Biochimica et Biophysica Acta, 942 (1988) 169–178
Elsevier

BBA 74059

Potassium dependence of sodium transport in frog skin

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(Received 1 February 1988)

Key words: Sodium ion flux; Ouabain; Potassium ion flux; Pump coupling ratio; Epithelium; (*R. pipiens*)

$^{22}Na^+$ and $^{42}K^+$ fluxes across the basolateral membrane of the isolated epithelium of frog skin were investigated with regard to dependence on K^+ in the basolateral solution. When K^+ was removed from the basolateral solution (K^+-free Ringer), there was a transient rise in short circuit current (I_{sc}) that could be eliminated by pretreatment with ouabain. Concurrently, the apparent sodium efflux across the basolateral membrane (J_{13}^{Na*}) showed either no change or an immediate (1–2 min) small decrease ($\approx$ 10%) that was followed by a small transient increase. K^+ fluxes showed either no change or a small decrease under these conditions. J_{13}^{Na*} was partially ouabain sensitive during all of the above treatments. Furosemide partially inhibited both sodium and potassium flux after K^+-free treatment. The pump, as defined by ouabain sensitivity of Na^+ flux, continued to work even after 20 minutes of K^+-free treatment. Pump activity may be maintained by potassium leaking from the cells that is recycled by the pump. However, the ouabain-sensitive transient rise in I_{sc} after K^+-free treatment cannot readily be explained by changes in either Na^+ or K^+ flux. A change in pump coupling ratio provides one explanation for these data.

Introduction

While it has been known for years that removal of K^+ from the basolateral solution tends to decrease net transepithelial Na^+ transport [1–9], the mechanisms that bring this about are not well understood. It has also been generally thought that K^+ uptake by the pump would be directly related to net transepithelial Na^+ transport. This was not found to be the case under most circumstances [6,10–13].

In toad urinary bladder, removal of K^+ caused an increase in short circuit current (I_{sc}) followed by a decrease. Sodium transport also showed a transient increase that was delayed relative to I_{sc}. This delay was thought to be due to isotope diffusion time through unstirred layers [6]. While the mechanism for the transient is not well understood, Robinson and Macknight [6] suggested that it might be related in part to stimulation of the sodium pump.

K^+ removal has been shown to hyperpolarize intracellular voltage initially [14,15] with depolarization relative to control values occurring after long time periods [15]. When K^+ was added back, there was either a further depolarization, or after longer depletion times, a hyperpolarization of the membrane voltage [5,15]. This hyperpolarization was thought to be due to reactivation of the Na^+ pump.

In contrast to what one might expect, intracellular K^+ was decreased by only 20% even after two hours of incubation in a K^+-free medium [16]. This observation led to the hypothesis that K^+ leaking out of the tissue was immediately recycled by the pump [16].

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It is clear from the above discussion that K^+ removal and restoration to the basolateral solution gives a complex response. This is not surprising in view of the fact that the simplest system with which we are dealing is at least a Na^+/K^+ exchange pump (pump current) in parellel with a K^+ channel (leak current). A change in K^+ concentration might be expected to cause opposing changes in pump current and leak current resulting in either an increase, a decrease, or no change in current flow (I_{sc}) across the basolateral membrane.

The purpose of the present study was to look at the effect of K^+-free conditions on Na^+ and K^+ fluxes across the basolateral membrane with the hope of providing some insight into the nature of the transient in I_{sc} observed upon K^+ removal. The principal finding was that K^+-free Ringer caused little immediate change in either Na^+ or K^+ flux. In addition, ouabain eliminated the I_{sc} transient caused by K^+ removal. This suggested a role for the Na^+ pump.

Methods

Abdominal skins of northern *Rana pipiens* (Lemberger, Oshkosh, WI) were used in all studies. Isolated epithelia were prepared using a modification of the splitting techniques of Aceves and Erlij [17] as described by Fisher et al. [18]. Details on the chambers and mounting procedures have been previously described [11,19,20]. The exposed surface area was 0.72 cm^2; the chamber volume was 0.6 ml. To minimize contamination of the solutions bathing the skin by leakage of K^+ from salt bridges, 1 mol/l NaCl salt bridges were used in all studies.

For sodium tracer studies, epithelia were short circuited for 30 min, after which ^{22}Na (3 μCi/ml, New England Nuclear, Boston, MA, U.S.A.) was added to one side of the chamber and its rate of appearance on the opposite side determined. Tracer equilibration of Na^+ in the intracellular pool is complete in 10–15 min [19,21,22]. Adopting the three compartment notation of Curran et al. [23], the scripts of the unidirectional tracer fluxes (J_{ij}) will refer to flux from compartment i to compartment j. Compartments 1, 2, and 3 are apical, cellular, and basolateral, respectively. Accordingly, J_{13}^{Na} is the unidirectional sodium flux from apical to basolateral solution, J_{23}^{Na} is the unidirectional sodium flux from the cell to the basolateral solution, and J_{32}^{Na} is the unidirectional sodium flux from the basolateral solution into the cell. The apparent J_{13}^{Na*} has been calculated from J_{23}^{Na} (tracer) assuming that the tracer specific activity inside the cell is the same as that in the loading (apical) solution. From one steady state to another, the apparent J_{13}^{Na*} will be equal to J_{13}^{Na}. During a transient, rapid changes in J_{23}^{Na} (tracer) will qualitatively reflect changes in Na^+ flux across the basolateral membrane unless or until there is a change in intracellular tracer specific activity for Na^+. Therefore, immediate changes in the reported J_{13}^{Na*} reflect changes in direction of Na^+ flux across the basolateral membrane that can be interpreted within a range of uncertainty bounded by the change in cell tracer specific activity *. See also Refs. 19, 20, and 24 and Results and Discussion.

For K^+ tracer experiments, $^{42}K^+$ (New England Nuclear, Boston, MA) was loaded into the tissue from the basolateral side for 3–4 hours prior to splitting [20]. The tissue was then mounted in the chamber and the rate of appearance of $^{42}K^+$ in the basolateral solution (J_{23}^{K}) was observed. Previous studies have shown that this K^+ originates from the transport pool, largely reflects K^+ moving through conductive mechanisms, and is highly correlated with I_{sc} after ouabain treatment [11,20,25]. Experiments were conducted such that all $^{42}K^+$ vials contained activity at least five times background.

Ouabain (Sigma, St. Louis, MO, U.S.A.) and furosemide (Hoechst-Roussel Pharmaceuticals, Somerville, NJ, U.S.A.) were used at final concentrations of 1.0 mmol/l.

The Ringer solution contained (in mmol/l): 100 NaCl, 2.0 $CaCl_2$, and 2.4 $KHCO_3$ (pH 8.1)

* The essential equations are as follows: $J_{13}^{Na*} = J_{23}^{Na}$ (tracer)/SA_1 by definition. J_{23}^{Na} (actual) = J_{23}^{Na} (tracer)/SA_2 where SA_1 and SA_2 are the specific activities of tracer in the apical and cellular compartments. Rearrangement gives J_{13}^{Na*}/J_{23}^{Na}(actual) = SA_2/SA_1. Therefore, J_{13}^{Na*} will qualitatively reflect changes in J_{23}^{Na}(actual) if changes in SA_2 are small since SA_1 is constant.

equilibrated with room air. K^+-free Ringer was made by substituting $NaHCO_3$ for $KHCO_3$. The K^+ concentration of the solution coming from the basolateral chamber after a 2-min incubation in K^+-free Ringer was measured and found to be less than 0.1 mmol/l. This is likely to represent a maximum value for K^+ since during an experiment the basolateral chamber is completely replaced by new K^+-free Ringer at 1-min intervals. This K^+ probably comes from the tissue and K^+ that may have been trapped in the current and voltage salt bridge ports. All experiments were done at room temperature. At the end of the control period, K^+-free solutions, solutions containing the drug, etc. were flushed directly into the basolateral chamber to begin the experimental period. Values are reported as the mean ± S.E. (N).

Results

K^+ dependence of Na^+ and K^+ flux

When K^+ was removed from the basolateral solution there was a transient rise in I_{sc} that reached a maximum in 2–5 min (Fig. 1a). Thereafter, there was a decline in I_{sc} to about 73% of control after 20 min. The simultaneously measured Na^+ tracer extrusion showed an immediate decrease to about 80% of control in Fig. 1a. Thereafter, there was a small increase in $J_{13}^{Na^*}$ that remained steady or in some experiments slightly declined. Out of the 13 skins examined, 12 showed a transient rise in I_{sc} similar to that in Fig. 1. One had essentially no increase (Fig. 3b). At three minutes the average increase in I_{sc} was to 116.9 ± 2.0% of control. This was a significant change ($P < 0.05$). Control was defined as the average of the three samples just prior to addition of K^+-free Ringer. Three of these skins showed distinct decreases in Na^+ flux similar to that in Fig. 1a. In seven skins there was no observable change in Na^+ flux in spite of rather large transients in I_{sc}. In three skins there was a slight increase in Na^+ flux that was still significantly less than the change in I_{sc}. See Fig. 3a. At 3 minutes the mean change in Na^+ flux was to 98.9 ± 2.4% of control. This was not a significant change.

When K^+ was returned to the basolateral solution there was a marked rise in $J_{13}^{Na^*}$ (Fig. 1a) with no change in I_{sc}. In a few tissues, there was a significant increase in I_{sc}, although in general there was no rise in I_{sc}. In addition to showing that return of K^+ restores the pump to a more efficient mode of Na^+ extrusion, it also demonstrates how quickly a change in turnover of the pump is reflected by a change in Na^+ tracer extrusion from the cell.

Since net current flow across the basolateral membrane (I_{sc}) is comprised of at least the pump current plus the leak current, it was of interest to examine the unidirectional efflux of K^+ from the cells across the basolateral membrane to get an

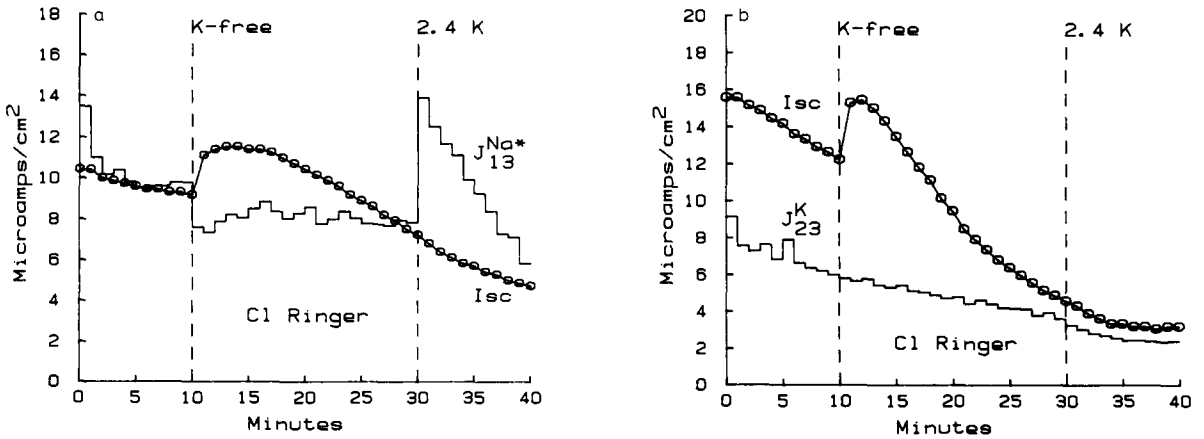

Fig. 1. Effect of K^+ removal and restoration on sodium (a) and potassium (b) fluxes and short circuit current across the basolateral membrane of frog skin epithelium. Short-circuit current (I_{sc}) and sodium flux ($J_{13}^{Na^*}$) are reported in $\mu A/cm^2$. Potassium flux (J_{23}^{K}) has been set equal to $I_{sc}/2$ at minute 10. See text for details.

index of the leak current. Previous studies have shown that this K^+ efflux approximates net K^+ flux through conductive pathways in parallel to the pump [11,20,25]. Since the intracellular specific activity of $^{42}K^+$ is not known [20], K^+ fluxes were presented in arbitrary units such that $J_{23}^K \cong I_{sc}/2$. Previous studies [11,20] have suggested that the coupling ratio of the Na^+ pump is about two Na^+ for every K^+ although this ratio may be transport rate dependent. This gives a reasonable estimate of changes of K^+ leak current under control conditions.

As shown in Fig. 1b, when K^+ was removed from the basolateral solution, I_{sc} went through the usual transient increase. There was no significant change in the rate of appearance of $^{42}K^+$ in the basolateral solution (J_{23}^K) when compared with control. Similar results were obtained in 11 other experiments. If the K^+ efflux is indeed about one half of the I_{sc} [20], it is clear that the transient rise in I_{sc} during the first few minutes cannot be explained by a rise in K^+ efflux. The hyperpolarization of the basolateral membrane observed in other studies [15] also suggests that if anything K^+ efflux would be decreased. It is also not likely that net K^+ flux through the K^+ channel is increased significantly by eliminating K^+ uptake through the channel. Previous studies have shown that ouabain blocks more than 95% of K^+ uptake (to less than 1 $\mu A/cm^2$ [11]) suggesting that K^+ uptake through the channel is small.

To test for ouabain dependence of the transient rise in I_{sc} and to look for a K^+-free solution effect on K^+ channels, K^+ was removed from the basolateral solution after pretreatment with ouabain (Fig. 2). After ouabain treatment, I_{sc} is largely carried by K^+ [20,25]. The transient rise in I_{sc} was not observed. Microelectrode studies have shown that there are no acute effects of ouabain on the resistance of the basolateral membrane (Ref. 19, unpublished observations). We have also shown that, after ouabain, changes in conductive K^+ efflux largely follow changes in I_{sc} [20,25]. These results suggest that the K^+ channels after a few minutes of ouabain should respond in a way similar to control tissues. As an additional check for time dependent ouabain effects on the K^+-free Ringer response, K^+-free Ringer and ouabain were added simultaneously to three skins. In all of these

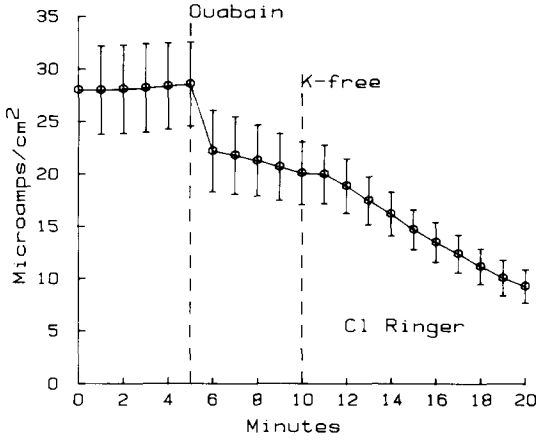

Fig. 2. Summary of the effect of ouabain (1 mmol/l) followed by K^+-free Ringer plus ouabain on I_{sc}. Bars represent one S.E. of the mean ($n = 4$).

experiments there was an immediate decrease in I_{sc} with no indication of a transient rise with the time course of minutes observed when K^+-free Ringer alone was added. When considered with the tracer studies, these results provide evidence arguing against an effect of K^+-free solutions on the leak current and suggest a role for the Na^+ pump in the transient rise in I_{sc} after K^+ removal in non-ouabain treated skins. A similar result was obtained by Robinson and Macknight on toad urinary bladder [7].

Ouabain and furosemide sensitivity in K^+-free solution

The following experiments were performed to test whether Na^+ extrusion into a K^+-free solution was still via the pump (i.e. ouabain sensitive) or via a furosemide-sensitive pathway. Even though the tissue is in a long term transient, the immediate effects of these treatments should give an indication of whether ions exit through drug-sensitive pathways. Ouabain caused a substantial decrease in I_{sc} and $J_{13}^{Na^*}$ (Fig. 3a) after 20 min of K^+-free treatment. Note the relatively large fractional inhibition of I_{sc} by ouabain. In five experiments, 2–3 minutes of ouabain decreased I_{sc} to $44.5 \pm 2.8\%$ of the immediately preceding value. Subsequent addition of furosemide caused a small but noticeable further decrease in $J_{13}^{Na^*}$ with no change in I_{sc}.

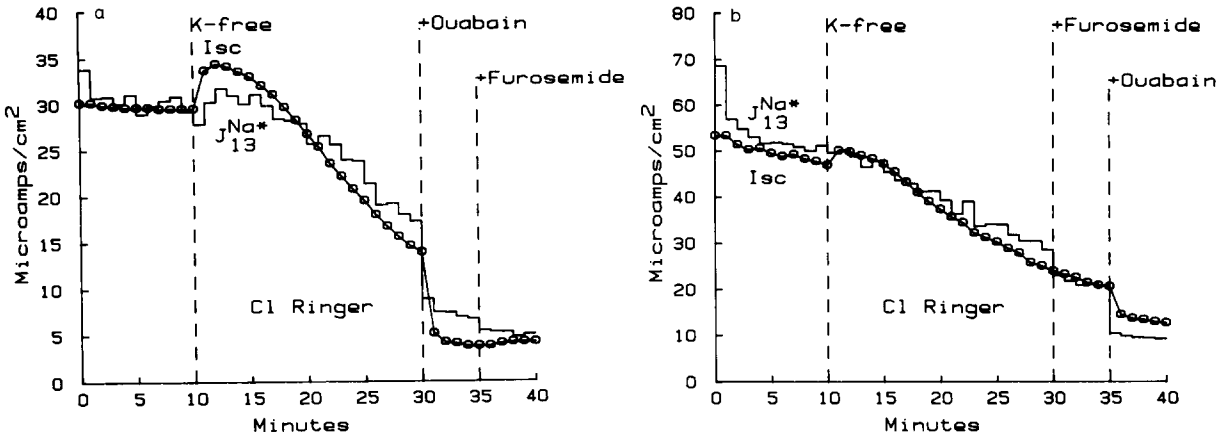

Fig. 3. Effect of ouabain followed by furosemide (a) and furosemide followed by ouabain (b) on sodium fluxes and I_{sc} under K^+-free conditions.

After 20 min in K^+-free Ringer, tissues were also treated with furosemide prior to ouabain. As shown in Fig. 3b, furosemide had no effect on I_{sc} but did not cause a small but significant decrease on J_{13}^{Na*}. Ouabain caused noticeable, additional inhibition.

K^+ fluxes were examined for ouabain and furosemide sensitivity. As shown in Fig. 4a, ouabain had a large effect on I_{sc} after K^+-free treatment. J_{23}^{K} was increased probably due to basolateral membrane depolarization. Subsequent addition of furosemide caused a decrease in J_{23}^{K} similar to its effects on J_{13}^{Na*}. In the experiment shown in Fig. 4b, furosemide alone caused a small decrease in I_{sc} and J_{23}^{K}. This effect of furosemide on I_{sc} was not consistently observed (see Fig. 3b) but it could reflect a decrease in pump activity due to a blockage of K^+ exit from the cell or a direct effect of furosemide on the pump. Ouabain caused a small increase in J_{23}^{K}, again probably due to membrane depolarization.

Since K^+-free conditions induced a significant furosemide-sensitive Na^+ flux, we considered the possibility that there might be a rapid increase in Na^+ influx across the basolateral membrane immediately after K^+ removal. An influx of Na^+ would load the pump causing increased pump activity perhaps leading to a transient increase in I_{sc}. To test for a furosemide sensitive influx (i.e. a Cl^- coupled influx) we pretreated the tissue with furosemide for 10 min prior to K^+ removal. In five experiments there was a small rise in I_{sc} and

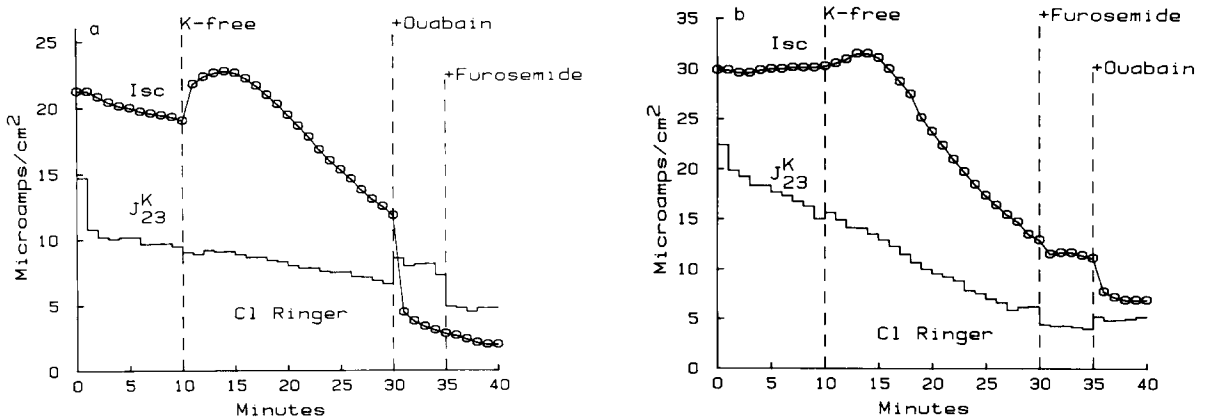

Fig. 4. Effect of ouabain followed by furosemide (a) and furosemide followed by ouabain (b) on potassium fluxes and I_{sc} under K^+-free conditions.

Na^+ flux after furosemide addition. Upon K^+ removal there was the usual rise in I_{sc} of about 15%. Concurrently there was a small drop in Na^+ flux that never exceeded the control value. These experiments suggest that a furosemide sensitive influx of Na^+ into the cell is not responsible for stimulating the pump and thus causing the transient.

Discussion

The purpose of the present study was to characterize I_{sc} and the Na^+ and K^+ fluxes across the basolateral membrane of frog skin immediately after K^+ removal from the basolateral solution. We have made estimates of Na^+ and K^+ fluxes from the corresponding tracer effluxes of these ions from the cells and compared them directly with the net current flow across the basolateral membrane. A primary assumption in these studies is that the intracellular tracer specific activity (while unknown) remains relatively constant during the first few minutes after perturbation. This will be considered in detail below. The results of these studies have a general pattern. During the first 5–10 min after K^+ was nominally removed from the basolateral solution, there was a transient increase in I_{sc} while sodium tracer fluxes showed either no change or small decreases. K^+ fluxes showed little change. It thus appears that there was a significant increase in I_{sc} that cannot readily be accounted for by changes in either Na^+ or K^+ fluxes. Pretreatment with ouabain prevented the transient rise in I_{sc}. Ouabain (after 20 min of K^+-free treatment) caused relatively large, immediate decreases in Na^+ flux and I_{sc} under these conditions. These results are similar to observations on I_{sc} and sodium fluxes made by Robinson and Macknight on toad urinary bladder [6,7].

When K^+ was returned to the basolateral solution there was a dramatic increase in Na^+ flux that reached a maximum in the first minute. This immediate rise in Na^+ flux is indicative of the rapid rate that solutions can be changed at the basolateral side due to minimal unstirred layers and how quickly a change in Na^+ flux can be detected. The pump has apparently been returned to a mode that rapidly extruded cellular Na^+ that had accumulated. The change in I_{sc} was less predictable. When K^+ was returned to the basolateral side, there was either no change or a transient rise. A rise in I_{sc} of course suggests increased electrogenic activity of the pump. When there is no increase in I_{sc}, the possibility of activation of a nonelectrogenic mode of the pump might be considered. There does not appear to be a consistent corresponding decrease in K^+ flux that might indicate a compensating decrease in K^+ current counterbalancing the expected increase in pump current.

Changes in short-circuit current

Short-circuit current in this preparation represents the net current flow across the apical or the basolateral membrane. Basolateral membrane current is comprised of a pump current, a K^+ leak current, plus current from any other ions that show a significant conductance and are not at equilibrium. Chloride is the dominant anion in this system but it probably does not make a significant contribution to I_{sc}. It has recently been shown that chloride crosses the basolateral membrane almost exclusively via electrically silent mechanisms [20,26,27].

The transient increase in I_{sc} was similar to that observed previously in toad bladder [6] and frog skin [9]. The probable primary difference here is that K^+-free conditions can be achieved more quickly and completely using the isolated epithelium. Previous studies of this preparation have shown that solution changes can be achieved within seconds [11,18–20]. The time course for the rise in I_{sc} is on the order of minutes, so it is not likely to be due to just a progressive washout of K^+ from the extracellular spaces.

The transient rise in I_{sc} could be eliminated by pretreatment with ouabain. This might reflect changes in K^+ permeability due to ouabain or it might reflect inhibition of a pump-mediated process. During the first several minutes, ouabain has no effect on basolateral membrane resistance in isolated epithelia of frog skin bathed with normal Ringer solution [19]. This argues against the possibility that secondary effects due to ouabain have reduced K^+ permeability and therefore eliminated a K^+-free effect on K^+ flux in the K^+ channel.

Estimates of changes in sodium flux from tracer flux

It is generally assumed, and has indeed been generally measured, that short-circuit current in frog skin in equal to net sodium transport across the apical and basolateral membrane in the steady state [17,19,21,22,24,28]. In this study, the overall averages of I_{sc} and $J_{13}^{Na^*}$ under control conditions were 26.0 ± 3.7 and 26.8 ± 3.5 $\mu A/cm^2$ ($N = 13$). The close agreement between these figures suggests that transepithelial sodium flux through nonconductive routes at the apical membrane and paracellular pathways is small.

It has also been implicitly assumed in the interpretation of the sodium flux across the basolateral membrane that the specific activity of sodium in the cells was equal to that in the apical loading solution. With no sodium recycling across the basolateral membrane in the steady state this would have to be true. However, it may be that there is a small amount of Na^+ recycling such that the specific activity of Na^+ inside the cells would be less than that in the loading solution [19,22]. More sodium would move through the pump but at a lower specific activity than that entering the cells from the apical solution. Then, at the steady state, I_{sc} would still equal $J_{13}^{Na^*}$ (net Na^+ flux across the basolateral membrane). Recent studies by Stoddard and Helman [22] have shown that there is a small but significant Na^+ permeability at the basolateral membrane of frog skin. Specific activity inside the cells would be expected to be about 70–80% of the specific activity of the loading solution. Calculations from their data suggest that there may be an additional 2–5 μA of Na^+ extruded by the pump in addition to that represented by I_{sc}. When I_{sc} is in the range of 25 μA as observed in this study, I_{sc} may underestimate by 10–20% the actual Na^+ extruded by the pump in the steady state.

During rapid changes in I_{sc} tracer extrusion across the basolateral membrane may not accurately reflect net Na^+ flux due to the inevitable lag of tracer equilibration behind changes in transport rate. However, an increase or a decrease in Na^+ efflux from the cell will be reflected as an increase or a decrease in tracer flux if there are no large immediate changes in intracellular specific activity. When the intracellular specific activity of tracer reaches a new steady state reflecting the balance of tracer and unlabeled Na^+ entry on the apical side and unlabeled Na^+ entry on the basolateral side, $J_{13}^{Na^*}$ will again reflect net Na^+ flux. However, during the time prior to any significant changes in intracellular specific activity qualitative conclusions can be drawn as to whether Na^+ flux has increased or decreased.

Estimates of changes in potassium flux from tracer flux

Potassium tracer fluxes must also be viewed with caution. Without a precise knowledge of intracellular specific activity we cannot quantitate K^+ efflux from $^{42}K^+$ measurements alone. However, previous studies on frog skin have suggested the K^+ current is about one half of the I_{sc} [11,20], allowing at least a gross estimate of K^+ flux in any given tissue normalizing in this way. It is unlikely that the specific activity of K^+ in the cells will change very much when they are bathed with a K^+-free Ringer. Therefore, we are relatively safe drawing qualitative conclusions from the $^{42}K^+$ data for immediate changes in flux.

Relationship of I_{sc} to Na^+ and K^+ fluxes and the Na^+ pump

In attempting to explain the nature of the transient in I_{sc} after K^+ removal, it is important to note that this transient was essentially eliminated by pretreatment with ouabain. Since ouabain blocks the major portion of both Na^+ and K^+ flux across the basolateral membrane, my attention has focused on these ions. Therefore, I have attempted to explain the transient taking into account factors which may influence ouabain sensitive Na^+ and K^+ fluxes.

Role of unstirred layers. After K^+-free treatment, there may be a significant amount of K^+ left in an unstirred layer; it may be that only small amounts of K^+ are required to maintain pump activity. Measurements of the nominally K^+-free solution coming from the basolateral chamber showed that a maximum of 0.1 mmol/l K^+ was still present. K^+ trapped in an unstirred layer would be expected to decrease in K^+-free conditions due to the large decrease in K^+ in the bathing solution and the small decrease in K^+ flux from the cells (Figs. 1a, 4a, 4b). Assuming an unstirred layer of 50–200 μm [17], a diffusion

constant for potassium of 10^{-5} cm^2/s, and the measured K^+ concentration in the K^+-free Ringer of 0.1 mmol/l, it can be calculated from the Fick diffusion equation that a concentration of 0.15–0.30 mmol/l K^+ is needed at the membrane to support a K^+ flux of 10 μA through the unstirred layer. This represents a 10–15-fold reduction in K^+ concentration at the membrane from that expected when normal Ringer is used. Other studies have estimated that the K_m for a K^+-dependent pump process is in the range of 3 mmol/l in epithelia other than frog skin [4,29], therefore a large decrease in Na^+ flux was expected when the tissue was bathed in K^+-free Ringer.

Comparison to ouabain effects. Potassium removal does not appear to have a similar effect to that of ouabain. While ouabain causes an immediate decrease in I_{sc} and Na^+ flux [19,20,24], K^+ removal caused a transient increase in I_{sc} and no change or a 10% decrease in J_{13}^{Na*} that remained at relatively constant levels for at least 20 minutes. This flux was ouabain sensitive. When K^+ was restored to the basolateral solution there was a rather large increase in J_{13}^{Na*} suggesting that K^+-free conditions had indeed slowed or at least altered the function of the pump. This observation is consistent with the long term observation of others that Na^+ increases inside the cells under these conditions [5,16].

Activation of other flux pathways. Furosemide is known to inhibit chloride-coupled Na^+ and K^+ cotransport pathways in frog skin [11,19,20,22,27, 30]. In the present study, it caused a significant inhibition of both Na^+ and K^+ fluxes after K^+-free treatment. This may represent inhibition of 'induced' neutral cotransporters previously observed after ouabain treatment [11,19,20]. An increase in Na^+ concentration inside the cells might serve to turn on this mechanism. There was also a large increase in the chemical gradient for K^+. This would be expected to increase K^+ flux through neutral mechanisms; it might even increase Na^+ flux if Na^+ is coupled to K^+. There are still additional routes of exit indicated by the significant rate of efflux observed even in the presence of ouabain and furosemide.

If the Na^+ pump is involved in volume regulation and K^+-free treatment reduces or alters the activity of the pump, there would be changes in cell volume. These changes might trigger compensating ion transport mechanisms in the cell's attempt to maintain constant volume [30]. Ussing [9], studying the frog skin, has shown that there are no changes in cell volume during the first 30 min of K^+-free treatment. The current transient occurs well within this time frame. Therefore, it is not likely that new volume regulatory fluxes are involved in the immediate response of the cells to K^+-free Ringer.

While there were no significant changes in volume even after long periods in K^+-free sulfate Ringer, there were small decreases in volume after 30 min in chloride Ringer. It was suggested that this might represent loss of KCl from the cells [9]. The furosemide sensitivity of the K^+ flux under K^+-free conditions observed in the present study is consistent with this suggestion.

Role of basolateral membrane voltage. When K^+ is removed from the basolateral solution, membrane voltage is hyperpolarized by about 20 mV [15]. This would clearly have effects on cation movement through channels and may have effects on ion movement through carriers. We have studied the effects of voltage on Na^+ flux in frog skin [19,31]. When membrane voltage is either hyperpolarized or depolarized there is very little change in Na^+ efflux from the cells in the first few minutes. (There are long term effects which may reflect changes in the Na^+ transport pool.) This suggests that the pump may act as a constant current source over this voltage range. We have also examined K^+ effluxes [20]. The flux was clearly voltage dependent as shown when the basolateral membrane voltage was depolarized by ouabain. This can also be seen in the present study (Fig. 4). Since K^+-free Ringer hyperpolarizes the membrane this will tend to decrease K^+ efflux. This is in the opposite direction than that needed to account for the transient increase in I_{sc}.

Role of the Na^+ pump. During the transient rise in I_{sc} there were no significant changes in Na^+ and K^+ fluxes. It does not seem likely that anion fluxes play a major role in the transient rise in I_{sc} considering the recent observation that chloride crosses the basolateral membrane almost exclusively via neutral mechanisms [20,26,27]. Ouabain elimination of the transient implies a role for the

sodium pump in this response. Since K^+ flux measurements suggest there was no change in K^+ current and there is no evidence for an important, ouabain-sensitive, third conductive pathway, a possible alternative is to have an increase in pump current. There are several ways for pump current to increase. If the Na^+/K^+ exchange ratio is fixed, pump current could be increased by increasing the turnover rate of each pump or by increasing the number of pumps. An increase in a furosemide sensitive Na^+ influx across the basolateral membrane does not appear to be responsible for increasing pump turnover (See Results). Both an increased turnover or increased number of pumps would necessarily result in an increase in Na^+ efflux. This was not observed. Another way for pump current to increase under these conditions would be to change the Na^+/K^+ coupling ratio. If fewer K^+ ions are taken up per turn of the pump, net charge transfer per cycle could be greatly increased with little or no change in Na^+ extrusion. This idea is supported by the fact that Na^+ flux did not increase and that there was a rather large inhibition by ouabain of I_{sc} after K^+-free Ringer treatment. The ouabain-inhibitable portion of I_{sc} represents a minimal estimate of pump current. Ouabain inhibited I_{sc} to 45% of control after K^+-free treatment, more than twice the inhibition observed under conditions of normal K^+ concentration [19]. This suggests that the pump current represents a larger fraction of I_{sc} than it does under control conditions. A similar, large ouabain-sensitive current was also observed under K^+ free conditions in toad urinary bladder [6].

The idea that pump stoichiometry is variable or even that Na^+ may be extruded without exchange for K^+ is not without precedent. Recent studies have shown that pump stoichiometry may vary in epithelia under various conditions [11,20,37–39]. Several modes of the pump have been demonstrated in red cells under conditions quite different from those used here [32]. In vesicles, Forgac and Chin [33] have demonstrated ATP-dependent, K^+-independent extrusion of Na^+ against a Na^+ gradient. The efficiency of this process was significantly reduced from the Na^+/K^+ exchange mode. Only 0.5 mole of Na^+ were transported per mole of ATP as opposed to 3 moles of Na^+ in the exchange mode. Blostein [34] has also demonstrated ATP-dependent electrogenic Na^+/Na^+ exchange. She suggested that Na^+ may be able to act like K^+ on the K^+ site. Cornelius and Skou [35] have demonstrated K^+-independent, ouabain-sensitive electrogenic Na^+ extrusion by reconstituted Na^+/K^+-ATPase. Borlinghaus et al. [36] have shown that the Na^+/K^+-ATPase is capable of electrogenic transfer of Na^+ in the absence of K^+. They also concluded that completion of the cycle is slow without K^+. In the present study if some of the pumps were operating in this mode this could account for the apparent increase in coupling ratio and for the eventual decline in transport rate.

Physiological implications

Contrary to what might have been expected for a Na^+/K^+ exchange pump, removal of K^+ from the basolateral solution did not cause an immediate decrease in I_{sc} or Na^+ flux. However, as indicated by longer term studies in frog skin and other tissues [3,5,6,15,16] and the persistent decline in transport with time in the present studies, it seems apparent that the Na^+ transporting mechanisms in epithelia cannot maintain long term high rates of Na^+ transport in the absence of basolateral K^+. In the short term it appears that the transport mechanisms are able to compensate for rather large changes in K^+ concentration while still maintaining Na^+ transport at relatively high levels. K^+ appears to be conserved by the cell as a result of voltage changes across the basolateral membrane and by some recycling of K^+ by the pump. While we cannot eliminate all other possible explanations, our data suggest that the pump coupling ratio is increased under K^+-free conditions.

Acknowledgements

I am grateful for the excellent technical assistance of Rebekah E. Woods. I thank Hoechst-Roussel Pharmaceuticals for the generous gift of furosemide. This research was supported by a grant in aid from the American Heart Association, Illinois Affiliate.

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