Na^+ and K^+ Transport at Basolateral Membranes of Epithelial Cells

III. Voltage Independence of Basolateral Membrane Na^+ Efflux

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ABSTRACT Na^+ efflux across basolateral membranes of isolated epithelia of frog skin was tested for voltage sensitivity. The intracellular Na^+ transport pool was loaded with ^{24}Na from the apical solution and the rate of isotope appearance in the basolateral solution (J_{2S}^{Na}) was measured at timed intervals of 30 s. Basolateral membrane voltage was depolarized by either 50 mM K^+, 5 mM Ba^{++}, or 80 mM NH_4^+. Whereas within 30 s ouabain caused inhibition of J_{2S}^{Na}, depolarization of V_b by 30–60 mV caused no significant change of J_{2S}^{Na}. Thus, both pump-mediated and leak Na^+ effluxes were voltage independent. Although the pumps are electrogenic, pump-mediated Na^+ efflux is voltage independent, perhaps because of a nonlinear relationship between pump current and transmembrane voltage. Voltage independence of the leak Na^+ efflux confirms a previous suggestion (Cox and Helman, 1983. *American Journal of Physiology.* 245:F312–F321) that basolateral membrane Na^+ leak fluxes are electroneutral.

INTRODUCTION

Active Na^+ transport in epithelia is maintained by a ouabain-sensitive Na,K-ATPase or Na^+ pump. Within seconds of treatment of isolated epithelia of frog skin with ouabain, basolateral membrane voltage is depolarized and Na^+ efflux from the cells is inhibited maximally, with little or no change of basolateral membrane electrical resistance (Cox and Helman, 1983). Accordingly, the pumps are electrogenic. This idea is supported by the finding that ouabain-inhibited Na^+ efflux is greater than ouabain-inhibited K^+ influx (Na/K exchange stoichiometry > 1) (Cox and Helman, 1986*a*, *b*). The studies reported here were done to determine whether Na^+ efflux via the pump was voltage sensitive.

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MATERIALS AND METHODS

Isolated epithelia were prepared from abdominal skins of *Rana pipiens* (Fisher et al., 1980) and short-circuited continuously while bathed in a Cl-HCO_3 Ringer solution containing 100 mM NaCl, 2.4 mM $KHCO_3$, and 2.0 mM $CaCl_2$. The short-circuit current (I_{sc}) was taken as a measure of the net electrical charge transfer at the apical and basolateral membranes of the cells. The unidirectional ^{22}Na tracer flux was used as a measure of the ^{22}Na efflux via pump and leak mechanisms at the basolateral membranes of the cells as described in detail previously (Cox and Helman, 1983). Tracer appearance in the basolateral solution was measured at 30-s intervals during three or more control periods and during three or more experimental periods. At zero time of the experimental periods, the basolateral membrane voltage was depolarized by either 50 mM K^+ (Fisher, 1979; Fisher et al., 1980), 5 mM Ba^{++} (Nagel, 1979), or 80 mM NH_4^+ (substitution for Na^+) to achieve near-maximal depolarizations of basolateral membrane voltage (V_b) by these agents. The pH of all solutions was ~8.1. V_b was measured directly in separate studies with intracellular microelectrode impalement of the cells (Helman and Fisher, 1977; Fisher et al., 1980) and the changes of V_b and I_{sc} were measured in parallel (see Fig. 1).

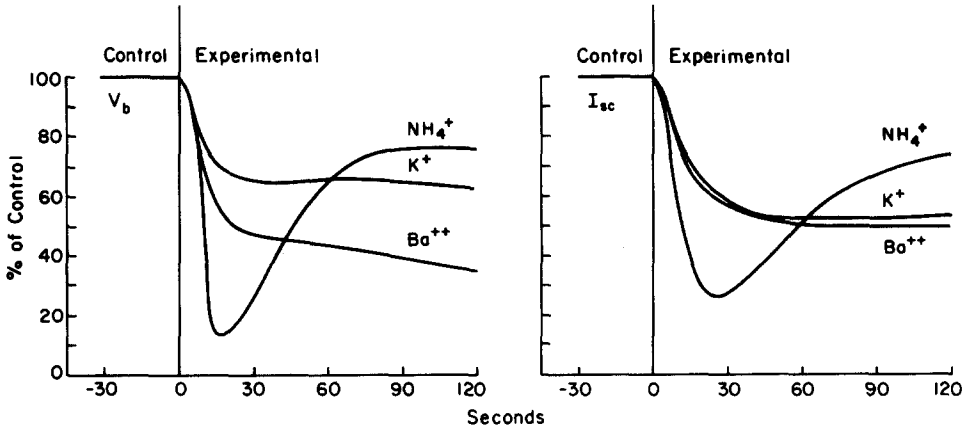

FIGURE 1. Changes of V_b and I_{sc} with the addition of 50 mM K^+, 5 mM Ba^{++}, or 80 mM NH_4^+ to the basolateral solution. Tissues were short-circuited continuously.

RESULTS

The results of typical microelectrode experiments are shown in Fig. 1. Within 30 s, K^+, Ba^{++}, and NH_4^+ caused depolarization of V_b. The "immediate" response to NH_4^+ was significantly greater but transient. The control values of I_{sc} and V_b are summarized in Table I, together with the experimental values of I_{sc} and V_b measured at 30 s (K^+ and Ba^{++}) and at the minimum I_{sc} and V_b after NH_4^+ (15–45 s). K^+, Ba^{++}, and NH_4^+ caused decreases of I_{sc} and V_b, with V_b falling, on the average, to 62.8, 46.8, and 15.2% of control, respectively.

Neither K^+, Ba^{++}, nor NH_4^+ depolarization of V_b caused an increase of Na^+ efflux from the cells as would be expected if Na^+ transport at basolateral membranes occurred via a voltage-dependent mechanism(s) (see Fig. 2). Indeed, within 30–60 s when V_b was depolarized and when cytosolic [Na^+] and tracer specific activity could be considered to remain essentially constant (Cox and Helman, 1983), Na^+ efflux via pumps and leaks either remained unchanged or

TABLE I

Changes of I_{sc} and V_b Caused by K^+, Ba^{++}, or NH_4^+

	I_{sc}	V_b
	$\mu A/cm^2$	mV
Control (5)	23.4±1.0	−75.4±5.4
50 mM K^+	14.5±1.0	−48.0±6.7
K^+/control (%)	62.2±3.9	62.8±5.2
Control (5)	28.1±4.4	−72.2±7.2
5 mM Ba^{++}	16.9±2.9	−32.6±4.0
Ba^{++}/control (%)	61.2±7.2	46.8±6.7
Control (6)	22.5±2.5	−91.7±8.3
80 mM NH_4^+	5.7±1.8	−13.2±10.3
NH_4^+/control (%)	23.8±4.8	15.2±8.6

Values are means ± SEM. For K^+ and Na^+, values were taken at 30 s. For NH_4^+, the minimum value at 15–45 s was used for purposes of summary.

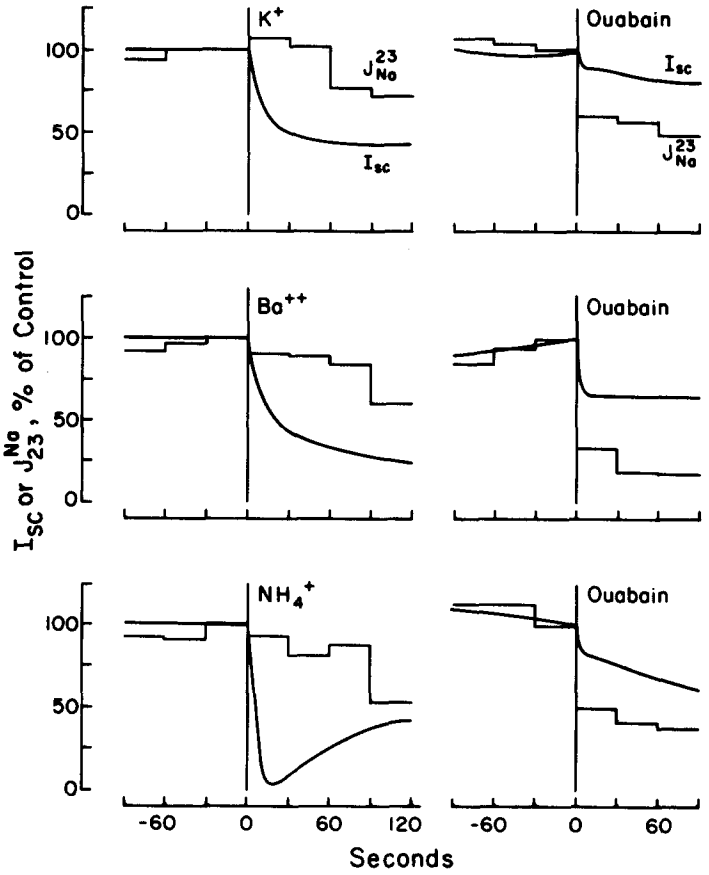

FIGURE 2. Changes of I_{sc} and J_{Na}^{23} caused by K^+, Ba^{++}, and NH_4^+ depolarization of V_b (left) and by 1 mM ouabain (right).

decreased slightly (Table II). To ensure that changes, if any, of Na^+ efflux would have been detected, the tissues were treated with ouabain (1 mM) after the experimental treatment was reversed and the control Na^+ efflux was measured again. Ouabain, within a few seconds at most, caused a large, readily observable inhibition of Na^+ efflux (see Fig. 2). Between 30 and 60 s, the I_{sc} was inhibited by ouabain on the average to 70.1% of control, whereas the Na^+ efflux was inhibited on the average to 30.4% of control (Table II). Therefore, despite finite unstirred layers, voltage-dependent changes of efflux would have been detected within 30–60 s of depolarization of basolateral membrane voltage.

Since ouabain induces a furosemide-sensitive component of J^{23}_{Na} (Cox and Helman, 1983), seven experiments were done to test additionally for voltage dependence of the post-ouabain J^{23}_{Na} that comprised under these conditions furosemide-sensitive and furosemide-insensitive Na^+ efflux. 3 min after ouabain inhibition of the Na,K-ATPase, either K^+ ($n = 2$), Ba^{++} ($n = 2$), or NH_4^+ ($n = 3$) was added to the basolateral solution. Within the first sampling period of 60 s,

TABLE II

Effect of K^+, Ba^{++}, and NH_4^+ on I_{sc} and FJ^{23}_{Na}

	I_{sc}	FJ^{23}_{Na}
	$\mu A/cm^2$	$\mu A/cm^2$
Control (18)	25.6±2.2	26.0±2.3
	Percent of control	
50 mM K^+ (6)	45.6±1.1	105.4±2.3
5 mM Ba^{++} (7)	40.3±2.2	89.6±4.3
80 mM NH_4^+ (5)	28.4±11.6	91.5±2.5
Ouabain (13)	70.1±2.3	30.4±2.5

the ouabain control I_{sc} was decreased from a mean of 12.5 ± 1.2 to 3.7 ± 0.6 $\mu A/cm^2$ (26.9 ± 4.8% of the ouabain control value), whereas J^{23}_{Na} was decreased from 7.2 ± 0.5 to 5.6 ± 0.6 $\mu A/cm^2$ (77.1 ± 6.7% of the ouabain control value). Thus, despite a marked decrease of the I_{sc} (secondary to depolarization of V_b), the ouabain-insensitive Na^+ efflux was decreased ~23% on the average, although an increase of J^{23}_{Na} was expected if Na^+ efflux occurred via an electrodiffusive mechanism. These observations lend further support to previous conclusions that Na^+ flux at the basolateral membranes of the cells occurs primarily via electroneutral mechanisms of transport (Cox and Helman, 1983; Stoddard and Helman, 1985).

DISCUSSION

Because changes of basolateral membrane flux can be measured rapidly in isolated epithelia of frog skin, we tested for voltage sensitivity of the Na^+ efflux via Na^+/K^+ pumps and parallel leak mechanisms. To the extent that the Cl-independent leak Na^+ efflux represents ~15–20% of the pump-mediated Na^+ efflux (Cox and Helman, 1983), it was clear that Na^+ efflux via the pump was for practical purposes voltage independent over the voltage range studied. Although NH_4^+, unlike Ba^{++} or K^+, caused a transient depolarization of V_b, it was

nevertheless clear that Na^+ efflux was not changed by depolarization of V_b in the range of 30–60 mV or more with NH_4^+. It was also clear that elevation of K^+ from 2.4 to 50 mM did not alter pump activity, as evidenced by the constancy of the Na^+ efflux. Thus, it must be presumed that pump-mediated K^+ influx is saturated at an extracellular K^+ concentration of 2.4 mM. Depolarization of V_b by Ba^{++} occurred at least in part by inhibition of K^+ conductance (Nagel, 1979). It is not known how NH_4^+ causes depolarization of V_b. Nevertheless, in the absence of changes of pump-mediated Na^+ efflux, neither Ba^{++} nor K^+ nor NH_4^+ appeared to influence pump-mediated Na^+ efflux, either chemically or electrically via changes of basolateral membrane voltage.

Although depolarization of V_b by K^+, Ba^{++}, and NH_4^+ (within 30–60 s) may be accompanied by changes of intracellular pH (or other factors) capable of influencing Na,K-ATPase activity (Eaton et al., 1984), we must conclude, in the absence of significant changes of Na^+ efflux, that either the pump is insensitive to changes of intracellular pH or, alternatively, that pH-dependent Na,K-ATPase sensitivity develops at times beyond the time frame of our measurements. As it is unlikely that Ba^{++}, K^+, or NH_4^+ causes precisely the same changes to the intracellular environment of the cells, we are compelled to the conclusion that pump-mediated and leak Na^+ effluxes are voltage independent. In epithelia bathed with Cl^--Ringer containing 2.4 mM HCO_3^-, 25% CO_2 added to the basolateral solution causes an ~14% decrease of J_{Na}^{23} (86.1 ± 1.3% of control [n = 6]), despite depolarization of V_b (within 10–15 s) to near 0 mV (Stoddard, 1984) and acidification of intracellular pH to <6.4 (Nunnally et al., 1983). Observations such as these confirm the notion that basolateral Na^+ efflux is voltage independent. It remains possible that increases of pump-mediated Na^+ efflux are balanced almost exactly by decreases of leak-mediated Na^+ efflux in response to depolarization of basolateral membrane voltage. We consider such a possibility rather unlikely, especially in view of the differences in agents used to depolarize V_b and the differences in the magnitudes of depolarizations and their time courses. Moreover, we cannot rule out absolutely the possibility that the procedures used here alter the "permeability" of the parallel shunt pathways to ^{24}Na and hence lead to a change or the absence of change of what is defined here as J_{Na}^{23}. To the extent that transepithelial voltage is constant ($V_T = 0$), and since the control ^{24}Na flux via shunt pathways is rather small, averaging ~0.05 $\mu A/cm^2$ (O'Neil and Helman, 1976), it would seem unlikely that changes of shunt ^{24}Na flux, if they occurred, could bias appreciably, if at all, the observations and thus the conclusions.

The observation of voltage independence of pump-mediated Na^+ efflux in an epithelium confirms and extends similar findings in other nonepithelial tissues (Thomas, 1972; Brinley and Mullins, 1974; Glynn, 1984). It has so far not been possible to voltage-clamp pump-containing basolateral membranes of intact epithelial cells over a larger range of voltage to test this idea further. In this regard, we chose to depolarize V_b via chemical means. We were limited to depolarization of V_b over larger ranges, but depolarization by 30–60 mV encompasses a reasonably large range of physiological voltages. Over this range of V_b, the pump appeared to be voltage independent, although compelling evidence exists for its

electrogenicity as in other tissues (Thomas, 1972; Brinley and Mullins, 1974; Helman et al., 1979; Cox et al., 1980; Cox and Helman, 1983; Glynn, 1984). Because ouabain causes essentially no immediate change of basolateral membrane resistance concurrent with inhibition of the Na,K-ATPase (Cox and Helman, 1983), it has been suggested that the pumps are rheogenic (sources of constant current). In this respect, voltage independence of the pump-mediated Na^+ efflux is consistent with this notion.

It remains unclear why the pump is voltage independent. If in fact the current-voltage relationship of the pump is linear through its reversal potential, then it may be presumed that the internal emf of the pump is sufficiently large that changes of 30–60 mV or more would cause little, if any, detectable change of Na^+ efflux. Alternatively and perhaps more likely, the current-voltage relationship of the pump is nonlinear, so that the pump appears to behave as a voltage-independent mechanism, at least in the physiological range of interest. Such ideas have been discussed previously and cannot be resolved further at present (De Weer, 1984).

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