J. Membrane Biol. 92, 37–46 (1986)

The Journal of
Membrane Biology

Cell Sodium Activity and Sodium Pump Function in Frog Skin

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Summary. Cell Na activity, a^c_{Na}, was measured in the short-circuited frog skin by simultaneous cell punctures from the apical surface with open-tip and Na-selective microelectrodes. Skins were bathed on the serosal surface with NaCl Ringer and, to reduce paracellular conductance, with $NaNO_3$ Ringer on the apical surface. Under control conditions a^c_{Na} averaged 8 ± 2 mM (n = 9, SD). Apical addition of amiloride (20 μM) or Na replacement reduced a^c_{Na} to 3 mM in 6–15 min. Sequential decreases in apical [Na] induced parallel reductions in a^c_{Na} and cell current, I_c. On restoring Na after several minutes of exposure to apical Na-free solution I_c rose rapidly (≲30 sec) to a stable value while a^c_{Na} increased exponentially, with a time constant of 1.8 ± 0.7 min (n = 8). Analysis of the time course of a^c_{Na} indicates that the pump Na flux is linearly related to a^c_{Na} in the range 2–12 mM. These results indicate that a^c_{Na} plays an important role in relating apical Na entry to basolateral active Na flux.

Key Words frog skin · cell Na activity · membrane potential · Na pump flux · Na microelectrodes

Introduction

Despite the central role of the basolateral Na pump in epithelial Na absorption, very little is known of the factors regulating its operation. It is generally assumed that the intracellular Na activity, a^c_{Na}, determines the rate of operation of the pump (Larsen, Fuchs & Lindemann, 1979; Nielsen, 1982*a*). However, some doubts about the generality of this relationship have recently been raised (Thomas, Suzuki, Thompson & Schultz, 1983). A successful direct technique in the investigation of the response of the Na pump to changes in a^c_{Na} has been to raise a^c_{Na} either by ionophoretic injection (Thomas, 1969) or by reversible pump inhibition (Thomas, 1972; Eisner, Lederer & Vaughan-Jones, 1984) and then simultaneously to follow the recovery of a^c_{Na} and pump current toward the steady state. Although these approaches are difficult to use in epithelia, it is possible to abolish Na entry and follow the rate of decrease in a^c_{Na}. In this way one can infer the relation between pump rate and a^c_{Na} (Thomas, 1972; Giráldez, 1984). An alternative approach, which we also use in this study of the short-circuited frog skin, is to suddenly restore Na after several minutes of exposure to an apical Na-free solution. Under these conditions, cell current (apical Na entry) rose rapidly, reaching a stable value in ≲30 sec, while a^c_{Na} increased exponentially with a time constant of ~1.8 min. Analysis of the time course of a^c_{Na} indicates that the basolateral Na flux is linearly related to a^c_{Na}, at least in the range 2–12 mM. A preliminary account of some of our results has been presented in abstract form (García-Díaz, Baxendale & Essig, 1985*a*).

Materials and Methods

Experiments were performed on abdominal skins of *Rana pipiens* (Northern variety) obtained from either Connecticut Valley Biological Supply Co. (Southampton, MA) or Kons (Germantown, WI). The experimental procedures and electronics were as described previously (García-Díaz, Baxendale, Klemperer & Essig, 1985*b*). In the experiments reported here the transepithelial voltage was clamped to zero. The inner side of the skin was perfused with NaCl Ringer solution of composition (in mM): NaCl 110, $CaCl_2$ 1, KOH 2.5 buffered to pH 7.8 with HEPES (3–4 mM). The outer solution was $NaNO_3$ Ringer, where all NaCl was replaced by $NaNO_3$. As shown previously (Nagel, García-Díaz & Essig, 1983; García-Díaz et al., 1985*b*), this reduces paracellular conductance and its response to inhibition of Na transport. Solutions with variable Na content were obtained by substituting tetramethylammonium (TMA) or N-methyl-D glucamine (NMDG) for Na. Amiloride (kindly supplied by Merck, Sharp, and Dohme, West Point, PA) was added to the apical solution to a final concentration of 20 μM.

Single-barreled Na-selective microelectrodes were fabricated according to the method described previously for K-selective microelectrodes (García-Díaz et al., 1985*b*). The Na-selec-

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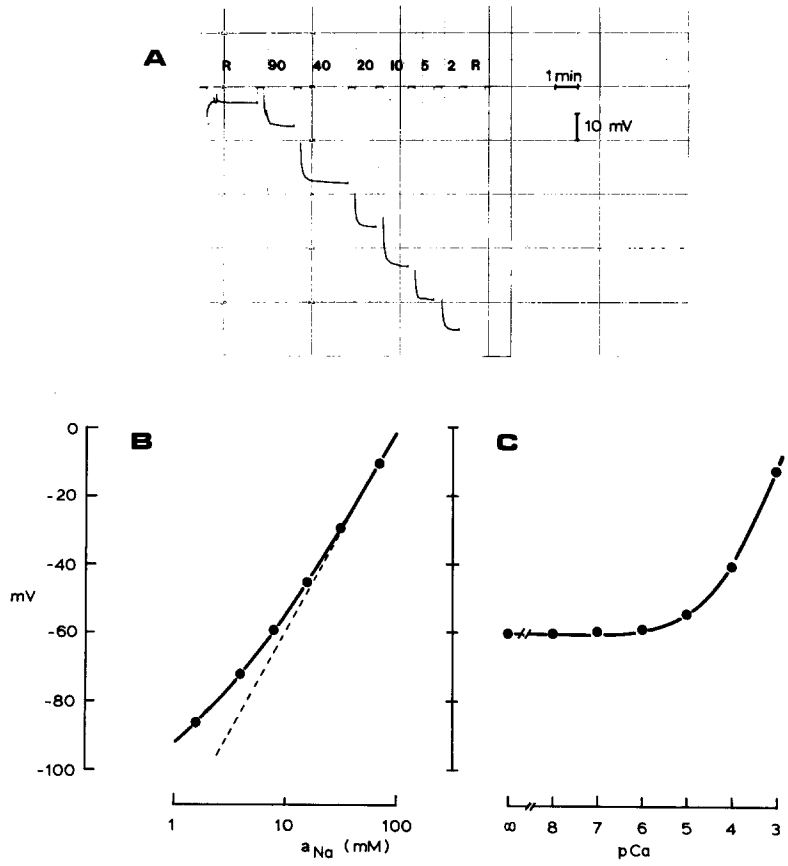

Fig. 1. Calibration of Na-selective microelectrodes. (*A*) Calibration in Ringer (*R*) and in solutions with [Na] + [K] = 125 mM and *p*Ca = 7.0. [Na] values in mM are shown above traces. The amplifier output is shorted during changes in solution. (*B*) Plot of Na-microelectrode voltage *vs.* Na activity for the calibration shown in *A*. The value in Ringer (110 mM Na, 1 mM Ca) is taken as zero. The dotted line is the Nernstian response (59 mV/decade). (*C*) Calibration of Na microelectrode in solutions with varying *p*Ca (at constant [Na] = 10 mM and [K] ~ 115 mM)

tive cocktail contained 10–12% neutral ligand ETH 227 (Sodium-Ionophore I, Fluka Chemical Corp., Hauppauge, NY) and 0.7–1.0% sodium tetraphenylborate in 3-nitro-*o*-xylene. We also made electrodes with a cocktail containing *o*-nitrophenyl octyl ether as a solvent but their response was considerably slower than that of electrodes made with 3-nitro-*o*-xylene. The backfilling solution employed was 0.2 M NaCl. The Na-selective electrodes were calibrated immediately after the experiment, and in some cases, also before impalement. Figure 1*A* and *B* shows a typical calibration in solutions of variable [Na]. In these solutions *p*Ca (free Ca concentration) was kept constant at 7.0 (EGTA 10 mM, $CaCl_2$ 5 mM, pH = 7.29) and Na was substituted for K ([Na] + [K] = 125 mM) so as to keep the ionic strength and activity coefficient (0.77) near constant. In the range of [Na] from 2 to 10 mM the K activity was 95-88 mM, close to the intracellular K activity measured in this preparation (García-Díaz et al., 1985*b*). The *p*Ca was kept at 7.0 to mimic the likely intracellular *p*Ca. Some Na microelectrodes were also calibrated in solutions with varying *p*Ca (at constant [Na] = 10 mM and [K] ≈ 115 mM) made according to the method described by Tsien and Rink (1981).[1] As shown in Fig. 1*C*, the Na microelectrode shows a strong dependence on *p*Ca for *p*Ca ≤ 5. This Ca effect has been observed previously (Bers & Ellis, 1982; Giráldez, 1984) as a discrepancy between readings in Ringer solutions (containing 1–2 mM Ca) and in calibrating solutions with a similar activity of Na but without any added Ca. However, for *p*Ca ≥ 6, the Na microelectrode hardly responds to *p*Ca. Thus, if intracellular *p*Ca does not decrease much below 6 under our experimental conditions, the measurement of cell Na activity, a^c_{Na}, will be unaffected.

All measurements of a^c_{Na} were made by simultaneous recording from two different cells of apical membrane potential, V_o, and Na microelectrode potential, V_{Na}, following the approach introduced by DeLong and Civan (1983) and employed previously by us to measure intracellular K activities (García-Díaz et al., 1985*b*). a^c_{Na} was calculated by direct interpolation of the value $V_{Na} - V_o$ from the calibration curve (with *p*Ca set to 7). V_o and V_{Na} (before impalement and in calibration) are set to zero in Ringer solution. In this way the effect of Ca (1 mM in Ringer) on the Na microelectrode is automatically corrected. Criteria for acceptance of impalements and of simultaneous recordings from two single barreled microelectrodes were as described previously (García-Díaz et al., 1985*b*) and as discussed below. Values given are mean ± SD.

[1] These solutions were checked with a Ca-selective PVC-membrane electrode containing the neutral ligand ETH 1001 (Calcium-Ionophore, Fluka Chemical Corp., Hauppauge, NY) fabricated according to the method of Affolter and Sigel (1979). The PVC Ca-electrode responded linearly (slope ~30 mV/decade) up to *p*Ca 7.5, and changed by 14 mV between *p*Ca 8 and infinity.

Results

The use of the two single-barreled microelectrodes placed in different cells allowed us to measure changes in a^c_{Na} during alteration of the composition

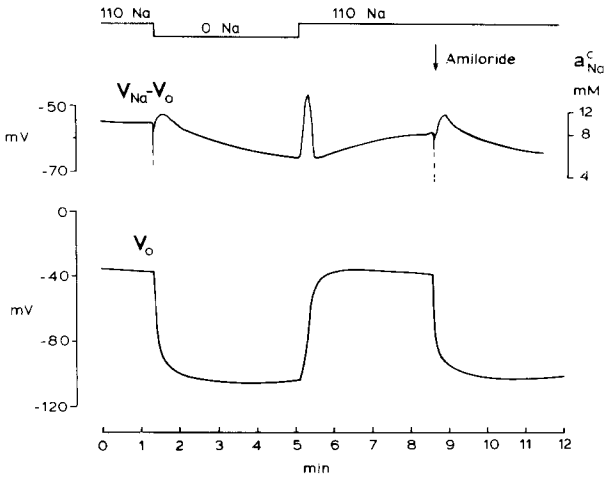

Fig. 2. Changes in cell Na activity and membrane potential after removal and restoration of apical Na (N-methyl D-glucamine substitution) and apical addition of amiloride (20 μM). The scale at the right side of the $V_{Na} - V_o$ trace shows the Na activity values derived from the calibration of the microelectrode (the same as shown in Fig. 1)

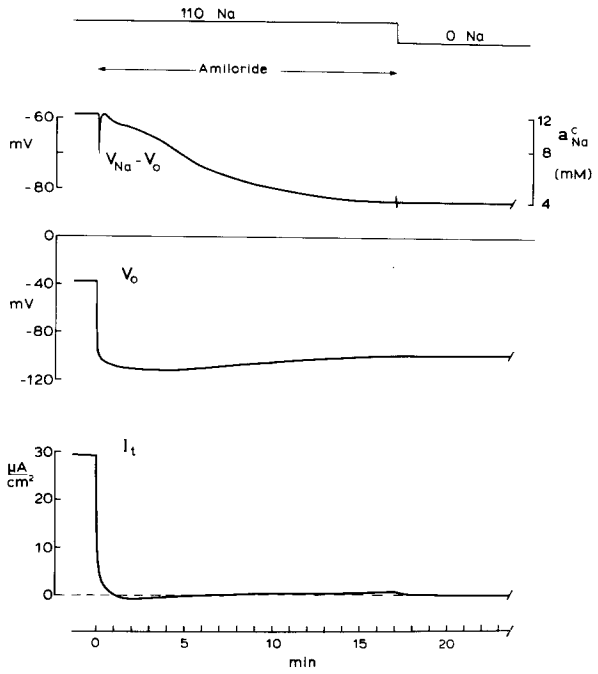

Fig. 3. Cell Na activity, membrane potential, and transepithelial current during inhibition of Na transport. Record continues in Fig. 4. Amiloride concentration: 20 μM. Na substituted by tetramethylammonium

of the apical solution, although it also introduced a complication. Immediately after the change in solutions there were transients in the $V_{Na} - V_o$ trace (*see* Fig. 2). These transients were not due entirely to the response time of the Na microelectrodes (<1 sec) since they were also observed when both microelectrodes were conventional open-tip electrodes recording V_o in two different cells (*see* Fig. 3 in García-Díaz et al., 1985*b*). Although the nature of the transients is unclear, we have attributed them to the presence of a time lag and different rate of change in membrane potential of two relatively distant cells after a change in solution. We based this explanation on the following observations: (i) the direction of the transients was reversed when the position of the microelectrodes was reversed; (ii) the magnitude of the transients was smaller when the microelectrodes were positioned closer to each other; (iii) the duration of the transients of $V_{Na} - V_o$ was about the same as the duration of the change in membrane potential (up to several minutes when washing out amiloride or removing apical Na).

One criterion for the acceptance of impalements when using two simultaneous intracellular recordings is an identical response to a fast change in membrane voltage (*see,* e.g., Giráldez, 1984; Giráldez & Ferreira, 1984). Also, when V_o is changed rapidly on restoring apical Na and after addition of amiloride, $V_{Na} - V_o$ should return to ±2 mV of the initial values after the fast transients (lasting ≤30 sec). When this did not happen, the impalements were assumed to be leaky and the results discarded (*see* García-Díaz et al., 1985*b*).

As shown in Fig. 2, a^c_{Na} decreases following apical Na removal, and it returns to near the initial value after restoration of Na. Application of amiloride also induces a decrease in a^c_{Na}. Removal of apical Na or application of amiloride elicited the usual hyperpolarizations in V_o (the same in both cases). Not shown in this figure are the decreases in transepithelial current, I_t, and conductance, g_t, and the increase to near unity of the apical voltage divider ratio, $F_o = \Delta V_o/\Delta V_t$, during removal of apical Na or addition of amiloride (*see,* e.g., Figs. 6 and 7 in García-Díaz et al., 1985*b*).

We were interested in analyzing the changes in a^c_{Na} that take place after sudden abolition or restoration of apical Na entry (i.e., cell current, I_c). It is possible to decrease I_c rapidly by addition of amiloride, but removal of this drug and the subsequent recovery of I_c usually takes several minutes. However, the recovery of apical Na transport on restoration of apical Na is a fast process as judged by the increase in I_t (~10–30 sec). Figures 3 and 4 show an experiment where several maneuvers were performed sequentially during the same cell impalement. On addition of amiloride I_t is inhibited within 1 min. After the initial fast transient in the $V_{Na} - V_o$ trace, a^c_{Na} decreases from 12 mM to a stable value of

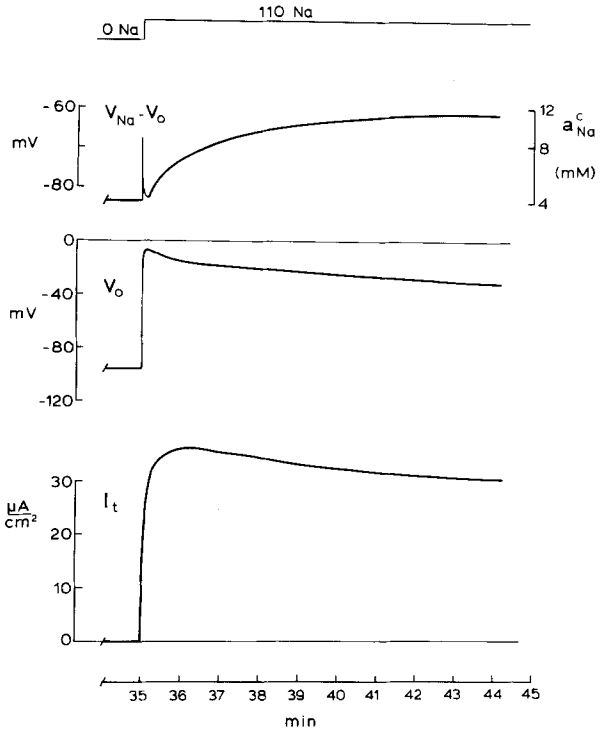

Fig. 4. Continuation of the record in Fig. 3. Note that the recorder speed is faster in this figure

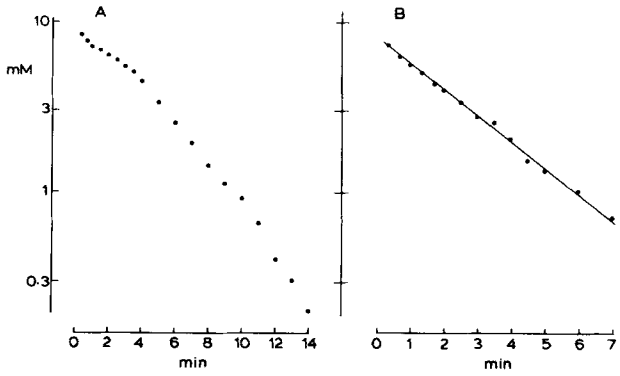

Fig. 5. Semilogarithmic plots of the time course of $|a_{Na}(t) - a_{Na}(\infty)|$ after addition of amiloride (*A*) and restoration of apical [Na] (*B*), for the experiment in Figs. 3 and 4. The time constant in *B* is 2.8 min. (Note the different time scales)

4 mM in ~15 min. The apical solution is then changed to one containing no amiloride and in which all Na is replaced by TMA, with no appreciable effect on a^c_{Na}. After 18 min, to allow for the removal of amiloride, the solution is changed again to normal Na Ringer (Fig. 4). The depolarization in V_o (~10 sec) and restoration of I_t (~20 sec) are very fast, while a^c_{Na} increases slowly toward the initial control value. In nine experiments the average a^c_{Na} was 3 ± 1 mM under apical Na-free conditions and increased to 8 ± 2 mM after restoration of Na (Table). In the presence of 20 μM amiloride the average steady-state value of a^c_{Na} was 3 ± 2 mM (n = 9).

The time courses of a^c_{Na} after inhibition and restoration of cell current are shown in Fig. 5 for the experiment of Figs. 3 and 4. The decrease in a^c_{Na} after addition of amiloride (Fig. 5*A*) can be fitted by more than one exponential. Although in some experiments a single exponential decay was observed, in 5 out of 9 experiments with amiloride (and also when a^c_{Na} was reduced by removal of apical Na) the behavior of a^c_{Na} was similar to that in Fig. 5*A*. The half-time for the decrease in a^c_{Na} after amiloride was 3.0 ± 0.9 min (n = 9). On the contrary, on restoration of apical Na, recovery of a^c_{Na} follows a single exponential (Fig. 5*B*), with a shorter time constant than those during inhibition of cell current. In eight out of nine experiments we found a single exponential increase in a^c_{Na} after restoration of apical Na with time constants ranging from 0.84 to 2.80 min (*see* Table).

In order to interpret the significance of the time course of a^c_{Na} it is necessary to know concurrent values of the cell current, I_c. In this regard it is of importance that with the use of Cl-free apical solutions paracellular conductance is greatly reduced (Nagel et al., 1983; García-Díaz et al., 1985*b*). Thus the paracellular current in the presence of the anion asymmetry is negligible, as shown by the near zero value of I_t in Fig. 3 after amiloride. In 22 experiments using $NaNO_3$ Ringer as the apical solution, I_t after amiloride was 1.5 ± 1.4 μA/cm². Thus, under these conditions, I_t was a good estimate of I_c. Even after apical Na replacement paracellular current is small, since as shown in Fig. 3 there is hardly a difference in I_t when amiloride is present or when apical Na is replaced by TMA. The average value of I_t after substitution of apical Na was −1.0 ± 1.8 μA/cm² (n = 22) with NMDG and 0.5 ± 1.5 μA/cm² (n = 10) with TMA.

Although I_t is a good approximation of I_c under the conditions of our experiments, the basolateral Na flux is equal to I_c only during the steady state, when there is no change in a^c_{Na}. In order to obtain the relation between a^c_{Na} and the basolateral Na flux during the steady state, we decreased apical [Na] in the sequence 110, 11, 3.3, 1.1 and 0.11 mM. Each of these solutions was perfused until a near constant value of a^c_{Na} was reached, usually in 4 to 8 min. Figure 6 shows one of these experiments. At each decrease in $[Na]_o$ there was a rapid hyperpolarization of V_o followed by a slow depolarization, while a^c_{Na} decreased to a near stable value in 4 to 5 min. Figure 7*A* shows the mean values of a^c_{Na} at each $[Na]_o$ for six experiments. The saturation in the relation between a^c_{Na} and $[Na]_o$ has also been observed

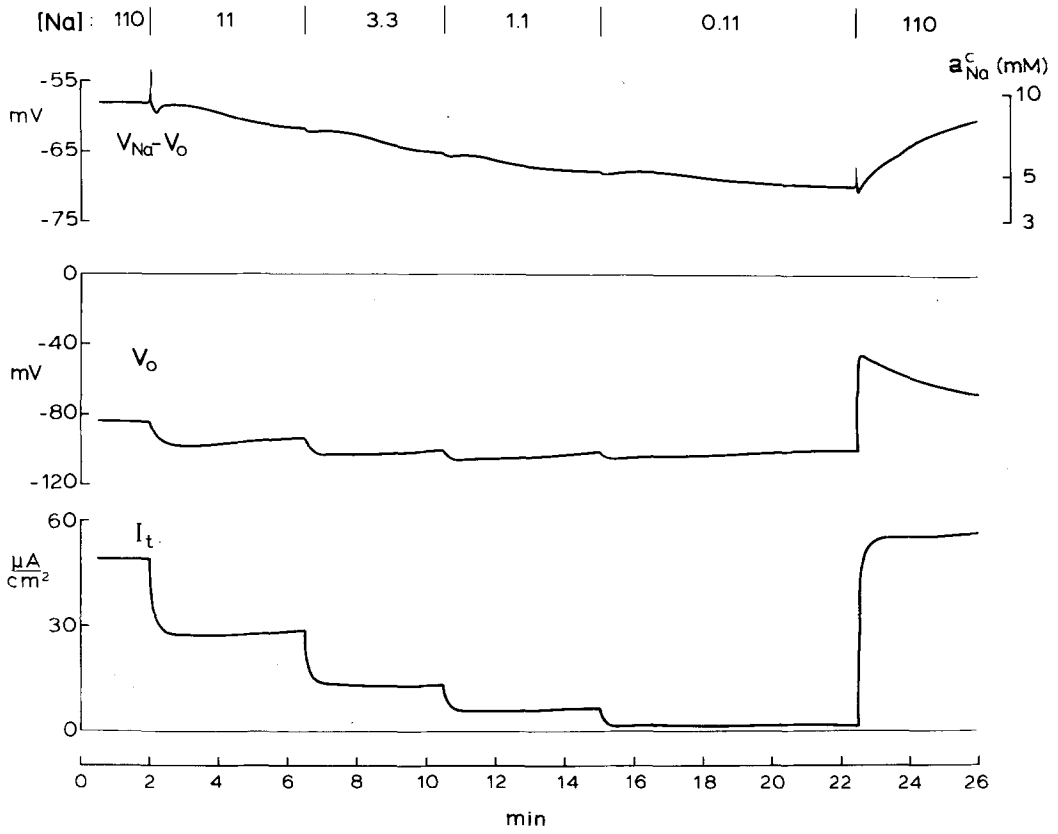

Fig. 6. Changes in Na activity, membrane potential, and transepithelial current in response to sequential decreases in apical [Na] (TMA substitution). The values of [Na], in mM, are shown on the top of the figure

by Harvey and Kernan (1984*b*) and agrees with the cell Na concentration measurements made under similar conditions with electron microscope analysis (Rick et al., 1984).

The relation between I_c and a^c_{Na} is shown in Fig. 7*B* for each experiment. Although there is a positive correlation between a^c_{Na} and I_c, linearity was observed in only four of the six experiments. In the linear cases the regression coefficient was significant at the $P < 0.01$ level and the slopes ranged from 3.6 to 10.7 μA cm^{-2} mM^{-1} (mean ± SD = 6 ± 3 μA cm^{-2} mM^{-1}).

Discussion

Contrary to the problem in measuring cell K activities (García-Díaz et al., 1985*b*), the sensitivity of the Na microelectrodes (~0.5 mM/mV) in the range of a^c_{Na} (~8 mM), allows the observation of systematic changes in a^c_{Na} during alteration of transcellular Na transport, when care is taken for the elimination of impalement artifacts.

The steady-state values of a^c_{Na} measured in this study, both in control (8–9 mM) and after inhibition of cell current with amiloride or removal of apical Na (3 mM), agree well with the average cellular Na *concentrations* measured by electron microprobe analysis: 13–16 mM and about 5 mM, respectively, in *R. esculenta* and *temporaria* (Rick et al., 1984, calculated using their estimate of 0.26 for dry wt/wet wt). In a previous study (Nagel, García-Díaz & Armstrong, 1981) the values of a^c_{Na} reported, both in control (14 mM) and after addition of amiloride (8 mM), were higher than the present ones. Also, in contrast to the present results, no change in a^c_{Na} was observed during the initial 5 min after addition of amiloride. These discrepancies are most likely due to the limited accuracy of the technique of successive impalements with open-tip and ion-selective microelectrodes employed at that time. In a recent study using double-barreled microelectrodes, Harvey and Kernan (1984*a* and *b*) reported values of a^c_{Na} of 10–12 mM for skins with $I_{sc} > 14$ μA/cm^2 and 2.8 mM for skins with $I_{sc} < 14$ μA/cm^2 under control conditions. After addition of amiloride or apical Na replacement they found a^c_{Na} to be 1–2 mM. The decreases in a^c_{Na} described by these authors were faster (completed within 3 min) than in our experiments (6–15 min). It is, however, not clear whether

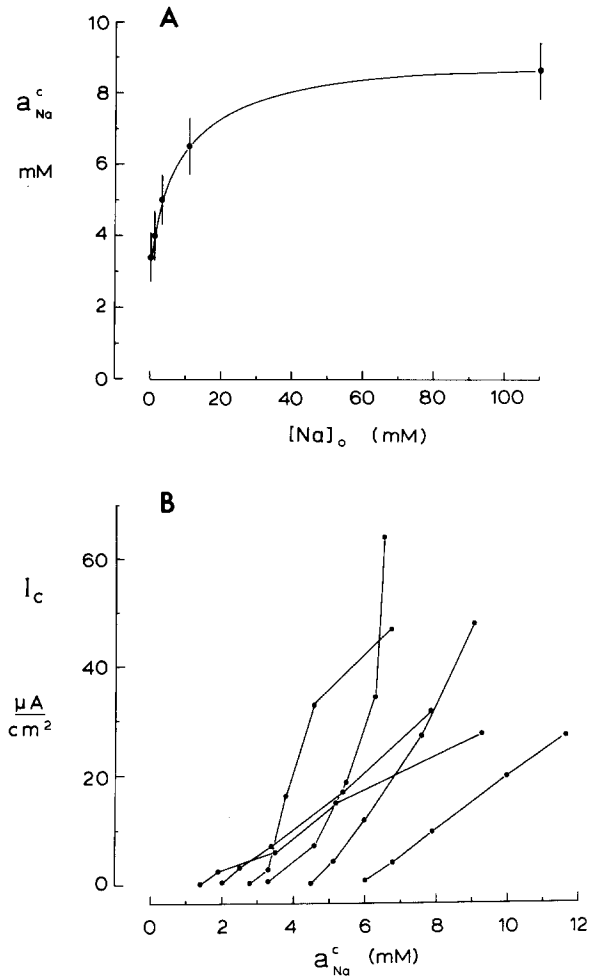

Fig. 7. (*A*) Cell Na activity dependence on apical [Na], for six experiments like that shown in Fig. 6. Vertical lines indicate SEM. (*B*) Relation between cell current and intracellular Na activity at the quasi-steady state. The lines join data points from the same experiment

their measurements were obtained at short circuit or open circuit (when cellular Na transport and a^c_{Na} are lower, *unpublished results*).

Harvey and Kernan (1984*a* and *b*) reported a correlation between a^c_{Na} and the short-circuit current when the observations from spontaneously transporting skins and others to which amiloride was added were pooled together. Our analysis of untreated skins did not show such a correlation. Nor did the electron microprobe measurements of Rick et al. (1984), who indicated that it was not possible to predict the cellular Na concentration from the transepithelial Na transport or vice versa. From our observations and those of Rick et al. it seems that other factors, in addition to a^c_{Na}, influence the overall rate of cellular Na transport.

The exponential time course of increase in a^c_{Na} after restoration of apical Na suggests a linear dependence of the basolateral Na flux on a^c_{Na}, as shown by the following analysis. On the assumption that there is no change in cell volume during these experiments,[2] the continuity equation states that

$$I^a_{Na} = I^b_{Na} + (F \cdot h/\gamma)(da^c_{Na}/dt) \quad (1)$$

where I^a_{Na} and I^b_{Na} are the net Na fluxes across the apical and basolateral membranes, respectively, expressed as currents ($\mu A/cm^2$), F is the Faraday constant, γ the cell activity coefficient for Na (taken as 0.77), and h is the ratio of volume to cross-sectional area of the epithelial cell layer. Inasmuch as Na is the only ion transported across the apical membrane $I^a_{Na} = I_c$. Assuming that the basolateral Na flux is linear in a^c_{Na} above a certain threshold value α, we have

$$I^b_{Na} = I_o + k(a^c_{Na} - \alpha), \qquad a^c_{Na} \geq \alpha \quad (2)$$

where I_o, k and α are constants for each skin. Equation (1) can then be written

$$da^c_{Na}/dt = (I_c - I_o + k\alpha - ka^c_{Na})/\theta \quad (3)$$

where $\theta = Fh/\gamma$. Assuming that I_c reaches its final steady value immediately after the perturbation, i.e., the change in I_c is stepwise, Eq. (3) can be written as

$$da^c_{Na}/dt = k[a^c_{Na}(\infty) - a^c_{Na}]/\theta \quad (4)$$

where $a^c_{Na}(\infty)$ is the final steady-state value of a^c_{Na}.

The general solution of Eq. (4) is

$$a^c_{Na}(t) - a^c_{Na}(\infty) = [a^c_{Na}(o) - a^c_{Na}(\infty)] \exp(-kt/\theta). \quad (5)$$

If at time zero I_c is abolished (for example, with application of amiloride) Eq. (5) becomes

$$a^c_{Na}(t) - a^c_{Na}(\infty) = (I_c/k) \exp(-kt/\theta). \quad (6)$$

On the other hand, for the case of restoration of I_c after several minutes of Na-free apical solution, Eq. (5) becomes

$$a^c_{Na}(t) - a^c_{Na}(\infty) = -(I_c/k) \exp(-kt/\theta). \quad (7)$$

In both cases the relaxation of a^c_{Na} toward $a^c_{Na}(\infty)$ follows a single exponential time course with the time constant given by

$$\tau = \theta/k. \quad (8)$$

[2] In frog urinary bladder, Davis and Finn (1985) found no change in cell volume after application of amiloride.

Several observations have suggested the existence of a basolateral Na leak pathway in parallel to the pump. Isotope studies (Candia & Reinach, 1977; Stoddard & Helman, 1985) have reported high levels of influx across the basolateral membrane of isolated skin. It is unclear, however, how much of this influx reflects a component of net leak, as opposed to Na : Na exchange. To the extent that net leak is small, near constant and/or linear, our previous analysis remains valid and the observed exponential time course of a^c_{Na} during restoration of apical Na indicates a linear dependence of the net *pump flux* on a^c_{Na}.

After application of amiloride there were several cases where the decrease in a^c_{Na} could not be fitted to a single exponential. There are two possible explanations for this different behavior. First, while I_c increases rapidly after restoration of apical Na (≤30 sec), the decrease in I_c on application of amiloride is slower, taking occasionally up to 2 min to reach 90% inhibition. Thus the assumption made to derive Eq. (4) may not be valid. In this regard it is worth noting that Nielsen (1982*b*) has reported a single exponential decrease of the isotopic Na flux from the cells to the basolateral solution in isolated epithelia of *R. temporaria* in association with a rapid response of the short-circuit current to amiloride. The half-time for this decrease (1.9 min) is somewhat shorter than our values for the decrease in a^c_{Na} after amiloride (3.0 ± 0.9 min). On the other hand, in similar experiments in *R. pipiens*, Cox and Helman (1983) observed more complex and slower decreases in basolateral Na efflux and short-circuit current after amiloride. Second, there may be an influence of the basolateral membrane potential on the Na pump operation. As shown in Fig. 3, on restoration of apical Na, $V_i(= -V_o)$ depolarized very rapidly (≤20 sec) to a low value (range 8 to 40 mV in these experiments) and then slowly repolarized during the increase in a^c_{Na} to final values in the range 30 to 60 mV. Thus during the time that a^c_{Na} increases V_i remains below 60 mV. On the contrary, after application of amiloride V_i increases to values between 100 and 120 mV and slowly depolarizes to values in the vicinity of 90 mV. Although several studies indicate that the Na pump operates independently of the membrane potential in a variety of animal cells (Glynn, 1984), including frog skin (Cox & Helman, 1986), Rakowski and De Weer (1982) have recently reported that hyperpolarization inhibits pump current in the squid axon, either under normal or reverse pump operation (*see also* De Weer & Rakowski, 1984). It is then possible that the large hyperpolarization of V_i during amiloride affects pump operation so that a^c_{Na} is no longer the only factor determining Na efflux through the pump.

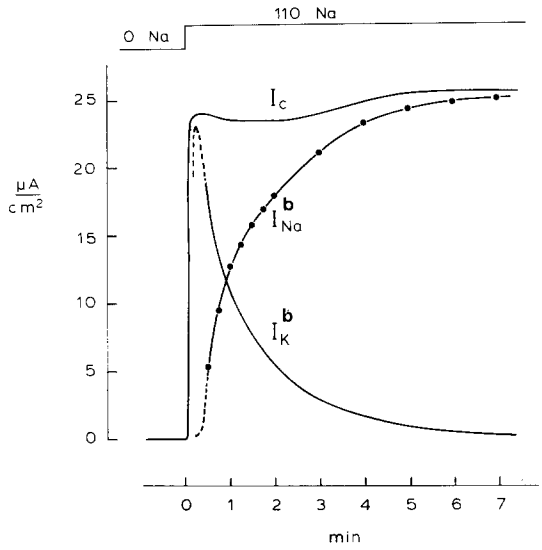

Fig. 8. Development of cell current, and basolateral Na and K net fluxes after restoration of apical Na. I^b_{Na} is calculated from Eq. (2) and the measured a^c_{Na}, as explained in the text. I^b_K is taken as $I_c - I^b_{Na}$

According to the previous analysis, the exponential time course of a^c_{Na} after restoration of apical Na suggests a linear dependence of Na pump flux on a^c_{Na}, at least in the range of a^c_{Na} studied (2–12 mM). This agrees with the behavior of the Na pump reported in a variety of cell types (Brinley & Mullins, 1968; Thomas, 1969, 1972; Garay & Garrahan, 1973; Eisner et al., 1984), although outside of this range of a^c_{Na} a departure from linearity is expected (Garay & Garrahan, 1973; Glitsch, Pusch & Venetz, 1976; Larsen et al., 1979; Nielsen, 1982*a*). In frog skins where Na transport has been increased above normal levels, the relation between Na pump efflux and a^c_{Na} (in the range 0.8 to 20 mM) is sigmoidal. Larsen et al. (1979) exposed the skin of *R. esculenta* to *p*-chloromercuribenzoate or benzimidazolylguanidine to increase apical permeability and varied Na transport by altering apical Na concentration. The relation between a^c_{Na} (calculated from the reversal potential of the K-depolarized skins) and the short-circuit current followed an *S*-shaped curve with a half-maximal saturation at 13 mM and maximum flux of 188 μA/cm². Essentially the same results were obtained by Nielsen (1982*a*) when the short-circuit current was correlated to the Na transport pool calculated from Na influx build-up experiments on the isolated epithelia of *R. temporaria*. In this case Na transport was altered by addition of amiloride, arginine vasotocin or arginine vasotocin plus theophylline. In rabbit urinary bladder Lewis, Wills and Eaton (1978) and Lewis and Wills (1983) have reported sigmoidal relations between a^c_{Na} and

Table. Transepithelial current, I_t, cell potential, V_o, and cell Na activity, a^c_{Na}, in the short-circuited frog skin in the absence and presence of apical Na

Exp. No.	$[Na]_o = 0$			$[Na] = 110$ mM			τ (min)	k (μA cm^{-2} mM^{-1})
	I_t (μA cm^{-2})	V_o (mV)	a^c_{Na} (mM)	I_t (μA cm^{-2})	V_o (mV)	a^c_{Na} (mM)		
1	−1.5	−90	2	27.5	−32	8	1.30	5.8
2	−1.2	−105	5	27.0	−38	11	1.44	8.7
3	−0.1	−90	4	25.5	−58	8	1.56	5.3
4	0.0	−98	2	21.7	−56	6	2.48	7.2
5	0.0	−108	1	26.5	−65	6	1.80	8.3
6	2.8	−96	3	33.5	−33	6	0.84	8.5
7	4.4	−84	1	57.0	−32	8	2.31	7.2
8	0.2	−100	4	31.0	−25	12	2.80	5.2
9	1.0	−100	4	57.0	−78	9	—	—
Mean			3			8	1.81	7.0
SD			1			2	0.66	1.4

Values of I_t, V_o, and a^c_{Na} are at the steady state, 10 to 15 min after removal or restoration of apical Na. τ is the time constant for the recovery of a^c_{Na} after restoration of apical Na, and k is the proportionality constant between a^c_{Na} and basolateral Na current, as defined by Eq. (2) in the text. In experiments 1 and 2 N-methyl-D-glucamine was substituted for Na. In all others tetramethylammonium was used.

pump current.[3] The latter was calculated by indirect methods using estimates of basolateral resistance and ionic permeabilities and the measurement of the transepithelial potential after step changes in apical [Na] in the presence of nystatin or after restoration of basolateral K.

The Table lists the values of τ and k (the latter calculated by extrapolating the semilogarithmic plots, as that in Fig. 5*B*, to intersect the ordinate) for the experiments of apical Na restoration. On average the rate constant k was found to be 7 μA cm^{-2} mM^{-1}. Estimates of k in different cell types differ, possibly reflecting the number of pump units per membrane area. In snail neurons, assuming a cell radius of 50 μm, k is estimated at ~20 μA cm^{-2} mM^{-1} from the data of Thomas (1972, Figs. 9*b* and 13*b*). In *Necturus* gallbladder, Giráldez (1984) reported a value of ~4 μA cm^{-2} mM^{-1}, although the cell volume changes described by this author do not permit attributing the observed changes in a^c_{Na} solely to proportional changes in the rate of function of the Na pump. The lowest values of k are found in squid axons: 6×10^{-2} μA cm^{-2} mM^{-1} (Brinley & Mullins, 1968) and 7.6×10^{-2} μA cm^{-2} mM^{-1} (Hodgkin & Keynes, 1956). It is also interesting to point out that from Eq. (8) one can calculate θ and thus h for each experiment. The values of h ranged between 34 and 84 μm, which compare reasonably well with the epithelial height measured optically in living skins (35–86 μm; MacRobbie & Ussing, 1961) or in freeze-dried sections (50 μm, Rick et al., 1984).

It is of interest to analyze the development of the basolateral Na flux after restoring Na to the apical solution. Naturally, since we are assuming a linear dependence of I^b_{Na} on a^c_{Na}, the increase in I^b_{Na} will be exponential with the same time constant as for the increase in a^c_{Na} (*see* Eq. (2)). Figure 8 shows the evolution of I^b_{Na} calculated from Eq. (2) for one of the experiments (taking $I^b_{Na} = 0$ for $t \leq 0$). The net basolateral K current (I^b_K) is taken as the difference $I_c - I^b_{Na}$. Thus during the initial period most of I_c is carried across the basolateral membrane by K ions leaving the cell. Note that during this period V_i becomes small (8–40 mV), and thus the diffusive efflux of K is large. (The K equilibrium potential is hardly affected by changes in cell current; García-Díaz et al., 1985*b*). With time V_i increases, reducing the driving force for K diffusion while at the same time the pump-mediated K influx increases. Both processes reduce the net K current. At the steady state, $I_c = I^b_{Na}$ and $I^b_K = 0$.

When apical [Na] was reduced in sequential steps, the relation between a^c_{Na} and $[Na]_o$ showed saturation (Fig. 7*A*). When $[Na]_o$ was reduced from 110 to 11 mM, the decrease in a^c_{Na} (2 ± 1 mM, SD, $n = 6$) was significant at the level $P < 0.05$. Rick et al. (1984, Fig. 6) and Harvey and Kernan (1984*b*, Fig. 7) found small decreases in cell Na when $[Na]_o$ was reduced to about 10 mM. Thomas et al. (1983) found that a^c_{Na} in *Necturus* urinary bladder, calculated by fitting the current-voltage (I–V) relation of the apical membrane to the Goldman-Hodgkin-Katz equa-

[3] Assuming negligible Na leak at the basolateral membrane, I^b_{Na} is equal to the pump-mediated Na flux. For a pump exchanging Na for K with stoichiometry ν(= K ions/Na ions), the pump current, I_p, is related to I^b_{Na} by $I_p = (1 - \nu)\, I^b_{Na}$.

tion, did not change significantly when apical [Na] was lowered from 45 to 5 mM. However, using the same approach in frog skins, Schoen and Erlij (1985) calculated that a^c_{Na} decreased from 25.1 to 17.2 mM in less than 1 min after $[Na]_o$ was reduced from 120 to 24 mM. We do not know the reasons for these discrepancies, but we would like to point out the indirect nature of the calculation of a^c_{Na} from the *I–V* relations and the assumptions involved, in particular that the paracellular *I–V* relation is unchanged by amiloride.

The quasi-steady state relations between a^c_{Na} and I_c shown in Fig. 7*B* were not always linear. It should be mentioned that, unlike the experiments with apical Na restoration, V_i reaches values in the vicinity of 100 mV during the sequential decreases in $[Na]_o$ and, as discussed above, this may affect pump operation. Although there is some scattering in the slopes of the quasi-linear plots in Fig. 7*B*, their values are close to the *k* values shown in the Table.

In summary, our experiments show that changes in cell Na correlate with changes in cell current. In a first approximation the relation between a^c_{Na} and Na pump flux can be considered linear in the range of a^c_{Na} 2–12 mM. These results strongly support the notion that a^c_{Na} is an important factor regulating the operation of the Na pump in epithelia. The role played by variables other than a^c_{Na}, particularly V_i, on pump operation is still to be determined.

This work was supported by National Institutes of Health Grant AM 29968, by U.S. Public Health Services Training Grant T32 AM 7053 (L.M.B.), and a Cystic Fibrosis Foundation Research Fellowship (G.K.).

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Received 9 December 1985; revised 3 April 1986