Repetitive Administration of Thyrotropin-Releasing Hormone Results in Small Elevations of Serum Thyroid Hormones and in Marked Inhibition of Thyrotropin Response

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ABSTRACT Repetitive administration of thyrotropin-releasing hormone (TRH) to human subjects was used to produce small elevations of endogenous serum triiodothyronine (T_3) and thyroxine (T_4) levels and thereby to determine the effect of these small elevations on the serum thyrotropin (TSH) response to subsequent doses of TRH. Each subject received 13 consecutive doses of 25 μg TRH at 4-h intervals. Serum T_3, T_4, and TSH levels were measured before the 1st, 7th, and 13th doses ("basal levels") and for the 4 h after each of these doses.

In 10 normal subjects, the mean TSH response fell from 14.6 μU/ml after the 1st TRH dose to 6.9 and 3.0 μU/ml after the 7th, and 13th doses. These falls in TSH response were accompanied by rises in the mean basal serum T_3 levels from 81 to 115 to 114 ng/100 ml (normal range, 70–150 ng/100 ml) and rises in the mean basal serum T_4 from 6.7 to 8.6 to 9.5 μg/100 ml (normal range, 5–11 μg/100 ml). These data suggest that TRH-induced TSH release is extremely sensitive to inhibition by small elevations, not above the normal ranges, of serum T_3 and T_4 of endogenous origin.

In four patients with primary hypothyroidism, the mean TSH responses were 92, 137, and 92 μU/ml after the 1st, 7th, and 13th TRH doses. The corresponding mean basal serum T_3 and T_4 levels at the times of these doses were 34, 30, and 32 ng/100 ml and 1.9, 1.9, and 1.7 μg/100 ml. These data show that repetitive administration of TRH does not result in progressively lower TSH responses in the absence of corresponding increases in serum T_3 and T_4 level. The progressive fall in TSH response observed in the normal subjects, therefore, was apparently due to the corresponding small increases in serum T_3 and T_4 levels and not to progressive depletion of pituitary TSH.

In two patients with presumed TRH deficiency, the TSH responses were blunted by repetitive TRH doses but only when the serum T_3 and T_4 levels increased to within the normal ranges. TRH deficiency was thus confirmed for the first time by producing euthyroidism by replacement of TRH.

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INTRODUCTION

Synthetic thyrotropin-releasing hormone (TRH) is a potent stimulus to the release of thyrotropin (TSH) in man (1–3). TRH-induced TSH release has already been shown to be impaired dramatically by small elevations of serum triiodothyronine (T_3) and thyroxine (T_4) levels produced by the administration of exogenous T_3 and T_4 (4). The objective of the present study was to determine the effect on TRH-induced TSH release of small elevations of serum T_3 and T_4 levels of endogenous origin.

A single intravenous dose of TRH has been shown to produce small elevations of serum T_3 and T_4 levels (5), presumably by stimulating TSH release. Repetitive intravenous administration of TRH was chosen, therefore, as a means whereby small elevations of serum T_3 and T_4 levels could be produced by increased endogenous secretion and whereby any effect of these elevations on the TSH response to successive doses of TRH could be measured simultaneously.

TABLE I

Pituitary Function Tests in Three Patients with Hypothyrotropic Hypothyroidism

Test	Normal range	L. C.	K. C.	M. J.
T_4, *μg/100 ml*	5–11 (6, 7)*	3.0	3.8	2.9
Free T_4, *ng/100 ml*	1.0–2.2‡	0.6	0.5	0.6
T_3, *ng/100 ml*	70–150 (6, 7)	50.0	58.0	37.0
TBG, *μg/100 ml*	15–32 (8)	22.0	17.0	29.0
Radioactive iodine uptake after TSH, %§	Increase above basal ≥15 (9)	16.0	16.0	18.0
Basal TSH, *μU/ml*	<2–8 (10)	4.4	5.6	3.0
Maximum ΔTSH after 400 μg TRH, *μU/ml*	6–33 (6, 7)	26.1	27.4	4.5
Basal prolactin, *ng/ml*	<1–15 (11)	8.4	‖	3.7
Maximum Δ prolactin after 400 μg TRH, *ng/ml*	20–120 (11)	102.0	‖	13.2
Maximum growth hormone after arginine, *ng/ml*	>7 (12)	39.3	5.4	2.5
Maximum deoxycortisol after metyrapone, *μg/100 ml*	>10 (13)	22.6	<1	<1

TBG, thyroxine-binding globulin.

* Numbers in parentheses refer to references for normal ranges.

‡ Normal range reported by BioScience Laboratories, Van Nuys, Calif. where test was performed.

§ 10 U bovine TSH once daily for 3 days.

‖ Not measurable because of prior administration of commercial preparations of vasopressin (14).

METHODS

Subjects. The normal subjects were 10 ambulatory women (aged 21–66) who had no illnesses nor were taking any medication known, or suspected, to effect thyroid hormone economy.

The four patients with primary hypothyroidism were two men and two women (aged 26–69) who were either previously untreated or had not taken thyroid medication for 8 wk before the study. The diagnosis of primary hypothyroidism was based on the findings in each patient of low serum T_3 and T_4 and an elevated serum TSH level.

The patients with hypothyrotropic hypothyroidism were two women and one man. L. C., a 24-yr old female, was diagnosed presumptively as having hypothyroidism due to idiopathic TRH deficiency. This diagnosis was based on the findings (Table I) of low serum T_3, T_4, and free T_4 levels, a normal thyroidal response to exogenous TSH, a normal serum TSH before TRH, and a normal serum TSH response to the standard 400 μg test dose of synthetic TRH. The radiologic appearance of the sella turcica was normal. Pituitary hormone secretion was otherwise normal: the serum growth hormone response to arginine infusion, the serum deoxycortisol response to oral metyrapone, the basal serum prolactin, and the prolactin response to TRH were all within the normal limits, and her menses occurred regularly at 28–30-day intervals.

K. C., a 25-yr old man, was also diagnosed presumptively as having hypothyroidism due to idiopathic TRH deficiency based on findings (Table I) similar to those in patient L. C. The radiologic appearance of the sella turcica was normal. Pituitary hormone secretion was otherwise subnormal: the serum growth hormone response to arginine infusion and the serum deoxycortisol response to oral metyrapone were subnormal; the serum testosterone was 311 ng/100 ml (normal, 400–1,200 ng/100 ml) in the presence of a serum luteinizing hormone (LH) of < 5 mIU/ml (normal, < 11 mIU/ml); the serum prolactin levels could not be assayed reliably because of the prior administration of commercial preparations of vasopressin for diabetes insipidus (14).

M. J., a 53-yr old woman was diagnosed presumptively as having hypothyroidism due to idiopathic pituitary insensitivity to TRH. This diagnosis was based on the findings (Table I) of low serum T_3, T_4, and free T_4 levels, a normal thyroidal response to exogenous TSH, a low-normal serum TSH before TRH, and a subnormal serum TSH response to the 400 μg test dose of synthetic TRH. The radiologic appearance of the sella turcica was normal. Pituitary hormone secretion was otherwise subnormal: the serum growth hormone response to arginine infusion and the serum deoxycortisol response to metyrapone were subnormal; and her serum LH, at age 53, 22 yr after vaginal hysterectomy, was 5 mIU/ml. The serum prolactin response to exogenous TRH was at the lower limit of normal compared with the response of euthyroid, normal subjects and also compared with the response of subjects with primary hypothyroidism (11). This prolactin response to TRH is consistent with the assumption that the anatomic site of this patient's hormonal abnormalities is the pituitary gland, rather than the hypothalamus.

Experimental design. Each subject and patient received 13 consecutive 25-μg doses of synthetic TRH at 4-h intervals. The TRH (Abbott Laboratories, North Chicago, Ill.)

was given intravenously as a bolus injection via an indwelling scalp-vein needle, which was flushed with saline and then filled with aqueous heparin after each TRH injection. Blood samples were drawn from the opposite arm before and for 4 h after the 1st, 7th, and 13th TRH doses. On each of these occasions blood samples were obtained at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min.

Analyses. Serum TSH (10), prolactin (14) (courtesy of Doctors L. S. Jacobs and W. H. Daughaday), and T_3 (15) were measured by radioimmunoassays. Serum T_4 (16) was measured by competitive protein binding. All samples for the determination of either TSH, T_3, or T_4 from any one subject were analyzed inthe same assay run.

All statistical analyses were made by use of the paired *t* test (17). When an individual serum T_3 level was below the lower limit of detection of the T_3 assay, 30 ng/100 ml, as occurred in the patients with primary hypothyroidism, the value was assumed to be 30 ng/100 ml for determining the mean serum T_3 levels in these patients. In reporting and discussing the results, the term "basal" is used to refer to zero time of any specified TRH dose. The term "maximum ΔTSH" is used to refer to the maximum increment in TSH above the basal level after any specified TRH dose.

RESULTS

Normal subjects. The serum TSH responses of the 10 normal subjects to the intravenous injection of 13 consecutive 25-μg doses of TRH at 4-h intervals are shown in Fig. 1. After the first dose of TRH the mean serum TSH rose from a basal level of 4.4±0.6 μU/ml (SEM) to a maximum of 18.5±2.3 μU/ml at 20 min. After the seventh dose of TRH the mean serum TSH rose from a basal level of 4.1±0.7 μU/ml to a maximum of 10.8±2.0 μU/ml. After the 13th dose of TRH the mean serum TSH rose from a basal level of 3.1±0.5 μU/ml to a maximum of 5.8±1.1 μU/ml. The fall in mean basal TSH levels from the 1st to the 13th test, 4.4–3.1 μU/ml, although small, was significant ($P <$ 0.02). The statistical significance of the fall in the TSH responses to TRH is described below.

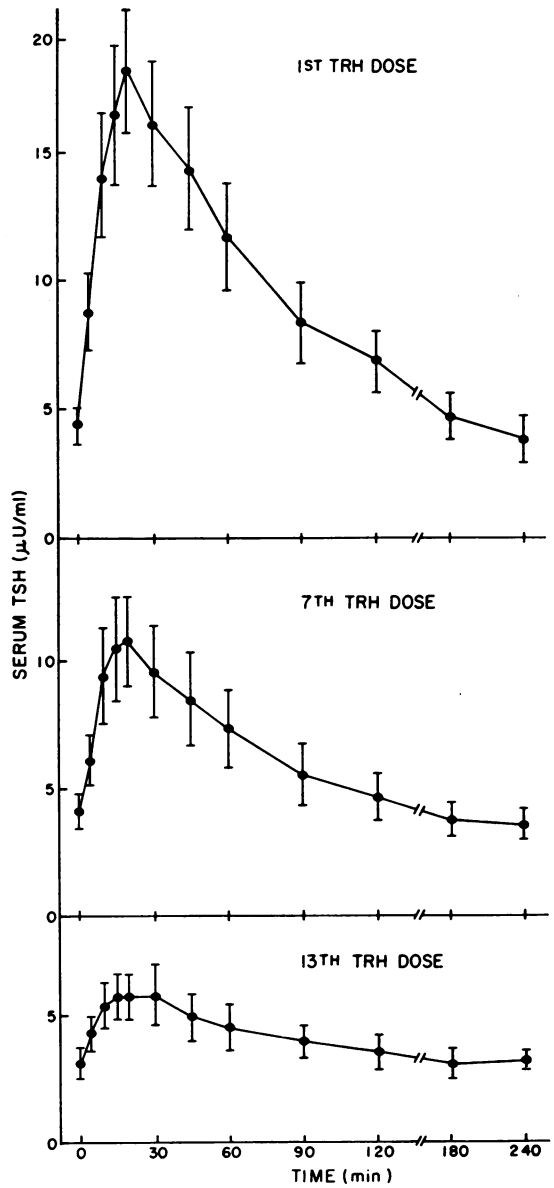

FIGURE 1 Mean serum TSH responses of 10 normal subjects to repetitive doses of TRH. Each subject was given 13 consecutive doses of 25 μg TRH at 4-h intervals, and the TSH response was measured after the 1st, 7th, and 13th doses. Vertical lines represent ±SEM.

TABLE II

Serum T_3 and T_4 Responses to Repetitive Doses of TRH in Normal Subjects

	Serum T_3					Serum T_4		
min	0	60	120	180	240	0	120	240
	ng/100 ml					*μg/100 ml*		
1st TRH dose								
Mean±SEM	81	82	106	104	95	6.7	7.4	7.3
	3	3	5	4	3	0.3	0.3	0.3
7th TRH dose								
Mean±SEM	115	108	114	105	103	8.6	8.3	8.1
	5	5	8	6	5	0.3	0.4	0.3
13th TRH dose								
Mean±SEM	114	107	106	101	101	9.5	9.1	9.2
	5	5	7	6	5	0.3	0.2	0.2

Mean serum T_3 and T_4 responses to repetitive doses of TRH of the 10 normal subjects whose TSH responses are shown in Fig. 1. The statistical significance of the 0 vs. 120 min values after the first TRH dose are serum T_3, $P < 0.001$; serum T_4, $P < 0.01$.

The serum T_3 and T_4 responses to the 1st, 7th, and 13th TRH doses are shown in Table II. After the first TRH dose the mean serum T_3 level rose to a peak 2 h after the dose. The peak level was 31% greater ($P <$ 0.001) than the base-line level. The mean serum T_4 level after the first TRH dose also reached a peak 2 h after the dose. The peak level was only 10% greater than the

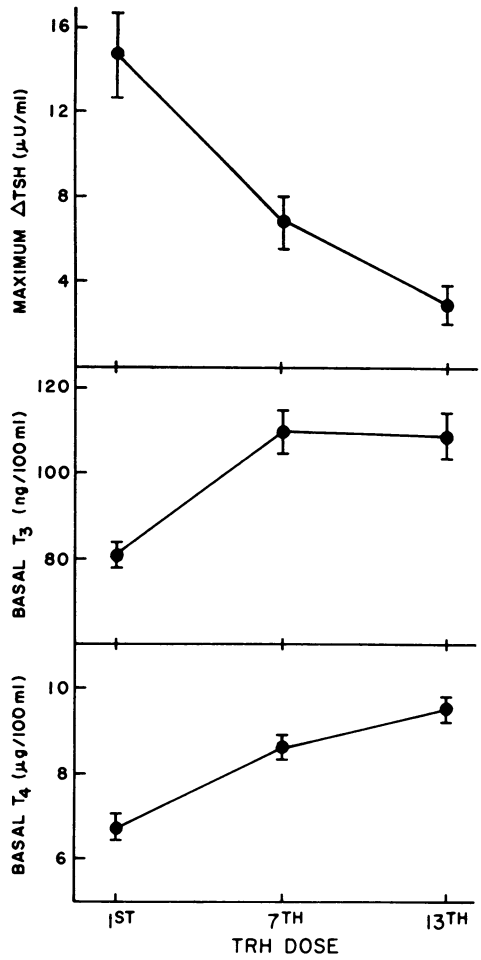

FIGURE 2 Correlation of the changes in maximum ΔTSH with the changes in basal serum T_3 and T_4 levels in response to repetitive doses of TRH in 10 normal subjects. "Basal" refers to the level just before a TRH dose, and "maximum Δ" refers to the maximum increment above the basal level during the 4 h after a TRH dose. Vertical lines represent ±SEM.

base-line level, but this increase was statistically significant ($P < 0.01$). After the 7th and 13th doses, however, the mean serum T_3 and T_4 levels, although starting from higher basal levels than at the time of the 1st dose, failed to rise above the basal levels. The 240-min serum T_3 and T_4 levels were, in fact, less ($P < 0.05$) than the corresponding basal values at the times of both the 7th and 13th doses.

Correlation of the mean basal serum T_3 and T_4 levels at the time of each dose with the maximum ΔTSH after that dose is shown in Fig. 2. At the time of the first dose, when the mean basal serum T_3 was 81±3 ng/100 ml and the mean basal serum T_4 was 6.7±0.3 μg/100 ml, the mean maximum ΔTSH was 14.6±2.2 μU/ml. At the time of the seventh dose, when the mean basal serum T_3 had risen to 115±5 ng/100 ml ($P < 0.001$) and the mean basal serum T_4 had risen to 8.6±0.3 μg/100 ml ($P < 0.01$), the mean maximum ΔTSH declined to 6.9±1.4 μU/ml ($P < 0.001$). At the time of the 13th dose, when the mean basal serum T_3 had remained virtually unchanged at 114±5 ng/100 ml and the mean basal serum T_4 had increased slightly to 9.5±0.3 μg/100 ml ($P < 0.05$), the mean maximum ΔTSH declined further to 3.0±0.7 μU/ml ($P < 0.005$).

Primary hypothyroidism. The serum TSH responses of the four patients with primary hypothyroidism to 13 consecutive 25-μg doses of TRH at 4-h intervals are shown in Fig. 3. After the first dose of TRH the mean serum TSH rose from a basal level of 148±39 μU/ml to a maximum of 212±61 μU/ml. After the seventh dose of TRH the mean serum TSH rose from a basal level of 99±25 μU/ml to a maximum of 230±75 μU/ml. After the 13th dose of TRH the mean TSH rose from 120±33 μU/ml to a maximum of 191±50 μU/ml.

No changes occurred in the mean serum T_3 and T_4 levels in these patients at any time during the 52 h study (Table III).

Correlation of the mean basal serum T_3 and T_4 levels at the time of each dose with the mean maximum ΔTSH

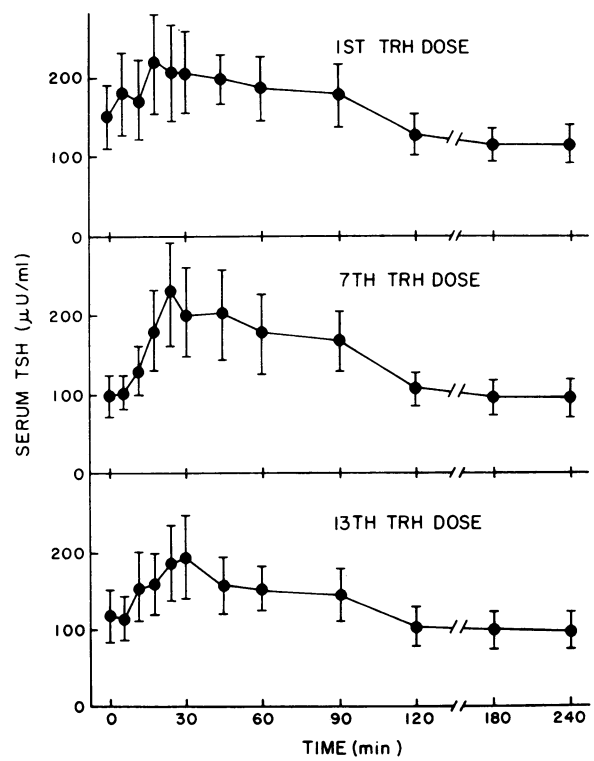

FIGURE 3 Mean serum TSH responses of four patients with primary hypothyroidism to repetitive doses of TRH. The procedure was the same as described for normal subjects in Fig. 1. Vertical lines represent ±SEM.

TABLE III

Serum T_3 and T_4 Responses to Repetitive Doses of TRH in Patients with Primary Hypothyroidism

	Serum T_3					Serum T_4		
min	0	60	120	180	240	0	120	240
	ng/100 ml					*μg/100 ml*		
1st TRH dose								
Mean±SEM	34	33	33	33	32	1.9	2.0	1.7
	2	2	1	1	1	0.4	0.5	0.3
7th TRH dose								
Mean±SEM	30	30	30	30	30	1.9	1.6	1.5
	0	0	0	0	0	0.4	0.5	0.4
13th TRH dose								
Mean±SEM	32	30	30	30	30	1.7	1.8	1.9
	1	0	0	0	0	0.4	0.5	0.5

Mean serum T_3 and T_4 levels after repetitive doses of TRH in the four patients with primary hypothyroidism whose TSH responses are shown in Fig. 3. No statistically significant changes occurred.

after that dose is shown in Fig. 4. In contrast to the normal subjects, these patients with primary hypothyroidism had neither increases in the mean basal serum T_3 and T_4 levels nor a fall in the mean maximum ΔTSH response to successive doses of TRH. The mean basal serum T_3 levels were 34±2, 30±1, and 32±1 ng/100 ml, and the mean basal serum T_4 levels were 1.9±0.4, 1.9±0.5, and 1.7±0.4 μg/100 ml at the times of the 1st, 7th, and 13th doses. The mean maximum ΔTSH responses were 92±29, 137±52, and 92±24 μU/ml, respectively.

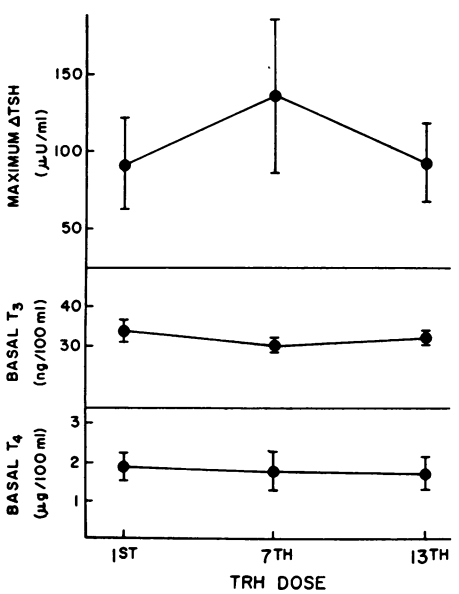

FIGURE 4 Correlation of the maximum ΔTSH with the basal serum T_3 and T_4 levels in four patients with primary hypothyroidism who received repetitive doses of TRH. "Basal" and "maximum Δ" are defined in Fig. 2. Vertical lines represent ±SEM.

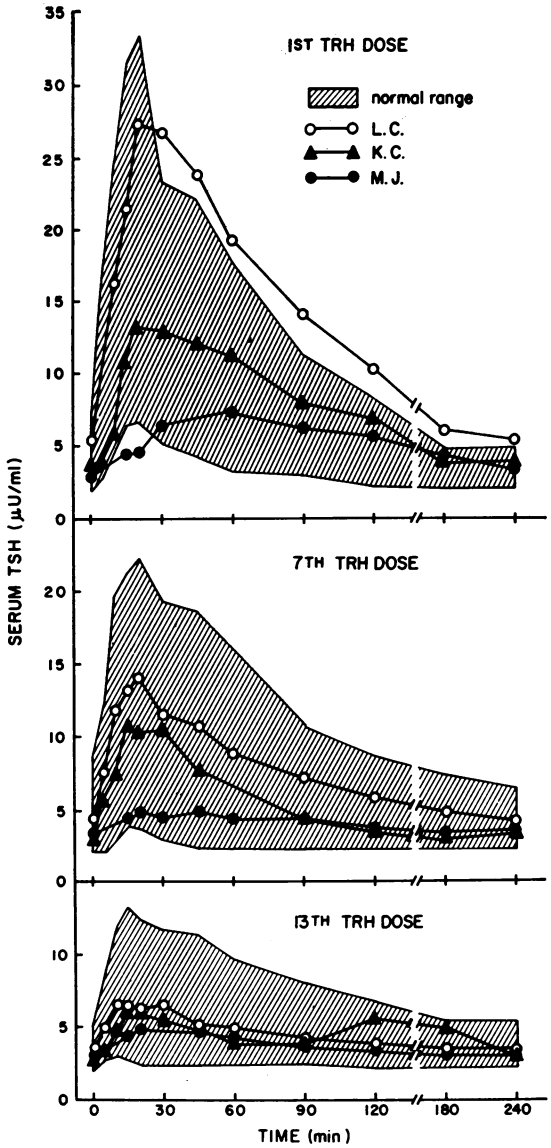

FIGURE 5 TSH responses of three patients with hypothyrotropic hypothyroidism, L. C., K. C., and M. J., to repetitive doses of TRH. The procedure was the same as described for normal subjects in Fig. 1. The hatched areas represent the range of responses of the 10 normal subjects whose mean TSH responses to TRH are shown in Fig. 1.

Patients with hypothyrotropic hypothyroidism. The serum TSH responses of the three patients with hypothyrotropic hypothyroidism, L. C., K. C., and M. J., to 13 consecutive 25-μg doses of TRH at 4-h intervals are shown in Fig. 5. L. C., who had presumed TRH deficiency, had a maximum TSH response after the first TRH dose of 27.0 μU/ml, at the upper limit of the normal range. Her maximum TSH responses fell progressively to 13.8 and then 6.5 μU/ml after the 7th and 13th TRH doses. These responses were also within the

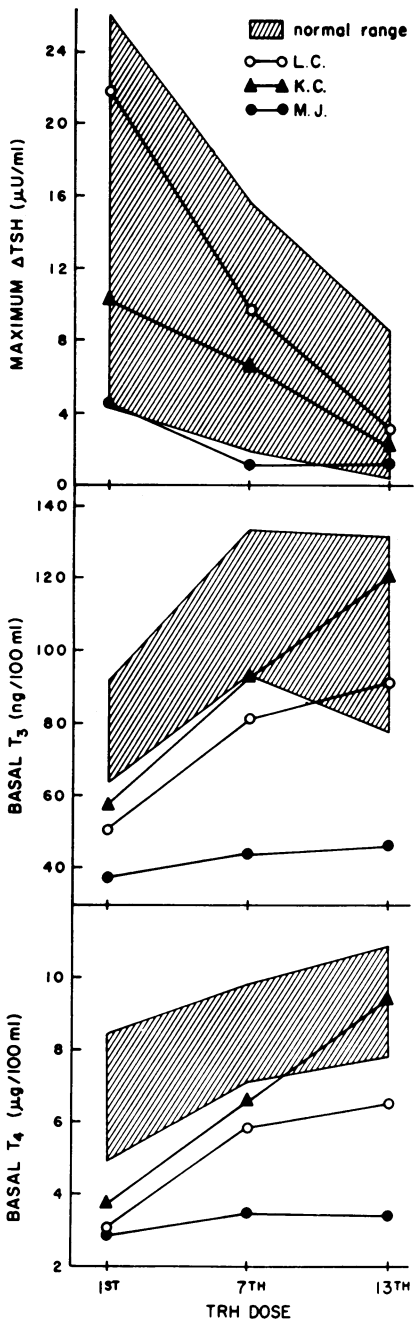

FIGURE 6 Correlation of the changes in maximum ΔTSH with the changes in basal serum T_3 and T_4 levels in response to repetitive doses of TRH in patients L. C., K. C., and J. J. The hatched areas represent the range of responses of the 10 normal subjects whose mean responses are shown in Fig. 2.

ranges of the normal subjects. K. C., who also had presumed TRH deficiency, had maximum TSH levels of 13.0, 10.3, and 6.4 μU/ml in response to the 1st, 7th, and 13th TRH doses. In contrast, M. J., who had presumed pituitary insensitivity to TRH, had a maximum TSH response to the first TRH dose of 7.5 μU/ml, at the lower limit of the normal range. Not only was her peak TSH response low in magnitude, but it was also delayed, occurring 45 min after the TRH. Her maximum TSH levels fell to 4.7 μU/ml after both the 7th and 13th TRH doses.

Correlation of the basal serum T_3 and T_4 levels at the time of each TRH dose in patients L. C., K. C., and M. J. with the maximum ΔTSH values after the dose is shown in Fig. 6. Like the normal subjects, these patients showed decreases in maximum ΔTSH as the basal T_3 and T_4 levels rose. They differed from the normal subjects, however, in other respects. L. C.'s basal serum T_3 and T_4 levels were initially subnormal: the T_3 was 50 ng/100 ml, and the serum T_4 was 3.0 μg/100 ml. At the time of the 13th TRH dose L. C.'s basal serum T_3 level had risen to 90 ng/100 ml, and her basal serum T_4 level had risen to 6.5 μg/100 ml, both within the initial basal ranges of the 10 normal subjects, as well as within the ranges of a larger normal population, 70–150 ng/100 ml for T_3 and 5–11 μg/100 ml for T_4 (6, 7). She had, therefore, been made "euthyroid" by 13 consecutive 4-h 25-μg TRH doses. Patient K. C. was also made chemically euthyroid by repetitive TRH administration. His basal serum T_3 levels rose from 58 to 92 to 120 ng/100 ml, and his basal serum T_4 levels rose from 3.8 to 6.6 to 9.3 μg/100 ml. Simultaneously his maximum ΔTSH responses fell from 10.2 to 6.5 to 3.2 μU/ml. M. J.'s initial basal T_3 and T_4 levels were also subnormal, 37 ng/100 ml and 2.9 μg/100 ml. At the time of the 13th dose the basal T_3 and T_4 levels had risen, to 46 ng/100 ml and 3.4 μg/100 ml, but not to within the initial basal ranges of the normal subjects or to within the ranges of normal of the general population. Even these small rises in serum T_3 and T_4 levels, however, were associated with a fall in M. J.'s maximum ΔTSH from a low level, 4.7 μU/ml, in response to the 1st TRH dose, to an even lower one, 1.3 μU/ml, in response to both the 7th and 13th doses.

DISCUSSION

The data presented here demonstrate the extreme sensitivity of TRH-stimulated TSH release to inhibition by small elevations of serum T_3 and T_4 of endogenous origin. Repetitive administration of 25-μg doses of TRH every 4 h to normal subjects produced a steady fall in the TSH response, the maximum ΔTSH responses after the 7th and 13th doses being 47 and 21% of that after the 1st dose. This striking fall in the TSH response to TRH was associated with rises in serum T_3 and T_4 levels but not above the normal ranges of either T_3 or T_4. Both the small rises in the serum T_3 and T_4 levels and the marked inhibition of TRH-induced TSH release

observed in the present study were quite similar in magnitude to those observed in a previous study in which these changes were produced by the administration of small quantities of exogenous T_3 and T_4. These similarities to the previous study, in which TRH was administered no more frequently than once every 3–4 wk (4), are evidence that the inhibition of TRH-induced TSH release observed in the present study was due to the small rises in serum T_3 and T_4 levels. Further, and more conclusive, evidence that the marked inhibition of TSH response in the normal subjects was due to the small rises in serum T_3 and T_4 levels is the data presented here from patients with primary hypothyroidism. These patients, who had no increases in serum T_3 and T_4 levels in response to repetitive TRH administration, had no decrease in TSH responses. This finding demonstrates that repetitive TRH administration, in the schedule used here, does not result in progressively lower TSH repsonses in the absence of rises in serum T_3 and T_4 levels. This finding, therefore, appears to exclude the possibility that the progressive fall in serum TSH response to repetitive TRH administration observed in the normal subjects was due to progressive depletion of pituitary TSH.

One implication of the data presented here is that production of elevations of serum T_3 and T_4 distinctly above the normal ranges due to supranormal secretion of endogenous TRH should be unlikely unless the endogenous TRH secretion were several orders of magnitude greater than normal.

The two patients with presumed hypothalamic hypothyroidism, L. C. and K. C., also demonstrated inhibition of TSH release by repetitive administration of TRH but only as the serum T_3 and T_4 levels rose into the normal ranges. TRH deficiency appears, therefore, to have been confirmed by producing chemical euthyroidism by replacement of the presumedly missing hormone. Several other patients, however, have been described who, like L. C., and K. C., had low serum thyroid hormone and low-normal basal TSH levels and normal TSH responses to a single dose of TRH (18–22).

The marked fall in the initially subnormal TSH response to TRH in M. J., the patient with presumed pituitary insensitivity to TRH, was accompanied by rises in serum T_3 and T_4, but rises so small that the final levels were still subnormal. That rises in serum T_3 and T_4 levels not sufficient to reach the normal ranges could cause such an inhibition of the TSH response to TRH demonstrates that the extreme sensitivity of TRH-induced TSH release to inhibition by small rises in endogenously secreted T_3 and T_4 was preserved in M. J., even though the sensitivity to TRH was diminished. Since stimulation of TSH release by TRH occurs by a different mechanism than inhibition of TSH release by thyroid hormones (23, 24), an abnormality of responsiveness to TRH without an abnormality of inhibition by thyroid hormones is not surprising.

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