Active Ion Transport in the Renal Proximal Tubule

III. The ATP Dependence of the Na Pump

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ABSTRACT The dependence of the Na pump activity of intact renal tubules on the ATP concentration was investigated using a suspension of rabbit cortical tubules. Rotenone (an inhibitor of mitochondrial oxidative phosphorylation) was used in graded fashion to alter the cellular ATP, and the Na pump activity was measured when the pump was stimulated by adding KCl to tubules suspended in a K⁺-free medium. The K⁺ uptake into the tubule was measured using an extracellular K⁺ electrode, and the oxygen consumption (Qₒₒ) was measured using a Clark-type oxygen electrode. The Na pump activity was found to have a linear, nonsaturating dependence on the ATP concentration. However, the Na,K-ATPase hydrolytic activity (assayed biochemically) of lysed proximal tubule membranes demonstrated saturation and had a Kₒ₅₀ value of 0.4 mM ATP. Presumably, unknown cytosolic factors present in the intact renal cell but not normally present in the biochemical assay accounted for the differences between the two measurements. The data suggest that an alteration in the intracellular ATP will result in a proportional change in active ion transport activity. Moreover, additional findings also suggest that the basal (non-transport-related) Qₒₒ may be redirected to support the proximal Na pump activity when transport activity is stressed. Thus, basal respiration is not invariant under all conditions, and ion transport activity appears to be maintained foremost among cellular ATP-dependent processes.

INTRODUCTION

The Na,K-ATPase depends on ATP as its metabolic substrate, although several other nucleotides may substitute (see Skou, 1974). In the proximal tubule, ATP is supplied mainly by mitochondrial oxidative phosphorylation, thus accounting for the tight coupling between Na transport and oxidative metabolism (Mandel...
and Balaban, 1981). The quantitative ATP dependence of the isolated Na,K-
ATPase enzyme has been extensively examined in many tissues, but studies
performed using more intact preparations are notably few in number. The rate
of ATP hydrolysis has been correlated with the rate of active ion transport in
red cell ghosts (Whittam and Ager, 1964) and perfused squid axons (Caldwell
et al., 1960), but similar studies have provided equivocal results in epithelial tissues.
Several investigators have reported a lack of correlation in the kidney between
Na transport and ATP content, which suggests that ATP did not play a role in
the link between metabolism and transport (Urbaitis and Kessler, 1971; Trimble
and Bowman, 1973; for review, see Kinne, 1979).

Several factors may account for the conflicting results that have been obtained.
In studies performed using whole kidneys, diffusional limitations may compro-
mise Na transport even before exposure to inhibitors, thus altering the apparent
degree of inhibition. Additionally, such limitations may restrict the ability of
(mitochondrial) inhibitors to reach their site of action at an effective concentra-
tion. In tissues and slices that may be limited by oxygen diffusion and which thus
have an anoxic core, an inhibitor-induced reduction of Na transport could serve
to reduce the relative state of anoxia. Under these conditions, the ATP content
might bear no obvious relationship to transport.

One problem involved in using whole kidneys for such studies involves the
heterogeneity of different renal segments. It is possible that an effect of an
inhibitor on one segment may be compensated by the activity of another segment,
making the interpretation of the results unclear. Another problem involves
sampling the tissue for ATP content. The extensive transport activity of the
kidney requires a rapid production of ATP. Without replacement, the ATP
content of the renal cell can support active transport for only a few seconds.
Thus, the hydrolytic activity of tissue samples must be rapidly quenched to avoid
a reduction in the ATP content, and this may have been a problem in some
studies (see Kinne, 1979).

In a recent study, Gullans et al. (1982) demonstrated that concentrations of
rotenone and antimycin A that maximally inhibited oxidative phosphorylation
of a suspension of rabbit proximal tubules also produced complete inhibition of
net fluid transport ($J_V$) in the isolated perfused proximal tubule. Moreover, a
submaximal concentration of rotenone (0.1 $\mu$M) reduced the rate of oxygen
consumption ($Q_{O_2}$), the ATP content, and fluid reabsorption ($J_V$) by about
the same degree (30–40%), which suggests that these processes are all interrelated.

The objective of the present study was to gain a more quantitative under-
standing of the dependence of the renal proximal Na pump on ATP in intact proximal
tubules. To avoid potential problems that may have affected other studies, a
relatively homogeneous suspension of proximal tubules was used along with a
sampling apparatus that allowed the hydrolytic activity of the tubules to be
rapidly quenched. Graded concentrations of rotenone were used to vary the
ATP levels within the proximal tubule, and the rates of oxygen consumption
and K$^+$ uptake were measured to monitor Na pump activity. Additionally, the
Na,K-ATPase enzyme activity of lysed proximal tubule membranes was assayed biochemically, and the results were compared with those obtained in the intact tubule. In addition to quantitating the ATP dependence of the Na pump, the findings suggest that basal respiration may be redirected to support the activity of the Na pump when it is stressed under certain conditions. This suggests that there is a hierarchy in the maintenance of renal function within the proximal tubule, such that ion transport activity is maintained preferentially over other ATP-dependent metabolic processes.

MATERIALS AND METHODS

Renal Tubule Suspension

Tubules were prepared by collagenase perfusion of female New Zealand White rabbits as described in the first paper (Soltoff and Mandel, 1984a). The tubules were resuspended in the normal solution after centrifugation upon a 25% Ficoll layer. Immediately before 37°C incubation, the tubules were washed three times by centrifugation and resuspension in a K+-free solution (in mM: 110 NaCl, 25 NaHCO3, 2 NaH2PO4, 1 CaCl2, 1 MgSO4, 5 glucose, 4 lactate, 1 alanine, 5 glutamate, 5 malate, 1 butyrate, and 0.6% dextran, pH 7.4). The tubules were incubated in this medium at 37°C for 15-20 min and gassed with a 95% O2/5% CO2 mixture. The tubules were then centrifuged and resuspended in fresh, cold, K+-free solution, incubated and gassed at 37°C for ~3 min, and added to a thermostatted chamber that contained a Clark-type oxygen electrode. Rotenone and K+ were subsequently added to the suspension (see Results).

Na,K-ATPase Activity

The Na,K-ATPase activity was measured at 37°C using lysed membranes of the normal tubule suspension as previously described (Soltoff and Mandel, 1984a). The total ATPase activity was measured by the rate of inorganic phosphate liberation using membranes incubated in a medium that contained 140 mM NaCl, 10 mM KCl, 0.125 mM EGTA, 20 mM Tris (pH 7.4), and 0-10 mM ATP·Na2 (Sigma Chemical Co., St. Louis, MO; vanadium-free). ATP and MgCl2 were present in equimolar concentrations. The Mg-ATPase activity was determined by incubating the membranes in the above mixture plus 10−5 M ouabain, and the Na,K-ATPase activity was defined as the difference between the total and the Mg-ATPase activities. Samples were assayed in triplicate, and appropriate blanks were used to measure the nonenzymatic hydrolysis of Pi.

RESULTS

Dependence of the Na,K-ATPase Enzyme Activity on ATP

As shown in Fig. 1, the Na,K-ATPase activity displayed a hyperbolic relationship as a function of ATP, reaching 90% Vmax at ~1.5 mM. The Na,K-ATPase activity is half-maximally activated by ~0.4 mM ATP. These results are similar to those reported for the Na,K-ATPase from other renal tissue (Kinsolving et al., 1963; Jørgensen, 1968; Braughler and Corder, 1977). At 7.5 and 10 mM, a slight decrease in activity was observed.
FIGURE 1. The dependence of the Na,K-ATPase activity of proximal tubule membranes on the concentration of ATP. The ATPase assay was performed at 37°C and pH 7.4. The total ATPase activity was determined in the presence of 140 mM NaCl, 10 mM KCl, 20 mM Tris-Cl, 0.125 mM EGTA, and 0–10 mM ATP. Na₂ (vanadium-free). ATP and MgCl₂ were present in equimolar concentrations. The Na,K-ATPase activity was defined as the difference between the total activity and the activity when ouabain (10⁻⁵ M) was present. Samples were assayed in triplicate, and appropriate blanks were used to measure the nonenzymatic hydrolysis of ATP. The values shown are the average of two experiments.

Dependence of the Na Pump on Intracellular ATP in Intact Tubules

PROTOCOL To investigate the dependence of the Na pump on intracellular ATP, the tubule suspension was exposed to graded amounts (0.03–0.25 μM) of rotenone, which inhibits the mitochondrial production of ATP by blocking the NADH dehydrogenase and can be used to lower the intracellular ATP content in a graded fashion (Gullans et al., 1982). The tubules were initially suspended in a K⁺-free medium to which rotenone was added. The Na pump activity was stimulated 2–3 min after rotenone by the addition of a KCl bolus sufficient to raise the extracellular K concentration to ~5 mM. The actual extracellular K concentration that was measured just before the addition of KCl was 0.24 ± 0.01 (n = 58) mM, which is well below the Kₐ₅ (1.3 mM) of the Na pump for K (Soltoff and Mandel, 1984b). In these experiments, three different parameters were monitored, as depicted in Fig. 2. In one series of experiments, the Qₒ was measured continuously, and duplicate samples for subsequent ATP determination were taken at four separate times, as follows: (a) before rotenone; (b) after rotenone, just before KCl addition; (c) after KCl addition, during the period of maximal respiratory stimulation; and (d) after the addition of ouabain (not shown in the figure).

In a separate series of experiments, the Qₒ was measured concurrently with the extracellular K concentration, which was monitored using a K⁺-sensitive ion electrode. The net rate of K uptake was calculated from the initial rate of extracellular K disappearance after the addition of KCl to the medium. After a new steady state was reached in the presence of extracellular K⁺ (not shown in the figure), ouabain was added, which caused net K release from the tubules. The K⁺ efflux (release) rate was calculated from the initial rate of the appearance of extracellular K. The first combination of measurements examined the de-
FIGURE 2. Increase in oxygen consumption (lower trace) and net uptake of extracellular K⁺ (upper trace) upon addition of KCl (5 mM bath concentration) to proximal tubules suspended in nominally K⁺-free medium. The extracellular K⁺ concentration was monitored using a K-sensitive ion electrode, and the oxygen consumption was monitored using a Clark-type oxygen electrode. After the addition of KCl (rapid increase in extracellular K⁺ concentration), a net K⁺ uptake is indicated by the disappearance of K⁺ from the medium. The K⁺ trace shown here is for illustrative purposes only. A stable trace was obtained ~6–8 s after the addition of KCl, and the net rate was usually calculated from the uptake during the next 7–10 s. The oxygen trace shows the effect of adding rotenone to a bath concentration of 5 × 10⁻⁸ M. The rates of oxygen consumption (in parentheses) were calculated from the rate of oxygen disappearance from the closed chamber and are given in units of nmol O₂/mg protein-min. Duplicate samples for determinations of ATP were taken before rotenone, after rotenone, after KCl, and after ouabain (not shown).

dependence of the respiratory transition on the ATP content, and the second enabled the relationship between K uptake and Qₒ₂ to be determined. The combined results from both of these studies enabled the rate of K uptake to be examined as a function of the ATP content.

ATP and Qₒ₂

Fig. 3 shows the ATP content as a function of rotenone concentration before KCl addition, immediately after KCl addition, and after ouabain. The mean ATP content of tubules incubated in nominally K⁺-free conditions before any additions was 8.06 ± 0.22 (n = 18) nmol/mg protein. The mean ATP content of tubules to which no rotenone was added was 7.73 ± 0.23 (n = 4). In the absence of extracellular K⁺, rotenone did not decrease the ATP content until the rotenone concentration exceeded 0.1 μM. However, the addition of KCl caused a significant decrease in the ATP content at all rotenone concentrations (0.05–0.2 μM), but did not significantly alter the ATP content in the absence of the drug. Thus, the effectiveness of rotenone as a metabolic inhibitor was more fully displayed when the demand for ATP by the active transport system was engaged by the addition of KCl. As also shown in this figure, after the addition of ouabain to the rotenone-treated tubules, the ATP content returned to levels
The ATP content of proximal tubules suspended in nominally K-free medium and exposed to different concentrations of rotenone and subsequent addition of 5 mM KCl and 10^{-4} M ouabain (see Fig. 2). Symbols and vertical bars represent the means ± SE of three or four determinations, each of which represents duplicate samples that were averaged. Where no vertical bars are indicated, the symbols represent one or two values, each of which represents the average of duplicate samples.

Close to the pre-KCl, post-rotenone values. Thus, in the presence of a partial metabolic insult (rotenone), the renal cell appears to be able to regulate its ATP content at a constant level in the absence of extensive demands made on the mitochondria for ATP support of Na pump activity.

In Fig. 4, the incremental KCl-induced stimulation of the rate of oxygen consumption (ΔQ_o2) is shown as a function of the ATP content corresponding to the period in which the maximal stimulation of Q_o2 was measured. A tight linear correlation (r = 0.98) between the ΔQ_o2 and the ATP content was obtained, which intersected the abscissa at 2.0 nmol ATP/mg protein, at which point no respiratory stimulation by KCl was noted.

**K^+ Uptake Rate and ΔQ_o2**

The effects of 0.03–0.25 μM rotenone on the KCl-induced respiratory stimulation (ΔQ_o2) and the net rate of K uptake by the tubules are shown in Fig. 5. Rotenone produced a concentration-dependent inhibition of both processes. The composite of all experiments (n = 55), in which the K uptake and Q_o2 were concurrently measured in the presence of different concentrations of rotenone, is shown in Fig. 6. The line drawn in the figure was generated by linear regression (r = 0.98) and has a slope of 8.22 and an intercept of 80.3 nmol K^+/mg protein·min.
FIGURE 4. The relationship between the ATP content and the incremental KCl-induced stimulation of respiration (ΔQo₂) of proximal tubules initially suspended in a K-free medium and exposed to different concentrations of rotenone. The ATP values represent the average of duplicate samples taken during the period of maximal respiratory stimulation after KCl addition (see Fig. 2). The closed and open circles represent samples collected from two different preparations of tubules. The ΔQo₂ values are the differences between the Qo₂ immediately before KCl addition and the maximum Qo₂ after KCl addition. The correlation is highly significant (r = 0.98) and gives an intercept value of 2.0 nmol ATP/mg protein by linear regression.

**K⁺ Uptake Rate and ATP**

By measuring the rate of K⁺ uptake and Qo₂ simultaneously in the suspension of intact tubules (Fig. 6), the K⁺ uptake rate could be calculated as a function of ATP (Fig. 7) from the ΔQo₂ vs. ATP relationship (Fig. 4). For each ΔQo₂ value in Fig. 4, the K uptake rate was calculated using the linearization of the results shown in Fig. 6. Thus, as indicated in Fig. 7, the K uptake rate was a linear function (r = 0.98) of the ATP content. The intracellular Na⁺ concentration during the K⁺-free (150 mM extracellular Na⁺) incubation presumably saturated the intracellular Na⁺ site of the pump (see Soltoff and Mandel, 1984b), so these results singularly reflect the dependence of the Na pump on ATP during conditions of maximal stimulation. The linear dependence of the Na pump activity on ATP found in the tubule suspension indicates that the Na pump is not saturated with ATP, a finding that is very different from the results that were obtained using the lysed proximal membranes to measure the Na,K-ATPase enzyme dependence on ATP, which is shown in Fig. 1 (see Discussion).

**K⁺ Content and K⁺ Leak Rate**

The total K content that was accumulated after the addition of KCl to the tubule suspension after rotenone is shown in Fig. 8. These values were calculated by the net appearance of K in the extracellular solution after 10⁻⁴ M ouabain was added to tubules that had reached steady state after the KCl-induced stimulation. Ouabain was added after the net accumulation of K by the tubules had been completed (i.e., the point at which the extracellular K concentration measured by the K-sensitive ion electrode did not change). Significant alterations in K
The rotenone concentration-dependent inhibition of the KCl-induced stimulation of oxygen consumption (upper figure) and initial rate of K⁺ uptake (lower figure) of proximal tubules initially suspended in K-free medium. The ΔQₒₛ values are the differences between the Qₒₛ immediately before KCl addition and the maximum Qₒₛ after the addition of KCl. Symbols and vertical bars represent the means ± SE of 4–15 determinations. Symbols without bars represent the average of two determinations. The data were collected from seven tubule preparations.

Content were observed only in the presence of rotenone concentrations of >0.1 μM, which diminished the content by ~25%.

The rate of K release from the tubules was also monitored from the initial rate of K appearance in the extracellular medium after the addition of ouabain to the suspension. Significant reductions in the rates of release were measured in the presence of high concentrations (≥0.1 μM) of rotenone, paralleling the reductions in K content. Under these conditions, the cellular ATP levels decrease, and this would be expected to inhibit the pump, leading to the observed decrease in K content. Since under steady state conditions the pump rate and the leak rate are equivalent and the intracellular K concentration is constant, it would be expected that both the pump and leak rates would be inhibited to the same extent.

**DISCUSSION**

It has been known for some time that active Na⁺ and K⁺ transport in aerobic tissues depends on energy from oxidative metabolism (for reviews, see Kinne, 1979; Mandel and Balaban, 1981). In the present studies, the coupling between ion transport and oxidative metabolism in the proximal tubule was used to
evaluate and alter active transport. ATP production was decreased by rotenone, which inhibits mitochondrial electron transport by binding on the oxygen side of NADH dehydrogenase (Singer, 1979). As shown in Fig. 3, the effect of rotenone on ATP content was highly dependent on the metabolic demand of the renal cells. In the absence of extracellular K*, the Na, K-ATPase is inhibited, and thus the mitochondria have sufficient reserve capacity to supply the diminished ATP requirements of the cell, despite partial inhibition by rotenone. At rotenone concentrations of >0.1 µM, the mitochondria are sufficiently inhibited.
FIGURE 8. The concentration-dependent effects of rotenone on the K⁺ content (lower figure) and initial rate of K⁺ release (upper figure) of proximal tubules in suspension. The tubules were initially suspended in a K-free medium. After the addition of KCl, the tubules reaccumulated K⁺. The reaccumulation was judged to be complete when the tubules no longer took up K⁺, as monitored by an extracellular K⁺-sensitive electrode. Ouabain (10⁻⁴ M) was then added to the tubules, resulting in a net release of K⁺ into the extracellular medium. The initial rate of K⁺ release was measured within the first 9–12 s after the addition of ouabain. The K⁺ content was calculated from the amount of K⁺ that was released from the tubules after the addition of ouabain. The point and vertical bars represent the means ± SE of three to eight determinations. Points without bars represent one or two determinations. The data were collected from three tubule preparations.

that even this rate of ATP production cannot be maintained. In contrast, upon stimulation of the Na pump by the introduction of K⁺, the ATP demand is of sufficient magnitude to stimulate the mitochondria to their maximal rate of ATP production (Harris et al., 1981; Soltoff and Mandel, 1984a). Any inhibition in the latter process would be expected to cause a drop in cellular ATP content, as was in fact observed in the presence of rotenone.

In a study by Gullans et al. (1982), the effects of different concentrations of rotenone were examined on the ATP content of proximal tubules in suspension respiring under normal (nonstimulated) conditions. As shown in Fig. 9, these changes assume an intermediate position between those measured in this study during the stimulation and the inhibition of the Na pump activity. The activity-dependent effect was particularly evident at 0.1 μM rotenone, for which the transition from low to intermediate to high activity was marked by a reduction in ATP from 88 to 70 to 46% of the control values, respectively.

Differences Between Intact Tubules and Membrane Preparation

The results obtained for the ATP dependence of the Na,K-ATPase enzyme activity in lysed proximal membranes (Fig. 1) are similar to those that have been reported by others in renal tissue using isolated enzyme preparations as well as homogenized tissue. In a variety of species, the $K_{0.5}$ ranged between 0.22 and
FIGURE 9. The rotenone concentration-dependent inhibition of the ATP content of proximal tubules in suspension in three different states of metabolic activity: inhibited (K⁺-free), normal (control), and stimulated (K⁺ stimulation). The results for the inhibited and stimulated states are from the present study, and were obtained immediately before and immediately after the Na pump was stimulated by KCl added to tubules suspended in a K-free medium. The control values are from Gullans et al. (1982), in which cortical tubules were maintained under normal (noninhibited, nonstimulated) conditions. The points and vertical bars represent the means ± SE of three or four determinations, each of which represents duplicate samples that were averaged. Where no vertical bars are indicated, the symbols represent one or two determinations, each of which was the average of duplicate samples. The data were collected from two tubule preparations. For simplicity, vertical bars are omitted from the data of Gullans et al. (1982).

~0.8 mM ATP (Jørgensen, 1968; Braughler and Corder, 1977; Kinsolving et al., 1963; Charney et al., 1975; Rodriguez et al., 1980).

In order to quantitatively compare the ATP dependence of the Na pump activity of the intact proximal tubule with that of the Na,K-ATPase of the membrane preparation (Fig. 1), three manipulations were performed on the data shown in Fig. 7, as follows. (a) The intracellular ATP contents were converted to concentrations by using an intracellular volume of 2.4 μl/mg protein (Soltoff and Mandel, 1984a). (b) The K uptake rates were converted to units of Na,K-ATPase activity by assuming a K/ATP stoichiometry of 2. (c) The K uptake rate prevailing before the addition of 5 mM KCl was added to the measured uptake rate, since this fraction also contributed to the Na pump activity. At an external K⁺ concentration of 0.2 mM, this prevailing rate can be estimated at ~15 nmol K⁺/mg protein·min (Harris et al., 1982), which, when added to the measured K⁺ uptake rate of Fig. 7, causes the line to intersect the origin.

The results recalculated from Fig. 7 are shown in Fig. 10, where they are compared with those from Fig. 1. The results from the intact tubules were quite different from those shown for the hydrolytic Na,K-ATPase activity of proximal tubules in two ways. (a) The Na pump activity in intact tubules had a linear dependence on the ATP concentration, and did not show any indication of saturation. (b) The quantitative dependence of each system on ATP was different. The linear dependence on ATP suggests that the Na pump of the intact proximal
FIGURE 10. The dependence of the Na,K-ATPase activity (solid line) of proximal tubule membranes and the Na pump activity (broken line) of intact proximal tubules on the ATP concentration. The Na,K-ATPase curve is the same as shown in Fig. 1. The Na pump activity of Fig. 7 was calculated in units of Na,K-ATPase activity by assuming a K/ATP stoichiometry of 2, and the ATP concentration of the tubules was calculated using an intracellular volume of 2.4 μl/mg protein. See text for further details.

A tubule is not saturated with ATP. Therefore, any alteration that increases or decreases the ATP will also increase or decrease the Na pump activity by the same relative degree. This finding is in line with the results of Gullans et al. (1982), who reported that a concentration of rotenone that reduced the ATP content of proximal tubules by 30% also caused a proportional reduction of fluid reabsorption (J) in the isolated perfused proximal convoluted tubule.

In these studies it is important to distinguish two separate properties: (a) the ATP concentration that is maintained by the proximal tubule, and (b) the respiratory capacity of the tubules. In the experiments described above, after the addition of KCl the tubules respired at their "maximal" capacity. This was limited, of course, by the inhibition imposed by rotenone on the rate of electron transport in the mitochondria. After the KCl-induced pump stimulation, the ATP level declined (Fig. 3) until the rate of ATP utilization matched the inhibited production rate.

These results can be compared with a previous study in which the addition of nystatin to tubules respiring under normal (nonstimulated) conditions resulted in a doubling of the QO₂ and only a small decrease in the ATP content (Harris et al., 1981). In those experiments, the stimulation of the Na pump by the nystatin-induced increase in intracellular Na engaged the full respiratory capacity of the tubules without substantially altering the ATP levels. Thus, the increase in the rate of ATP production was able to almost fully meet the increased demand for ATP. This demonstrated that the proximal tubule normally has a substantial respiratory reserve with which to meet an increase in ATP utilization by the Na pump. However, the results of the rotenone experiments described here indicate that the cells do not possess a saturating concentration of ATP. This suggests that cellular metabolism may play a controlling role in the regulation of Na transport, such that metabolic or pathological conditions that alter
metabolism so as to change the production of ATP will similarly affect renal Na transport.

Since the Na pump is not saturated with ATP in the intact tubule, it is not possible to evaluate the ATP dependence in terms of alterations in a $K_{0.5}$ value. Nevertheless, the affinity of the Na pump for ATP appears to be much smaller than that of the Na,K-ATPase measured in the lysed membrane preparation. Moreover, the maximum Na,K-ATPase activity ($\approx 340$ nmol P$_i$/mg protein·min) for the proximal membranes was approximately twice the calculated maximum Na,K-ATPase activity ($\approx 185$ nmol P$_i$/mg protein·min) of the tubules in suspension. Thus, there are real quantitative differences between the results obtained in this study using the lysed membranes (or by others using the purified Na,K-ATPase) under specific, well-defined assay conditions, and those obtained using a preparation that may be regulated in a more physiological manner. These differences may be due to cytosolic factors that are present in the intact tubule but which are lacking when the enzyme activity of the membrane is determined biochemically. Either singly or in combination, the following agents might account for these differences: ADP, inorganic phosphate (P$_i$), vanadate, and Mg$^{2+}$.

ADP AND P$_i$: ADP and P$_i$ have been found to inhibit the Na pump activity in intact cells (Garay and Garrahan, 1975; De Weer, 1970), and to inhibit Na,K-ATPase activity in membrane preparations (Moake et al., 1970; Hexum et al., 1970; Post et al., 1965). Hexum et al. (1970) found that ADP was a competitive inhibitor ($K_i = 0.45$ mM), and Garay and Garrahan (1975) and Hexum et al. (1970) reported that P$_i$ was a noncompetitive inhibitor ($K_i = 17$ and $23$ mM, respectively) of the Na pump/Na,K-ATPase. Since the Na,K-ATPase can be driven in reverse to synthesize ATP from ADP and P$_i$ (Garrahan and Glynn, 1966), it is not surprising that the products of ATP hydrolysis can inhibit the forward reaction.

Of interest is whether ADP and P$_i$ are present at sufficient intracellular concentrations to account for the apparent alteration in Na pump activity in the experiments described here. Studies in various tissues suggest that under normal conditions the majority of the intracellular ADP is localized in the mitochondria, while most of the ATP is found in the cytosol (Akerboom et al., 1978). In the present studies with rotenone, the KCl-induced Na pump stimulation diminished the ATP content by $\approx 2$–$4$ nmol/mg protein (Fig. 3). Presumably, the reduction in cytosolic ATP occurred with a concomitant increase in cytosolic ADP and P$_i$. The extent of this increase depends on the “buffering” power of the mitochondria and binding sites for these substances. Clearly, concentrations of ADP on the order of the remaining concentration of ATP could account for a substantial increase in the $K_{0.5}$ of the Na pump for ATP.

As to whether there is sufficient intracellular phosphate in the renal tubules to account for the depression in the maximal activity of the Na,K-ATPase, there are similar concerns regarding compartmentation. Renal cortical tubules bathed in 2 mM extracellular P$_i$ had $\approx 10$ nmol P$_i$/mg protein (Kreusser et al., 1980), from which one can calculate an intracellular concentration of $\approx 4$ mM, a concentration well below the $K_i$ for P$_i$. Moreover, since cellular P$_i$ is concentrated
within the mitochondria (Akerboom et al., 1978), the cytosolic concentration would be less. $^{31}$P nuclear magnetic resonance studies have measured cytosolic Pi concentrations of <1 mM in intact renal tissue (Freeman et al., 1983). Thus, it would not appear that this substance could account for the differences in the results shown in Fig. 10.

**Vanadate**  
Vanadate is a natural compound that is a potent inhibitor of the Na,K-ATPase in a variety of tissues, including the kidney. At a physiological concentration of Mg$^{2+}$ (0.6 mM), the $K_v$ for dog renal Na,K-ATPase was 0.25 pM vanadate (Cantley et al., 1977), a concentration that has been found in renal tissue (Post et al., 1979). Similar concentrations have been measured in various other tissues (see Cantley et al., 1977). In the kidney, vanadate has been reported to inhibit primarily the proximal tubule (Higashi and Bello-Reuss, 1980). Experiments with renal Na,K-ATPase (Charney et al., 1975) showed that vanadate decreased the $V_{max}$ and the $K_{0.5}$ for ATP by ~70%, although a saturating dependence on ATP was still observed. In addition, the inhibitory effect of vanadate increases as the $K^+$ is increased, resulting in decreased Na,K-ATPase activity at high $K^+$ concentrations (Grantham and Glynn, 1979; Beaugé et al., 1980). These effects of vanadate contrast sharply with the linear dependence on ATP (Fig. 7) and the saturation of pump activity that was observed as a function of extracellular $K^+$ (Soltoff and Mandel, 1984b, Fig. 8) exhibited by this tubule preparation. Thus, it seems unlikely that endogenous vanadate could account for the observed differences between the Na,K-ATPase and the Na pump. Moreover, exogenous vanadate causes a reduction of the Na pump-related $Q_0^S$ of tubules in suspension (Soltoff, S. P., and L. J. Mandel, unpublished observations), leaving open to question whether endogenous vanadate is present in amounts sufficient to affect the Na pump activity.

**Mg$^{2+}$**  
Mg-ATP is the energetic substrate of the Na,K-ATPase (Hexum et al., 1970). Binding studies have demonstrated that the Na,K-ATPase has at least two sites that can bind ATP: a high-affinity site having a $K_m$ near 1 $\mu$M, and a site of lower affinity with a $K_m$ of ~0.5 mM (Robinson, 1976). Since both Mg$^{2+}$ and ATP can compete for the lower-affinity site, an excess of one or the other will inhibit the pump activity by inhibiting the binding of Mg-ATP (Hexum et al., 1970; Robinson, 1974). In kinetic studies of the Na,K-ATPase, it has generally been observed that a 1:1 ratio is optimal (Wheeler and Whittam, 1962), although that may not hold at all concentrations (Schoner et al., 1967).

Whether alterations in the Mg$^{2+}$/ATP ratio can play a regulatory role in vivo is uncertain. In various tissues of vertebrates and invertebrates, the total Mg$^{2+}$ and ATP contents are correlated because of the presence of Mg-ATP, and Mg$^{2+}$ is always present in excess of ATP (Burton, 1980). As mentioned above, the KCl-induced stimulations of the Na pump resulted in a large decrease of ATP (i.e., Mg-ATP). The change in free Mg$^{2+}$ upon ATP hydrolysis depends on the availability of the products of hydrolysis to bind Mg$^{2+}$, as well as on the buffering capacity of other cellular components. Without knowing the fate of the Mg$^{2+}$ that was formerly bound to ATP, it is difficult to evaluate whether the decrease in ATP initiated a Mg$^{2+}$-dependent alteration in pump activity. If most of the Mg$^{2+}$ from the 2–4 nmol ATP/mg protein that was hydrolyzed remained free,
approximately doubling the concentration of free Mg$^{2+}$, the affinity of the Na pump for ATP could be altered.

**OTHER FACTORS** A possibility that requires evaluation is whether a diffusional limitation exists within the proximal cell, such that the ATP concentration at the Na pump is very much different (lower) than the average cellular value that is measured. This problem is difficult to evaluate quantitatively because of the complex morphology of these cells. However, the high concentration of mitochondria present in proximal tubules, and their close proximity to the basolateral membrane (Kaissling and Kriz, 1979) makes it unlikely that such a diffusional limitation exists in these cells. Similarly, the ADP concentration at the Na pump site is probably determined primarily by compartmentation and binding, as discussed earlier, rather than by diffusion between the pump and the mitochondria.

The slope of the linear dependence in Fig. 10, but not the maximal value of Na pump activity that was observed, depends on the accuracy of the intracellular volume measurement used to calculate the ATP concentration. Although the volume measurement was made under conditions different from the rotenone experiments presented here, it would take about an eightfold error in this estimation to render the linear ATP dependence of the intact tubules quantitatively similar to the initial "linear" component of the Na,K-ATPase hydrolytic activity.

**CONCLUSIONS** The preceding discussion describes several possibilities to account for the quantitative differences in the ATP dependence of the Na pump and the Na,K-ATPase activities shown in Fig. 10. The most likely candidates are ADP and Mg$^{2+}$, although unknown cellular factors are probably also involved. These conclusions must remain speculative until methods are developed to accurately measure the free cytoplasmic concentrations of these substances.

**Basal Metabolism**

A question that has been debated for a long time in the epithelial literature is whether or not the basal metabolic rate is invariant when ion transport is altered (Weiner and Maffly, 1978; Mandel and Balaban, 1981). Given the penchant of investigators for measuring ion/O$_2$ stoichiometries by using maneuvers that stimulate and inhibit net transport, this is a very critical question. However, alterations of basal metabolism during Na pump stimulation are difficult to determine by conventional means because, as Heisenberg's uncertainty principle states, the process that one attempts to observe is altered by attempts to measure it. To measure the basal component of respiration, ouabain is usually added to the tubule suspension. However, if the basal Q$_{O_2}$ is altered only during stressed conditions, the raison d'être of this effect no longer exists in the presence of ouabain. As indicated in Fig. 3, the KCl-induced pump stimulation produced a decrease in ATP, which could provide a strong impetus for "borrowing" a portion of basal Q$_{O_2}$ to support pump activity. In the presence of ouabain, the ATP content was much greater, close to pre-KCl levels, and thus there is no metabolic need to dip into the basal Q$_{O_2}$. Other means must therefore be used.
to determine alterations in basal metabolism during conditions of metabolic stress, as described below.

The results shown in Fig. 6 may shed some light on this point since the linearized K⁺ uptake rate vs. incremental Qₒₛ relationship does not pass through the origin. When these results are extrapolated to the ordinate, it appears that 80 nmol K⁺/mg protein-min is taken up into the tubules without any change in Qₒₛ. Two pieces of evidence suggest that most of this uptake is active: (a) it corresponds to an accumulation of 33 mM K⁺/min within the cell in the presence of 5 mM K⁺ in the extracellular medium; and (b) Figs. 7 and 10 indicate that the K⁺ uptake measured in these experiments is linearly related to the cellular ATP content. Therefore, the active transport of K⁺ without a corresponding change in Qₒₛ may indicate that a portion of basal respiration was applied to support the Na pump rather than to support basal metabolic work. Thus, the total support of K⁺ transport would encompass not only the KCl-induced respiratory transition (ΔQₒₛ) but also a portion of the basal Qₒₛ that was measured during the K⁺-free exposure (see Fig. 11). This suggests that when stressed under these conditions, the proximal tubule is able to borrow or recruit a portion of basal respiration for the support of transport activity in order to maintain this aspect of renal function. The amount borrowed can be estimated from the extrapolated intercept of the abscissa of Fig. 6, which is ~10 nmol Qₒₛ/mg protein-min. Although this is just an estimate, it is a plausible value since the ouabain-insensitive respiration averaged ~12.5 nmol O₂/mg protein-min.

This interpretation of the data affects the calculation of the K⁺/O₂ ratio. The slope of the line shown in Fig. 6 suggests that the K⁺/O₂ ratio does not change

![Diagram](image)

**Figure 11.** The Qₒₛ that usually goes to the support of basal metabolism may be used to support transport. The KCl-induced Qₒₛ is made up of a basal portion (the difference between no respiration and the respiration before KCl addition) and a Na pump-related portion (the difference between the KCl-stimulated respiration and the respiration before KCl). Under certain conditions, a portion (shaded region) of the basal Qₒₛ may be applied to support the Na pump rather than being used entirely to support basal metabolic processes.
The rotenone concentration-dependent apparent increase of the K/O2 ratio of proximal tubules in suspension. The K/O2 ratio represents the initial K+ uptake rate divided by the incremental respiratory stimulation (ΔQO2) produced by KCI addition to tubules suspended in K-free medium in the presence of rotenone. The plot was obtained by calculating the K/O2 ratio of the data shown in Fig. 6 and expressing it in terms of the rotenone concentration. Points and vertical bars represent the means ± SE of 4–15 determinations. Where no bars are indicated, the points present two determinations.

as the stimulation of the Na pump is diminished by rotenone. However, if the K+/O2 ratio of each sample is calculated without first subtracting the intercept value, an apparent increase in this ratio is seen in the presence of increasing concentrations of rotenone. Such a calculation is equivalent to the slope of a line drawn from the origin in Fig. 6 to the values obtained for each sample. With increasing concentrations of rotenone, the apparent K+/O2 increases to larger and larger values, shown in Fig. 12, as the constant value of K uptake (80 nmol/mg protein-min) contributes a larger and larger portion to the measured K uptake. The K+/O2 values in the range of 8–12 are similar to those reported previously (Harris et al., 1980, 1982) and can be explained in terms of the known stoichiometries for K+/ATP and ATP/O2. However, values >12 are difficult to explain since they require the assumption of unphysiologically large stoichiometries for the latter two ratios. This problem is eliminated by subtracting the amount borrowed and thus obtaining a constant K+/O2 ratio.

The results shown in Fig. 6 contrast with those obtained by Harris et al. (1982), who stimulated the Na pump in a graded manner by exposing tubules suspended in nominally K+-free solution to different concentrations of K+. Using values from their study, a graph of the K uptake rate vs. ΔQO2 is a linear relationship that passes through the origin, and thus a fixed component of basal
respiration is not displayed. However, these results and those presented in this communication may be reconciled if it is assumed that when tubules that were not metabolically compromised (e.g., by rotenone) were maximally stimulated by high concentrations of \( K^+ \), they borrowed substantial amounts from basal respiration, but that those tubules which were stimulated to a lesser degree by low \( K^+ \) concentrations were better able to meet the metabolic requirements for the support of active transport and borrowed much less. Thus, with increasing concentrations of \( K^+ \), a larger and larger quantity of basal respiration was recruited to support the higher levels of Na pump activity. An important consequence of this interpretation is that measurements of the \( K^+ / O_2 \) ratio using KCl-induced transitions of the \( Q_o \) and K uptake rate are not as straightforward as they appear to be. The \( K^+ / O_2 \) ratio may be overestimated from the K concentration-dependent stimulation of Na pump activity unless alterations in basal metabolism are taken into account.

Borrowing from basal respiration suggests that there can be competition between transport-related and non-transport-related processes for the ATP that is produced by the mitochondria. Moreover, it suggests that metabolic support of the Na pump was achieved at the expense of the non-transport-related metabolic activities. Direct support for this supposition comes from the work of Gullans et al. (1982), who studied gluconeogenesis and active transport in proximal tubules. These authors found that normally the tubules have sufficient energy to support both ATP-dependent processes; however, when active transport was greatly stressed by the addition of nystatin, gluconeogenesis was greatly inhibited. Thus, the present studies suggest that under conditions of metabolic stress, there is a hierarchy in the maintenance of energy-dependent renal processes, such that active ion transport is maintained preferentially over other ATP-dependent processes.

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