The Effect of Ouabain on Thyrotropin-stimulated Respiration of Thyroid Slices*

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Previous studies on isolated thyroid cell membranes (1) revealed the presence of an adenosine triphosphatase (ATPase) activity in these membranes which showed features characteristic of Na⁺ and K⁺ transport by intact cells, and which was enhanced by thyrotropin (TSH). This ATPase activity was stimulated by Na⁺ and K⁺ and required both ions together, was inhibited by ouabain, was Mg⁺⁺-requiring, and hydrolyzed adenosine triphosphate in preference to inosine triphosphate. The hydrolysis of ATP by the cell membranes in the presence of TSH seemed in all respects to represent an augmentation of this "Na⁺-K⁺ pump," and it was suggested that the primary effect of TSH may be on Na⁺ transport in the intact thyroid cell. Wolff and Halmi (2) have likewise demonstrated an Na⁺-K⁺-stimulated, ouabain-sensitive ATPase in the "nuclear" (cell membrane-containing) fraction of thyroid homogenates and have shown that it is enhanced by stimulation in vitro with TSH.

Stimulation in vitro by TSH of the oxygen consumption of thyroid slices has been reported by Paal (3), Anderson and Alt (4), Canzanelli and Rapport (5), and Freinkel (6), and the question arises as to whether this stimulatory effect of TSH on respiration is likewise inhibited by ouabain and dependent upon the presence of sodium. In the present investigation those factors found to influence thyroid cell membrane ATPase were studied in relation to their effect on cellular respiration. It was observed that the responses in cellular respiration closely paralleled changes known to occur in ATPase activity.

EXPERIMENTAL PROCEDURE

Thyroid glands were obtained from freshly slaughtered calves at the abattoir and brought to the laboratory packed in ice. The glands were then freed of connective tissue investments and sliced with a Stadie-Riggs microtome into slices approximately 0.3 mm in thickness. Slices were serially rinsed for several seconds in chilled Krebs-Ringer-phosphate medium, quickly blotted on filter paper, and weighed on a Roller-Smith torsion balance. In the experiments involving respiration in an Na⁺-free medium, the slices were washed four times for 5 minutes each in a solution of Krebs-Ringer-phosphate in which NaCl was replaced by 0.25 M sucrose and sodium phosphate buffer by potassium phosphate buffer. The weighed amounts of tissue slices (250 to 300 mg) were introduced into the main compartment of Warburg vessels containing 2.7 ml of Krebs-Ringer-phosphate medium (0.131 M NaCl, 0.005 M KCl, 0.0012 M MgSO₄, 0.008 M CaCl₂, and 0.010 M sodium phosphate buffer, pH 7.4) with 10 M Na⁺ glucose. This was the standard incubation medium, except where otherwise indicated, and all reagent solutions added were prepared in this medium. TSH ("Thytropin," Armour, Lot X8060) was added before incubation to yield a final concentration of 0.2 U.S.P. unit per ml. All operations before incubation were performed at 0° except the weighing.

For incubation the vessels were gassed with 100% oxygen, and center wells were filled with 2 N NaOH and fluted strips of filter paper. After temperature equilibration at 37° for 15 minutes, manometric readings were obtained at 10 minute intervals.

For all studies the incubations were performed in duplicate. Oxygen consumption was estimated in terms of microliters of O₂ consumed per hour per mg, wet weight. Ouabain was obtained commercially. All other reagents were of reagent grade and were prepared in water purified by passage through ion exchange columns.

RESULTS

Effect of TSH—Table I summarizes the results obtained in the measurement of oxygen consumption of calf thyroid slices in various media with and without TSH present. In 14 experiments with standard medium, a mean of 0.46 μl per mg per hour was obtained, which is slightly higher than the value of 0.39 obtained by Freinkel (6) for sheep thyroid slices. In the presence of 0.2 U.S.P. unit of TSH per ml of the standard medium, the value rose to a mean of 0.79, a mean increase of 72 ± 13% (p < 0.01). This elevation is 4 times the value of 18.7 ± 3% found by Freinkel for sheep thyroid slices respiring in the presence of 0.1 U.S.P. unit of TSH per ml. Respiration was elevated by TSH to approximately the same degree in the absence of glucose.

Cation Requirements—Approximately half of the total ATPase activity found in the isolated thyroid cell membranes was stimulated by Na⁺ and K⁺ ions, and required both ions together: in the absence of one ion this activity could not be demonstrated. The stimulation of this ATPase activity by TSH likewise did not occur in the absence of either ion. Table I shows the oxygen consumption for thyroid slices which were thoroughly washed and incubated in a medium in which Na⁺ was replaced by isotonic sucrose. The exclusion of Na⁺ did not alter O₂ consumption significantly, but no significant elevation occurred in the presence of TSH in this medium. Other slices washed in the same manner and allowed to respire with Na⁺ present showed standard increases in respiration in the presence of TSH.

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1 The abbreviation used is: TSH, thyrotropin.
Effect of Ouabain on TSH-stimulated Respiration

TABLE I

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Medium</th>
<th>Oxygen consumption</th>
<th>Increase with TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µl/mg wet tissue/hr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>+ TSH</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Standard</td>
<td>0.46 ± 0.06</td>
<td>72 ± 13</td>
</tr>
<tr>
<td>4</td>
<td>Standard minus glucose</td>
<td>0.46</td>
<td>68</td>
</tr>
<tr>
<td>4</td>
<td>Standard minus Na⁺</td>
<td>0.44</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>(sucrose)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>40 mM K⁺</td>
<td>0.52</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>100 mM Na⁺</td>
<td>0.58</td>
<td>19</td>
</tr>
<tr>
<td>1</td>
<td>Standard minus Mg⁺</td>
<td>0.44</td>
<td>55</td>
</tr>
</tbody>
</table>

* Standard deviation.

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Fig. 1. The effect of ouabain on the TSH-stimulated respiration of calf thyroid slices in "standard" medium.

At high K⁺ concentrations the absolute amount by which the thyroid cell membrane ATPase was stimulated by TSH was lower than that at 5 mM K⁺. Table I shows that increasing the K⁺ ion concentration while decreasing the Na⁺ ion concentration to maintain a total concentration of 145 mM produced a significantly decreased degree of stimulation of respiration by TSH. The smaller increases parallel the smaller amount of TSH-stimulated ATPase obtained with cell membranes at these concentrations. The exclusion of Mg⁺ did not reduce oxygen consumption significantly with and without TSH. It was not possible to demonstrate a rise in the basic oxygen consumption with increasing concentrations of Na⁺ or a fall in the absence of Na⁺, as has been shown in brain (7).

Effect of Ouabain—The work of Schatzmann (8) and Glynn (9) has shown that the cardiac glycosides, including ouabain, inhibit the active transport of K⁺ by human erythrocytes without affecting glycolysis. Ouabain inhibits the Na⁺-K⁺-stimulated ATPase of the thyroid cell membrane (1, 2) and prevents the stimulation of this activity by TSH (1). In Fig. 1 is seen the inhibition by ouabain of the TSH-stimulated respiration over a wide concentration range. Half-maximal inhibition occurred at about 6 × 10⁻⁷ M ouabain. This is the same value as found for ouabain inhibition of TSH-stimulated ATPase in thyroid cell membranes (1).

It was shown by Wolff and Halmi (2) that increasing levels of K⁺ could reduce the inhibition of "nuclear fraction" Na⁺-K⁺-stimulated ATPase produced by 2 × 10⁻⁷ M ouabain. In the present studies the ouabain inhibition of TSH-stimulated respiration was less in the presence of increasing levels of K⁺, as shown in Fig. 2. The inhibition produced by concentrations of ouabain greater than 10⁻⁶ M did not change in the presence of increasing levels of K⁺.

Time Course of Respiratory Stimulation—The rates of respiration in the control studies were in all cases linear for at least 30 minutes beyond the usual 1-hour incubation period. Fig. 3 shows the rate of respiration for a single thyroid slice, and is representative of the pattern observed in five experiments. At the times indicated, TSH, ouabain, and K⁺ were added. Slight stimulation by TSH was manifest in 10 minutes, and during the 40- to 50-minute interval the rate of oxygen consumption was...
70 ± 12% (standard deviation) greater than the control value in each experiment. The inhibitory effect of ouabain was apparent in the first 5 minutes after its addition, and during the 60- to 70-minute interval the rate of respiration was 31 ± 11% greater than the control rate. In the presence of $5 \times 10^{-7} \text{M}$ ouabain the rate of respiration rose to 62 ± 13% above the control rate in the final 10-minute interval after the addition of K+. This rise did not occur with higher concentrations of ouabain.

DISCUSSION

These studies have been designed to demonstrate that responses in cellular respiration parallel the responses of the Na$^+$-K$^+$-stimulated ATPase which have been previously characterized in isolated thyroid cell membranes. In view of the difficulty in dealing with the cation-rich colloid, no attempt has been made to measure actual fluxes of cations into and out of cells. The response in cellular respiration to TSH stimulation is that which would be predicted from increased rates of ATP hydrolysis with increased levels of the respiratory stimulant ADP regenerated at the cell membrane: (a) TSH stimulates Na$^+$-K$^+$-stimulated membrane ATPase and also produces a marked stimulation in cellular respiration; (b) in the absence of Na$^+$ from the medium, TSH fails to stimulate membrane ATPase or cellular respiration; (c) high levels of K$^+$ reduce proportionally the response of the ATPase and cellular respiration to TSH; (d) ouabain inhibits the TSH stimulation of ATPase and of cellular respiration, and the ouabain concentration for half-maximal inhibition is approximately the same for each; (e) in the presence of increasing levels of K$^+$ the ouabain inhibition of the Na$^+$-K$^+$-stimulated ATPase as well as of the TSH stimulation is decreased.

The close parallelism of these effects is consistent with the idea that the primary effect of TSH is to enhance the activity of the Na$^+$-K$^+$-stimulated ATPase in the cell membrane (1). Although these preliminary findings do not distinguish between primary and secondary events, the fact that TSH stimulates membrane ATPase in a cell-free nonrespiring system suggests that membrane ATPase stimulation may be the more direct effect of TSH. Although aerobic oxidation maintains a supply of energy in the form of ATP, the Na$^+$ K$^+$-stimulated ATPase as a generator of the respiratory stimulant ADP would appear to regulate partly the rate of respiration of the thyroid slices according to its use of this energy. In this sense the Na$^+$ K$^+$-stimulated ATPase and respiration are tightly coupled and act as pacemakers for each other. Such a relationship exists in brain (7), and Whittam has shown that about 40% of the respiration of brain cortex depends upon activation by Na$^+$ ions. Final proof of such a system operating in the thyroid must await further studies on active cation transport.

SUMMARY

Thyrotropin-stimulated respiration of thyroid slices is inhibited by ouabain over a wide concentration range, with half-maximal inhibition occurring at about $6 \times 10^{-7} \text{M}$ ouabain. This ouabain inhibition is not obtained at higher K$^+$ levels. Thyrotropin fails to stimulate the respiration of thyroid slices in the absence of Na$^+$ from the medium, and its stimulation is proportionally reduced by high levels of K$^+$. These responses in cellular respiration closely parallel those responses of the Na$^+$-K$^+$-stimulated adenosine triphosphatase previously characterized in isolated thyroid cell membranes.

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REFERENCES

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