Stimulation in Vitro of Pathways of Glucose Oxidation in Thyroid by Thyroid-stimulating Hormone

JAMES B. FIELD, IRA PASTAN, PHYLLIS JOHNSON, AND BETTY HERRING

From the Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, United States Public Health Service, National Institutes of Health, Bethesda 14, Maryland

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Despite many observations on morphologic and biochemical changes in the thyroid after the administration of thyroid-stimulating hormone, its exact mechanism of action is still obscure (1, 2). The earliest in vitro effect previously reported was a small increase in oxygen consumption 10 minutes after the addition of TSH to thyroid slices (3). TSH has also been found to increase phospholipid synthesis and iodide trapping, organization of iodide, and thyroid hormone release, but these effects have been somewhat delayed (4, 5).

In a preliminary report we noted evidence for the existence of the hexose monophosphate pathway for glucose metabolism in thyroid slices and the ability of TSH to increase glucose oxidation (6). Stimulation of glucose oxidation was evident 5 minutes after the addition of TSH. Although not as dramatic as its effect on glucose-1-C¹⁴ oxidation, there was also some increase in glucose-6-C¹⁴ oxidation to C¹⁴O₂ when TSH was present. This effect of TSH was not elicited when ACTH, prolactin or growth hormone were tested. The purpose of this communication is to elaborate on this observation as well as to present some information regarding possible mechanism of action.

EXPERIMENTAL PROCEDURE

Calf thyroids were obtained at the abattoir and kept on ice until sliced. Within an hour after the animals were killed, slices varying between 100 and 200 mg were made with a Stadie-Riggs slicer, lightly blotted on filter paper and weighed on a torsion balance. Each slice was placed in a 25-ml Erlenmeyer flask containing 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4), 0.5 µC of either glucose-1-C¹⁴ or glucose-6-C¹⁴ (purchased from the National Bureau of Standards), and either 2 or 5 mg of glucose. All substances tested were dissolved in buffer. The flasks were gassed with 95% O₂ and 5% CO₂ and incubated in a Dubnoff metabolic shaker at 37° for 45 minutes. At the end of the incubation period there is still evidence of a stimulating effect of TSH, although by this time the difference between the control and stimulated slices is not as great as at earlier times. Previously we noted a small effect from one preparation of FSH which could be explained on the basis of its known contamination with a small amount of TSH (6). Another preparation of FSH which contained very little TSH gave no stimulation (Table I).

Table I indicates that TSH stimulates the oxidation of glucose-1-C¹⁴ and glucose-6-C¹⁴ to C¹⁴O₂. Even at the end of a 4-hour incubation period there is still evidence of a stimulating effect of TSH, although by this time the difference between the control and stimulated slices is not as great as at earlier times. Previously we noted a small effect from one preparation of FSH which could be explained on the basis of its known contamination with a small amount of TSH (6). Another preparation of FSH which contained very little TSH gave no stimulation (Table I). Although TSH caused an increase in glucose-1-C¹⁴ oxidation to C¹⁴O₂ in thyroid slices, it was without effect on liver and testis (Table II).

Table III indicates that other drugs which are known to interfere with thyroidal iodine metabolism do not appear to effect the conversion of glucose-C¹⁴ to C¹⁴O₂. Although propylthiouracil by itself had no effect on glucose-1-C¹⁴ oxidation to C¹⁴O₂ in thyroid slices, it did appear to inhibit partially the stimulating effect of TSH. Other antithyroid drugs did not modify the TSH effect (Table III). Glucose oxidation was not altered by the addition of 100 µg of either thyroxine or triiodothyronine to the medium. The response of glucose oxidation to C¹⁴O₂ to

1 The abbreviations used are: TSH, thyroid-stimulating hormone; ACTH, adrenocorticotropic; FSH, follicle-stimulating hormone.
various amounts of TSH is indicated in Table IV. This effect of TSH is not limited to calf thyroid slices, since the same result has been obtained with slices from normal human thyroid. In this latter case it was also possible to observe stimulation within 5 minutes of the addition of TSH. It is most unlikely that the effect of TSH is a nonspecific protein effect, since other proteins such as albumin, insulin, and plasma proteins produced no stimulation of glucose oxidation to $\text{CO}_2$ and in some cases there was a suggestion of an inhibitory action (Table IV).

In an attempt to delineate the mechanism of this TSH effect, the action of the hormone was measured on glucose uptake by thyroid slices. In these studies the period of incubation was 4 hours. Experiments of shorter duration led to inconclusive results. Table V demonstrates that TSH does increase glucose uptake by thyroid slices. Although this same effect can be produced by insulin, it is not a nonspecific protein effect, since albumin and ACTH were inactive. There was no increase in the combined activity of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of thyroid slices incubated with TSH as compared with control slices. Activity in the control slices averaged 105 units per g (99 to 109) as compared to 103 units per g (91 to 114) in the TSH-treated slices.

Thyroid slices obtained from a rabbit treated with propyl-

**Table II**

_Lack of effect of TSH on $\text{CO}_2$ production in liver and testis_

Glucose concentration in the flasks was 5 mg/2 ml and there were 479,000 c.p.m. of glucose-1-$\text{C}^4$ and 438,000 c.p.m. of glucose-6-$\text{C}^4$ added to the appropriate flasks. The FSH was a gift of Dr. Robert Bates, National Institutes of Health, and contained less than 0.002 units of TSH per mg. The results are the averages of two experiments.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount per flask</th>
<th>Glucose-1-$\text{C}^4$</th>
<th>Glucose-6-$\text{C}^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4,000</td>
<td>725</td>
<td>30,200</td>
</tr>
<tr>
<td>TSH</td>
<td>28,500</td>
<td>215</td>
<td>40,600</td>
</tr>
<tr>
<td>FSH</td>
<td>3,550</td>
<td>585</td>
<td>20,200</td>
</tr>
</tbody>
</table>

**Table IV**

_Effect of anti-thyroid drugs alone and on TSH stimulation of $\text{CO}_2$ production_

Glucose concentration was 5 mg/2 ml and results of three different sets of experiments are included. The variability in the counts in $\text{CO}_2$ in the control experiments is accounted for by the fact that in each set of experiments, a thyroid gland from a different animal was used. The numbers are the averages of two duplicate determinations. In the first set of experiments there were 395,000 c.p.m. of glucose-1-$\text{C}^4$ and 430,000 c.p.m. of glucose-6-$\text{C}^4$ added to the appropriate flasks. In the other two sets there were 480,000 c.p.m. of glucose-1-$\text{C}^4$ and 440,000 c.p.m. of glucose-6-$\text{C}^4$ added to the appropriate flasks. Boiled thyroid slices were heated at 100° in buffer for 4 minutes.
primary effect on glucose metabolism. Insulin also increases glucose uptake by thyroid slices, this does not appear to be specific, since albumin and ACTH were unable to increase glucose uptake. The failure of TSH to increase the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase is not surprising, since it is more likely that the level of TPN controls the activity of the hexose monophosphate pathway (13).

**Table IV**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount per flask</th>
<th>Glucose uptake*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.45 ± 0.29 (3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Normal Plasma</td>
<td>2.95 ± 0.22 (4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.70 ± 0.30 (4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TSH</td>
<td>3.64 ± 0.26 (4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Insulin</td>
<td>3.40 ± 0.08 (3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ACTH</td>
<td>4.45 ± 0.29 (3)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Mean ± standard error.
† Number of determinations.

### Table V

Effect of TSH, insulin, albumin, and ACTH on 4-hour glucose uptake by thyroid slices

Glucose concentration was 2 mg/2 ml. Two different experiments are included in the table. *p* values are calculated comparing the test substance with albumin. In the control experiment no protein was added to the buffer.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount per flask</th>
<th>Glucose uptake*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.95 (2)‡</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>3.70 ± 0.30 (4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TSH</td>
<td>3.64 ± 0.26 (4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Insulin</td>
<td>3.40 ± 0.08 (3)</td>
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When antithyroid drugs such as propylthiouracil, thiocyanate, perchlorate, and iodide were tested, no change in glucose oxidation to C\(^{14}\)O\(_2\) was observed. This suggests that the mechanism of action of these drugs lies elsewhere. It is not surprising that antithyroid drugs did not inhibit glucose metabolism, since thyroids from animals treated with these drugs manifest clear-cut evidence of growth and hypertrophy. If glucose metabolism were being suppressed, it would be very difficult to explain continued growth. Although propylthiouracil appeared to inhibit somewhat the stimulatory effect of TSH, none of the other antithyroid drugs behaved in the same way. It is of interest that thyroid slices from a propylthiouracil-treated rabbit metabolized glucose more rapidly than thyroid slices from normal animals, and that the addition of TSH to the medium was without further effect. Presumably the gland of the propylthiouracil-treated animal was already under maximal endogenous TSH stimulation.

It is realized that the amount of TSH necessary to produce these effects is large when considered in relation to the amount probably present in vivo, but the specificity of its action argues for this being of physiologic importance. Human thyroid slices were also stimulated by TSH and the effect was manifest within 5 minutes, indicating that the phenomenon may be general for thyroid in different species. At the present time the meaning and significance of the slight inhibition of glucose oxidation in the presence of other proteins such as albumin and plasma is not clear. Indeed these substances were added to prevent loss of small amounts of TSH through adsorption to glassware.

Although TSH stimulates glucose uptake by thyroid slices during a 4-hour incubation, it seems unlikely that this is its primary effect on glucose metabolism. Insulin also increases glucose uptake by thyroid slices, yet there is no increased oxidation of glucose to C\(^{14}\)O\(_2\), as shown in Table IV. The insulin effect is presumably mediated by an increase in glucose transport into the cell (12). Were this the mechanism of action of TSH one would expect insulin also to stimulate glucose oxidation to C\(^{14}\)O\(_2\). This difference suggests that TSH stimulates glucose oxidation by some other means, and that the increased glucose uptake is secondary. It further suggests that in the thyroid the rate-limiting step for glucose oxidation is not the rate of entry of glucose into the cell. The effect of TSH on glucose uptake appears to be specific, since albumin and ACTH were unable to increase glucose uptake. The failure of TSH to increase the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase is not surprising, since it is more likely that the level of TPN controls the activity of the hexose monophosphate pathway (13).

**SUMMARY**

Thyroid-stimulating hormone in vitro is capable of stimulating oxidation of glucose-1-C\(^{14}\), and to a lesser extent of glucose-6-C\(^{14}\), to C\(^{14}\)O\(_2\) in thyroid slices. This effect appears to be specific, since adrenocorticotropic, prolactin, growth hormone, and follicle-stimulating hormone were inactive and thyroid-stimulating hormone had no effect on liver or testis slices. Since an effect was apparent within 5 minutes after the addition of thyroid-stimulating hormone, it is suggested that this might be the primary action of the hormone on the thyroid gland and its effect on phospholipid synthesis and iodine metabolism are secondary. None of the anti-thyroid drugs interfered with glucose oxidation to CO\(_2\); although there was some inhibition of the thyroid-stimulating hormone effect when propylthiouracil was present.

Although thyroid-stimulating hormone causes an increased glucose uptake by thyroid slices, this does not appear to be the mode of action of the hormone, since insulin also increases glucose uptake but does not stimulate glucose oxidation. Thyroid-stimulating hormone does not increase the levels of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase.
REFERENCES
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