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Potassium Transport in the Rabbit Renal Proximal Tubule: Effects of Barium, Ouabain, Valinomycin, and Other Ionophores

Stephen P. Soltoff* and Lazaro J. Mandel
Department of Physiology, Duke University, Durham, North Carolina 27710

Summary. Potassium fluxes in a suspension of rabbit proximal tubules were monitored using a potassium-sensitive extracellular electrode. Ouabain (10^{-4} M) and barium (5 mM) were used to selectively quantitate the potassium efflux pathway (105 ± 5 nmol K^+ · mg protein^{-1} · min^{-1}) and the sodium pump-related potassium influx (108 ± 7), respectively. These equal and opposite fluxes suggest that potassium accumulation in the cell occurs mainly through the sodium pump and that potassium efflux occurs mainly through barium-sensitive potassium channels. Thus the activity of the sodium pump (Na,K-ATPase) in the basolateral membrane of the proximal tubule is balanced by the efflux of potassium, presumably across the basolateral membrane, which has a high potassium permeability. In addition, the effect of valinomycin and other ionophores was examined on potassium fluxes and several metabolic parameters [oxygen consumption (QO_2), ATP content]. The addition of valinomycin to the tubules produced a net efflux of potassium which was quantitatively equivalent to the efflux produced by the addition of ouabain. The valinomycin-induced efflux was mainly due to the activity of valinomycin as a mitochondrial uncoupler, which indirectly inhibited the sodium pump by allowing a rapid reduction of the intracellular ATP. Amphotericin, nystatin, and monensin all produced large net releases of intracellular potassium. The action of the ionophores could be localized to the plasma or mitochondrial membrane and classified into three groups, as follows: (a) those which demonstrated full mitochondrial uncoupler activity (FCCP, valinomycin), (b) those which had no uncoupler activity (amphotericin B, nystatin); and (c) those which displayed partial uncoupler activity (monensin, nigericin).

Key Words proximal tubule · potassium flux · ionophore · ouabain · barium · ATP · QO_2

Introduction

The basolateral membrane of the rabbit proximal tubule contains the Na,K-ATPase (sodium pump), which maintains the intracellular potassium concentration above electrochemical equilibrium. This membrane is also characterized by a high permeability to potassium, whereas the luminal membrane appears to be largely potassium impermeable in this tissue (Biagi et al., 1981). Under steady-state conditions the intracellular potassium concentration remains at a constant level because the uptake of potassium by the sodium pump is equivalent to the efflux of potassium through the passive leak pathway(s) (pump = leak) (Tosteson & Hoffman, 1960). In a previous study (Soltoff & Mandel, 1984*a*) we reported that the ouabain-sensitive uptake of ^{42}K by a suspension of rabbit proximal tubules demonstrated single exponential kinetics at 37 and 25°C and thus, extracellular potassium appeared to communicate with a single intracellular compartment. In the present communication we report on further studies of potassium fluxes in the rabbit proximal tubule, and include separate measurements of the leak pathway as well as sodium pump-mediated transport. Net fluxes were monitored using a potassium-sensitive electrode by measuring the appearance or disappearance of potassium from the extracellular medium (*see* Materials and Methods). Ouabain, an inhibitor of the sodium pump, and barium, which has been demonstrated to decrease the basolateral membrane potassium conductance in various epithelia (Nagel, 1979; Biagi et al., 1981; Moffett & Koch, 1985), were used to selectively quantitate the leak and pump fluxes, respectively. In addition, we examined the effects of valinomycin, a potassium-selective ionophore, on potassium fluxes in this tissue, and also report on the effects of other ionophores on several transport and metabolic parameters of the proximal tubule.

Valinomycin is a cyclic peptide that has been used to investigate the involvement of potassium gradients, membrane potentials, and the K permeability across the membranes of cells and organelles (*see* Pressman, 1976). Its effects on biological mem-

* *Present address:* Dept. of Physiology, Tufts University School of Medicine, 136 Harrison Ave., Boston, Massachusetts 02111.

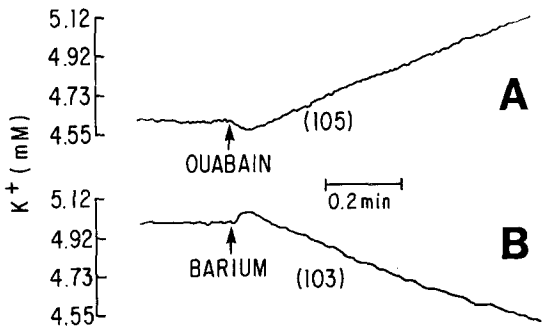

Fig. 1. Typical effect of 10^{-4} M ouabain (trace *A*) or 5 mM barium (trace *B*) on the net potassium flux of rabbit proximal tubules in suspension. Numbers in parentheses (nmol K^+ · mg protein^{-1} · min^{-1}) refer to the rate of potassium release or uptake from the extracellular medium

branes were originally studied using isolated mitochondria, in which it was found to promote potassium uptake and to uncouple oxidative phosphorylation from respiration (McMurray & Begg, 1959). Thus, one focus of our studies was to attempt to separate the ionophoric properties of valinomycin at the plasma membrane from its potential action as a mictochondrial uncoupler. The other ionophores that were examined in this study have varying cation selectivities (*see* Reed, 1979). In total, three different classes of antibiotic ionophores were examined: polyene antibiotics (nystatin, amphotericin B), carboxylic ionophores (monensin, nigericin), and neutral ionophores (valinomycin). By measuring the effect of the ionophores on the rate of oxygen consumption, ATP content, and potassium flux of the proximal tubule, we were able to distinguish whether the actions of the ionophores could be localized to the plasma membrane, the mitochondrial membrane, or a combination of both.

Materials and Methods

Tubule Preparation

The protocol for obtaining proximal tubules has been presented elsewhere (Soltoff & Mandel, 1984*a*). In brief, tubules with open lumens were obtained from New Zealand White rabbits by perfusing the kidneys *in situ* with a slightly hypertonic solution containing collagenase (Worthington Biochemical). Cellular debris and nonpatent tubules were separated by centrifugation on a layer of Ficoll (40,000 mol wt) (Pharmacia). After several washes, the final preparation of tubules was suspended at a concentration of 4 to 6 mg protein/ml in a solution containing the following composition (mM): 115 NaCl, 5 KCl, 1 $MgSO_4$, 2 NaH_2PO_4, 25 $NaHCO_3$, 1 $CaCl_2$, 5 glucose, 4 Na lactate, 1 alanine, 1 butyrate, and 0.6% dextran (T40, Pharmacia), pH 7.4.

Potassium Fluxes and Oxygen Consumption Measurements

All experiments were performed at 37°C. The tubules were incubated at this temperature and equilibrated with a 95% O_2/5% CO_2 gas mixture for 15 to 20 min prior to all measurements. The net release and uptake of potassium by the tubules was monitored using a potassium-sensitive extracellular electrode (Orion, Cambridge, MA). An increase or decrease in extracellular potassium was interpreted as net cellular K efflux or influx, respectively. The oxygen consumption (QO_2) of the tubule suspension was monitored polarographically by the disappearance of oxygen from a closed chamber using a Clark-type oxygen electrode. After the addition of ionophores to the tubule suspension, the initial linear stimulation of oxygen consumption observed during the first minute of exposure was used to calculate the rate of oxygen consumption. The initial potassium efflux rates were calculated for a period of about 0.3 min commencing after about a 0.1-min delay due to transient noise created by the addition of the ionophore. Aside from FCCP, the ionophores did not alter the response of the potassium electrode except to cause an initial minor positive offset voltage, after which (in the absence of tubules) a constant voltage (potassium concentration) was measured. However, in the absence of tubules, the addition of FCCP produced a large steadily decreasing voltage, which artifactually represented a decrease in the potassium concentration of the solution. In the presence of tubules, FCCP produced an apparent loss in the tubule potassium content, but since the change was of a similar magnitude but opposite direction to that measured in the absence of tubules, reliable quantitation could not be performed.

ATP Content

In one series of experiments the effects of several ionophores on the ATP content of the tubules and the rate of oxygen consumption were determined simultaneously. In these experiments, tubules incubated as one batch were apportioned into two 1.6-ml thermostated chambers which each contained an oxygen electrode. Ouabain was added 1 min prior to ionophore addition. The ionophore was added to one chamber, and the dissolving vehicle (dimethylsulfoxide or ethanol) was added to the other. A 0.5-ml sample from each chamber was subsequently taken 2 min after the ionophore addition. The samples were added to equal volumes of ice-cold 6% perchloric acid/1 mM EDTA. The ATP content of the neutralized deproteinated extract was determined using a hexokinase-linked fluorometric assay (Lowry & Passoneau, 1972). In a separate series of experiments, the time course of the effect of several ionophores on the ATP content was monitored. In these studies, a special thermostated chamber which permitted rapid sampling was used (Balaban et al., 1980). These samples were treated as described above.

Statistics

All measurements were normalized to protein content. Protein was determined by the biuret procedure (Gornall et al., 1949) using bovine serum albumin as the standard.

All data are presented as the mean ± standard error (*n*, number of samples).

Chemicals

Nigericin (Calbiochem), FCCP (Sigma), valinomycin (Sigma), and monensin (Calbiochem) were dissolved in ethanol. Nystatin (Calbiochem) and amphotericin B (Sigma) were dissolved in dimethylsulfoxide (Fisher). Ouabain (Sigma) was dissolved in distilled water. All other chemicals were of reagent grade.

Results

Potassium Flux Through the Pump and Leak Pathways

The potassium flux through the leak pathway was measured by adding ouabain (10^{-4} M) to the proximal tubule suspension and measuring the initial rate of potassium efflux from the tubules (Fig. 1, curve *A*). This allowed the rate of potassium efflux to be measured without the contribution of the equal but opposite potassium influx mediated by the sodium pump. The potassium efflux rate measured in this manner was 105 ± 5 (n = 11) nmol K/mg protein · min. To monitor the sodium pump-mediated potassium uptake, barium was employed. Barium has been shown to block the potassium leak pathway in a variety of epithelial tissue, including frog skin (Nagel, 1979), insect midgut (Moffett & Koch, 1985), turtle (Kirk & Dawson, 1983) and rabbit (Wills, Zeiske & Van Driessche, 1982) colon, and the rabbit proximal tubule (Biagi et al., 1981). The addition of 5 mM $BaCl_2$ to the tubule suspension promoted the net influx of potassium (Fig. 1, curve *B*). The initial rate of potassium uptake, calculated from the decrease in extracellular potassium during the first 30 sec of barium exposure, was 98 ± 2 (n = 6) nmol K/mg protein · min, not significantly different from the measured potassium efflux. Thus, the equal and opposite contributions of the sodium pump and the potassium leak pathway of the proximal tubule maintain potassium homeostasis within the cell. All these measurements of initial flux rate were made rapidly (within 30 sec), to minimize changes in basolateral membrane potential that occur in a time scale of minutes when ouabain is applied (Biagi et al., 1981; Planelles et al., 1981).

Valinomycin

The effects of valinomycin on tubular potassium fluxes and QO_2 are shown in Fig. 2. Prior to the addition of valinomycin, the tubules respired at their normal rate (about 30 nmol O_2/mg protein · min) and the extracellular potassium concentration was stable since the efflux and influx of potassium

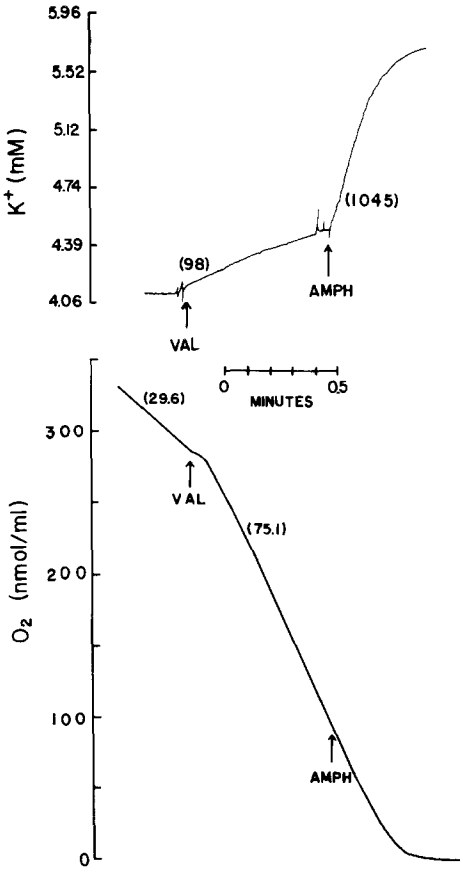

Fig. 2. Effect of valinomycin (7.2 μM) and amphotericin B (51.1 μM) on the rate of oxygen consumption (QO_2) and net potassium flux in a suspension of rabbit proximal tubules. The QO_2 (nmol O_2 · mg protein^{-1} · min^{-1}) and the potassium flux (nmol K^+ · mg protein^{-1} · min^{-1} were measured simultaneously

across the plasma membrane were equal to each other. The intracellular potassium concentration in these tubules is about 125 mM (Soltoff & Mandel, 1984*a*), and thus there is normally a substantial chemical potassium gradient across the plasma membrane. Upon the addition of 6 μM valinomycin, the QO_2 increased about 150% above the normal rate, and a net potassium efflux (increase in extracellular potassium concentration) of 98 nmol K/mg protein · min was observed. The stimulation of QO_2 by valinomycin was not significantly different between about 0.3 and 7 μM (*not shown*).

The net potassium efflux induced by valinomycin could be caused by an increase in the plasma membrane permeability to potassium. However, if this were the case, the increase was not maximal. As shown in Fig. 2, the addition of amphotericin B to the tubules promoted about a 10-fold larger increase in the potassium release rate than did the

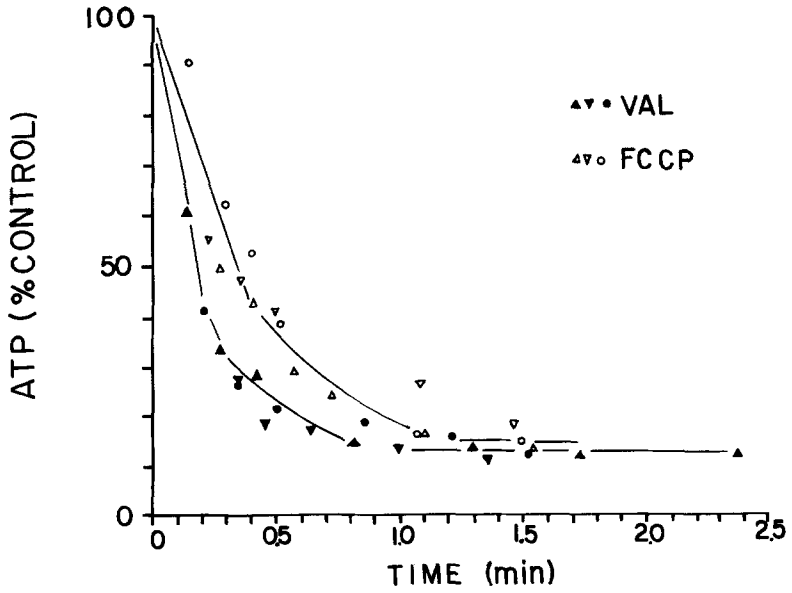

Fig. 3. Effect of valinomycin (6.1 μM) and FCCP (3.3 μM) on the ATP content of the rabbit proximal tubule in suspension. The different symbols refer to individual trials performed using batches of tubules taken from the same preparation

Table 1. Effect of valinomycin (6 μM) and ouabain (10^{-4} M), added singly or simultaneously, on the net potassium flux of rabbit proximal tubule. Both agents promoted a net efflux (release)

	K^+ release rate (nmol K^+ · mg protein^{-1} · min^{-1})
A. Valinomycin	108 ± 7 (11)
Ouabain	105 ± 5 (11)
B. Valinomycin	115 ± 9 (7)
Ouabain	111 ± 6 (6)
Ouabain + valinomycin	123 ± 7 (6)

prior addition of valinomycin, and the QO_2 was not further increased. On the other hand, the valinomycin-induced net potassium efflux could be caused by metabolic inhibition of the sodium pump since valinomycin, which is known to be a mitochondrial uncoupler, might diminish the production of ATP. This would reduce the rate of potassium uptake through the sodium pump and thereby allow the efflux of potassium through the leak pathway to be expressed. In addition, patch-clamp studies have demonstrated that some cells have a potassium channel that is blocked by intracellular ATP (Cook & Hales, 1984). In order to distinguish among these possibilities, a series of experiments was performed to compare the potassium efflux induced by valinomycin to that observed when the sodium pump was inhibited by ouabain.

The results of these experiments are listed in Table 1. In the first series, the net potassium efflux rates produced by valinomycin and ouabain were compared. The initial efflux rates promoted by valinomycin (108 ± 7 nmol K/mg protein · min) and ouabain (105 ± 5) were indistinguishable. Furthermore, the next set of experiments showed that their effects were not additive. The combined exposure of valinomycin and ouabain promoted a net potassium release of 123 ± 7 nmol K/mg protein · min, while valinomycin and ouabain added separately to the tubule suspension produced efflux rates of 115 ± 9 and 111 ± 6, respectively. A test for significance indicated that only the pair of ouabain *vs.* ouabain + valinomycin was significantly different ($P < 0.02$). These results strongly suggest that the major effect of valinomycin on potassium efflux occurs via the release of intracellular potassium by inhibiting the sodium pump. The additional efflux induced by valinomycin in the presence of ouabain could reflect the ionophoric effect of valinomycin on the plasma membrane, as discussed later.

In order to evaluate the metabolic effects of valinomycin, its effect on the ATP content of the tubules was compared to that of FCCP, a potent uncoupler of mitochondrial oxidative phosphorylation. The results are shown in Fig. 3. FCCP and valinomycin both promoted the rapid depletion of the ATP content to about 10% of the initial level. The effect of valinomycin ($t_{1/2}$ = 0.17 min, n = 3) was faster than that of FCCP ($t_{1/2}$ = 0.32 min). These results suggest that ATP synthesis by the mitochondria is rapidly inhibited by these agents, and that the sodium pump activity is rapidly reduced as the remaining ATP is consumed by the sodium pump and other exergonic cellular processes. Unfortunately, it was not possible to directly compare the effects of FCCP and valinomycin on the rate of potassium efflux. FCCP, as well as 1799, another

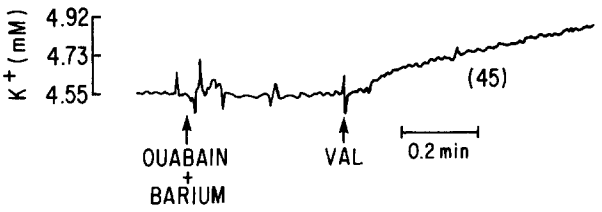

Fig. 4. Effect of valinomycin (6 μM) added to the tubule suspension after the prior simultaneous addition of barium (5 mM) and ouabain (10^{-4} M). Number in parentheses is the rate of potassium efflux (nmol K^+ · mg protein^{-1} · min^{-1})

mitochondrial uncoupler, produced large artifacts in the response of the potassium-sensitive electrode (*not shown*).

Although the action of valinomycin on potassium efflux appeared to be due to its effectiveness as a mitochondrial uncoupler, additional experiments were performed in an attempt to detect a valinomycin-induced increase in the potassium permeability of the plasma membrane. These experiments utilized the contrasting effects of barium and ouabain on the potassium fluxes of the proximal tubule, which were described above (Fig. 1). The simultaneous addition of ouabain (10^{-4} M) and barium (5 mM) blocked both the pump-related potassium influx as well as the leak-related potassium efflux, and the extracellular potassium concentration remained stable, indicating the absence of any net fluxes (Fig. 4). Under these conditions, the addition of valinomycin produced a much smaller efflux (40 ± 5 nmol K/mg protein · min, n = 13) compared to valinomycin (111 ± 10, n = 10) in the absence of other agents. Presumably, this value represents the ionophoric action of valinomycin, that is, the net potassium efflux due to an increase in the potassium permeability of the plasma membrane, but could also be due to a different mechanism, such as the activation of a voltage-dependent potassium channel, or an ATP-sensitive potassium channel.

Other Ionophores

Several other types of ionophores were compared to valinomycin and FCCP in terms of their effect on the QO_2 and ATP content of the tubules.

QO_2

As shown in Table 2, all the ionophores tested increased the QO_2. However, their respective mechanisms of action varied and were distinguished by their localization to the mitochondrial membrane and/or the plasma membrane. At one extreme is the mitochondrial uncoupling action exemplified by FCCP, which produces a large increase in QO_2 that is unaffected by ouabain. At the other extreme is the alteration of the ionic permeability of the plasma membrane, and is exemplified by nystatin, which increases the QO_2 by stimulating the Na,K-ATPase activity through increased entry of sodium into the tubules (Harris et al., 1981). The stimulation of QO_2 elicited by nystatin is completely abolished by ouabain, demonstrating that this ionophore exerts no uncoupling effect on the mitochondria. Amphotericin B appears to be identical to nystatin in its action, which is to be expected from the preference of polyene antibiotics for cholesterol-containing membranes (Cass et al., 1970) such as the plasma membrane. The other three ionophores displayed a combination of the mitochondrial uncoupling and the plasma membrane ionophoric effects, since QO_2 was increased in the presence of ouabain but not as much as in the absence of ouabain.

It was possible that the QO_2 responses to several of the ionophores in the presence of ouabain were affected by alterations in the intracellular ionic milieu that were produced by inhibition of the sodium pump. In the results shown in the second column of Table 2, the ionophores were added 1 min after ouabain. This interval is a sufficient time to considerably alter the sodium and potassium contents of the tubules (Soltoff & Mandel, 1984*b*). Therefore, the effects on QO_2 were also examined

Table 2. Effect of several ionophores on QO_2 in the presence and absence of ouabain (10^{-4} M)[a]

	QO_2 (% control)		
	Ionophore	Ouabain	Ionophore + ouabain
FCCP (3.3 μM)	275 ± 8 (6)	260 ± 6 (6)	270 ± 7 (6)
Valinomycin (6 μM)	205 ± 5 (8)	177 ± 6 (6)	204 ± 9 (4)
Monensin (0.6 μM)	225 ± 6 (8)	180 ± 4 (8)	184 ± 9 (8)
Nigericin (41 μM)	241 ± 6 (10)	127 ± 7 (6)	133 ± 7 (7)
Amphotericin B (43 μM)	178 (2)	42 (2)	
Nystatin (0.43 μM)	185 ± 9 (4)	39 ± 1 (8)	
Control	100	40 ± 1 (11)	

[a] The ionophores were added alone (ionophore), 1 min after ouabain (ouabain), or simultaneously with ouabain (ionophore + ouabain). The QO_2 response was calculated in terms of the QO_2 (27.7 ± 0.2 nmol O_2 · mg protein^{-1} · min^{-1}, n = 110) that was measured prior to any addition.

Table 3. Effect of several ionophores on the ATP content and QO_2 of rabbit proximal tubules in the presence of 10^{-4} M ouabain[a]

	ATP (% control)	QO_2 (% control)
FCCP	13 ± 1 (7)	261 ± 13 (7)
Valinomycin	13 ± 2 (7)	201 ± 15 (7)
Monensin	93 ± 4 (7)	171 ± 2 (7)
Nigericin	71 ± 2 (7)	139 ± 6 (7)
Nystatin	87 ± 4 (8)	45 ± 2 (6)

[a] The data are normalized to paired samples to which the dissolving vehicle (DMSO or ethanol) was added. The ionophore concentrations are identical to those found in Table 2. The control (no ionophore) values were 7.8 ± 0.1 nmol ATP · mg protein^{-1} (n = 36) and 27.9 ± 0.3 nmol O_2 · mg protein^{-1} · min^{-1} (n = 20).

under sodium pump-inhibited conditions in which the intracellular ionic conditions were not greatly altered. To accomplish this, the ionophores were added to the tubule preparation simultaneously with ouabain (Table 2, column 3). With the exception of valinomycin, the effects on QO_2 were the same as when ouabain was added 1 min prior to the ionophores. For valinomycin, the stimulation (104%) produced by this procedure was identical to that produced in the absence of ouabain (105%). However, valinomycin increased QO_2 significantly less (77%) when the ionophore was added in the presence of ouabain after a 1-min delay. It is most likely that the lower response obtained when ouabain was added first was due to the diminished extramitochondrial (cytosolic) potassium concentration. Since valinomycin promotes a coupled potassium uptake—H^+ efflux in isolated mitochondria (Pressman, 1965)—lowering the extramitochondrial/intramitochondrial potassium gradient would diminish the uncoupler activity. In agreement with this, it was observed that the valinomycin-stimulated QO_2 (62.4 ± 27 nmol O_2/mg protein · min, n = 3) obtained when tubules were suspended in about 5 mM extracellular potassium was more than twice the QO_2 (26.8 nmol O_2/mg protein · min, n = 2) obtained when valinomycin was added to tubules suspended in a potassium-free solution. The latter condition probably reduces the cytosolic potassium concentration to a greater degree than the mitochondrial potassium (Soltoff & Mandel, 1984*b*).

ATP

The effect of these ionophores on the ATP content of the proximal tubules was examined to more fully identify the potential uncoupler activity. Ouabain (10^{-4} M) was added 1 min prior to the ionophores, and ATP samples were collected 2 min after the ionophores were added. The rates of oxygen consumption of the tubule suspensions from which the ATP samples were taken were also measured. The results are listed in Table 3. Valinomycin and FCCP produced the largest decreases in ATP, causing a 90% reduction in the ATP content. There results were similar to those at comparable times shown in Fig. 3. Nystatin and monensin produced the smallest changes, reducing the ATP content by only 15 and 18%, respectively. Nigericin produced a 28% reduction.

Potassium Fluxes

In addition to the net potassium efflux promoted by valinomycin, the addition of amphotericin, nystatin, and monensin also produced large net releases of potassium from the proximal tubule suspension. The release rates were concentration-dependent, and at the concentrations used in this study (about 10, 100, and 100 μg/mg protein, respectively) the rates of net potassium efflux were about 10 to 15 times larger than the rates produced by valinomycin.

Discussion

In this paper, we describe the effects of several commonly used ionophores on the rabbit proximal tubule. Ionophores have been successfully employed by numerous investigators to probe ion transport functions of membranes as diverse as lipid bilayers (Cass et al., 1970), mitochondrial membranes (Pressman, 1976), and plasma membranes (Mendoza et al., 1980). We and others have previously utilized nystatin (Harris et al., 1981; Soltoff & Mandel, 1984*a*,*b*) and amphotericin B (Spring & Giebisch, 1977), polyene antibiotics which bind selectively to sterol-containing membranes, to increase the permeability of the plasma membrane to sodium and to thereby stimulate sodium pump activity in the kidney. Other ionophores such as monensin (Mendoza et al., 1980), valinomycin (Smith & Robinson, 1981), and nigericin (Nakhoul & Boron, 1985) have also been used to increase the plasma membrane permeability to cations. However, these ionophores may have multiple effects on intact cells due to their concomitant action on membranes other than the plasma membrane, most notably the mitochondria and other organelles. With this in mind, we studied the effects of valinomycin and various ionophores on several metabolic and ion transport parameters of the rabbit proximal tubule. The data support the close functional relationship between the mitochondria and the sodium pump

(Na,K-ATPase), and evaluate the interaction between the pump flux and the leak flux in this renal preparation. Several classes of ionophores were examined, and the relative degree to which they uncouple the mitochondria was evaluated.

K Fluxes

Under steady-state conditions the rate of potassium influx is equivalent to the rate of potassium efflux. The flux measurements obtained in this study by the single addition of barium or ouabain (Fig. 1) were 98 ± 2 nmol K/mg protein · min for the pump flux and 105 ± 5 for the leak flux. These rates can be compared to others previously obtained using this preparation. The ouabain-sensitive rate of ^{42}K uptake was 89 ± 4 nmol K/mg protein · min under steady-state conditions at 37°C (Soltoff & Mandel, 1984*a*), a value close to that measured by the potassium-sensitive electrode method reported here.

The addition of valinomycin to the proximal tubule suspension produced a net potassium efflux of about 110 nmol K/mg protein · min (Table 1). Because this rate was nearly identical to that produced by ouabain, and since the effects of valinomycin and ouabain were not additive, the fluxes produced by valinomycin and ouabain appeared to be produced by the same mechanism—inhibition of the sodium pump activity. The addition of valinomycin to the suspension of tubules resulted in a rapid and severe decrease in the ATP content (Fig. 3). Thus, due to its metabolic effect as an uncoupler of mitochondrial oxidative phosphorylation, valinomycin appeared to inhibit the sodium pump to the same extent as ouabain (but *see below*). Valinomycin has also been observed to produce reductions in the ATP content of other tissues, including hepatocytes (Kristensen, 1980), lymphocytes (Arslan et al., 1981), Ehrlich ascites cells (Levinson, 1967), and fibroblasts (Yamanishi, 1984).

The inhibition of the sodium pump activity by valinomycin that we observed is in contrast to the results of Capasso et al. (1985), who found that this ionophore stimulated J_v (volume flow) in the proximal tubule of thyroidectomized rats, presumably by stimulating the activity of the sodium pump. The concentration used in the rat studies (0.9 μM) was about 15% of the concentration used in the present study, but was within the range in which we observed a maximal stimulation of the QO_2 by valinomycin (*see* Results). Differences between the two studies may be due to the sensitivity of different animal species to valinomycin.

It must be acknowledged, however, that the effect of valinomycin may not be as straightforward as the results in Table 1 would appear to indicate. The potassium fluxes measured by the extracellular potassium-sensitive electrode represent net fluxes. In the analysis of the results it was assumed that only two compartments (intracellular and extracellular) existed. However, as mentioned above, valinomycin promoted the uptake of potassium (and release of H^+) in isolated mitochondria (Pressman, 1965). Thus, the net effect of valinomycin may represent a balance between potassium influx across the mitochondrial membranes and efflux across the plasma membrane. A second consideration that must be evaluated pertains to the kinetics of ATP depletion promoted by valinomycin. During the time that the measurement of the initial rate of potassium efflux was made, between 0.1 to 0.4 min after the addition of valinomycin, the ATP content dropped from about 60 to 20% of the normal value. This should produce a concomitant relative reduction in the activity of the sodium pump (Soltoff & Mandel, 1984*c*), but not reduce it completely. Thus, the valinomycin-induced potassium efflux may actually represent the net flux of the potassium leak pathway, the (reduced) sodium pump, and the ionophoric activity on the plasma membrane. It is possible that the last two of these compensate each other to result in a net potassium efflux rate equal to that obtained in the presence of ouabain. Also, as mentioned above, it is possible that valinomycin alters voltage-dependent potassium channels. The small flux induced by valinomycin after barium and ouabain (Fig. 4), indicates that an additional potassium pathway may be stimulated. Nevertheless, it appears that the main effect of valinomycin in the proximal tubule is to uncouple the mitochondria. Its effectiveness as a plasma membrane ionophore appears to be much less than that of the polyene antibiotics. In a recent report valinomycin was found to inhibit the Ca-ATPase activity of skeletal muscle sarcoplasmic reticulum, an effect that was attributed to a direct interaction with membrane protein (Davidson & Berman, 1985). In this study we did not examine whether valinomycin directly inhibits the Na,K-ATPase activity, but the actions described above appear to account for all of its effects.

The effects of barium on the proximal tubules deserve further discussion. In two separate studies which employed intracellular potassium-sensitive microelectrodes, barium was reported to increase (Biagi et al., 1981) and to decrease (Planelles et al., 1981) the intracellular potassium activity in the proximal tubule. Both studies observed that barium decreased the potassium conductance across the membrane and caused depolarization. The decrease in potassium activity was attributed to the barium-promoted increase in the potassium driving force (the difference between V_B, the potential difference across the basolateral membrane, and E_K, the potassium equilibrium potential across the basolateral

membrane) being larger than the barium-promoted decrease in the potassium conductance of the basolateral membrane (Planelles et al., 1981). In the studies presented here, the addition of 5 mM barium to the tubule suspension promoted the net uptake of potassium, presumably due to the block of the potassium leak pathway. However, after several minutes the net uptake diminished, and the extracellular potassium concentration became steady (*not shown*). The intracellular potassium content was increased by an average of 115 ± 1 nmol K/mg protein (n = 6), an increase of about 30% above normal. Volume measurements were not made, so it is not known how this affected the intracellular potassium concentration. Biagi et al. (1981) reported that 0.5 mM barium, a concentration one-tenth of that used in the present studies, produced about a 10% increase in the activity of intracellular potassium in the rabbit proximal straight tubule. Therefore, it appears that the addition of barium to the tubule suspension promoted a new steady state with a higher potassium gradient and a lower potassium leak rate. At the concentration (5 mM) used, barium may not completely block the potassium leak pathways when the potassium gradient across the membrane becomes greater than normal, at which point potassium efflux may again equal potassium influx. After the intracellular potassium content was increased to a new steady state by barium, the addition of valinomycin produced a net potassium efflux of 79 ± 5 nmol K/mg protein · min (n = 8). This rate is larger than that produced by valinomycin in the combined presence of ouabain and barium, and may represent the ionophoric action of valinomycin in the presence of a larger potassium gradient across the plasma membrane.

Degree of Respiratory Uncoupling

With the exception of the polyene antibiotics nystatin and amphotericin, all of the ionophores examined in this study were observed to have mitochondrial uncoupling activity. Their relative degree of uncoupling can be categorized on the basis of their effects on QO_2 (Table 2). FCCP can be categorized as having 100% uncoupling activity. Relative to the other ionophores, the addition of FCCP produced the maximum stimulation of oxygen consumption. Since this stimulation was identical in the presence and absence of ouabain, the entire QO_2 was a measurement of uncoupled respiration. At the other extreme, nystatin and amphotericin can be classified as producing 0% uncoupler activity. The stimulation of QO_2 that was observed upon the addition of these antibiotics was due solely to the increase in the cation permeability of the plasma membrane and the subsequent activation of the sodium pump. The increase in QO_2 was attributed to fully coupled respiration, since no stimulation was observed when the sodium pump activity was blocked by ouabain. Monensin and nigericin demonstrated intermediate uncoupler activities. In the presence of ouabain, they stimulated the QO_2 less than in its absence, demonstrating that part of the stimulation was mediated by ionic activation of the sodium pump. Also, the uncoupled stimulation of QO_2 (ouabain present) by monensin and nigericin was less than the maximum produced by FCCP. The stimulation of QO_2 by valinomycin appeared to be due entirely to its action as a mitochondrial uncoupler, since there was no difference whether ouabain was present or absent. In addition, the rapid ATP depletion observed in the presence of valinomycin attested to its uncoupling action. The results also suggest that the selectivity of valinomycin for potassium compared to sodium is much larger than that of nigericin, which displayed partial activity as a sodium ionophore by virtue of its partial stimulation of the sodium pump. Amphotericin and nystatin are known to have little selectivity between sodium and potassium, thus accounting for the simultaneous increase in intracellular sodium (*not shown*) and efflux of intracellular potassium (Fig. 2) produced by these ionophores.

ATP Effects

The decrease in the ATP contents obtained after FCCP or valinomycin addition (Fig. 3) is typical of those expected from mitochondrial uncouplers. However, the results obtained with the other ionophores are a little surprising because there is no apparent correlation between the degree of uncoupling and the alteration of the ATP content. For instance, monensin produces almost as much apparent uncoupling activity (as judged by the QO_2 in the presence of ouabain) as valinomycin; however, its effect on ATP resembles that of nystatin rather than valinomycin (Table 3). Also, nystatin did not display any apparent uncoupling activity as judged by the QO_2 response, but a slight reduction in the ATP content was observed.

In the cases of monensin and nigericin, these results may reflect the highly nonlinear relationship between the electrical potential across the mitochondrial inner membrane and the mitochondrial QO_2 (Nicholls, 1979). This potential reflects the ability of the mitochondria to produce ATP. Varying degrees of uncoupling may produce similar QO_2's while affecting the mitochondrial potential and oxidative phosphorylation differentially. In contrast, the slight decrease in ATP observed with

nystatin may be due to other indirect effects, such as cellular and possibly mitochondrial swelling that occur by accumulation of NaCl (Soltoff & Mandel, 1984*b*).

In summary, we have tested the effects of some commonly used ionophores on the proximal renal tubule and found a variety of responses. Of the ionophores tested, only nystatin and amphotericin B appeared to be specific for the plasma membrane. All the other ionophores also produced varying degrees of mitochondrial uncoupling. Of these, valinomycin was especially interesting since its main action on the tubules could be attributed to mitochondrial uncoupling. These studies emphasize the importance of understanding metabolic as well as transport effects of compounds with possible multiple sites of action, such as the ionophores. These studies also provide separate quantitation of the transport of potassium through the pump and leak pathways in the rabbit proximal tubule.

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