Relation between Na-K-ATPase activity and respiratory rate in the rat kidney

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Silva, Patricio, Jorge Torretti, John P. Hayslett, and Franklin H. Epstein. Relation between Na-K-ATPase activity and respiratory rate in the rat kidney. Am. J. Physiol. 230(5), 1432-1438, 1976. - The relation between Na-K-ATPase activity in homogenates of rat kidney and oxygen consumption in kidney slices was studied by employing different physiological maneuvers known to change the activity of renal Na-K-ATPase. Treatment of euthyroid rats with 3,5,3'-triiodo-1-thyronine increased Na-K-ATPase activity, sodium-dependent oxygen consumption (QO2[t]), and para-aminohippurate (PAH) accumulation by kidney slices without changing glomerular filtration rate or net sodium reabsorption by the intact kidney. Treatment with methylprednisolone also increased Na-K-ATPase, QO2[t], and PAH transport. Chronic potassium loading, on the other hand, increased renal Na-K-ATPase to the same degree as the first two procedures, but QO2[t] and PAH accumulation were unchanged. Partial nephrectomy induced an increase in the activity of Na-K-ATPase in homogenates of the remaining kidney fragment, but QO2[t] did not change significantly and PAH uptake was unaltered. An increase in the activity of Na-K-ATPase in kidney homogenates is therefore not necessarily associated with a parallel change in oxygen consumption by the intact cell.

oxidaive metabolism is linked to the rate of ATP consumption by a series of feedback mechanisms that are in direct relation with the ratio of ATP/ADP. The hydrolysis of ATP by Na-K-ATPase and other ATPases produces ADP which in turn regulates the oxidative metabolism of the mitochondrion and regeneration of ATP (24, 41).

The active transport of sodium and potassium is coupled with and depends on metabolism. This dependence has been widely illustrated by the reduction in electrolyte transport found in various tissues after exposure to metabolic inhibitors (11, 26, 34, 38-40). The tight coupling between electrolyte transport and metabolism is further exemplified by the changes in tissue metabolism that attend experimental alterations of electrolyte transport. When the rate of electrolyte transport is reduced by omitting sodium or decreasing its concentration in the solution bathing tissue slices or the mucosal side of the toad bladder, the oxidative metabolism of these tissues is also reduced (3, 12, 27). The converse is also true; when electrolyte transport is stimulated, the rate of metabolism rises as well (12, 13, 16, 28, 35).

The linkage between cellular metabolism and ion transport mediated by Na-K-ATPase has evoked the suggestion that a primary increase in the amount of "transport enzyme" per cell (as measured by the activity of cellular homogenates) might itself stimulate an increase in cellular respiration. Such a sequence has been postulated to account for the calorigenic action of thyroid hormone (16). On the other hand, an increase in the activity of Na-K-ATPase in tissue homogenates might not necessarily be reflected in the rate of Na-K-dependent hydrolysis of ATP in intact cells and hence in cellular respiration or heat production.

In the present series of experiments, the relation between oxidative metabolism and Na-K-ATPase was studied further. The rat kidney was chosen because it has a high content of Na-K-ATPase that can be readily modified by diverse experimental manipulations.

METHODS

Male Sprague-Dawley rats weighing 140–200 g were used for all the experiments. The animals were kept two to a cage and allowed free access to food and water unless otherwise specified. The animals were divided into four experimental groups. Group I was composed of rats receiving 3,5,3'-triiodo-1-thyronine (T3) (Sigma Chemical Company, St. Louis), dissolved in 0.5 mM NaOH, 50 µg/100 g body weight ip, every 48 h, for a total of five doses. The animals were studied 24 h after the last injection. Controls, run simultaneously, received intraperitoneal injections of equal volume of 0.5 mM NaOH. Group II rats received intramuscular injections of methylprednisolone (Depo-Medrol, Upjohn, Kalamazoo, Mich.) 3 mg/100 g body wt, daily, for 3 days. The animals were studied 24 h after the last dose. Controls, run simultaneously, received intramuscular injections of saline. Group III was composed of rats whose renal mass was reduced by approximately 75%. This was performed as a two-step procedure under ether anesthesia. A subcapsular purse-string suture was placed around the middle section of the left kidney, taking care to avoid the hilar structures, and tightened in place. The inferior section of the kidney was then severed with a razor blade. The following day the right
kidney was removed. Sham-operated rats served as controls. The animals were studied 7 days after the right nephrectomy. Group IV rats were fed a high-potassium diet for a period of 7 days (diet composition: sucrose 43%, casein 26%, lard 9%, corn oil 4%, vitamin diet fortification mixture 1.8%, normal mineral mixture 2.7%, KCl 13%, NaCl 0.5%) and in addition were given 0.1 M KCl to drink. Pair-fed controls receiving a normal diet were studied simultaneously.

Na-K-ATPase assay. The kidneys were removed under light ether anesthesia and placed in ice-cold 0.9% saline. The kidneys were then cleaned, stripped of their capsules, and weighed. A sagittal hemisection was performed and the cortex, red medulla, and white medulla (papilla) were identified and dissected with a pair of fine scissors, discarding the tissue adjacent to the border, and returned to the ice-cold saline. When all kidneys were dissected, the pieces of cortex and red medulla were lightly blotted with filter paper, weighed, and then homogenized with a Teflon pestle in a glass homogenizer immersed in ice in a 20/1 (vol/wt) solution containing 0.25 M sucrose, 6 mM EDTA, 20 mM imidazole, and 2.4 mM sodium deoxycholate (added just before use), pH 6.8. The homogenate was then filtered through a double layer of surgical gauze, and after 45 min, assayed for enzyme activity. Na-K-ATPase activity was defined as the amount of inorganic phosphate liberated in the presence and absence of potassium, corrected for the spontaneous nonenzymatic breakdown of ATP. Total ATPase was determined in a 5-ml reaction mixture containing NaCl 100 mM, KCl 20 mM, imidazole buffer pH 7.8, 10 mM, MgCl2 6 mM, ATP disodium salt (Sigma Chemical Company), 6 mM, and enzyme suspension enough to bring the final protein concentration to 0.005-0.01 mg/ml. The reaction was started by the addition of ATP and MgCl2, shaken in a constant temperature water bath at 37°C for 15 min, and stopped by the addition of 1 ml of 35% (wt/vol) trichloroacetic acid. After centrifugation the inorganic phosphate liberated was measured by the method of Fiske and SubbaRow (8), the optical density reading at 660 µm in a 300N Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) equipped with a quick-sampling cell. The activity of the enzyme was expressed as micromoles of inorganic phosphate liberated per milligram of protein per hour. Determination of protein in the homogenates was carried out according to the method of Lowry, Rosebrough, Farr, and Randall (29) using crystalline bovine albumin (Sigma Chemical Company) dissolved in homogenizing solution as a standard.

Oxygen uptake studies. Different animals from those used for the Na-K-ATPase assay but otherwise similarly treated were used. The animals were sacrificed one at a time and the kidneys removed, placed on a 0.9% saline-moistened filter paper on top of a petri dish cover, sitting on crushed ice. Free-hand kidney cortex slices were obtained from both kidneys and placed in ice-cold oxygenated Krebs-Ringer-phosphate. Half of the slices were used for the PAH uptake studies. Approximately 20 min later, the slices were transferred to regular Warburg flasks containing 3 ml of Krebs-Ringer-phosphate with 10 mM glucose, 0.2 ml of 10% KOH in the center well, and gassed with O2. The slices were allowed to equilibrate for 30 min under constant agitation at 37°C in a Branowi respirometer, and recordings of the pressure changes were made at 20-min intervals for the following 2 h. In some experiments sodium was left out of the incubation media to inhibit the Na pump, maintaining osmotic activity with choline chloride. The slices were then removed from the flasks, lightly blotted with filter paper, and weighed in a torsion balance. The results were expressed as microliters of O2 consumed per gram of wet tissue weight per hour.

\( p \)-Aminohippurate uptake. The method used here was essentially the same as that described by Cross and Taggart (4). The freshly prepared slices were kept in ice-cold oxygenated Krebs-Ringer-phosphate for approximately 30 min. The slices were then transferred to 25-ml beakers containing 3 ml of Krebs-Ringer-phosphate (NaCl 110 mM, KCl 4 mM, MgSO4 1 mM, CaCl2 1 mM, phosphate buffer pH 7.4 20 mM, and PAH 7.4 × 10^{-3} M) with an oxygen gas phase and placed in a Dubnoff metabolic shaking incubator at 37°C for 60 min.

Following incubation, all slices were immediately blotted on filter paper, weighed, and transferred to conical glass homogenizer tubes containing 5 ml of 10% TCA. PAH was determined as described by Smith et al. (37), and the slice/medium (S/M) ratio for PAH is reported.

Clearance studies. Animals prepared in the same way but different from those employed for the above studies were used. The animals were anesthetized with Inactin (Fromonta) 100 mg/kg body wt ip. Tracheostomy was performed and a PE-50 polyethylene catheter was inserted into the right external jugular vein, right carotid artery, and urinary bladder. Immediately after surgery, 3% of the body weight of 0.9% NaCl was given over 20 min, followed by a priming dose of 10 µCi of [carboxyl-14C]inulin (New England Nuclear Corp.) in 0.2 ml of saline. A sustaining infusion of 0.02 ml/min containing 10 µCi of [14C]inulin per milliliter was continued for the duration of the experiment. Thirty minutes after the priming dose of inulin, three 10-min consecutive clearance periods were collected. Blood withdrawn through the carotid artery at the midpoint of each period was analyzed for radioactivity, sodium, and potassium. Urine was collected in 1-ml pipettes graduated in 1/100 and analyzed for radioactivity, sodium, and potassium. The results were pooled and averaged for each animal.

All results are expressed as the mean ± standard error. Statistical comparison was made using the Student's t test wherever applicable.

RESULTS

3,5,3'-Triiodo-1-thyronine studies (group I, Table 1). A dose of T3 of 50 µg/100 g every other day for a total of 5 times produced an increase in Na-K-ATPase activity of 21 and 24% above control values in whole homogenates of cortex and outer medulla of the kidney (11.8 ± 0.7 vs. 14.3 ± 0.2, P < 0.01 and 28.4 ± 2.0 vs. 35.2 ± 1.6, P < 0.01, respectively). A similar increase in the activity of this enzyme in whole homogenates of rat kidney after only three injections of equal amounts of T3 has been
previously reported (16). In that study, however, no association of the enzyme preparation to plasma membrane induction in the enzyme was present after further purification of the enzyme preparation to plasma membrane fractions.

The activity of Mg-ATPase, another membrane-bound enzyme, remained unchanged after treatment with T3 (control 44.1 ± 1.1 and 47.5 ± 2.3; T3 treated 42.0 ± 1.8 in cortex and medulla, respectively). This is in agreement with the report referred to above (16), which demonstrated a selective increase in other membrane-bound enzymes, Mg-ATPase and 5'-nucleotidase.

The in vivo administration of thyroid hormones causes an increase in oxygen consumption of kidney slices that can be measured in vitro (2). In keeping with this effect, the administration of T3 in these experiments was accompanied by a 23% increase in the oxygen uptake of renal cortex slices (control 456.9 ± 8.2; T3 treated 561.2 ± 9.7, P < 0.001). As shown in Table 2 the Na-dependent oxygen uptake, as measured by removing the Na from the incubation medium, accounts for 50% of the increase in uptake induced by T3, the remainder being non-Na dependent.

Thyroidectomy has been shown to decrease the PAH uptake of rat kidney cortex slices (7, 32). T3 administered in vivo, on the other hand, is capable of increasing the S/M ratio of PAH in weanling rats (14) while competing for its transport in vitro. In the present study, we found that T3 is also able to stimulate the transport of PAH in adult rats. An increase of almost 70% in the S/M ratio was observed, as depicted in Table 3.

Reg. medium 456.9 ± 8.2 561.2 ± 9.7 104.3 < .001
(6) (6)

No Na+ medium 416.6 ± 14.3 464.3 ± 40.7 47.7 NS
(6) (6)

P < .01 < .01

Values are μl O2/g wet wt per h ± SE. Numbers in parentheses are numbers of observations. Dose of T3 as in Table 1.

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T3 has been reported to increase the glomerular filtration rate and sodium reabsorption in rats (20) and sodium transport in isolated toad bladders (10, 31). In the present experiments, however, T3 in a dose that stimulated Na-K-ATPase activity in the cortex and outer medulla of the kidney did not modify glomerular filtration rate or sodium transport (Table 4). Since developing animals respond more readily to the stimulation by thyroid hormones, the same clearance studies were conducted in a group of weanling rats. As in adult rats, no change in either GFR or tubular sodium reabsorption per gram of wet kidney weight was found (Table 4).

Methylprednisolone studies (group II). Previously published studies from this laboratory have shown that methylprednisolone is a very powerful stimulator of the activity of renal Na-K-ATPase at slightly lower dosage than the one used in the present study (18). As in those studies, the activity of Na-K-ATPase in the present series of experiments (Table 1) rose by 37 and 47% in whole homogenates of kidney cortex and outer medulla, respectively (control vs. methylprednisolone: 12.1 ± 0.7
vs. 16.6 ± 0.6, \( P < 0.01 \) and 28.3 ± 1.8 vs. 41.7 ± 1.2, \( P < 0.01 \)). Again, this increase in the activity of Na-K-ATPase was not accompanied by a concomitant change in Mg-ATPase (control: 41.3 ± 1.6 and 50.9 ± 3.4; methylprednisolone 43.9 ± 1.0 and 52.6 ± 1.1).

The effect of chronic stimulation with methylprednisolone is not limited to increasing the specific activity of Na-K-ATPase. An increase of similar magnitude, 40%, vs. prednisolone is not limited to increasing the specific activity of Na-K-ATPase. An increase of similar magnitude, 40%, again, this increase in the activity of Na-K-ATPase was not accompanied by a concomitant change in Mg-ATPase (control: 41.3 ± 1.6 and 50.9 ± 3.4; methylprednisolone 740.2 ± 27.5, \( P < 0.0005 \), Table 5). Removing sodium from the medium is accompanied by a significant reduction in oxygen utilization of about 50%, indicating that, as for \( T_3 \), half of the increase in oxygen uptake induced by methylprednisolone is dependent on sodium transport.

In parallel with the increase in Na K-ATPase and renal cortical oxygen consumption, methylprednisolone stimulated the uptake of PAH by kidney cortex slices by about 32%, as shown in Table 3.

Glomerular filtration rate and net sodium reabsorption are greatly increased after the chronic administration of methylprednisolone (5, 18). This effect is clearly shown in Table 4 where methylprednisolone is shown to increase glomerular filtration rate and sodium reabsorption by 33 and 32%, respectively.

Compensatory hypertrophy studies (group III). After renal mass is reduced, the remaining kidney tissue undergoes compensatory growth, whereas glomerular filtration rate and tubular reabsorption of sodium increase per unit of remaining kidney (18, 19, 21). Na-K-ATPase activity rises in close association with the increase in the rate of sodium reabsorption (18). In the present series of experiments, animals subjected to experimental conditions similar to those previously reported from this laboratory showed a 34 and 24% increase in Na-K-ATPase activity in cortex and outer medulla (control vs. nephrectomized: 12.3 ± 0.7 vs. 15.7 ± 9.0, \( P < 0.005 \), and 30.3 ± 0.9 vs. 39.2 ± 1.4, \( P < 0.005 \), Table 1). There was no change in Mg-ATPase activity. Renal oxygen consumption increases in the contralateral kidney immediately following nephrectomy and slowly returns to normal after a period of approximately 10 days when the remaining renal tissue has hypertrophied (6). In the present series of experiments, a very modest, albeit significant, increase of 10% in oxygen uptake by kidney cortex slices was observed 7 days after nephrectomy, at a time when reabsorptive sodium transport, total oxygen consumption, and the activity of Na-K-ATPase in kidney homogenates are still elevated (Table 3 and 4), the uptake of p-aminohippuric acid by kidney cortex slices of remaining renal tissue was not different from slices obtained from control animals.

Potassium loading studies (group IV). Potassium loading is associated with an increase in Na-K-ATPase activity in whole homogenates of kidney cortex and outer medulla (36). In the present series of experiments, these studies were repeated and the results are included in Table 1. Potassium loading for 1 wk increases the activity of Na-K-ATPase by 30% in the cortex and 29% in the outer medulla. This increase is limited to the Na-K-ATPase fraction, without changes in the Mg-ATPase fraction.

Oxygen consumption in kidney cortex slices of rats subjected to potassium loading was not different from that of control animals (Table 7). No change in the p-enzyme Na-K-ATPase and respiratory rate.

\[ 75\% \text{ reduction in renal mass (Table 6). Removing sodium from the incubation medium results in a reduction in oxygen uptake. Unlike the two preceding models, however, the sodium-dependent oxygen uptake (QO}_2(\text{I}) \text{ was not significantly affected by compensatory hypertrophy.} \]

The uptake of p-aminohippuric acid by cortex slices of hypertrophying kidneys rises rapidly within 48 h after nephrectomy and with equal speed returns to normal (9). In the present experiments performed 1 wk after nephrectomy, at a time when reabsorptive sodium transport, total oxygen consumption, and the activity of Na-K-ATPase in kidney homogenates are still elevated (Table 3 and 4), the uptake of p-aminohippuric acid by kidney cortex slices of remaining renal tissue was not different from slices obtained from control animals.

### Table 5. Effect of potassium loading on oxygen uptake by rat kidney cortex slices

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>K⁺ loaded</th>
<th>Δ</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg. media</td>
<td>467.8 ± 10.0</td>
<td>495.8 ± 21.5</td>
<td>28.0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Na⁺ media</td>
<td>415.6 ± 22.9</td>
<td>434.5 ± 16.6</td>
<td>18.9</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QO₂[t]</td>
<td>52.2 ± 18.0</td>
<td>61.3 ± 9.3</td>
<td>0.1</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( P \) < .05 < .025

Values are \( μl \) QO₂/g wet wt per h ± SE. Numbers in parentheses are numbers of observations. Potassium loading as in Table 1.

### Table 6. PAH uptake by rat kidney cortex slices

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_3 )</td>
<td>7.74 ± 0.65</td>
<td>13.01 ± 1.08</td>
<td>&lt; .0025</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td></td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>8.88 ± 0.53</td>
<td>11.76 ± 0.49</td>
<td>&lt; .0005</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td></td>
</tr>
<tr>
<td>Potassium loaded</td>
<td>8.34 ± 0.68</td>
<td>8.80 ± 0.88</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td></td>
</tr>
<tr>
<td>Nephrectomized</td>
<td>8.62 ± 0.58</td>
<td>8.95 ± 0.45</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td></td>
</tr>
</tbody>
</table>

Values are the average S/M ratio ± SE. Numbers in parentheses are numbers of observations. Treatments as in Table 1.

### Table 7. Renal function in \( T_3 \)-treated adult and weanling rats, methylprednisolone-treated rats, and potassium-loaded rats

<table>
<thead>
<tr>
<th></th>
<th>( n )</th>
<th>( O₂K ), min/mg per g Wet Kidney Weight</th>
<th>Tubular Reabsorption of Sodium, ( μg/min ) per g Wet Kidney Weight</th>
<th>Excretion of Potassium, ( μg/min ) per g Wet Kidney Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1.06 ± 0.16</td>
<td>160.6 ± 21.6</td>
<td>0.88 ± 0.28</td>
</tr>
<tr>
<td>( T_3 )</td>
<td>6</td>
<td>1.13 ± 0.30</td>
<td>176.7 ± 41.9</td>
<td>0.56 ± 0.31</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>6</td>
<td>1.46 ± 0.21*</td>
<td>217.5 ± 29.9*</td>
<td>1.01 ± 0.23</td>
</tr>
<tr>
<td>Chronic potassium loading</td>
<td>6</td>
<td>1.24 ± 0.35</td>
<td>195.7 ± 56.0</td>
<td>3.00 ± 2.0*</td>
</tr>
<tr>
<td>Weanling rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>1.05 ± 0.17</td>
<td>160.1 ± 22.8</td>
<td>0.76 ± 0.37</td>
</tr>
<tr>
<td>( T_3 )</td>
<td>6</td>
<td>1.15 ± 0.11</td>
<td>160.1 ± 22.8</td>
<td>0.76 ± 0.37</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( * P < 0.01 \). \( + P < 0.05 \). Treatments as in Table 1.
that of the control animals.

**DISCUSSION**

The active transport of sodium and potassium across the cell membrane is coupled to cell respiration in such a fashion that changes in the active uptake of potassium and extrusion of sodium in slices of kidney cortex are attended by parallel changes in the rats of oxygen consumption (38-40). The respiratory rate of rabbit kidney homogenates has been shown to vary in the same direction as that of Na-K-ATPase after exposure to ouabain or calcium ions (3). Analogous results have been shown in the toad bladder, in which amiloride or ouabain causes reductions in both net sodium transport and the metabolic rate (12, 13, 27). Ouabain has also been shown to decrease both sodium reabsorption and the rate of metabolism in the kidney of intact animals (34). The opposite effect has also been documented. Stimulation of sodium transport in the toad bladder by the addition of vasopressin or aldosterone is associated with a rise in metabolism (12, 13, 28, 40).

Recently, Ismail-Beigi and Edelman (15-17) suggested an important relation between the action of thyroid hormones on cell respiration and the active transport of sodium across cell membranes. These authors found that oxygen consumption of tissue slices and Na-K-ATPase activity in homogenates of liver and kidney varied directly with the change in thyroid status of hypothyroid and euthyroid rats. They concluded that thyroid hormones acted to increase the activity of Na-K-ATPase, and so increased the activity of the sodium pump, thus accounting for their calorigenic action (16). A reasonable correlate of this hypothesis is that an increase in the activity of Na-K-ATPase, as measured in cell homogenates, can be expected to produce an increase in the active and energy-requiring transport of sodium and potassium in intact cells. While this might well be true, there is no a priori reason for it to be the rule. The extent to which the capacity of the transport ATPase is actually utilized in the cell must depend on a number of modifying factors, such as the availability of substrates and ions to the enzyme at its location in the plasma membrane, the possible influence of allosteric inhibitors, etc. The difference in oxygen consumption of tissue slices with and without sodium in the external medium (\(Q_{O_2}[t]\), shown to be equivalent to the difference obtained by adding ouabain to or omitting potassium from the medium (3, 15)), may be taken as a guide to the energy actually expended by transport processes related to Na-K-ATPase. A crucial test would therefore be to increase the activity of Na-K-ATPase in tissue homogenate in a variety of ways and to see if this was invariably accompanied by an increase in \(Q_{O_2}[t]\). In the present series of experiments, this relation was studied using four different physiological maneuvers known to increase the activity of renal Na-K-ATPase.

Administration of triiodothyronine to euthyroid rats increased the specific activity of Na-K-ATPase in both cortex and medulla and also increased the \(Q_{O_2}[t]\) in slices of kidney cortex. Transport of \(p\)-aminophenylurate was increased in kidney slices as well. The extent to which the capacity of the transport ATPase is actually utilized in the cell must depend on a number of modifying factors, such as the availability of substrates and ions to the enzyme at its location in the plasma membrane, the possible influence of allosteric inhibitors, etc. The difference in oxygen consumption of tissue slices with and without sodium in the external medium (\(Q_{O_2}[t]\), shown to be equivalent to the difference obtained by adding ouabain to or omitting potassium from the medium (3, 15)), may be taken as a guide to the energy actually expended by transport processes related to Na-K-ATPase. A crucial test would therefore be to increase the activity of Na-K-ATPase in tissue homogenate in a variety of ways and to see if this was invariably accompanied by an increase in \(Q_{O_2}[t]\). In the present series of experiments, this relation was studied using four different physiological maneuvers known to increase the activity of renal Na-K-ATPase.

Treatment with methylprednisolone is followed in rats by an increase in glomerular filtration rate, increased filtered load of sodium, and higher rates of sodium reabsorption per gram of wet kidney weight. These changes are accompanied by a substantial rise in the specific activity of renal Na-K-ATPase (18). As the present report shows, these changes are also attended by an increase of similar magnitude in the rate of oxygen consumption.

In compensatory renal hypertrophy, the activity of renal Na-K-ATPase rises in step with the increase in sodium reabsorption by the remnant kidney (18, 19). Oxygen consumption by the remaining renal tissue is found to increase after the initial reduction of the renal mass and eventually to return to normal as renal hypertrophy develops, this series of events taking approximately 10 days in the rat (6). In the present experiments, carried out 7 days after nephrectomy, total oxygen uptake showed a small but significant rise. However, despite an increase in cortical Na-K-ATPase of approximately the same magnitude as that produced by T3 or methylprednisolone, the oxygen consumption attributable to sodium transport (\(Q_{O_2}[t]\)) was not significantly affected by compensatory hypertrophy. PAH uptake by slices of kidney cortex was likewise unchanged.

Chronic potassium loading in rats is associated with an adaptive increase in the specific activity of renal Na-K-ATPase of approximately the same magnitude as that following compensatory hypertrophy (Table 1). Nevertheless, respiratory rate was not increased after potassium loading, and PAH uptake was also unchanged.

These results indicate that an increase in Na K ATPase activity in kidney homogenates may or may not be attended by an increase in \(Q_{O_2}[t]\) or \(Q_{O_2}[t]\) of kidney slices. An increase in transport enzyme activity in homogenates of kidney tissue is therefore not a sufficient explanation for the increase in renal metabolic rate that accompanies hyperthyroidism. A distinction should be made between the activity observed in vitro in broken-cell homogenates or membrane fragments and the activity of the enzyme in the intact working cell, organ, or tissue slice. While much evidence links the latter to respiratory or metabolic rate, the former, representing maximum possible enzymatic activity under ideal conditions, need not be so linked.

A possible clue to the present findings may lie with the measurements of PAH uptake. Increases in Na-K-ATPase activity were associated with an increase in transport-associated oxygen uptake when PAH uptake by kidney slices was also increased (as in T3 administration and methylprednisolone treatment). When PAH uptake was unchanged (after potassium loading or 75% nephrectomy), \(Q_{O_2}[t]\) was also unaffected, even though cortical Na-K-ATPase rose. Since the proximal tubule is the only part of the nephron capable of transporting PAH, it seems possible that potassium loading and partial nephrectomy influenced only the distal tubule, whereas T3 and methylprednisolone altered the proximal tubule as well. The bulk of kidney cortex is com-
posed of proximal tubules (25), and it is thus conceivable that changes in distal tubular enzymatic activity, though detectable as an increase in whole homogenates of kidney cortex, might not produce a perceptible increase in respiratory rate of cortical slices. Further evidence along these lines must await the assay of Na-K-ATPase in single-nephron segments by microtechniques.

These experiments also bear on a tangential issue concerning the mechanism of action of thyroid hormone on renal Na-K-ATPase. There has been some difference of opinion as to whether the effect of thyroid hormones on Na-K-ATPase in the kidney is a primary one or secondary to the changes in GFR and sodium reabsorption that occur in myxedema and hyperthyroidism. Katz and Lindheimer (20) suggested that the deficiency in renal Na-K-ATPase in hypothyroidism was an adaptive response to decreased reabsorptive sodium load rather than an effect of the hormone deficiency per se. The present experiments indicate, on the other hand, that when large doses of thyroid hormone are given to either adult or weanling euthyroid rats, renal Na-K-ATPase rises appreciably, even though there is no detectable change in GFR or total sodium reabsorption. It therefore seems reasonable to interpret the effect of hyperthyroidism on renal Na-K-ATPase as a direct effect of the hormone rather than one secondary to any change in the net reabsorptive transport of sodium.

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