The pituitary TSH response to TRH is inversely related to the plasma TSH concentration and directly related to the pituitary TSH content during hypothyroidism in the rat

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Abstract. We studied the effects of degree and duration of hypothyroidism on the pituitary TSH concentration and the pituitary TSH secretory response to TRH. Varying degrees of hypothyroidism were achieved in thyroparathyroidectomized rats (THYREX) by continuous sc infusion of T₃ (0.2, 0.3, 0.4, or 0.5 µg/100 g · day) or T₄ (0.6, 1.2, or 1.8 µg/100 g · day). While T₃ was more potent than T₄, both resulted in a dose-dependent suppression of the post-thyroidectomy rise in TSH. After 7 or 14 days of severe hypothyroidism (non-replaced THYREX rats) the pituitary TSH secretory response to TRH (250 ng/100 g body weight, iv) was found to be decreased when compared to that of euthyroid rats. Decreasing the degree of hypothyroidism increased the pituitary secretory response to TRH and the pituitary TSH content. The results indicate that in the hypothyroid rat: 1) severe hypothyroidism results in a blunted pituitary TSH response to TRH through 14 days after thyroidectomy, 2) at 7 and 14 days after thyroidectomy the pituitary TSH response to exogenous TRH is inversely related to the basal plasma TSH concentration, 3) the pituitary TSH concentration increases with the duration of hypothyroidism, 4) the pituitary TSH content is increased by low rates of thyroid hormone replacement, and 5) the pituitary TSH response to exogenous TRH is directly related to the pituitary TSH content.

The secretion of TSH is primarily regulated by two opposing factors: 1) a net stimulation of pituitary TSH secretion which is the result of the interaction among at least three hypothalamic factors (TRH, somatostatin and dopamine), and 2) the negative feedback effects of the thyroid hormones, T₃ and T₄ (Morley 1981). At the pituitary, the inhibitory effect of thyroid hormones is in competition with the stimulatory effect of TRH from the hypothalamus. According to this model, changes in the circulating concentrations of thyroid hormones result in reciprocal changes in the basal concentration of TSH and in the sensitivity of the pituitary to TRH. In euthyroid humans and animals, administration of thyroid hormones decreases plasma TSH levels, and blunts the pituitary TSH response to TRH (Bowers et al. 1967; Snyder & Utiger 1972; Brozmanova et al. 1980). Conversely, in humans and animals a decrease in thyroid hormones increases plasma TSH (Reichlin et al. 1970; Vagenakis et al. 1974; Saberi & Utiger 1975; Larsen & Frumess 1977; Connors & Hedge 1980, 1981a,b), but an exaggerated pituitary TSH response has only been demonstrated in humans (Vagenakis et al. 1974; Saberi & Utiger 1975). In contrast, we have previously shown that in severely hypothyroid rats the pituitary TSH secretory response to TRH is either blunted or similar to that of euthyroid controls (Connors et al. 1984). Lemarchand-Beraud & Berthier (1981) have reported a similar effect in rats made hypothyroid by 7 days of PTU administration. These studies suggest that in the hypothyroid rat the pituitary secretory response to TRH is not inversely related to the amount of feedback inhibition exerted by thyroid hormones at the pituitary. The aim of the present study was to further assess the effects of hypothyroidism on the pituitary.
TSH secretory response to TRH by establishing varying degrees of hypothyroidism by continuous infusion of T₃ or T₄ to thyroidectomized rats.

Materials and Methods
Throughout these experiments female Sprague-Dawley rats (Hilltop Labs Animals Inc, Scottsdale, PA) weighing 170–220 g were used. All animals were maintained on Purina rat chow and tap water, with the drinking water of the THYREX rats supplemented with 1% CaCl₂. Seven or 14 days prior to TRH administration, rats were surgically thyroparathyroidectomized under ether anaesthesia. Within 2 h of thyroparathyroidectomy, Alzet osmotic minipumps (Alza Corp, Palo Alto, CA) containing thyroid hormone replacement were implanted sc between the scapulae under light ether anaesthesia. Intact euthyroid control rats were left untreated. In the first experiment, rats received infusions of T₃ (0.2, 0.3, 0.4 or 0.5 µg/100 g·day) or T₄ (0.6, 1.2 or 1.8 µg/100 g·day) in 0.01 N NaOH, 5% rat serum, in saline for 7 days. In the second experiment, infusion of T₃ or T₄ was maintained for 14 days. In these animals, after 7 days minipumps were removed and fresh minipumps containing the appropriate thyroid hormone replacement were implanted in the same site. In both experiments, four days prior to TRH administration rats were anaesthetized with ketamine (24 mg/100 g body weight, ip) and sodium pentobarbital (2 mg/100 g body weight, ip) and prepared with chronic intra-atrial catheters (Connors et al. 1984).

TRH stimulation
TRH was administered, and sequential blood samples were obtained via the intra-atrial catheters in unaesthetized, unstressed rats. Rats received a bolus injection of a submaximal dose of TRH (250 ng/100 g body weight) in saline at 7 or 14 days after thyroidectomy and the initiation of thyroid hormone replacement. Blood samples were collected prior to (time 0) and at 5, 15, 30 and 45 min after the administration of TRH. Heparinized plasma samples were frozen and stored at −15°C until assayed for TSH. The change in the plasma TSH concentration (Δ pTSH) was calculated by subtracting the basal plasma TSH concentration (time 0) from the plasma TSH concentrations after injection of TRH. The area under the Δ pTSH curve for individual rats was estimated with the use of the trapezoidal rule for approximation of integrals.

Pituitary TSH concentration
Pituitary TSH was extracted as previously described (Connors et al. 1984). Briefly, the extraction was carried out in an ice bath at 0°C. Ice-cold 2.0% NaCl, pH 7.0 (1.0 mg:20 ml tissue: saline) was added to the tissue samples. The samples were then sonicated (Braunsonic 1510, Braun Instruments, San Francisco, CA) for 10–15 sec at 300 W to disrupt the cells. The pH of the sonicate was adjusted to 4.5 by addition of 0.1 N HCl. The sonicate was then centrifuged at 1500 x g for 45 min at 0°C and the supernatant assayed for TSH. Under these conditions, approximately 80% of the immunoreactive TSH is recovered in the acid-saline supernatant. Data are presented as µg of TSH per mg of tissue (wet weight).

Radioimmunoassay
Plasma and tissue extracts were assayed for TSH using RIA materials provided by the NIADDK National Hormone and Pituitary Program and Dr A. F. Parlow, using NIADDK rat TSH-RP-1 (0.22 U/mg) as the standard. Carrier-free ¹²⁵I for iodination of TSH was purchased from Amersham (Arlington Heights, IL). Specific RIAs for T₃ and T₄ were performed with antisera kindly provided by Dr P. R. Larsen (Boston, MA). [¹²⁵I]T₄ (SA 100 mCi/µg) was obtained from Industrial Nuclear Co (St. Louis, MO). RIA results were analyzed on an Apple II plus microcomputer using a weighted logit-log RIA data processing program. Data from separate assays were pooled only if the interassay variation was sufficiently small (indicated by the results of replicates of two plasma pools run in every assay) as determined by the quality control parameters of Rodbard (1974).

Statistical analysis
Tests for homogeneity of variance were performed using the F max procedure described by Winer (1971). Using the Dunnett’s modified t statistic the effects of thyroid hormone replacement were assessed by comparing the replaced animals to intact control or non-replaced THYREX rats (Winer 1971). The effects of duration of hormone replacement (7 vs 14 days) were analysed using analyses of variance. Tests of simple main effects were used to test for significant differences between two groups. The size of the region of rejection of the null hypothesis was set by an alpha error of 5% (Winer 1971).

Results
Seven and 14 days after thyroidectomy, the plasma T₃ concentrations in non-replaced THYREX rats fell to levels below our limits of detection (Fig. 1). When compared to that of non-replaced THYREX rats, continuous infusion of T₃ (0.2, 0.3, 0.4 or 0.5 µg/100 g·day) or T₄ (0.6, 1.2 or 1.8 µg/100 g·day) for 7 or 14 days increased the plasma concentration of T₃ in a dose-dependent manner (Fig. 1). However, after
7 or 14 days of replacement, plasma T₃ concentrations were less than that of euthyroid controls in all groups except for those rats receiving 0.5 µg T₃/100 g · day for 7 days (Fig. 1A, left panel). It should be noted, however, that the plasma T₃ levels illustrated in Fig. 1A and 1B, were determined in separate T₃ assays. Unfortunately, the inter-assay variation was large enough to account for the difference in the control levels, illustrated in Fig. 1. This prohibited the statistical comparisons of plasma T₃ levels between groups infused for 7 or 14 days.

In the non-replaced THYREX rats and in those receiving continuous T₃ infusions, the plasma T₄ concentrations fell to and remained below our limit of detection (Fig. 2, left panel). Continuous infusion of T₄ for 7 or 14 days resulted in a dose-dependent increase in the plasma concentration of T₄ (Fig. 2, right panel). Infusion of 1.2 or 1.8 µgT₄/100 g · day for 7 days resulted in plasma T₄ concentrations which were not different from those of euthyroid control rats. However, at all infusion rates, after 14 days of replacement the plasma T₄ concentrations were significantly less than those of euthyroid controls. In addition, plasma T₄ concentrations of rats infused with 0.6

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**Fig. 1.**

Plasma concentrations of T₃ (mean ± SEM) in THYREX rats receiving replacement doses of T₃ (0.2, 0.3, 0.4, or 0.5 µg/100 g · day) or T₄ (0.6, 1.2, or 1.8 µg/100 g · day), for 7 (A) or 14 (B) days. Plasma concentrations T₃ in non-replaced THYREX rats (7 or 14 days) are presented as the 0.0 dose of T₃. The shaded area represents the mean (± SE) plasma concentration of T₃ of control rats. The dashed line represents the minimal detectable dose (MDD) of the assay. The number of animals in each group ranged from 5 to 9.
Fig. 2.
Plasma concentrations of T₄ (mean ± SEM) in THYREX rats receiving replacement doses of T₃ (0.2, 0.3, 0.4, or 0.5 µg/100 g · day; left panel) or T₄ (0.6, 1.2, or 1.8 µg/100 g · day; right panel) for 7 or 14 days. The shaded area represents the mean (±SE) plasma concentration of T₄ of control rats. The dashed line represents the minimal detectable dose (MDD) of the assay. The number of animals in each group ranged from 5 to 9.

Fig. 3.
Plasma concentrations of TSH (mean ± SEM) in THYREX rats receiving replacement doses of T₃ (0.2, 0.3, 0.4, or 0.5 µg/100 g · day; left panel) or T₄ (0.6, 1.2, or 1.8 µg/100 g · day; right panel) for 7 or 14 days. The shaded area represents the mean (±SE) plasma concentration of TSH of control rats. The number of animals in each group ranged from 5 to 9.
Pituitary TSH concentrations (mean ± SEM) in THYREX rats receiving replacement doses of T₃ (0.2, 0.3, 0.4, or 0.5 µg/100 g · day; left panel) or T₄ (0.6, 1.2, or 1.8 µg/100 g · day; right panel) for 7 or 14 days. The shaded area represents the mean (± SE) pituitary TSH concentration of control rats. Data are presented as µg TSH per mg tissue (wet weight). The number of animals in each group ranged from 5 to 9.

or 1.2 µgT₄/100 g · day for 14 days were significantly less than those in rats infused with T₄ at the same rates for 7 days.

Continuous infusion of T₃ or T₄ for 7 or 14 days resulted in a dose-dependent inhibition of the post-thyroidectomy rise in plasma TSH (Fig. 3). After 7 days of replacement only the highest infusion rates of T₃ (0.4 or 0.5 µg/100 g · day) or T₄ (1.8 µg/100 g · day) were sufficient to block a significant rise in plasma TSH. After 14 days of replacement, plasma TSH concentrations of all THYREX rats (with or without replacement) were significantly greater than the corresponding concentrations after 7 days of replacement. However, the dose-dependent inhibition of plasma TSH was maintained.

In 7 day THYREX rats (with or without replacement), the pituitary TSH concentrations were significantly less than that of euthyroid controls (Fig. 4). Pituitary TSH concentrations in THYREX rats, with or without replacement, increased from 7 to 14 days after thyroidectomy. Infusion of T₃ had little apparent effect on pituitary TSH concentration, with only one group (0.5 µg T₃ for 7 days) showing a significant increase above that of non-replaced THYREX rats (Fig. 4, left panel). Infusion of T₄ resulted in a dose-dependent increase in pituitary TSH concentration when compared to those of non-replaced THYREX rats at both 7 and 14 days (Fig. 4, right panel). At 7 days, T₄ replacement rates of 1.2 or 1.8 µgT₄/100 g · day resulted in pituitary TSH concentrations which were not different from those of euthyroid controls.

Plasma samples for TSH determination were collected immediately prior to (time 0) the injection of TRH (250 ng/100 g body weight) and at 5, 15, 30 and 45 min thereafter. The area under the Δ pITSH curve was calculated for each animal and was used as an estimate of the amount of TSH secreted in response to TRH (Table 1). In response to TRH stimulation, non-replaced THYREX rats secreted significantly less TSH than did euthyroid controls (Table 1). Infusion of T₃ or T₄ in THYREX rats for 7 or 14 days resulted in a significant increase in the amount of
TSH secreted in response to TRH when compared to that of non-replaced THYREX rats (P's < 0.05). There was no qualitative difference in the action of T₃ or T₄ on the pituitary TSH response to TRH (Table 1). Therefore, we plotted the pituitary TSH secretory response to TRH of individual rats as a function of basal plasma TSH (Fig. 5A and B) and pituitary TSH concentrations (Fig. 5C and D). In hypothyroid rats (i.e., those with elevated basal plasma TSH's), the TSH response to TRH was negatively correlated with basal plasma TSH (P < 0.05) at both 7 and 14 days of replacement. All non-replaced THYREX rats displayed a blunted TSH response to TRH. Throughout the range of pituitary TSH concentrations, the TSH response to TRH was positively correlated with pituitary TSH concentrations after both 7 or 14 days of replacement (Fig. 5C and D).

### Discussion

In the present study we examined the effects of the degree and duration of hypothyroidism on the pituitary TSH secretory response to TRH by establishing varying degrees of hypothyroidism by continuous infusion of T₃ or T₄ to THYREX rats for 7 and 14 days. The plasma T₄ concentrations resulting from continuous replacement were slightly lower in rats receiving T₄ for 14 days than in those receiving T₄ for only 7 days. This may, in part, be due to inter-assay variability (<10%). Other sources of the observed variability might include the increase in body weight and changes in T₄ metabolic clearance rate (MCR) with constant T₄ replacement of increasing duration. The variability of the plasma thyroid hormone levels at 7 and 14 days may have contributed to the differences observed in the plasma and pituitary TSH concentrations at these times. The duration of the hypothyroid state also appears to contribute to this variability. In each of the thyroidectomized groups, with or without thyroid hormone replacement, the plasma and pituitary TSH concentrations are greater at 14 days. The results of Spira et al. (1979) and our own previous observations (Connors et al. 1984) have demonstrated that both the plasma TSH concentration and the pituitary TSH content are increasing during this period in hypothyroid rats. While T₃ was quantitatively more potent than T₄, both resulted in a dose-dependent suppression of the post-thyroidectomy rise in plasma TSH. These data are consistent with previous reports which indicate that continuous infusions or bolus injections of T₃ or T₄ have an inhibitory effect on basal TSH secretion in the hypothyroid rat (Reichlin et al. 1970; Larsen & Frumess 1977; Connors & Hedge 1980).

### Table 1

Area (μg TSH·min/ml) under the ApltSH curve following iv injection of TRH (250 ng/100 g·body weight).

<table>
<thead>
<tr>
<th>Control</th>
<th>0.0</th>
<th>Thyroid hormone infusion rate (μg/100 g·body weight)</th>
<th>T₃</th>
<th>T₄</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.3</td>
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<td>7 day experiment</td>
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<td>64.3</td>
<td>14.5</td>
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<td>40.4</td>
<td>40.7</td>
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<tr>
<td>± 5.1</td>
<td>± 3.2</td>
<td></td>
<td>± 13.4</td>
<td>± 6.1</td>
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<td>N 6</td>
<td>5</td>
<td></td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>14 day experiment</td>
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<td></td>
<td>46.6</td>
<td>15.6</td>
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<tr>
<td>± 6.8</td>
<td>± 4.5</td>
<td></td>
<td>± 6.9</td>
<td>± 15.1</td>
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<tr>
<td>N 7</td>
<td>8</td>
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a: P < 0.05 vs control. b: P < 0.05 vs THYREX (no replacement).
Both T₄ and T₃ replacement led to inhibition of the post-thyroidectomy rise in plasma TSH. Therefore, we used the basal plasma TSH concentration as an index of the degree of hypothyroidism and the amount of feedback inhibition exerted by plasma T₃ and/or T₄ at the pituitary. We have previously shown that the severity of hypothyroidism does not affect the MCR of TSH (Connors et al. 1984). Therefore the area under the ΔplTSH curve is directly proportional to the amount of TSH secreted in response to TRH and this was used as the index of the pituitary responsiveness to TRH.

Under euthyroid and hyperthyroid conditions, the amount of TSH secreted in response to TRH is inversely related to the amount of inhibition exerted by thyroid hormones at the pituitary (Morley 1981). However, it is clear from the present results that in the rat this relationship does not hold under hypothyroid conditions. In

![Image of graphs showing scatterplots of the relationship between area under the ΔplTSH curve and basal plasma TSH concentrations (panels A and B) and pituitary TSH concentrations (panel C and D) in non-replaced THYREX rats (□) and THYREX rats receiving replacement doses of T₃ (○) or T₄ (Δ) for 7 or 14 days. The linear relationship between area and basal plasma TSH or pituitary TS concentrations is illustrated by the straight line in each panel. In panel A only hypothyroid animals (i.e., those with basal plasma TSH concentrations greater than those of control animals) were used in the calculations of the correlation coefficient. The shaded areas represent the means and the 95% confidence intervals for control rats.

**Fig. 5.**
this and a previous study (Connors et al. 1984), we have shown that the pituitary TSH response to TRH is not increased in thyroidectomized rats receiving no replacement. While thyroidectomy resulted in an elevation of basal TSH, the TSH response to exogenous TRH was decreased when compared to that of euthyroid controls. This is consistent with a previous report of a decreased pituitary TSH response to TRH in rats made hypothyroid by treatment with propylthiouracil for 7 days (Larsen & Frumess 1977). More interestingly, our data indicate that as the severity of hypothyroidism is decreased (i.e., as the post-thyroidectomy rise in the basal plasma TSH concentration is suppressed by thyroid hormone replacement), the pituitary TSH concentration and the TSH response to TRH increase toward those of euthyroid animals. In fact, in some rats the TSH response to TRH was even greater than that of euthyroid controls. This augmentation of TSH secretion in THYREX rats by continuous infusion of thyroid hormones is consistent with results obtained previously in our laboratory (Connors & Hedge 1981a and two unpublished experiments using slightly different replacement doses of T₄ and T₃). In all cases the results indicate that low rates of thyroid hormone replacement after thyroidectomy increase the pituitary TSH content and the TSH response to TRH. The present results are also consistent with those in which small amounts of thyroid hormone replacement were found to augment goitrogenesis in rats given antithyroid drugs (Sellers et al. 1953; Teir et al. 1956).

The mechanism(s) involved in the restoration of the pituitary TSH concentration and the increase in the pituitary TSH response to TRH associated with mild hypothyroidism are unclear. It is clear that the pituitary TSH response to TRH is not determined solely by the reduced feedback inhibition at the level of the pituitary. The positive correlation between pituitary TSH concentration and responsiveness in hypothyroid rats suggests that the restoration of the pituitary TSH response to TRH is associated with the restoration of pituitary TSH concentration. The pituitary TSH concentration is determined by a balance between TSH synthesis, degradation, and release. An increase in the pituitary TSH concentration in hypothyroid rats might result from an increase in TSH synthesis or a decrease in intrapituitary TSH degradation, such that TSH accumulates despite the increase in TSH release. Such an increase in TSH content may lag considerably behind the increase in TSH secretion (Spira et al. 1979) after an abrupt initiation of hypothyroidism (e.g. after surgical thyroidectomy). This increase in pituitary TSH concentration was enhanced by the replacement of thyroid hormones. The increase in the pituitary TSH concentration in rats receiving thyroid hormone replacement may result from an alteration in the hypothalamic input to the pituitary and/or a direct effect of thyroid hormones on TSH synthesis and release at the pituitary. While TRH stimulates the synthesis of TSH when added to dispersed pituitary cells (Marshall et al. 1981), little is known about the effect of TRH on TSH synthesis in vivo. Similarly, the effect of thyroid hormones, or their absence, on the hypothalamic input to the pituitary is unclear (Morley 1981). Thyroid hormones have been reported to have a negative feedback (Yamada & Greer 1959), positive feedback (Reichlin et al. 1972), or no effect (Bassiri & Utiger 1974) on the hypothalamic control of pituitary TSH secretion. When added to dispersed pituitary cells in vitro, thyroid hormones directly inhibit (Gershengorn 1978; Marshall et al. 1981) or stimulate (Gershengorn 1978) TSH synthesis. Of particular interest is the observation by Gershengorn (1978) that low levels of thyroid hormones stimulate, while high levels inhibit, the production rate of TSH when added to dispersed mouse pituitary tumour cells. This is consistent with in vivo studies in which low amounts of thyroid hormones increase the pituitary TSH concentration (Bakke & Lawrence 1964), and the TSH production rate (D'Angelo et al. 1976). Together, these studies suggest that the increased pituitary TSH concentration observed in the present study may be the result of a stimulatory effect of these low levels of thyroid hormones on TSH synthesis. Alternatively, the increase in pituitary TSH concentration may be the result of separate effects of thyroid hormones on TSH synthesis and release. That is, both TSH synthesis and release are inhibited by thyroid hormone, but thresholds may differ, which can lead to conditions in which one effect may predominate. Thus, the pituitary TSH responsiveness to TRH during hypothyroidism appears to be determined, in part, by the pituitary TSH content which is increased by thyroid hormone replacement.

In conclusion, the results of the present study...
are not consistent with a model of TSH control in which the response of the pituitary to TRH is simply a function of the amount of feedback inhibition exerted by thyroid hormones at the pituitary. The results of the present study indicate that in the hypothyroid rat: 1) severe hypothyroidism results in a blunted pituitary TSH response to TRH through 14 days after thyroidectomy, 2) at 7 and 14 days after thyroidectomy the pituitary response to exogenous TRH is inversely related to the basal plasma TSH concentration, 3) the pituitary TSH concentration increases with the duration of hypothyroidism, 4) the pituitary TSH concentration is increased by low rates of thyroid hormone replacement, and 5) the pituitary TSH response to exogenous TRH is directly related to the pituitary TSH content.

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