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(Citation)
The Kobe journal of the medical sciences,18(4):215-228

(Issue Date)
1972-12

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/0100489003

Kobe J. Med. Sci. 18, 215-228, December 1972

EFFECT OF SYNTHETIC THYROTROPIN-RELEASING FACTOR (TRF) ON PITUITARY TSH SECRETION IN MAN, WITH SPECIAL REFERENCE TO THE EVALUATION FOR TRF TEST

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Indexing Words

hypothalamus; thyrotropin-releasing factor (TRF); pituitary; thyroid; TSH

Makoto ŌTSUKI and Shigeaki BABA. *Effect of Synthetic Thyrotropin-Releasing Factor (TRF) on Pituitary TSH Secretion in Man, with Special Reference to the Evaluation for TRF Test.* Kobe J. Med. Sci. 18, 215-228, December 1972 ——It is valuable to use synthetic thyrotropin-releasing factor for diagnosis and treatment of hypothalamic-pituitary disorders because of the specific action on TSH release induced by synthetic TRF from the anterior pituitary gland. We investigated about the standardization of "TRF test". TRF was administered in one of the four ways including, (i) a single intravenous injection of 25-1,000 μg, (ii) intravenous infusion over a period of 30 min to 8 hr, (iii) subcutaneous injection of 100-200 μg or, (iv) single oral dose of 1 - 10 mg. It is recommended to use the single intravenous injection as a routine TRF test because of the easiness of administration and the reliability of TRF absorption. Six different doses between 25 and 800 μg of TRF were administered to 40 normal subjects. The mean maximal rises in plasma TSH showed a dose-related response which was linear for the log of the dose in the range of 50 μg to 400 μg. In utilizing TRF as a test for pituitary thyrotropin reserve, we are currently giving doses of TRF at the extremes of the linear portion of the dose response curve which produced clear but significantly different elevation of plasma TSH; a person who failed to respond at 50 μg of TRF was administered 400 μg of TRF one week later. Although intravenous single injection was employed as a screening test for TSH secretion, based upon the results mentioned above, the mode and dose of TRF administration and the pretreatment should be studied further for a more precise method of pituitary TSH reserve.

INTRODUCTION

The hypothalamus has known to control the release of thyrotropin from the anterior pituitary gland by means of neurohumoral agent which has been called thyrotropin-releasing factor (TRF). The enthusiastic studies concerning TRF have revealed its chemical properties and biological activity,[4, 7, 10] and TRF has been synthesized in several laboratories. It is valuable to use synthetic TRF for diagnosis and treatment of hypothalamic-pituitary disorders because of the specific action on TSH release induced by synthetic TRF from the anterior pituitary gland in normal subjects. In this paper the studies concerning the standardization of TRF test for the pituitary TSH reserve will be presented.

Received for publication September 28, 1972
Authors' names in Japanese: 大槻真, 馬場茂明

MATERIALS AND METHODS

A) *Thyrotropin Releasing Factor (TRF)*

Thyrotropin-releasing factor used in this study was synthesized following the method of Gillessen et al[12] with a slight modification. It was presented as pyro-glutamyl-histidyl-proline amide in the form of acetate salt. Each ampoule contained TRF 50, 100, 200 and 500 μg as dry powder, which was immediately dissolved before use in 2.0 ml of steril distilled water with mannitol 25 mg. TRF activity of this preparation was determined with *in vivo* and *in viro* experiments.

B) *Side Effect Attending TRF Administration*

Transient nausea, a "queasy feeling" in the abdomen, a feeling of facial flushing and an urge to urinate were observed in about 10 per cent of the subjects. These symptoms began almost immediately after TRF injection and lasted several minutes. There was no relationship between the occurrence of side effect and doses of TRF, nor the magnitude of plasma TSH response. No change occurred in pulse rate, blood pressure, or respiration in any subjects. Estimations of hemoglobin, white cell counts and liver function tests were unchanged.

C) *Subjects*

Synthetic TRF was administered to 60 normal volunteers. The first 20 including the authors were healthy male volunteers who had no clinical or biochemical evidence of the thyroid disease. Subsequent volunteers were informed of the purpose of this study and of the experience of those who had received synthetic TRF. In addition to healthy subjects, TRF was given to 120 patients with hyperthyroidism, primary hypothyroidism, operated pituitary chromophobe adenoma, acromegaly, diabetes insipidus and long-term corticosteroid treated subjects. Almost all the subjects were fasted overnight and kept sitting throughout the test which was performed about 9: 00 a. m. Some were performed after breakfast.

D) *Assay*

i) *Immunoassay of Human Thyrotropin*

Plasma TSH concentrations were measured by means of radioimmunoassay using the double antibody technique. Purified human TSH preparation for labeling with radioactive iodine and antiserum to human TSH were supplied by the National Institute of Health Endocrinology Study Section. The Human Thyrotropin Research Standard A was used as a standard. TSH was labeled with ^{125}I by the method of Greenwood et al.[11] Standards were diluted in the buffer containing plasma from pituitarigenic hypothyroid subjects; thus all tubes in the assay contained the same volume of plasma. The minimum detectable level of plasma TSH was 1.7 μU/ml in our laboratory.

ii) *Other Hormone Assay*

The serum protein bound iodine (PBI) was measured by the autoanalyzer

technique. Serum thyroxine level was measured by competitive protein binding assay. Indirect estimation of serum thyroid hormone concentration was performed with T_3 resin sponge uptake (T_3 RSU) (Triosorb : Dinabot R. I. Lab., Ltd.). Plasma HGH was measured by radioimmunoassay using the double antibody technique after Schalch and Parker,[31] serum cortisol by a fluorometric modification according to DeMoor et al.[8]

RESULTS

I. On the Mode of TRF Administration

TRF was administered in one of the four ways including (i) a single intravenous injection of 25 - 1,000 μg given over 30 sec, (ii) 200 μg and 1,000 μg infusion in 100 - 500 ml of saline over a period of 30 min to 8 hr, (iii) subcutaneous injection of 200 μg or (iv) a single oral dose of 1 - 10 mg.

Intravenous single injection of synthetic TRF stimulates a rise in plasma TSH levels in all normal subjects. A detectable plasma TSH rise was observed within 5 min and further rise in the following 20 min. Peak level occurred at 10 - 30 min after TRF injection with gradual fall over the next 120 min (Fig. 1). The

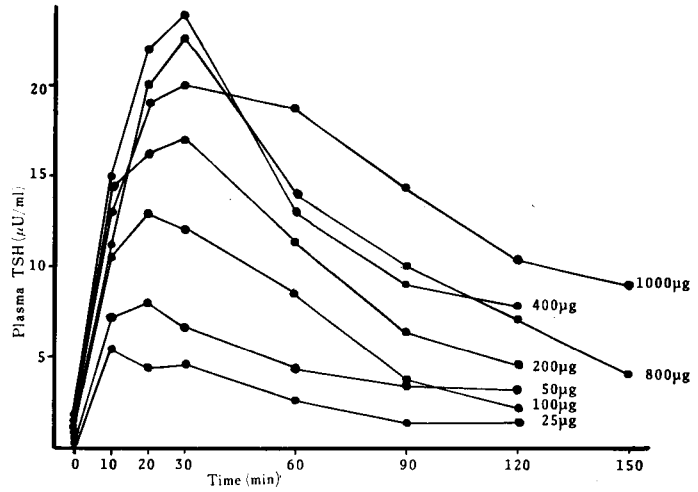

Fig. 1 Time course of plasma TSH response to the intravenous injection of synthetic TRF in normal subjects. Each point represents the mean of the values in the subjects.

response to 100 μg TRF in normal females appeared to be greater than in males (Fig. 2). Difference in the response to TRF administered by a prolonged infusion (30 - 120 min) of 200 μg TRF in 100 - 300 ml saline was investigated in 5 euthyroid males (Fig. 3). A peak plasma TSH value was observed in all cases immediately after the end of the TRF infusion, which was followed by a gradual fall to nearly basal values over the next 150 min. These responses were greater than those seen in the same subjects after rapid intravenous injection of the same dose.

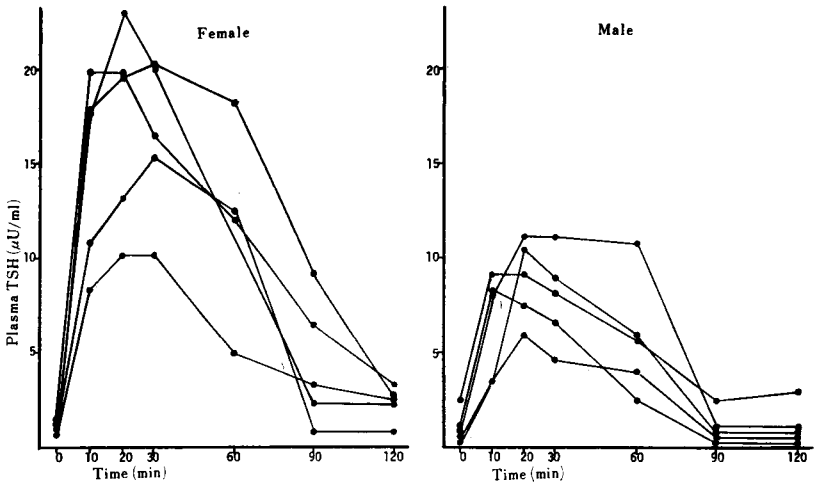

Fig. 2 The effect of sex on TSH response to 100 μg TRF in normal subjects.

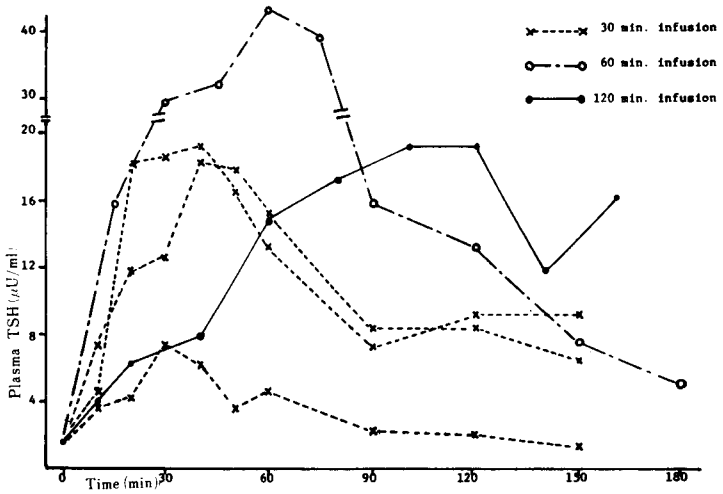

Fig. 3 Effect of 200 μg TRF infused in 100 - 300 ml saline over a period of 30 - 120 min.

When 1,000 μg TRF were infused over an eight hour period, plasma TSH rose from an undetectable level to peaks at about 4 and 7 hr followed by declines in the next 1 - 2 hr in spite of the continuous TRF infusion. Serum thyroxine of one case (M. O.: male) rose from 4.7 μg/dl to a peak of 7.6 μg/dl at 4.5 hr followed by a decline in the next 3.5 hr. Whereas in the other case (M. O.: female), serum thyroxine rose from 8.0 μg/dl to peaks of 12.1 μg/dl at 5 hr and 14.3 μg/dl at 8.5 hr. Although these results may be due to a diminished sensitivity of the pituitary to a rise of circulating thyroxine induced by the elevated TSH levels after the TRF infusion, or may indicate the limit of TSH release from the anterior pituitary gland,

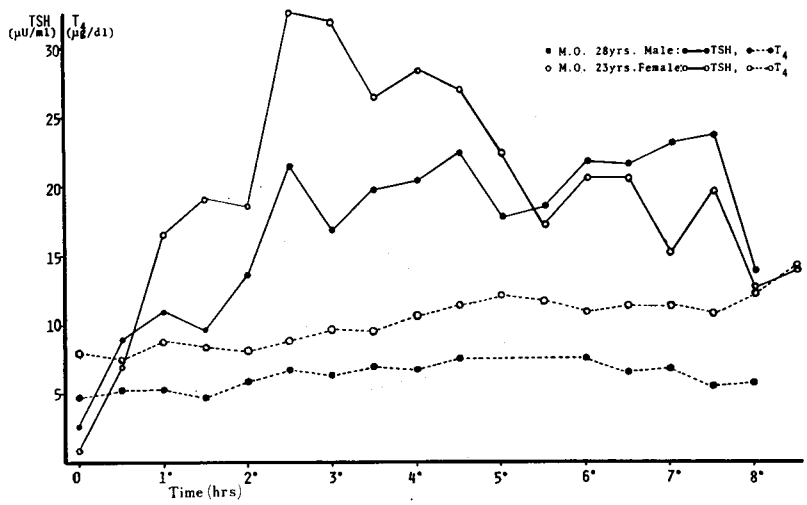

Fig. 4 Effect of 1,000 μg TRF infused in 500 ml saline over a period of 8 hr on plasma TSH and serum thyroxine levels.

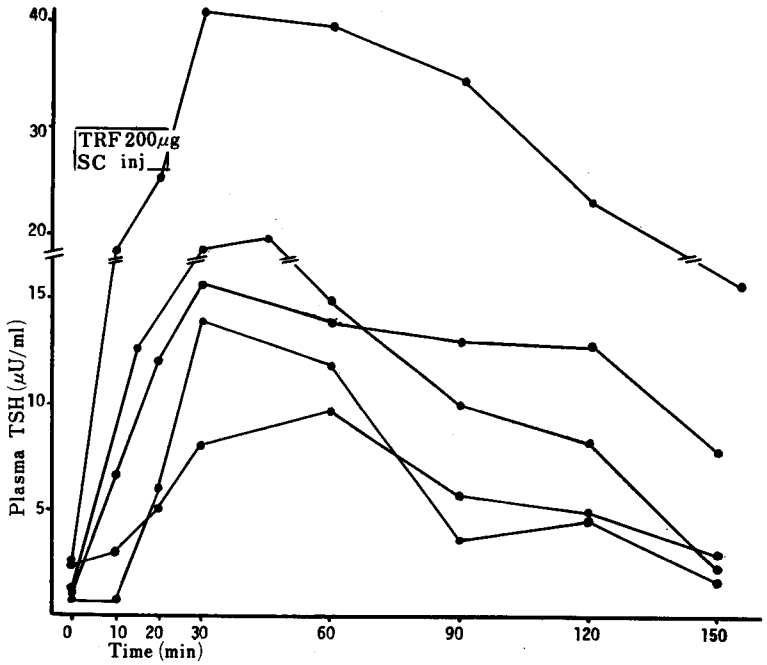

Fig. 5 Effect of 200 μg TRF injected subcutaneously on plasma TSH levels in 5 euthyroid subjects.

further studies will be necessary to confirm these effects of TRF (Fig 4).

Subcutaneous administration of 200 μg TRF in 2.0 ml of steril distilled water produced a prompt plasma TSH rise. Peak level occurred at 30 - 60 min after TRF injection, which was followed by a gradual fall to nearly basal values over the next 150 min (Fig. 5).

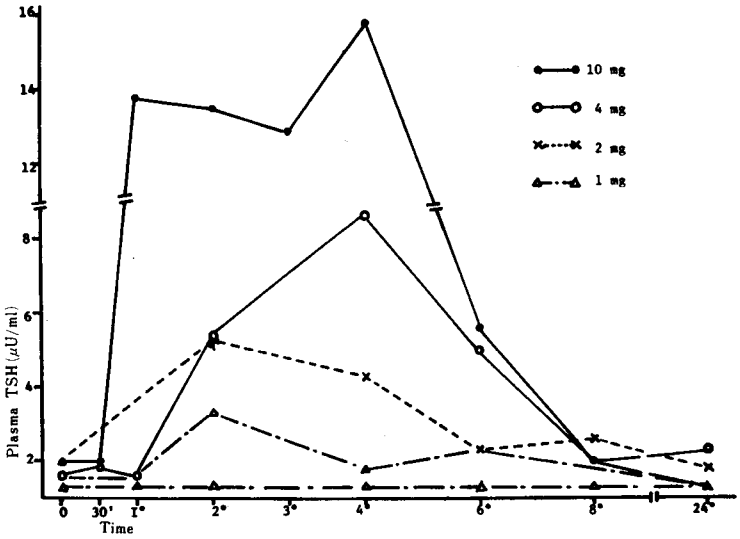

Fig. 6 Effect of single oral dose of one to 10 mg TRF on plasma TSH levels in 7 euthyroid subjects.

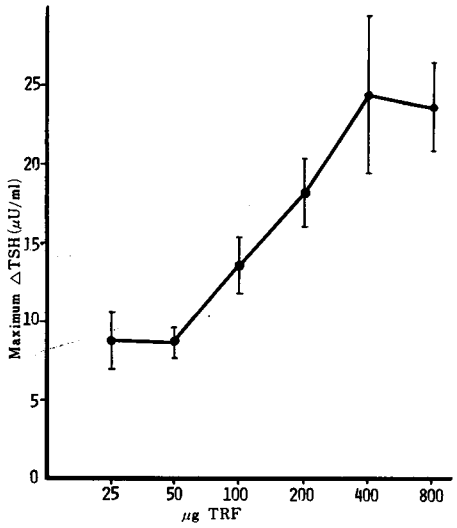

Fig. 7 Mean maximal increases in plasma TSH in euthyroid subjects given synthetic TRF doses of 25 to 800 μg.

A single oral dose of 1 - 10 mg TRF was administered to 7 euthyroid subjects. 4 mg or more of synthetic TRF caused more prolonged elevation of TSH levels than those seen after intravenous administration, with a TSH peak 2 to 4 hr after TRF ingestion (Fig. 6). Certain cases showed two peaks of TSH response to TRF administered orally or infused intravenously.

II. On the Dose of TRF Administration

Six different doses between 25 and 800 μg of TRF (25, 50, 100, 200, 400 and 800 μg) were administered to 44 euthyroid volunteers. The mean maximal increases in plasma TSH showed a dose-related response which was linear for the log of the dose in the range of 50 to 400 μg (Fig. 7). The responses to 25 and 50 μg were similar, and also the responses to 400 and 800 μg were not significantly different.

III. On the Indices of Response to TRF

PBI and T_3 RSU were measured in 16 euthyroid subjects who showed normal TSH response to 50 - 1,000 μg TRF. One μg/100 ml or more rise of PBI was observed in 6 out of 16 subjects at 90 to 120 min after intravenous TRF administration, while T_3 RSU remained unchanged (Table 1). The prolonged rise in the serum thyroxine level stimulated by oral administration of TRF was observed and its peak was 8 hr after application of TRF. Although plasma TSH induced by 1,000 μg TRF administered intravenously increased to just the same level as by oral administration, serum

Table 1 Effect of TRF on serum PBI levels in euthyroid subjects.

Intravenous single push				Time after TRF administration (min)*					
	Dose of TRF	Age	Sex	0	30′	60′	90′	120′	150′
1.	50μg	26	M.	3.4	3.4	3.4	4.4	4.8	
2.	〃	27	M.	5.0	5.2	5.4	5.1	5.3	
3.	〃	29	M.	4.2	3.6	3.6	3.8	3.4	
4.	〃	37	M.	4.8	4.6	5.2	7.2	5.4	
5.	〃	30	M.	4.8		5.0	5.4	5.4	
6.	〃	22	F.	4.2		4.8	4.8	5.2	
7.	100μg	27	M.	6.2		6.2	6.2	6.2	
8.	〃	28	M.	5.2		5.6	4.4	4.6	
9.	〃	21	F.	6.1		5.8	5.8	6.0	
10.	〃	22	F.	4.0		4.0	4.4	4.4	
11.	200μg	22	F.	5.9		6.7	6.8	6.8	
12.	〃	38	M.	3.0	3.8	3.6	4.0	3.8	
13.	800μg	21	F.	7.0	7.0	6.8	7.0	7.2	7.0
14.	1000μg	23	F.	6.6	6.8	6.6	7.0	6.8	7.0
15.	〃	27	M.	5.0	4.8	5.0	5.6	6.1	6.0
16.	〃	28	M.	6.0	6.0	6.2	7.6	7.4	7.0

*PBI values in μg/100 ml

Table 2 Serum thyroxine response to TRF.

(I) Intravenous single injection

Dose	Age	Sex		Time after TRF injection (min) 0	30′	60′	90′	120′	150′
1,000μg	28	M.	TSH*:	2.6	18.2	18.2	11.2	7.9	8.9
			T_4**:	8.9	9.6	10.2	9.7	9.8	9.8
1,000μg	27	M.	TSH :	1.7	23.4	20.5	17.2	15.2	10.6
			T_4 :	7.6	7.0	7.4	6.8	8.2	8.1
1,000μg	23	F.	TSH :	1.7	18.8	17.8	15.2	7.9	7.6
			T_4 :	10.3	10.9	10.2	10.2	8.8	10.5

(II) Oral administration

Dose	Age	Sex		Time after TRF ingestion (hr) 0	1°	2°	3°	4°	6°	8°	24°
5mg	29	M.	TSH :	3.0	18.2	19.8	19.5	22.4	12.5	19.5	5.9
			T_4 :	9.7	9.8	9.2	9.6	10.2	10.3	11.3	10.6
10mg	38	M.	TSH :	2.0	13.8	13.5	12.9	15.8	5.6	2.0	1.7
			T_4 :	7.0	6.5	7.6	7.8	8.7	9.2	9.9	7.9

(III) Infusion over an eight hour period

Dose	Name	Age	Sex		Time from the start of infusion (hr) 0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
1,000μg	M.O.	28	M.	TSH :	2.6	8.9	10.9	9.6	13.5	21.5	16.8	19.8	20.4	22.4
				T_4 :	4.7	5.3	5.3	4.8	6.0	6.8	6.4	7.0	6.8	7.6
1,000μg	M.O.	24	F.	TSH :	1.7	6.9	16.5	19.1	18.5	33.0	32.3	26.4	28.4	27.1
				T_4 :	8.0	7.5	8.9	8.4	8.1	8.9	9.6	9.5	10.6	11.3

					5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5
1,000μg	M.O.	28	M.	TSH :	17.8	18.5	21.7	21.6	23.2	23.7	13.8	
				T_4 :	—	—	7.5	6.6	6.8	5.6	5.8	
1,000μg	M.O.	24	F.	TSH :	22.4	17.2	20.5	20.5	15.2	19.8	12.5	13.9
				T_4 :	12.1	11.7	11.0	11.4	11.4	10.7	12.4	14.3

*TSH values in μU/ml

**Thyroxine (T_4) values in μg/100ml normal range 5.0~13.0

thyroxine did not increase (Table 2). It is well said that plasma TSH immunoassay was to be the most accurate indicator for intravenous single injection of TRF.

Other endocrine effects of TRF on plasma levels of human growth hormone and cortisol have been also investigated. We have found no significant response of these hormones to TRF.

IV. On the Decision Time after TRF Administration

As the time course of TSH response to TRF varied with the mode of TRF administration, it should be separately investigated. In the cases with intravenous single injection of TRF, blood samples were collected before and at 10, 20, 30 and 60 min after the injection. These intervals of sampling were used because the maximum ⊿TSH increment following TRF administration occurred at 5 min in one subject, at 10 min in 6, at 15 min in 6, at 20 min in 14 and at 30 min in 13.

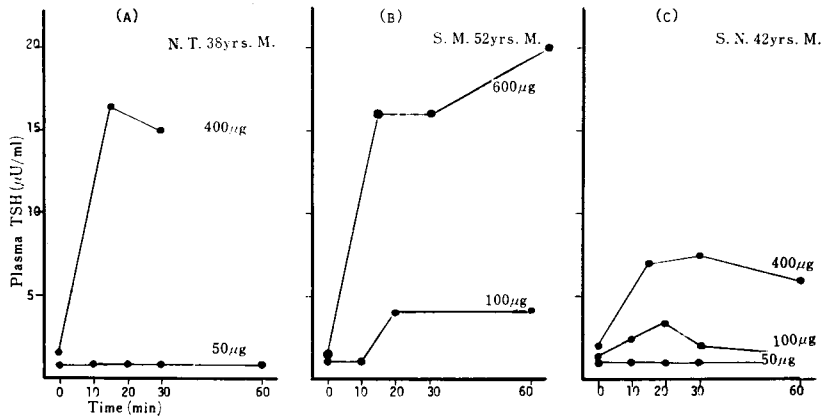

Fig 8 Effect of increasing dose of TRF on plasma TSH in cases with pituitary chromophobe adenoma (A, B) and a long-term corticosteroid administration (C).

Table 3 Effect of increasing dose of TRF on plasma TSH levels in cases with endocrinopathies.

Name	Age	Sex	Diagnosis	Dose of TRF	TSH(μU/ml) response to TRF 0	10(15)20′	30′	60′	BMR (%)	PBI (μg/100ml)	T3RSU (%)
A. N. T.*	38	M.	Pituitary chromophobe adenoma	50μg	<1.7	<1.7 <1.7	<1.7	<1.7	−21		24.9
				400μg	<1.7	16.5	14.0				
B. S. M.**	52	M.	Pituitary chromophobe adenoma	100μg	<1.7	<1.7 4.0		4.0	−9	4.2	28.8
				600μg	<1.7	16.0	16.0	20.0			
C. S. N.***	42	M.	Bronchogenic carcinoma	50μg	<1.7	<1.7 3.3	2.0	<1.7		5.7	35.4
				100μg	<1.7	2.4 3.3	2.0	1.7			
				400μg	2.5	6.9	7.3	5.9			

* Diagnosis was ascertained by operation.

** This is a case receiving long-term (2 years), low-dosage (receiving 30 mEq of cortisol per day) steroid therapy.

*** At least two weeks were separated between the TRF tests.

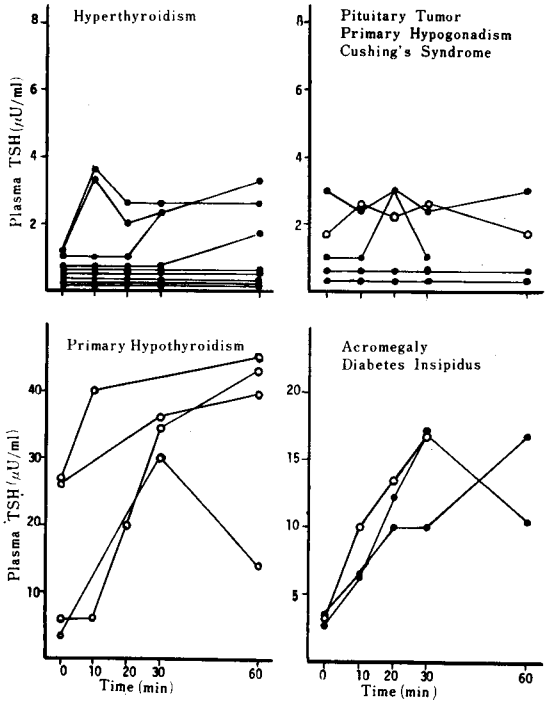

Fig. 9 Various types of TSH response to TRF.

V. Effect of TRF on Plasma TSH Levels in Cases with Endocrinopathies

Two patients (A, B) with hypopituitarism secondary to an operation to remove a pituitary chromophobe adenoma and a patient (C) receiving a long-term cortico-steroid administration did not respond to TRF stimulation with 50 μg (A, C) and 100 μg (B). However they showed definite response to 400 μg (A, C) and 600 μg (B) TRF (Fig. 8, Table 3).

We observed three patterns of TSH response to TRF in cases with hypothalamo-pituitary disorders as well as other endocrinopathies; (i) normal TSH response just the same as euthyroid subjects, (ii) no or slight response, seen in the cases with pituitary disorders and hyperthyroidism, and (iii) hyper-response, that is, TSH increases continuously even at 60 min after TRF administration (Fig. 9).

DISCUSSION

The availability of pure synthetic TRF promoted a number of investigators to test its biological activtiy in man. However, no report has presented the studies of dose response curve and route of administration for synthetic TRF in a large number of euthyroid subjects. Such investigations would be necessary before "TRF test" could be standardized.

As the mode of TRF administration to man, intravenous,[1, 2, 6, 9, 14, 15, 16, 18, 24, 25, 30] intramuscular[20] and oral routes[15, 16, 19, 20, 24, 25] were reported. TRF also produced a significant release of TSH from the anterior pituitary glands of mice and rats administered by intravenous, subcutaneous, intraperitoneal, intramuscular and oral routes. Considering the report by Redding et al[27] ; intramuscular injection of TRF in a volume of 0.1 ml causes a delay in the absorption and presumably provides a greater opportunity for the inactivation of TRF by plasma and/or enzymes from damaged tissues, resulting in a poor response as compared with the same dose given intravenously, whereas in a volume of 10 μl it is quickly absorbed into the blood, and the response identical with the one following the intravenous administration of TRF, we did not investigate the route of intramuscular administration. Subcutaneous injection of TRF resulted in an identical response with that following the intravenous administration of TRF. However, the route of subcutaneous administration was not useful as a routine test because of the wide individual variation in response. It also seemed to be difficult to use oral administration of TRF as a routine test, because the TSH response to TRF administered orally was delayed and the rate of absorption of TRF varied with individuals. The studies reported here showed that the administration of synthetic TRF by intravenous, subcutaneous and oral routes produced a significant release of TSH. The intravenous administration of TRF produced the greatest response in the shortest time as compared with the other methods of administration. We decided to use intravenous administration as a routine TRF test because of the stability of TSH response to TRF and the reliability of TRF administration. Although intravenous infusion of TRF produced greater responses than intravenous single injection, the latter is convenient as a routine test because of the easiness of administration.

About the dose of TRF administration, we could not find agreement with any respects. Including the purity of synthetic TRF preparation, there are many factors about the difference of TRF used. Minimum effective dose of TRF seemed to be less than 25 μg from our observations. Finding a dose-related increase of TSH output up to 200 μg of TRF, Köbberling et al[20] recommended 200 μg for routine test. Ormston et al,[24] Mühlen et al,[33] Karlberg et al[18] and Rothenbuchner et al[28] also used 200 μg of TRF. On the other hand, Hall et al,[14] Hershman and Pittman[16] and Gual et al[13] proposed 500 μg or more of TRF. Using six different doses of TRF between 25 and 800 μg, we found a significant dose-related increases of TSH release up to 400 μg of TRF. It was suspected that there must be a limit about pituitary TSH reserve, because in some cases the TSH responses to 800 and 1,000 μg TRF were poorer than to 400 μg TRF. In utlilizing TRF as a test for pituitary TSH reserve, the authors may recommend to use 50 - 100 μg of TRF for a routine TRF test, because these doses of TRF lead to a dose-related increase of radioimmunologically measurable TSH and the response dose not reach the maximum level. However, to detect the degree and the character of TSH deficiency found by using these doses of TRF, it should be considered to increase the dose of TRF and to use the other modes of administration, such as intravenous infusion. PBI and T_3 RSU were measured in 16 euthyroid subjects who showed normal TSH responses to 50 - 1,000 μg TRF. One μg/100ml or more rise of PBI was observed in 6 out of 16 normal

subjects at 90 to 120 min after intravenous TRF administration. Concerning T_3 RSU responses to TRF, it has been reported that no or slight response occurred after intravenous administration just as our observation.[30] These findings suggest that plasma TSH immunoassay was to be the most accurate indicator for intravenous single administration of TRF. Our observation showed that TRF was active when administered orally to euthyroid subjects in doses of 4 mg or more. It produced a rise in plasma TSH which was followed by an increase in serum thyroxine levels. Oral administration of TRF with estimation of thyroxine levels may therefore be useful as an indirect test of pituitary TSH reserve, when TSH assays are not available.

Other endocrine effects of TRF on plasma levels of HGH and cortisol have been investigated. We have found no significant responses of these hormones to TRF. Several authors reported a HGH increase to TRF administration,[2,18,19] but a lack of HGH response has also been observed.[6,9,19,25] In any way, the mechanism of the effect of TRF on HGH release is considered to be indirect one. There are different reports about the effect of TRF on plasma cortisol levels; some observed a decrease in circulating cortisol level,[2,28] while others have found no changes[9,19,25,29] or even increases of plasma cortisol.[6,18] From available data, it seems that TRF has no effect on the release of serum LH, FSH and insulin.[2,6,19,25] Recent reports have demonstrated that the ability of TRF to stimulate prolactin release was not limited to rat pituitary tumor cells[32] or bovine pituitary pieces *in vitro*,[21] but extended to normal man *in vivo*.[17] About other hormonal effects of TRF further studies should be done before any definite conclusions are reached.

Attempts to develop such a "stress" test for pituitary TSH resevre have previously been unsuccessful.[14,23,26] Administration of TRF causes a rise in plasma TSH and so should provide a useful test of the function and reserve capacity of TSH secretion of the anterior pituitary gland. Although partial and complete deficiencies of TSH secretion of the anterior pituitary gland are considered to exist, the clinical and biochemical diagnosis of minor degrees of pituitary TSH deficiency was particularly difficult. The usefulness of TRF as a test of functional integrity of the pituitary for TSH secretion was demonstrated by our patients who did not react with increased plasma TSH to 50 or 100 μg but to 400 or 600 μg of TRF. If we use only large doses of TRF, it is dangerous to overlook the partial deficiency of TSH reserve and to consider that the pituitary is quite normal. Although intravenous single push method was employed as a screening test for TSH secretion, based upon the results mentioned above, in the cases with low or no TSH increase following factors should be taken into account for more precise method for pituitary TSH reserve test; mode and dose of TRF administration.

We observed three patterns of TSH response to TRF in the cases with hypothalamo-pituitary disorders as well as other endocrinopathies; normal response, hyporesponse and hyper-response. These results indicate that the response of plasma TSH induced by TRF is modified by other hormones. Thus, it is necessary to consider the endocrine conditions carefully before coming to decision of the responsiveness to TRF.

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