

Sequential changes in the pituitary thyroid axis after chronic TRH administration: effects on euthyroid and thyroxine treated female rats

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Abstract. The effect of chronic oral thyrotrophin-releasing hormone (TRH) administration on thyrotrophin (TSH), L-triiodothyronine (T_3) and L-thyroxine (T_4) serum levels, pituitary TSH concentration and serum response to acute TRH injection, has been studied in female rats under different thyroidal conditions: sham-operated control animals, and thyroidectomized animals receiving 25 μ g L- T_4 /100 g body weight/day. After 30 days, these groups were divided into two subgroups (6-10 animals per group), one receiving the aforementioned treatment and the other the same plus 2 mg TRH/10 ml distilled water (DW), as drinking water.

TRH-treated sham-operated animals showed significantly reduced serum and pituitary TSH levels and increased serum T_3 levels at most of the times studied (1, 6, 10, 18 and 34 days of oral TRH or DW administration), and a transient elevation in serum T_4 between day 1 and 6. Thyroidectomized-L- T_4 -treated animals showed increased serum and pituitary TSH levels throughout the treatment and reduced T_3 and T_4 serum levels at the beginning, as compared to thyroidectomized-L- T_4 -treated animals.

TSH response to iv TRH administration on the 10th day of oral TRH administration was reduced in controls chronically treated with oral TRH as compared to non-treated controls, and was increased in thyroidectomized-

L- T_4 -treated animals on chronic TRH vs the same group on oral DW.

These results suggest that chronic TRH administration can stimulate TRH synthesis in vivo, bypassing the inhibitory effects of thyroid hormones, the increased pituitary TSH reserve being responsible for the partial restoration of a response to acute TRH injection in the thyroidectomized-L- T_4 -treated animals.

Studies carried out both in humans and in rats have demonstrated that chronic administration or multiple doses of TRH induce severe changes in the pituitary-thyroid axis (Snyder & Utiger 1973; Rabello et al. 1974; Nakagawa 1975; D'Angelo et al. 1975; Frey & Haug 1977; Lifschitz et al. 1978; Staub et al. 1978; Soji 1978; Nemeroff et al. 1980). In humans and normal rats these changes can be summarized as a reduction in basal serum TSH levels, an increase in thyroid hormone circulating levels and diminished TSH response to further TRH stimulation. Various explanations have been proposed for the interpretation of these findings: thyroid hormone-mediated pituitary inhibition (Snyder & Utiger 1973), ultrashort negative TSH feedback (Staub et al. 1978; Liu & Hedge 1983), decreased pituitary TSH reserve (Rabello et al. 1974; Nakagawa 1975), and reduction in the number of pituitary TRH receptors (Nakagawa 1975; Staub et al. 1978). It is also well known that in hyperthyroid humans and animals, the thyrotroph response to TRH is blunted, the degree of TSH response being closely related to the T_4 serum levels (Bowers et al. 1967; Snyder & Utiger 1972;

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Averill 1974). Nevertheless, the amount of TRH administered also plays an important role in the degree of response (Lifschitz et al. 1978; Padmanabhan et al. 1981). The aim of the present report has been to study the sequential effects of chronic TRH administration (given orally, to obtain a prolonged stimulation), on the pituitary-thyroid axis in normal animals and in animals rendered hyperthyroid by L-T₄ administration, to ascertain whether high TRH doses can bypass the inhibitory effects of thyroid hormone on thyrotroph function, and whether the effects of chronic TRH administration on thyrotroph function described by others are mainly due to thyroid activation.

Materials and Methods

Animals

Female Sprague-Dawley rats were used in all the experiments. They were housed six per cage with automatically controlled temperature (20–23°C) and light-dark cycle (08.00–20.00 h). They were fed laboratory rat chow and weighed every 3 days.

Experimental design

Twenty-four day old female rats were surgically thyroidectomized or sham-operated. L-T₄ or ip saline administration treatment was started 24 h later. Sham-operated controls (C) received saline, while thyroidectomized animals received 25 µg L-T₄/100 g body weight/day (T + 25).

After 30 days on this treatment ('0' time), each group was divided into two subgroups which were put on either distilled water (DW) or TRH (2 mg/10 mg DW) as drinking solution. The resulting groups were C, C + TRH, T + 25 + TRH. L-T₄ was obtained from Sigma, and TRH was kindly donated by Prem, Spain. Animals were sacrificed by decapitation on days 1, 6, 10, 18 and 34 of oral DW or TRH administration. Bottles with drinking solutions were removed from the cages 1 h prior to sacrifice. Sacrifice was carried out between 10.00 and 11.00 h.

On the tenth day of oral TRH or DW administration, an acute TRH administration test was performed by injecting 2 µg TRH/100 g body weight into the tail vein. Blood samples were taken from the tail vein at 0, 15, 30 and 60 min. This test was only done on animals to be sacrificed after 34 days of oral TRH administration.

Samples

After decapitation, the pituitaries were weighed and put into plastic tubes containing 1 ml DW. They were placed in an ice-bath and homogenized by sonication. They were then kept at -20°C until TSH assay was performed. Trunk or tail blood samples were allowed to clot and were then centrifuged. The sera were stored at -20°C until TSH, T₃ and T₄ assays were performed.

Assays

TSH was measured by RIA using the immunoreactants kindly provided by the Rat Pituitary Hormone Distribution Program (NIADDK, NIH). Antibody-bound and free hormone were separated by protein A-bearing *Staphylococcus aureus* (Cowan Strain, Sigma). Since RIA

Table 1.
Effect of DW or TRH oral administration on pituitary weight in control (C) and thyroidectomized-L-T₄ treated rats (T + 25).

	Time (days) on DW or TRH				
	1	6	10	18	34
C	10.05 ± 0.4 (6)	10.6 ± 0.3 (10)	10.1 ± 0.4 (10)	12.1 ± 0.5 (10)	12.3 ± 0.6 (9)
C + TRH	10.2 ± 0.4 (10)	11.4 ± 0.4 (10)	10.7 ± 0.4 (10)	10.8 ± 0.4 (10)	12.4 ± 0.6 (9)
T + 25	9.67 ± 0.54 (6)	9.7 ± 0.3 (8)	10.0 ± 0.6 (8)	11.2 ± 0.7 (10)	11.2 ± 0.3 (9)
T + 25 + TRH	9.5 ± 0.4 (10)	11.8 ± 0.3 (10) ***	11.2 ± 0.5 (10)	13.1 ± 0.5 (10) *	12.5 ± 0.7 (10)

Values are expressed as means (mg) ± SEM. Numbers in parentheses below data indicate the number of animals studied. Asterisks below data indicate the statistical differences between animals on TRH vs the same group on DW. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

standard curves were done in buffer and not in TSH-free serum, values may overestimate the real TSH values, as stated by Obregón et al. (1978).

Serum T_3 and T_4 were measured by RIA, as described elsewhere (Obregón et al. 1978, 1979) using antibodies from Henning, L- T_2 and L- T_3 were used for radioiodination and L- T_3 and L- T_4 as reference. Antibody-bound and free hormone were separated using polyethyleneglycol (Carbowax 6000), and bovine gamma globulin.

Statistics

Data are expressed as means (6–10 animals) \pm SE. Comparisons between groups were done using the non-paired Student's *t*-test.

Results

Table 1 shows that no differences in pituitary weight were present between C and T+25. Oral TRH induced a slight increase in this parameter in

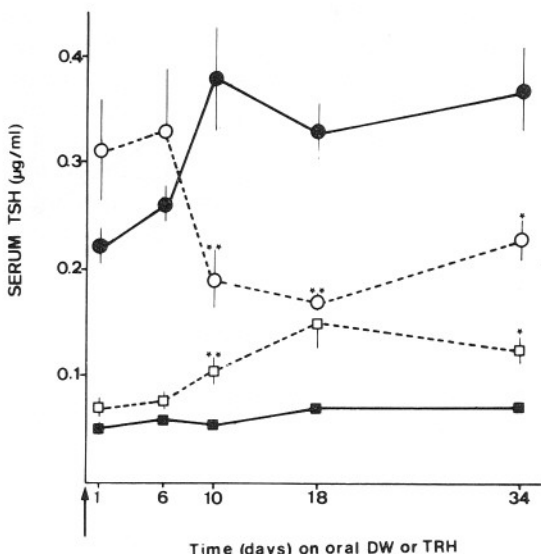


Fig. 2.

Serum TSH concentration. Representation and symbols are as defined in Fig. 1.

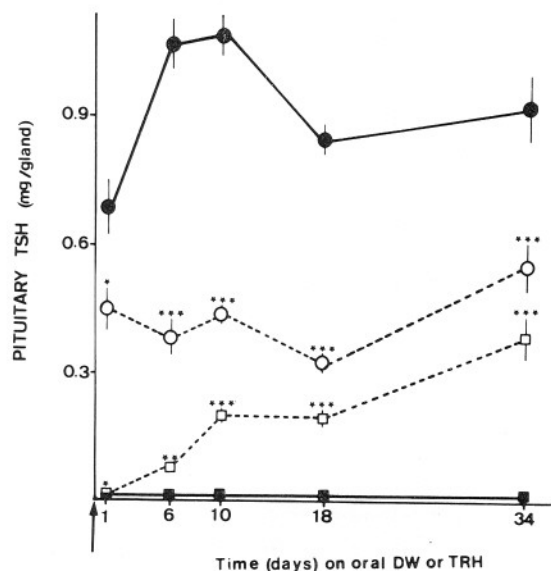


Fig. 1.

Pituitary TSH content in sham-operated control animals and thyroidectomized animals treated with 25 µg of L- T_4 /100 g body weight/day at different times of oral administration of TRH (2 mg/10 ml DW). Arrow indicates when TRH treatment was started. ●—● Control animals on oral DW (C); ○—○ control animals on oral TRH (C + TRH); ■—■ thyroidectomized L- T_4 treated animals on oral DW (T + 25); □—□ thyroidectomized L- T_4 treated animals on oral TRH (T + 25 + TRH);

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

T + 25 + TRH vs T + 25 at days 6 and 18, while no differences were observed between C and C + TRH. Pituitary TSH content (Fig. 1) was always much lower in T + 25 animals than in C, the differences being highly significant ($P < 0.001$, the comparisons are not shown in the figure). From the first day of TRH administration, there was a considerable decrease in pituitary TSH in the C + TRH group. In group T + 25 + TRH there was a progressive increase in this parameter from 15.1 µg on the first day to 385 µg on day 34, the differences being significant vs T + 25 at all the times studied. As expected, serum TSH values were very low in T + 25 (Fig. 2). TSH values increased in T + 25 + TRH vs T + 25, being statistically significant at days 10 and 34.

Serum T_4 values (Fig. 3) of T + 25 animals were about twice those of controls, the differences being highly significant ($P < 0.001$, the comparisons are not shown in the figure). Increased levels of this hormone were also seen in C + TRH groups at days 1 and 6 of TRH treatment, which then decreased to attain normal values on day 10. By contrast, lower T_4 levels were seen in T + 25 + TRH than in T + 25 during the first 10 days.

Serum T_3 values (Fig. 4) in T + 25 were significantly higher than those of C at the beginning of TRH treatment ($P < 0.001$, comparison not shown

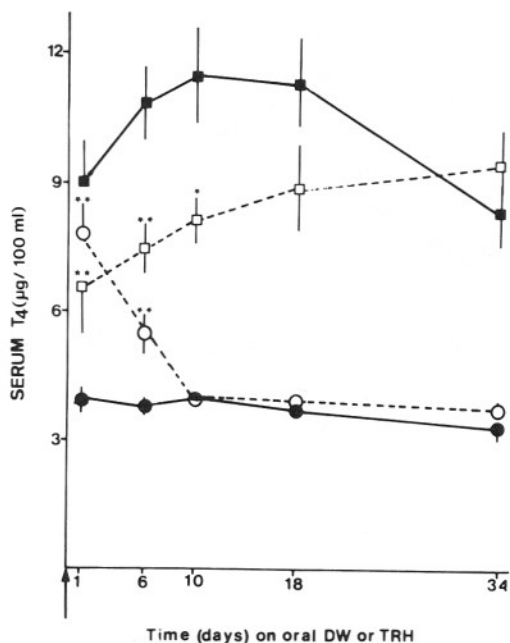


Fig. 3.

Serum T₄ concentration. Representation and symbols are as defined in Fig. 1.

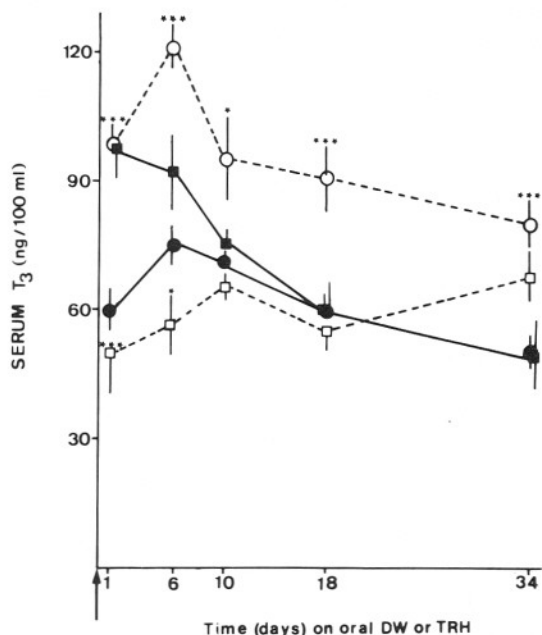


Fig. 4.

Serum T₃ concentration. Representation and symbols are as defined in Fig. 1.

in the figure). They decline progressively, so that no differences between T + 25 and C were present at any further time. Oral TRH administration induced an elevation in serum T₃ levels in C + TRH vs C at all the times studied. By contrast, serum T₃ values in T + 25 + TRH were significantly lower than those in T + 25 at days 1 and 6 of TRH administration.

The serum TSH response to an iv bolus of TRH (2 µg/100 g body weight), at 10 days on oral TRH or DW administration was reduced in the C + TRH group as compared to C (Fig. 5). TSH values in C + TRH animals injected with saline were lower than those of C injected with saline ($P < 0.05$ at all the times, comparison not shown in the figure). In group T + 25, the response to iv TRH administration was abolished. However, oral TRH administration to group T + 25 + TRH induced the presence of a response, although the TSH values attained were significantly lower than those of C + TRH ($P < 0.01$, comparison not shown in the figure).

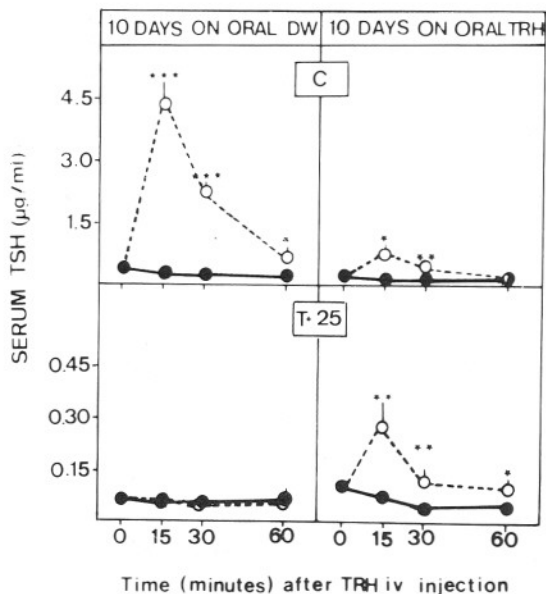


Fig. 5.

Serum TSH (µg/ml) response to TRH (2 µg/100 g body weight) in injection in control (C) and thyroidectomized animals treated with 25 µg of L-T₄/100 g body weight (T + 25). ○—○ TRH injected, ●—● saline injected. Left panel: animals on oral DW for 10 days. Right panel: animals on TRH for 10 days. Asterisks represent statistically significant differences between animals injected with saline and animals injected with TRH. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Discussion

The main aspect of the present study is the finding of a positive effect of chronic TRH administration on TSH secretion in rats treated with large doses of L-T₄. This finding is in apparent contradiction to the effects of the tripeptide on normal rats, described by others and confirmed in the present paper.

Although it has been demonstrated that administration of TRH or thyroid hormones, both in vivo and in vitro, causes a reduction in pituitary TRH receptors (Gershengorn 1978; Perrone & Hinkle 1978; Hinkle et al. 1981; Wilber & Seibel 1973; Hinkle & Tashjian 1975), the high doses used in this study would have been sufficient to stimulate the secretion in group C+TRH, since T₃ values remained high during the 34 days of treatment. On the other hand, these animals responded to acute TRH administration although their response was lower than those of C. Since it is not documented that TRH can act directly on the thyroid, these facts are consistent with the existence of transient TSH elevations that we have not been able to detect, these elevations leading to thyroid activation and pituitary TSH depletion.

Elevations in serum TSH in group C+TRH might therefore be present during TRH treatment at times other than when the animals were sacrificed. The explanation for this hypothesis lies in the fact that the rat drinks 85–95% of its daily intake of water at night (Kuribara et al. 1978), this time being when maximal thyrotroph stimulation and thyroid hormone should be present. As the half life of thyroid hormones is longer than that of TSH (Pittman 1979; Griessen & Lemarchand-Béraud 1973; Silva & Larsen 1978; Pastor & Jolin 1983), their blood levels should remain higher than those of the latter, blunting TSH secretion and the response of this hormone to acute TRH administration. This would explain the high serum T₃ levels and the observed decreased serum TSH.

The increased serum and pituitary TSH seen in group T+25+TRH vs group T+25 indicate that TRH has been able to stimulate the pituitary thyrotroph, overcoming the effects of thyroid hormones, an effect not seen in vitro (Hinkle & Goh 1982). Therefore the high TRH doses given in this study should have been sufficient to reach the pituitary, thereby stimulating TSH synthesis. This would partially replenish the TSH reserve, making possible the increase in TSH values and the pre-

sence of a response to iv TRH injection. It could be argued that these effects of TRH on TSH may be due not to the direct action of TRH but to the lower thyroid hormone levels seen in T+25+TRH vs T+25. Nevertheless, we have observed (data not shown) that when sham-thyroidectomized T₄-treated rats are administered with TRH as described here, they show increased serum T₃ and TSH and increased pituitary TSH as compared to animals on oral DW. We therefore conclude that TRH mediated induction in TSH synthesis in animals treated with L-T₄ and oral TRH is the primary factor in the partial restoration of thyrotroph function.

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