Enhanced Plasma GH Responses to Simultaneous Administration of TRH and GHRH in Patients with Primary Hypothyroidism

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Abstract. The mechanism of aberrant GH responses to TRH was indirectly evaluated in 7 patients with primary hypothyroidism. All patients showed GH response to TRH. When TRH was administered together with GHRH, the plasma GH response was much greater than after a single administration of TRH or GHRH (TRH+GHRH vs. TRH or GHRH: max. Δ GH, 16.4 ± 3.2 vs. 5.4 ± 1.3 or 6.0 ± 0.8 $\mu\text{g/L}$; AUC, 1282.7 ± 234.7 vs. 384.0 ± 95.0 or 441.8 ± 66.2 , both $P < 0.01$). The combined administration of TRH and GHRH caused an additive, but not a synergistic, GH response. In contrast, 8 normal subjects showed neither any plasma GH response to TRH nor enhancement by TRH of GHRH-induced GH response following combined administration. It is concluded that the sites of action of TRH seemed to be different from GHRH in patients with primary hypothyroidism.

Key words: Plasma GH, TRH, GHRH, Primary hypothyroidism

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PARADOXICAL growth hormone (GH) increase following TRH injection is often observed in various kinds of disorders, e.g. patients with acromegaly, anorexia nervosa, chronic renal failure, chronic hepatic failure, depression, schizophrenia, and primary hypothyroidism [1]. Although acromegalic pituitary adenoma possesses TRH receptors, it is not clear whether TRH receptor expression occurs in other disorders since these aberrant GH responses disappear in patients with anorexia nervosa and depression after treatment [1, 2]. It is reported that TRH and GHRH stimulate GH secretion from the rat pituitary gland through different intracellular transduction mechanisms [3–5]. In this connection, we have observed a synergistic GH increase in patients with acromegaly after the simultaneous administration of TRH

and GHRH [6]. In rats, GH releasing activity of TRH is enhanced by GHRH pretreatment [7]. In this study we therefore examined plasma GH response to combined administration of TRH and GHRH in patients with primary hypothyroidism to indirectly evaluate the mechanism of action of TRH.

Materials and Methods

Seven untreated patients with primary hypothyroidism (2 males and 5 females, aged 23–72 yr), who had an aberrant GH response to TRH were studied (Table 1). As a control, 8 normal subjects (4 males and 4 females, aged 19–26 yr) were employed in this study. Informed consent was obtained from each subject.

Hypothyroid patients had decreased plasma thyroid hormone and increased basal plasma TSH, and exaggerated TSH responses to TRH (Table 1).

Among these patients, the titer of thyroglobulin autoantibody was high in 3 patients (# 5–7) and

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that of microsome autoantibody was high in 6 patients (# 1, 2, 4–7).

After an overnight fast, all subjects were given TRH (500 µg, iv) or GHRH (100 µg, iv) individually or in combination, and blood sampling was performed –30, 0 min before, and 15, 30, 45, 60, 90 and 120 min after the injection of the above agents. The AUC (area under the response curve) over the baseline was calculated by trapezoidal integration.

Plasma GH, PRL, TSH, T_3 , T_4 and free T_4 were measured in duplicate with a commercial RIA kit (GH: Dainabot, Tokyo, free T_4 : DPC, Tokyo), IRMA kit (PRL: Daiichi, Tokyo) and immunofluorometric assay kit (TSH, T_3 , T_4 : Pharmacia, Uppsala), respectively. The sensitivity to GH, PRL, TSH, T_3 , T_4 and free T_4 was 0.2 µg/L, 0.3 µg/L, 0.02 mU/L, 0.1 nmol/L, 2.6 nmol/L, and 0.1 pmol/L, and the intra- and interassay coefficients of variation were 4.1 and 4.7% for GH, 3.2 and 5.6% for PRL, 4.0 and 5.6% for TSH, 3.0 and 6.1% for T_4 , 2.9 and 6.7% for T_3 , and 5.5 and 7.6% for free T_4 , respectively. All samples from an individual subject were analyzed in the same assay.

Data are expressed as the mean ± SEM. Statistical analysis was carried out by analysis of variance followed by the Student/Newman-Keuls test and Fisher's randomization test.

Results

Plasma GH responses to TRH and GHRH individually or in the combination in 7 patients with primary hypothyroidism and in 8 normal subjects

The mean peak GH value after individual ad-

ministration of TRH or GHRH was much greater than the basal value (basal vs peak: TRH, 1.1 ± 0.5 vs. 6.6 ± 1.4 ; GHRH, 1.6 ± 0.7 vs. 7.7 ± 1.3 µg/L, both $P < 0.01$). When GHRH was administered together with TRH, the plasma GH response was much greater than the response after single administration in terms of the mean value at 60, 90, 120 min ($P < 0.05$ – 0.01) and the mean peak value (TRH + GHRH, 18.0 ± 3.7 µg/L; vs. TRH, $P < 0.01$, vs. GHRH, $P < 0.05$) (Fig. 1, left panel). The AUC for the combined administration of TRH and GHRH (1282.7 ± 234.7 µg/Lxmin) was also much greater than that for TRH (384.0 ± 95.0 , $P < 0.01$) or GHRH (441.8 ± 66.2 , $P < 0.01$) (Fig. 1), but we could not find a significant difference between GH response to the combined administration of TRH and GHRH and sum of the response to a single administration of TRH and GHRH (max. Δ GH: 16.4 ± 3.2 vs. 11.0 ± 1.7 µg/L; AUC: 1282.7 ± 234.7 vs. 825.8 ± 153.2 µg/Lxmin, both $P = \text{NS}$).

The eight normal subjects did not show any response of plasma GH to the single administration of TRH, and these subjects did not show any enhancement of the response to the combined administration of TRH and GHRH, while the response pattern was somewhat suppressed until 60 min and enhanced thereafter by the combined administration (Fig. 2).

The peak plasma GH response to GHRH of hypothyroid patients was much lower than that of normal subjects (32.2 ± 6.8 , $P < 0.01$).

Discussion

In this study, patients with primary hypothyroidism showed an impaired GH response to

Table 1. Clinical and laboratory data in 7 patients with primary hypothyroidism

Subjects	Age (yr)	Sex	T_3 (nmol/L)	T_4 (nmol/L)	free T_4 (pmol/L)	Responses to TRH			
						TSH (mU/L)		GH (µg/L)	
						Basal	Peak	Basal	Peak
1	23	F	0.69	15.4	1.3	805.2	2428.8	0.2	5.0
2	29	F	0.83	32.2	6.4	38.8	308.2	0.3	2.6
3	34	F	0.73	21.9	1.3	194.7	398.4	3.2	6.8
4	39	F	0.88	43.8	6.6	6.9	87.8	0.4	4.6
5	65	M	0.15	3.9	—	570.2	838.0	1.3	5.3
6	67	M	0.48	42.5	1.3	13.7	57.4	0.2	7.7
7	72	F	0.30	9.0	1.0	159.1	324.9	2.4	14.8

The normal range of T_4 , T_3 , free T_4 , basal and peak TSH to TRH and basal GH is 57.9 to 193.1 nmol/L, 0.92 to 2.77 nmol/L, 10.2 to 25.1 pmol/L, 0.3 to 3.0 mU/L, 8 to 27 mU/L and below 5 µg/L, respectively.

Fig. 1. Plasma GH response to TRH or GHRH individually and in combination in 7 patients with primary hypothyroidism. The AUC (area under the curve) over the baseline was calculated by trapezoidal integration. TRH + GHRH vs. TRH or GHRH, * $P < 0.05$; ** $P < 0.01$.

Fig. 2. Plasma GH response to TRH or GHRH individually or in combination in 8 normal subjects.

GHRH but aberrant plasma GH response to TRH, and this response was enhanced by the simultaneous administration of GHRH. However, normal subjects showed neither any GH response to TRH nor any enhanced response to the combined administration of TRH and GHRH.

It is well known that thyroid hormone stimulates the synthesis and secretion of GH and also increases GH gene transcription and GH mRNA

[8]. Hypothyroidism therefore depletes pituitary GH, inhibits GH secretion and causes severe growth retardation [8]. Nevertheless, patients with hypothyroidism often show abnormal GH responses to TRH as was seen in this study [1, 9, 10].

The exact mechanism of such aberrant GH responses to TRH is not clear, but it is reported that acromegalic patients, due to the ectopic GHRH secreting tumor, and who usually have pituitary hyperplasia, show a GH response to TRH, and such a response disappears after the removal of the ectopic tumor [11, 12]. It has also been demonstrated that acromegalic patients with constitutively activated adenylate cyclase-protein kinase A system by the mutation of stimulatory GTP (Gs) binding protein show exaggerated GH response to exogenous TRH [13]. In addition, TRH-induced GH release from rat pituitary cells is enhanced by GHRH during the perfusion experiments [7]. It is also reported that plasma GH response to TRH is more prominent in hypothyroid rats than in euthyroid rats, and that GHRH release from the hypothalamus *in vitro* is increased in hypothyroid rats [5, 8]. These phenomena suggest that the paradoxical GH responses to TRH are related to the exposure of pituitary somatotrophs to GHRH.

TRH stimulates GH release from the stored pool

mainly via the activation of the phosphatidyl inositol- Ca^{++} system [4, 5, 14]. It is therefore probable that significant amounts of GH are stored even in hypofunctional somatotrophs of patients with primary hypothyroidism. Relating to this, Valcavi *et al.* have observed that pyridostigmine (a cholinesterase inhibitor) or arginine, which stimulates GH secretion through the inhibition of hypothalamic somatostatin release, enhanced the plasma GH responses to GHRH in patients with primary hypothyroidism, and they have indicated the considerable somatotroph secretory capacity of these patients [15].

In contrast, GHRH stimulates the secretion of GH primarily from the newly synthesized pool mainly through activation of the stimulatory GTP binding (G_s) protein-adenylate cyclase system [14]. The blunted GH response to GHRH seen in hypothyroid patients might be closely related to disorders of hormone synthesis [8, 16, 17].

Taken together, it is conceivable that the enhanced GH responses to the simultaneous administration of TRH and GHRH are induced by the stimulation of the distinct intracellular transduction pathways. There may be no interaction between the pathways since the GH response to the combined administration was additive, but not synergistic.

In normal subjects, when GHRH was administered together with TRH, the plasma GH response was somewhat suppressed for 60 min and enhanced thereafter. Although the exact reason is not clear, it is possible that TRH exerts some inhibitory effects on GHRH-induced GH release, as GH responses to secretagogues were suppressed by TRH infusion [18, 19], and caused postinhibitory GH increases.

Further studies are required to determine, 1) if normal somatotrophs possess TRH receptors in a masked state, and 2) if such receptors are unmasked in the several pathologic states.

In conclusion, abnormal GH response to TRH seen in patients with primary hypothyroidism appears to act by means of a mechanism distinct from GHRH.

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References

1. Müller EE, Nistico G (1989) Thyrotropin-releasing hormone. In: Brain Messengers and The Pituitary. Academic Press, San Diego, pp 322–335.
2. Maeda K, Kato Y, Yamaguchi N, Chihara K, Ohgo S, Iwasaki Y, Yoshimoto Y, Moridera K, Kuromaru S, Imura H (1976) Growth hormone release following thyrotropin-releasing hormone injection into patients with anorexia nervosa. *Acta Endocrinol (Copenh)* 81: 1–8.
3. Brazeau P, Ling N, Esch F, Bohlen P, Mougin G, Guillemin R (1982) Somatocrinin (growth hormone releasing factor) *in vitro* activity; Ca^{2+} involvement, cAMP mediated action and additivity of effect with PGE_2 . *Biochem Biophys Res Commun* 109: 588–594.
4. Rebecchi MJ, Kolesnick RN, Gershengorn MC (1983) TRH stimulates rapid loss of phosphatidylinositol and its conversion to 1,2-diacyl-glycerol and phosphatidic acid in rat mammatropic pituitary cells. *J Biol Chem* 258: 227–234.
5. Szabo M (1986) TRH and GRF stimulate release of growth hormone through different mechanisms. *Am J Physiol* 250: E512–517.
6. Utsumi A, Hanew K, Sugawara A, Simizu Y, Murakami O, Yoshinaga K (1991) Plasma GH responses to combined administrations of hypothalamic hormones in patients with acromegaly. 73rd Annual Meeting of The Amer Endocrine Soc Abst. # 206.
7. Borges JLC, Uskavitch DR, Kaiser DL, Cronin MJ, Evans WS, Thorner MO (1983) Human pancreatic growth hormone releasing factor-40 (hpGRF-40) allows stimulation of GH release by TRH. *Endocrinology* 113: 1519–1521.
8. Miki N, Ono M, Hizuka N, Aoki T, Demura H (1992) Thyroid hormone modulation of the hypothalamic growth hormone (GH)-releasing factor-pituitary GH axis in the rat. *J Clin Invest* 90: 113–120.
9. Collu R, Leboeuf G, Letarte J, Ducharme J-R (1977) Increase in plasma growth hormone levels following thyrotropin-releasing hormone injection in

- children with primary hypothyroidism. *J Clin Endocrinol Metab* 44: 743–747.
10. Faggiano M, Criscuolo T, Graziani M, Iorio S, Pisano G, Sinisi AA, Bellastella A (1985) Persistent TRH-induced growth hormone release after short-term L-thyroxine replacement therapy in primary congenital hypothyroidism. *Clin Endocrinol (Oxf)* 23: 61–66.
 11. Thorner MO, Perryman RL, Cronin ML, Rogol AD, Draznin M, Johanson A, Vale W, Horvath E, Kovacs K (1982) Somatotroph hyperplasia: successful treatment of acromegaly by removal of a pancreatic islet tumor secreting a growth hormone-releasing factor. *J Clin Invest* 70: 965–977.
 12. Frohman LA, Thominet J, Szabo M, Berelowitz M (1984) Growth hormone hypersecretion, acromegaly, and extrapituitary tumors: Ectopic production of growth hormone releasing factor. In: Camanni F, Müller EE (eds) *Pituitary Hyperfunction*. Raven Press, New York, pp 343–353.
 13. Spada A, Arosio M, Bochicchio D, Bazzoni N, Valler L, Bassetti M, Faglia G (1990) Clinical, biochemical, and morphological correlates in patients bearing growth hormone-secreting pituitary tumors with or without constitutively active adenylate cyclase. *J Clin Endocrinol Metab* 71: 1421–1426.
 14. Stachura ME, Tyler JM, Farmer PK (1985) Human pancreatic growth hormone-releasing factor-44 differentially stimulates release of stored and newly synthesized rat growth hormone *in vitro*. *Endocrinology* 116: 698–706.
 15. Valcavi R, Valente F, Dieguez C, Zini M, Procopio M, Portoli I, Ghigo E (1993) Evidence against depletion of the growth hormone (GH)-releasable pool in human primary hypothyroidism: studies with GH-releasing hormone, pyridostigmine, and arginine. *J Clin Endocrinol Metab* 77: 616–620.
 16. Martial JA, Baxter JD, Goodman HM, Seeburg PH (1977) Regulation of growth hormone messenger RNA by thyroid and glucocorticoid hormones. *Proc Natl Acad Sci USA* 74: 1816–1820.
 17. Katakami H, Downs TR, Frohman LA (1986) Decreased hypothalamic growth hormone-releasing hormone content and pituitary responsiveness in hypothyroidism. *J Clin Invest* 77: 1704–1711.
 18. Maeda K, Kato Y, Chihara K, Ohgo S, Iwasaki Y, Imura H (1975) Suppression by thyrotropin-releasing hormone (TRH) of human growth hormone release induced by L-dopa. *J Clin Endocrinol Metab* 41: 408–411.
 19. Maeda K, Kato Y, Chihara K, Ohgo S, Iwasaki Y, Abe H, Imura H (1976) Suppression by thyrotropin-releasing hormone (TRH) of growth hormone release induced by arginine and insulin-induced hypoglycemia in man. *J Clin Endocrinol Metab* 43: 453–456.