

# Interaction between the Basolateral $K^+$ and Apical $Na^+$ Conductances in *Necturus* Urinary Bladder

JEFFERY R. DEMAREST and ARTHUR L. FINN

From the Departments of Medicine and Physiology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

**ABSTRACT** Experimental modulation of the apical membrane  $Na^+$  conductance or basolateral membrane  $Na^+$ - $K^+$  pump activity has been shown to result in parallel changes in the basolateral  $K^+$  conductance in a number of epithelia. To determine whether modulation of the basolateral  $K^+$  conductance would result in parallel changes in apical  $Na^+$  conductance and basolateral pump activity, *Necturus* urinary bladders stripped of serosal muscle and connective tissue were impaled through their basolateral membranes with microelectrodes in experiments that allowed rapid serosal solution changes. Exposure of the basolateral membrane to the  $K^+$  channel blockers  $Ba^{2+}$  (0.5 mM/liter),  $Cs^+$  (10 mM/liter), or  $Rb^+$  (10 mM/liter) increased the basolateral resistance ( $R_b$ ) by >75% in each case. The increases in  $R_b$  were accompanied simultaneously by significant increases in apical resistance ( $R_a$ ) of >20% and decreases in transepithelial  $Na^+$  transport. The increases in  $R_a$ , measured as slope resistances, cannot be attributed to nonlinearity of the  $I$ - $V$  relationship of the apical membrane, since the measured cell membrane potentials with the  $K^+$  channel blockers present were not significantly different from those resulting from increasing serosal  $K^+$ , a maneuver that did not affect  $R_a$ . Thus, blocking the  $K^+$  conductance causes a reduction in net  $Na^+$  transport by reducing  $K^+$  exit from the cell and simultaneously reducing  $Na^+$  entry into the cell. Close correlations between the calculated short-circuit current and the apical and basolateral conductances were preserved after the basolateral  $K^+$  conductance pathways had been blocked. Thus, the interaction between the basolateral and apical conductances revealed by blocking the basolateral  $K^+$  channels is part of a network of feedback relationships that normally serves to maintain cellular homeostasis during changes in the rate of transepithelial  $Na^+$  transport.

## INTRODUCTION

For some time it has been known that there are important feedback mechanisms that couple the passive membrane permeabilities to the activity of the  $Na^+$  pump in  $Na^+$ -transporting epithelia (Schultz, 1981; Diamond, 1982). MacRobbie and

Address reprint requests to Dr. Arthur L. Finn, Depts. of Medicine and Physiology, University of North Carolina at Chapel Hill, Old Clinic Bldg. 226H, Chapel Hill, NC 27514. Dr. Demarest's present address is Physiology-Anatomy Dept., University of California, Berkeley, CA 94720.

Ussing (1961) were the first to show that inhibition of the  $\text{Na}^+$  pump caused a decrease in both the apical and basolateral ion permeabilities in the frog skin. This finding has subsequently been confirmed by other investigators using isotopic tracers and intracellular microelectrode techniques (Chase and Al-Awqati, 1979; Helman et al., 1979). Reuss and Finn (1975) were the first to report electrical interactions between the apical and basolateral membranes that could not be attributed to changes in current flow through the parallel shunt pathway. The alteration of the apical membrane electromotive force (emf) caused by the application of mucosal amiloride or by changing the ionic composition of the mucosal bath resulted in a rapid change in the basolateral membrane emf (Reuss and Finn, 1975; Finn and Reuss, 1978; Narvarte and Finn, 1980). More recently (Davis and Finn, 1982*a, b*), it has been shown that inhibition of the apical membrane  $\text{Na}^+$  channel results in the inhibition of the basolateral  $\text{K}^+$  conductance. These interactions have been interpreted as homeostatic regulatory mechanisms that serve to maintain steady state intracellular ionic concentrations and cell volume during changes in the rates of transcellular ion and water flux.

In the preceding article (Demarest and Finn, 1987), we demonstrated that the dominant factor determining the membrane potential in the basolateral membrane of the *Necturus* urinary bladder is a highly selective  $\text{K}^+$  conductance. Other studies have shown that the activity of basolateral  $\text{K}^+$  channels of epithelia is dependent on the volume of the cells and can be regulated by hormones (Nagel and Crabbe, 1980; Davis and Finn, 1982*a*; Maruyama and Petersen, 1982; Lau et al., 1984). Both effects appear to be mediated through changes in intracellular  $\text{Ca}^{2+}$  (Maruyama and Petersen, 1984; Davis and Finn, 1985). The central role that such  $\text{K}^+$  conductance pathways have in the widely accepted Koefoed-Johnson and Ussing (1958) model of transepithelial  $\text{Na}^+$  transport raises the question of whether the  $\text{K}^+$  channel is an important site for the regulation of  $\text{Na}^+$  transport. Is there a tight coupling between the basolateral  $\text{K}^+$  and apical  $\text{Na}^+$  channels?  $\text{Ba}^{2+}$ , a known blocker of  $\text{K}^+$  channels, has been shown to inhibit net  $\text{Na}^+$  transport by epithelia, but its site of action has been a matter of dispute (Ramsay et al., 1976; Nagel, 1979; Hardcastle et al., 1983). In this study,  $\text{Ba}^{2+}$  and several other  $\text{K}^+$  channel blockers have been used to investigate interactions between the passive membrane permeabilities of *Necturus* urinary bladder. Blocking the basolateral  $\text{K}^+$  conductance results in an immediate reduction of apical  $\text{Na}^+$  conductance that is not mediated through changes in membrane potential. Preliminary reports of these studies have been presented elsewhere (Demarest and Finn, 1983, 1984).

#### METHODS

Urinary bladders from male *Necturus maculosus* (Nasco Biological Supply, Ft. Atkinson, WI) were mounted horizontally, serosal side up, in an open-topped Lucite chamber that was placed on the stage of the inverted microscope (Diavert, E. Leitz, Inc., Rockleigh, NJ) used to view the epithelial cells at 320 $\times$  with bright-field illumination during the experiments. Details of the experimental methods are described in the preceding article (Demarest and Finn, 1987). Briefly, most of the serosal muscle and connective tissue was removed from the basolateral surface of the epithelial cell layer and microelectrode

impalements were made across the basolateral membranes of the cells. Both sides of the epithelium were continuously perfused using a system that allowed rapid changes in the composition of the serosal bathing solution.

### *Solutions*

The *Necturus* Ringer solution had the following composition (mM/liter): 95 NaCl, 10 NaHCO<sub>3</sub>, 2.0 CaCl<sub>2</sub>, 1.0 MgCl<sub>2</sub>, 1.19 K<sub>2</sub>HPO<sub>4</sub>, 0.11 KH<sub>2</sub>PO<sub>4</sub>, 5 glucose. The total osmotic concentration was 210 mosmol/kg and the pH was 7.9 when equilibrated with 99% O<sub>2</sub>, 1% CO<sub>2</sub>. In solutions with a higher-than-normal [K<sup>+</sup>] (i.e., >2.5 mM), KCl was substituted for NaCl to obtain the concentrations indicated in the text. RbCl and CsCl were substituted for NaCl as indicated in the text. Amiloride (a gift from Merck, Sharp & Dohme Research Laboratories, West Point, PA) was dissolved in *Necturus* Ringer at a final concentration of 10<sup>-4</sup> M. Verapamil (Calbiochem-Behring Corp., La Jolla, CA) was dissolved in *Necturus* Ringer at a final concentration of 10<sup>-4</sup> M. The experiments were performed at room temperature (23 ± 1 °C).

### *Electrical Measurements*

Electrical measurements and circuit analysis were made as described in the preceding article (Demarest and Finn, 1987).

The emf of the shunt pathway was assumed to be zero when the solutions on the two sides of the epithelium were identical; experiments employing nonsymmetrical solutions are discussed in the Results.

### *Statistics*

All mean values are given with standard errors (mean ± SE). Comparisons between means were made using the *t* test for paired data. Coefficients and intercepts for least-squares regression lines are given with standard deviations and were compared using analysis of variance.

## RESULTS

### *Effects of Ba<sup>2+</sup> on the Measured Electrical Properties*

The addition of 0.5 mM Ba<sup>2+</sup> to the serosal solution (Fig. 1) caused a rapid depolarization of  $V_{cs}$ , which reached a new steady state in ~7 s. Simultaneously,  $V_{mc}$  was shifted in the negative direction. The changes in the membrane potentials were accompanied by a decrease in the ratio of the deflections of the potentials resulting from transepithelial current pulses ( $R_a/R_b$ ), which indicated an increase in the relative resistance of the basolateral membrane. 11 experiments, in which measurements were made in the quasi-steady state 30 s after the addition of 0.5 mM/liter serosal Ba<sup>2+</sup>, are summarized in Table I. From the significant decrease in  $V_{ms}$  and the increase in  $R_t$ , it can be calculated that the short-circuit current fell from 29 ± 6 to 20 ± 4 μA·cm<sup>-2</sup> ( $P < 0.01$ ,  $n = 11$ ), which indicates a significant inhibition of net Na<sup>+</sup> transport. Higher concentrations of serosal Ba<sup>2+</sup> did not produce significantly greater effects (e.g., in eight experiments, 1.0 mM/liter Ba<sup>2+</sup> depolarized  $V_{cs}$  to 44.7 ± 3.3 mV and reduced  $R_a/R_b$  to 3.66 ± 0.55; these effects were not significantly different from those shown in Table I).

Increasing serosal K from 2.5 to 50 mM/liter depolarized  $V_{cs}$  by 32.7 ± 2.5 mV, increased  $R_a/R_b$  from 3.27 ± 0.60 to 6.36 ± 0.55, and decreased  $R_t$  by >20% (Demarest and Finn, 1987). These effects of increased serosal K<sup>+</sup> were

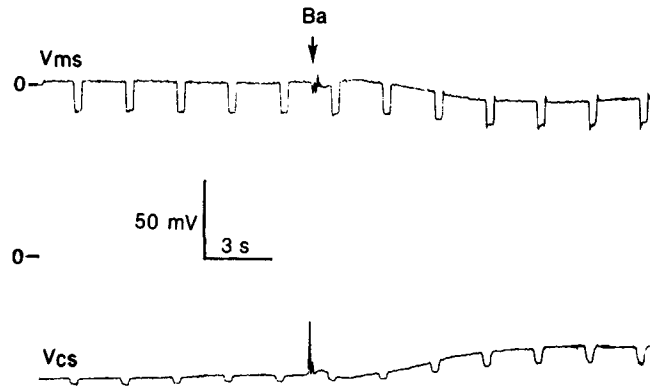


FIGURE 1. The effect of  $\text{Ba}^{2+}$  on the cell membrane potentials. The record starts with a microelectrode in the cell. The upper trace is the apical ( $V_{mc}$ ) and the lower trace is the basolateral ( $V_{cs}$ ) membrane potential. Upward is positive for both traces, which were measured with reference to the serosal bathing solution as ground. At the arrow, the serosal solution was changed to Ringer with 0.5 mM/liter  $\text{BaCl}_2$ . The repeated downward deflections in the traces were due to transepithelial current pulses ( $5 \mu\text{A}\cdot\text{cm}^{-2}$  for 500 ms). The ratio of the deflections ( $\Delta V_{mc}/\Delta V_{cs} = R_a/R_b$ ) decreased, which indicates an increase in the relative resistance of the basolateral membrane.

significantly attenuated in the presence of serosal  $\text{Ba}^{2+}$  ( $V_{cs}$  was depolarized by  $13.3 \pm 2.9$  mV,  $R_a/R_b$  increased from  $1.72 \pm 0.19$  to  $3.42 \pm 0.42$ , and  $R_t$  decreased by 8%) as compared with its absence, which is consistent with partial blockade of the basolateral  $\text{K}^+$  conductance by  $\text{Ba}^{2+}$ .

#### *Effects of $\text{Ba}^{2+}$ on the Membrane Resistances and Electromotive Forces*

The values of  $R_a$ ,  $R_b$ ,  $E_a$ , and  $E_b$ , calculated from the data of Table I before and in the steady state after  $\text{Ba}^{2+}$ , are shown in Table II. In a separate set of experiments on five bladders,  $\text{Ba}^{2+}$  was found to have no significant effect on the shunt resistance,  $R_s$ , which was  $6.24 \pm 1.25$   $\text{k}\Omega\cdot\text{cm}^2$  before and  $6.26 \pm 1.37$  after serosal  $\text{Ba}^{2+}$ .  $\text{Ba}^{2+}$  caused an increase in  $R_b$  and a depolarization of  $E_b$ .

TABLE I  
*Effects of Serosal  $\text{Ba}^{2+}$  (0.5 mM) on the  
Transepithelial and Cellular Electrical Properties*

	$V_{ms}$	$V_{mc}$	$V_{cs}$	$R_a/R_b$	$R_t$
		mV			$\text{k}\Omega\cdot\text{cm}^2$
Control	$-86.6 \pm 5.8$	$-16.5 \pm 5.1$	$-68.8 \pm 3.9$	$5.25 \pm 1.36$	$4.16 \pm 0.69$
$\text{Ba}^{2+}$	$-73.3 \pm 5.9$	$-32.7 \pm 5.3$	$-40.1 \pm 3.9$	$3.16 \pm 1.15$	$4.81 \pm 0.78$
$\Delta$	$13.4 \pm 1.8$	$-16.7 \pm 2.0$	$28.7 \pm 2.9$	$2.09 \pm 0.38$	$0.64 \pm 0.15$
<i>P</i>	$<0.001$	$<0.001$	$<0.001$	$<0.001$	$<0.01$

Measurements were made by continuously recording in single cells. Values are means  $\pm$  SE for 11 bladders.  $\Delta$  is the difference between control and  $\text{Ba}^{2+}$ -treated tissues.

TABLE II  
*Effect of Serosal Ba<sup>2+</sup> (0.5 mM) on the Cell  
 Membrane Resistances and Electromotive Forces*

	$R_a$	$R_b$	$E_a$	$E_b$
	$k\Omega \cdot \text{cm}^2$	$k\Omega \cdot \text{cm}^2$	$\text{mV}$	$\text{mV}$
Control	$7.82 \pm 1.88$	$1.74 \pm 0.37$	$-93 \pm 17$	$-86 \pm 5$
Ba <sup>2+</sup>	$9.82 \pm 2.53$	$3.95 \pm 0.98$	$-112 \pm 19$	$-73 \pm 8$
$\Delta$	$2.00 \pm 0.85$	$2.21 \pm 0.73$	$18 \pm 2$	$14 \pm 4$
$P$	$<0.05$	$<0.02$	$<0.001$	$<0.02$

Values were calculated from the data in Table I as described in the text.  $R_a$  was  $8.24 \text{ k}\Omega \cdot \text{cm}^2$ .  $\Delta$  is the difference between control and Ba<sup>2+</sup>-treated tissues.

Table III shows the effects of increased serosal K<sup>+</sup> on the membrane resistances and emf's of Ba<sup>2+</sup>-treated bladders. In the absence of serosal Ba<sup>2+</sup>, 50 mM/liter serosal K<sup>+</sup> did not significantly affect  $R_a$  (see Fig. 2) or  $E_a$ , but reduced  $R_b$  by  $>50\%$  and depolarized  $E_b$  by  $53 \pm 5 \text{ mV}$  (Demarest and Finn, 1987). However, in the presence of serosal Ba<sup>2+</sup> (Table III), the effect on  $E_b$  was significantly attenuated. These data indicate that Ba<sup>2+</sup> significantly reduces the transference number of the basolateral membrane for K<sup>+</sup> (Demarest and Finn, 1987). Thus, the effect of Ba<sup>2+</sup> on  $R_b$  can be attributed to a partial block of the basolateral K<sup>+</sup> conductance. However, serosal Ba<sup>2+</sup> also caused a significant increase in  $R_a$  and a hyperpolarization of  $E_a$  (Table II). Fig. 2 shows that these increases are not simply due to a nonlinear current-voltage relationship of the apical membrane, since the values of  $V_{mc}$  before and after either 50 mM/liter serosal K<sup>+</sup> or 0.5 mM/liter serosal Ba<sup>2+</sup> were not significantly different, yet increased K<sup>+</sup> did not change  $R_a$  significantly. Thus, blocking the basolateral K<sup>+</sup> conductance resulted in a decrease in apical Na<sup>+</sup> permeability that was reflected as an inhibition of the  $I_{sc}$ . The Ba<sup>2+</sup>-induced hyperpolarization of  $E_a$  is consistent with a drop in

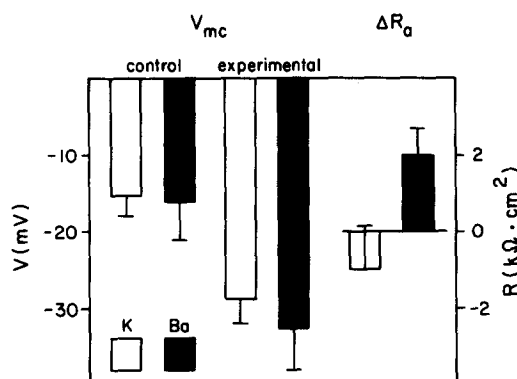


FIGURE 2. Comparison of the effects of 50 mM K<sup>+</sup> and 0.5 mM Ba<sup>2+</sup> on  $V_{mc}$  and the change in  $R_a$  ( $\Delta R_a$ ). Although the values of  $V_{mc}$  after K<sup>+</sup> or Ba<sup>2+</sup> were not significantly different, only Ba<sup>2+</sup> resulted in a significant ( $P < 0.05$ ) increase in  $R_a$ .

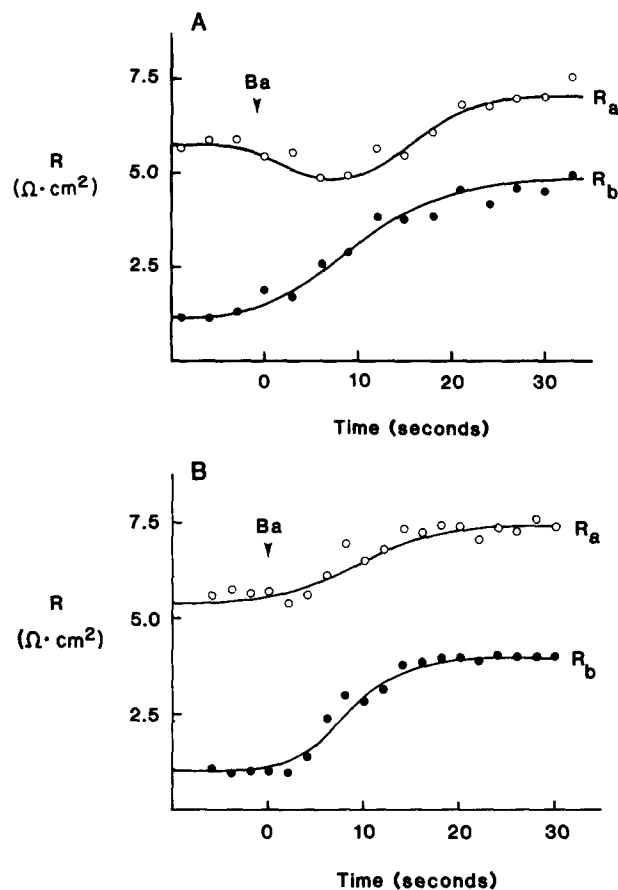


FIGURE 3. Time course of the changes in the cell membrane resistances caused by  $Ba^{2+}$ . Two types of time course for  $R_a$  were observed. (A) In bladders with transepithelial potentials,  $V_{ms}$ , greater than  $\sim 70$  mV,  $R_a$  exhibited a biphasic response. (B) In bladders with  $V_{ms}$  less than  $\sim 70$  mV,  $R_a$  increased monotonically to a quasi-steady state.  $R_b$  increased monotonically in all bladders.

TABLE III  
Effect of Increasing Serosal  $K^+$  to 50 mM on the Cell  
Membrane Resistances and Electromotive Forces of  $Ba^{2+}$ -treated Bladders

	$R_a$	$R_b$	$E_a$	$E_b$
	$k\Omega \cdot cm^2$	$k\Omega \cdot cm^2$	mV	mV
$Ba^{2+}$	$5.97 \pm 1.63$	$3.26 \pm 0.58$	$-81 \pm 12$	$-80 \pm 4$
$Ba^{2+} + 50 \text{ mM } [K^+]_s$	$5.27 \pm 1.16$	$1.52 \pm 0.31$	$-71 \pm 14$	$-41 \pm 3$
$\Delta$	$0.90 \pm 0.62$	$1.73 \pm 0.37$	$9 \pm 11$	$39 \pm 2$
$P$	NS	$<0.005$	NS	$<0.001$

$n = 6$ . NS, no significant difference.  $\Delta$  is the difference between tissues treated with  $Ba^{2+}$  alone and those to which both  $Ba^{2+}$  and  $K^+$  were added.

intracellular Na<sup>+</sup> resulting from the continued activity of the Na<sup>+</sup>-K<sup>+</sup> ATPase while the rate of apical Na<sup>+</sup> entry was decreasing.<sup>1</sup>

*Time Course of the Ba<sup>2+</sup> Effects on the Cell Membrane Resistances*

Two different time courses of Ba<sup>2+</sup>-induced changes in  $R_a$  were encountered that were dependent upon the value of  $V_{mc}$  after Ba<sup>2+</sup>. If the Ba<sup>2+</sup>-induced value of  $V_{mc}$  was more negative than about -10 mV,  $R_a$  exhibited a biphasic response (Fig. 3A). It first decreased to a minimum at  $9.6 \pm 0.8$  s, which was significantly ( $P < 0.01$ ) lower than control. By  $20.4 \pm 2.4$  s,  $R_a$  had returned to a level not significantly different from control, and then continued to increase to a quasi-steady state significantly ( $P < 0.05$ ) higher than control by 30 s. In bladders with a Ba<sup>2+</sup>-induced  $V_{mc}$  more positive than -10 mV,  $R_a$  increased monotonically to a quasi-steady state significantly ( $P < 0.05$ ) higher than control by 20 s (Fig. 3B). The Ba<sup>2+</sup>-induced increase of  $R_b$ , hyperpolarization of  $E_a$ , and depolarization of

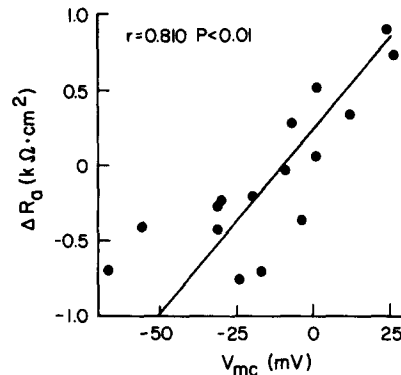


FIGURE 4. Correlation between the change in  $R_a$ ,  $\Delta R_a$ , and  $V_{mc}$  at 10 s after serosal Ba<sup>2+</sup>. There was a significant correlation ( $r = 0.810$ ,  $P < 0.001$ ) between  $\Delta R_a$  and  $V_{mc}$ . The solid line ( $\Delta R_a = 0.016 \pm 0.003$ ,  $V_{mc} = -1.466 \pm 0.310$ ;  $P < 0.001$ ,  $n = 16$ ) was fitted to the data by least-squares regression.

$E_b$  (Table II) were monotonic in all cases and reached steady state levels by 20 s. Note that the Ba<sup>2+</sup>-induced changes in  $V_{mc}$  and  $V_{cs}$  had reached their steady state levels within 5–7 s (Fig. 1).

There was a significant correlation ( $r = 0.810$ ,  $P < 0.01$ ) between the change in  $R_a$  ( $\Delta R_a$ ), and  $V_{mc}$  at 10 s after Ba<sup>2+</sup> (Fig. 4), but no significant correlation ( $r = 0.063$ ,  $P > 0.5$ ) between the quasi-steady state values. In addition, there was no significant difference between the mean steady state values of  $R_a$  for bladders that exhibited biphasic ( $R_a = 8.15 \pm 1.80$  k $\Omega \cdot \text{cm}^2$ ) and monophasic ( $R_a = 8.79 \pm 2.10$  k $\Omega \cdot \text{cm}^2$ ) time courses.

<sup>1</sup> In the high- $V_{ms}$  winter bladders of Table II,  $E_a$  appears to be exclusively an Na<sup>+</sup> emf (Demarest and Finn, 1987). Calculating the intracellular Na<sup>+</sup> concentration from the values of  $E_a$  (Table II) using the Nernst equation gives a value of 2.6 mM/liter under control conditions and 1.2 mM/liter after serosal Ba<sup>2+</sup>. The former value for control conditions is about half of that estimated in previous studies on *Necturus* urinary bladder by fitting the constant field equation to the current-voltage relationship of the amiloride-sensitive apical Na<sup>+</sup> pathway (Fromter et al., 1977; Thomas et al., 1983).

TABLE IV  
Effect of Verapamil (100  $\mu$ M) on the Response to Serosal  $Ba^{2+}$

	Without verapamil			With verapamil		
	$V_{cs}$	$R_a/R_b$	$R_t$	$V_{cs}$	$R_a/R_b$	$R_t$
	mV		$k\Omega \cdot cm^2$	mV		$k\Omega \cdot cm^2$
Control	$-68.7 \pm 1.8$	$3.72 \pm 0.75$	$1.76 \pm 0.42$	$-65.3 \pm 2.8$	$3.15 \pm 0.52$	$1.79 \pm 0.43$
$Ba^{2+}$	$-37.4 \pm 2.9$	$1.90 \pm 0.31$	$1.98 \pm 0.44$	$-31.6 \pm 2.3$	$1.60 \pm 0.36$	$2.14 \pm 0.41$
$\Delta$	$31.3 \pm 1.8$	$1.82 \pm 0.77$	$0.22 \pm 0.06$	$33.8 \pm 1.0$	$1.55 \pm 0.32$	$0.35 \pm 0.13$

$n = 4$ .  $\Delta$  is the difference between control and  $Ba^{2+}$ -treated tissues.

#### Effects of Verapamil on the $Ba^{2+}$ Response

Verapamil blocks Ca channels that exhibit high conductances for  $Ba^{2+}$  (Hagiwara and Byerly, 1981). A verapamil-sensitive Ca channel has been reported in isolated toad urinary bladder cells (Humes et al., 1980). To determine whether the effect of  $Ba^{2+}$  on  $R_a$  was due to  $Ba^{2+}$  entry into the cells, the effects of  $Ba^{2+}$  were examined in bladders treated with verapamil (100  $\mu$ M). Table IV shows that there was no significant effect of serosal verapamil on the electrical properties or the responses of the bladders to  $Ba^{2+}$ . Furthermore, the effects of  $Ba^{2+}$  were found to be completely reversible for the short exposure times investigated in this study (<10 min). These data suggest that  $Ba^{2+}$  does not exert its effects by entering the cells.

#### Effects of $Cs^+$ and $Rb^+$ on the Cell Membrane Resistances and Electromotive Forces

Fig. 5 shows the effects on  $V_{cs}$  of raising serosal  $K^+$  from 2.5 to 50 mM/liter

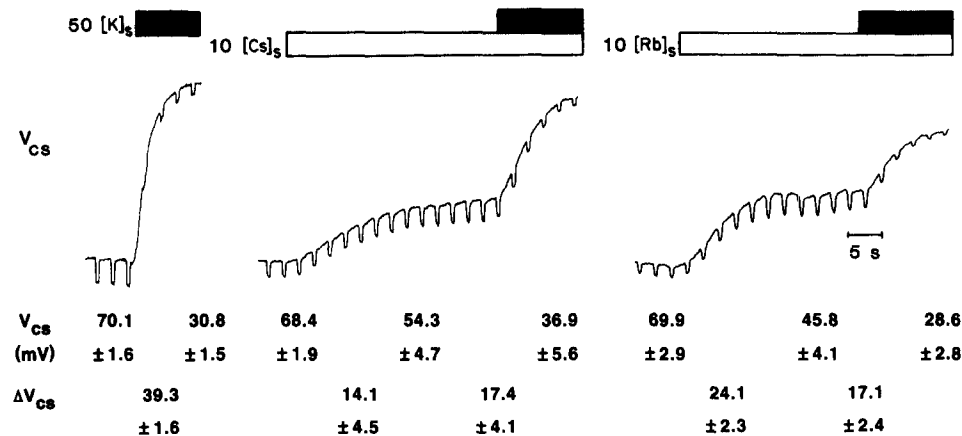


FIGURE 5. The response of  $V_{cs}$  to 50 mM/liter serosal  $K^+$  under control conditions (left), in the presence of 10 mM/liter serosal  $Cs^+$  (center), and in the presence of 10 mM/liter serosal  $Rb^+$  (right). Records of  $V_{cs}$  for three cells in different bladders are shown. Each record starts with a microelectrode in the cell. The horizontal filled bars at the top of the figure indicate the time periods when serosal  $K^+$  was elevated from 2.5 to 50 mM/liter. The open bars indicate the presence of 10 mM/liter  $Cs^+$  (center) or 10 mM/liter  $Rb^+$  (right). The mean values of  $V_{cs}$  and the change in  $V_{cs}$ ,  $\Delta V_{cs}$ , are shown across the bottom of the figure.



TABLE V  
*Effects of Serosal Cs (10 mM) on Cell  
 Membrane Resistance and Electromotive Forces*

	$R_a$	$R_b$	$E_a$	$E_b$
	$k\Omega \cdot cm^2$	$k\Omega \cdot cm^2$	$mV$	$mV$
Control	7.30±1.18	1.78±0.28	-101±10	-96±4
Cs	9.14±1.38	3.15±0.32	-108±10	-93±5
$\Delta$	1.84±0.50	1.37±0.32	-7±8	3±4
<i>P</i>	<0.01	<0.01	NS	NS

$n = 11$ . NS, no significant difference.  $\Delta$  is the difference between control and Cs-treated tissues.

under control conditions and in the presence of 10 mM/liter Cs<sup>+</sup> or Rb<sup>+</sup>. Both ions significantly reduced the effect of increasing serosal K<sup>+</sup>. The high-K<sup>+</sup>-induced changes in  $V_{cs}$  in the presence of either Cs or Rb were not significantly different from one another, even though the depolarization of  $V_{cs}$  caused by Cs<sup>+</sup> was significantly smaller than that caused by Rb<sup>+</sup> (Fig. 5). The depolarizations of  $V_{cs}$  caused by 10 mM/liter Cs<sup>+</sup> and Rb<sup>+</sup> were not significantly different from the depolarizations caused by 47.5 mM/liter of these ions (Demarest and Finn, 1987). Thus, 10 mM/liter of either ion is sufficient to produce a maximal effect. Cs<sup>+</sup> and Rb<sup>+</sup> caused significantly smaller hyperpolarizations of  $V_{mc}$  than Ba<sup>2+</sup>. Rb<sup>+</sup> hyperpolarized  $V_{mc}$  by  $14.9 \pm 1.7$  mV and Cs<sup>+</sup> hyperpolarized  $V_{mc}$  by only  $9.7 \pm 1.3$  mV, or slightly more than half of the hyperpolarization caused by Ba<sup>2+</sup> (Table I). Both ions significantly inhibited Na<sup>+</sup> transport. Cs<sup>+</sup> reduced the  $I_{sc}$  by  $19 \pm 4\%$  ( $P < 0.05$ ,  $n = 11$ ) and Rb<sup>+</sup> reduced the  $I_{sc}$  by  $22 \pm 4\%$  ( $P < 0.05$ ,  $n = 9$ ). However, neither ion was as effective at depolarizing  $V_{cs}$  or inhibiting the  $I_{sc}$  as 0.5 mM/liter Ba<sup>2+</sup> (Table I). As was the case for Ba<sup>2+</sup>, the effects of Cs<sup>+</sup> and Rb<sup>+</sup> were completely reversible.

Tables V and VI demonstrate that both Cs<sup>+</sup> and Rb<sup>+</sup> at 10 mM/liter were as effective as Ba<sup>2+</sup> in increasing  $R_b$  and  $R_a$ . Although small depolarizations of  $E_b$  and hyperpolarizations of  $E_a$  were produced by Cs<sup>+</sup> and Rb<sup>+</sup>, none of the effects was significant. The effects of Cs<sup>+</sup> and Rb<sup>+</sup> are consistent with the ability of these ions to reduce the transference number of the basolateral membrane for K<sup>+</sup> by competitively blocking the K<sup>+</sup> conductance pathways (Demarest and Finn, 1987).

TABLE VI  
*Effects of Serosal Rb (10 mM) on Cell  
 Membrane Resistances and Electromotive Forces*

	$R_a$	$R_b$	$E_a$	$E_b$
	$k\Omega \cdot cm^2$	$k\Omega \cdot cm^2$	$mV$	$mV$
Control	7.23±1.21	1.92±0.48	-92±19	-104±11
Rb	8.65±1.31	3.93±0.56	-107±15	-92±11
$\Delta$	1.42±0.60	2.01±0.38	-15±9	12±8
<i>P</i>	<0.05	<0.001	NS	NS

$n = 9$ . NS, no significant difference.  $\Delta$  is the difference between control and Rb-treated tissues.

*Relationship between the Na<sup>+</sup> Transport and Membrane Conductances*

The relationship between the calculated  $I_{sc}$  and the apical ( $G_a$ ) and basolateral ( $G_b$ ) membrane conductances for Ba<sup>2+</sup>-treated bladders are shown in Figs. 6 and 7. The lines connect points measured in the same cell before and after serosal Ba<sup>2+</sup>. There are highly significant correlations between the  $I_{sc}$  and  $G_a$  and  $G_b$  both before and after Ba<sup>2+</sup> exposure. The correlation coefficients for each membrane were not significantly affected by Ba<sup>2+</sup>.

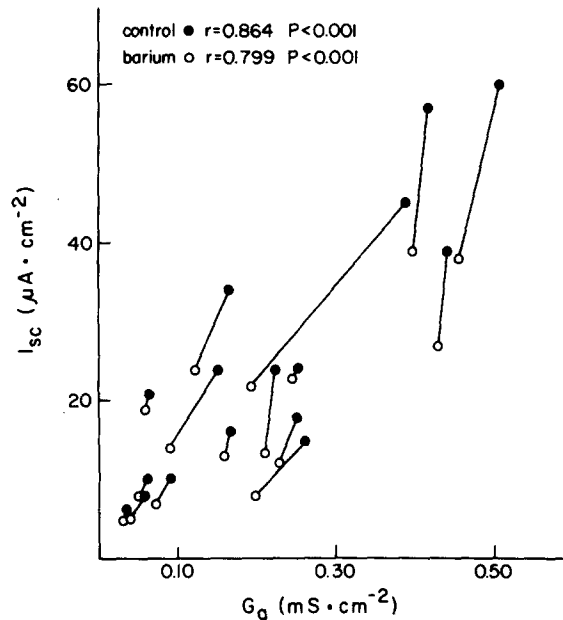


FIGURE 6. Relationship between the short-circuit current ( $I_{sc}$ ) and the apical membrane conductance ( $G_a$ ) in the absence (open circles) and presence (filled circles) of 0.5 mM/liter serosal Ba<sup>2+</sup>. The lines connect points for the two conditions measured in the same cell for 16 different bladders. The Ba<sup>2+</sup> values are for the quasi-steady state >25 s after Ba<sup>2+</sup> exposure.

Table VII shows the slopes and y-intercepts for regression lines fitted to the  $I_{sc}$ ,  $G_a$ , and  $G_b$  data for all of the bladders inhibited with Ba<sup>2+</sup>, Cs<sup>+</sup>, or Rb<sup>+</sup>. All of the regressions were highly significant and none of the y-intercepts was significantly different from zero. Blocking the basolateral K<sup>+</sup> conductance did not significantly alter the regression coefficients for either membrane. Thus, blocking the K<sup>+</sup> conductance shifts  $G_a$ ,  $G_b$ , and  $I_{sc}$  to lower values along the lines that describe the bladder-to-bladder variability, without changing the slopes or y-intercepts of these relationships.<sup>2</sup>

<sup>2</sup> The calculated  $I_{sc}$  is used here merely as an index of Na<sup>+</sup> transport rate, i.e., pump activity. Since the  $I_{sc}$  is not included in the equivalent circuit that we have used, the slopes and intercepts of the relationships between the  $I_{sc}$  and the membrane conductances have not been interpreted further.

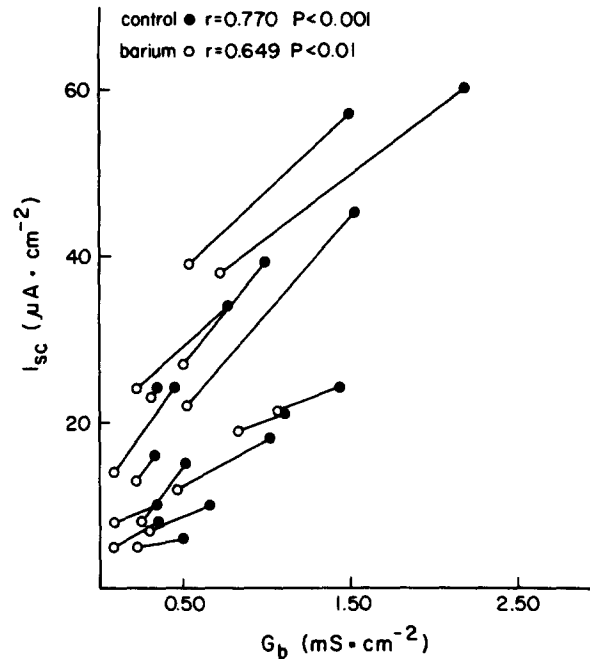


FIGURE 7. Relationship between the short-circuit current ( $I_{sc}$ ) and the basolateral membrane conductance ( $G_b$ ) in the absence (open circles) and presence (filled circles) of 0.5 mM/liter serosal  $Ba^{2+}$ . The lines connect points for the two conditions measured in the same cell for 16 different bladders. The  $Ba^{2+}$  values are for the quasi-steady state >25 s after  $Ba^{2+}$  exposure.

DISCUSSION

We have previously shown (Demarest and Finn, 1987) that the electrical properties of the basolateral membrane of *Necturus* urinary bladder are dominated by  $K^+$ -selective conductances and those of the apical membrane are dominated by an amiloride-sensitive  $Na^+$  channel. The location of the  $K^+$  channels in the same membrane as the receptors for many hormones and their known dependence on a number of putative intracellular mediators and "second messengers," such as  $Ca^{2+}$ , voltage, pH, etc., would seem to make the  $K^+$  channels an ideal primary site for the regulation of transepithelial ion transport by hormones.

TABLE VII  
*Regression Analysis of  $I_{sc}$  vs. Membrane Conductance*

	Slope		Intercept		Regression P	
	Control	Blocked	Control	Blocked	Control	Blocked
$I_{sc}$ vs. $G_a$	93.6±13.0	61.1±12.2	6.7±9.1	9.0±8.0	<0.001	<0.001
	NS*		NS			
$I_{sc}$ vs. $G_b$	14.9±3.1	27.1±6.0	12.2±11.1	10.4±8.9	<0.001	<0.002
	NS		NS			

\* P levels for F test for the difference between control and blocked conditions.

Indeed, several studies of hormonal stimulation of transepithelial ion transport have shown that the hormones produce their effects by simultaneously increasing the conductance of both the apical and basolateral cell membranes (Nagel and Crabbe, 1980; Smith and Frizzell, 1984; Demarest and Machen, 1985). Further, the modulation of the  $K^+$  channels by changes in cell volume (Davis and Finn, 1982*a, b*; Lau et al., 1984) suggest that they may serve as the sensor for the activation of volume-regulatory mechanisms. We have examined the effects of experimental modulation of the  $K^+$  conductance on the transepithelial  $Na^+$  transport rate and apical membrane  $Na^+$  conductance in order to determine whether the  $K^+$  conductance has the properties of a regulatory site.

*Inhibition of the Apical  $Na^+$  Channel by Basolateral  $K^+$  Channel Block*

The data presented in Tables II, V, and VI indicate that blocking the basolateral  $K^+$  pathways results in blockage of the apical  $Na^+$  channel. Thus, the inhibition of net transepithelial  $Na^+$  transport caused by the  $K^+$  channel blockers is the result of their direct inhibition of  $K^+$  exit from the cells across the basolateral membrane and a presumably indirect effect to inhibit  $Na^+$  entry into the cells across the apical membrane. This effect is not mediated by the change in  $V_{mc}$  after  $K^+$  channel block (Table I), since its magnitude is not sufficient to account either for the reduction of  $Na^+$  entry into the cells owing to a reduction of the electrochemical gradient for  $Na^+$  or for the observed increase of  $R_a$  (which might be predicted if the apical membrane channels are voltage dependent) (Fig. 2; see also Demarest and Finn, 1987). In addition, even though  $Cs^+$  caused a hyperpolarization of  $V_{mc}$  that was only 58% of that caused by  $Ba^{2+}$ , exposure to either ion increased  $R_a$  by about the same amount (compare Tables II and V), which provides further evidence that the increase in  $R_a$  resulting from the  $K^+$  channel blockers is not a function of the change in  $V_{mc}$ .

Since amiloride increases  $R_a/R_b$  of all bladders by ~20-fold, regardless of the value of  $V_{mc}$  (Demarest and Finn, 1987), it is clear that the  $Na^+$  conductance is the dominant conductance in the apical membrane of all bladders. Thus, a possible explanation for the transient decrease in  $R_a$  shown in Fig. 3*A* would be the opening of a small voltage-dependent apical conductance that would be masked in the steady state by the simultaneous decrease in the amiloride-sensitive apical  $Na^+$  conductance. Experiments to test this hypothesis, e.g., by studying the effects of voltage-clamping  $V_{mc}$  on the time course of the  $R_a$  response to  $Ba^{2+}$ , and to further characterize the conductance were beyond the scope of the present study. However, this does not affect the conclusions presented in this article, since the steady state responses of the two groups of bladders are not significantly different.

The interaction demonstrated here between the basolateral  $K^+$  channels and the apical  $Na^+$  channels is the reciprocal of the one demonstrated by Davis and Finn (1982*a, b*), who showed in the toad and frog urinary bladder that apical amiloride resulted in an inhibition not only of apical  $Na^+$  conductance but of the basolateral  $K^+$  conductance as well. Close correlations between the rate of transepithelial ion transport and the apical and basolateral membrane conductances have been observed in *Necturus* urinary bladder and a number of other

epithelia (Fromter and Gebler, 1977; Finn and Reuss, 1978; Narvarte and Finn, 1980; Schultz, 1981; Thomas et al., 1983). These interrelationships appear to be parts of a feedback regulatory mechanism involving the passive permeabilities of the apical and basolateral membranes and the Na<sup>+</sup>-K<sup>+</sup> ATPase, which allows for the maintenance of intracellular ionic and volume homeostasis when the rate of transepithelial ion transport changes. In the present study, the close relationships between the rate of transepithelial transport and the membrane conductances were preserved after partial basolateral K<sup>+</sup> channel block (Figs. 6 and 7 and Table VII), which suggests that experimental manipulation of the basolateral K<sup>+</sup> conductance caused an activation of the regulatory mechanism(s) that functions under normal conditions in these cells. In support of this interpretation are the observations that blocking the basolateral K<sup>+</sup> channels in rabbit proximal tubules with Ba<sup>2+</sup> does not result in a significant change in intracellular K<sup>+</sup> activity or cell volume (Biagi et al., 1981; Welling et al., 1985).

#### *Mechanisms That Are Not Involved in the Membrane Interaction*

Although the reduction of  $G_a$  caused by blocking the basolateral K<sup>+</sup> conductance appears to be a manifestation of an intrinsic regulatory mechanism of the urinary bladder cells, the mechanism of the membrane interaction remains obscure. However, our data indicate that several factors can be ruled out as possible mediators. It cannot, as stated above, be attributed to voltage alone, since K<sup>+</sup>-induced membrane potential changes not significantly different from those resulting from Ba<sup>2+</sup> block of the K<sup>+</sup> channel do not cause a significant change in  $R_a$  (Fig. 2, and see below). Further, the smaller changes in  $V_{mc}$  produced by Rb<sup>+</sup> or Cs<sup>+</sup> block were associated with increases in  $R_a$  equivalent to that caused by Ba<sup>2+</sup> (Fig. 5 and Tables II, V, and VI). In addition, these data indicate that the increase in  $R_a$  is not simply due to a shift of the measured slope resistance along a nonlinear (Goldman-like) current-voltage curve of the apical membrane.

An alternative way of expressing the same ideas is to compute the change in net flux across the apical membrane that would be expected if voltage alone were the determinant. To do this, one can use the flux form of the constant field equation, as follows (from Schultz, 1980):

$$I_{sc} = \frac{P_{Na} F V_{mc}}{RT} \left[ \frac{[Na]_m - [Na]_c \exp(FV_{mc}/RT)}{1 - \exp(FV_{mc}/RT)} \right],$$

where  $I_{sc}$  is the short-circuit current (equal to net transepithelial or transapical Na transport),  $P_{Na}$  is the apical membrane Na permeability,  $[Na]_m$  is the mucosal Na concentration (105 meq/liter),  $[Na]_c$  is cell Na concentration (5 meq/liter) (for the purpose of this argument, variations in this value from 2 to 15 have no effect on the conclusion, especially if it is assumed to be constant for the short period after the changes in serosal solution), and  $R$ ,  $T$ , and  $F$  have their usual meanings. By using the measured apical membrane voltage before and after changes in the serosal solution, it is possible to compute the expected changes in the short-circuit current and compare them with those observed (or, in this case, determined as the quotient of the open-circuit transepithelial potential,  $V_{ms}$ , and

the transepithelial resistance,  $R_t$ ), if one makes the critical assumption that  $P_{Na}$  remains unchanged. The predicted effect of the addition of 50 mM  $K^+$  is a reduction of the short-circuit current by 33%, whereas the observed change was not different from zero. After  $Ba^{2+}$  addition, the predicted change was a reduction by 37%, and in this case there was an average decrease of 27%.

It is evident, then, that the effects of these solution changes (and the same is true if one makes the calculations after  $Rb^+$  or  $Cs^+$  addition) on apical membrane resistance cannot be explained by invoking the constant field equation, and that at least under these circumstances, this model does not provide a sufficient description of the behavior of the apical membrane  $Na^+$  conductance.<sup>3</sup> This is so not only for the effects of  $Ba^{2+}$  and other  $K^+$  channel blockers on the apical  $Na^+$  channel, but it also seems to be the case for the effects of  $K^+$ . Here, as shown above, there was no effect whatsoever on the apical  $Na^+$  conductance, despite the large depolarization.

Intracellular  $Na^+$  and  $Ca^{2+}$  have both been implicated as regulators of the amiloride-sensitive  $Na^+$  channel (Schultz, 1981; Chase, 1984). To account for the observed increase in  $R_a$  through self-regulation of the amiloride-sensitive  $Na^+$  channel by  $Na^+$ , an increased intracellular  $Na^+$  would be required; however, the increase in  $E_a$  (predominantly an  $Na^+$  emf) after  $Ba^{2+}$  (Table II) is consistent with a decrease of intracellular  $Na^+$ . Indeed, such a change by itself might be expected to produce a decrease in  $R_a$  (Fuchs et al., 1977), and not the observed increase.

It has been proposed that cell  $Ca^{2+}$  is regulated in epithelia, at least in part through the operation of an electrogenic  $Na^+-Ca^{2+}$  exchanger located in the basolateral membrane (Grinstein and Erlij, 1978; Chase and Al-Awqati, 1981). Concentrations of  $Ba^{2+}$  comparable to those used in the present study effectively block such an exchanger in cardiac sarcolemma vesicles and canine erythrocytes (Trosper and Philipson, 1983; Parker, J. C., personal communication). Thus, although  $Ba^{2+}$  clearly blocks the basolateral  $K^+$  channel, the membrane interaction could be mediated independently through a rise in intracellular  $Ca^{2+}$  caused by the direct effect of  $Ba^{2+}$  on the  $Na^+-Ca^{2+}$  exchanger. However, it seems unlikely that  $Rb^+$  or  $Cs^+$  could act through such a mechanism. All three ions could, in theory, interfere with the operation of the exchanger by depolarizing the basolateral membrane potential, since the exchanger is poised with only a small electrochemical driving force favoring  $Ca^{2+}$  extrusion (Chase, 1984); however, the voltage independence of the membrane interaction obviates this explanation (see above). Thus, it is unlikely that the membrane interaction is the result of an increase of intracellular  $Ca^{2+}$  caused by the inhibition of an  $Na^+-Ca^{2+}$  exchange mechanism, although the possibility of an increase in intracellular  $Ca^{2+}$  brought about through some other mechanism cannot be eliminated. For example,  $Ba^{2+}$  has been reported to increase cyclic AMP (Grill, 1978) and alter phospholipid metabolism (Best and Malaisse, 1984), in association with its ability to promote insulin secretion by pancreatic cells, a  $Ca^{2+}$ -dependent process.

<sup>3</sup> It is evident that the assumption of a constant  $P_{Na}$  is wrong, since we measured the apical membrane conductance (which in this tissue is a good measure of  $P_{Na}$ ; see accompanying article). Therefore, the constant field equation does not adequately describe this epithelium under these conditions.

Alternatively, Ba<sup>2+</sup> could enter the cells and either directly substitute for Ca<sup>2+</sup> or cause Ca<sup>2+</sup> release from intracellular stores (Hardcastle et al., 1983). A verapamil-sensitive Ca<sup>2+</sup> entry has been demonstrated in isolated toad bladder epithelial cells (Humes et al., 1980), and Ba<sup>2+</sup> is known to permeate the verapamil-inhibitable Ca<sup>2+</sup> channels of excitable cells more easily than Ca<sup>2+</sup> itself (Hagiwara and Byerly, 1981). However, the reversibility of the effects of Ba<sup>2+</sup> and the lack of any effect of verapamil on the Ba<sup>2+</sup> responses (Table IV) suggest that Ba<sup>2+</sup> does not exert its effects by entering the cells. Further evidence against Ba<sup>2+</sup> entry into epithelial cells comes from a recent study in the polyene-treated frog skin (Nielsen, 1979). In this preparation, the apical membrane Na<sup>+</sup> selectivity and amiloride sensitivity are totally destroyed by apical exposure to a polyene antibiotic, so that there is essentially no barrier to Na<sup>+</sup> entry, and transepithelial measurements, therefore, at least in principle, yield information on the characteristics of the basolateral membrane alone. Using this preparation, the stoichiometry of the Na<sup>+</sup>-K<sup>+</sup> pump was determined in the presence of serosal Ba<sup>2+</sup> (Nielsen, 1979). If Ba<sup>2+</sup> were entering the cells, an inhibition of transport would be expected, since Ba<sup>2+</sup> inhibits the isolated Na<sup>+</sup>-K<sup>+</sup> ATPase by competing with Mg<sup>2+</sup> at a normally intracellular-facing site on the enzyme (Rendi and Uhr, 1964); however, no significant inhibition of the  $I_{sc}$  was observed for 90 min after serosal Ba<sup>2+</sup> (see Table VIII of Nielsen, 1979). Again, there is no evidence to suggest that Rb<sup>+</sup> or Cs<sup>+</sup> could act through such mechanisms.

In summary, we have demonstrated the existence of a feedback interaction between the basolateral K<sup>+</sup> conductance and the amiloride-sensitive apical Na<sup>+</sup> conductance of *Necturus* urinary bladder. Inhibition of the conductance of the K<sup>+</sup> channels with Ba<sup>2+</sup>, Cs<sup>+</sup>, or Rb<sup>+</sup> results in a rapid and simultaneous inhibition of the apical Na<sup>+</sup> channel, which is reflected as an increase in  $R_a$ . The intracellular signal that couples the activity of these two conductance pathways has not been identified. However, we have shown that the interaction cannot be attributed to voltage changes across the cell membranes and that the K<sup>+</sup> channel blockers are not interacting directly with the Na<sup>+</sup> channel, since Ba<sup>2+</sup> does not enter the cells and there is no evidence for the interaction of Rb<sup>+</sup> or Cs<sup>+</sup> with the amiloride-sensitive Na<sup>+</sup> channel. Further, our data indicate that the increase in  $R_a$  is not due to inhibition of the Na<sup>+</sup> channel by an increase in intracellular Na<sup>+</sup>, but rather that intracellular Na<sup>+</sup> probably falls after basolateral K<sup>+</sup> channel block, as reflected by an increase in  $E_a$ . While our data do not rule out the possibility that the increase in  $R_a$  was due to an increase in intracellular Ca<sup>2+</sup>, they suggest that such an increase could not be due to the inhibition of a basolateral Na<sup>+</sup>-Ca<sup>2+</sup> exchanger. The membrane interaction demonstrated here is part of a network of regulatory mechanisms that serve to maintain intracellular ionic and volume homeostasis during changes in the rate of transepithelial ion transport. Finally, our data suggest that the K<sup>+</sup> channels in the basolateral membranes of epithelia may be important regulatory sites for the control of transepithelial Na<sup>+</sup> absorption in a manner analogous to their proposed role in the regulation of Cl<sup>-</sup> transport in secretory epithelia (Petersen and Maruyama, 1984).

We would like to thank Drs. H. S. Chase and L. P. Sullivan for making copies of their manuscripts available to us before publication.

This study was supported by National Institutes of Health grants AM 17854 and AM 07047.

Original version received 22 October 1985 and accepted version received 26 October 1986.

#### REFERENCES

- Best, L., and W. J. Malaisse. 1984. Effects of ionophore A23187 and  $Ba^{2+}$  ions on labelling of phospholipids in rat pancreatic islets. *Cell Calcium*. 5:65-75.
- Biagi, B., M. Sohtell, and G. Giebisch. 1981. Intracellular potassium activity in the rabbit proximal straight tubule. *American Journal of Physiology*. 241:F677-F686.
- Chase, H. S., Jr. 1984. Does calcium couple the apical and basolateral membrane permeabilities in epithelia? *American Journal of Physiology*. 247:F869-F876.
- Chase, H. S., Jr., and Q. Al-Awqati. 1979. Removal of ambient  $K^+$  inhibits net  $Na^+$  transport in toad urinary bladder by reducing  $Na^+$  permeability of the luminal border. *Nature*. 281:494-495.
- Chase, H. S., Jr., and Q. Al-Awqati. 1981. Regulation of the luminal sodium permeability of the toad urinary bladder by intracellular sodium and calcium. Role of sodium-calcium exchange in the basolateral membrane. *Journal of General Physiology*. 77:693-712.
- Davis, C. W., and A. L. Finn. 1982a. Sodium transport inhibition by amiloride reduces basolateral membrane potassium conductance in tight epithelia. *Science*. 216:525-527.
- Davis, C. W., and A. L. Finn. 1982b. Sodium transport effects on the basolateral membrane in the toad urinary bladder. *Journal of General Physiology*. 80:733-751.
- Davis, C. W., and A. L. Finn. 1985. Cell volume regulation in frog urinary bladder. *Federation Proceedings*. 44:2520-2525.
- Demarest, J. R., and A. L. Finn. 1983. Serosal  $Ba^{2+}$  increases apical membrane resistance in *Necturus* urinary bladder. *Federation Proceedings*. 42:1282. (Abstr.)
- Demarest, J. R., and A. L. Finn. 1984. Basolateral potassium channel block and interactions between the cell membranes of *Necturus* urinary bladder. *Journal of General Physiology*. 84:28a. (Abstr.)
- Demarest, J. R., and A. L. Finn. 1987. Characterization of the basolateral membrane conductance of *Necturus* urinary bladder. *Journal of General Physiology*. 89:541-562.
- Demarest, J. R., and T. E. Machen. 1985. Microelectrode measurements from oxyntic cells in intact *Necturus* gastric mucosa. *American Journal of Physiology*. 249:C535-C540.
- Diamond, J. M. 1982. Transcellular cross talk between epithelial cell membranes. *Nature*. 300:683-685.
- Finn, A. L., and L. Reuss. 1978. Electrical interaction between apical and basolateral cell membranes in the toad urinary bladder epithelium: evidence for rheogenic sodium extrusion. In *Membrane Transport Processes*. J. F. Hoffman, editor. Raven Press, New York. 1:229-241.
- Fromter, E., and B. Gebler. 1977. Electrical properties of amphibian urinary epithelia. III. The cell membrane resistance and the effect of amiloride. *Pflügers Archiv*. 371:99-108.
- Fuchs, W., E. H. Larsen, and B. Lindemann. 1977. Current-voltage curve of sodium channels and concentration dependence of sodium permeability in frog skin. *Journal of Physiology*. 267:137-166.
- Grill, V. 1978.  $Ba^{2+}$  stimulates accumulation of cyclic AMP in rat pancreatic islets. *Biochemical and Biophysical Research Communications*. 82:750-758.
- Grinstein, S., and D. Erlij. 1978. Intracellular calcium and the regulation of sodium transport in the frog skin. *Proceedings of the Royal Society of London, Series B*. 202:353-360.



- Hagiwara, S., and L. Byerly. 1981. Calcium channel. *Annual Review of Neurosciences*. 4:69–125.
- Hardcastle, J., P. T. Hardcastle, and J. M. Noble. 1983. The effect of barium chloride on intestinal secretion in the rat. *Journal of Physiology*. 344:69–80.
- Helman, S. I., W. Nagel, and R. S. Fisher. 1979. Ouabain on active transepithelial sodium transport in the frog skin. Studies with microelectrodes. *Journal of General Physiology*. 74:105–127.
- Humes, H. D., C. F. Simmons, and B. M. Brenner. 1980. Effect of verapamil on the hydroosmotic response to antidiuretic hormone in toad urinary bladder. *American Journal of Physiology*. 239:F250–F257.
- Koefoed-Johnsen, V., and H. H. Ussing. 1958. The nature of the frog skin potential. *Acta Physiologica Scandinavica*. 42:298–308.
- Lau, K. R., R. L. Hudson, and S. G. Schultz. 1984. Cell swelling increases a barium-inhibitable potassium conductance in the basolateral membrane of *Necturus* small intestine. *Proceedings of the National Academy of Sciences*. 81:3591–3594.
- MacRobbie, E. A. C., and H. H. Ussing. 1961. Osmotic behavior of the epithelial cells of frog skin. *Acta Physiologica Scandinavica*. 53:348–365.
- Maruyama, Y., and O. H. Petersen. 1982. Cholecystokinin activation of single-channel currents is mediated by internal messenger in pancreatic acinar cells. *Nature*. 300:61–63.
- Maruyama, Y., and O. H. Petersen. 1984. Control of K<sup>+</sup> conductance by cholecystokinin and Ca<sup>2+</sup> in single pancreatic acinar cells studied by the patch-clamp technique. *Journal of Membrane Biology*. 79:293–300.
- Nagel, W. 1979. Inhibition of potassium conductance by barium in the frog skin epithelium. *Biochimica et Biophysica Acta*. 552:346–357.
- Nagel, W., and J. Crabbe. 1980. Mechanism of action of aldosterone on active transport across toad skin. *Pflügers Archiv*. 385:181–187.
- Narvarte, J., and A. L. Finn. 1980. Microelectrode studies in toad urinary bladder epithelium. Effects of Na concentration changes in the mucosal solution on equivalent electromotive forces. *Journal of General Physiology*. 75:323–344.
- Nielsen, R. 1979. Coupled transepithelial sodium and potassium transport across isolated frog skin: effect of ouabain, amiloride and the polyene antibiotic flipin. *Journal of Membrane Biology*. 51:161–184.
- Petersen, O. H., and Y. Maruyama. 1984. Calcium-activated potassium channels and their role in secretion. *Nature*. 307:693–696.
- Ramsay, A. G., D. L. Gallagher, R. L. Shoemaker, and G. Sachs. 1976. Barium inhibition of sodium ion transport in the toad bladder. *Biochimica et Biophysica Acta*. 436:617–627.
- Rendi, R., and M. L. Uhr. 1964. Sodium, potassium-requiring adenosinetriphosphatase activity. I. Purification and properties. *Biochimica et Biophysica Acta*. 89:520–531.
- Reuss, L., and A. L. Finn. 1975. Dependence of serosal membrane potential on mucosal membrane potential in toad urinary bladder. *Biophysical Journal*. 15:71–75.
- Schultz, S. G. 1980. *Basic Principles of Membrane Transport*. Cambridge University Press, Cambridge, England. 24–25.
- Schultz, S. G. 1981. Homocellular regulatory mechanisms in sodium-transporting epithelia: avoidance of extinction by “flush through.” *American Journal of Physiology*. 241:F579–F590.
- Smith, P. L., and R. A. Frizzell. 1984. Chloride secretion by canine tracheal epithelium. IV. Basolateral membrane K permeability parallels secretion rate. *Journal of Membrane Biology*. 77:187–199.

- Thomas, S. R., Y. Suzuki, S. M. Thompson, and S. G. Schultz. 1983. Electrophysiology of *Necturus* urinary bladder. I. "Instantaneous" current-voltage relations in the presence of varying mucosal sodium concentrations. *Journal of Membrane Biology*. 73:157-175.
- Trosper, T. L., and K. D. Philipson. 1983. Effects of divalent and trivalent cations of Na<sup>+</sup>-Ca<sup>2+</sup> exchange in cardiac sarcolemmal vesicles. *Biochimica et Biophysica Acta*. 731:63-68.
- Welling, P. A., M. A. Linshaw, and L. P. Sullivan. 1985. Effect of barium on cell volume regulation in rabbit proximal straight tubules. *American Journal of Physiology*. 249:F20-F27.