

Pituitary-Thyroid Responses to 4-Hour Constant Infusions of Thyrotropin Releasing Hormone in Man

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ABSTRACT. Constant iv infusions of TRH for 4 h were administered to normal men in dosages of 0.5 (n = 5), 2.0 (n = 10) and 5.0 (n = 4) $\mu\text{g}/\text{min}$ and to women in a dosage of 0.5 $\mu\text{g}/\text{min}$ (n = 4). Control 4-h saline infusions were performed in 4 men and 4 women. Measurements at regular intervals were made for serum levels of TSH, PRL, T_4 , T_3 and 3,3',5'-triiodo-L-thyronine (reverse triiodothyronine or rT_3).

Serum levels of TSH increased in 2 phases. The first phase started within 5 min after the infusions began and reached a plateau at approximately 50 min which lasted until 90 min. After 90 min, a second phase of increase in serum TSH levels occurred which continued until 120 to 150 min. The two phases of increase could reflect the existence of

two pools of TSH in the pituitary, one requiring longer stimulation for release than the other, as has been suggested for LH. In contrast, serum levels of PRL during the same infusions reached maximal values by 30 to 45 min and then declined gradually in spite of continued TRH stimulation, with no second phase of increase.

Serum levels of T_3 were significantly increased by 180 min during the infusion of all TRH dosages used. Levels of T_4 were significantly increased by 240 min during the 2.0 and 5.0 $\mu\text{g}/\text{min}$ infusions. No significant increases were found in rT_3 . These results are consistent with the interpretation that the major source of circulating rT_3 is not the thyroid, but peripheral deiodination of T_4 . (*J Clin Endocrinol Metab* 45: 981, 1977)

SEVERAL protein hormones including insulin (1), glucagon (2) and LH (3) have been shown to be secreted in a biphasic pattern during constant stimulation by an appropriate agent. Of pituitary hormones, LH has been demonstrated to increase in two phases during constant luteinizing hormone-releasing hormone (LHRH) stimulation in normal men (3,4), women (5,6) and experimental animals (7). The biphasic pattern of LH increase has been interpreted as evidence for the existence of two pools of pituitary LH, one acutely releasable and the other requiring longer LHRH stimulation for release (3). It has been suggested that

the first pool may represent presynthesized hormone available for immediate release while the second may require new synthesis of LH or of a protein necessary for its release (3). In contrast, serum FSH levels during the same LHRH infusions increased in a gradual, progressive manner, without a detectably biphasic pattern of increase.

The present study was undertaken to determine the patterns of secretion of two other pituitary hormones, TSH and PRL, during 4-h constant infusions of thyrotropin releasing hormone (TRH) in man. In addition, the thyroxine (T_4), 3,5,3'-triiodo-L-thyronine (T_3) and 3,3',5'-triiodo-L-thyronine (reverse triiodothyronine or rT_3) responses to the TSH increases were measured.

Materials and Methods

Subjects

Fourteen normal men, aged 18 to 33, and 7 normal women, aged 18 to 32, were studied. Many subjects received more than one infusion, study days being separated by at least 2 weeks.

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The women were all menstruating regularly and were studied on day 3 to 5 of their cycles. None of the subjects had a history of endocrine or serious medical disease or was receiving any medication.

Infusions

TRH (supplied courtesy of Roche, Australia Ltd.) was administered to the men in dosages of 0.5 $\mu\text{g}/\text{min}$ ($n = 5$), 2.0 $\mu\text{g}/\text{min}$ ($n = 10$) and 5.0 $\mu\text{g}/\text{min}$ ($n = 4$). Four women received TRH in a dosage of 0.5 $\mu\text{g}/\text{min}$. TRH was diluted in 100 ml of 0.9% saline containing 1% human serum albumin. Control infusions of 0.9% saline with 1% human serum albumin and no TRH were administered to 4 men and 4 women. All infusions were administered over 4 h into an arm vein, using an LKB infusion pump (a small pump employing a rotor to advance liquids along the infusion tubing at a predetermined rate). Studies were begun between 0800 and 1000 hours; subjects were allowed to eat as they wished and were usually supine throughout the infusions.

Blood sampling and hormone assays

Blood was obtained from an indwelling needle in an arm vein. Three samples were drawn at 15 min intervals before the infusions; sampling was continued at 15 min intervals during the saline and the majority of the TRH infusions. In 4 TRH studies using 2.0 $\mu\text{g}/\text{min}$ into men, sampling was at 5 min intervals for 150 min, then 30 min intervals until the end of the infusions. Blood was allowed to clot at room temperature, then centrifuged and the serum was stored at -20 C .

Serum TSH was measured by radioimmunoassay as described previously (8). PRL was measured by radioimmunoassay (9) using the VLS #3 reagents supplied by NIAMDD. The sensitivity of this assay in our laboratory is 0.7 ng/ml, the intra-assay variability is 6% and the inter-assay variability is 26% (mean of coefficients of variation of high, mid and low range pools in 10 consecutive assays). Normal values for men are 13 ± 3.4 ng/ml ($n = 34$), and for menstruating women are 17.2 ± 5.7 ($n = 36$) (mean \pm SD). T_3 was measured by radioimmunoassay of unextracted serum against standards assayed with an equivalent volume of charcoal-treated hormone-free serum, using a rabbit antibody show-

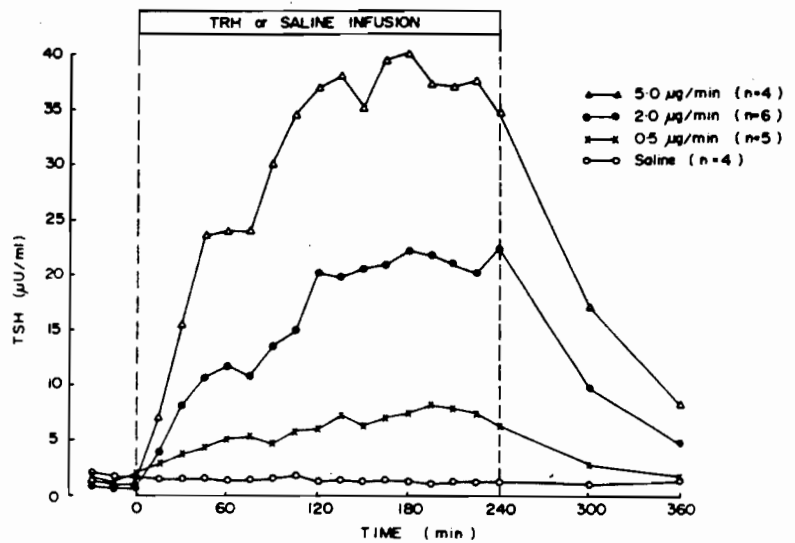
ing 0.1% cross-reaction with T_4 . Merthiolate, 2 mg/ml was used to inhibit binding of T_3 to serum proteins (10). Normal values were 75 to 175 ng/100 ml ($n = 100$). rT_3 was measured by radioimmunoassay of ethanol-extracted serum (11), with similar extraction of standards prepared in hormone-free serum. The rabbit antibody showed 0.12% cross-reaction with T_4 ; rT_3 results were corrected for the T_4 content of each sample. The normal range for rT_3 was $<5\text{--}35$ ng/100 ml ($n = 80$) using L- rT_3 (Henning, Berlin) as the reference standard. T_4 was measured by Ames Tetralute; normal values were 4 to 11 $\mu\text{g}/100$ ml ($n = 120$). All samples for each subject were included in a single assay for each hormone being measured. Statistical analysis was by Student's t test for paired observations (12), and by analysis of variance and t tests as described previously (4).

Results

Serum TSH levels both in men and women, were increased above basal values by 15 min during TRH infusions at all dosages used (Figs. 1, 2, and Table 1). A progressive increase in TSH levels continued until 30 to 50 min after which essentially stable levels were maintained until approximately 90 min. Around 90 min, in all studies except the 0.5 $\mu\text{g}/\text{min}$ infusions in men, a second phase of increase in TSH levels began. This second phase of increasing TSH lasted until 120 to 150 min, following which levels generally remained stable until the end of the infusions, when they declined sharply. At the 0.5 $\mu\text{g}/\text{min}$ dosage in men, a second phase of TSH increase was apparent in some, but not all, studies.

When TSH was determined at 5 min intervals, a clearer delineation of the two phases of increase was obtained (Fig. 2). There was an early sharp rise lasting until 30 to 60 min, followed by stable levels until approximately 90 min, when a second phase of increase occurred. Statistical analysis revealed that there was a significant increase in TSH levels between 0 and 50 min ($P < 0.001$), no significant change between 50 and 90 min ($P > 0.2$) and a second signifi-

FIG. 1. Mean levels of serum TSH before, during and after iv infusions of TRH at various dosages or of saline into normal men.



cant increase between 90 and 120 min ($P < 0.05$).

Serum PRL levels increased rapidly during the first 15 to 30 min of the TRH infusions, reaching peak levels by 30 to 45 min (Fig. 3 and Table 1). Thereafter, in spite of continued TRH infusions, PRL levels declined gradually until the end of the infusions when they fell more rapidly. In contrast to serum TSH levels during the same infusions, no second phase of increase in PRL was found.

A clear dose-response relationship between TRH administered and serum TSH levels was found in the men (Fig. 1). The dose-response relationship was less clear for PRL; the highest dosage (5.0 µg/min) did not yield a greater response than the next highest dosage (2.0 µg/min). Serum levels of both TSH and PRL were greater in the women during the 0.5 µg/min TRH infusion than in the men at the same dosage (Figs. 1, 3, and Table 1).

Serum T_3 levels in the men increased significantly ($P < 0.05$) by 180 min during the infusion of each TRH dosage used (Table 2). If T_3 results were grouped together for all TRH dosages, a highly significant ($P < 0.01$) increase occurred by 120 min. No significant change occurred in T_3 during saline

administration. Serum levels of T_4 increased significantly ($P < 0.05$) by 240 min during the 2.0 and 5.0 µg/min infusions, and no increase occurred during saline infusions (Table 2). Although there were increases in serum rT_3 levels during TRH infusions at

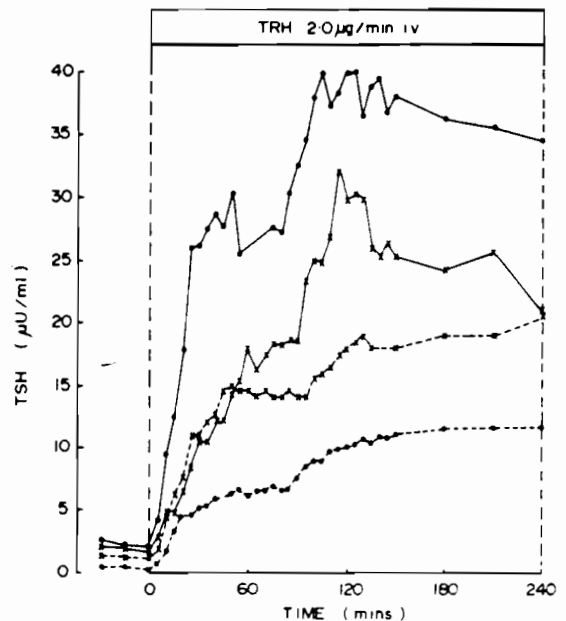


FIG. 2. Serum levels of TSH in 4 normal men before and during TRH infusions of 2.0 µg/min for 4 h.

TABLE 1. Mean (\pm SE) serum levels of TSH and PRL before and during infusions of TRH (0.5 μ g/min, n = 4) or saline (n = 4) into normal women

	-20	-10	0	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240 min	
TSH (μU/ml)																				
Saline																				
	1.3	1.6	1.2	0.9	0.8	0.8	0.7	0.7	0.6	0.7	0.7	0.6	0.7	0.7	0.7	0.9	0.6	0.7	0.6	
	± 0.2	± 0.15	± 0.15	± 0.2	± 0.2	± 0.2	± 0.15	± 0.2	± 0.2	± 0.15	± 0.1	± 0.15	± 0.15	± 0.15	± 0.15	± 0.15	± 0.1	± 0.1	± 0.1	
TRH																				
	2.0	1.7	1.2	5.9	12.3	15.3	15.0	15.5	15.7	18.9	19.4	22.8	21.6	22.8	23.0	23.3	23.8	22.4	23.0	
	± 0.6	± 0.8	± 0.5	± 1.2	± 2.1	± 2.0	± 2.0	± 2.1	± 2.6	± 2.2	± 2.7	± 4.5	± 3.8	± 4.0	± 3.6	± 3.9	± 3.8	± 2.6	± 3.0	
PRL (ng/ml)																				
Saline																				
	10.3	7.1	8.7	8.6	8.2	10.0	8.4	10.4	8.9	9.1	9.2	9.4	9.7	9.3	9.5	9.8	11.3	13.3	12.2	
	± 1.8	± 0.8	± 1.5	± 1.9	± 1.5	± 1.5	± 0.4	± 1.6	± 1.4	± 1.7	± 1.6	± 1.4	± 1.3	± 1.3	± 1.2	± 1.4	± 1.5	± 1.6	± 1.6	
TRH																				
	10.8	11.6	12.4	21.0	26.7	24.7	24.1	24.7	24.5	26.0	26.1	23.1	22.1	23.0	22.5	22.4	21.5	22.9	22.6	
	± 1.4	± 2.3	± 2.0	± 1.4	± 3.0	± 3.0	± 2.7	± 2.6	± 3.6	± 3.7	± 4.0	± 2.0	± 2.3	± 2.1	± 1.5	± 1.6	± 1.2	± 1.9	± 1.1	

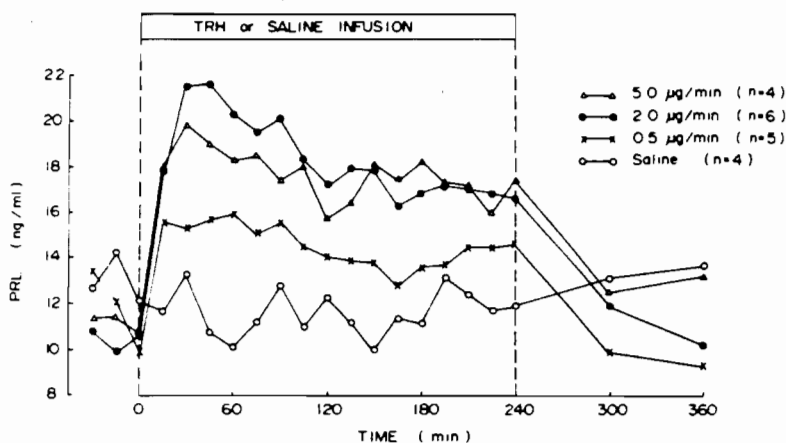
all dosages (and during the saline infusions), none of these increases reached statistical significance.

Discussion

The results of the present study demonstrate that serum levels of TSH increase in two phases during constant administration of TRH to normal men and women. We interpret this pattern of increase as evidence for the existence of two pools of pituitary TSH, one available for immediate release and the other requiring longer stimulation for release. Similar patterns of hormone secretion have been described for LH (3), insulin (1) and glucagon (2) and the concept of two pools of hormone within the pituitary or pancreas has been suggested in each instance. For LH, the concept of two pools has been supported by the findings that the hormonal milieu may affect one pool differently from the other (6) and that the ratio of bio- to immuno-activity increases with time following the onset of LHRH stimulation (13). Wang *et al.* (6) have demonstrated that rising estrogen concentrations during the normal menstrual cycle are associated with a greater increase in responsivity of hormone secretion from the second or later pool of LH when compared with secretion from the first pool. Dufau *et al.* (13) have shown that although the ratio of bio- to immunoreactivity of LH is essentially 1.0 immediately after LHRH stimulation in women, this ratio increases with time following LHRH to as high as 4.1. These results suggest that LH released from the second pool has a higher ratio of biologic to immunologic activity than that released from the first pool (13). Similar studies have not been reported for TSH.

Evidence is accumulating from animal studies that new protein synthesis is important in the second phase of LH secretion, but not the first. Chemical inhibitors of protein synthesis will markedly and selectively suppress the second phase of LH secretion during constant LHRH administration both *in vivo* (14) and *in vitro* (15). In addition, the "self-priming" effect of LHRH (16), which probably depends upon a mobiliza-

FIG. 3. Mean levels of serum PRL before, during and after iv infusions of TRH at various dosages or of saline into normal men.



tion of the second pool of pituitary LH, can be blocked by inhibitors of protein synthesis (17). By analogy with these findings for LH, it is possible that the first phase of TSH secretion could represent pre-synthesized hormone stored in the gland, while the second could reflect new synthesis of TSH or of a protein necessary for the release of TSH. There is evidence from studies in mice that the early release of TSH in response to TRH does not require protein synthesis (18). Alternatively, other aspects of the secretory mechanisms of TSH such as TRH binding to receptors or secretory granule formation and transport may be important in regulating the two phases of release.

Several other groups have reported serum TSH levels during constant infusions of TRH. The largest studies of constant TRH infusions with a duration near that of the present work have been reported by Gonzalez-Barcena *et al.* (19) and Wartofsky *et al.* (20). Neither group has described 2 phases of TSH increase, probably due to blood sampling at intervals of 30 min or greater. Even with this interval, however, the data of Wartofsky *et al.* (20) in normal subjects is suggestive of 2 phases of increase. The clearest delineation of 2 phases of increase in the present study was found with a 5 min sampling interval. Gonzalez-Barcena *et al.* (19) found a marked decline in serum TSH levels following 2 h of TRH infusion (6.7 µg/min). This apparent development of pituitary refractoriness to

TRH has not been confirmed either by Wartofsky *et al.* (20) or in the present study, possibly due to differences in TRH infu-

TABLE 2. Mean (\pm SE) serum levels of thyroid hormones before and during infusions of TRH at various dosages or of saline into normal men

	Basal	60	120	180	240 min
T₃ (ng/100 ml)					
Saline (n=4)	115 \pm 5	108 \pm 4	108 \pm 3	106 \pm 5	110 \pm 5
TRH					
0.5 (n=5)	95 \pm 17	93 \pm 15	115 \pm 13	132* \pm 13	139* \pm 11
2.0 (n=6)	116 \pm 4	124 \pm 4	134 \pm 7	157* \pm 11	167* \pm 10
5.0 (n=4)	82 \pm 7	93 \pm 8	127 \pm 16	140* \pm 22	162* \pm 28
rT₃ (ng/100 ml)					
Saline	7.6 \pm 2.7	—	11.3 \pm 2.2	—	12.9 \pm 3.3
TRH					
0.5	9.2 \pm 0.5	—	11.6 \pm 1.5	—	11.8 \pm 2.3
2.0	13.1 \pm 2.2	—	14.6 \pm 2.6	—	14.1 \pm 2.4
5.0	13.8 \pm 2.8	—	17.3 \pm 3.0	—	17.5 \pm 2.7
T₄ (µg/100 ml)					
Saline	6.3 \pm 0.4	—	5.8 \pm 0.5	—	5.6 \pm 0.4
TRH					
0.5	6.4 \pm 1.2	—	7.1 \pm 1.1	—	7.6 \pm 1.2
2.0	6.2 \pm 0.7	—	6.8 \pm 0.5	—	7.7* \pm 0.5
5.0	5.9 \pm 0.7	—	6.2 \pm 0.5	—	7.2* \pm 0.4

* Denotes significance at less than 0.05 level by paired *t* test.

sion rates. In 2 subjects, Otsuki *et al.* found prolonged TSH elevations for as long as 8 h during constant TRH administration of 2 $\mu\text{g}/\text{min}$ (21).

Serum levels of PRL did not reveal evidence of 2 phases of increase. Instead, PRL levels increased rapidly to reach peak levels by 30 to 45 min and then declined gradually in spite of continued TRH stimulation until 240 min. A similar pattern of PRL levels during prolonged, constant TRH infusions has been reported by Wartofsky *et al.* (20). The fact that constant administration of TRH does not produce two discernible phases of PRL increase *in vivo* does not exclude the possibility that there is more than one pool of pituitary PRL. Indeed, in certain situations, the synthesis and release of PRL may occur independently (22). The results of the present study suggest that under constant, 4-h stimulation, the rates or mechanisms of hormone synthesis and incorporation into a releasable pool differ for PRL when compared with LH and TSH.

The increases in serum TSH in the present study caused significant increases in serum T_3 levels by 120 min and T_4 by 240 min. These results are consistent with those of several previous studies using single injection or oral administration of TRH (23). The fact that serum TSH levels remained at near-maximal levels or increased, between 120 and 240 min while T_3 levels were increased, implies that the negative feedback effect of small increases in serum T_3 on TSH responsiveness requires longer than 60 to 120 min to begin, a conclusion that agrees with the work of Parks *et al.* (24) and Wartofsky *et al.* (20).

In spite of the increases found in serum levels of T_3 and T_4 , no significant increases were found in serum levels of rT_3 . Although rT_3 has been shown to be present in human thyroglobulin, it is in much lower concentration than T_4 or T_3 (11), so thyroid stimulation by TSH might be expected to yield relatively small changes in serum rT_3 . Chopra *et al.* (25) have demonstrated that the thyroid stimulation produced by the neo-

natal TSH surge produces increases in T_3 and T_4 , but not rT_3 . Similar results were found with exogenous TSH administration to a few normal adults (25). The present study demonstrates that large increases in endogenous TSH over a 4-h period in normal men are ineffective in significantly increasing serum rT_3 levels. These results are consistent with the interpretation that the major source of circulating rT_3 is not the thyroid but may be peripheral deiodination of T_4 (26).

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