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

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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, organification of iodide, and thyroid hormone release, but these effects have been somewhat delayed (4, 5).

In a preliminary note we reported 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-Cl4 oxidation, there was also some increase in glucose-6-Cl4 oxidation to C1402 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-Cl4 or glucose-6-Cl4 (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, the reaction was stopped by the addition of 0.2 ml of 10 N H2SO4. The flasks were then shaken for a further 60 minutes at room temperature to trap C1402 in the hyamine. The hyamine containing C1402 was then quantitatively transferred to a counting vial. Small amounts of 0.4% diphenyloxazole in toluene were used to rinse out the center well and then added to the hyamine in the counting vial. The volume in the counting vial was made up to 15 ml with 0.4% diphenyloxazole in toluene and then the vials were counted in a Packard liquid scintillation counter. Results are expressed as counts per min per g wet weight of tissue per 45 minutes.

In studies in which glucose uptake was measured, the incubation flasks contained 2 mg of unlabeled glucose in 2 ml of Krebs-Ringer bicarbonate buffer. Incubations were carried out for 4 hours, at the end of which time duplicate 0.5-ml aliquots of the incubation medium were removed for glucose determinations. Protein-free filtrates were made according to the method of Somogyi (7) and the glucose was determined with the use of glucose oxidase (Glucostat, Worthington Biochemical Corporation) (8). A control flask without a thyroid slice was included as a measure of the initial glucose concentration. The results are expressed as glucose disappearing from the medium per g wet weight of thyroid slices per 4 hours.

Glucose 6-phosphate dehydrogenase and 6-phosphoglucoseomerase dehydrogenase were assayed by the method of Glock and McLean (9). A unit of enzyme activity is defined as the quantity which, at 20°C and pH 7.6, reduces 0.01 µmole of TPN per minute. The TSH preparations used were either gifts from Dr. Peter Condiffe and Dr. Robert Bates or purchased from Armour and Company (Thytropar). Prolactin, FSH, and growth hormone were gifts of the Endocrine Study Section, National Institutes of Health.

RESULTS

Table I indicates that TSH stimulates the oxidation of glucose-1-Cl4 and glucose-6-Cl4 to C1402. 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-Cl4 oxidation to C1402 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 affect the conversion of glucose-C14 to C1402. Although propylthiouracil by itself had no effect on glucose-1-C14 oxidation to C1402 in thyroid slices, it did appear to inhibit partially the stimulatory effect of TSH. Other antithyroid drugs did not modify the TSH effect (Table III). Glucose oxidation was not altered by the addition of 10 µc of either thyroxine or triiodothyronine to the medium. The response of glucose oxidation to C1402 to
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 C\textsubscript{14}O\textsubscript{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 106 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 propylthiouracil were without effect when added to the slices from the treated rabbit.
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during a 4-hour incubation, it seems unlikely that this is its

Indeed these substances were added to prevent loss of
the presence of other proteins such as albumin and plasma is not

5 minutes, indicating that the phenomenon may be general for
and significance of the slight inhibition of glucose oxidation in
probably present in vivo, but the specificity of its action argues
for this being of physiologic importance. Human thyroid slices
these effects is large when considered in relation to the amount
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

TABLE IV

Effect of other proteins and graded doses of TSH on
C\textsuperscript{14}O\textsubscript{2} production

Four different sets of experiments are included and the results
are averages of two values. In the first three the glucose concentra-
tion was 2 mg/2 ml and there were 450,000 c.p.m. of glucose-
1-C\textsuperscript{14} and glucose-6-C\textsuperscript{14} added to the appropriate flasks, whereas
in the last set the glucose concentration was 5 mg/2 ml and 505,000
c.p.m. of glucose-1-C\textsuperscript{14} and 545,000 c.p.m. of glucose-6-C\textsuperscript{14} were
present in the appropriate flasks. For each set of experiments a
thyroid gland from a different animal was used.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Amount per flask</th>
<th>Glucose-1-C\textsuperscript{14} (c.p.m./g)</th>
<th>Glucose-6-C\textsuperscript{14} (c.p.m./g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.1 ml</td>
<td>4,410</td>
<td>1,588</td>
</tr>
<tr>
<td>Normal Plasma</td>
<td>1 mg</td>
<td>3,480</td>
<td>1,710</td>
</tr>
<tr>
<td>Control</td>
<td>1 mg</td>
<td>4,935</td>
<td>1,095</td>
</tr>
<tr>
<td>Albumin</td>
<td>1 mg</td>
<td>2,995</td>
<td>685</td>
</tr>
<tr>
<td>Control</td>
<td>1 unit (0.04 mg)</td>
<td>6,470</td>
<td>1,140</td>
</tr>
<tr>
<td>Insulin</td>
<td>1 unit (1 mg)</td>
<td>4,588</td>
<td>954</td>
</tr>
<tr>
<td>TSH</td>
<td>1 unit</td>
<td>10,950</td>
<td>1,735</td>
</tr>
<tr>
<td>Control</td>
<td>1 unit</td>
<td>4,480</td>
<td>1,740</td>
</tr>
<tr>
<td>TSH</td>
<td>0.1 unit</td>
<td>17,200</td>
<td>2,615</td>
</tr>
<tr>
<td>TSH</td>
<td>0.01 unit</td>
<td>8,410</td>
<td>1,705</td>
</tr>
<tr>
<td>TSH</td>
<td>0.001 unit</td>
<td>4,790</td>
<td>1,320</td>
</tr>
</tbody>
</table>

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 experi-
ments 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>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 mg</td>
<td>2.95 (2)†</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Albumin</td>
<td>1 unit</td>
<td>3.70 ± 0.30 (4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TSH</td>
<td>1 unit</td>
<td>3.64 ± 0.26 (4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Albumin</td>
<td>2 mg</td>
<td>4.30 ± 0.08 (3)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ACTH</td>
<td>1 mg</td>
<td>4.45 ± 0.29 (3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TSH</td>
<td>1 unit</td>
<td>5.36 ± 0.09 (3)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Mean ± standard error.
† Number of determinations.

glucose uptake by thyroid slices, yet there is no increased oxida-
tion of glucose to C\textsuperscript{14}O\textsubscript{2} (Table IV). The insulin effect is pre-
sumably 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\textsuperscript{14}O\textsubscript{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 ap-
pears to be specific, since albumin and ACTH were unable to
increase glucose uptake. The failure of TSH to increase the
activities of glucose 6-phosphat dehydrogenase and 6-phos-
phogluconate dehydrogenase is not surprising, since it is more likely
that the level of TPN controls the activity of the hexose mono-
phosphate pathway (13).

SUMMARY

Thyroid-stimulating hormone in vitro is capable of stimulating
oxidation of glucose-1-C\textsuperscript{14}, and to a lesser extent of glucose-6-C\textsuperscript{14},
to C\textsuperscript{14}O\textsubscript{2} in thyroid slices. This effect appears to be specific,
since adrenocorticotropin, prolactin, growth hormone, and fol-
lie-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\textsubscript{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
uprate but does not stimulate glucose oxidation. Thyroid-
stimulating hormone does not increase the levels of glucose 6-
phosphate dehydrogenase and 6-phosphogluconate dehydro-
genases.
REFERENCES

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