EFFECTS OF SUBSTITUTION WITH THYROXINE ON THE THYROTROPHIN (TSH) RESPONSE TO THYROTROPHIN-RELEASING HORMONE (TRH) IN SEVERE PRIMARY MYXOEDEMA AND IN MILD HYPOTHYROIDISM FOLLOWING PROLONGED THYROSTATIC THERAPY

By

ABSTRACT

The evolution of the thyrotrophin (TSH) response after 400 μg thyrotrophin-releasing hormone (TRH) was investigated during recovery of hypothyroidism. In 14 myxoedematous patients, replacement therapy with thyroxine (T₄) caused characteristic changes. Before treatment a relatively blunted pattern with mΔTSH¹ of 51.2 ± 30.6 mU/l was found. Two and 4 weeks after treatment an enhanced response was evolved with values for mΔTSH of 87.7 ± 43.4 and 96.0 ± 48.0 mU/l (P < 0.02 respectively < 0.01). Comparison of the TSH curve showed significant increases in ΔTSH during the first 4 weeks of treatment at 20, 40 and 60 min compared to corresponding measurements in the basal state.

In 17 overtreated hyperthyroid patients with mild, mostly chemical hypothyroidism the influence of T₄ replacement on TSH responses at different TSH levels was studied. At the stage of mild TSH elevation (5–10 mU/l)

¹ Basal TSH level prior to injection of TRH: basal TSH.
Maximal TSH concentration after TRH injection: mTSH.
Increase above the basal value: ΔTSH.
Maximal increase above basal TSH value: mΔTSH.
Maximal ΔTSH as percentage of basal TSH: % mΔTSH.
mÅTSH before and during substitution were 12.6 ± 6.7 and 25.9 ± 15.3 mU/l, respectively ($P < 0.01$). Moderate TSH elevation (10–25 mU/l) was accompanied by mÅTSH of 25.4 ± 13.4, respectively 44.5 ± 22.9 mU/l ($P < 0.01$) while high TSH values (> 26 mU/l) had responses of 39.1 ± 16.0 and 92.5 ± 22.0 mU/l ($P < 0.001$). In these patients the initial blunting of TSH responses could not be attributed to the foregoing toxicosis, since follow up during 6–15 weeks of protracted hypothyroidism did not show any tendency to enhancement of relative release (% mÅTSH).

These findings suggest that in the early stage of replacement therapy of hypothyroidism, either pituitary TSH is accumulating or that endogenous TRH is declining.

In primary hypothyroidism, elevated levels of serum TSH (Odell et al. 1967) and an exaggerated response to TRH administration is a constant finding (Hershman & Pittman 1971; Shenkman et al. 1972; Fleischer et al. 1972). The relative rise in serum THS is however found to be equal or less than that in euthyroid subjects.

Thyroid hormone(s) suppress the TSH response (in euthyroid subjects) within several hours (Azizi et al. 1975; Wenzel et al. 1975). The effect on the TSH response by TRH in primary hypothyroidism has not been extensively reported.

We have studied the sequential changes in the TSH response during gradually increasing substitution with thyroxine. We compared a group of severely myxoedematous patients with longstanding hypothyroidism with a group of overtreated hyperthyroid patients, who had had hypothyroidism for only a few weeks and were metabolically and clinically marginally myxoedematous.

**MATERIALS AND METHODS**

**Patients**

**Group A**

All patients from our clinical and out patient population, who showed a clear cut picture of primary myxoedema, joined this group after laboratory studies had confirmed primary hypothyroidism. During the last 3 years 14 patients (12 females, 2 males) were studied according to the following protocol. After basal investigations of thyroid function, including a standard TRH test, replacement therapy was started with sodium-l-thyroxine 50 µg daily with a fortnightly increment of 50 µg in dosage until an adequate substitution was reached. Every 2 weeks the condition of the patients was re-evaluated.

**Group B**

Seventeen thyrotoxic patients (11 females, 6 males) with neither eye symptoms or substantial goitre were treated over a prolonged period with either 40 mg carbimazol or 400 mg propylthiouracil. In the course of this treatment basal TSH was estimated
every 2–3 weeks. When an elevated level was discovered the patient entered group B. In the majority of patients, suppressive therapy was tolerated without complaints ascribable to hypothyroidism, growing goitre or eye symptoms. Substitution could thus be postponed (for at least 2 weeks) allowing multiple investigations while the patient remained hypothyroid.

Basal investigations included clinical index (Billewicz et al. 1969), serum thyroxine (T4), serum triiodothyronine (T3) and a standard TRH test. Thereafter replacement therapy was started while suppressive medication was continued as before. The replacement scheme and management were in principle the same as for patients from group A.

**Group B'**

A subgroup of 12 patients without goitre and good tolerance to the hypothyroid state continued suppressive therapy for 6–15 weeks prior to substitution therapy. During this period the patients were kept to a 5 weekly protocol. The pre-substitution phase was continued, till T4 levels fell below 25 nmol/l, with the exception of one patient (G. T.). In this patient the substitution was started with a T4 level of 52 nmol/l. This procedure was designed to study the changes in the TSH curve over a period of progressing hypothyroidism.

**Methods**

After an overnight fast an intravenous cannula was inserted for administration of TRH and withdrawal of blood-samples. Synthetic TRH (Roche), 400 µg was injected as a bolus and blood collected at 0, 20, 40, 60 and 120 min.

TSH and T3 were measured by radioimmunoassay (TSH expressed in mU of first Int. Stand.) and T4 by competitive protein binding. All other investigations were done by standard techniques. For statistical analysis Student’s t-test was used.

**Table 1.**

Clinical and biochemical data in group A and B patients.

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>T4 nmol/l</th>
<th>T3 nmol/l</th>
<th>TSH mU/l</th>
<th>Cholesterol mmol/l</th>
<th>Billewicz index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A (n = 14)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± sd</td>
<td>64.6 ± 12.5</td>
<td>7.4 ± 7.8</td>
<td>0.62 ± 0.45</td>
<td>95.7 ± 43.1</td>
<td>9.5 ± 3.6</td>
<td>+36 ± 19</td>
</tr>
<tr>
<td>Range</td>
<td>41–84</td>
<td>0–36</td>
<td>0–1.3</td>
<td>25–183</td>
<td>5.9–17.7</td>
<td>-11–+62</td>
</tr>
<tr>
<td><strong>Group B (n = 17)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± sd</td>
<td>68.1 ± 9.5</td>
<td>14.6 ± 12.3</td>
<td>1.70 ± 0.67</td>
<td>32.3 ± 31.0</td>
<td>7.6 ± 1.8</td>
<td>-16 ± 20</td>
</tr>
<tr>
<td>Range</td>
<td>49–83</td>
<td>0–52</td>
<td>0.4–2.8</td>
<td>6–110</td>
<td>3.4–10.6</td>
<td>-47–+20</td>
</tr>
<tr>
<td>Normal values</td>
<td>–</td>
<td>60–142</td>
<td>1.3–3.0</td>
<td>0–3</td>
<td>3.5–7.0</td>
<td>&lt; -24</td>
</tr>
</tbody>
</table>

T4: Serum thyroxine. T3: Serum triiodothyronine.

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Table 2.
Effect of replacement therapy with l-thyroxine on basal TSH, TSH response to TRH, serum T₄ and serum T₃, in group A.

<table>
<thead>
<tr>
<th>Test</th>
<th>0. before replacement</th>
<th>1: 2 weeks on therapy</th>
<th>2: 4 weeks on therapy</th>
<th>3: 6 weeks on therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>basal TSH mU/l</td>
<td>mTSH</td>
<td>mΔTSH</td>
<td>basal TSH mU/l</td>
</tr>
<tr>
<td>Mean</td>
<td>95.7</td>
<td>146.9</td>
<td>51.2</td>
<td>96.9</td>
</tr>
<tr>
<td>sd</td>
<td>43.1</td>
<td>49.4</td>
<td>30.6</td>
<td>49.3</td>
</tr>
<tr>
<td>P*</td>
<td>&lt; 0.02</td>
<td>&lt; 0.05</td>
<td>NS</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>T₄ nmol/l</td>
<td>7.4 ± 7.8</td>
<td>32.6 ± 16.0</td>
<td>68.1 ± 25.7</td>
<td>101.8 ± 49.5</td>
</tr>
<tr>
<td>T₃ nmol/l</td>
<td>0.62 ± 0.45</td>
<td>1.06 ± 0.64</td>
<td>1.54 ± 0.53</td>
<td>1.87 ± 0.61</td>
</tr>
</tbody>
</table>

T₄ and T₃ mean ± 1 sd. Normal range of TSH 0–3 mU/l.

* P-value for the difference from test 0.

NS: Not significant.

basal TSH: Basal TSH level prior to injection of TRH.
mTSH: Maximal response of TSH after TRH-injection.
mΔTSH: Maximal increase above basal TSH value.
RESULTS

Group A

Basal data for this group are summarized in Table 1. These 14 patients had a high clinical index, high levels of serum cholesterol and very low levels of T4 and of T3 to a lesser extent.

The TRH test results are shown in Table 2 and Fig. 1. These results are numbered as follows. Zero: just before replacement therapy, test 1: 2 weeks after the start of l-thyroxine, test 2: 4 weeks after starting and so on. During replacement basal TSH declined from test 0 before substitution to test 3 after 6 weeks of substitution from 95.7 ± 43.1 to 26.6 ± 19.8 mU/l. T4 increased

![Graph of TSH responses to TRH injection]

**Fig. 1.**

Effect of replacement therapy in patients of group A on TSH responses (mean ± sd) to 400 µg TRH iv. P-values indicate significance of differences of ΔTSH from test 0 at successive time intervals after TRH injection. Shaded areas and numbers in rectangles represent mean integrated surfaces of ΔTSH at different stages. Surface areas of test 1 and test 2 were significantly higher than the result in test 0 (P-values respectively < 0.02 and < 0.01). ΔTSH: Increase above basal value.
gradually in this period from 7.4 ± 7.8 to 101.8 ± 49.5 nmol/l and T₃ from 0.62 ± 0.45 to 1.87 ± 0.61 nmol/l. In test 1 after 2 weeks of substitution the maximum TSH response (mTSH) had increased to 184.6 ± 84.6 mU/l and mTSH to 87.7 ± 43.4 mU/l. In test 2 the rise in mTSH of 96.0 ± 48.0 mU/l was relatively more pronounced since basal TSH levels were already declining.

In test 3 both basal and mTSH were lower than in test 0, but the proportion between them had essentially changed. All together 13 of 14 patients demonstrated an increase in mTSH on substitution in comparison with the results of test 0.

When considering the total TSH curve significant increase in ΔTSH were found in test 1 and 2 at 20, 40, 60 and 120 min after TRH stimulation. A significant increase in the integrated surface area under the curves of ΔTSH were found in test 1 and 2 when compared with test 0 (respectively P < 0.02 and P < 0.01) (Fig. 1).

**Group B**

The clinical indices, serum cholesterol and values for T₄ and T₃ (just prior to replacement therapy) are summarized in Table 1. These 17 patients were clinically and metabolically not severely myxoedematous (mean Billewicz index –16). Their serum T₄ was very low with 14.6 ± 12.3 nmol/l, but their serum T₃ was still within the normal range i.e. 1.7 ± 0.67 nmol/l.

There was much more heterogeneity in basal TSH in these patients both before and after substitution therapy than in the primary hypothyroid patients. Moreover, many patients had rapidly declining levels of serum TSH after substitution so that comparison of mTSH obtained at moments when basal levels were widely scattered proved to be impossible. The approach of within patient comparison of responses before and after replacement, as we used in group A did not appear feasible.

In this group all test results were divided into three groups of approximately similar basal TSH values. Each group of test results in the pre-substitution period was compared with the "matched" basal TSH group during replacement therapy.

This arrangement made it possible to compare a large collection of TRH-test results with and without thyroxine therapy although the patient populations in the compared groups were not identical.

The setting of hypophyseal feedback at the time of testing, is characterised by two principal influences; these are the level of thyroid hormones and the time interval before and after the starting of replacement. Thyroid hormone levels show the expected variations as hypothyroidism first progresses and later is ameliorated by therapy (Fig. 2). In general the intervals on the time scale before and after the start of replacement, were longer when basal TSH
Comparison of responses obtained before and during substitution with L-sodium-thyroxine in group B subjects. The test results are divided in 3 groups regarding bas. TSH levels. Responses before substitution are compared with those in the matched basal TSH state during L-sodium-thyroxine. The significance of differences between m.TSH before and during substitution is given by the P-values. For differences in increments (m./TSH empty bars) see text. Values for level of thyroid hormone concentrations at the moment of testing are given in the lower part of the figure.

was 3–10 mU/l, while the tests at the higher levels of basal TSH lay closer to this point on both sides.

In Fig. 2 the left bars and column represent results, when basal TSH was mildly elevated (3–10 mU/l). Tests were done in 13 patients before substitution and 11 during replacement therapy. Ten patients are represented in both situations. In 23 tests before replacement basal TSH was 6.6 ± 2.0 mU/l, mTSH 19.2 ± 8.0 mU/l and m1/2TSH 12.6 ± 6.7 mU/l.

During replacement 19 test results at the same basal level of 6.4 ± 2.1 mU/l, showed significantly higher values for mTSH and m1/2TSH with 32.4 ± 16.7 (P < 0.05) and 25.9 ± 15.3 mU/l (P < 0.01), respectively.
Table 3.
Influence of protraction of induced hypothyroid state on basal TSH and per cent rise (‰ mΔTSH) of TSH after TRH in previous toxic patients (group B').

<table>
<thead>
<tr>
<th>Patient</th>
<th>Test</th>
<th>V</th>
<th>IV</th>
<th>III</th>
<th>II</th>
<th>I</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td></td>
<td>15 weeks</td>
<td>12 weeks</td>
<td>9 weeks</td>
<td>6 weeks</td>
<td>3 weeks</td>
<td>0</td>
</tr>
<tr>
<td>Patient</td>
<td>basal TSH mU/l</td>
<td>‰ mΔTSH</td>
<td>basal TSH mU/l</td>
<td>‰ mΔTSH</td>
<td>basal TSH mU/l</td>
<td>‰ mΔTSH</td>
<td>basal TSH mU/l</td>
</tr>
<tr>
<td>E. P.</td>
<td>3</td>
<td>110</td>
<td>9</td>
<td>127</td>
<td>28</td>
<td>132</td>
<td>42</td>
</tr>
<tr>
<td>W. S.</td>
<td>4</td>
<td>225</td>
<td>22</td>
<td>155</td>
<td>35</td>
<td>129</td>
<td>26</td>
</tr>
<tr>
<td>C. S.</td>
<td>7</td>
<td>233</td>
<td>7</td>
<td>229</td>
<td>11</td>
<td>173</td>
<td>10</td>
</tr>
<tr>
<td>G. O.</td>
<td>10</td>
<td>129</td>
<td>16</td>
<td>63</td>
<td>30</td>
<td>83</td>
<td>34</td>
</tr>
<tr>
<td>R. V.</td>
<td>5</td>
<td>144</td>
<td>10</td>
<td>280</td>
<td>11</td>
<td>300</td>
<td>18</td>
</tr>
<tr>
<td>L. B.</td>
<td>6</td>
<td>206</td>
<td>8</td>
<td>225</td>
<td>11</td>
<td>255</td>
<td>21</td>
</tr>
<tr>
<td>C. Q.</td>
<td>6</td>
<td>209</td>
<td>5</td>
<td>357</td>
<td>7</td>
<td>385</td>
<td></td>
</tr>
<tr>
<td></td>
<td>J. S.</td>
<td>G. T.</td>
<td>C. P.</td>
<td>C. L.</td>
<td>G. D.</td>
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<tr>
<td>Mean</td>
<td>8.5</td>
<td>168</td>
<td>15.5</td>
<td>141</td>
<td>15.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sd</td>
<td>-</td>
<td>-</td>
<td>13.0</td>
<td>45.6</td>
<td>10.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4 nmol/l</td>
<td>45.5</td>
<td>36</td>
<td>55 ± 30.2</td>
<td>41.8 ± 22.4</td>
<td>28.5 ± 15.0</td>
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</tr>
<tr>
<td>± sd</td>
<td>2.6</td>
<td>1.9</td>
<td>2.5 ± 0.5</td>
<td>2.2 ± 0.5</td>
<td>2.0 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3 nmol/l</td>
<td>6</td>
<td>181</td>
<td>8</td>
<td>189</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>± sd</td>
<td>7</td>
<td>78</td>
<td>16</td>
<td>75</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>167</td>
<td>18</td>
<td>189</td>
<td>32</td>
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<tr>
<td></td>
<td>7</td>
<td>79</td>
<td>16</td>
<td>31</td>
<td>21</td>
<td></td>
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<td>20</td>
<td>75</td>
<td>74</td>
<td>39</td>
<td>110</td>
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<tr>
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<td></td>
<td></td>
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<td></td>
<td>36</td>
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</tbody>
</table>
In Fig. 2 the middle bars and column show the effect of substitution at the stage of moderately elevated TSH levels (11–25 mU/l). Eighteen tests on 13 patients were performed before thyroxine medication and on 10 patients 17 tests, during thyroxine medication. There were 9 patients who are represented in both circumstances. Before replacement basal TSH was 16.7 ± 3.8 mU/l, mTSH 42.2 ± 14.1 mU/l and mΔTSH 25.4 ± 13.4 mU/l. The same basal TSH of 17.4 ± 4.6 mU/l during substitution was accompanied by a significantly higher levels of mTSH of 61.8 ± 25.5 mU/l (P < 0.05) and mΔTSH of 44.5 ± 22.9 mU/l (P < 0.01).

In Fig. 2 the right bars and column represent results obtained at the stage of a high basal TSH (> 25 mU/l). Fourteen tests in 6 patients were performed before replacement and 12 tests in 5 patients during thyroxine therapy. Four patients in this subgroup are represented in both study conditions.

Before replacement basal TSH was 52.3 ± 25.8 mU/l, mTSH 91.4 ± 35.7 and mΔTSH 39.1 ± 16.0 mU/l. An identical basal TSH of 53.6 ± 25.3 mU/l was during substitution accompanied by a mTSH of 146.1 ± 28.4 mU/l (P < 0.01) and a mΔTSH of 92.5 ± 22.0 mU/l (P < 0.001).

**Group B**

Because all tests before substitution therapy were, in a temporal sense, performed more closely to the previously existing thyrotoxic state, the question whether this had any remaining effect on TSH response was evaluated. The results in 12 of 17 patients in group B, who were followed during a period varying from 6 to 15 weeks in the hypothyroid state, are displayed in Table 3. The test results are numbered: zero = just before replacement therapy, I = 3 weeks before therapy, II = 6 weeks before and so on.

In this part of our study an attempt is made to compare TRH response at successive stages of hypothyroidism with increasing levels of basal TSH. The only way to compare responses with highly divergent values for TSH is to consider the % mΔTSH instead of the absolute values (as was done with all other results).

Gradual increases in basal TSH as expected in progressive hypothyroidism were accompanied with increases in maximal and ΔTSH levels. However, the % mΔTSH showed no tendency to rise as the interval from the preceding thyrotoxicosis increased.

**DISCUSSION**

Comparison of TRH test results before and after the administration of l-thyroxine in hypothyroid patients, revealed how a relatively blunted response of TSH release, did change in the following characteristic way. During the
first month of therapy basal TSH tended to decrease, but m₄TSH rose significantly. This phenomenon had been observed earlier in a few isolated cases (Ridgway et al. 1973; Wartofsky et al. 1976).

The finding that patients without clinical myxoedema (group B) demonstrated the same sequential response pattern as those in group A, deserves special attention. The argument that in group B, blunting was caused by preceding thyrotoxicosis, appears false because of the observations in the longitudinal study of group B'. In this pre-substitution phase of slowly progressive hypothyroidism, the relative TSH response on TRH remained in principle unchanged (m₄TSH constant in Table 3) notwithstanding a growing time interval of continuous hypothyroidism.

The mechanism responsible for the observed effect of thyroxine on the TSH response in hypothyroidism cannot be defined with certainty. A simple metabolic effect on the pituitary gland does not appear likely, because of the observations on patients in group B, without clinical myxoedema and T₃ levels within the normal range. In spite of this and of a hypothyroidism of proven short duration, blunting and enhancement of the TSH response was found just as it was in the patients with primary hypothyroidism.

Moreover, if enhancement were an expression of transient increased TSH synthesis, one would not expect to find it, one month after the initiation of L-thyroxine substitution when nearly normal thyroid hormone levels had already resulted in a considerable decline of basal TSH release (Fig. 1, test 2). However, on the basis of our results a definite conclusion on the influence of thyroxine on TSH synthesis is impossible.

The increased responses after TRH during the first weeks of substitution could be explained by an increased TSH content of the hypophysis or by an increase in the sensitivity of the releasing mechanism. We offer the following hypothetical explanations:

1. In the initial phase of replacement when low doses of thyroxine were used, the negative feedback on TSH secretion was accompanied by an increase in storage of TSH in the anterior pituitary and thus an increase in the releasable fraction on TRH-stimulation. This would correspond with the experimental results of D'Angelo (1969) and D'Angelo et al. (1976) who could demonstrate accumulation of TSH on injecting subphysiological amounts of thyroxine in hypothyroid rats, the so-called “pituitary TSH rebound”. These investigators explain their findings with asynchrony between production and secretion in the early phase of substitution, the background of which remains unresolved.

2. Patients tested before substitution had very high levels of endogenous TRH, either circulating or only at the receptor level of the pituitary gland. This would make them less responsive to exogenous TRH given in the standard dose. Substitution with L-thyroxine is followed by the well known
negative feedback on TSH synthesis, but perhaps also by a negative feedback on TRH synthesis in the hypothalamus. If this last process has an earlier effect, then re-testing after 2–4 weeks of replacement could reveal a state, whereby endogenous TRH secretion is suppressed more effectively than TSH secretion. This would provide a higher sensitivity for exogenous TRH demonstrable as a less blunted response curve.

The reason for high levels of endogenous TRH, present in both groups of hypothyroid patients, can be explained either by the very low levels of circulating T4 which they have in common, or by a preceding rapid disappearance of T4 which surely was the case in group B. According to this concept, blunting of the TSH response can be expected when T4 levels are very low or rapidly declining. In contrary to patients with mild hypothyroidism and stable levels of circulating hormones, where maximal responses of several hundred per cent are the rule (Gordin et al. 1973; Evered et al. 1973).

This hypothesis regarding the influence of thyroid status on hypothalamic function is in contrast to that postulated by Reichlin (1975). This was based on measurements of TRH excretion in the urine of hypothyroid rats (Jackson et al. 1974). On the other hand Montoya et al. (1975) could not find any change in blood TRH and hypothalamic TRH content after drug induced hypothyroidism in the rat. Recently Mitsuma et al. (1976) found high levels of circulating TRH in myxoedematous patients, which returned to normal during substitution therapy. Because of the conflicting results about the possible positive, negative or absent of feedback of thyroid hormones on the TRH secretion and the technical problems of the TRH radioimmunoassays, Reichlin (1978) recently concluded, that at present it is impossible to draw any conclusions, regarding the influence of thyroid hormones on TRH secretion.

Further clarification of the hormonal background and causes of the changing pattern in TRH response must await the development of a reliable radioimmunoassay for TRH with which will allow dynamic changes (during substitution) to be measured.

REFERENCES


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