BRIEF COMMUNICATION

INTRACELLULAR Na^+ ACTIVITY AS A FUNCTION OF Na^+ TRANSPORT RATE ACROSS A TIGHT EPITHELIUM

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ABSTRACT Liquid Na^+ resin microelectrodes were used to measure intracellular Na^+ activities ($a_i^{Na^+}$) in the mammalian tight epithelium, rabbit urinary bladder. $a_i^{Na^+}$ averaged 7 ± 1 mM and was independent of Na^+ transport rate over the range of 2 to 8 $\mu A/\mu F$. (1 mF ≃ 1 cm^2 apical membrane area.) After Na^+ loading the cells the Na^+ pump in the basolateral membrane was measurably electrogenic. A method for shielding the Na^+-sensitive microelectrodes is described which increases the response time and eliminates an electrical shunting artifact.

A primary function of most mammalian epithelia is the absorption of sodium ions. Movement of Na^+ across epithelial cells is generally thought to require a passive entry step across the apical membrane and active extrusion across the basolateral membrane. We now report direct evidence for this hypothesis from measurements of intracellular Na^+ activity ($a_i^{Na^+}$) obtained with Na^+-sensitive microelectrodes (1, 2). We also address the following questions: is $a_i^{Na^+}$ altered as a function of Na^+ transport rate? If so, what is the nature and time-course of this alteration?

Experiments were performed on urinary bladders from 16 male New Zealand white rabbits. Nine rabbits were fed a low Na^+ diet (Ralston Purina Co., Richmond, Ind.) for 13–15 d before the experiments; the others received normal rabbit chow (Ralston Purina Co.). The purpose of the low Na^+ diet was to stimulate the transport rate for this ion (3, 4). After removal of the bladder from the animal, muscle layers were gently dissected away. The epithelium was then mounted vertically between lucite chambers and bathed on both sides with NaCl Ringer's solution. Bathing solutions were well stirred, oxygenated, maintained at 37°C and pH 7.4, and (unless otherwise noted) consisted of [NaCl] = 111.2 mM, [KCl] = 7 mM, [Na HCO_3] = 25 mM, [NaH_2PO_4] = 1.2 mM, [$MgSO_4$] = 1.2 mM, [$CaCl_2$] = 2 mM, and 11 mM glucose. (Throughout this paper brackets will denote concentration, and parentheses will indicate activity.) Procedures for avoiding edge damage, electrical methods, and microelectrode impalement criteria were the same as reported previously (4, 5). All potential readings were made with a digital printer (Newport Research Corp., Fountain Valley, Calif.; model 810) or a microprocessor-interfaced analog-to-digital converter (North Star Computers, Berkeley, Calif.; model Horizon II) with an accuracy of ± 0.1 mV.

Microelectrodes were pulled from thin-walled glass capillary tubing (Corning Glass Works, Science Products Div., Corning, N.Y.; #7740:o.d. = 1.2 mm; i.d. = 0.9 mm) on a horizontal pipette puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan; model PD-5) to a tip diameter of < 1 μm (tip resistance = 20–40 MΩ when filled with 3 M KCl solution). Ion-sensitive microelectrodes were constructed identically, except that these were siliconized before use (5, 8). For potassium-sensitive microelectrodes, liquid K^+ exchanger resin (Corning Medical Products Div., Medfield, Mass., #477317) was placed in the tip and the remaining portion of the microelectrode was backfilled with 0.5 M KCl solution (7). For Na^+-sensitive microelectrodes the same resin was mixed (50% vol/vol) with neutral Na^+ ionophore resin (1, 2, 6) and the remainder was filled with 0.5 M NaCl solution. All microelectrode impalements were from the luminal surface and were referenced to the serosal bath. Ion-sensitive microelectrodes were calibrated before and after each use for response to decade changes in $a_i^{Na^+}$ ("slope") and sensitivity to competing ions ("selectivity"). To calculate intracellular activities a modified version of Walker's equation was used (5, 7; Table I). Reference potentials for ion activity calculations were basolateral membrane potentials (V_{bl}) taken with a conventional microelectrode from surrounding cells either simultaneously or immediately before and after the ion-sensitive microelectrode measurements. To estimate sensitivity to competing ions, ion-sensitive microelectrodes were calibrated in a regime of pure and mixed salt solutions and in NaCl Ringer's solution at 37°C and at room temperature. The response of the Na^+ microelectrode in 100 mM KCl was chosen to calculate the microelectrode selectivity ratio for Na^+:K^+, as this concentration of K^+ approached previously measured a_i^K values (5). This value was similar to that obtained in mixed salt solutions or NaCl Ringer's solution. Na^+ microelectrodes were nearly equally sensitive to Ca^{+2}. However, since Ca^{+2} is probably quite low under most physiological conditions, interference from this ion was negligible.

Under control conditions, intracellular K^+ was calculated as 89 ± 9 mM (n = 32). This value was not statistically different from our earlier measurements (5). Initial measurements with Na^+-sensitive microelectrodes (see Table I ["unshielded microelectrodes"] produced a mean $a_i^{Na^+}$ = 19 ± 3 mM (n = 54). In contrast to results from K^+-sensitive or conventional

TABLE I
COMPARISON OF SHIELDED AND UNSHIELDED Na^+-SENSITIVE MICROELECTRODE CHARACTERISTICS

	Microelectrode characteristics		Measurements in urinary bladder	
	Na^+:K^+ selectivity	Slope (millivolts per decade change in Na^+ activity)	Calculated activity	Resistance ratio (R_a/R_{bl})
Unshielded N = 11	14:1	57 ± 1.7	19 ± 2.5	4 ± 1.1
Shielded N = 8	26:1	60 ± 0.6	7 ± 0.7	15 ± 1.5

Na^+ activity equation: $a_i^{Na^+} = (a_o^{Na^+} + K_{K/Na} \cdot a_o^{K^+}) \exp(nF/RT)(V_{Na} - V_{bl}) - K_{K/Na} \cdot a_i^{K^+}$, where $K_{K/Na}$ is the sensitivity to competing ions, V_{Na} is the Na^+-sensitive microelectrode reading, and n, F, R, and T have their usual meanings.

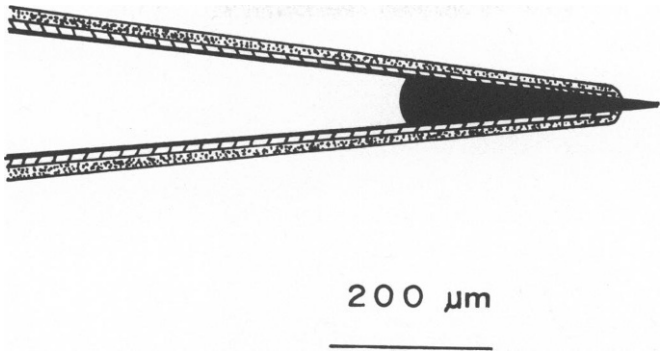

FIGURE 1 Method for shielding ion-sensitive microelectrodes. This diagram shows an Na^+-sensitive microelectrode in cross section. The microelectrode is first filled with exchanger resin (dark region) and backfilled with salt solution (clear area). The microelectrode outer surface is then coated to within ~ 50 µm of the tip with a layer of electrically conductive silver paint (electrodag 416; Acheson Colloids Co., Huron, Mich.; shown as hatched region) by passing the electrode through a bubble of the paint. The paint is next covered by a layer of insulating material (M coat D, Micro-Measurements Div., Vishay Intertechnology Inc., Romulus, Mich.; shown as stippled area). The microelectrode is shielded by connecting the silver paint to a follower amplifier which drives the paint to the same potential as the microelectrode. This procedure increased the performance characteristics of the microelectrode (see text). In addition, a significant current loop between the microelectrode tip and the glass wall was eliminated.

microelectrodes, resistance ratios (defined as the ratio of apical membrane resistance to basolateral membrane resistance or "α") were low. The average value obtained from Na^+-sensitive microelectrodes was 4, as compared with the usual mean of 15 to 20. This finding suggested the possibility of a shunt pathway in parallel to the Na^+ exchange resin. We also found that the electrical resistance of the resin was large ($>10^{10}\Omega$; see also reference 6) and comparable to the electrical resistance of the microelectrode glass. Thus the glass resistance might have attenuated the microelectrode potential. A consequence of this shunt would be an overestimation of $a_i^{Na^+}$ and an underestimation of α.

To reduce this artifact, Na^+-sensitive microelectrodes were shielded according to the method shown in Fig. 1. By driving the conductive layer of the shield to the same potential as the microelectrode tip, we were able to eliminate significant shunt pathways through the microelectrode glass. A comparison of shielded and unshielded microelectrodes produced normal resistance ratios and reduced the calculated $a_i^{Na^+}$ by approximately threefold. In addition, these microelectrodes showed improved response times. This difference is shown in Fig. 2, which compares the responses of the same Na^+-sensitive microelectrode with the shield connected or disconnected. The average $a_i^{Na^+}$ measured with shielded microelectrodes was 7 ± 1 mM (n = 76). These results are summarized in Tables I and II. The calculated equilibrium potential for Na^+ was +76 mV (cell interior positive).

A second aspect of these studies was to investigate alterations in $a_i^{Na^+}$ as a function of Na^+ transport rate. Two alterations were examined; specifically, these were increased transport rate (induced by low Na^+ diet), and transport inhibition. To inhibit transport, bladders were either treated with 10^{-4}M ouabain on the serosal side or the bathing solutions were replaced with potassium-free Ringer's solutions (or "zero K^+ solution"; K^+ was replaced by Na^+). The results of transport stimulation are summarized in Table II. As expected, the transepithelial

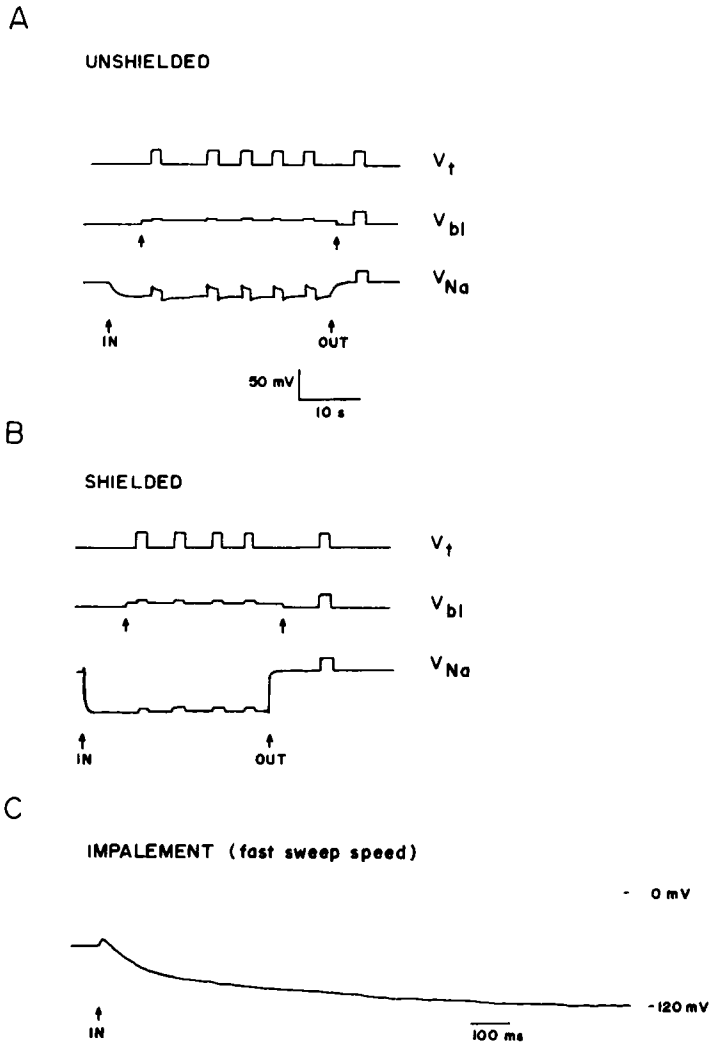

FIGURE 2 Recordings from a single Na^+-sensitive microelectrode. In *B* and *C* the driven shield is connected; in *A* it is disconnected. *A* and *B* show transepithelial potential (V_t), basolateral membrane potential (V_{bl}) (recorded from an adjacent cell with a conventional microelectrode), and the Na^+-sensitive microelectrode potential (V_{Na}) *C* illustrates V_{Na} (shield-connected) at a faster recording speed. The initial resting value of all traces was equal to V_t (-59 mV in this case). Square steps in *A* and *B* reflect voltage responses to current pulses passed transepithelially. Note that the change in V_{Na} upon impalement is smaller and slower in *A* than in *B*. In addition, the voltage deflection of V_{Na} produced by a square current pulse is greater in *A* than in *B*, indicating shunting by the glass of the ion-sensitive microelectrode. (Voltages were measured within ± 0.1 mV with the aid of a digitial voltmeter. For average values, see text and Table II.) The shielded impalement (*B*) shows a rapid deflection upon entry that is equal to the basolateral membrane potential. This initial deflection is shown more clearly in panel *C*. The deflection is not seen when the transepithelial potential is less than V_{bl} (either spontaneously or artifically by voltage clamp).

TABLE II

INTRACELLULAR Na^+ ACTIVITIES AND ELECTRICAL PROPERTIES OF BLADDERS FROM NORMAL AND Na^+-DEPELTED RABBITS

	V_t (mV)	I_{sc} ($\mu A/\mu F$)*	V_{bl} (mV)	Calculated intracellular activity	
				Na^+	K^+
Control $N = 7$	−45 ± 2.2	2 ± 0.5	−53 ± 1.8	7 ± 1.2	89 ± 9.5
Low Na^+ diet $N = 9$	−58 ± 4.3‡	4 ± 1.1§	−56 ± 1.3	6 ± 0.9	97 ± 8.7

*1 $\mu F \simeq 1$ cm^2 apical membrane area.
‡$p < 0.05$.
§$p < 0.005$.

potential difference (V_t) and short-circuit current (I_{sc}) were significantly increased by low Na^+ diet. Since I_{sc} in the bladder is completely accounted for by net Na^+ absorption, such an increase in I_{sc} indicates increased Na^+ transport and therefore increased Na^+ entry. However, $a_i^{Na^+}$, $a_i^{K^+}$, and V_{bl} were not significantly changed. Consequently, under these conditions of increased Na^+ transport, the extrusion mechanism (i.e., the Na^+-K^+ pump) was not saturated, as intracellular Na^+ remained relatively constant.

In contrast, inhibition of Na^+ transport caused a two-stage increase in $a_i^{Na^+}$. The first change was a rapid fivefold increase which occurred during the first 10 min, followed by a

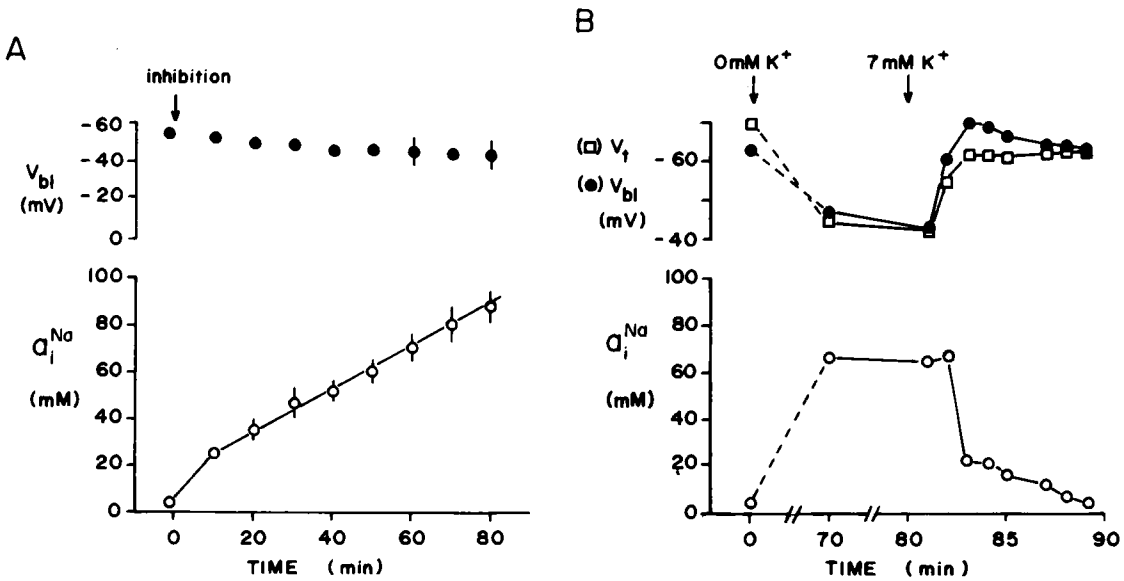

FIGURE 3 Intracellular Na^+ as a function of decreased (*A*) or increased (*B*) transport rate. (*A*) Mean a_i^{Na} rose in two stages after inhibition of the Na^+-K^+ pump (see text). V_{bl}, in contrast, depolarized gradually. Bars indicate standard error of the mean ($n = 5$). (*B*) An example of stimulation of the pump in Na^+-loaded cells. At $t = 0$ the bladder was bathed on both sides with K^+-free solution (see text). At $t = 80$, 7 mM KCl was added to both sides. $a_i^{Na^+}$ returned to control values in < 10 min.

second slower increase which appeared to be linear for the duration of the inhibition. Our preliminary calculations indicate that the Na^+ permeability of the apical membrane is reduced during the first 20 min. As we have previously reported (5), $a_i^{K^+}$ remained relatively constant, then decreased after 20 min. V_{bl} showed a small gradual depolarization. These results are illustrated in Fig. 3 *A*.

Upon addition of 7 mM KCl to zero K^+ treated bladders, marked electrogenicity of the Na^+-K^+ pump was found. An example is shown in Fig. 3 *B*. During the first minute after K^+ application, V_{bl} hyperpolarized by 19 ± 3.7 mV, yet $a_i^{Na^+}$ and $a_i^{K^+}$ were not significantly changed ($a_i^{Na^+}$ = 72 ± 7.5 mM, $a_i^{K^+}$ = 45 ± 5.3 mM, n = 3). Within 8 min, $a_i^{Na^+}$ was reduced to initial control levels of 5 ± 0.9 mM. $a_i^{K^+}$ returned to initial control values (85 ± 6.3 mM) after 15–20 min.

The importance of these findings is threefold. First, these measurements provide direct evidence that Na^+ entry into mammalian epithelial cells is a passive process and that its extrusion is active. Secondly, the Na^+-K^+ pump in this epithelium has a high capacity. At increased transport levels (which were as much as four times higher than control values in some cases), intracellular Na^+ levels were not significantly altered. Moreover, when Na^+-loaded cells were provided with external K^+, the Na^+-K^+ pump was able to rapidly re-establish Na^+ gradients. Lastly, these results provide further evidence that the Na^+-K^+ pump in epithelial membranes is electrogenic under certain conditions, possibly indicating that the coupling ratio of the pump is dependent on the level of $a_i^{Na^+}$.

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