J. Membrane Biol. 84, 1–7 (1985)

The Journal of
Membrane Biology

Effects of Intracellular Sodium and Potassium Iontophoresis on Membrane Potentials and Resistances in Toad Urinary Bladder

Javier Narvarte and Arthur L. Finn
Departments of Medicine and Physiology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

Summary. Glass microelectrodes were used to measure membrane potentials and the ratio of apical to basolateral membrane resistances before and after the passage of current from the potential-recording microelectrode to ground, in toad urinary bladder epithelium, in order to iontophorese cations into the cell. After application of the current, there was a transient change in the tip potential of the microelectrode. This artifact was measured with the microelectrode in the mucosal medium and was subtracted from the potential recorded in the cell. The serosal medium was bathed by Ringer's solution containing 51.5 mM K^+ to minimize any current-induced increase of K^+ in the unstirred layer. Under those conditions, both Na^+ and K^+ iontophoresis caused a significant hyperpolarization of basolateral membrane potential (V_{cs}) and a significant increase in the ratio of apical to basolateral membrane resistances (R_a/R_b). When bladders were exposed to amiloride in the mucosal solution, Na^+ iontophoresis caused the basolateral membrane to hyperpolarize, but no significant changes were observed in R_a/R_b. When Na^+ was injected in the presence of serosal ouabain, V_{cs} depolarized and R_a/R_b increased. K^+ iontophoresis caused the basolateral membrane potential to hyperpolarize in the presence of ouabain but R_a/R_b did not change significantly. These results indicate that the Na^+ pump in toad bladder is rheogenic, that apical Na^+ conductance is sensitive to the cell levels of Na^+ and K^+ and that the basolateral membrane is K^+ permeable.

Key Words toad bladder · intracellular iontophoresis · sodium pump · membrane potentials · sodium transport · rheogenic pump

Introduction

The urinary bladder of the toad is a typical tight [8] epithelium that transports sodium actively from the urinary (mucosal or apical) to the blood (serosal or basolateral) side [14]. The active transport of Na^+ across the basolateral membrane (that provides the driving force for transepithelial Na^+ transport) [14], is thought to be mediated by the Na^+, K^+-ATPase [13] and is linked to the uptake of K^+ by the cell. Several lines of evidence have led to the suggestion that the Na pump in nonpolar cells [22] as well as in epithelial tissues [11–13] is rheogenic, that is, that the ratio of Na^+ to K^+ ions transported is larger than one, and that therefore the pump contributes directly to the basolateral membrane potential by creating a current across that membrane.

Several experimental procedures have been employed to demonstrate the existence of rheogenic Na transport. One used most widely is to load the cells with Na^+ [22]. Since the rate of active Na extrusion is dependent in some way on intracellular Na concentration, raising cell Na^+ over some range will increase the number of Na^+ ions transported by the pump, therefore enhancing any effect of pump current on the membrane potential [22]. One of the ways to increase cell Na^+ is by intracellular electrical injection (iontophoresis) [21, 22].

It has been previously shown that the apical membrane in toad urinary bladder is mostly Na^+ selective [15] while the basolateral membrane is almost certainly potassium selective [9]. Furthermore, it has been recently proposed that the activity of the basolateral Na^+ pump is linked to the sodium conductance of the apical membrane by a negative feedback regulatory mechanism that keeps the steady-state cell Na^+ content constant [23]. The signal linking pump activity and Na^+ entry is thought to be cell Na^+ activity, i.e., there would be an inverse relationship between cell Na^+ activity and the permeability of the apical membrane to Na^+ [23, 24], perhaps through the intermediacy of cell calcium [5].

We studied the effects of intracellular iontophoresis on the membrane potentials and resistances of toad urinary bladder epithelium. We found that intracellular iontophoresis of Na^+ hyperpolarizes the basolateral membrane potential, indicating that the sodium pump is rheogenic. Furthermore, our results suggest that apical Na^+ conductance is sensitive to cell levels of both Na^+ and K^+.

Materials and Methods

Toads (*Bufo marinus*) were obtained from the Jacques Weil Co. (Rayne, La.), the National Reagents Co. (Bridgeport, Conn.), or

the Southwestern Scientific Co. (Tucson, Ariz.) and kept in running water. Urinary bladders were mounted between the two halves of a Lucite® chamber, as previously described [15, 17], with Ringer's solution bathing both sides of the tissue at room temperature (22 ± 1°C).

Solutions

Standard Ringer's solution had the following composition (mM): NaCl 110, KCl 2.4, $NaHCO_3$ 2.4, $CaCl_2$ 0.9, glucose 5.5, pH about 8.5, gassed with room air. In experiments with high serosal K^+, K^+ was raised by isosmolar substitution for Na^+. Amiloride (a gift from Merck, Sharp and Dohme Research Laboratories, West Point, Pa.) was dissolved in the Ringer's solution to a final concentration of 10^{-5} M, and ouabain (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 5×10^{-4} M. Verapamil HCl (a gift from Knoll Pharmaceutical Co., Wippeny, N.J.) was added to the serosal solution to a final concentration of 10^{-5} M to inhibit smooth muscle contractions, so that the microelectrode could be kept intracellularly for longer periods. It has been previously reported that at that concentration verapamil either does not change the short-circuit current significantly [10] or causes only a modest decrease in that parameter [2]. In our experiments, verapamil did not affect V_{ms} or R_t.

Electrical Measurements

A) Transepithelial Potential (V_{ms}) and Resistance (R_t)

V_{ms} was measured with a high-impedance electrometer connected to both bathing media with Ag-AgCl pellets and agar-Ringer's bridges. R_t was measured from the change in V_{ms} produced by a transepithelial DC pulse [17].

B) Cell Membrane Potentials

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) threaded with fiberglass (W-P Instruments, Inc., New Haven, Conn.) and filled with 4 M potassium acetate, 4 M sodium acetate, or 3 M lithium chloride. The microelectrodes were beveled [4] to tip resistances of 10 to 50 MΩ and tip potentials were less than 5 mV. The impalements were performed with a motor-driven micromanipulator under visual control with an inverted, phase-contrast microscope (Leitz Wetzlar, W. Germany) at 200×. The criteria for successful impalements were as previously reported [17]. Potentials were measured with M-4 electrometer probes (W-P Instruments, Inc., New Haven, Conn.) and displayed on a storage oscilloscope (Tektronix, Inc., Beaverton, Ore). An Ag-AgCl electrode connected to the serosal solution by means of a short agar-Ringer's bridge was grounded and used as reference.

C) Ratio of Cell Membrane Resistances (R_a/R_b)

The ratio of the apical (R_a) to the basolateral (R_b) cell membrane resistances was measured as the ratio of voltage deflections produced by a transepithelial pulse with the microelectrode in a cell; i.e., $\frac{R_a}{R_b} = \frac{\Delta V_{mc}}{\Delta V_{cs}}$ [15].

D) Polarity Convention

For convenience, all potentials will be presented with the mucosal solution as reference. In this way, when the polarity of the tissue is as observed in control conditions (cell positive to the mucosal and negative to the serosal medium), all potentials have a positive sign.

E) Statistics

All results are expressed as means ± SEM. Differences between means were analyzed with the paired Student's *t*-Test.

Iontophoresis

The same glass microelectrode was used for injecting a cation into the cell (by making the tip of the microelectrode positive with respect to the grounded serosal electrode) and for recording the membrane potentials and the value of R_a/R_b before and after the iontophoresis. As previously reported [1], passing current through a microelectrode brings about a transient change in the microelectrode tip potential; its magnitude was 4 to 12 mV immediately after the injection and decayed thereafter, vanishing within 20 to 60 sec. To correct for this artifact, we measured it with the electrode in the mucosal solution, and then subtracted the observed values from the potentials recorded with the electrode in a cell, after passing the same amount of current for the same length of time. In doing so, we assumed that the electrode polarization was the same inside and outside the cell [1]. Microelectrodes with large artifact potentials were rejected, and the results of a given intracellular iontophoresis were accepted only if the extracellularly measured artifact potential was unchanged after the intracellular injection. A Med 80 computer (Nicolet Instruments, Madison, Wis.) was used for delivering the pulses to the microelectrode and also for acquiring and processing data. The experimental protocol was as follows:

1) The computer was triggered to record the baseline potential difference between the microelectrode and the serosal solution and between the macroelectrode and the serosal solution, while passing transepithelial current pulses through the current macroelectrodes.

2) Upon a second trigger pulse, the computer put out a four-second square voltage pulse through the microelectrode, making it positive with respect to the grounded serosal solution.

3) After a delay of one second following the pulse, the computer recorded and stored for four seconds the potential difference measured by the microelectrode and by the macroelectrode, with respect to the serosal solution.

4) Immediately after this, transepithelial DC current pulses were passed again and the potentials measured by the micro- and macroelectrodes were recorded.

The sequence 1) to 4) was performed first with the microelectrode out of the cell and then with the microelectrode in the cell. The change in potential induced by the current injection with the electrode in the cell was subtracted by the computer, point by point, from that recorded with the electrode out of the cell, so that the true change in membrane potential elicited by the ionic injection could be determined. In this way, we had the values for V_{ms}, R_t, R_a/R_b, V_{mc} and V_{cs} before and after the iontophoresis. The injection of current was performed by connecting

the microelectrode to a BB-1 Breakaway Box (W-P Instruments, New Haven, Conn.). The amount of current injected was not measured routinely because it was found to be very close to $i = \frac{V}{R + 22}$, both with the electrode in and out of the cell, where i is the current injected, V is the potential difference applied to the BB-1 Breakaway Box, R is the resistance of the microelectrode and 22 (MΩ) is the resistance of the BB-1 Breakaway Box. Given the difficulty in maintaining a microelectrode intracellularly for long periods of time in toad urinary bladder epithelial cells, we chose to perform the iontophoresis for a period of four seconds, recording the resulting change in membrane potential for another period of four seconds. Preliminary experiments showed that the effective currents were of the order of 10^{-7} A. (For comparison, the current due to leakage of K^+ from an intracellular 3 M KCl filled microelectrode would be at most of the order of 10^{-9} A [3, 7, 16]). In the experiments reported here, we used currents of 1.8 to 4 × 10^{-7} A.

Figure 1 illustrates how the artifact potential was subtracted to obtain the change in membrane potential due to iontophoresis. The bottom trace shows the potential recorded with the microelectrode in the mucosal solution after the passage of current. Its value one second after the end of the pulse was 35 mV, and the baseline value was 42 mV. This depolarization of 7 mV represents the change in tip potential induced by the current. The potential across the basolateral membrane recorded after the injection is shown by the middle trace. Its values before and one second after the iontophoresis were 33 and 31 mV, respectively. If we consider the change in tip potential obtained from the bottom trace, the change in basolateral membrane potential due to iontophoresis was 5 mV one second after the pulse, and in the hyperpolarizing direction. This is shown by the upper trace, which represents the change in V_{cs} due to Na iontophoresis, after correcting for electrode polarization. We see that in this experiment Na electrophoresis caused hyperpolarization of the basolateral membrane that was largest immediately after the pulse; its magnitude decreased subsequently, as V_{cs} relaxed towards its baseline value.

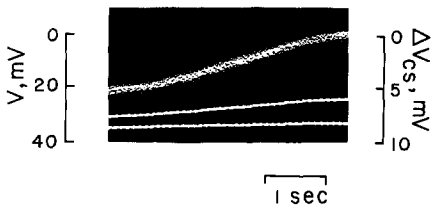

Fig. 1. Voltage traces. The bottom trace is the potential recorded beginning one second after passing a pulse through the recording microelectrode, which is in the mucosal solution. The middle trace is obtained one second after another pulse, with the microelectrode in a cell. To obtain the upper trace, the baseline value of each of the bottom two traces is subtracted from the respective potential and the difference between the two corrected values is the upper trace. The left-hand ordinate refers to the bottom two traces, and the right-hand ordinate to the upper trace. *See* text for details

Theoretical Considerations

In these experiments, we passed current between the microelectrode and ground, so that the current crossed cell membranes. It is likely that most of the current flowing from the microelectrode to the cell was carried by the cation of the salt filling the microelectrode[1] (*see* Discussion). However, we do not know which ions carried the current across the cell membranes. Given the relatively high K^+ concentration in the cell and the high K^+ conductance of the basolateral membrane we can predict that a sizeable portion of the current would be carried by K^+ across the basolateral membrane. However, since the apical membrane presents a high Na^+ conductance, at least part of the current must be carried by Na across that membrane. Therefore, one would expect that the injection of Na^+ increases cell Na^+ and decreases cell K^+, probably also increasing the K^+ concentration in the serosal unstirred layer. Such a result would almost certainly lead to depolarization of V_{cs}, because of the K^+ selectivity of the membrane, and stimulation of a neutral pump by Na^+ would only restore cell K^+ and Na^+ gradients; thus no hyperpolarization of V_{cs} would be expected. If any hyperpolarization is observed, it would have to be due to the operation of a rheogenic pump. The injection of K^+, on the other hand, would increase cell K^+ and decrease cell Na^+, probably hyperpolarizing V_{cs} because of the increased K^+ gradient across the basolateral membrane. Finally, no changes in V_{ms} are expected in these experiments, because the injection of ions will affect the membrane potentials only of the injected cell and at most a few neighboring cells according to the degree of cell-to-cell coupling. Since the transepithelial potential is determined by the whole epithelium, the V_{ms} of the injected cell is "clamped" by the rest of the epithelium so that changes equal in magnitude and opposite in direction will be expected for V_{mc} and V_{cs}. Thus, we must use experimental maneuvers to distinguish the membrane to which the primary effect of iontophoresis is exerted.

Results

Figure 2 shows the mean values of V_{cs} and R_a/R_b before and one second (V_{cs}) or five seconds (R_a/R_b) following intracellular iontophoresis with Na^+ or K^+, with normal Ringer's in both bathing media. It is clear that neither Na^+ nor K^+ iontophoresis had any significant effect on V_{cs} or R_a/R_b. As stated above, if during the iontophoresis a significant portion of the current is carried out of the cell by K^+, the possibility exists that an increase in K^+ concen-

[1] 100 nl droplets (composition (mM) NaCl 50, KCl 10) were suspended in mineral oil and impaled with glass microelectrodes (resistance 10 to 22 MΩ, measured in standard Ringer's solution) filled with 4 M potassium acetate or with 4 M sodium acetate (*see* Materials and Methods). The tip of the microelectrode was made positive with respect to a grounded tungsten electrode immersed in the droplet, and current was passed between the microelectrode and ground (1 to 6 × 10^{-7} A) for periods of 1 to 30 min. The IR drop through a 1 KΩ resistor was monitored continuously during the injection of current. Na or K concentrations were measured in each droplet after the passage of current and compared with the Na^+ or K^+ concentration calculated by assuming that all of the current was carried out of the microelectrode by the respective cation. The ratio (measured concentration/calculated concentration) was not significantly different from 1.0 both for Na^+ and for K^+ injections (0.98 ± 0.03, 3 Na^+ injections and 2 K^+ injections).

Table 1. Effect of Na^+ and K^+ iontophoresis on membrane potentials and on the ratio of resistances of toad urinary bladder epithelium[a]

	Na^+ Iontophoresis				K^+ Iontophoresis			
	V_{mc} (mV)	V_{cs} (mV)	R_a/R_b	n (tissues, cells)	V_{mc} (mV)	V_{cs} (mV)	R_a/R_b	n (tissues, cells)
Before iontophoresis	20.9 ± 2.3	22.0 ± 1.5	2.86 ± 0.63	13, 21	20.3 ± 1.7	16.0 ± 0.9	1.62 ± 0.23	3, 13
After iontophoresis	17.0 ± 2.4	25.9 ± 2.0	4.14 ± 0.67		13.5 ± 1.7	22.4 ± 1.3	2.24 ± 0.35	
Δ	−3.9 ± 0.9	3.9 ± 0.9	1.28 ± 0.23		−6.8 ± 1.3	6.4 ± 1.4	0.62 ± 0.24	
p	<0.001	<0.001	<0.001		<0.001	<0.001	<0.05	

[a] Serosal K concentration was 51.5 mM. Δ = value after iontophoresis − value before iontophoresis.

Table 2. Effect of Na^+ and K^+ iontophoresis on V_{mc}, V_{cs}, and R_a/R_b of bladders exposed to 10^{-5} M amiloride on the mucosal side[a]

	Na^+ Iontophoresis				K^+ Iontophoresis			
	V_{mc} (mV)	V_{cs} (mV)	(R_a/R_b)	n (tissues, cells)	V_{mc} (mV)	V_{cs} (mV)	(R_a/R_b)	n (tissues, cells)
Before iontophoresis	−27.0 ± 3.7	7.5 ± 1.2	5.54 ± 0.76	4, 13	−4.9 ± 2.0	6.3 ± 1.4	4.58 ± 0.44	3, 11
After iontophoresis	−30.4 ± 3.7	11.3 ± 1.5	5.94 ± 0.99		−6.7 ± 2.8	8.2 ± 1.8	5.10 ± 0.68	
Δ	−3.4 ± 0.9	3.8 ± 0.9	0.40 ± 0.98		−1.8 ± 1.0	1.9 ± 1.0	0.52 ± 0.47	
p	<0.01	<0.01	>0.50		>0.05	>0.05	>0.20	

[a] *See* footnote in Table 1.

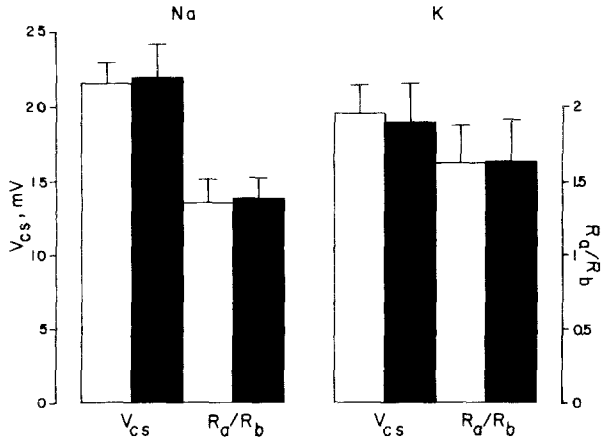

Fig. 2. Effects of sodium and potassium iontophoresis with normal Ringer's on both sides. The open bars give the mean values (± SEM) before, and the closed bars after, iontophoresis

tration in the serosal unstirred layer depolarizes V_{cs}, masking the hyperpolarization that would be expected following an increase in cell K^+ or an enhanced pump rate. The experiments were repeated with the concentration of K^+ raised to 51.5 mM in the serosal medium (by isosmolar substitution for Na^+) to minimize any current-induced increase of K^+ in the unstirred layer. Table 1 shows that under these conditions, both Na^+ and K^+ iontophoresis caused a highly significant hyperpolarization of V_{cs}, and an increase in R_a/R_b. As expected, the membrane potentials changed in opposite directions, so that the change in V_{cs} could be secondary to a depolarization of V_{mc} induced by the increase in cell Na^+ brought about by Na^+ iontophoresis (as opposed to a primary hyperpolarization of V_{cs}). Table 2 shows the effect of Na^+ and K^+ iontophoresis on tissues previously exposed to 10^{-5} M amiloride in the mucosal solution. Under these conditions, the conductance of the apical membrane to Na^+ is essentially zero [14] and changes in cell Na^+ concentration should not affect V_{mc}. However, Na^+ iontophoresis still hyperpolarized V_{cs} and depolarized V_{mc}, suggesting that the effect of Na^+ iontophoresis is on the basolateral membrane. Furthermore, when amiloride was present, Na^+ iontophoresis brought about no changes in R_a/R_b, and K^+ iontophoresis caused no significant changes in either membrane potentials or R_a/R_b. If the hyperpolarizing effect of Na^+ iontophoresis on V_{cs} is due to stimulation of rheogenic Na^+ pumping, Na^+ injection in the presence of the pump inhibitor ouabain should not elicit

Table 3. Effect of Na^+ and K^+ iontophoresis on V_{mc}, V_{cs}, and R_a/R_b of bladders incubated with 5×10^{-4} M ouabain in the serosal medium[a]

	Na^+ Iontophoresis				K^+ Iontophoresis			
	V_{mc} (mV)	V_{cs} (mV)	(R_a/R_b)	n (tissues, cells)	V_{mc} (mV)	V_{cs} (mV)	(R_a/R_b)	n (tissues, cells)
Before iontophoresis	7.1 ± 1.7	15.5 ± 0.7	2.72 ± 0.21	4, 15	15.8 ± 1.3	10.1 ± 1.0	2.03 ± 0.26	4, 14
After iontophoresis	10.4 ± 1.2	11.9 ± 1.3	3.54 ± 0.29		11.6 ± 1.5	14.2 ± 1.6	2.22 ± 0.41	
Δ	3.4 ± 0.8	−3.6 ± 0.9	0.82 ± 0.31		4.3 ± 1.2	4.1 ± 1.1	0.25 ± 0.33	
p	<0.001	<0.01	<0.05		<0.01	<0.01	>0.20	

[a] *See* footnote in Table 1.

such a hyperpolarization of V_{cs}. Table 3 shows the effect of Na^+ and K^+ iontophoresis on membrane potentials and R_a/R_b of bladders incubated with ouabain in the serosal medium. In these conditions Na^+ iontophoresis caused V_{cs} to depolarize significantly instead of hyperpolarizing as observed when no ouabain was present, and led to a significant increase in R_a/R_b. On the other hand, K^+ iontophoresis still caused V_{cs} to hyperpolarize significantly, although the mean change was somewhat smaller than that observed when ouabain was not present, and had no significant effect on R_a/R_b.

Some of the effects of iontophoresis of Na^+ and K^+ on membrane potentials and resistances could be due to a nonspecific effect of the passage of current across the cell membranes. To rule out that possibility, injections of Li^+ were performed using the same conditions and similar currents to those used for K^+ and Na^+ iontophoresis. Li^+ iontophoresis caused no significant changes in membrane potentials: V_{mc} hyperpolarized by 0.5 ± 0.6 mV ($P > 0.20$) and V_{cs} depolarized by 0.6 ± 0.5 mV ($P > 0.20$) (4 bladders, 15 cells), and there was a small decrease in R_a/R_b of 0.28 ± 0.12 ($P > 0.05$).

Discussion

Assumptions

a) The basic assumption in these experiments is that the current-induced polarization of the microelectrode is the same inside and outside the cell. We cannot prove that that is true. However, even if this artifactual polarization were different inside and outside the cell it could not explain the qualitatively different results obtained with the different ions and inhibitors. That is, the error involved (the difference in polarization inside and outside the cell) should be the same regardless of the electrolyte filling the electrode; furthermore, it should not vary with inhibitors of transport.

b) The fact that current crosses the cell membranes during injection complicates the interpretation of these results, since cell composition changes not only because of the ion being injected, but also because of the possibility that other intracellular ions carry current across the membranes. Furthermore, the concentration of ions in the extracellular unstirred layers is perturbed. Since K^+ is the main intracellular cation and the basolateral membrane is K^+ selective [10], the passage of outward current between the microelectrode and ground increases the K^+ concentration in the serosal unstirred layer and depolarizes V_{cs} due to the decrease in the K^+ gradient across the basolateral membrane. To minimize this change in gradient induced by the iontophoresis, we raised the concentration of K^+ in the serosal medium. It is true that by increasing K^+ we are probably changing the cell K^+ levels [18] and kinetic properties of the sodium pump [20]. However, such changes would only affect the baseline electrical parameters and the absolute change in those parameters brought about by iontophoresis but they could not explain the different results obtained with different ions and transport inhibitors.

c) We also assumed that amiloride can be used to distinguish between the apical or the basolateral membranes being the locus for the primary effect of a given experimental maneuver. It has been previously shown that the apical membrane is mostly Na^+ selective [15] and that the major effect of amiloride is to block the conductance of that membrane to Na^+ [14]. Thus, in the presence of amiloride, V_{mc} and apical Na^+ conductance are not expected to be affected by changes in the composition of the cell, so that under those conditions changes in membrane potentials and R_a/R_b brought about by iontophoresis are due to a primary effect on the basola-

teral membrane. The fact that basolateral membrane conductance also decreases upon addition of amiloride to the mucosal medium (probably because of a decrease in basolateral K^+ conductance) [6, 19] does not invalidate the above assumption.

d) The currents used to inject ions in these experiments were of the order of 10^{-7} A. By doing iontophoresis into 100 nl droplets we have shown that at least under those conditions current is carried out of the electrode almost exclusively by the cation; that is, the transport number across the electrode tip is not significantly different from one for the cation of the electrolyte filling the microelectrode when the tip is made positive with respect to ground[1]. If the volume of a cell is of the order of 10^3 μm^3 [6], the injected current was enough to increase cell Na^+ by about two orders of magnitude and K^+ by one order of magnitude. Of course the actual increase in cell concentration is much smaller, because the current flows out of the cell to the mucosal solution, to the serosal solution and to other cells and possibly also because water enters the cell. The net increase in cell content for the ion being injected will depend mostly on which ions carry the current out of the cell. For example, if during K^+ iontophoresis all the current is carried out of the cell by K^+, no increase in $[K^+]_c$ will be expected. In any case, all electrical measurements returned to baseline levels within 10 sec after the injections.

Changes in Membrane Potentials

No significant changes in membrane potentials were found when K^+ and Na^+ iontophoresis were performed with a normal $[K^+]$ in the serosal medium. However, when $[K^+]_s$ was increased both Na^+ and K^+ iontophoresis caused significant hyperpolarization of V_{cs}. This suggests strongly that the failure to see hyperpolarization under control conditions is due to depolarization of V_{cs} induced by increased K^+ concentration in the serosal unstirred layer (brought about by current-induced K^+ flow out of the cell through the basolateral membrane). By increasing $[K^+]$ in the serosal medium, the change in K^+ in the serosal unstirred layer and the attendant depolarization of V_{cs} were minimized, so that the hyperpolarization due to iontophoresis was unmasked. However, the possibility also exists that the increase in serosal K^+ increased basolateral K^+ conductance, resulting in more current being carried out of the cell by K^+ during the injection and in a larger increase of Na^+ concentration in the cell after Na^+ iontophoresis. That this is not the only mechanism is shown by the fact that the injection of K^+ also resulted in hyperpolarization of V_{cs} when serosal $[K^+]$ was increased.

The fact that increased *serosal* K^+ made the iontophoresis-induced hyperpolarization of V_{cs} apparent suggests that the basolateral membrane was the site of the primary effect of iontophoresis. However, the possibility also exists that the first effect was on V_{mc}, as explained above. That this is not the case is shown by the experiments with amiloride. When the iontophoresis of Na^+ was repeated in the presence of mucosal amiloride a hyperpolarization of V_{cs} was still observed, indicating that the basolateral membrane was indeed the site of the primary effect. The fact that K^+ iontophoresis caused no significant hyperpolarization of V_{cs} in the presence of amiloride indicates that under those conditions virtually all of the injected current was carried out of the cell by K^+ because the apical membrane conductance was essentially zero and no increase in cell K^+ was expected to occur.

If the hyperpolarization of V_{cs} induced by Na^+ iontophoresis is due to stimulation of the Na^+ pump it should be blocked by ouabain [14, 21]. Table 3 shows that when ouabain was present in the serosal medium, Na^+ iontophoresis caused V_{cs} to depolarize instead of hyperpolarize. Under those conditions, the hyperpolarizing effect of Na^+ injection was prevented by the inhibition of the Na^+ pump, and the cell K^+ depletion brought about by the injection of Na^+ led to depolarization of V_{cs}. However, the injection of K^+ still had a hyperpolarizing effect on V_{cs}, showing that the K^+-induced hyperpolarization of V_{cs} is a passive process that does not depend on the activity of the Na pump, and that the basolateral membrane is K permeable. Thus, Na^+ iontophoresis hyperpolarizes V_{cs} by stimulating the Na^+ pump; as explained above, the stimulation of a neutral pump would be expected only to restore the K and Na gradients dissipated during the iontophoresis, and no hyperpolarization of V_{cs} would be expected. These results are best explained by stimulation of rheogenic Na^+ extrusion upon loading the cells with Na^+. They do not tell us if the pump is rheogenic when cell Na^+ concentration is normal.

Changes in R_a/R_b

When Na^+ and K^+ iontophoresis were done with normal serosal K^+ concentration, no significant changes were found in R_a/R_b. However, upon increasing the concentration of K^+ in the serosal medium both Na^+ and K^+ iontophoresis brought about significant increases in the resistance ratio. That this result was due to an increase of R_a and not to a decrease in R_b is shown by the fact that in the presence of amiloride both Na^+ and K^+ iontophoresis

failed to increase R_a/R_b significantly. If the increase in R_a/R_b were due to an effect on R_b, it should still be present with amiloride.

In the presence of ouabain, the change in R_a/R_b elicited by Na iontophoresis was smaller than in control conditions. This finding supports the previously proposed effect of $[Na^+]_c$ on apical sodium conductance (g_a^{Na}), that is, decreasing $[Na^+]_c$ raises g_a^{Na}. Ouabain has been shown to increase $[Na^+]_c$ and R_a [14, 15], so that under those conditions an increase in $[Na^+]_c$ would be expected to be less effective in changing g_a^{Na} and R_a. This also suggests that the effect of Na^+ iontophoresis on R_a is at the same locus as ouabain, namely, g_a^{Na}. Sodium iontophoresis may also lead to an increase in cell calcium via stimulation of a Na^+-Ca^{++} exchange mechanism at the basolateral border [5]; such a mechanism would also be expected to increase R_a. Further experiments are under way to clarify this point.

In the presence of ouabain, K^+ caused no significant increase of R_a/R_b. This is probably related to the fact that ouabain, as noted above, by itself causes a rise in R_a, so that K^+ injection can have no further effect.

The experiments with lithium indicate that the results shown above cannot be explained by nonspecific current-dependent mechanisms. Nonspecific effects of current injection should not depend on the ion injected, or on the presence of ouabain or of amiloride. The absence of any significant change in R_a/R_b or membrane potentials after Li^+ iontophoresis indicates that the changes observed with both Na^+ and K^+ must be due to specific effects of the ions. In addition, these data indicate that Li^+ is not a sodium (or potassium) substitute in this tissue.

In summary, we have shown that intracellular injection of Na hyperpolarizes the basolateral membrane most probably by stimulation of a rheogenic Na pump, that the cellular iontophoresis of K^+ also hyperpolarizes the basolateral membrane, as expected for a potassium-selective membrane, and that the intracellular injection of either Na^+ or K^+ increases the resistance of the apical membrane, probably by decreasing the conductance of that membrane to sodium.

References

1. Araki, T., Ito, M., Oscarson, O. 1961. Anion permeability of the synaptic and non-synaptic motoneurone membrane. *J. Physiol. (London)* **159:**410–435
2. Bentley, P.J. 1974. Effects of verapamil on the short-circuit current of an epithelial membrane: The toad urinary bladder. *J. Pharmacol. Exp. Ther.* **189:**563–569
3. Blatt, M.R., Slayman, C.L. 1983. KCl leakage from microelectrodes and its impact on the membrane parameters of a nonexcitable cell. *J. Membrane Biol.* **72:**223–234
4. Brown, K.T., Flaming, D.G. 1974. Beveling of fine micropipette electrodes by a rapid precision method. *Science* **185:**693–695
5. Chase, H.S., Al-Awqati, Q. 1981. Regulation of the sodium permeability of the luminal border of toad bladder by intracellular sodium and calcium. Role of sodium-calcium exchange in the basolateral membrane. *J. Gen. Physiol.* **77:**693–712
6. Davis, C.W., Finn, A.L. 1982. Sodium transport effects on the basolateral membrane in toad urinary bladder. *J. Gen. Physiol.* **80:**733–751
7. Fromm, M., Schultz, S.G. 1981. Some properties of KCl-filled microelectrodes: Correlation of potassium "leakage" with tip resistance. *J. Membrane Biol.* **62:**239–244
8. Frömter, E., Diamond, J.M. 1972. Route of passive ion permeation in epithelia. *Nature New Biol.* **235:**9–13
9. Gatzy, J.T., Clarkson, T.W. 1965. The effect of mucosal and serosal solution cations on bioelectric properties of the isolated toad bladder. *J. Gen. Physiol.* **48:**647–671
10. Gordon, L.G.M. 1978. Cellular and shunt conductances of toad bladder epithelium. *J. Membrane Biol.* **44:**309–319
11. Kirk, K.L., Halm, D.R., Dawson, D.C. 1980. Active sodium transport by turtle colon via an electrogenic Na-K exchange pump. *Nature (London)* **287:**237–239
12. Lewis, S.A., Wills, N.K. 1983. Apical membrane permeability and kinetic properties of the sodium pump in rabbit urinary bladder. *J. Physiol. (London)* **341:**169–184
13. Lewis, S.A., Wills, N.K., Eaton, D.C. 1978. Basolateral membrane potential of a tight epithelium: Ionic diffusion and electrogenic pumps. *J. Membrane Biol.* **41:**117–148
14. Macknight, A.D.C., DiBona, D.R., Leaf, A. 1980. Sodium transport across toad urinary bladder: A model "tight" epithelium. *Physiol. Rev.* **60:**615–715
15. Narvarte, J., Finn, A.L. 1980. Microelectrode studies in toad urinary bladder epithelium. Effects of Na concentration changes in the mucosal solution on equivalent electromotive forces. *J. Gen. Physiol.* **75:**323–344
16. Nastuk, W., Hodgkin, A. 1950. The electrical activity of single muscle fibers. *J. Cell. Comp. Physiol.* **35:**39–74
17. Reuss, L., Finn, A.L. 1974. Passive electrical properties of toad urinary bladder epithelium. Intracellular electrical coupling and transepithelial cellular and shunt conductances. *J. Gen. Physiol.* **64:**1–25
18. Robinson, B.A., Macknight, A.D.C. 1976. Relationships between serosal medium potassium concentration and sodium transport in toad urinary bladder: II. Effects of different medium potassium concentrations on epithelial cell composition. *J. Membrane Biol.* **26:**239–268
19. Schultz, S.G. 1982. Homocellular regulatory mechanisms in sodium-transporting epithelia: An extension of the Koefoed-Johnsen-Ussing model. *Semin. Nephrol.* **2:**343–347
20. Stekhoven, F.S., Bonting, S.L. 1981. Transport adenosine triphosphatases: Properties and functions. *Physiol. Rev.* **61:**1–76
21. Thomas, R.C. 1969. Membrane current and intracellular sodium changes in a snail neurone during extrusion of injected sodium. *J. Physiol. (London)* **201:**495–514
22. Thomas, R.C. 1972. Electrogenic sodium pump in nerve and muscle cells. *Physiol. Rev.* **52:**563–594
23. Turnheim, K., Frizzell, R.A., Schultz, S.G. 1978. Interaction between cell sodium and the amiloride-sensitive sodium entry step in rabbit colon. *J. Membrane Biol.* **39:**233–256
24. Wills, N.K., Lewis, S.A. 1980. Intracellular Na^+ activity as a function of Na^+ transport rate across a tight epithelium. *Biophys. J.* **30:**181–186

Received 9 October 1984