THYROGLOBULIN IN SERUM AFTER TSH STIMULATION IN HYPERTHYROIDISM

By

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ABSTRACT

Thyroglobulin in serum was demonstrated by a haemagglutination-inhibition technique and a reversed haemagglutination technique. Circulating thyroglobulin was found in 12 of 15 hyperthyroid patients — both before and during treatment with methylthiouracil — but in none of 12 euthyroid subjects. In three hyperthyroid patients it was not possible to determine thyroglobulin, as thyroglobulin antibody was present in the serum.

After TSH stimulation thyroglobulin appeared in the serum of nine of the 12 euthyroid subjects and the thyroglobulin level increased in five of the 12 untreated and six of the 12 treated hyperthyroid patients. The serum thyroxine, T₃ uptake in resin and ¹³¹I uptake in the thyroid gland at 4 and 24 h were increased after TSH stimulation in all the euthyroid cases; the hyperthyroid patients (both before and during treatment), however, only showed a slight but significant increase in serum thyroxine and 4 h ¹³¹I uptake, while the T₃ uptake in resin and the 24 h ¹³¹I uptake did not rise at all.

LATS was found in serum of only five of 15 untreated hyperthyroid patients. No significant changes in the LATS content could be detected during treatment.

The increased content of thyroglobulin in the serum of hyperthyroid patients seems to be due neither to greater sensitivity to TSH nor to the influence of LATS.

Previous studies of serum thyroglobulin by Torrigiani et al. (1969) using a radioimmunoassay technique have demonstrated elevated thyroglobulin levels in untreated and treated hyperthyroid patients as compared to normals. We
have made similar observations in hyperthyroid patients using a reversed haemagglutination technique (Hjort et al. 1970).

Animal experiments by Daniel et al. (1967) showed increased content of thyroglobulin in the lymph draining from the thyroid gland following injections of TSH. These results might suggest that the presence of thyroglobulin in the serum of antithyroid treated patients could in some cases be due to increased TSH stimulation (Odell et al. 1965). In untreated hyperthyroid patients an increased content of thyroglobulin in the serum, however, can not be explained by this mechanism as the TSH concentration in the blood of these patients is lower than in normal subjects (Odell et al. 1965).

The purpose of the present study has been to investigate the effect of TSH on the serum concentration of thyroglobulin in euthyroid controls and in patients with hyperthyroidism.

MATERIAL AND METHODS

The series studied consisted of 12 control subjects (5 men and 7 women aged 40–80; mean 60 years) and 15 patients (2 men and 13 women aged 38–68; mean 55 years) with untreated hyperthyroidism. Four had exophthalmos, 9 had diffuse goitre and 2 had adenomas. The hyperthyroid patients were re-tested after treatment for two months with methylthiouracil (100 mg three times daily). In all cases the thyroid gland was stimulated with 4 U. S. P. units of TSH (Ferring) im for three consecutive days at about 5 p.m.

In all stimulated subjects without circulating thyroglobulin antibody a comparison was made between the thyroglobulin findings immediately before the stimulation experiment and the results obtained on the day after the last TSH injection.

Two independent methods were used for the detection of thyroglobulin: a regular haemagglutination-inhibition technique using thyroglobulin-coated sheep erythrocytes and a reversed haemagglutination technique using red cells coated with highly purified rabbit antibody against human thyroglobulin. The simultaneous testing by two such methods working in opposite directions, i.e. an inhibiting and an agglutinating system, was used in order to ensure the highest possible degree of specificity. However, it must be pointed out that not only intact thyroglobulin, but also split products with intact antigenic groups may react in these tests. Furthermore, neither of the techniques can reveal circulating thyroglobulin when thyroglobulin antibody is present in the serum (as was the case in three of the 15 hyperthyroid patients).

The haemagglutination-inhibition technique was carried out in a micro-titre system in the conventional way with mixtures of a constant "minimal" amount of thyroglobulin antibody and serial dilutions of the sera to be tested. After incubation at 37°C overnight, thyroglobulin-coated cells were added to detect whether the serum had contained substances capable of neutralizing the "minimal" amount of thyroglobulin antibody.

The reversed haemagglutination test was also performed in a micro-titre system with 0.025 ml volumes, but otherwise essentially as previously described (Hjort 1968).

The two techniques used for the demonstration of thyroglobulin components were set up with such initial dilutions that they had approximately the same sensitivity,
both being capable of detecting thyroglobulin concentrations in the serum down to about 0.05 μg per ml. Even if the two methods did not always show absolute parallelism, a strong reaction with one test and a negative reaction with the other were never observed. The greatest discrepancy found between the two tests was in a single serum which yielded a negative inhibition reaction and a titre of 20 in the reversed haemagglutination test.

Demonstration of thyroglobulin was considered of significance only when both test systems were positive, and a reaction in only one of the tests was recorded a ± reaction. Similarly, changes in the thyroglobulin levels after stimulation with TSH were considered to be of significance only if both test systems reflected the same type of changes (always increases), and doubtful if merely one of the tests revealed a change.

Serum thyroxine was determined by the method of Murphy (1965) before and 20 h after the last TSH injection and, at the same time, the uptake of labelled T₃ in resin (Triosorb®, Abbott) incubated with the patients serum was tested. Furthermore the uptake of ¹³¹I in the gland was determined 4 and 24 h after a dose of 10–20 μCi ¹³¹I had been given both before stimulation and 16 h after the last TSH injection.

In the hyperthyroid patients the serum concentrations of LATS were determined before and during treatment. Guinea-pigs given intraperitoneal injections of 20 μCi ¹³¹I were used. Blood samples were taken 3 days later by cardiac puncture, after which 1 ml serum was injected intraperitoneally. Blood samples were taken again by cardiac puncture 3 and 20 h later. The serum concentrations of LATS were estimated on the basis of the ratio of the radioactivities present in the blood samples at 20 and 3 h after the serum injection (Friis 1969). If this ratio was greater than 150 per cent, LATS was definitely considered to be present whereas a ratio ranging between 125 and 150 per cent was regarded as doubtfully positive.

RESULTS

1. The effect of TSH on the occurrence of thyroglobulin in serum

The results are shown in Table 1. As far as the findings before stimulation are concerned, there is a striking difference between the normal subjects and the patients with thyrotoxicosis. Thus, at the beginning of the experiment all the thyrotoxic patients, but none of the normal subjects had significant levels of circulating thyroglobulin. In the latter group, the only findings were two doubtful reactions.

The time for the second blood sample for the determination of thyroglobulin – i.e. the day after the last TSH injection – was chosen after a preliminary experiment with TSH stimulation in four normal subjects. Serum samples were collected daily for the first four days of the experiment and again on the eighth day. One of the test subjects revealed a rather marked response: thus from being negative before the experiment both tests for thyroglobulin changed to positive on day three, and maximum titres were found on day four (titres of 40 in the reversed agglutination test and 16 in the inhibition test, respectively). Two subjects revealed weaker responses, but
Table 1.

Serum thyroglobulin before and after stimulation with TSH (only subjects without thyroglobulin antibody).

<table>
<thead>
<tr>
<th>Total No.</th>
<th>Thyroglobulin findings before stimulation</th>
<th>Changes in thyroglobulin titres after stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Thyrotoxic patients</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Before treatment</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>During treatment</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

also with maximum on day four, while in the fourth case only a doubtful reaction was observed.

As seen in Table 1 stimulation with TSH for three days was followed by a significant increase in the thyroglobulin levels in nine of the 12 normal subjects and in five of the 12 toxic patients when tested before treatment was started. Furthermore, in each of these two groups an insignificant increase was observed in two cases. On the other hand, decreases were not seen in any of the cases. Repeated administration of TSH to the patients with thyrotoxicosis after they had been treated for two months resulted in changes in the thyroglobulin concentrations very similar to those observed before treatment was started.

The tendency to increase in the concentration of circulating thyroglobulin after stimulation with TSH seemed more marked in normal subjects than in thyrotoxic patients; however, statistical analysis ($\chi^2$ test) revealed that the difference in reactivity between the two groups was not significant ($0.1 > P > 0.05$).

2. The effect of TSH on the thyroid-function tests

The effect of TSH stimulation on the level of serum thyroxine, the uptake of $T_3$ in resin and the uptake of $^{131}I$ in the thyroid gland at 4 and 24 h are shown in Table 2.

A considerable response to TSH stimulation was seen in all the 12 normal subjects, and the means of each of the parameters revealed highly significant changes.

In the untreated hyperthyroid patients no statistically significant changes in the uptake of $T_3$ in resin or in the 24 h thyroid uptake were found. On the
Table 2.
The effect of TSH stimulation, 4 U.S.P. units daily for three consecutive days, on serum thyroxine, T<sub>3</sub> uptake in resin and <sup>131</sup>I uptake in the thyroid glands of normal subjects, and untreated and treated hyperthyroid patients.

<table>
<thead>
<tr>
<th></th>
<th>Euthyroid</th>
<th>Untreated hyperthyroid</th>
<th>Treated hyperthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before TSH</td>
<td>Difference</td>
<td>Before TSH</td>
</tr>
<tr>
<td>Serum thyroxine</td>
<td>9.0 ± 2.5</td>
<td>+11.2</td>
<td>14.7 ± 3.5</td>
</tr>
<tr>
<td>(4.5–13.5 μg/100 ml)</td>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(0.02 &lt; P &lt; 0.05)</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; uptake in resin</td>
<td>26.6 ± 4.5</td>
<td>+5.8</td>
<td>39.0 ± 6.2</td>
</tr>
<tr>
<td>(25–35 %)</td>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &gt; 0.1)</td>
</tr>
<tr>
<td>13&lt;sup&gt;1&lt;/sup&gt;I uptake at 4 h</td>
<td>24.5 ± 7.4</td>
<td>+21.3</td>
<td>56.2 ± 19.6</td>
</tr>
<tr>
<td>(15–45 %)</td>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(0.02 &lt; P &lt; 0.05)</td>
</tr>
<tr>
<td>13&lt;sup&gt;1&lt;/sup&gt;I uptake at 24 h</td>
<td>40.1 ± 11.6</td>
<td>+22.9</td>
<td>64.1 ± 16.6</td>
</tr>
<tr>
<td>(30–70 %)</td>
<td></td>
<td>(P &lt; 0.001)</td>
<td>(P &gt; 0.1)</td>
</tr>
</tbody>
</table>
other hand, the increases in the 4 h uptake of $^{131}$I and in serum thyroxine were significant, but much smaller than in the normal subjects.

Moreover in the hyperthyroid patients treated with methylthiouracil for two months, significant increases in the serum level of thyroxine and the 4 h uptake of $^{131}$I were observed after stimulation, while the T$_3$ uptake in resin and the 24 h uptake in the thyroid were completely unchanged. All patients were clinically euthyroid, although a few patients had slightly reduced thyroid parameters presumably due to overdosage.

3. Serum LATS

In the 15 patients with hyperthyroidism the LATS factor was determined both before and in most cases one, two and three months after the start of treatment with methylthiouracil. Before treatment was started a positive LATS reaction ($> 150$ per cent) was found in only three patients and a doubtfully positive test (125–150 per cent) in the serum from two patients. During antithyroid treatment the LATS reaction returned to normal in the five patients, while seven patients revealed a transient positive LATS reaction during antithyroid treatment. Statistical analysis (Student's $t$-test for paired samples) of the mean values showed no significant changes in the LATS titres during treatment ($P > 0.1$).

No relation was found between the occurrence of LATS and the serum concentration of thyroglobulin before or after stimulation with TSH.

**DISCUSSION**

Torrigiani *et al.* (1969) found amounts of thyroglobulin in serum ranging from 10 ng/ml to 150 ng/ml in 60 to 70 per cent of normal subjects using radioimmunoassay technique with a sensitivity of 10 ng/ml. Van Herle *et al.* (1973) have recently confirmed these results with a similar, but slightly more sensitive radioimmunoassay though these investigators generally found lower thyroglobulin levels in normal subjects (up to 20.7 ng/ml). The sensitivity of our technique is 50 ng/ml which explains why thyroglobulin was not found in the 12 euthyroid subjects. Torrigiani *et al.* (1969) found concentrations of thyroglobulin between 43 and 1800 ng/ml in 23 of 24 untreated hyperthyroid patients and between 100 and 275 ng/ml in six patients treated with carbimazole which is in accordance with our results.

Our investigations indicate that the concentration of thyroglobulin in the serum of normal subjects increases after TSH stimulation, which is in accordance with both animal experiments (Daniel *et al.* 1967) and recent experiences on euthyroid subjects (Uller *et al.* 1973). In hyperthyroid patients,
both before and during treatment, only a small increase or no increase at all was found in the concentration of serum thyroglobulin. In addition the generally used parameters for the thyroid function showed a weaker response to TSH than in normal subjects. Werner et al. (1955) obtained similar results when estimating PBI and the 24 h $^{131}$I uptake in the thyroid gland after stimulation with TSH in hyperthyroid patients.

In hyperthyroidism the functional capacity of the thyroid gland is probably maximally utilized, which may explain the decreased response after TSH in these patients. Levy et al. (1953) were of the opinion, that the weaker response in antithyroid-treated hyperthyroid patients could be explained by blockade of hormone synthesis, and that TSH stimulation cannot overcome this blockade. Contrary to this explanation are the results of Mitchell et al. (1961) who found that euthyroid subjects on a similar dose of antithyroid drugs showed a strong reaction in the thyroid parameters after stimulation with the same dose of TSH.

With reference to Torrigiani et al. (1969) the excretion of thyroglobulin in hyperthyroidism is due to LATS stimulation of the thyroid gland. In this study, however, LATS in the blood was only found in five out of 15 untreated hyperthyroid patients similar to other findings of Friis (1969), Kriss et al. (1967) and Major & Munro (1962). No relation was found between the occurrence of LATS and thyroglobulin in serum before or after stimulation with TSH, nor could any significant changes in the content of LATS in the blood be detected during antithyroid treatment.

The cause of the increased content of thyroglobulin in serum from hyperthyroid patients has not yet been explained. Since neither an increased sensitivity to TSH nor the influence of LATS seems to be the cause, a third still unknown factor in hyperthyroidism might be responsible for the increased release of thyroglobulin.

REFERENCES


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