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EFFECT OF Li AND OF OTHER IONS ON Na TRANSPORT IN EPITHELIAL CELLS OF FROG SKIN

Thomas U.L. Biber and Terry L. Mullen

Department of Physiology, Medical College of Virginia, Richmond, Virginia 23298

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We have used the isolated frog skin preparation of Rana pipiens as a model membrane to investigate the effect of Li^+ and of other monatomic ions on Na^+ transport in epithelial cells. We found that addition of Li^+, K^+, Rb^+ and Cs^+ causes a decrease in Na^+ absorption whereas addition of Br^- and I^- results in an increase in Na^+ absorption.

The experiments were done by mounting the frog skin in a chamber which is specifically designed to prevent damage to the tissue where it is attached to the chamber (1). Under control conditions, the solutions bathing the blood side (basolateral side) and the external side (apical side) of the epithelial cells were identical, containing 15 mM NaCl, 100 mM choline chloride (chCl), 2.5 mM $KHCO_3$ and 1 mM $CaCl_2$. After completion of the control measurements, the 100 mM chCl in the external solution was replaced by an equimolar amount of a monatomic test salt. This allowed us to examine whether the addition of a given ion changed the Na^+ transport when the Na^+ concentration remained 15 mM on both sides of the epithelial cells. Except for the brief (<5 sec) interruptions needed for bath changes, the potential across the epithelial cells (PD) was maintained at zero throughout the experiment by applying an automatic voltage clamping device. Therefore, the electrochemical potential for Na^+ remained equal on both sides of the epithelial cells during the experiment. This experimental setup has the advantages that any net movement of Na^+ across the epithelial cells could be assigned to active transport (other forces for net Na^+ movement were not present) and that the current measured under these conditions, the so-called short-circuit current (I_o), is equal to net Na^+ movement provided no other ion is transported across the cells (2). The experimental design allowed direct determination of the two unidirectional Na^+ fluxes, namely the absorptive Na^+ flux (Na influx) from the apical to the basolateral side of the cells (i.e. from external bath to "blood side" bath) and the backflux of Na^+ (Na efflux) which proceeds in opposite direction across the cells. The Na^+ fluxes were measured with ^{22}Na by using standard techniques involving liquid scintillation spectrometry (1,3).

The change in Na^+ flux observed in this study began immediately after the introduction of the test salt into the external bath and was readily reversible upon return to control conditions. This suggested that the changes in absorption of Na^+ (i.e. Na influx) could be due to alterations in the properties of the apical cell membrane, particularly since, under

many conditions, the entry of Na^+ across the apical cell membrane (Na uptake) is the rate-limiting step for absorption of Na across the entire epithelium (4,5).

The relative ability of the substituent ions to inhibit Na influx was, in decreasing order, $Li^+ > K^+ > Rb^+ > Cs^+ > choline^+$ and the relative ability to stimulate was, in decreasing order, $I^- > Br^- > Cl^-$ (see Figure 1). In general, changes in Na efflux followed the same pattern except that the stimulation of Na efflux was larger in presence of Br^- than in presence of I^- (the changes in presence of Rb^+ and Cs^+ were not significant). Under the conditions tested, the net Na flux (Na influx minus Na efflux) was for all practical purpose equal to the Na influx since the Na efflux was only a very small fraction of the Na influx. For example, under control conditions the Na efflux was 0.023 ± 0.002 $\mu eq/cm^2 hr$, whereas the Na influx was 1.004 ± 0.048 $\mu eq/cm^2 hr$ (64 observations for each average). With the exception of Li^+, the sequence of changes in Na influx is identical to the sequence of changes in I_0 and in PD (Figure 1). Of all the cations tested, Li^+ produced the strongest inhibition of Na^+ transport. Li^+ is transported actively in the inward direction (i.e. from apical to basolateral side) across the epithelial cells of the frog skin (6) by a process involving at least two major steps: (1) the entry of Li^+ into the cells across the apical cell membrane (Li uptake) and (2) the extrusion of Li^+ from the cell across the basolateral cell membrane on the other side of the cell. It has been known for some time that Na and Li uptake across the apical cell membrane are closely related. Previous experiments have shown that the Na uptake ex-

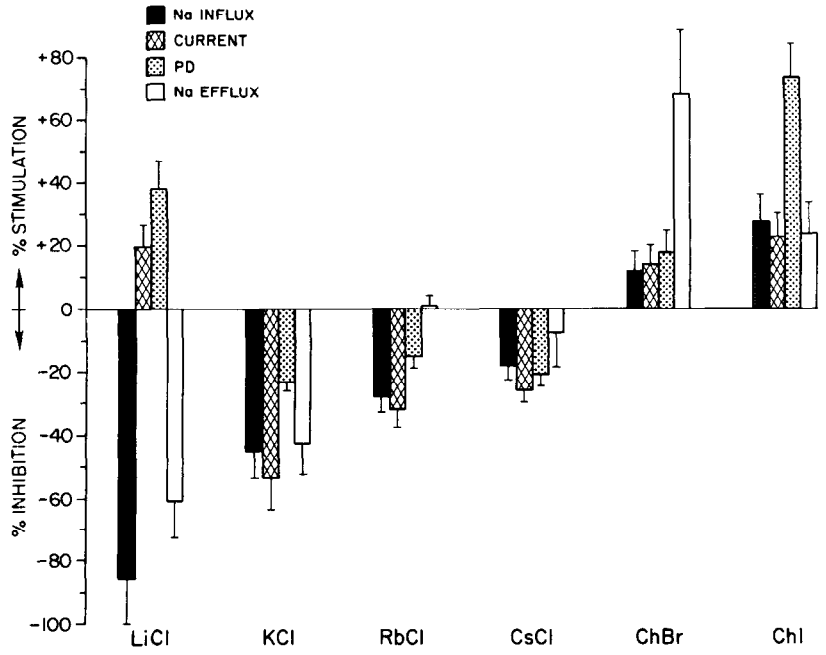

Figure 1. Average percentage changes ± SEM after substitution of 100 mM chCl by different test salts. Number of observations for Na influx, Na efflux, PD and short-circuit current measurements was 8, 16, 24 and 24, respectively.

hibits saturation kinetics and can be described as:

$$\text{Na uptake} = \{(J^m\ Na_{ext})/(K_{Na} + Na_{ext})\} + (\alpha Na_{ext})$$

where J^m is the maximal Na uptake, K_{Na} is an "apparent Michaelis constant", α is a permeability coefficient and Na_{ext} is the Na concentration in the external bath (7). In this equation, the last term, αNa_{ext} describes a linear component which is very small when compared to the saturating component (first term in equation). J^m and K_{Na} were determined to be 4.0 $\mu eq/cm^2 hr$ and 14.3 mM, respectively. In the same study it was discovered that when Li^+ is added to the external bath, Na uptake is inhibited competitively with an inhibitor constant for Li^+, K_{Li}, of about 25 mM (7). This suggests that Na^+ and Li^+ share a common pathway or site for entrance into the cells. Another explanation, that inhibition of the Na uptake is exclusively the result of a Li^+-induced shift in cell membrane potential (8) is not tenable since the degree of inhibition is vastly different from the one calculated from the magnitude of the cell membrane potential shift. Li^+ uptake proceeds along a favorable electrochemical gradient since, in the short-circuited preparation, the intracellular potential is substantially negative, even in presence of high Li^+ levels in the external bath (8). In fact such potentials account for the observed accumulation of Li^+ to ten times the level in the external bath (9,10). On the other hand the extrusion of Li^+ across the basolateral cell membrane must be assigned to active transport since this transfer proceeds against an electrochemical gradient.

Figure 1 indicates that Li^+ proceeds in an inward direction at a considerable rate when added to the external bath, since the current increased by 20% instead of decreasing in proportion to the Na influx to 15% of the control values. From this and from earlier work which showed that net movement of Li^+ is accompanied by an equivalent current (6) one must conclude that Li^+ absorption proceeded at a slightly higher rate than the Na influx under control conditions (0.6 $\mu eq/cm^2 hr$). It should be noted here that the experiments with Li^+ were the only ones in which short-circuit current differed from the Na influx.

Studies done on toad urinary bladder indicate that Li^+ cannot be transported against as great an electrochemical gradient as Na (11). This limited capability for reabsorption against a gradient has been used by Hayslett and Kashgarian (12) as possible explanation for their observation that, in the rat kidney, no Li^+ is reabsorbed in the distal parts of the nephron in which Li^+ faces a large adverse electrochemical gradient, whereas 75% of the filtered Li^+ is reabsorbed in more proximal parts of the nephron.

This study shows that K^+ causes a 45% inhibition of Na influx, presumably because it inhibits Na transport at the apical cell membrane. This view is supported by other experiments in which inhibition of Na uptake by K^+ was observed in direct measurements of Na entry across the apical cell membrane (13).

The nature of the interaction of ions other than Li^+ with Na uptake remains to be de-

dermined. It is interesting to note that both the decrease in inhibition of Na influx in the cation series as well as the increase in stimulation of Na influx in the anion series are correlated with the size of the radius of the crystal of the different ions (see Figure 2). This suggests that dimensional properties of these ions are an important factor in the interaction with Na transport. Further studies are under way to try to characterize this interaction more fully.

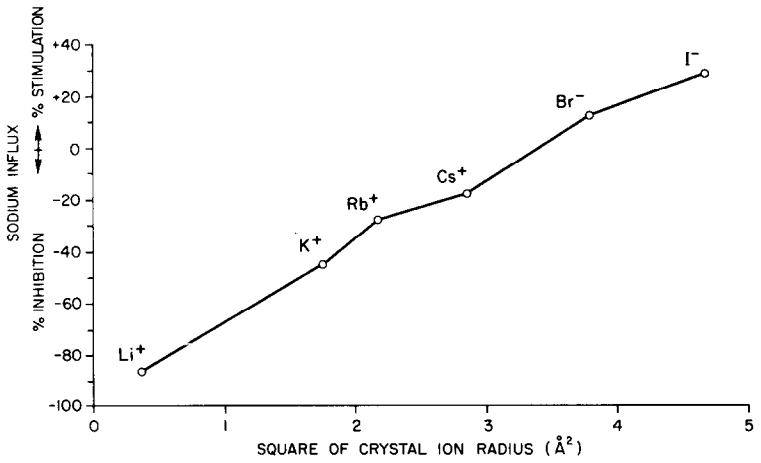

Figure 2. Changes in Na influx shown in Figure 1 plotted against the square of the crystal ion radius.

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