Active Ion Transport in the Renal Proximal Tubule

II. Ionic Dependence of the Na Pump

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ABSTRACT The dependence of Na pump activity on intracellular and extracellular Na\(^+\) and K\(^+\) was investigated using a suspension of rabbit cortical tubules that contained mostly (86\%) proximal tubules. The ouabain-sensitive rate of respiration (\(Q_{o2}\)) was used to measure the Na pump activity of intact tubules, and the Na,K-ATPase hydrolytic activity was measured using lysed proximal tubule membranes. The dependence (\(K_{0.5}\)) of the Na pump on intracellular Na\(^+\) was affected by the relative intracellular concentration of K\(^+\), ranging from ~10 to 15 mM at low K\(^+\) and increasing to ~30 mM as the intracellular K\(^+\) was increased. The Na pump had a \(K_{0.5}\) for extracellular K\(^+\) of 1.3 mM in the presence of saturating concentrations of intracellular Na\(^+\). Measurements of the Na,K-ATPase activity under comparable conditions rendered similar values for the \(K_{0.5}\) of Na\(^+\) and K\(^+\). The Na pump activity in the intact tubules saturated as a function of extracellular Na at ~80 mM Na, with a \(K_{0.5}\) of 30 mM. Since Na pump activity under these conditions could be further stimulated by increasing Na\(^+\) entry with the cationophore nystatin, these values pertain to the Na\(^+\) entry step and not to the Na\(^+\) dependence of the intracellular Na\(^+\) site. When tubules were exposed to different extracellular K\(^+\) concentrations and the intracellular Na\(^+\) concentration was subsaturating, the Na pump had an apparent \(K_{0.5}\) of 0.4 mM for extracellular K. Under normal physiological conditions, the Na pump is unsaturated with respect to intracellular Na\(^+\), and indirect analysis suggests that the proximal cell may have an intracellular Na\(^+\) concentration of ~35 mM.

INTRODUCTION

Since its discovery by Skou (1957), the ubiquitous nature of the Na,K-ATPase in physiological systems has been matched only by the ubiquitous publications concerning this enzyme. As an object of study, it has received cardinal focus in physiology, as well as in biochemistry and pharmacology. As an enzyme, it has...
been isolated from many tissues and studied with respect to its dependence on Na\(^+\), K\(^+\) and ATP. Far fewer studies have examined these dependencies for the Na pump (the biological counterpart of the Na,K-ATPase enzyme) to determine how it functions in intact cells. Studies using the isolated Na,K-ATPase have proved to be extremely useful. However, alterations of the surrounding membrane, lack of sidedness in the ionic compartmentation, and incomplete replication of the intracellular conditions leave open to question whether the results obtained with the isolated enzyme are quantitatively identical to those obtainable under more intact conditions.

A partial listing of results that have been obtained in intact and broken membrane or purified preparations is provided in Table 1, with particular emphasis on results obtained using renal tissue. The concentration of Na\(^+\) required for half-maximal activation (\(K_{0.5}\)) of the Na pump/Na,K-ATPase activity

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Preparation</th>
<th>Measurement</th>
<th>Na</th>
<th>K</th>
<th>Notes*</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Red blood cell</td>
<td>Membrane</td>
<td>Na,K-ATPase</td>
<td>24</td>
<td>3</td>
<td>Na + K = 145 mM</td>
<td>Post et al., 1960</td>
</tr>
<tr>
<td>Human</td>
<td>Intact</td>
<td>Ouabain-sensitive</td>
<td>20</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Na + K movement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Intact</td>
<td>Ouabain-sensitive</td>
<td>20</td>
<td>2.1</td>
<td>Na + K = 150 mM</td>
<td>Post and Jolly, 1957</td>
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<tr>
<td></td>
<td></td>
<td>Na + K movement</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Na efflux</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Brain</td>
<td>Microsomes</td>
<td>Na,K-ATPase</td>
<td>57</td>
<td></td>
<td>Na + K = 150 mM</td>
<td>Skou, 1974</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Nonrenal epithelia</td>
<td>Isolated intact</td>
<td>K-sensitive Na efflux</td>
<td>7</td>
<td>1.1</td>
<td></td>
<td>Saito and Wright, 1982</td>
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<tr>
<td>Frog choroid plexus</td>
<td>Isolated intact</td>
<td>Short-circuit current</td>
<td>5.5</td>
<td></td>
<td>Activity (not conc)</td>
<td>Eaton, 1981</td>
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<td>Rabbit urinary bladder</td>
<td>Microsomes</td>
<td>Na,K-ATPase</td>
<td></td>
<td>58</td>
<td>Na + K = 150 mM</td>
<td>Jørgensen, 1968</td>
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<tr>
<td>Rat whole kidney</td>
<td>Microsomes</td>
<td>Na,K-ATPase</td>
<td></td>
<td>57</td>
<td>Na + K = 150 mM</td>
<td>Jørgensen, 1980</td>
</tr>
<tr>
<td></td>
<td>Homogenate</td>
<td>Na,K-ATPase</td>
<td>25</td>
<td>2</td>
<td>Na + K = 150 mM</td>
<td>Wheeler and Whittam, 1962</td>
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<tr>
<td>Rabbit cortex</td>
<td>Microsomes</td>
<td>Na,K-ATPase</td>
<td>17</td>
<td>0.5</td>
<td>Na + K = 20 mM K</td>
<td>Katz and Lindheime, 1975</td>
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<td></td>
<td>Purified</td>
<td>Na,K-ATPase</td>
<td>16</td>
<td>1.5</td>
<td>20 mM K</td>
<td>Braughler and Corder, 1977</td>
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<td></td>
<td>Homogenate</td>
<td>Na,K-ATPase</td>
<td>7, 12, 30</td>
<td>2</td>
<td>K = 5, 20, or 80 mM</td>
<td>Kinsolving et al., 1963</td>
</tr>
<tr>
<td></td>
<td>Single tubule</td>
<td>Na,K-ATPase</td>
<td></td>
<td></td>
<td></td>
<td>Doucet et al., 1979</td>
</tr>
<tr>
<td></td>
<td>Perfused</td>
<td>(J_s)</td>
<td>0.85</td>
<td></td>
<td></td>
<td>Sasaki et al., 1985</td>
</tr>
<tr>
<td></td>
<td>Membrane perfused</td>
<td>Na,K-ATPase</td>
<td>45</td>
<td>1.5</td>
<td>Na + K = 150 M</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Intact suspension</td>
<td>Na,K-ATPase</td>
<td>50</td>
<td>1.5</td>
<td></td>
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* Ionic conditions of measurement.
ranges from below 10 to almost 40 mM. In general, red blood cells and tight epithelia (rabbit urinary bladder), have a lower \( K_{0.5} \) value than do leaky epithelia. In a study in which the \( Na^+ \) and \( K^+ \) concentrations were reciprocally varied to maintain isotonicity at 150 mM (Jørgensen, 1980), the \( Na^+ \) dependence of the \( Na,K\)-ATPase activity of purified renal medullary enzyme was sigmoidal. The \( Na^+/K^+ \) concentration ratio (in mM) for half-maximal activity was 37/113, and the \( Na,K\)-ATPase activity doubled when the \( Na^+/K^+ \) ratio was increased from 20/130 to 30/120, concentrations close to the expected values of intact tissue. If the same relationship were applicable in the intact tissue, physiological alterations in the intracellular \( Na \) concentration could greatly affect \( Na \) transport. As indicated in Table I, the \( K_{0.5} \) is much lower in renal tissue when the \( Na,K\)-ATPase activity is measured in the presence of a fixed (low) \( K \) concentration (Katz and Lindheimer, 1975; Braughler and Corder, 1977).

A relatively large number of renal studies have examined the dependence of active \( Na \) and fluid reabsorption (\( J_f \)) on the extracellular \( K^+ \) concentration because of the apparent ease with which it can be altered. Although the \( Na,K\)-ATPase has an absolute dependence for \( K^+ \), the studies of intact kidney preparations have produced conflicting results. In perfused \textit{Necturus} kidneys (Giebisch et al., 1973) and guinea pig kidney cortex slices (Whittembury and Proverbio, 1970), the persistence of active \( Na \) reabsorption when extracellular \( K \) was removed led investigators to propose that there exists a second, non-\( K \)-linked \( Na \) pump at the basolateral membrane. However, it was observed that in the isolated perfused rabbit proximal tubule, removal of \( K \) from the peritubular bathing solution caused the rate of fluid reabsorption and the transtubular potential difference to fall to zero (Burg and Green, 1976; Cardinal and Duchesneau, 1978; Sasaki et al., 1983). In studies that used the perfused rabbit proximal tubule, \( J_f \) was found to vary with the peritubular \( K \) concentration (Cardinal and Duchesneau, 1978; Sasaki et al., 1983), demonstrating a \( K_{0.5} \) of 0.85 mM (Sasaki et al., 1983). Net fluid absorption was not significantly altered when the intraluminal \( K \) concentration was varied between 0 and 10 mM (Cardinal and Duchesneau, 1978). Since slices and intact tissues have diffusional limitations that may have inhibited the complete removal of \( K \), it is possible that the residual active transport in the \textit{Necturus} and guinea pig preparations may be explained by the maintenance of a residual \( Na,K\)-ATPase activity caused by the incomplete removal of extracellular \( K \).

The present study examined the effects of alterations of concentrations of \( Na^+ \) and \( K^+ \), both intracellular and extracellular, on the \( Na \) pump (measured as the ouabain-sensitive or extracellular \( K \)-sensitive \( Q_{0.5} \)) in intact rabbit proximal tubules in suspension. The following paper (Soltoff and Mandel, 1984b) examines the effects of alterations of the intracellular ATP. For comparison, the \( Na,K\)-ATPase enzyme activity was measured using lysed proximal tubules. These studies demonstrate how the interaction of ions on both sides of the membrane affects \( Na \) pump activity, as well as how constituents of the intracellular milieu may account for differences observed in measurements made under intact and nonintact conditions. The results support the identical nature of the \( Na \) pump and the \( Na,K\)-ATPase, and demonstrate the influence of the cellular milieu on
the Na pump activity, as well as providing detailed information regarding the activity of the renal Na pump under physiological and pathophysiological conditions.

MATERIALS AND METHODS

The protocol used to obtain renal tubules is outlined in the previous paper (Soltof and Mandel, 1984a). In brief, female New Zealand White rabbits were perfused with a hypertonic solution that contained collagenase, and the cortex was dissected from each kidney to obtain separated renal tubules that were mainly proximal in origin. The tubules were observed to have open lumens, and thus appeared capable of transepithelial transport. In order to remove nonvital cells and debris, the tubules were centrifuged on a layer of Ficoll. After Ficoll, the tubules were suspended three times in the appropriate solution by centrifugation and replacement of the supernatant with cold (0–4°C) fresh medium (Table II). In experiments in which the extracellular medium was varied by alterations in Na and/or K, the appropriate solution was substituted beginning with the tubule resuspensions after Ficoll. In experiments in which the Na concentration of the extracellular medium was varied, choline was used in place of Na, and the Na⁺ concentration was varied by mixing solutions A and C. In experiments in which tubules were exposed to K⁺-free solution, K⁺ was replaced by equimolar Na⁺.

All experiments were conducted at 37°C. Unless indicated otherwise, the tubules were incubated at 37°C for 15–20 min and gassed with a 95% O₂/5% CO₂ mixture before all

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Experimental Solutions</th>
</tr>
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<tbody>
<tr>
<td>Solution</td>
<td>A</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>295</td>
</tr>
<tr>
<td>NaCl</td>
<td>105</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>—</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>25</td>
</tr>
<tr>
<td>Choline HCO₃</td>
<td>—</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>2</td>
</tr>
<tr>
<td>H₃PO₄</td>
<td>—</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1</td>
</tr>
<tr>
<td>KCl</td>
<td>5</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>—</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1</td>
</tr>
<tr>
<td>Glucose</td>
<td>5</td>
</tr>
<tr>
<td>Lactate</td>
<td>4</td>
</tr>
<tr>
<td>Alanine</td>
<td>1</td>
</tr>
<tr>
<td>Glutamate</td>
<td>5</td>
</tr>
<tr>
<td>Malate</td>
<td>5</td>
</tr>
<tr>
<td>Butyrate</td>
<td>1</td>
</tr>
<tr>
<td>HEPES</td>
<td>—</td>
</tr>
<tr>
<td>Dextran</td>
<td>0.6%</td>
</tr>
<tr>
<td>EGTA</td>
<td>—</td>
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<tr>
<td>pH</td>
<td>7.4</td>
</tr>
</tbody>
</table>

* In several experiments where noted, KCl was not present (NaCl was substituted).
† The substrate was buffered with NaOH (solutions A and B), TMA hydroxide (C), or KOH (D).
‡ HEPES was buffered with KOH.
measurements. The suspension was then transferred to thermostatted chambers that were magnetically stirred and the respiratory rates were measured using a Clark-type oxygen electrode. Tubules exposed to a K'-free medium were resuspended in fresh cold medium one additional time after the 37°C incubation, and then were reincubated at 37°C for 3–5 min before measuring the respiratory rates. Samples of the tubule suspension were occasionally taken for an analysis of tissue Na and K content at the end of the final incubation period.

Tubules that were used to measure the state 3 (ADP-stimulated) response of the mitochondria after digitonin permeabilization were centrifuged and resuspended in solution D twice immediately before use, as described in Soltoff and Mandel (1984a). These tubules were then incubated for 3–5 min at 37°C and gassed with 100% O₂ before digitonin exposure and measurement of the ADP-stimulated $Q_o$. In experiments in which the state 3 incubation medium contained Na, equimolar Na was substituted for K in solution D.

In one study, net K movement was measured using an extracellular K-sensitive ion electrode (Orion Research, Inc., Cambridge, MA), as described by Harris et al. (1980). All other conditions and techniques are as reported in Soltoff and Mandel (1984a). Unless denoted otherwise, all values are shown as means ± SE.

RESULTS

Relationship Between Extracellular Na Concentration and Oxygen Consumption

The dependence of the steady state ouabain-sensitive rate of oxygen consumption ($Q_o$) of the tubule suspension on the extracellular Na⁺ concentration is shown in Fig. 1. The ouabain-sensitive $Q_o$, which is a measure of Na,K-ATPase-mediated transport, was a saturable function of the external Na⁺ concentration. Saturation occurred at ~80 mM Na⁺, and the $K_{0.5}$ value for this function was ~30 mM. Under physiological conditions, the entry of Na⁺ across the luminal membrane is the rate-limiting step of Na,K-ATPase-mediated transport in the proximal tubule, and the Na pump is not saturated with respect to intracellular Na⁺ (Stroup et al., 1974; Spring and Giebisch, 1977). Indeed, if the membrane

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** The dependence of the ouabain-sensitive rates of oxygen consumption ($Q_o$) of proximal tubules in suspension on the concentration of extracellular Na⁺. At each Na⁺ concentration, the rate of oxygen consumption ($Q_o$) was first monitored in the absence of ouabain, and then ouabain (10⁻⁴ M) was added to the tubules to determine the ouabain-insensitive $Q_o$. The difference between the two values is the $ΔQ_o$. The points and bars represent the means ± SE of 3–12 individual determinations taken from seven tubule preparations. Where no bars are shown, $n = 2$. The line was drawn by eye.
permeability to Na\(^+\) is increased (e.g., by exposure of the tubules to the antibiotic nystatin) at any of the external Na\(^+\) concentrations shown here, it results in a stimulation of the Na pump caused by an increase in the concentration of the intracellular Na\(^+\) (see below). Thus, the curve shown in Fig. 1 represents the dependence of the luminal entry of Na\(^+\) on the extracellular Na\(^+\) concentration. Under these conditions, the saturation that is observed pertains to the Na entry step phenomenon and does not indicate the saturation of the pump site with intracellular Na\(^+\).

**Dependence of the Na Pump on Intracellular Na Concentration**

Because of the tight coupling between oxygen consumption and Na,K-ATPase-mediated transport (Mandel and Balaban, 1981), Na pump activity can also be monitored by the transient incremental stimulation of oxygen consumption that occurs upon the addition of a KCl bolus into a suspension of tubules bathed in a nominally K\(^+\)-free medium (Balaban et al., 1980; Harris et al., 1980). To study the effect of different concentrations of intracellular Na\(^+\) on the Na pump, tubules were exposed to K\(^+\)-free solutions in the presence of different concentrations of extracellular Na\(^+\). Because the Na pump was greatly inhibited under these conditions, the intracellular Na\(^+\) concentration presumably equaled or approached the extracellular concentration. Thus, the incremental changes in respiration upon KCl addition (5 mM extracellular concentration) to tubules bathed in different concentrations of extracellular Na\(^+\) reflected the Na pump activity at different intracellular Na\(^+\) concentrations. The respiratory response as a function of Na\(^+\), shown in Fig. 2, was an S-shaped curve that had a foot

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**Figure 2.** The KCl-induced stimulation of the rate of oxygen consumption of proximal tubules suspended in a nominally K-free solution in the presence of different concentrations of Na\(^+\). The \(\Delta Q_{O_2}\) value represents the difference in the \(Q_{O_2}\) before and after the addition of KCl to a 5-mM concentration. The Na\(^+\) concentration was varied by mixing solutions B and C (Table II), except that NaCl or choline chloride was substituted for KCl. The points and bars represent the means ± SE of 3–16 determinations taken from 9 tubule preparations. Where no bars are shown, the standard error is less than the size of the points. The line was drawn by eye.
Dependence of the Na,K-ATPase on Na Concentration: Effect of K

The Na,K-ATPase activity of membranes from lysed proximal tubules was examined as a function of Na concentration under two conditions, as follows: (a) the concentration of Na\(^+\) plus K\(^+\) was kept constant (150 mM) such that the K\(^+\) concentration in the K\(^+\)-free samples was 0.1-0.2 mM. To calculate the tissue K\(^+\) content of the 5 mM K\(^+\) samples, the contribution of trapped extracellular solution was subtracted from the total extracted ion content by assuming an extracellular space of 2 \(\mu\)l/mg protein (Soltoff and Mandel, 1984a). The points and bars represent the means ± SE of three to eight determinations from five tubule preparations. Where no bars are shown, \(n = 2\). The line drawn for the K\(^+\)-free samples is the mean value of all samples (\(n = 46\)). The line for the 5 mM K\(^+\) samples was drawn by eye.

between 0 and 5 mM, saturated at ~100 mM, and was half-maximally activated at ~30 mM Na\(^+\).

Although the solutions themselves did not contain any K\(^+\), ~0.1-0.2 mM extracellular K\(^+\) was measured during the incubation of tubules in these solutions. This is a concentration much smaller than the \(K_{0.5} (1.3\) mM) of the proximal tubule Na pump for extracellular K\(^+\) (see below). Fig. 3 illustrates that exposure of the tubules to K\(^+\)-free media containing different concentrations of Na\(^+\) diminished, but did not deplete, their K\(^+\) content. Thus, the low extracellular K\(^+\) concentration that persisted under these conditions was probably due to K\(^+\) leaking out of the proximal cell.

Assuming an intracellular water space of 2.4 \(\mu\)l/mg protein (Soltoff and Mandel, 1984a), the average intracellular concentration of K\(^+\) calculated from Fig. 3 was ~30 mM during K\(^+\)-free exposure, although it is not clear how much of this K\(^+\) was freely exchangeable (see Soltoff and Mandel, 1984a). In red blood cells, the dependence of the Na pump activity on intracellular Na\(^+\) is known to be affected by the concentration of intracellular K (Garay and Garrahan, 1973; Knight and Welt, 1974). This effect has also been observed for the Na,K-ATPase hydrolytic activity that is measured under nonintact conditions (e.g., see Kinsolving et al., 1963). To better understand the significance of the results shown in Fig. 2, this effect of K was studied in the proximal tubule. The next two sections describe these studies in both nonintact (Na,K-ATPase hydrolytic activity) and intact (Na pump-related \(Q_{O_2}\)) conditions.
concentration was increased as the Na\(^+\) concentration was reduced; and (b) the K\(^+\) concentration was maintained at 10 mM, and the Na\(^+\) concentration was varied by the substitution of Tris-Cl for NaCl. As shown in Fig. 4, the two conditions produced activities that had different \(K_{0.5}\) values (\(\sim 17\) and 43 mM, respectively), but had about the same \(V_{\text{max}}\) (260–280 nmol Pi/mg protein-min). These results emphasize the effect of K on the \(K_{0.5}\) for Na\(^+\), which is attributable to a competition of Na\(^+\) and K\(^+\) at the intracellular Na\(^+\) site. The \(K_{0.5}\) obtained when Na\(^+\) was varied by equimolar substitution with K\(^+\) is similar to that obtained in kidney (Kinsolving, 1963; Jørgensen, 1968, 1980) and brain (Skou, 1974) when the Na,K-ATPase activity was measured under equivalent conditions.

**Effect of Intracellular K on the Dependence of the Na Pump for Intracellular Na**

The results shown in Fig. 2 represent the response of the Na pump to different concentrations of intracellular Na\(^+\) in the presence of an intracellular K concentration (\(\sim 30\) mM) that is lower than normal because of the protocol (K\(^+\)-free exposure) that was used. In an attempt to reduce the inhibitory effects of intracellular K\(^+\) to an even greater extent, intracellular K\(^+\) was lowered further by adding nystatin to tubules incubated in the presence of 5 mM K\(^+\) and various

![Figure 4](https://example.com/figure4.png)

**Figure 4.** The dependence of the Na,K-ATPase activity of proximal tubule membranes on the concentration of Na\(^+\) in the presence of different concentrations of K\(^+\). In one case (left curve), the K\(^+\) concentration was maintained at 10 mM, and Na\(^+\) was varied by replacement of NaCl with Tris chloride. In the other case (right curve), Na\(^+\) was varied by replacement with K\(^+\), such that the sum of Na\(^+\) + K\(^+\) was kept constant at 150 mM. The ATPase assay was performed at 37°C and pH 7.4. The total ATPase activity was determined in the presence of 20 mM Tris-Cl, 0.125 mM EGTA, 5 mM ATP·K\(_2\), and 5.5 mM MgCl\(_2\), under the ionic conditions described above. The Na,K-ATPase was defined as the difference between the total activity and the activity in the additional presence of 10\(^{-3}\) M ouabain. The membranes were obtained from a single preparation of tubules. Samples were assayed in duplicate, and appropriate blanks were used to measure the nonenzymatic hydrolysis of Pi.
concentrations of Na⁺. Nystatin is a polyene antibiotic that increases the permeability of sterol-containing membranes, such as the plasma membrane, to Na⁺ and K⁺ (Cass and Dalmark, 1973). Thus, nystatin will collapse the Na⁺ and K⁺ electrochemical gradients across the plasma membrane, promoting a decrease in the intracellular K⁺ and an increase in the intracellular Na⁺ to levels approximating the extracellular K⁺ and Na⁺ concentrations. Although direct measurements of the intracellular Na⁺ concentration were attempted, it was observed that nystatin promoted both an increased Na⁺ (chemical) content and cellular swelling (probably by the accumulation of NaCl). Since the cellular volume would be expected to depend on the extracellular Na⁺ concentration in the presence of nystatin, the calculation of the intracellular concentration would require an accurate volume determination at each extracellular Na⁺ concentration. The uncertainty in measuring the cell volume in this preparation (see Soltoff and Mandel, 1984a) precludes the accurate measurement of the Na⁺ concentration under these conditions. Indirect evidence to support the equilibration of intracellular Na⁺ in the presence of nystatin includes the rapid equilibration of K⁺ (see below) and the findings of other investigators using nystatin or amphotericin B (e.g., see Graf and Giebisch, 1979).

**Figure 5.** The stimulation of the rate of oxygen consumption (Q₀₂) and the release of intracellular K⁺ promoted by the addition of nystatin (80 μg/mg protein) to proximal tubules suspended in a solution that contained 150 mM Na⁺ and 5.2 mM K⁺. The Q₀₂ (in parentheses, in units of nmol O₂/mg protein·min) was measured using a Clark-type oxygen electrode, and K⁺ was monitored by an extracellular K⁺-sensitive ion electrode. The tissue K⁺ content of the tubules was calculated from the amount of K⁺ that was released into the extracellular solution upon the addition of nystatin.
FIGURE 6. The ouabain-sensitive nystatin-stimulated rates of oxygen consumption of proximal tubules suspended in different concentrations of extracellular Na\(^+\) and 5 mM K\(^+\). Tubules were incubated at each Na\(^+\) concentration and placed in the oxygen electrode chambers in the absence of any additional agents. Nystatin (0.05–0.08 mg/mg protein) and ouabain (10\(^{-4}\) M) were subsequently added separately to the same chamber. The \(\Delta Q_{O_2}\) values are the differences between the stimulated \(Q_{O_2}\) in the presence of nystatin and the inhibited \(Q_{O_2}\) in the presence of ouabain. Each point represents a single determination of \(\Delta Q_{O_2}\). The data were obtained from four tubule preparations. The line was drawn by eye.

An example of the effect of nystatin upon respiration and cellular K\(^+\) distribution is shown in Fig. 5. At 150 mM extracellular Na\(^+\) and 5.2 mM K\(^+\), the addition of nystatin caused about a doubling of the normal rate of respiration and resulted in a depletion of intracellular K\(^+\), which was recorded as an increase

FIGURE 7. The ouabain-sensitive nystatin-stimulated rates of oxygen consumption of proximal tubules suspended in different concentrations of extracellular Na\(^+\) and either 5 or 50 mM K\(^+\). The protocol and solutions were identical to those used in Fig. 6, except that the additional KCl was substituted for NaCl. The data were obtained from a single preparation of tubules and represent a typical experiment (n = 3). Each point represents a single determination of \(\Delta Q_{O_2}\). The lines were drawn by eye.
in the extracellular K* concentration that was monitored by a K ion-selective electrode. Thus, the response of the Na pump to various concentrations of intracellular Na* at exceptionally low concentrations of intracellular K* was characterized by measuring the ouabain-sensitive nystatin-stimulated rate of oxygen consumption of tubules incubated in the presence of different concentrations of Na* and \( \sim 5 \) mM K*. As shown in Fig. 6, the \( K_{0.5} \) of the pump for intracellular Na* was decreased to \( \sim 10-15 \) mM when the intracellular K* was reduced in this manner. This value is about one-third to one-half of the concentration that was necessary to half-maximally stimulate the pump at larger concentrations of K* (see Fig. 2). An additional demonstration of the K effect was achieved by comparing the ouabain-sensitive nystatin stimulation of respiration in the presence of 5 and 50 mM K, since the latter condition will produce a larger intracellular K concentration. As shown in Fig. 7, the response in the presence of 50 mM K was shifted to the right of the response obtained in the presence of 5 mM K. Thus, the Na pump of the rabbit proximal tubule displayed a marked sensitivity to the intracellular K* concentration in a manner similar to the red cell. Presumably this effect was due to the competition of K* and Na* at the Na* transport site of the Na,K-ATPase (Garay and Garrahan, 1973). Taken together, the similarity between the effects and the \( K_{0.5} \) values illustrated in Figs. 2 and 6 and those observed in Fig. 4 suggests that under the appropriate conditions the kinetics of the Na pump in the "intact" proximal tubule epithelium were remarkably similar to those of the Na,K-ATPase enzyme.

Dependence of the Na Pump on Extracellular K and the Na,K-ATPase on K

The dependence of the Na pump rate on the extracellular K* concentration was examined by measuring the incremental respiratory stimulation \( (\Delta Q_{O_2}) \) upon the addition of different concentrations of KCl to a tubule suspension incubated in a 150-mM Na*/K*-free solution. This is shown in Fig. 8 along with the hydrolytic Na,K-ATPase activity that was measured in the presence of different concentrations of K* (and 140–150 mM Na*) using membranes obtained from a different suspension of proximal tubules. There is an excellent correlation between the two different measurements of Na,K-ATPase activity; indeed, the two functions are nearly superimposable. The \( K_{0.5} \) is \( \sim 1.3 \) mM in both cases. This finding is similar to that obtained by Harris et al. (1982), who demonstrated the similarity of the incremental respiratory response \( (\Delta Q_{O_2}) \) to the initial rate of K* uptake (measured by an extracellular K*-sensitive electrode) upon the addition of different concentrations of KCl to tubules exposed to 150 mM Na*/K*-free medium. Taken together, these complementary findings indicate that there is an extremely tight coupling between the Na,K-ATPase and the mitochondria in the proximal tubule at different levels of Na pump activation by extracellular K.

Stimulation of the Na Pump by Extracellular Monovalent Cations

The relative ability of various external monoivalent cations to stimulate the Na pump in tubules exposed to K*-free media was examined by measuring the incremental respiratory stimulation produced when the cations were added as a bolus to produce a 5-mM extracellular concentration. In these experiments,
Figure 8. The K⁺ concentration-dependent stimulation of the rates of oxygen consumption (filled circles) of proximal tubules initially suspended in K-free (150 mM Na⁺) solution. Also shown is the K⁺ concentration dependence of the Na,K-ATPase activity (open circles) of lysed proximal membranes. The ΔQo₂ value represents the difference in the Qo₂ before and after the addition of a bolus of KCl sufficient to render the concentrations shown on the abscissa; each point represents one to four determinations obtained from two tubule preparations. Membranes for the measurement of Na,K-ATPase activity were obtained from a single tubule preparation. The Na,K-ATPase activity was determined in the presence of 20 mM Tris-Cl, 0.125 mM EGTA, 5 mM ATP·Na₂ (vanadium-free), 5.5 mM MgCl₂, and 140 mM NaCl. Equimolar KCl was substituted for NaCl. Samples were assayed in triplicate. Other procedures were identical to those described in Fig. 4. The 100% values were as follows: Na,K-ATPase, 283.7 nmol Pᵢ/mg protein-min; ΔQo₂, 27.1 ± 0.9 (4) nmol O₂/mg protein-min.

Separate aliquots of the tubule suspension were washed and incubated in a K⁺-free solution and apportioned into three chambers, one of which received a bolus of KCl and the chloride salt of one of the cations. As shown in Fig. 9, the relative stimulation sequence was: K⁺ > Rb⁺ > NH₄⁺ > Cs⁺ > Li⁺. The incremental

Figure 9. Incremental stimulations of the rate of oxygen consumption induced by the 5-mM addition of monovalent cations to a suspension of proximal tubules suspended in nominally K-free solution (solution B, Table II). The difference in the Qo₂ before and after the addition of the cations was compared with the K⁺ response that was obtained concurrently in a separate chamber with an aliquot of the same suspension (see text). The data were obtained from a single preparation of tubules. The mean value for the K⁺-induced stimulation was 26.0 ± 0.2 (9) nmol O₂/mg protein-min.
stimulations were calculated as the differences between the rate of oxygen consumption during K⁺-free exposure and the stimulated initial rate of oxygen consumption upon the addition of the cation.

Dependence of Steady State Na Pump Activity on Extracellular K: Effect of Intracellular Na

To examine the activity of the Na pump under steady state conditions at various concentrations of extracellular K⁺, the ouabain-sensitive rate of oxygen consumption was measured in the presence of different extracellular K⁺ concentrations. The tubules in this study were incubated for ~15 min in the presence of different concentrations of extracellular K⁺ before the measurement of oxygen consumption. Thus, unlike the transitional response (ΔQₒₒ) represented in Fig. 8, in which the extracellular K⁺ was acutely varied, these conditions may approximate the in vivo response of the Na pump to different concentrations of extracellular K⁺. The results are shown in Fig. 10, along with the chemical Na⁺ and K⁺ tissue contents that were measured in the same experiments. Under these conditions, the steady state Na pump activity was maximal in the presence of ≥1 mM K⁺ concentrations, and half-maximal activity was attained in the presence of ~0.4 mM K⁺.

Although these results appear to indicate that the Kₒₒ of the Na pump for extracellular K⁺ under steady state conditions was altered (compare Figs. 8 and 10), the apparent change is due to differences in the concentration of intracellular Na⁺ in the two conditions. The stimulatory responses produced by K⁺ shown in Fig. 8 were obtained using tubules in which the Na pump was initially saturated with intracellular Na⁺ but deficient in extracellular K⁺, and thus extracellular K⁺ was the singular modifier of pump activity. For the results shown in Fig. 10, the Na pump was saturated with intracellular Na⁺ only at very low concentrations of extracellular K⁺ and the low Na pump activity was due mainly to the low extracellular K⁺ concentration. However, in the presence of a larger (up to 1 mM) extracellular K⁺ concentration, the greater activity of the Na pump maintained a subsaturating intracellular Na⁺ concentration, and thus the pump activity was limited by both the extracellular K⁺ and the intracellular Na⁺. In the presence of ≥1 mM K⁺ concentrations, the steady state activity of the Na pump was limited only by the intracellular Na⁺ concentration, such that an increase in intracellular Na⁺ (e.g., by exposure to nystatin) would result in a stimulation of the Na pump. Thus, the results obtained under steady state conditions indicate that under physiological conditions the Na pump activity of the proximal tubule remains constant between 1 and 10 mM extracellular K⁺.

Ionic Dependence of the State 3 Response

The effect of varying intracellular cationic concentrations on the ability of the renal mitochondria to respond to saturating concentrations of ADP (the state 3 response) was tested. The state 3 respiratory response of digitonin-treated tubules was measured as a function of reciprocal changes made in the incubation medium: the K⁺ concentration was decreased as the Na⁺ concentration was increased, so that the sum of the two cations was kept constant at 150 mM. Digitonin is a
Figure 10. The steady state ouabain-sensitive rates of oxygen consumption (lower figure) and the Na and K ion contents (upper figure) of proximal tubules suspended in the presence of different concentrations of K⁺. The tubules were equilibrated for ~15 min at 37°C in solutions containing different concentrations of K⁺ (solution A, with KCl substituted for NaCl). A sample was then taken for the intracellular and extracellular electrolyte determination, and the \( Q_{O_2} \) was measured first in the absence and then in the presence of 10⁻⁴ M ouabain. The abscissa represents the extracellular K⁺ concentration that was measured by atomic absorption spectrophotometry. The tissue ion contents that are shown were corrected for trapped extracellular ions by assuming an extracellular volume of 2 \( \mu l/mg \) protein (Soltoff and Mandel, 1984a). Each point represents a single determination of the ion content and the average of two determinations of the \( Q_{O_2} \). The lines were drawn by eye.

detergent that selectively permeabilizes cholesterol-containing membranes, such as the plasma membrane, but does not significantly affect the mitochondria. Thus, after digitonin treatment the extramitochondrial milieu should be quite similar to the extracellular incubation medium. As shown in Fig. 11, there was a decline in the state 3 response by ~25% as the Na⁺ was increased to 140 mM. These results are in agreement with isolated mitochondria studies, which demonstrated that the presence of K⁺ was necessary for optimal respiration and that high concentrations of Na⁺ had some inhibitory effects on mitochondrial respiration (Pressman and Lardy, 1952, 1955; Weiner, 1980).
The results from this study include the following. (a) The Na pump of the rabbit proximal tubule is not saturated with intracellular Na\(^+\) when tubules are exposed to physiological and subphysiological concentrations of extracellular Na\(^+\). (b) In the presence of a saturating concentration of intracellular Na\(^+\), the \(K_{0.5}\) of the Na pump for extracellular K\(^+\) (1.3 mM) is equivalent to the \(K_{0.5}\) of the Na,K-ATPase hydrolytic activity in the presence of a high concentration of Na\(^+\). (c) The \(K_{0.5}\) of the Na pump for Na\(^+\) depends on the intracellular K\(^+\) concentration: it ranges from \(\sim 10-15\) mM in the presence of very low K\(^+\) to \(\sim 30\) mM in the presence of higher K\(^+\). The Na,K-ATPase hydrolytic activity responded qualitatively in the same manner to these ionic conditions.

These results support the identical nature of the Na pump and the Na,K-ATPase in the proximal tubule. They also demonstrate the importance of recognizing all the variables that affect the Na,K-ATPase (e.g., the effect of intracellular K\(^+\) on the dependence of the Na pump on intracellular Na\(^+\)).

Several previous investigators have focused their attention on the luminal entry of Na\(^+\) in the proximal tubule and its dependence on the external Na\(^+\) concentration. The replacement of Na\(^+\) in the bath and perfusate with choline, tetramethylammonium, or lithium caused fluid reabsorption to fall to near zero in the isolated perfused rabbit proximal tubule (Burg and Green, 1976). In micropерfusion studies of the rat proximal tubule, it was reported that Na\(^+\) flux did not vary between 155 and 300 mM external Na\(^+\) (Baldamus et al., 1969). In the rabbit proximal tubule, net Na\(^+\) transport was constant between 115 and 150 mM external Na\(^+\); however, it was decreased at concentrations of 80, 180,
and 220 mM (Corman et al., 1980). In the present study, Na" entry (characterized by the ouabain-sensitive $Q_{0s}$) was found to be a saturable function of the extracellular Na", with a $K_{0.5}$ of ~30 mM Na" (Fig. 1). This observation is very similar to that made for the perfused Necturus proximal tubule by Spring and Giebisch (1977), who found that net fluid reabsorption and net Na" flux were saturable functions of the perfusate Na" concentration, with a $K_m$ of 35-39 mM. Spring and Giebisch also reported that net fluid reabsorption and Na flux were linearly dependent on the intracellular Na" concentration with no indication of saturation; however, these authors only examined variations in intracellular Na" up to ~45 mM. In the present study, the Na pump activity was an S-shaped function of the presumed intracellular Na" (see Fig. 2), which saturated at ~80-100 mM. The present studies agree with those of Györy and Lingard (1976) in rat proximal tubule, for which a saturating sigmoidal dependence was also observed.

A comparison of Figs. 2 and 6 with Fig. 4 indicates that the Na pump activity of the proximal tubules in suspension and the Na,K-ATPase activity of lysed proximal membranes had a similar dependence on Na", and that K" (at the internal Na" site) affected each activity in the same way. Similarly, in the red blood cell the $K_{0.5}$ of the Na pump for intracellular Na" in red cell ghosts was close to that obtained for the $K_{0.5}$ of the Na,K-ATPase enzymatic activity measured in a broken cell preparation (Post et al., 1960), and intracellular K" inhibited the Na pump activity in cells with low intracellular Na" concentrations (Garay and Garrahan, 1975; Knight and Welt, 1974). Thus, the intracellular Na"/K" ratio, as well as the absolute intracellular Na" concentration, plays an important role in determining the activity of the Na pump in both the red blood cell and the renal proximal tubule.

The dependence of the Na pump on intracellular Na shown in Fig. 2 is quantitatively accurate only if the abscissa represents both the intracellular and the extracellular Na concentrations. Assuming that the cytosolic compartment is isotonic with the extracellular compartment, the fact that there is cellular K present during exposure of the tubules to K"-free solution (Fig. 3) indicates that intracellular Na did not fully equilibrate with extracellular Na. The true dependence on intracellular Na would then be represented by a curve shifted to the left of the one shown in Fig. 2. It was calculated above that the remaining K was present in an ~30-mM concentration. However, in the previous paper (Soltoff and Mandel, 1984a), it was suggested that a portion of the extracted K content is compartmentalized within the mitochondria, which would mean that the true remaining cytosolic K was ~30 mM. Thus, the maximum shift in the curve shown in Fig. 2 would be 20% (30 mM out of 150 mM), but it may actually be much less.

The $K_{0.5}$ value of ~30 mM intracellular Na" for the Na pump of the proximal tubule is high compared with values of 10-12 mM for the red cell (Garay and Garrahan, 1973) and even lower values for rabbit urinary bladder (Eaton, 1981). It is not always recognized that the $K_{0.5}$ values for Na" may be very different in different tissues. The greatly different $K_{0.5}$ values for Na" in the red blood cell and the kidney indicate that although they qualitatively respond similarly to
alterations in ionic conditions, the Na,K-ATPase is somehow different in each tissue. It would be of great interest to understand whether this difference was due to the physicochemical nature of the enzyme or to some unknown modifying factor(s).

The K⁺ concentration dependence of the Na,K-ATPase in proximal membrane fragments was nearly identical to that of the Na pump in the intact tubule suspension, as shown in Fig. 8. In both preparations, the internal Na⁺ transport site was exposed to saturating concentrations of Na⁺, but in the tubule suspension this site was additionally exposed to a larger K⁺ concentration (see Fig. 3). The near-superimposability of the two functions may indicate that the external K⁺ concentration in the intercellular clefts was not substantially larger than the concentration that was measured in the bulk external solution under nominally K⁺-free conditions. A large, unmeasurable extracellular K⁺ concentration would produce a shift of the respiratory response curve in Fig. 8 to the left of the enzymatic activity curve. This concentration can be estimated by noting that ouabain reduced the rate of oxygen consumption of tubules in nominally K⁺-free (150 mM Na⁺) media by ~5 nmol O₂/mg protein-min. This decrement was ~15% [5/(28 + 5)] of the calculated full respiratory response of the addition of 5 mM K⁺ to these tubules. From Fig. 8, this percentage of the Na,K-ATPase enzymatic activity corresponds to a K⁺ concentration of ~0.3 mM, which is very close to the amount that was measured in the bulk extracellular solution for the intact tubules. The \( K_{0.5} \) of both the Na pump and the Na,K-ATPase activity for (extracellular) K⁺ was ~1.3 mM, which is similar to previous reports for renal preparations (Braughler and Corder, 1977; Wheeler and Whittam, 1962; Doucet et al., 1979), intact proximal tubules in suspension (Harris et al., 1982), and perfused rabbit proximal tubules (Sasaki et al., 1983).

The respiratory stimulation of tubules exposed to a nominally K⁺-free medium upon exposure to various cations shown in Fig. 9 was an attempt to determine the preference of the external K⁺ site for monovalent cations. No effort was made to measure the \( K_{0.5} \) or \( V_{\text{max}} \) to determine the relative affinity or activity of the pump for these agents. The effect of various cations on the Na pump of red blood cells produced different rankings for the \( K_m \) and \( V_{\text{max}} \) values. The preference for enzyme activity was NH₄⁺ > K⁺ > Rb⁺ > Cs⁺ > Li⁺, but in order of the highest affinity it was K⁺ > Rb⁺ > NH₄⁺ > Cs⁺ > Li⁺ (Skou, 1960). Thus, the order of the response of the proximal tubules, which was K⁺ > Rb⁺ > NH₄⁺ > Cs⁺ > Li⁺, refers only to the specific conditions under which the experiment was performed, that is, the relative Na pump activity produced by a 5-mM extracellular concentration of these cations.

Accurate chemical measurements of the intracellular Na⁺ concentration in these tubules are very difficult to obtain because the open tubular lumens trap a large volume of extracellular fluid, and conventional extracellular isotopic marker techniques are imprecise (Soltoff and Mandel, 1984a). To overcome these difficulties, an indirect method was used to estimate the intracellular Na⁺ concentration under physiological conditions (150 mM extracellular Na⁺). In this method, the ouabain-sensitive \( Q_{\text{O}_{2}} \) of unknown intracellular Na⁺ concentrations (Fig. 1) was compared with the \( Q_{\text{O}_{2}} \) response of known Na⁺ concentrations
The ouabain-sensitive KCl-stimulated rates of oxygen consumption of proximal tubules suspended in different concentrations of Na⁺. The data shown (upper curve) are the ouabain-sensitive component of the data shown in Fig. 2. Also shown (lower curve) is the steady state ouabain-sensitive Q₀ of cortical tubules suspended in different concentrations of Na⁺, redrawn from Fig. 1. The saturation value of the latter curve may be extrapolated to a point on the former curve that corresponds to a Na⁺ concentration of ~35 mM. See text for further information.

(Fig. 2). However, for the latter function, instead of using the transitory stimulation of oxygen consumption produced by the addition of KCl to tubules exposed to a K⁺-free medium (i.e., K⁺-stimulated Q₀ minus K⁺-free Q₀, shown in Fig. 2), the ouabain-sensitive component was used (i.e., K⁺-stimulated Q₀ minus ouabain Q₀). As shown in Fig. 12, this comparison indicated that the intracellular Na⁺ concentration of proximal tubules exposed to physiological concentrations of extracellular Na⁺ may be ~35 mM.

The results of this kind of analysis must remain speculative since it is based on the assumption that the extracellular Na⁺ concentration reflected by the K⁺-stimulated Q₀ is an accurate representation of the intracellular Na⁺. As discussed earlier, residual active transport activity will produce lower concentrations of intracellular Na⁺ than the values presumed in Figs. 2 and 12, and this would shift the activity curve for “known” values of intracellular Na to the left. However, the protocol used to obtain this curve caused the reduction of the K content of the tubules below physiological levels. A larger K concentration would shift this curve to the right in Fig. 12 because of the competition between intracellular K and Na. These two effects may cancel one another, such that the value that was calculated (35 mM) by this approach may be a fairly good estimate of the physiological Na concentration of the tubules in suspension.

In summary, the ionic dependence of the Na pump in the intact tubule is similar to the Na,K-ATPase hydrolytic activity of broken proximal tubule membranes when relevant ionic factors are recognized. In contrast, in the next paper (Soltoff and Mandel, 1984b), the behavior of the intact tubule was not identical to that observed in broken membranes when the ATP dependence was examined, probably because not all relevant cytosolic factors controlling Na pump activity at the ATP site are known.
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