TRF Test in Patients with Hypothalamic-Pituitary Disorders

MAKOTO OTSUKI, MAKOTO TATEIWA AND SHIGEAKI BABA

*The Second Department of Internal Medicine,
Kobe University School of Medicine, Kobe*

Synopsis

TRF test with 50–100 μ g of synthetic TRF was performed in 57 patients with hypothalamic-pituitary disorders. According to the degree and the pattern of TSH response, they were classified as an absent, subnormal, normal or supernormal response and/or delayed pattern of TSH response. 9 of 21 patients with pituitary chromophobe adenoma showed an absent or a subnormal response. Six of them were hypothyroid. 21 of 23 patients with hypothalamic lesions had normal or supernormal TSH response. 5 with a normal response and all of the six with a supernormal response showed clinical or biochemical evidence of hypothyroidism. Since the pituitary of these cases remained intact from tumor invasion, they should be postulated as hypothalamic hypothyroidism. A delayed pattern of TSH response to TRF, where the peak TSH response occurred after 20 min, was observed in 17 of 23 in hypothalamic lesions. 6 of 7 patients with diabetes insipidus showed just the same pattern of TSH response as observed in the patients with determined hypothalamic lesions. This delayed pattern of response and normal or supernormal response to a small dose of TRF, such as 50–100 μ g, could be used to distinguish hypothalamic lesions from pituitary diseases.

Administration of synthetic TRF has been recently introduced as a test of hypothalamic pituitary thyroid function (Bowers *et al.*, 1970; Burgus *et al.*, 1969; Folkers *et al.*, 1969; Hall *et al.*, 1970, 1972; Hershman *et al.*, 1970; Fleisher *et al.*, 1970; Karberg *et al.*, 1971; Otsuki, 1971; Otsuki *et al.*, 1972, 1973a, b; Saito *et al.*, 1971; Sakoda *et al.*, 1970). In normal euthyroid subjects, intravenous administration of synthetic TRF causes a consistent rise of plasma TSH levels. We reported on the standardization of TRF test and the normal range of TSH response to a standard intravenous dose of TRF and its application to the diagnosis of hypothalamic pituitary disorders (Otsuki, 1971; Otsuki *et al.*, 1973a, b). There are only several reports on 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.

Materials and Methods

1) Thyrotropin Releasing Factor (TRF)

Thyrotropin releasing factor used in this study was synthesized following the method of Gillissen *et al.* (1970) 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 were fasted overnight and remained seated throughout the TRF test which was performed early in the morning. Venous blood was withdrawn at zero time for the estimation of TSH and routine thyroid function tests, and 50 or 100 μ g of TRF 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. A highly purified human TSH and anti-human TSH serum provided from National Pituitary Agency and Human Thyrotropin Research Standard A from National Institute for Medical Research were used. The minimum detectable level of TSH was approximately 0.7–1.7 μ U/ml in our laboratory.

Routine thyroid function tests included the serum protein bound iodine (PBI; Auto Analyzer methods), 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 hr and basal metabolic rate.

Plasma GHG was measured by radioimmunoassay using the double antibody technique after Schalch and Parker (1964), serum cortisol by a fluorometric modification according to DeMoor *et al.* (1960).

4) Clinical Subjects

Twenty-two males and 22 females between sixteen and 37 yr of age agreed to act as controls. To determine the effect of age, 50 and 400 μ g of TRF were also given to 12 males and 8 females over 70 yr of age. The volunteers were euthyroid with normal circulating levels of thyroid hormone. Subsequent volunteers were informed of the purpose of this study and of the experiences of those who had previously received synthetic TRF. After obtaining consent, the TRF test was performed. Fifty-seven patients with

hypothalamic-pituitary disorders were given TRF in doses of 50–100 μ g. These patients were divided into 4 groups as shown in Table 1.

Results

1) Control Subjects

Seven doses of TRF (25, 50, 100, 200, 400, 800 and 1000 μ g) were administered to 44 euthyroid volunteers. The mean maximum TSH increases were linearly related to the log of the dose of TRF between 50–400 μ g (Otsuki *et al.*, 1972; 1973a, b). To determine the effect of age, 50 and 400 μ g of TRF were administered to 20 subjects ranging from 70 to 88 yr old. The response to 50 μ g of TRF in the aged subjects was similar to that in the young, while the response of 400 μ g of TRF in the aged was smaller than those in the young (Table 2).

We used 50 or 100 μ g of TRF as a test dose for pituitary TSH reserve according to the results reported previously (Otsuki, 1971; Otsuki *et al.*, 1972, 1973a, b). In normal subjects a detectable rise of plasma TSH was observed within 5 min and a further rise followed over the next 15 min. Peak level occurred at 10 to 20 min after 50–100 μ g of TRF.

Table 1. Patients with hypothalamic-pituitary disorders

	Number of patients	
Group 1.		
Pituitary chromophobe adenoma*	21	
Group 2.		
Acromegaly	6	
Group 3.		
Lesions of the hypothalamus	23	
Craniopharyngioma*		15
Pinealoma		5
Meningioma of the region of the optic chiasma*		2
Tumor of the floor of the third ventricle		1
Group 4.		
Diabetes insipidus**	7	
Total	57	

* Diagnosis was ascertained by operation.

** Clinically diagnosed as diabetes insipidus except nephrogenic diabetes insipidus.

Table 2. Plasma TSH response of TRF in normal subjects

Dose of TRF	Number of subjects	Age (yr)	Time after TRF injection (min)							Maximum Δ TSH
			0	10	20	30	60	90	120	
25 μ g	4 M.	2	<1.7*	4.2	3.8	3.5	2.1	1.7	1.7	8.8
	F. 2	22-30		± 1.2	± 1.5	± 1.7	± 0.4			± 1.9
50 μ g	10 M.	5	<1.7 — 3.6*	6.9	8.0	6.7	4.4	3.2	3.0	7.9
	F. 5	22-37		± 0.8	± 0.8	± 0.5	± 0.4	± 0.3	± 0.5	± 0.9
50 μ g	10 M.	5	<1.7 — 5.2*	5.7	8.0	7.1	5.4			7.0
	F. 5	71-88		± 1.2	± 1.7	± 0.8	± 0.8			± 0.8
100 μ g	10 M.	5	<1.7 — 2.6*	10.7	13.1	12.2	8.6	3.3	2.2	13.0
	F. 5	18-28		± 1.7	± 1.7	± 1.4	± 1.7	± 0.5	± 0.7	± 1.7
200 μ g	6 M.	3	<1.7 — 2.3*	14.4	16.1	17.1	11.3	6.3	4.5	17.8
	F. 3	16-38		± 3.4	± 2.9	± 3.3	± 3.8	± 2.3	± 3.8	± 2.3
400 μ g	5 M.	2	<1.7 — 3.6*	14.8	22.0	23.8	12.7	9.0	7.7	22.9
	F. 3	19-34		± 3.1	± 5.3	± 6.1	± 4.5	± 4.3	± 3.4	± 5.3
400 μ g	10 M.	5	<1.7 — 5.3*	14.8 $\pm$ 2.5**		18.5	12.8			15.1
	F. 5	70-80				± 3.2	± 3.0			± 2.8
800 μ g	6 M.	3	<1.7 — 2.0**	11.1	20.6	22.8	13.6	9.8	7.3	23.1
	F. 3	19-28		± 3.5	± 4.2	± 4.0	± 1.3	± 1.4	± 2.4	± 2.7
1000 μ g***	3 M.	2	<1.7 — 2.6*	13.0	19.0	20.1	18.8	14.5	10.3	
	F. 1	24-29								

TSH values in μ U/ml (mean $\pm$ SE). The minimum detectable value is 1.7 μ U/ml.

* Basal values of plasma TSH are represented as the ranges in euthyroid subjects receiving TRF.

** 15 min after TRF injection.

*** The plasma TSH values are represented as the mean.

The maximum value of Δ TSH response of 50 μ g TRF was not different between males and females: the maximum levels of Δ TSH were between 4.2 and 10.8 μ U/ml in males and 4.3 and 9.3 μ U/ml in females. However, females showed a greater maximum Δ TSH increases than males to 100 μ g of TRF: the maximum value of Δ TSH in males was between 4.9 and 9.5 μ U/ml, while in females 8.7–21.4 μ U/ml.

Table 3. Definition of response interms of w TSH.

	Dose of TRF	
	50 μ g	100 μ g*
Absent	< 2.0	< 2.0
Subnormal	2.0 — 4.0	2.0 — 4.0
Normal	4.0 — 11.0	4.0 — 22.0
Supernormal	> 11.0	> 22.0

TSH values (μ U/ml) were represented as the increments above basal level.

* Although the maximum Δ TSH increases were different between males and females, the same criteria were employed because of the small number of the control subjects.

2) 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 shown in Table 3: (i) absent, (ii) subnormal, (iii) normal and (iv) supernormal.

As mentioned above, in all normal subjects the peak TSH level occurred at 10 to 20 min, cases of which the peak TSH values were obtained later than 20 min were defined as a delayed response.

3) Patients

The results of the TRF test were summarised in Table 4.

Group 1. (21 patients with pituitary chromophobe adenoma)

12 patients responded normally and 4 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. 5 patients with a normal response and 3 with a subnormal response showed a delayed rise

Table 4. TSH response to TRF test

	Absent	Sub-normal	Normal	Super-normal
Group 1				
Pituitary chromophobe adenoma				
Treated*	5(0)	4(3)	8(40)	
Untreated	0	0	3(1)	
Group 2				
Acromegaly	0	1(1)	5(4)	
Group 3				
Lesions of the hypothalamus				
Treated**	2(0)†	0	10(8)	4(4)
Untreated	0	0	5(3)	2(2)
Group 4				
Diabetes insipidus	0	1(0)	2(2)	4(4)

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

* They underwent an operation of craniotomy.

** 13 with craniopharyngioma, 3 with pinealoma and each one with meningioma of the region of optic chiasma and tumor of the floor of the third ventricle underwent an operation of craniotomy.

† These two patients had a long history, more than 10 years, and underwent an operation of intradural craniotomy twice and were irradiated with the telecobalt.

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 hypothyroid. 5 of 6 acromegalic patients showed a delayed TSH response.

Group 3. (23 patients with hypothalamic lesions)

Of the 15 craniopharyngioma 11 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 twice undergone an operation of intradural craniotomy and was irradiated with use of the telecobalt, it was supposed that not only hypothalamus but also pituitary was destroyed in these patients. Of the other 8 with hypothalamic lesions, 4 responded normally and 4 showed a supernormal response of 50 μ g TRF. All of the six patients with a supernormal response were hypothyroid, however, they showed a normal response to exogenous TSH (Thyropar; Armour, Ltd.) (Table 5).

Table 5. Hypothalamic-pituitary thyroid function

Name	Age (yrs)	Sex	Clinical Diagnosis	TSH (μ U/ml) response to TRF				
				0.	10' (15')	20'	30'	60'
K. Y.	6	M.	Craniopharyngioma**	1.7	—	—	29.6	—
I. N.†	37	M.	Craniopharyngioma**	1.7	22.8	20.1	25.1	23.1
K. K.	26	M.	Pinealoma**	1.7	18.5		18.5	12.5
K. H.	16	M.	Pinealoma***	2.3	13.2	23.7	28.1	23.1
K. O.	50	F.	Meningioma of optic chiasma**	1.7	12.8	14.2	22.2	15.2
T. T.	36	F.	Tumor of the floor of the third ventricle***	3.6	34.7		52.8	27.4

The TRF dose was 50 μ g iv single injection in all cases except one (†) who received 100 μ g.

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

** Diagnosis was ascertained by operation.

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

One responded subnormally, 2 showed a normal response and 4 showed a supernormal response. Each one with subnormal and normal response had biochemical evidence of hypothyroidism. 4 with a supernormal response were euthyroid. In 6 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 (Bowers *et al.*, 1967; Mittler *et al.*, 1969; Wilber, 1971). Bowers *et al.* (1967) reported that a small amount of TRF depleted the stores of pituitary TSH, while a larger amount of TRF did not change the pituitary TSH contents and concluded that only a larger amount of TRF could stimulate both TSH synthesis and release. From these observations, it may be impossible that the newly synthesized TSH is released within 60 min

(Bowers *et al.*, 1967; Wilber, 1971) after these small doses of TRF, such as 50 or 100 μ g. So that the magnitude of the changes in plasma TSH levels after iv administration of 50–100 μ g of synthetic TRF seems to reflect the reserve pituitary TSH contents. A log-linear dose related response was found within the range of 50 to 400 μ g of TRF given as a single iv injection (Otsuki *et al.*, 1972, 1973a, b). However, there was a wide individual variation in TSH response to TRF, especially to a large dose of TRF, as shown in Table 2. TSH responses of 400 μ g TRF in aged euthyroid subjects showed wider individual variations than in young subjects. As a whole, they had a reduced TSH response of 400 μ g TRF. However, the responses of 50 μ g TRF were just similar to those in the young. In utilizing TRF for pituitary TSH reserve test, we employed a relatively small dose of TRF such as 50–100 μ g (Otsuki, 1971; Otsuki *et al.*, 1972, 1973a, b). As the peak level occurred at 10 to 20 min after 50–100 μ g of TRF in all control subjects, we arbitrarily defined as a delayed type of TSH response when the peak response was observed at 30 min or after. This definition must be limited only when a

in patients with supernormal TSH response

BMR (%)	PBI (μ g/dl)	T ₃ RSU (%)	TSH test*				Insulin Hypoglycemia			
				0.	24°	48°		0.	30'	60'
−36	3.8	24.0					B. G.: HGH:	57 12.8	28 5.2	52 2.4
−36	3.1	25.1	T ₄ :	3.4	6.4	5.5	B. G.: HGH:	57 2.7	10 1.2	16 1.1
−25	1.8	22.6	PBI: T ₄ :	1.8 1.4	2.0 1.7	2.2 2.8	B. G.: HGH: C. S.:	84 0.6 2.2	32 0.5 5.6	75 0.7 5.6
−20	2.6	20.4	PBI: T ₄ :	2.6 4.0	5.5 8.5	7.8 11.9	B. G.: HGH:	60 6.1	26 —	34 7.0
−22	4.4	22.2	PBI: T ₄ :	4.4 6.4	8.2 12.1	8.8 13.7	B. G.: HGH: C. S.:	96 1.1 14.0	42 1.0 12.0	70 6.0 35.0
−21	4.5(T ₄)	25.3	T ₄ :	4.5	5.7	5.7	B. G.: HGH:	96 5.2	44 3.8	82 5.1

*** Diagnosis was confirmed by PVG.

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

small amount of TRF (100 μ g or less) was used, because, as shown in Table 2, a prolonged period was required for the maximum TSH response when larger doses of TRF (200 μ g or more) were administered.

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 thyrotroph cells; a decrease of the sensitivity to TRF due to long-standing deficiency of the endogenous TRF, or the existence of too many free receptor sites to TRF because of lack of endogenous TRF, or a reduction of the effective receptor sites to TRF (Grant *et al.*, 1972; Wilber & Seibel, 1972). Recent studies in rats revealed that hypothalamic lesions resulted in decreases in serum thyroxine, PBI and TSH levels, but that the thyrotroph cells did not atrophy nor did the TSH concentration in the pituitary gland decrease (D'Angelo, 1958; Martin *et al.*, 1970). These animals with hypothalamic lesions responded to goitrogen administration (D'Angelo 1958) or to thyroidectomy (Martin *et al.*, 1970) with a definite but blunted rise in serum TSH. This indicates some autonomy or regulation of thyrotroph function by factors other than TRF and suggests that these cells do not atrophy because of loss of hypothalamic stimulation. From these observations, it seems to be quite natural that the patients with hypothalamic lesions responded to a small dose of TRF which acts on the anterior pituitary gland directly. 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. However, some patients with an incomplete defect of pituitary thyrotrophs could have undetectable baseline TSH levels but still show a normal TSH response to a large pharmacological dose of TRF as we showed previously (Otsuki *et al.*, 1972, 1973b). Hence TRF test with a small dose, such as 50–100 μ g, can be a sensitive test of impaired TSH reserve and can make a difference between pituitary diseases and hypothalamic lesions.

Although we used a small dose of TRF, half of the patients with pituitary chromophobe adenoma responded normally. The results showed that the pituitary had a great TSH reserve and that its destruction was not "all or none".

In acromegaly, TSH reserve was almost normal; 5 of 6 showed a normal response. Hall *et al.* (1972) 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 acromegaly may be accompanied by impaired TSH reserve (Hall *et al.*, 1972).

In patients with hypothalamic lesions without major pituitary lesions, a normal TSH response to TRF in 15 patients and a supernormal response in 6 were observed. Moreover, 17 of 21 showed the delayed pattern of TSH response. Although six patients with supernormal TSH response and 5 with normal response had clinical and biochemical evidence of hypothyroidism, as shown in Table 5, baseline plasma TSH levels were low, thyroid autoantibody was negative and responded normally to exogenous TSH. From these results, the existence of both hypothalamic lesions and primary hypothyroidism in the same subjects could be denied. Hence the hypothyroidism observed in these patients was due to lack of effective endogenous TRF secretion. These patients with hypothalamic lesions had a normal TSH concentration in the pituitary gland in spite of the deficiency of endogenous TRF (D'Angelo, 1958; Martin *et al.*, 1970), which resulted in a supernormal TSH response induced by a small dose of exogenous TRF. From these findings it seems to be possible to postulate for the criteria of hypothalamic (tertiary) hypothyroidism: i) the existence of clinical and biochemical evidence of hypothyroidism, 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 "small" dose of TRF, such as 50 μ g.

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. This pattern of TSH response was just the same as that seen in the patients with determined hypothalamic lesions. According to the pattern of TSH response, 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 before coming to decision.

We described the usefulness of TRF test with a small dose 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 patients with pituitary diseases who showed an absent or a subnormal response. However, the delayed pattern of TSH response was also found in 8 of 21 with pituitary chromophobe adenoma. 7 of 8 who showed the delayed pattern of response had been subjected to local treatment, whereas only one with untreated pituitary disease had a delayed response. This difference may indicate hypothalamic dysfunction or vascular damage resulting from the local treatment.

References

- Bowers, C. Y., A. V. Schally, D. S. Schalch, C. Gual, A. J. Kastin and K. Folkers (1970). *Biochem. Biophys. Res. Comm.* **39**, 352.
- Bowers, C. Y., A. V. Schally, G. A. Reynolds, and W. D. Hawley (1967). *Endocrinology* **81**, 741.
- Burgus, R., T. F. Dunn, D. Desiderio, and R. Guillemin (1969). *C. R. Acad. Sci. (Paris)* **269**, 1870.
- D'Angelo, S. A. (1958). *J. Endocrinol.* **17**, 286.
- DeMoor, P., O. Steeno, M. Raskin, and A. Hendrikx (1960). *Acta Endocrinol. (Kbh)* **33**, 297.
- Fleischer, N., R. Burgus, W. Vale, T. F. Dunn, and R. Guillemin (1970). *J. Clin. Endocrinol.* **31**, 109.
- Folkers, K., F. Enzmann, J. Bller, C. Y. Bowers, and A. V. Schally (1969). *Biochem. Biophys. Res. Comm.* **37**, 123.
- Gillessen, D., A. M. Felix, W. Largier, and R. O. Studer (1970). *Helv. Chim. Acta* **53**, 63.
- Grant, G., W. Vale, and R. Guillemin (1972). IV International Congress of Endocrinology (Abstracts) 84.
- Hall, R., J. Amos, R. Garry, and R. L. Buxton (1970). *Brit. Med. J.* **2**, 274.
- Hall, R., B. J. Ormston, G. M. Besser, and R. J. Cryer (1972). *Lancet* **1**, 759.
- Hershman, J. M., and J. A. Pittman, Jr. (1970). *J. Clin. Endocrinol.* **31**, 457.
- Karlberg, R., S. Almqvit, and S. Werner (1971). *Acta Endocrinol. (Kbh.)* **67**, 288.
- Martin, J. B., R. Boshans, and S. Reichlin (1970). *Endocrinology* **87**, 1032.
- Mittler, J. C., T. W. Redding, and A. V. Schally (1969). *Proc. Soc. Exp. Biol. Med.* **130**, 406.
- Ormston, B. J., R. Garry, R. J. Cryer, and G. M. Besser (1971). *Lancet* **2**, 10.
- Otsuki, M. (1971). *Folia Endocrinol. Japon.* **47**, 301 (In Japanese).
- Otsuki, M., M. Sakoda, and S. Baba (1972). *Kobe J. Med. Sci.* **18**, 215.
- Otsuki, M., and S. Baba (1973a). *J. Clin. Endocrinol.* **36**, 95.
- Otsuki, M., H. Mori, S. Baba, and N. Hiroshige (1973b). *Acta Endocrinol. (Kbh.)* (in press)
- Saito, S., K. Abe, H. Yoshida, T. Kaneko, E. Nakamura, N. Shimizu, and N. Yanaihara (1971). *Endocrinol. Japon.* **18**, 101.
- Sakoda, M., M. Otsuki, N. Hiroshige, K. Kanao, A. Yagi, and M. Honda (1970). *Ibid.* **17**, 541.
- Schalch, D. S., and M. L. Parker (1964). *Nature* **203**, 1142.
- Wilber, J. F. (1971). *Endocrinology* **89**, 873.
- Wilber, J. F., and M. J. Seibel (1972). IV International Congress of Endocrinology (Abstracts) 85.