INTRA VE N OUS AND PER ORAL TRH ST IMUL AT ION IN SPORA D I C A TOXIC GOITRE

By

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ABSTRACT
Thyrotrophin releasing hormone (TRH) stimulation test with 200 μg iv was performed in 35 patients with atoxic sporadic goitre. In 23 patients with diffuse goitre 7 showed a lack of increase in serum thyrotrophin (TSH) at a significantly increased frequency compared to controls (P = 0.0028). In 4 patients with solitary nodules 2 showed no significant response to TRH (negative), while 3 of the 8 patients with multinodular goitres had negative TRH test. Only 6 of the 12 TRH negative patients also had non-suppressible 131I uptake following T3. No significant difference in age and thyroid parameters was found between the TRH negative and TRH positive patients. In 7 TRH negative patients the test was repeated with 400 μg TRH but all remained negative. Five of these patients were given TRH perorally 80 mg daily for 2 weeks resulting in a significant increase in serum T4 and T3. No detectable increase in TSH was found. The response to iv bovine TSH in 4 TRH negative patients was found to be normal, suggesting that there was normal thyroid sensitivity to TSH. Our findings suggest that patients with TRH negative atoxic goitre can release biological active TSH following prolonged TRH stimulation. The high frequency of a negative standard TRH test in atoxic goitre seems to diminish the diagnostic value of the standard TRH test.

Several studies on the thyrotrophin (TSH) response to intravenous doses of thyrotrophin releasing hormone (TRH) in clinical euthyroid patients with sporadic atoxic goitre have demonstrated a lack of response in a varying percentage of the patients studied (Pickardt et al. 1973; Ridgway et al. 1973; Dige-Petersen & Hummer 1974).
In most studies a very high incidence (50–80%) of negative response following standard doses of TRH in patients with solitary autonomous nodules was found (Karlberg 1973; Ridgway et al. 1973; Dige-Petersen & Hummer 1974) in contrast to the findings in patients with diffuse atoxic goitre in whom normal increase in serum TSH has been found in most cases (Beckers et al. 1972; Dige-Petersen & Hummer 1974; Rothenbuchner et al. 1974). The purpose of the present study has been to evaluate the effect of different doses of TRH intravenously and repeated perorally TRH doses in patients with atoxic goitre in whom a lacking of TSH response to a standard TRH stimulation test was found.

**MATERIAL AND METHODS**

The material comprised a total of 35 consecutive patients with sporadic atoxic goitre in our clinic. All patients had a palpable goitre and by a clinical evaluation judged to be euthyroid (no characteristic symptoms of hyperthyroidism: eye symptoms, heart symptoms, tremor, weight loss).

The following laboratory tests were within normal range in all patients: serum thyroxine (T₄) (Murphy 1965) (normal range: 5.0–11.0 μg/100 ml), serum triiodothyronine (T₃) (Kirkegaard et al. 1974) (normal range: ≤ 60 years: 85–189 ng/100 ml; > 60 years: 13–139 ng/100 ml), T₃ resin uptake (Triosorb, Abbott) (normal range: 0.80–1.20). After the conclusion of the study each patient has been followed for at least one and a half year without significant changes in clinical symptoms or laboratory values. Evaluated by 99mTc scintigrams the material was divided into three groups: diffuse goitre, multinodular goitre and solitary adenoma. The group with diffuse goitre consisted of 5 males and 18 females, aged 21 to 73 years (mean: 49 years). One male and 7 females aged 19 to 71 years (mean: 49 years) had a multinodular goitre, whereas solitary adenomas were found in 4 females aged 28 to 64 years (mean: 44 years).

In all patients a standard iv TRH (200 μg) stimulation test with estimation of serum TSH at 0, 20 and 60 min was performed (Lauridsen et al. 1974), (normal range: basal serum TSH < 4.9 μU/ml and maximal increase in serum TSH (Δ max TSH) ≥ 2.0 μU/ml). The 131I uptake in thyroid gland after 4 and 24 h was also measured before and after suppression with peroral T₃ (20 μg 4 times daily for 7 days) (Friis 1963). In patients with a negative (i.e. an increase in serum TSH less than 2.0 μU/ml) TRH test, the stimulation was repeated with 400 μg TRH iv. Patients who did not respond to the increased dose of TRH received a peroral stimulation with 20 mg TRH 4 times a day (8 a.m., noon, 4 and 8 p.m.) for 14 days. Blood samples for estimation of serum T₃, T₄, TSH and T₃ resin uptake were drawn before and after the prolonged TRH stimulation. In 1 euthyroid control patient and in 1 euthyroid patient with diffuse goitre and negative standard TRH test, serum samples were obtained at 4 to 8 h intervals on day 1–9, 12 and 14 during the peroral TRH stimulation test. In order to evaluate a possible increased sensitivity of the thyroid to TSH, a TSH stimulation test (Faber et al. 1976) was performed 3 months after the peroral TRH stimulation. The effect of TSH was evaluated by the increase in serum T₃ 1, 2, 3 and 4 h after the iv injection of 7.5 mU bovine TSH (Ferring) per kg body weight.
Table 1.
The outcome of TRH test and $T_3$ suppression test in 35 patients with different types of atoxic goitre.

<table>
<thead>
<tr>
<th></th>
<th>Diffuse goitre</th>
<th>Multinodular goitre</th>
<th>Solitary adenoma</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>23</td>
<td>8</td>
<td>4</td>
<td>60</td>
</tr>
<tr>
<td>Negative TRH-test*</td>
<td>7**</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$T_3$ non-suppressible</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>

* TSH response $< 2.0 \mu U/ml$.

** Compared to controls: $P = 0.0028$ (Fisher’s exact test).

RESULTS

In Table 1 the total material is divided into three groups with different types of goitre, and the results of the standard TRH stimulation test and the $T_3$ suppression of the $^{131}I$ uptake are given. The mean values of serum $T_4$, serum $T_3$, $T_3$ resin uptake, serum TSH and $^{131}I$ uptake did not differ significantly from controls in any of the groups. One third of the TSH values were below

Table 2.
The relation between the outcome of TRH test and thyroid parameters (mean $\pm$ sd) in 35 patients with atoxic goitre.

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Age years</th>
<th>Serum $T_4$ µg/100 ml</th>
<th>Serum $T_3$ ng/100 ml</th>
<th>$T_3$ resin uptake arb. units</th>
<th>Serum TSH µU/ml</th>
<th>$24$ h $^{131}I$ uptake $%$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive TRH-test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_3$ suppressible</td>
<td>23</td>
<td>43±16</td>
<td>8.4±1.7</td>
<td>119±36</td>
<td>1.06±0.14</td>
<td>2.3±2.7</td>
<td>48.7±15.8</td>
</tr>
<tr>
<td><strong>Negative TRH-test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_3$ suppressible</td>
<td>6</td>
<td>49±12</td>
<td>7.9±1.4</td>
<td>115±36</td>
<td>1.01±0.08</td>
<td>1.6±1.2</td>
<td>41.3±6.4</td>
</tr>
<tr>
<td>$T_3$ non-suppressible</td>
<td>6</td>
<td>49±16</td>
<td>8.5±1.6</td>
<td>142±25</td>
<td>0.96±0.18</td>
<td>1.7±0.4</td>
<td>44.0±16.8</td>
</tr>
</tbody>
</table>

* TSH response $< 2.0 \mu U/ml$. 

510
the detection limit, a figure similar to that found in the controls. The relations
between the outcome of the TRH test and the other parameters of thyroid
function studied are given in Table 2. In all patients with positive TRH test
(mean $\Delta$ max TSH: 10.3 $\mu$U/ml $\pm$ 10.4 (sd)) a normal $T_3$ suppression test was
found.

In 12 patients with negative TRH test (mean $\Delta$ max TSH: 0.6 $\mu$U/ml $\pm$ 0.6
(sd)) only half of them were also non-suppressible with $T_3$. No significant
difference in serum $T_4$, serum $T_3$, $T_3$ resin uptake, serum TSH, $^{131}$I uptake
or age could be demonstrated between the groups.

In 7 of the 12 TRH negative patients the TRH test was repeated with a
double dose (400 $\mu$g iv) of TRH but no measurable response in serum TSH
was obtained. In 4 of these patients the $^{131}$I uptake was not suppressible with
$T_3$. One patient had a single adenoma, while diffuse and multinodular goitres
occurred in 4 and 2 patients, respectively. In 5 of the 7 patients a prolonged
peroral TRH stimulation was performed. The thyroid parameters before and
after 2 weeks of peroral TRH are given in Table 3. Serum $T_4$ and serum $T_3$
rose significantly, while no significant increase in serum TSH could be
demonstrated.

In addition one control and 1 patient with an atoxic, TRH negative, diffuse
goitre were studied in more detail with repeated blood sampling during the
day from 8 a.m. to 10 p.m. The data are given in Fig. 1. The patient with
TRH negative goitre showed a response in serum $T_3$ similar to that of the
control patient. The first 3 days serum $T_3$ increased with spikes following each
dose of TRH and then declined to level somewhat higher than the pre-value.
Serum $T_4$ values showed a steady rise without significant variations during the
day to a level on the 3rd day and then remained elevated during the period
of TSH medication. In both subjects serum TSH increased during the first
2–3 days, but the rise in the TRH negative patients was less in spite of a more

\textit{Table 3.}

Thyroid parameters (mean $\pm$ sd) in 5 patients with atoxic goitre and negative TRH test
before and and after 20 mg TRH po 4 times a day for 14 days.

<table>
<thead>
<tr>
<th>Serum $T_4$</th>
<th>Serum $T_3$</th>
<th>$T_3$ resin uptake</th>
<th>Serum TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$g/100 ml</td>
<td>ng/100 ml</td>
<td>arb. units</td>
<td>$\mu$U/ml</td>
</tr>
<tr>
<td>Before TRH</td>
<td>7.0 $\pm$ 1.5</td>
<td>118 $\pm$ 21</td>
<td>0.91 $\pm$ 0.08</td>
</tr>
<tr>
<td>After TRH</td>
<td>10.4 $\pm$ 2.6$^a$</td>
<td>142 $\pm$ 25$^{**}$</td>
<td>0.99 $\pm$ 0.09</td>
</tr>
</tbody>
</table>

$^a$ $P < 0.01$. $^{**} P < 0.001$.

(Student's $t$-test for paired data).
Serum T3, T4 and TSH in a control subject and a patient with atoxic, TRH negative goitre during peroral TRH medication (20 mg 4 times a day for 14 days). Serum TSH values within the shaded area are below the detection limit of the assay.

pronounced T4 and T3 release. After the 3rd day the serum TSH was below the detection limit of the assay (1.0 µU/ml) in both subjects.

The response to intravenous bovine TSH (7.5 mU/kg) was estimated in 4 of the TRH negative patients. The increase in serum T3 during 4 h was almost identical to the values found in 15 normal controls.

**DISCUSSION**

The high frequency of negative standard TRH tests in patients with atoxic nodular goitre is in accordance with previous studies by Karlberg (1973), Pickardt et al. (1973), Ridgway et al. (1973) and Dige-Petersen & Hummer (1974). However, it has not been previously described that patients with sporadic atoxic goitre have a significant higher frequency (30 %) of negative TRH tests than found in controls (Table 1). This high frequency (95 % confidence limits: 13–53 %) seems to diminish the diagnostic value of the standard TRH test in the diagnosis of Graves' disease. When comparing the standard TRH test with the T3 suppression test agreement was found in all cases, except six in which the TRH test was negative though 131I uptake could be suppressed
with T₃. This may suggest that the T₃ suppression test is more reliable in establishing euthyroidism in patients with goitre than the TRH test. The follow-up for 1½ year seems to rule out the possibility of incipient hyperthyroidism in the group studied.

Considering the possible explanations of our finding of a high frequency of negative TRH tests in atoxic goitres, it seems unlikely that the lack of response to a standard TRH dose can be explained by insufficient stimulation since stimulation with a double dose (400 μg) of TRH also gave negative result. In order to study further the release of TSH and the effect on the thyroid hormone release in these patients a prolonged stimulation with 80 mg TRH for 2 weeks was performed. A very slight increase in serum TSH was noted in one patient (Fig. 1) during the first few days, whereas no TSH was detectable after 14 days of TRH medication. However, TSH secretion must have been stimulated in all patients, since highly significant increases were found in both serum T₃ and T₄ and remained elevated during TRH medication.

These findings are in accordance with those described in controls during peroral medication with TRH for 1 week (Rabello et al. 1974). The absence of increase in serum TSH after the 3rd day of peroral TRH in the 2 subjects studied in detail may be due to suppression of the pituitary by the elevated serum levels of thyroid hormones. As the pituitary in the TRH negative patients is able to secrete some amounts of biological active TSH thus maintaining the elevated levels of T₃ and T₄, it seems possible that the TSH secreted does not react normally with the antibodies (National Pituitary Agency, N. I. H.) used in the present assay. A divergency between immunological and biological active TSH has recently been demonstrated in pituitaries from patients with asymptomatic, atrophic thyroiditis (Vanhaelst et al. 1975). Another explanation for the negative standard TRH test may be that a physiological significant increase in serum TSH was undetectable in the assay used (Kohler et al. 1973). Our finding could also be in agreement with the assumption that the thyroid in atoxic sporadic goitre has an increased sensitivity to TSH (Bray 1968). Our patients as judged by the ¹³¹I uptake had no iodine deficiency, and our preliminary data on T₃ response to iv bovine TSH showed an apparently normal sensitivity of the thyroid to TSH. However, further studies are necessary to support the hypothesis that some patients with sporadic atoxic goitre secrete an immunological abnormal but biological active TSH.

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REFERENCES


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