Relation Between Pituitary Responsiveness to Thyrotropin Releasing Hormone and Serum Thyroid Hormones in Hyperthyroidism

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Synopsis

Seventy-five TRH tests were carried out in 61 subjects with hyperthyroidism before and in the course of treatment; the results were correlated with serum T_4 and T_3 concentrations. In certain subjects, T_3 suppression tests were done along with the TRH test. All subjects with elevated T_4 and T_3 (group 1) did not show a TSH response to TRH. Eleven out of 13 subjects with normal T_4 and elevated T_3 (group 2) did not show TSH response. In 22 subjects with normal T_4 and T_3 (group 3), 40.9% (9/22) did respond and 59.1% (13/22) did not respond to TRH. In 7 subjects with low T_4 and/or T_3 (group 4), 4 did not respond and 3 did respond to TRH. In each group, there were no significant differences in age and sex of the subjects, mode of therapy, serum thyroid hormones (T_4 , T_3) and BMR between responders and non-responders to TRH. Sixteen subjects underwent T_3 suppression tests. Twelve subjects with no or low TSH response to TRH did not show suppression, while all 4 subjects with normal or exaggerated TSH response showed suppression. The results indicate that serum thyroid hormone levels are not the sole determinant of the TSH response to TRH and confirm that the suppressibility of the thyroid gland by T_3 appears to require the presence of sufficient TSH secretion from the pituitary. Several mechanisms of variable TSH response to TRH in the course of treatment of hyperthyroidism were discussed.

Serum thyrotropic hormone (TSH) concentration rises after the administration of thyrotropin-releasing hormone (TRH) to hypothyroid and euthyroid subjects. In contrast, patients with untreated hyperthyroidism do not show a TSH response to TRH, presumably resulting from suppression of the pituitary thyrotropic cells by increased thyroid hormones in blood (Haigler *et al.*, 1971; Ormston *et al.*, 1971).

The TSH response to TRH in patients rendered clinically euthyroid by therapy remains controversial. Shenkman *et al.* and Ormston *et al.* ascribed failure of TSH response to TRH in certain subjects to elevated serum triiodothyronine (T_3) (Shenkman *et*

al., 1973; Ormston *et al.*, 1971). Two patients described by Lawton *et al.* showed lack of TSH response to TRH together with non-suppressibility of thyroid function after T_3 in the presence of normal serum total thyroxine (T_4), free T_4 , and total T_3 concentration. (Lawton *et al.*, 1971) One subject reported by Hershman *et al.* showed a small TSH response to TRH in the face of elevated serum T_4 . (Hershman and Pittman, 1970).

In the present report, the incidence of TSH unresponsiveness to TRH was sought in a larger number of subjects with treated and untreated hyperthyroidism and the relation between TSH and serum thyroid hormones was investigated. Results of T_3 suppression tests obtained in certain subjects were correlated with those of TSH response to TRH as well.

Materials and Methods

Sixty-one subjects were included in the study. The diagnosis of diffuse toxic goiter was made by clinical criteria. Patients were thyrotoxic, under partial control, euthyroid or hypothyroid at the time of study. Five subjects were diagnosed as "euthyroid Graves' disease" at the time of TRH tests. Their clinical data are given in the footnotes of Tables 2 and 3. In 12 subjects, TSH response to TRH was estimated more than once during the course of the treatment: 10 subjects were tested twice, one subject 4 times, one subject 6 times. After blood sample was drawn, 500 μ g (or 2,000 μ g when specified) of TRH was administered intravenously. Three additional blood samples were drawn after 30, 60 and 120 min. Serum TSH was measured by double antibody radioimmunoassay modified to retain high sensitivity as described by Patel *et al.* (Patel *et al.*, 1971). The assay was sensitive enough to detect TSH as low as 0.5 μ U/ml and to distinguish difference of 0.5 μ U in the range between 0.5 and 10 μ U/ml. Serum T₄ was measured by competitive protein binding assay, using Res-o-mat T₄ kit (Mallinckrodt Co.). The results were expressed as T₄-iodine (T₄-I) μ g/100 ml. Serum T₃ was measured by radioimmunoassay described by Chopra *et al.* (Chopra *et al.*, 1972). The antibody against T₃ was raised in rabbit according to Gharib *et al.* (Gharib *et al.*, 1971). The crossreactivity with T₄ of the antibody was 0.01%. T₃ resin sponge uptake (RT₃U) was measured using Triosorb test kit (Dainabot Laboratory). Free thyroxine index (FT₄ index) was calculated according to the formula described by Hamada *et al.*, i.e.,

$$\text{FT}_4 \text{ index} = \frac{\text{T}_4\text{-I} \times \text{RT}_3\text{U}}{1 - 0.6 \times \text{RT}_3\text{U}}$$

(Hamada *et al.*, 1970)

T₃ suppression tests were performed as described by Werner *et al.* (Werner and Spooner, 1955). Lack of suppression after administration of 75 μ g T₃ for 8 days was defined as uptake more than 36% of the initial uptake.

Results

Seventy-five TRH tests with 500 μ g TRH were carried out in 61 subjects. They were divided into 4 groups according to serum T₄ and T₃ levels at the time of TRH tests. Group 1 consisted of 33 subjects, whose serum T₄ and T₃ were elevated; Group 2, 13 subjects

with normal T₄ and elevated T₃; Group 3, 22 subjects with normal T₄ and T₃; Group 4, 7 subjects with reduced T₄ and/or T₃. Herein, normal ranges of T₄, T₃ and RT₃U were defined as 3.0–7.2 μ g/100 ml (T₄-I), 90–190 ng/100 ml (T₃) and 26–39% (RT₃U), based on tests of 40 euthyroid hospital employees. Serum TSH at 30 min after 500 μ g TRH i.v. administration ranges between 10 and 40 μ U/ml in normal subjects (Kumahara *et al.*, 1971). When more than one TRH test was carried out in a single subject in the course of therapy, results of the first TRH test were cited to avoid repetition of the same case in one group.

All subjects in group 1 failed to respond to TRH. Eleven out of 13 subjects in group 2 failed to respond to TRH while two showed normal response (Table 1). In group 3, variable TSH responses to TRH were observed: 13 subjects showed low or no response and 9 normal or supranormal responses (Table 2). When comparisons were made of age, T₄, FT₄-index, T₃, BMR and modes of therapy between responders and non-responders, no differences were observed. Variable TSH response to TRH was noted in group 4 as well: 4 subjects showed no response and 3 supernormal responses. Here again, no obvious differences were observed in serum thyroid hormones (Table 3). Without exception, subjects who failed to respond to TRH always had low baseline TSH, i.e., less than 0.5 μ U/ml. As illustrated in typical cases, variable responses were observed during the course of therapy when studied repeatedly (Figs. 1 and 2).

No response or subnormal response was noted during thyrotoxic and euthyroid phases. During the hypothyroid phase, however, the responses were supranormal. Five subjects were challenged with 2,000 μ g as well as 500 μ g TRH. TSH responses to 2,000 μ g TRH were no greater than those after 500 μ g TRH (Table 4). Two subjects in group 2 underwent T₃ suppression tests. All failed to show suppression (Table 1). Twelve subjects in group

Table 1. Clinical data and TSH response of subjects with normal T_4 and elevated T_3 (Group 2)

Subjects	Age	Sex	TSH-response to TRH (μ U/ml)		T_4 -I (μ g/100 ml)	RT_3 U (%)	FT_4 Index	T_3 (ng/100 ml)	BMR (%)	T_3 - suppres- sion test*	Therapy**
			0	30 min							
non or low responder											
I. I.	39	M	<0.5	<0.5	7.2	41	3.92	460	+19		MMI
Y. H.	37	F	<0.5	<0.5	5.6	38	2.76	330	+16		PTU
S. F.	35	F	<0.5	<0.5	5.7	31	2.17	275	+12		untreated
T. O.	21	F	<0.5	<0.5	5.5	33	2.26	263			MMI
U. T.	23	F	<0.5	<0.5	6.7	30	2.45	254	+33	—	untreated
K. K.	39	F	0.5	0.7	5.3	28	1.78	250	+31		untreated
H. S.	24	M	<0.5	<0.5	5.7	29	2.00	230	-13	—	MMI
M. F.	18	F	<0.5	<0.5	4.0	27	1.29	218	+9		MMI
A. Y.	28	F	<0.5	<0.5	5.0	25	1.47	200	+6		MMI
A. M.	18	F	<0.5	<0.5	3.6	29	1.26	195	+3		MMI
T. Ok	11	F	1.3	5.8	5.5	31	2.09	192	-9		MMI
Mean	31.6				5.76	30.8	2.22	276.5	+12.9		
$\pm$ SEM	± 4.0				± 0.33	± 1.2	± 0.20	± 22.9	± 4.6		
Normal responder											
Y. Y.	20	F	1.8	16.5	5.5	28	1.85	212	+14		Surgery
T. T.	23	F	1.8	12.0	6.5	31	2.48	203	+4		MMI
Mean	22				6.0	29.5	2.17	207.5	+9.0		

*—: non-suppressible

**MMI: 1-methyl-2-mercaptoimidazole

PTU : 6-propyl-2-thiouracil

3 had T_3 suppression tests. There was normal suppression in 4 subjects, whose TSH responses to TRH were either normal or supranormal. On the other hand, 8 subjects who failed to show suppression to T_3 had no or subnormal TSH response to TRH (Table 2).

Discussion

All subjects in group 1 showed no TSH response to TRH. Eleven out of 13 subjects in group 2 did not show TSH response to TRH. These results were comparable with others (Haigler *et al.*, 1971; Ormston *et al.*, 1971; Shenkman *et al.*, 1973). In contrast to previous reports, however, 2 subjects in group 2, with supranormal serum T_3 concentration showed TSH response to TRH and 59.1% (13/22 cases) of group 3 with normal serum

T_3 concentration did not show TSH response. Moreover, 4 out of 7 subjects with reduced T_4 and/or T_3 (group 4) did not show TSH response to TRH.

The present radioimmunoassay of serum TSH as described earlier was highly sensitive. Therefore, the difference between positive TSH response and lack of response represents suppression of pituitary TSH secretion and is not an artifact resulting from insensitive radioimmunoassay of TSH. It is unlikely that the pituitary of TRH unresponsive subjects is irreversibly altered. Subjects unresponsive to TRH at one time became capable of responding to a second TRH test when their serum T_4 and T_3 were further reduced. The destruction of TRH was accelerated when it was incubated in plasma obtained from hypophysectomized rats receiving T_3 (Redding and Schally, 1969). Lack of TSH re-

Table 2. Clinical data and TSH response of subjects with normal T₄ and T₃ (Group 3)

Subjects	Age	Sex	TSH response to TRH (μ U/ml)		T ₄ -I (μ g/100 ml)	RT ₃ U (%)	FT ₄ Index	T ₃ (ng/100 ml)	BMR (%)	T ₃ -suppression test ⁽¹⁾	Therapy ⁽²⁾
			0	30 min							
non or low responder											
K. N.	39	F	<0.5	1.6	6.5	38	3.20	190	+50		Untreated ⁽³⁾
M. K.	36	M	0.5	<0.5	5.0	32	1.98	190	-7	-	Untreated ⁽⁴⁾
K. M.	20	F	<0.5	<0.5	6.3	38	3.10	178	+12	-	Untreated ⁽⁵⁾
T. Og	24	M	<0.5	<0.5	5.4	30	1.98	135			MMI
K. Y.	27	F	<0.5	<0.5	6.1	26	1.88	170	+9	-	Surgery
T. F.	23	F	<0.5	<0.5	5.7	31	2.17	168	+5		PTU
Ky N.	40	F	<0.5	<0.5	4.6	30	1.68	155	+21		RI and MMI
M. D.	36	F	<0.5	0.6	5.9	25	1.74	146	+3	-	Surgery
Y. T.	16	M	0.7	0.7	5.7	30	2.09	127	+1	-	MMI
I. I.	39	M	<0.5	<0.5	3.0	27	0.97	123	+1	-	MMI
F. A.	25	F	0.7	2.1	5.9	30	2.16	141	+11	-	Surgery
Ka N.	36	F	0.8	6.8	4.3	27	1.39	122	+30	-	PTU
T. O.	21	F	1.2	6.3	4.6	24	1.29	130	+7		MMI
Mean	29.4				5.26	29.8	1.97	151.9	+11.9		
$\pm$ SEM	± 2.3				± 0.27	± 1.2	± 0.17	± 6.6	± 4.3		
normal or supranormal responder											
M. Y.	23	F	1.3	19	6.7	27	2.16	176	+5		MMI
M. N.	20	F	2.8	24	5.4	24	1.51	168	+9	+	Surgery
Ak M.	27	F	2.5	40	5.9	23	1.57	165	-10	+	Surgery
Y. S.	40	M	1.7	23	5.6	26	1.73	153	-5		Untreated ⁽⁶⁾
Y. M.	23	F	3.0	28	6.1	25	1.79	145	+14		Surgery
M. H.	24	M	2.2	27	4.2	35	1.86	186	-8		Surgery
N. A.	22	F	1.4	19	5.3	27	1.71	127	+6	+	Surgery
M. Ko	33	M	2.5	35	6.7	30	2.45	95			MMI
S. Y.	44	F	7.8	103	5.4	25	1.59	151	-9	+	MMI
Mean	27.4				5.52	26.3	1.73	149.1	+0.3		
$\pm$ SEM	± 2.6				± 0.28	± 1.2	± 0.12	± 8.2	± 2.74		

(1) +: suppressible —: non suppressible

(2) MMI: 1-methyl-2-mercaptoimidazole PTU: 6-propyl-2-thiouracil RI: radioiodine

(3) previously T₄ 8.3 μ g/100 ml; now clinically euthyroid without therapy(4) previously PBI 13.6 μ g/dl, RT₃U 43% BMR + 38%; now clinically euthyroid without therapy(5) exophthalmos, and non suppressible by T₃ suppression test

(6) exophthalmos, LATS (+), TRC (+) and histologically Graves' disease with Hashimoto's disease

sponse to TRH seen in the present study, however, is hardly explained by the accelerated destruction of injected TRH because of failure of an augmented dose (2,000 μ g) to elicit positive response.

Age and sex were similarly distributed in subjects with positive TSH response and those without the response in groups 2, 3 and 4.

The modes of therapy employed (medical, surgical and radio-thyroidectomy) were similar between TRH responders and non-responders. No significant differences in serum T₄, T₃, RT₃U, FT₄ index and BMR were observed between TRH responders and non-responders in groups 2, 3 and 4. As free T₄ fraction is normalized after resumption of

Table 3. Clinical data and TSH response of subjects with low T_4 and/or T_3 (Group 4)

Subjects	Age	Sex	TSH-response to TRH (μ U/ml)		T ₄ -I (μ g/100 ml)	RT ₃ U (%)	FT ₄ Index	T ₃ (ng/100 ml)	BMR (%)	Therapy*
			0	30 min						
non-responder										
F. Y.	24	F	<0.5	<0.5	2.0	26	0.62	107	+7	MMI
M.M.	53	M	<0.5	<0.5	1.4	23	0.37	98	+15	MMI
D. A.	47	M	<0.5	<0.5	3.4	39	1.73	86	-3	MMI
H. N.	28	M	<0.5	<0.5	6.9	33	2.84	84	+14	Untreated**
Mean	38				3.4	30.3	1.39	93.8	+8.3	
supranormal responder										
I. I.	39	M	31	84	1.1	23	0.29	95		MMI
K.N.	43	F	113	205	0.6	27	0.19	73		MMI
F. F.	62	F	110	174	0.6	23	0.16	50	-7	MMI
Mean	48				0.76	24.3	0.21	72.7		

* MMI: 1-methyl-2-mercaptoimidazole

PTU: 6-propyl-2-thiouracil

** Previously, non suppressible by T_3 suppression test, BMR + 34% T_4 -I 9.8 μ g/100 ml, RT_3U 33%; now clinically euthyroid without therapy

euthyroid state as shown by Braverman *et al.*, free T_4 or T_3 , even if measured, there seems to be no difference between TRH responders and non-responders in groups 3 and 4 (Braverman *et al.*, 1968).

The present study showed the following: (1) A few subjects showed TSH response to TRH while their serum T_3 was still elevated. (2) more than half of the patients whose serum T_4 and T_3 was normal or reduced, had no TSH response to TRH, (3) the difference of TSH response to TRH could not be ascribed to the differences in age, sex, mode of treatment, and usual clinical parameters of thyroid function. The cause of this variable responsiveness of TSH to TRH in the course of treatment for thyrotoxicosis is conceivable multifarious.

Firstly, it has been shown by Bowers *et al.* (Bowers *et al.*, 1967) that pituitary responsiveness to TRH is inhibited by thyroid hormones. This inhibition, however, could be overcome by employing an augmented dosage of TRH. In the present study, we failed to

elicit positive TRH response using 2,000 μ g TRH in subjects who did not show a TSH response to a smaller dose of TRH. Secondly, Wilber *et al.* showed that the inhibition of TRH stimulated TSH release in the presence of normal T_4 and T_3 levels obtained 48 hr after a single oral administration of thyroid hormone (Wilber *et al.*, 1972). We have found similar results 24 hr after a single intravenous injection of thyroid hormone (Azukizawa and Miyai, unpublished observation). Vagenakis *et al.* reported recently that there was pituitary unresponsiveness to TRH despite subnormal serum T_4 and T_3 concentrations for 2-5 weeks following prolonged suppressive thyroid therapy (Vagenakis *et al.*, 1973). In these three instances, plasma T_4 and/or T_3 binding proteins did not seem to be altered so as to make their free fractions increase or decrease.

Additionally, two out of 13 subjects in group 2 gave positive TSH response to TRH. On scrutiny, however, their serum T_3 was elevated but much less so than the rest of the group. Minimal elevation of serum T_3 might

Case K.N. 39F

Fig. 1.

Table 4. TSH response to 500 μ g and 2,000 μ g TRH administration

Subjects	Age	Sex	TRH (μ g)	TSH response to TRH (μ U/ml)			
				0	30	60	120 min
F. I.	26	M	500	<2	<2	<2	<2
			2,000	<2	<2	<2	<2
T. O.	21	F	500	<1	<1	<1	<1
			2,000	<1	<1	<1	<1
T. S.	36	M	500	<1	<1	<1	<1
			2,000	<1	<1	<1	<1
Ka N.	36	F	500	<1	6.8	4.0	<1
			2,000	<1	4.8	3.7	1.8
S. Y.	44	F	500	7.8	103	100	51
			2,000	1.1	58	82	88

Case I.I. 39M

Fig. 2.

Fig. 1 and 2. Results of repeated TRH tests in the course of treatment and relationship with other parameters of thyroid functions (case K. N. and I. I.).

not be sufficient to suppress TSH response to TRH in certain individuals. Three subjects in group 4 with low serum T_4 and T_3 failed to respond to TRH. This finding rather suggests that set points of negative feedback regulation of TSH by T_3 and/or T_4 vary in individuals. Alternatively, there seem to be a variable time lag between normalization of circulating thyroid hormones and the recovery of responsiveness of pituitary thyrotropic cells to TRH, although the evidence has not been obtained in the present study. Conceivably, a certain amount of time has to elapse before the recovery of TSH responsiveness to TRH; during this time the pituitary content of thyroid hormones may become depleted. Kumahara *et al.* reported reduced TSH content in the pituitary obtained from patients who died of thyrotoxic crisis (Kumahara *et al.*, 1967). These observations suggest that the

store of TSH in the pituitary is reduced in the subjects unresponsive to TRH administration. In the present study subjects who did not show TSH response to TRH had always very low baseline serum TSH concentration, i.e., less than $0.5 \mu\text{U/ml}$, while TRH responders had higher baseline serum TSH.

However, these low basal values of serum TSH concentration cannot be related directly to the reduction of the pituitary TSH content, because Wilber *et al.* reported transient discrepancy between serum TSH concentration and pituitary TSH content in rats treated with PTU (Wilber and Utiger, 1967).

Lastly, dissociation of thyroid hormones and their secretion rate is a possibility. Woeber *et al.* demonstrated that serum T_3 turnover remained accelerated in 4 subjects who were treated for hyperthyroidism (Woeber *et al.*, 1970). This suggests that the hyperthyroid

state may be manifested by accelerated turnover of T_3 in the face of normal serum T_4 and T_3 in certain subjects. In such subjects, however, TSH release from the pituitary may be inhibited.

All 10 subjects with low or no TSH response to TRH did not show suppression by T_3 , whereas all 4 subjects with normal or exaggerated TSH response to TRH showed normal suppression of function by administered T_3 . This result is comparable with those of 2 patients studied by Lawton *et al.* Positive suppression tests appear to require presence of sufficient TSH secretion from the pituitary.

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References

- Braverman, K. E., A. E. Foster and S. H. Ingbar (1968). *J. Clin. Invest.* **47**, 1349.
- Bowers, C. Y., A. V. Schally, G. A. Reynolds and W. D. Hawley (1967). *Endocrinology* **81**, 741.
- Chopra, I. J., R. S. Ho and R. Lam (1972). *J. Lab. Clin. Med.* **80**, 729.
- Gharib, H., R. J. Ryan, W. E. Mayberry and T. Hockert (1971). *J. Clin. Endocrinol. Metab.* **33**, 509.
- Haigler, E. D. Jr., J. A. Pittman Jr., J. M. Hershman and C. M. Baugh (1971). *J. Clin. Endocrinol. Metab.* **33**, 573.
- Hamada, S., T. Nakagawa, T. Mori and K. Torizuka (1970). *J. Clin. Endocrinol. Metab.* **31**, 166.
- Hershman, J. M. and J. A. Pittman Jr. (1970). *J. Clin. Endocrinol. Metab.* **31**, 457.
- Kumahara, Y., H. Iwatsubo, K. Miyai, H. Masui, M. Fukuchi and H. Abe (1967). *J. Clin. Endocrinol. Metab.* **27**, 333.
- Kumahara, Y., K. Miyai and M. Azukizawa (1971). *Med. J. Osaka Univ.* **22**, 97.
- Lawton, N. F., R. P. Ekins and J. D. N. Nabarro (1971). *Lancet*. **2**, 14.
- Ormston, B. J., R. Barry, R. J. Cryer, G. M. Besser and R. Hall (1971). *Lancet*. **2**, 10.
- Patel, Y. C., H. G. Burger and B. Hudson (1971). *J. Clin. Endocrinol. Metab.* **33**, 768.
- Redding, T. W. and A. V. Schally (1969). *Proc. Soc. Exp. Biol. Med.* **131**, 420.
- Shenkman, L., T. Mitsuma and C. S. Hollander (1973). *J. Clin. Invest.* **52**, 205.
- Vagenakis, A., Fi. Azizi, G. Portnay, J. DeRidder, L. Braverman and S. H. Ingbar (1973). *Abstract of the 49th Meeting, American Thyroid Association Inc.*
- Werner, S. C. and M. Spooner (1955). *Bull. N. Y. Acad. Med.* **31**, 137.
- Wilber, J. F., A. Jaffer, L. Jacobs, R. D. Utiger and N. Freinkel (1972). *Horm. Metab. Res.* **4**, 508.
- Wilber, J. F. and R. D. Utiger (1967). *Endocrinology* **81**, 145.
- Woeber, K. A., R. J. Sobel, S. H. Ingbar and K. Sterling (1970). *J. Clin. Invest.* **49**, 643.