THE USEFULNESS OF THYROTROPIN-RELEASING FACTOR (TRF) TEST IN PATIENTS WITH HYPOTHALAMIC-PITUITARY DISORDERS

OTSUKI, Makoto

(Citation)
The Kobe journal of the medical sciences,19(1):13-22

(Issue Date)
1973-03

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/0100488974

Kobe J. Med. Sci. 19, 13-22, March 1973

THE USEFULNESS OF THYROTROPIN-RELEASING FACTOR (TRF) TEST IN PATIENTS WITH HYPOTHALAMIC-PITUITARY DISORDERS

Makoto OTSUKI, Makoto TATEIWA, Hidetaro MORI and Shigeaki BABA

The Second Department of Internal Medicine Kobe University School of Medicine

Indexing Words

thyrotropin-releasing factor (TRF); TRF test; craniopharyngioma; pituitary chromophobe adenoma; acromegaly; diabetes insipidus; hypothalamic hypothyroidism

Makoto OTSUKI, Makoto TATEIWA, Hidetaro MORI and Shigeaki BABA. *The Usefulness of Thyrotropin-Releasing Factor (TRF) Test in Patients with Hypothalamic-Pituitary Disorders.* Kobe J. Med. Sci. 19, 13-22, March 1973——Intravenous administration of synthetic TRF has been used as a test of hypothalamic-pituitary function. Changes of plasma TSH levels were measured after administration of 50 - 100 μg of TRF to 55 patients with hypothalamic-pituitary disorders. Of 21 patients with pituitary chromophobe adenoma, 11 had a normal TSH response. Five of 10 patients with absent or subnormal response showed clinical or biochemical evidence of hypothyroidism. Of 20 patients with hypothalamic lesions 12 showed a normal response, and 6 responded supernormally. A delayed response to TRF, where the maximum TSH response was observed after 30 min, occurred in 16 of 18. This delayed pattern of response and normal or supernormal response to small doses of TRF, such as 50 - 100 μg, can be used to recognize hypothalamic lesions and to differentiate hypothalamic lesions from pituitary diseases.

INTRODUCTION

Administration of synthetic TRF has been recently introduced as a test of hypothalamic pituitary thyroid function.[2, 5, 9, 12-16] In normal euthyroid subjects intravenous administration of synthetic TRF causes a consistent rise of plasma TSH levels. We reported about the standardization of TRF test, the normal range of TSH response to a standard intravenous dose of TRF, and its application to the diagnosis of hypothalamc-pituitary disorders.[13-16] There are only several reports about the TSH response to TRF in small groups of patients with hypothalamic-pituitary disorders. This paper describes the usefulness of the TRF test in a large group of patients with hypothalamic-pituitary disorders.

Received for publication December 12, 1972
Authors' names in Japanese: 大槻 真, 立岩 誠, 森頴太郎, 馬場茂明

MATERIALS AND METHODS

1) *Thyrotropin Releasing Factor (TRF)*

The thyrotropin releasing factor used in this study was synthesized following the method of Gillessen et al.[6] with a slight modification. It was presented as pyroglutamyl-histidyl-proline amide in the form of acetate salt.

2) *TRF Test*

Almost all the subjects had been fasted overnight and remained seated throughout the TRF test which was performed early in the next morning. Venous blood was withdrawn at zero time for the estimation of TSH and routine thyroid function tests, and 50 or 100 μg of TRF in 2 ml saline solution was rapidly administered intravenously through the same needle. Blood samples were subsequently obtained for TSH estimation at 10, 20, 30 and 60 min.

3) *Plasma TSH and Other Hormone Assays*

Plasma TSH concentrations were measured by a double-antibody radioimmunoassay. Results were expressed in terms of μU of MRC Standard A Human TSH. The minimum detectable level was 1.7 μU/ml in our laboratory.

Routine thyroid function tests included the serum protein bound iodine (PBI; Autoanalyzer method), the total serum thyroxine level (Tetrasorb test; Dinabot R.I. Lab. Ltd.), the T_3 resin sponge uptake (Triosorb test; Dinabot R.I. Ltd.), the thyroidal uptake of ^{131}I at 24 hours and basal metabolic rate.

Plasma HGH was measured by radioimmunoassay using the double antibody technique after Schalch and Parker,[17] serum cortisol by a fluorometric modification according to DeMoor et al.[4]

4) *Clinical Subjects*

The normal range of responses of plasma TSH to 50 and 100 μg of TRF was studied in 10 males and 10 females aged 16 to 37 years.

The patients with hypothalamic-pituitary diseases were divided into 4 groups as shown on Table 1.

RESULTS

Control Subjects

We used 50 or 100 μg of TRF as a test dose according to the results reported previously.[13, 14, 16] In normal subjects a detectable rise of plasma TSH was observed within 5 min and further rise followed over the next 15 min. Peak levels occurred at 10 to 20 min after injection of 50 - 100 μg of TRF. The maximum value of ΔTSH in this study was between 4.2 μU/ml and 10.8 μU/ml to 50 μg of TRF and from 4.9 μU/ml to 21.4 μU/ml to 100 μg of TRF, respectively (Table 2). We did not find any

Table 1 Patients with hypothalamic-pituitary disorders.

	number	dose of TRF (μg)
Group 1		
Pituitary chromophobe adenoma*	21	100
Group 2		
Acromegaly	6	100
Group 3		
Hypothalamic lesions		
Craniopharyngioma*	13	50***
Pinealoma	5	50
Meningioma of the region of optic chiasma*	2	50
Tumor of the floor of the third ventricle	1	50
Group 4		
Diabetes insipidus**	7	50

* Diagnosed histologically after operation.
** Clinically diagnosed diabetes insipidus except nephrogenic diabetes insipidus.
*** Some cases were administered 100 μg of TRF.

Table 2 Plasma TSH response to TRF in normal subjects.

Dose of TRF (μg)	Number of subjects	Age(yr)	Time after TRF injection(min) 0	10	20	30	60	90	120
25	4 M. 2 F. 2	22-30	<1.7*	4.2±1.2	3.8±1.5	3.5±1.7	2.1±0.4	1.7	1.7
50	10 M. 5 F. 5	22-37	2.6±0.4	6.9±0.8	8.0±0.8	6.7±0.5	4.4±0.4	3.2±0.3	3.0±0.5
100	10 M. 5 F. 5	18-28	1.7±0.2	10.7±1.7	13.1±1.7	12.2±1.4	8.6±1.7	3.3+0.5	2.2±0.7
200	6 M. 3 F. 3	16-38	<1.7*	14.4±3.4	16.1±2.9	17.1±3.3	11.3±3.8	6.3±2.3	4.5±3.8
400	5 M. 2 F. 3	19-34	2.0±0.4	14.8±3.1	22.0±6.2	23.8±6.1	12.7±4.5	9.0±4.3	7.7±3.4
800	6 M. 3 F. 3	19-28	<1.7*	11.1±3.5	20.6±4.2	22.8±4.0	13.6±1.3	9.8±1.4	7.3±2.4
1,000**	3 M. 2	24-29	<1.7*	13.0	19.0	20.1	18.8	14.5	10.3

TSH values in μU/ml (mean±SEM).
* The plasma TSH levels were less than the minimum detectable value (1.7μU/ml).
** The plasma TSH values were represented as the mean.

difference in TSH responses to 50 μg of TRF between males and females. However, females showed a maximum ΔTSH increase greater than males to 100 μg of TRF.

Definition of Response

On the basis of these results in the control subjects the responses of plasma TSH in the TRF test were arbitrarily defined as, (i) absent (a rise of plasma ΔTSH less than 2.0 μU/ml), (ii) subnormal (a rise of plasma ΔTSH between 2.0 and 4.0 μU/ml), (iii) normal (a rise of plasma ΔTSH of 4.0 μU/ml or more) and (iv) supernormal (a rise of plasma ΔTSH greater than 11.0 μU/ml to 50 μg of TRF and 22.0 μU/ml to 100 μg of TRF, respectively).

As mentioned above, in all normal subjects peak TSH levels occurred at 10 to 20 min; cases in which the peak TSH values were obtained after 20 min were defined as a delayed response, for the present.

Patients

The results of the TRF test were summarized on Table 3.

Table 3 TSH response in the TRF test.

	Absent	Subnormal	Normal	Supernormal
Group 1				
Pituitary chromophobe Adenoma:				
Treated	5(0*)	5(3*)	8(4*)	
Untreated	0	0	3(1*)	
Group 2				
Acromegaly		1(1*)	5(4*)	
Group 3				
Lesions of the hypothalamus	2(0*)		12(10*)	6(6*)
Group 4				
Diabetes insipidus		1(0)	2(2*)	4(4*)

* The number of delayed responses in each group is shown in parentheses.

Group 1 (21 patients with pituitary chromophobe adenoma)

Eleven patients responded normally and 5 showed a subnormal response, one of whom had clinical and biochemical evidence of hypothyroidism. There were 5 with an absent response, all of whom were hypothyroid cases. Six patients with a normal response and 2 with a subnormal response showed a delayed rise of TSH.

Group 2 (6 patients with untreated acromegaly)

One had a subnormal TSH response and the other 5 responded normally, one of whom was the hypothyroid case. Five of the 6 acromegalic patients showed a delayed TSH response.

Group 3 (19 patients with hypothalamic lesions)

Of the 13 cases with craniopharyngioma 9 responded normally, 2 had a supernormal response and 2 showed an absent response. As these 2 patients who showed thyroid hypofunction and an absent TSH response to TRF had a long history, more than 10 years, of this disease and had been operated twice, it was supposed that not only hypothalamus but also pituitary was destroyed. Of the other 5 with hypothalamic lesions, one responded normally and 4 showed a supernormal response to 50 μg of TRF. All of the six patients with a supernormal response were hypothyroid, however, they showed a normal response to exogenous TSH (Thytropar ; Armour, Ltd.) (Table 4).

Group 4 (7 patients with clinical evidence of diabetes insipidus)

Two patients showed a normal response and 4 showed a supernormal response. Two patients with normal response had biochemical evidence of hypothyroidism. In 6 patients of 7 delayed TSH responses were observed.

DISCUSSION

The mechanism of the action of TRF on the pituitary gland was poorly understood. TRF appeared to stimulate not only the release of preformed TSH but also the biosynthesis of new TSH.[1, 11, 19] Bowers et al.[1] reported that the small amounts of TRF depleted the pituitary stores of TSH, while the larger amounts of TRF did not change the pituitary TSH contents, and concluded that only the larger amounts of TRF can stimulate both TSH synthesis and release. From these observations, it may be impossible that the newly synthesized TSH is released within 60 min after the injection of these small amounts of TRF, such as 50 to 100 μg. So that the magnitude of the changes in plasma TSH levels after iv administration of 50 - 100 μg of synthetic TRF seems to reflect the reserve pituitary TSH contents.

There was a wide individual variation in TSH response to TRF as shown on Table 2. However, a log-linear dose-related response was found for the range of 50

Table 4 Hypothalamic-pituitary thyroid function in patients with supernormal TSH response.

Name	Age (yr)	Sex	Clinical diagnosis	TSH (μU/ml) response to TRF 0	10′(15′)	20′	30′	60′	BMR (%)	PBI (μg/dl)	T_3RSU (%)	TSH test* 0	24°	48°	Insulin hypoglycemia	0.	30′	60′
	6	M.	Craniopharyngioma**	<1.7	—	—	29.6	—	−36	3.8	24.0				#B.G.:	57	28	52
															HGH:	12.8	5.2	2.4
+	37	M.	Craniopharyngioma**	<1.7	22.8		20.1	25.1 23.1	−36	3.1	25.1	T_4:	3.4	6.4 5.5	B.G. :	57	10	16
															HGH:	2.7	1.2	1.1
.	26	M.	Pinealoma**	<1.7	18.5		18.5	12.5	−25	1.8	22.6	PBI:	1.8	2.0 2.2	B.G. :	84	32	75
												T_4:	1.4	1.7 2.8	HGH:	0.6	0.5	0.7
															C.S. :	2.2	5.6	5.6
.	16	M.	Pinealoma***	2.3	13.2		23.7	28.1 23.1	−20	2.6	20.4	PBI:	2.6	5.5 7.8	B.G. :	60	26	34
												T_4:	4.0	8.5 11.9	HGH:	6.1	—	7.0
.	50	F.	Meningioma of optic chiasma**	<1.7	12.8		14.2	22.2 15.2	−22	4.4	22.2	PBI:	4.4	8.2 8.8	B.G. :	96	42	70
												T_4:	6.4	12.1 13.7	HGH:	1.1	1.0	6.0
															C.S. :	14.0	12.0	35.0
.	36	F.	Tumor of the floor of the third ventricle***	3.6	34.7		52.8	27.4	−21	4.5(T_4)	25.3	T_4:	4.5	5.7 5.7	B.G. :	96	44	82
															HGH:	5.2	3.8	5.1

The TRF dose was 50 μg iv single injection in all cases except one(+) who received 100 μg.

* 10 USP of bovine TSH (Thytropar; Armour, Ltd.) was injected im.

** Diagnosis was ascertained by operation.

*** Diagnosis was confirmed by PVG.

B.G. represents blood glucose level (mg/dl), HGH represents plasma growth hormone level (mμg/ml), C.S. represents plasma cortisol level (μg/dl).

to 400 μg of TRF given as a single iv injection.[14-16] Haigler et al.[7] and Snyder and Utiger[18] reported similar results. In utilizing TRF for pituitary TSH reserve test, we employed a relatively small dose of TRF such as 50 - 100 μg. As the peak level occurred at 10 to 20 min after injection of 50 - 100 μg of TRF in all control subjects, we arbitrarily defined the case in which the peak level was observed at 30 min or after as a delayed type of TSH response.

This definition must be limited only when the small amounts of TRF (100 μg or less) were used, because, as shown on Table 2, the more time was necessary for the maximum TSH response when larger doses of TRF (200 μg or more) were administered.

Recent studies in rats showed that hypothalamic lesions resulted in decreases in serum T_4, PBI and TSH levels, but that the thyrotroph cells did not atrophy nor the TSH concentrations in the pituitary gland decrease.[8, 10] These animals with hypothalamic lesions responded to thyroidectomy[10] or to goitrogen administration[8] with a definite but blunted rise in serum TSH. From these observations, it seemed to be quite natural that the patients with hypothalamic lesions respond to the TRF which acts on the anterior pituitary gland directly.

The delayed rise in plasma TSH levels observed in the majority of patients with hypothalamic lesions may be a consequence of the abnormality of the cell membrane of the thyrotrophs; decreases of the sensitivity to TRF due to longstanding deficiency of the endogenous TRF, or reduction of the receptor sites to TRF. On the other hand, in the patients with pituitary disorders, TSH responses to TRF may be reduced or absent due to loss of thyrotroph cells. Hence TRF test with a relatively small dose, such as 50 - 100 μg, can be a sensitive test of impaired TSH reserve and can make a difference between pituitary diseases and hypothalamic lesions.

Although we used relatively small doses of TRF, half of the patients with pituitary chromophobe adenoma responded normally, which showed that the pituitary had a great TSH reserve and that its destruction was not a "all or none" way. Little or no rise in plasma TSH levels was observed following the injection of 100 μg of TRF in 5 patients with pituitary chromophobe adenoma who had a longstanding pituitary insufficiency and secondary hypothyroidism.

In acromegaly, TSH reserve was almost normal; 5 patients out of 6 showed a normal response. Hall et al.[8] reported that TSH reserve was often impaired in acromegalics. This difference may be due to treatment. None of our patients had been treated. Effective treatment of acremegaly may be accompanied by the impaired TSH reserve.[8]

In patients with hypothalamic lesions without major pituitary lesions, a normal TSH response to TRF in 12 patients and a supernormal response in 6 were observed. Moreover, 16 of 18 patients showed the delayed pattern of TSH response. Although six patients with supernormal TSH responses had clinical and biochemical evidence of hypothyroidism, as shown on Table 3, basal plasma TSH levels were low, thyroid autoantibodies were negative and responded normally to exogenous TSH. From these results, existence of both hypothalamic lesions and primary hypothyroidism in the same patient could be denied. Hence the hypothyroidism observed in these patients was due to lack of effective endogenous TRF secretion. These patients with

hypothalamic lesions showed supernormal TSH responses to a small dose of exogenous TRF because of the normal TSH concentrations in the pituitary gland in spite of the deficiency of endogenous TRF.[8, 10] From these findings, it seems to be possible to postulate for the criteria of hypothalamic (tertiary) hypothyroidism: i) existence of clinical and biochemical evidence of hypothroidism, ii) with normal thyroid gland, which can respond to exogenous TSH normally and iii) with normal pituitary TSH reserve, in other words, normal or supernormal TSH response to a relatively "small" dose of TRF, such as 50 μg.

Of the seven patients who were diagnosed as diabetes insipidus clinically, 2 showed a normal response and 4 had a supernormal response. Moreover, 6 of the seven patients showed a delayed rise of TSH. These patterns of TSH responses were just the same as those seen in the patients with determined hypothalamic lesions. According to the pattern of TSH response, the diabetes insipidus observed in this study was probably induced by hypothalamic lesions. However, the effect of ADH deficiency on TSH response to TRF should be considered.

We described the usefulness of TRF test with small doses as a sensitive test for pituitary TSH reserve. The delayed pattern of TSH response and normal or supernormal response to such a small dose of TRF indicate the presence of hypothalamic lesions and can differentiate them from pituitary diseases. However a delayed TSH response was also found in 8 of 21 of the pituitary chromophobe adenoma. Seven of the 8 patients who showed a delayed response had been subjected to local treatment, whereas only one with untreated pituitary diseases had a delayed TSH response. This difference may indicate hypothalamic dysfunction or vascular damage resulting from the local treatment.

All the patients with suspected tertiary hypothyroidism showed no HGH increase with insulin hypoglycemia, while plasma cortisol showed normal increase. These results may indicate that these patients had deficiency of TRF and presumably growth-hormone releasing factor, because these two releasing factors were supposed to exist about the same area of hypothalamus or the order of deficiency of pituitary hormones.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. N. Hiroshige, and Mr. K. Kanao, Sumitomo Hospital, Osaka for excellent technical assistance.

REFERENCES

1. Bowers, C. Y., Schally, A. V., Reynold, G. A. and Hawley, W. D. Endocrinol. 1967. 81. 741/747. Interaction of 1-thyroxine or 1-triiodothyronine and thyrotropin-releasing factor on the release and synthesis of thyrotropin from the anterior pituitary gland of mice.
2. Burgus, R., Dunn, T. F., Desiderio, D. and Guillemin, R. C. R. Acad. Sc. Paris. 1969. 269. 1870/1873. Structure moléculaire du facteur hypothalamique hypophysiotrope TRF d' origine ovine: Mise en évidence par spetrométrie de masse de la séquence PCA-His-Pro-NH_2.
3. D'Angelo, S. A. J. Endocrinol. 1958. 17. 286/299. Role of the hypothalamus in pituitary thyroid interplay.
4. DeMoor, P., Steeno, O., Raskin, M. and Hendrikx, A. Acta Endocrinol. (Kbh.) 1960. 33. 297/307. Fluorimetric determination of free plasma 11-hydroxycorticosteroids in man.
5. Folkers, K., Enzmann, F., Boler, J., Bowers, C. Y. and Schally, A. V. Biochem. Biophys. Res. Commun. 1969. 37. 123/126. Discovery of modification of the synthetic tripeptide-sequence of the thyrotropin releasing hormone having activity.
6. Gillessen, D., Felix, A. M., Largier, W. and Studer, R. O. Helv. Chim. Acta 1970. 53. 63/72. Synthese des "Thyrotropin-releasing" Hormone (TRH) (Schaf) und verwandeter Peptide.
7. Haigler, E. D. Jr., Pittman, J. A. Jr., Hershman, J. M. and Baugh, C. M. J. Clin. Endocrinol. Metab. 1971. 33. 573/581. Direct evaluation of pituitary thyrotropin reserve utilizing synthetic thyrotropin releasing hormone.
8. Hall, R., Ormston, B. J., Besser, G. M. and Cryer, R. J. Lancet 1972. 1. 759/763. The thyrotropin-releasing hormone test in diseases of the pituitary and hypothalamus.
9. Karlberg, R., Almqvit, S. and Werner, S. Acta Endocrinol. (Kbh.) 1971. 67. 288/302. Effect of synthetic pyroglutamyl-histidyl proline amide on serum levels of thyrotrophin, cortisol, growth hormone, insulin and PBI in normalsubjects and patients with pituitary and thyroid disorders.
10. Martin, J. B., Boshans, R. and Reichlin, S. Endocrinol. 1970. 87. 1032/1040. Feedback regulation of TSH secretion in rats with hypothalamic lesions.
11. Mittler, J. C., Redding, T. W. and Schally, A. V. Proc. Soc. Exp. Biol. Med. 1969. 130. 406/409. Stimulation of thyrotropin (TSH) secretion by TSH-releasing factor (TRF) in organ cultures of anterior pituitary.
12. Ormston, B. J., Garry, R., Cryer, R. J. and Besser, G. M. Lancet 1971. 2. 10/14. Thyrotropin releasing hormone as a thyroid function test.
13. Otsuki, M. Folia Endocrinol. Jap. 1971. 47. 301/321. Effect of synthetic thyrotropin-releasing factor (TRF) on pituitary TSH secretion in man with special reference to the evaluation for TRF test (in Japanese).
14. Otsuki, M. and Baba, S. Kobe J. Med. Sci. 1972. 18. 215/228. Effect of synthetic thyrotropin-releasing factor (TRF) on pituitary TSH secretion in man, with special reference to the the evaluation for TRF test.
15. Otsuki, M., Mori, H., Baba, S. and Hiroshige, N. Acta Endocrinol. (Kbh.) 1973. 73. 233/249. Effect of synthetic thyrotropin-releasing factor on pituitary TSH secretion in normal subjects and patients with hypothalamic-pituitary disorders.
16. Otsuki, M. and Baba, S. J. Clin. Endocrinol. Metab. 1973. 36. 95/102. Influence of glucocorticoids on TRF-induced TSH response in man.

17. Schalch, D. S. and Parker, M. L. Nature 1964. 203. 1141/1142. A sensitive double antibody immunoassy for human growth hormone in plasma.
18. Snyder, P. J. and Utiger, R. D. J. Clin. Endocrinol. Metab. 1972. 34. 380/385. Response to thyrotropin releasing Hormone (TRH) in normal man.
19. Wilber, J. F. Endocrinol. 1971. 89. 873/877. Stimulation of ^{14}C-glucosamine and ^{14}C-alanine incorporation into thyrotropin by synthetic thyrotropin-releasing hormone.