc}/\Delta V_{cs}$, measured during the passage of transepithelial current or a brief change in clamp command voltage. The shunt resistance (R_s) was taken to equal total tissue resistance in the steady state after the mucosal addition of 10^{-4} M amiloride (Ussing and Windhager, 1964).

According to the equivalent circuit for the epithelium shown in Fig. 1,

$$R_t = \frac{(R_a + R_b)R_s}{R_a + R_b + R_s}. \tag{1}$$

Thus, from R_t, R_s, and R_a/R_b, the resistances of the apical and basolateral membranes can be calculated:

$$R_a = \frac{(R_a/R_b)R_sR_t}{(R_a/R_b + 1)(R_s - R_t)}, \quad \text{and} \tag{2}$$

$$R_b = \frac{R_sR_t}{(R_a/R_b + 1)(R_s - R_t)}. \tag{3}$$

The validity of these calculations rests on the assumptions that there is a negligible cellular conductance in the presence of high concentrations of amiloride and that R_s remains constant during Na transport inhibition with amiloride.

In Fig. 1, the current source of a voltage clamp is incorporated in the equivalent circuit. Total currents through the clamp (I_e), the shunt (I_s), and the cellular (I_c)

[1] Measurement of resistance in this manner may lead to an error in the determination of the membrane emf's if the current-voltage curve of the membrane is nonlinear. This arises from the fact that Eqs. 4 and 5 are strictly true only if the chord resistance is used instead of the actually measured slope resistance (Finkelstein and Mauro, 1977). However, since our measurements of resistance are made under open-circuit conditions where the membrane potentials are not far from the predicted sodium and potassium equilibrium potentials, and since in this tissue the resistance at open circuit is not different from that at short circuit, the current-voltage curve must be nearly linear in this range. Thus, slope and chord resistances will be equal or nearly so, and emf's calculated from Eqs. 4–8 will not be subject to this kind of error.

limbs are depicted as arrows, the heads of which indicate the direction of positive current flow. For example, cell current in the M-to-S direction is defined as positive. Each cell membrane of the epithelium is represented as a battery in series with a resistor. In the experiments reported here, identical Ringer's solution bathes both sides of the tissue, so that the emf of the paracellular shunt is assumed to be zero. Under open-circuit conditions, the following equations define the measured potentials:

$$V_{mc} = V_a - I_c R_a, \quad \text{and} \tag{4}$$

$$V_{cs} = V_b - I_c R_b. \tag{5}$$

The only current that flows in open circuit is the internal current (I_i), the source of which is the membrane emf's. Since $I_e = 0$, all other currents are identical, i.e., $I_i =$

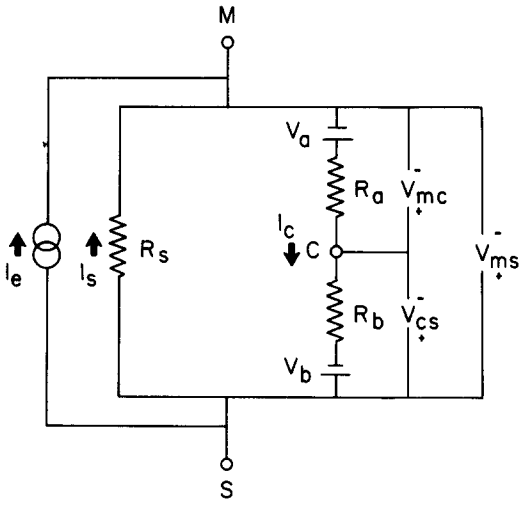

FIGURE 1. An equivalent electrical circuit representing toad urinary bladder epithelium with the current source of a voltage clamp incorporated. V_a, R_a, V_b, and R_b are emf's and resistances of the apical and basolateral cell membranes, respectively, and R_s is the resistance of the paracellular or shunt path. V_{ms}, V_{mc}, and V_{cs} are measured potentials across the epithelium, and the apical and basolateral membranes, respectively. Arrows represent net currents: I_e, external or clamping current; I_c, I_s, currents in the cell and shunt paths, respectively.

$I_c = I_s$. This current can be measured because its driving force through the shunt path is only the transepithelial potential:

$$I_s = V_{ms}/R_s. \tag{6}$$

Thus, the emf's can be calculated from the measured values by substituting Eq. 6 into Eqs. 4 and 5:

$$V_a = V_{mc} + V_{ms}R_a/R_s, \quad \text{and} \tag{7}$$

$$V_b = V_{cs} + V_{ms}R_b/R_s. \tag{8}$$

Under closed-circuit conditions (that is, whenever current flows in the external circuit; this occurs whenever the tissue is clamped at a potential other than the spontaneous open-circuit value), current flows from two sources, the clamp circuit and the membrane emf's. Under these conditions, it cannot be assumed that the internal current is identical to that seen under open-circuit conditions. This is not a limitation in the present experiments, however, since we do not study the tissues in the transition from open- to closed-circuit conditions.

The external current is partitioned into cellular (I_e^c), and shunt (I_e^s) components:

$$I_e = I_e^c + I_e^s. \tag{9}$$

The partitioning is determined solely by the relative resistances of the cellular and shunt pathways and is given by:

$$I_e^c = \frac{R_s I_e}{R_a + R_b + R_s}. \tag{10}$$

The total currents in each limb are given as the algebraic sums of the individual currents:

$$I_c = I_i + I_e^c; \tag{11}$$

$$I_s = I_i - I_e^s. \tag{12}$$

The total shunt current (under both open- and closed-circuit conditions) is given by Eq. 6; combining this with Eq. 12, we have

$$I_i = V_{ms}/R_s + I_e^s. \tag{13}$$

From Eqs. 9, 11, and 12 (or as deduced directly from Fig. 1 by Kirchhoff's current law):

$$I_c = I_e + I_s. \tag{14}$$

Finally, the emf's under closed-circuit conditions can be calculated by substituting Eqs. 6 and 14 into Eqs. 4 and 5:

$$V_a = V_{mc} + I_e R_a + V_{ms} R_a/R_s, \quad \text{and} \tag{15}$$

$$V_b = V_{cs} + I_e R_b + V_{ms} R_b/R_s. \tag{16}$$

Experimental Protocol

In these experiments, we were concerned with the changes in cell membrane potentials and resistances and in the internal and external currents, during the transition between a Na-transporting steady state and a nontransporting steady state. After cell impalement, in open circuit or with the apical membrane voltage clamped, the mucosal superfusate was switched to one containing amiloride, and the changes in the desired parameters were observed. Several minutes later, with amiloride still present, R_s was estimated as noted above. With this value, currents, cell membrane resistances, and emf's were calculated from the preceding record for each period in which a transepithelial current pulse or a change in the voltage-clamp command had occurred (see Figs. 2 and 8).

RESULTS

In 10 tissues, R_t and R_s were 8.5 ± 0.8 (mean ± SE) and 15.1 ± 1.5 kΩ·cm^2, respectively. V_{ms} was sufficiently high (64.9 ± 5.2 mV) to assume negligible apical membrane conductance in the presence of saturating doses of amiloride (Narvarte and Finn, 1980*a*).[2] Additional observations on the effects of amiloride on cell membrane potentials and R_a/R_b under open- and closed-circuit conditions were made on more than 20 tissues, all with $V_{ms} > 30$ mV and $R_t > 3$ kΩ·cm^2.

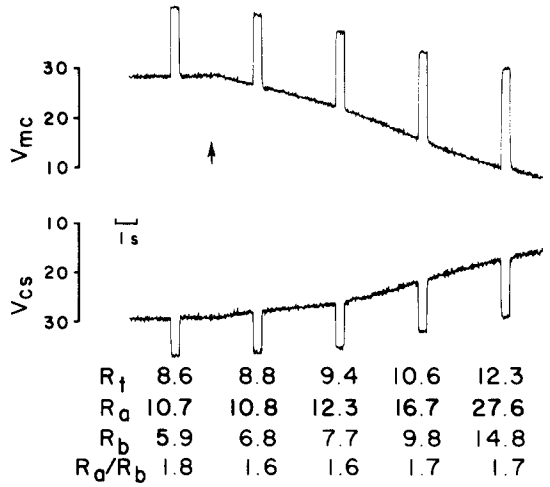

FIGURE 2. Open circuit: effects of amiloride on cell membrane potentials. The record starts with a microelectrode in a cell; amiloride is added at the arrow. Deflections in the traces are the voltage responses to periodic constant current pulses. In the tabular portion of the figure, the circuit resistances are given, with each column representing those calculated for its associated current pulse. The resistances were calculated using a value of R_s (17.8 kΩ·cm^2) measured at steady state after the termination of intracellular data collection. Units: potentials, mV; resistances, kΩ·cm^2. (Reprinted from Davis and Finn [1982] with the permission of *Science* [copyright 1982 by the American Association for the Advancement of Science].)

Effects of Amiloride on Cell Membrane Electrical Parameters: Open Circuit

When the mucosal superfusate is switched from Ringer to Ringer plus amiloride, both cell membrane potentials, V_{mc} and V_{cs}, depolarize (Fig. 2; Reuss and Finn, 1975*b*). In the experiment depicted in the figure, I_c decreased from 3.2 to 1.6 μA·cm^{-2} during the period of intracellular recording and was

[2] Although the resistance of the cell pathway is very high under these conditions, enough of the external current crosses the cells to allow sizeable voltage deflections across both cell membranes; in fact, only very small currents would be necessary, because of high resistances. In addition, if one assumes that a "typical" tissue has values given by the means listed (R_t = 8.5 kΩ·cm^2 before, and 15.1 after, amiloride), then in the control state, R_t = 8.5, ($R_a + R_b$) = 19.4, and R_s = 15.1. If as much as 10% of the cell conductance remains 5 min after adding amiloride, the calculated control values for ($R_a + R_b$) and R_s become 17.5 and 16.5, respectively. Such a discrepancy is too small to affect any of our conclusions.

zero in the steady state following amiloride addition. The decrease in I_c would, if it were the only change, cause a hyperpolarization of V_{cs} (see Eq. 5 and Fig. 1). A second unexpected change in the basolateral membrane was that the response of V_{cs} to an intermittent transepithelial current pulse (the magnitude of which remained unchanged throughout the experiment) increased after amiloride. This increase in ΔV_{cs} after amiloride, at a time when the proportion of applied current that takes the cellular pathway is decreased (because of the effect of amiloride on cell resistance), indicates an increase in R_b; that R_a/R_b does not change initially after amiloride indicates that R_a and R_b increase with the same time course.[3] According to the calculations (Fig. 2), R_b increased from 5.9 to 14.8 $k\Omega \cdot cm^2$ during the course of the record. Both membrane emf's decreased after the application of amiloride. In the record depicted in

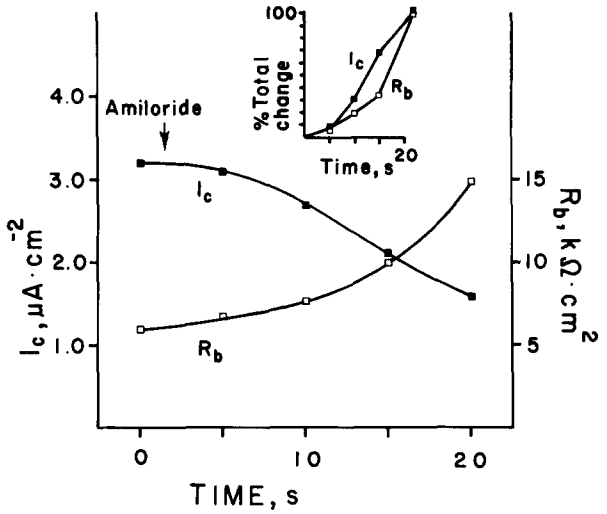

FIGURE 3. Open circuit: effects of amiloride on cell current and basolateral membrane resistance. I_c and R_b were calculated from data in Fig. 2. Inset: I_c and R_b are plotted as percentages of their total respective changes to emphasize their temporal relationship.

Fig. 2, V_a decreased from 62.8 to 49.7 mV and V_b decreased from 47.9 to 40.2 mV.

Finn and Reuss (1978) and Narvarte and Finn (1980*a*) attributed the depolarization of V_{cs} to a decrease in pump rheogenicity. However, since under open-circuit conditions (Eq. 5)

$$V_{cs} = V_b - I_c R_b,$$

either a decrease in emf or an increase in resistance could cause a decrease in V_{cs}. The changes in I_c and R_b, calculated from the data shown in Fig. 2, are plotted in Fig. 3 as functions of time after amiloride addition. The figure

[3] In nine tissues, the change in R_a/R_b from a control value of 1.5 ± 0.2, measured 2 s after amiloride application, was 0.04 ± 0.17 (not significantly different from zero); in the same period R_t increased by 44 ± 15%.

depicts a consistent observation: the changes in I_c and R_b follow different time courses, with I_c decreasing faster than R_b increases during transport inhibition (see Fig. 3, inset). An ohmic response of the basolateral membrane potential to the initial decrease in current would be a hyperpolarization. However, V_{cs} depolarizes, which indicates depolarization of V_b in addition to the change in the *I-R* drop.

Fig. 4 shows the results of an experiment in which the tissue was initially superfused by Ringer plus amiloride. Upon the removal of amiloride, both V_{mc} and V_{cs} hyperpolarize, and both ΔV_{mc} and ΔV_{cs} decrease, with R_a/R_b again remaining essentially constant for the duration of the record. Since the proportion of applied current that takes the transcellular path is increased after amiloride removal, the decrease in ΔV_{cs} indicates a decrease in R_b. Values for individual cell membrane resistances cannot be obtained in these experiments because when $R_s \approx R_t$, as in an amiloride steady state, R_a and R_b become immeasurably large (see Eqs. 2 and 3).

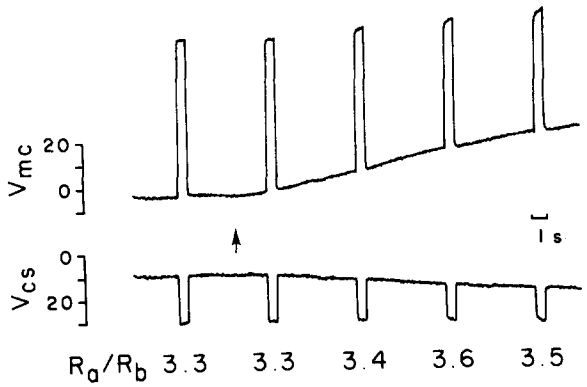

FIGURE 4. Open circuit: effects of amiloride removal on cell membrane potentials. The record starts with a microelectrode in a cell; amiloride had been added to the mucosal medium before the impalement. At the arrow, the superfusate is switched to Ringer without amiloride.

Mean steady state values for R_a/R_b reported previously from this laboratory were 1.4–1.7 for urinary bladders bathed by Ringer and 4.5–5.5 for those exposed to mucosal Na-free Ringer or to amiloride (Reuss and Finn, 1974; Narvarte and Finn, 1980*a*). Sudou and Hoshi (1977) reported values of 2.0 and 4.5, respectively, for *Bufo vulgaris* urinary bladder. Prolonged microelectrode impalements in four tissues were used to answer the question of when, after amiloride, R_a/R_b changes from low to high values. In all of them, R_a/R_b remained constant for 1–2 min, then changed to high values. This change was a gradual process consuming some 2–3 min in two tissues. In the others (Fig. 5), the transition occurred more rapidly.

Cell Membrane Voltage Clamp: Baseline Data

Under closed-circuit conditions ($I_e \neq 0$), changes in external current can be equated with changes in Na transport. Therefore, to investigate the relation-

ships between V_{cs}, R_b, and Na transport, we voltage clamped the apical membrane with external current and determined the effect of amiloride on the clamping current (below).

Fig. 6 depicts the steady state cell membrane parameters in open circuit

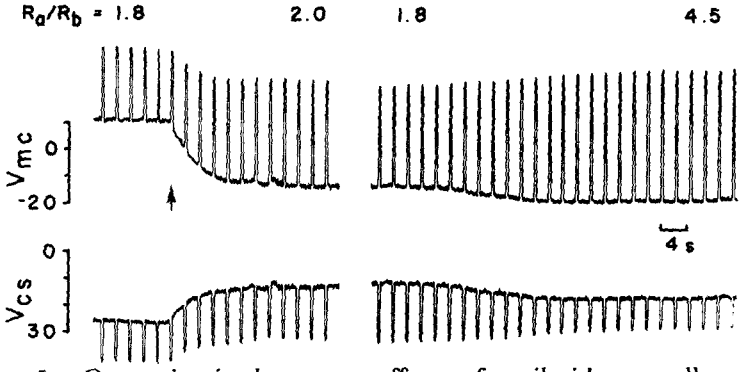

FIGURE 5. Open circuit: long-term effects of amiloride on cell membrane potentials and the ratio of resistances (R_a/R_b). The record starts with a microelectrode in a cell; amiloride is added at the arrow. The gap in the record represents an elapsed time of 90 s.

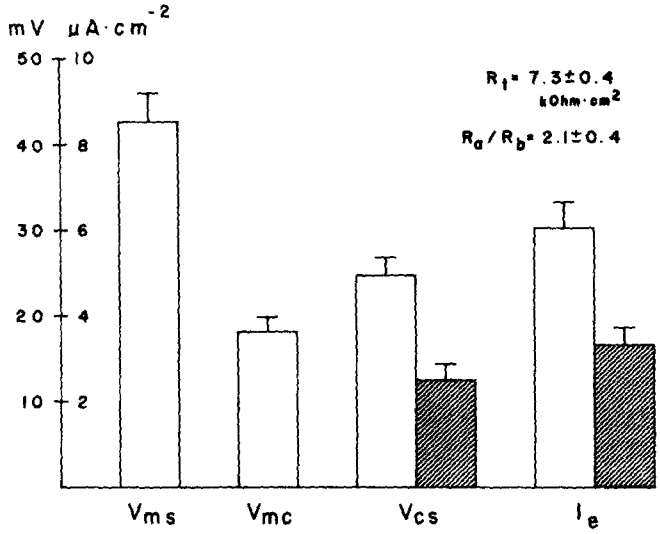

FIGURE 6. Open and closed circuit: a comparison of cell membrane steady state electrical parameters in open circuit (open bars) and in the same cells in closed circuit (shaded bars), where external current is used to voltage clamp the apical membrane potential (V_{mc}) to zero. Data are presented as the mean ± SE (13 impalements in 3 tissues).

and in the same cells with V_{mc} clamped to zero. The current (I_e) needed to hold V_{mc} at 0 mV was approximately one-half the short-circuit current. The clamping current caused a depolarization of V_{cs} to a value about one-half of its open-circuit value. Both of these changes from the open-circuit state are

consistent with the ratio of resistances, R_a/R_b, of 2.1, measured in these tissues (Fig. 6). As shown in Fig. 7, increasing I_e (by decreasing the V_{mc} command voltage below open-circuit potential) causes a depolarization of V_{cs}, whereas decreasing I_e has the opposite effect. Thus, these changes in external current have the predicted effects on V_{cs}, based on an ohmic response of R_b.

Effects of Amiloride on Cell Membrane Parameters: Voltage-Clamp Data

Results quite different from those in Fig. 7 were obtained when external current was decreased by inhibiting transport with V_{mc} held constant. Fig. 8 shows the results of one such experiment in which V_{mc} was clamped to zero for the duration of the record, and the effects of amiloride on the V_{mc}-clamping

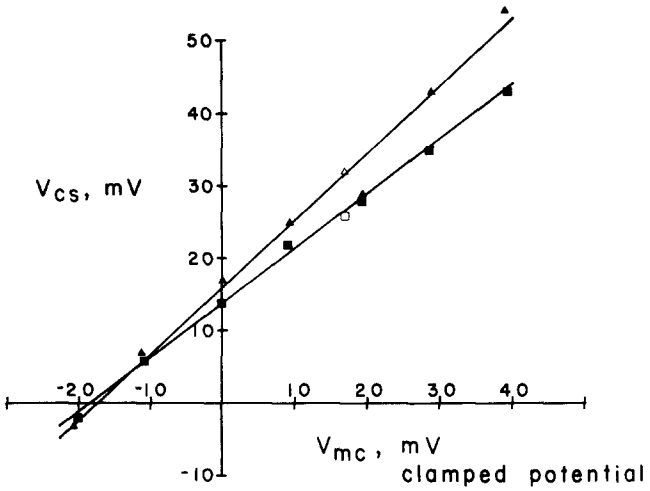

FIGURE 7. Closed circuit: effects of voltage clamping the apical membrane on the basolateral membrane potential (V_{cs}). V_{mc} was varied by varying the voltage-clamp command, and the effect of the V_{mc}-clamping current on V_{cs} was examined. Data are from two cells in the same preparation. Open symbols designate open circuit potentials in the respective cells.

current and V_{cs} were recorded. When the tissue was exposed to amiloride, the current necessary to maintain V_{mc} at zero was reduced. An ohmic response of V_{cs} to the decrease in I_e would be a hyperpolarization, as shown in Fig. 7; as Fig. 8 shows, however, V_{cs} depolarizes slightly. In all tissues studied, V_{cs} remained constant or depolarized during the amiloride inhibition of V_{mc}-clamping current; hyperpolarization of V_{cs} has never been observed under these conditions. This same behavior of V_{cs} during inhibition of V_{mc} -clamping current is observed when V_{mc} is clamped to potentials other than zero, in either the positive or negative direction, when graded amounts of ouabain are used to inhibit the Na pump progressively (12 observations in 2 tissues), or when the serosal potassium concentration is increased to 10 mM (6 observations in 2 tissues).

The deflections in the current trace (ΔI_e) of Fig. 8 represent the current responses to brief changes in the V_{mc}-clamp command from 0 to 20 mV. Amiloride caused a reduction in ΔI_e, consistent with the increase in R_a. Note, however, that ΔV_{cs}, responding to ΔI_e, is not reduced as would be expected of a simple resistor at the basolateral membrane. The constancy of ΔV_{cs} in these experiments again indicates an increase in R_b as Na transport is inhibited. In the record shown in Fig. 8, R_b increased from 7.8 $k\Omega \cdot cm^2$ before amiloride addition to 33.1 $k\Omega \cdot cm^2$ at the end of the record, similar to the results in open circuit in the same tissue (Fig. 2).

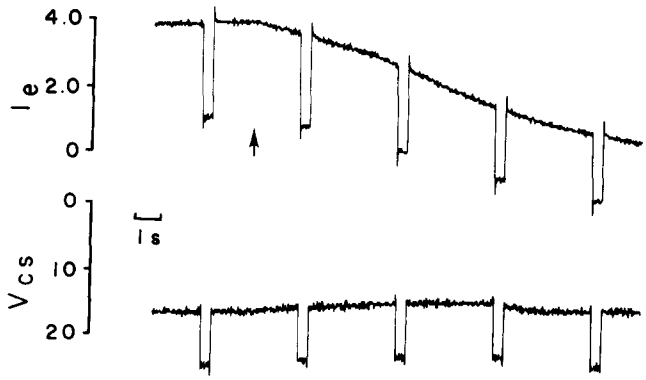

FIGURE 8. Closed circuit, $V_{mc} = 0$: effects of amiloride on external clamping current (I_e) and basolateral membrane potential (V_{cs}) when V_{mc} is clamped to zero. Deflections in the traces represent the current and potential responses to brief changes in the V_{mc}-clamp command from 0 to 20 mV. In the tabular portion of the figure, the circuit resistances are given as in Fig. 2 (R_s = 16.1 $k\Omega \cdot cm^2$). The tissue is the same as in Fig. 2.

In Fig. 9, the changes in I_e^c, I_i, I_c, and R_b calculated from the data in Fig. 8 are plotted as functions of time after amiloride. The figure shows that the currents in the cellular pathway follow time courses after amiloride that are quite different from that of R_b, with all the currents undergoing their major decreases before the major change in R_b.

Both cell membrane emf's decrease during transport inhibition, similar to their behavior in open circuit, but then increase to very high values. The increases occur at the same time as the late rapid rise in membrane resistances and are thus related to the artifactual rise in calculated membrane resistances as R_t approaches R_s (see above and Eqs. 7 and 8).

Thus, our data indicate that the basolateral membrane exhibits nonohmic

behavior during Na transport inhibition. To see whether the apical membrane behaves in a less complex way, we clamped V_{cs} instead of V_{mc} and studied the effects of amiloride on the V_{cs}-clamping current and V_{mc}. Fig. 10 shows the results of one such experiment. At the beginning of the record, the mucosal

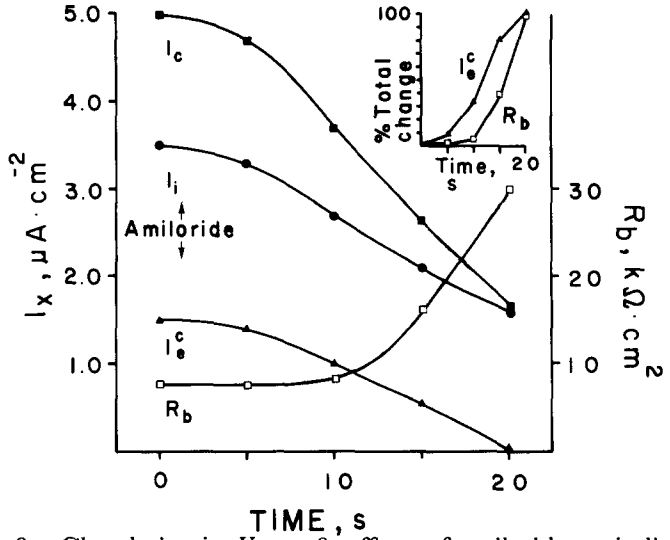

FIGURE 9. Closed circuit, $V_{mc} = 0$: effects of amiloride on individual (I_i, I_e^c) and net (I_c) transcellular currents and on basolateral membrane resistance. Data are calculated from data in Fig. 8. Inset: I_e^c and R_b are plotted as percentages of their total respective changes to emphasize their temporal relationship.

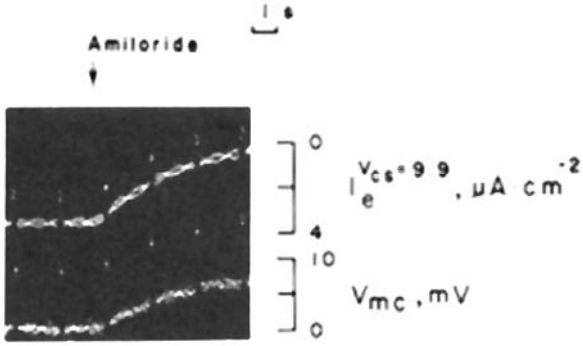

FIGURE 10. Closed circuit, $V_{cs} = 0$: effects of amiloride on external clamping current (I_e) and apical membrane potential (V_{mc}) when V_{cs} is clamped to a value (9.9 mV) such that $V_{mc} = 0$ at the beginning of the record. Deflections in the traces represent current and potential responses to brief 10-mV changes of the V_{cs}-clamp command.

surface is superfused with Ringer. Instead of clamping V_{cs} to 0 mV, we clamped it to a value such that V_{mc} was depolarized to 0 mV at the beginning of the experiment. This maneuver allowed the initial conditions of these experiments to approximate those in the V_{mc}-clamp experiments; however, in

the V_{cs}-clamp experiments, V_{mc} is "floating" (i.e., free to change), rather than V_{cs}. The results shown in Fig. 10 are typical: when the superfusate is switched to Ringer plus amiloride, the V_{cs}-clamping current is decreased and V_{mc} responds to the change in I_e in a way predicted for an ohmic resistor, i.e., it hyperpolarizes. R_b increases in these experiments, as in the V_{mc}-clamp and the open-circuit experiments, as seen from the decreasing ΔI_e in response to the intermittent changes in the V_{cs}-clamp command voltage (10 mV). Since R_b changes even when V_{cs} is held constant as transport is inhibited by amiloride, the change in basolateral membrane resistance cannot be attributed simply to a change in voltage.

DISCUSSION

Correlation between Na Transport and Basolateral Membrane Potential

In epithelial tissues such as *Necturus* urinary bladder (Frömter and Gebler, 1977) and rabbit colon (Schultz et al., 1977), mucosal application of amiloride under open-circuit conditions causes a hyperpolarization of V_{cs}, as would be predicted from a decrease in internal current flow (see Fig. 1 and Eq. 5). As first observed by Reuss and Finn (1975*a*, *b*) and later by Sudou and Hoshi (1977), however, this is not the case for toad urinary bladder. From the data in Fig. 2, we calculate (from Eq. 6, with $I_c = I_s$ at open circuit) that for each millivolt change in V_{ms}, the change in I_c would be 5.6×10^{-2} $\mu A/cm^2$ which, if there were no changes in R_b and V_b after amiloride, would hyperpolarize V_{cs} by 0.33 mV. As shown by Reuss and Finn (1975*b*), and in Fig. 2, however, V_{cs} depolarizes after amiloride. Thus, the change in V_{cs} in toad urinary bladder after amiloride addition cannot be explained solely by a decrease in internal current.

The observed changes in V_{cs} seem to be correlated with changes in transepithelial Na transport, a notion that is supported by the following experimental observations: (*a*) abrupt addition of amiloride causes rapid decreases in both short-circuit current (Bentley, 1968) and open-circuit V_{cs} (Reuss and Finn, 1975*b*; Fig. 2); (*b*) graded reductions of mucosal Na in the steady state lead to graded decreases in V_{cs} (Reuss and Finn, 1975*b*; Narvarte and Finn, 1980*a*); (*c*) increasing apical membrane Na conductance (and transepithelial sodium transport) by substituting I^- for Cl^- in the mucosal medium causes an increase in V_{cs} (in the absence of amiloride), whereas decreasing apical Na conductance by substituting $SO_4^=$ for Cl^- causes a decrease in V_{cs} (Narvarte and Finn, 1980*b*); (*d*) constant transepithelial current applied in a direction that would increase cellular Na entry across the apical membrane enhances the hyperpolarizing V_{cs} response to the introduction of Na to a Na-free mucosal medium, and opposing current inhibits the V_{cs} response (Finn and Reuss, 1978); (*e*) after suppression of Na transport by amiloride, V_{cs} does not change when V_{mc} is hyperpolarized by replacing mucosal Cl^- with the less permeant I^- (Narvarte and Finn, 1980*b*); and (*f*) an amiloride-induced decrease in clamping current (V_{mc}-clamp) has either no effect on V_{cs} or depolarizes it (Figs. 8 and 9, and see below). Possible mechanisms for these changes in V_{cs} are discussed below.

Basolateral Membrane Resistance

The method of measurement of cell membrane resistances used in this study requires the assumption that in the presence of maximal concentrations of amiloride cellular conductance is negligible and shunt conductance is unchanged from normal. The former condition is approached in toad urinary bladders with baseline $V_{ms} > 50$ mV (Narvarte and Finn, 1980*a*), a condition met for tissues used in our cell membrane resistance studies (range: 54–100 mV). That shunt resistance is unchanged with amiloride is shown by the observation that the drug does not affect the serosa-to-mucosa (paracellular) fluxes of K, Na, and Cl (Hong and Essig, 1976; O'Neil and Helman, 1976; A. L. Finn, unpublished data). It would thus seem that the estimation of R_s as R_t in the steady state following amiloride addition is valid, and indeed, calculation of cell membrane resistances using this method gives values that agree favorably with those derived by other means (Finn et al., 1980).

Frömter and Gebler (1977) and Nagel and Crabbé (1980) have observed a correlation between R_b and short-circuit current in *Necturus* urinary bladder and in the aldosterone-stimulated frog skin, respectively. Narvarte and Finn (1980*a*) showed that R_b was increased in the steady state after the reduction of mucosal Na to 2.4 mM in toad urinary bladder, and that the change in R_b occurred rapidly. We have found that in toad urinary bladder R_b is also dependent upon Na transport when the latter is inhibited by amiloride. In open circuit after mucosal amiloride addition, R_b increases with a time course similar to the increase in R_a (Figs. 2 and 5). Furthermore, we have recently shown, using the noninvasive cell volume measuring technique of Spring and Hope (1978), that basolateral membrane K permeability decreases after the addition of amiloride (Davis and Finn, 1982). Since the basolateral membrane is predominantly K conductive, the electrophysiologic and cell volume data are complementary, and we conclude that R_b increases through a decrease in K permeability. Although R_b increases during Na transport inhibition, it can be seen from the apical membrane voltage-clamp studies (Figs. 8 and 9) that it increases with a time course that is delayed with respect to that of I_e^c. Thus, R_b is not directly sensitive to, but changes as a secondary consequence of, changes in Na transport. In other high-resistance epithelia, R_a/R_b increases rapidly after amiloride addition (*Necturus* urinary bladder: Frömter and Gebler, 1977; rabbit colon: Schultz et al., 1977); such as increase does not preclude an increase in R_b.

As shown in Fig. 5, the ratio of membrane resistances in toad urinary bladder does not increase to its steady state value for some time after Na transport is reduced to a negligible value, as both R_a and R_b increase in concert, initially. If it is assumed that R_a is maximal shortly after exposure to a saturating dose of amiloride, the increase in R_a/R_b must indicate a secondary decrease in R_b. The reason for the delayed increase in R_a/R_b is not clear. A reasonable assumption is that this cellular effect is in the Na pathway since amiloride addition or Na removal is required to demonstrate it. Note, however, that the time course of R_a/R_b is not identical to the time course of Na transport. For instance, removal of amiloride or the introduction of Na to a

Na-free mucosal solution leads to increases in Na transport well before R_a/R_b begins to decrease (see Fig. 1, Reuss and Finn, 1975*a*; Fig. 4 in this paper). Thus, the late changes in R_b and in R_a/R_b must be related to changes in the Na pool or to some other parameter(s) whose properties change more slowly than, but are dependent on, the rate of Na transport. Like impalements of toad urinary bladder cells in the steady state (Narvarte and Finn, 1980*a*; Finn et al., 1980), none of these changes can be artifactually related to leaks around the microelectrode, for the following reasons: (*a*) R_a and R_a/R_b are stable immediately on impalement (within 100 ms) and remain essentially unchanged for up to 30 min in the absence of amiloride, and (*b*) the late rise in R_a/R_b after amiloride (Figs. 2 and 5) is mirrored by a late fall when amiloride is removed, and all changes are reversible and repeatable in the same cell. Furthermore, Warncke and Lindemann (1981), using a noninvasive electrophysiological technique (they made inferences about the relative membrane resistances from the measurement of transepithelial capacitance), reported values of R_a/R_b in toad bladder that were similar to those we find with microelectrodes ($R_a/R_b \approx 1.2$ in their Fig. 3). They also show that R_b decreases after stimulation of Na transport by antidiuretic hormone, a result that is entirely consistent with the observations of this study.

Currents in Open and Closed Circuit

Figs. 3 and 9 depict the changes in the transcellular currents during Na transport inhibition. In open circuit, I_c is driven by both cellular emf's, and there is no information as to what ions carry this current across either the cell or shunt pathway. Thus, when amiloride decreases both V_a and V_b, and hence I_c, the latter change cannot be equated with the change in Na transport. However, since amiloride affects only net sodium transport, and since at least a portion of the external current during the transepithelial voltage clamps is carried by Na, the amiloride-induced change in I_e^c when V_{mc} is clamped can be equated directly with a change in Na transport. As shown in Fig. 9, when V_{mc} is clamped to zero, amiloride causes a rapid decrease in I_e^c. Furthermore, the decrease in I_e^c occurs at a more rapid rate than the change in R_b. Since changes in I_e^c induced by voltage clamping in the absence of amiloride cause predictable responses in V_{cs} (Fig. 7), deviations from the expected ohmic responses in V_{cs} during Na transport inhibition must be due to changes in other circuit parameters.

We have shown that the initial depolarization of V_{cs} induced by amiloride is due to a decrease in V_b. This element represents the Thevenin equivalent emf and therefore includes contributions from each of the ionic conductances of the basolateral membrane and from any current source, such as a rheogenic Na/K pump. Were G_K ($= 1/R_K$) the only ionic conductance, the lack of hyperpolarization of V_{cs} during the amiloride-induced decrease in I_e^c could only be interpreted as being due to a decrease in pump current output; that is, V_{cs} would hyperpolarize unless the decrease in I_e^c was matched by a simultaneous decrease in pump current independent of any changes in G_K. Although K is the main permeant species at the basolateral membrane, V_b

(~50 mV) is far removed from E_K (~90 mV), so that other conductances must be present. Furthermore, we have recently shown that the substitution of K for Na in the serosal medium of frog urinary bladder leads to cell swelling, which indicates a significant permeability of the basolateral membrane to both K and Cl (Davis and Finn, 1982). Given these permeabilities (and, in addition, there may be a small but significant Na conductance of the basolateral membrane), a decrease in G_K alone could cause depolarization of V_b by increasing the relative contribution of other conductances with emf's oriented opposite to E_K. Nonetheless, our data are consistent with the notion that the Na/K pump is rheogenic, and the observation that I_e^c decreases faster than R_b increases (Fig. 9) may indicate that a rheogenic Na pump is decreasing its current output before changes in R_b; any stronger statement regarding pump rheogenicity is not warranted at this time. There is, however, a variety of other experimental evidence for the presence of a rheogenic Na pump in high-resistance epithelia. Nagel (1980) has presented electrophysiologic evidence for such a system in frog skin; alteration of apical membrane permeability with polyene antibiotics has led to evidence for a basolateral rheogenic Na pump in rabbit urinary bladder (Lewis et al., 1978) and turtle colon (Kirk et al., 1980); finally, Lewis and Wills (1981) have examined the kinetics of the Na pump in rabbit urinary bladder by measuring intracellular Na activity as a function of transport and arrived at a similar conclusion.

If the Na pump in high-resistance epithelia is rheogenic, one can explain the surprising observation in toad urinary bladder (Reuss and Finn, 1975*b*) that V_{cs} depolarized "immediately" after the mucosal application of amiloride. Although the expected hyperpolarization of V_{cs} is observed after mucosal amiloride application in *Necturus* urinary bladder (Frömter and Gebler, 1977), rabbit colon (calculated from Fig. 3 in Schultz et al., 1977), and frog skin (Nagel, 1980), in all published records of amiloride effects, regardless of species, a secondary depolarization is also observed (Reuss and Finn, 1975*b*, Fig. 2; Frömter and Gebler, 1977, Fig. 2; Nagel, 1980, Fig. 2; this paper, Figs. 2 and 8). It is thus plausible that the apparent lack of an initial hyperpolarization of V_{cs} after amiloride in toad urinary bladder is simply a matter of degree, i.e., the secondary depolarization of the basolateral membrane caused by the cessation of the Na pump occurs more rapidly in toad urinary bladder than in the other amphibian tissues, so that the depolarization begins before a hyperpolarization is evident. As mentioned above, in tissues with a high shunt resistance, the expected hyperpolarization of V_{cs} is only a fraction of a millivolt for each millivolt change in V_{ms}. Since the onset of the change in V_{cs} begins 10–50 ms after the onset of the change in V_{mc} (Reuss and Finn, 1975*b*), the decrease in V_{ms} at this time is minimal, and hence it is not surprising that the hyperpolarization of V_{cs} is undetectable. In support of this thesis, Nagel (1980) found that the duration of hyperpolarization between the application of amiloride and the onset of the secondary depolarization of the cell potential (short-circuit conditions) could be varied experimentally, by varying the time the tissue was amiloride free; the longer this period and hence the more Na accumulated in the tissue, the longer the hyperpolarization plateau after

amiloride reapplication. These results suggest a direct relationship between the amount of Na in the tissue and the time required for cessation of pump activity after amiloride addition. We further note that there is a rough correlation between cell size and the duration of the post-amiloride hyperpolarization plateau in various tissues. Thus, in frog skin, functionally a syncytium of several cell layers (Rick et al., 1978), the plateau lasts for minutes (Nagel, 1980); in *Necturus* urinary bladder, a single-layered epithelium with large cells, the plateau lasts for seconds (Frömter and Gebler, 1977); and in toad urinary bladder, with smaller cells, the plateau appears to be nonexistent, since depolarization begins within milliseconds of the onset of amiloride-induced changes in the apical membrane.[4]

The principal finding in this series of studies is that R_b increases during inhibition of Na transport. As noted above, most of this change occurs after the major change in transport. Since K is the main permeant species at the basolateral membrane, the increase in R_b may indicate a significant decrease in K conductance during the inhibition of Na transport. This conclusion is strongly supported by recent studies of cell volume regulation and of the responses of cell volume to high serosal K (Davis and Finn, 1982). In those studies it was shown that the K permeability of the basolateral membrane (as determined by K-dependent volume regulation and KCl-induced swelling) is greatly reduced by the addition of amiloride to the mucosal bathing medium. Such a mechanism would prevent a decrease in cell K after Na transport inhibition, as has been observed in frog skin (Rick et al., 1978), and may prove to be a common feature in Na-transporting epithelia. It is also clear that the basolateral membrane cannot be modeled as a simple (ohmic) resistor-emf combination. If a change in current is brought about by clamping the apical membrane at a series of different potentials, the resistance remains constant. On the other hand, transport-induced changes in current do not lead to proportionate changes in potential. It is important to point out, however, that whereas the basolateral membrane is nonohmic, this does not seem to be the case for the apical membrane. As shown in Fig. 10, when the basolateral membrane voltage is clamped, changes in transepithelial current induced by amiloride lead to changes in apical membrane voltage that are, to a first approximation, those expected for an ohmic element.

The data presented here indicate that the simple equivalent circuit model of the epithelium (Fig. 1) is inadequate; minimum additional requirements are that V_b be partitioned into a current source representing the Na pump, that parallel emf's be added for each ionic conductance, and that R_b be

[4] There are sexual differences in both morphological and electrophysiological parameters in *Necturus* urinary bladder (Lefevre et al., 1977; Higgins et al., 1977). From the stair-step potential profile of their record (Frömter and Gebler, 1977, Fig. 2), we deduce that it represents a male (Higgins et al., 1977) for which we calculate cell volumes on the order of 8,000 μm^3 from the micrographs of Lefevre et al. (1977) (volumes of cells from females are on the order of 5,000 μm^3). From the micrographs of Bobrycki et al. (1981) we estimate the volume of toad urinary bladder cells to be on the order of 1,500 μm^3, similar to the 2,100-μm^3 volumes we have measured in frog urinary bladder (Davis and Finn, 1981).

designated a variable resistor controlled in some way by the rate of Na transport.

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