Comparative Study on the Responses of Plasma GH to Synthetic GH-Releasing Factor and Other Stimulatory and Inhibitory Agents in Patients with Acromegaly

KUNIIHIKO HANEW, SHUICHI SATO, ATSUSHI SASAKI,
YASUYUKI SHIMIZU, OSAMU MURAKAMI and KAORU
YOSHINAGA

*The Second Department of Internal Medicine, Tohoku
University School of Medicine, Sendai 980*

HANEW, K., SATO, S., SASAKI, A., SHIMIZU, Y., MURAKAMI, O. and YOSHINAGA, K. *Comparative Study on the Responses of Plasma GH to Synthetic GH-Releasing Factor and Other Stimulatory and Inhibitory Agents in Patients with Acromegaly*. Tohoku J. exp. Med., 1985, **145** (2), 161-166 — Ten patients with active acromegaly received synthetic human pancreatic GH releasing factor (hpGHRF-44), and the GH responses to GHRF were compared with the basal GH levels and with the GH responses to arginine, TRH, LH-RH, somatostatin, and L-dopa. There were no significant relationships between the plasma GH responses to GHRF and the results of the stimulatory and inhibitory tests mentioned above, except that the responses to GHRF showed weak positive or negative correlations with the responses to LH-RH ($r = +0.56$) or with the basal GH levels ($r = -0.57$). These results indicate that acromegalic somatotrophs would have GHRF receptors as well as several other receptors to hypothalamic hormones and to dopamine, and that there might be no distinct relationship between the somatotroph sensitivities to GHRF and to other stimulatory or inhibitory stimuli. Further studies are required regarding the weak correlations between the response to GHRF and that to LH-RH or basal GH value. ——— GH; acromegaly; GHRF; TRH; arginine; LH-RH; L-dopa; somatostatin

It is well known that acromegalic patients often respond to exogenous stimuli with an increase or decrease in GH release (Hanew et al. 1980). As TRH, LH-RH, somatostatin and dopamine modulate the GH secretion from acromegalic pituitary either in vivo or in vitro, the presence of respective receptors has been postulated (Irie and Tsushima 1972; Rubin et al. 1973; Besser et al. 1974; Camanni et al. 1977; Matsukura et al. 1977; Ishibashi and Yamaji 1978; Peillon et al. 1979; Webb et al. 1983).

Recently, synthetic human pancreatic GH-releasing factor (designated

hpGHRF-44 and -40) became available to use, and it was demonstrated that the peptides can specifically stimulate GH secretion from human and rat pituitaries in vivo as well as in vitro (Brazeau et al. 1982; Guillemin et al. 1982; Rivier et al. 1982; Thorner et al. 1983). Furthermore, it has been demonstrated that hpGHRF-44 can stimulate pituitary GH secretion in patients with acromegaly (Lamberts et al. 1984; Shibasaki et al. 1984). These results indicate the presence of GHRF receptors in their somatotrophs.

It is of interest to study the relationships between the GH responses to hpGHRF-44 and those to other stimulatory and inhibitory agents in acromegalic patients, since Liuzzi et al. (1974) have already reported the mutual relationships between the responses to TRH and to dopaminergic agents in these patients. In the present study, the responses to hpGHRF-44 and those to several other agents were compared; i.e. arginine (which is believed to stimulate GH release via the hypothalamic GHRF release), GIH (GH-inhibiting hormone: somatostatin; the native GH regulator similar to GHRF), TRH, LH-RH, and L-dopa. The relationships between GH responses to hpGHRF-44 and the basal GH levels were also examined.

MATERIALS AND METHODS

Subjects

Ten patients with active acromegaly, 6 males and 4 females, aged 26-62 years, were studied after receiving informed consent. Every patient had an elevated plasma GH level (>6 ng/ml), acral enlargement, perspiration and enlarged sella turcica. None had been under any medication or treatment.

Procedures

After an overnight fast, the patients had i.v. catheters placed in each arm and all tests were started between 8 and 9 a.m. Each test was performed with an interval of 3 to 7 days.

Synthetic hpGHRF-44 (Peninsula Lab., CA) was dissolved in physiological saline, and aliquots were sterilized by passage through a $0.22 \mu\text{m}$ millipore filter. hpGHRF-44 (100 μg) was administered intravenously and blood samples were collected at -30, 0 min before and at 15 min intervals over 150 min after the injection. GIH (somatostatin, Protein Research Foundation, Osaka; 500 μg), arginine (10% L-arginine-HCl, Morishita, Osaka; 0.5 g/kg BW), TRH (Tanabe Pharmaceutical Co., Tokyo; 500 μg), LH-RH (Daiichi Pharmaceutical Co., 100 μg), and L-dopa (Sankyo Pharmaceutical Co., 500 mg) were used in the present study. Blood sampling and administration of these agents were accomplished as described previously (Hanew et al. 1977, 1980). Plasma GH was measured in duplicate according to the methods described previously (Hanew et al. 1980).

Regression analysis was performed using the Spearman rank correlation coefficient. Statistical analysis was carried out using Student's *t* test.

RESULTS

Comparisons of plasma GH responses to hpGHRF-44 and other related agents in acromegaly

The mean basal GH value ranged from 9.2 ng/ml to 61.8 ng/ml. The

TABLE 1. Comparisons of plasma GH responses to hpGHRF-44 and to other related agents in 10 patients with acromegaly

Subjects	Age (years)	Sex	Basal GH*	hpGHRF-44	GIH	Arginine	TRH	LH-RH	L-Dopa
1	53	F	21.8	+ 1277.1	-88.7	+ 138.3	+ 871.5	+ 743.4	-64.6
2	59	F	7.8	+ 1069.2	-83.5	+ 79.8	+ 127.1	+ 46.6	-86.2
3	34	M	15.8	+ 487.3	-65.6	+ 49.5	+ 523.2	+ 27.2	-37.4
4	58	M	38.5	+ 431.5	-77.2	+ 313.0	+ 162.7	+ 357.5	-39.3
5	62	F	40.5	+ 323.2	-68.5	+ 208.3	+ 1345.1	+ 377.6	-37.0
6	57	M	23.5	+ 228.9	-87.6	+ 128.7	+ 631.3	+ 70.9	-16.2
7	38	M	12.0	+ 180.8	-97.8	+ 36.4	+ 369.7	+ 8.0	-71.3
8	26	M	55.5	+ 49.1	-70.0	+ 16.0	+ 50.5	+ 58.9	-75.3
9	26	F	64.6	+ 43.7	-44.2	+ 70.3	+ 748.3	+ 81.3	-41.2
10	38	M	71.6	+ 33.9	-74.7	+ 44.7	+ 369.5	- 1.0	-24.8
Mean				+ 412.5	-75.8	+ 108.5	+ 519.9	+ 177.0	-49.3
S.E.M.				137.1	4.8	29.2	125.4	76.6	7.4
r†					- 0.35	+ 0.21	+ 0.07	+ 0.56	- 0.41
p					NS	NS	NS	NS	NS

* The basal value (ng/ml) at hpGHRF-44 test.

† Correlation coefficient between hpGHRF-44 test and other tests.

NS = not significant.

maximal plasma GH responses to hpGHRF-44 varied widely, as reported by Shibasaki et al. (1984), from 33.9% to 1277.1% exceeding the basal value (Table 1). There appeared to be a slight negative correlation between the basal GH value and the GH response to hpGHRF-44 ($r = -0.57$), although it was statistically insignificant. After the administration of arginine, plasma GH increased slightly from 16.0% to 313.0%, but there was no clear correlation between the responses to arginine and to hpGHRF-44 ($r = +0.21$, $p = \text{not significant: NS}$) (Table 1). TRH caused a marked GH increase (range: 50.5%–1345.1%), and again there was no significant relationship between the responses to TRH and to hpGHRF-44 ($r = +0.07$, $p = \text{NS}$). Interestingly, there was a weak positive correlation between the responses to LH-RH and to hpGHRF-44 ($r = +0.56$) although it was not strong enough to show statistical significance. No correlation was found between the GH increase induced by hpGHRF-44 and the GH decreases induced by GIH or L-dopa ($r = -0.35$ and -0.41 , respectively, $p = \text{NS}$) (Table 1).

DISCUSSION

We could not find any significant relationships between the plasma GH responses to hpGHRF-44 and those to other stimulatory and inhibitory agents, although the responses to hpGHRF-44 showed weak positive or negative correlations between the responses to LH-RH or the basal GH levels.

As it has been believed that arginine stimulates GH secretion via the release of hypothalamic GHRH (Pecile et al. 1972; Martin 1973; Merimee and Rabin 1973), GHRH center is probably operating in arginine responsive cases (six cases in our series judged by the criteria of 50% or more increase above the basal levels). However, no correlation was found between the GH responses to arginine and hpGHRF-44. One possible explanation for such a discrepancy might be the difference in sensitivities of hypothalamic GHRH neurons to arginine and of pituitary somatotrophs to GHRH.

Of special interest is the weak negative correlation between the GH response to hpGHRF-44 and the basal GH level together with the lowest responses to the GHRF observed in three cases with highest GH values. From these results, the following questions arise: 1) whether negative feedback mechanism is operating between GHRH secretion and plasma GH level, 2) whether autoregulation of somatotroph function by GH itself exists or not in acromegalic subjects (Müller et al. 1977), 3) whether tumor somatotrophs with high secretory activity are less sensitive to GHRH.

There was observed a weak, though not statistically significant, correlation between the responses to hpGHRF-44 and to LH-RH. There is, however, no resemblance between hpGHRF-44 and LH-RH in their chemical structures. Therefore, further studies are necessary to elucidate the meaning of this phenomenon.

No relationship was found between the responses to GHRF and to TRH, GIH,

and L-dopa, although Lamberts et al. (1984) have observed an interaction of hpGHRF-44 and GIH on GH release in cultured pituitary tumor cells from acromegalic patients. These findings would suggest that tumor somatotrophs have corresponding receptors to hypothalamic hormones and to dopamine, but there are no mutual relationships between GHRH receptors and other native and non-native receptors in terms of somatotroph sensitivities to exogenous stimuli.

In conclusion, somatotrophs of acromegaly would have the GHRH receptors as well as several other receptors, and there might be no distinct relationships in the somatotroph sensitivities between hpGHRF-44 and other stimulatory and inhibitory factors. Regarding the weak positive correlation between the GH responses to hpGHRF-44 and to LH-RH, and weak negative correlation between the responses to GHRF and the basal GH levels further studies are required to elucidate its mechanisms and significance.

Acknowledgments

This work was partly supported by a grant from the Intractable Disease Division, Public Health Bureau, Ministry of Health and Welfare, Japan.

References

- 1) Besser, G.M., Mortimer, C.H., Carr, D., Schally, A.V., Coy, D.H., Evered, D., Kastin, A.J., Tunbridge, W.M.G., Thorner, M.O. & Hall, R. (1974) Growth hormone release inhibiting hormone in acromegaly. *Brit. med. J.*, **1**, 352-355.
- 2) Brazeau, P., Ling, N., Böhlen, P., Esch, F., Ying, S.Y. & Guillemin, R. (1982) Growth hormone releasing factor, somatocrinin, release pituitary growth hormone in vitro. *Proc. nat. Acad. Sci. USA.*, **79**, 7909-7913.
- 3) Camanni, F., Massara, F., Belforte, L., Rosatello, A. & Molinatti, G.M. (1977) Effect of dopamine on plasma growth hormone and prolactin levels in normal and acromegalic subjects. *J. clin. Endocr.*, **44**, 465-473.
- 4) Guillemin, R., Brazeau, P., Böhlen, P., Esch, F., Ling, N. & Wehrenberg, W.B. (1982) Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science*, **218**, 585-589.
- 5) Hanew, K., Aida, M., Tano, T. & Yoshinaga, K. (1977) Abnormal growth hormone responses to L-dopa and thyrotropin-releasing hormone in patients with acromegaly. *Tohoku J. exp. Med.*, **121**, 197-206.
- 6) Hanew, K., Kokubun, M., Sasaki, A., Mouri, T. & Yoshinaga, K. (1980) The spectrum of pituitary growth hormone responses to pharmacological stimuli in acromegaly. *J. clin. Endocr.*, **51**, 292-297.
- 7) Irie, M. & Tsushima, T. (1972) Increase of serum growth hormone concentration following thyrotropin-releasing hormone injection in patients with acromegaly or gigantism. *J. clin. Endocr.*, **35**, 97-100.
- 8) Ishibashi, M. & Yamaji, T. (1978) Effect of thyrotropin-releasing hormone and bromocriptine on growth hormone and prolactin secretion in perfused pituitary adenoma tissues of acromegaly. *J. clin. Endocr.*, **47**, 1251-1256.
- 9) Liuzzi, A., Chiodini, P.G., Botalla, L., Silvestrini, F. & Müller, E.E. (1974) Growth hormone (GH) releasing activity of TRH and GH lowering effect of dopaminergic drugs in acromegaly: Homogeneity in the two responses. *J. clin. Endocr.*, **39**, 871-876.
- 10) Lamberts, S.W.J., Verlenn, T. & Oosterom, R. (1984) The interrelationship between

- the effects of somatostatin and human pancreatic growth hormone releasing factor on growth hormone release by cultured pituitary tumor cells from patients with acromegaly. *J. clin. Endocr.*, **58**, 250-254.
- 11) Martin, J.B. (1973) Neural regulation of growth hormone secretion. *New Engl. J. Med.*, **288**, 1384-1393.
 - 12) Matsukura, S., Kakita, T., Hirata, Y., Yoshimi, H., Fukase, M., Iwasaki, Y., Kato, Y. & Imura, H. (1977) Adenylate cyclase of GH and ACTH producing tumors of human : Activation by non-specific hormones and other bioactive substances. *J. clin. Endocr.*, **44**, 392-397.
 - 13) Merimee, T.J. & Rabin, D. (1973) A survey of growth hormone secretion and action. *Metabolism*, **22**, 1235-1251.
 - 14) Müller, E.E., Nistico, G. & Scapagnini, U. (1977) *Neurotransmitters and Anterior Pituitary Function*. Academic Press, New York, pp. 149-162.
 - 15) Pecile, A., Müller, E.E., Felici, M. & Netti, C. (1972) Nervous system participation in growth hormone release from anterior pituitary gland. In: *Growth and Growth Hormone*, edited by A. Pecile & E.E. Müller, Excerpta Medica, Amsterdam, pp. 261-270.
 - 16) Peillon, F., Cesselin, F., Bression, D., Zygelman, N., Brandi, A.M., Nousbaum, A. & Mauborgne, A. (1979) In vitro effect of dopamine and L-dopa on prolactin and growth hormone release from human pituitary adenomas. *J. clin. Endocr.*, **49**, 737-741.
 - 17) Rivier, J., Spiess, J., Thorner, M. & Vale, W. (1982) Characterization of a growth hormone-releasing factor from a human pancreatic islet tumor. *Nature*, **300**, 276-278.
 - 18) Rubin, A.L., Levin, S.R., Bernstein, R.I., Tyrrel, J.B., Noacco, C., Forsham, P.H. (1973) Stimulation of growth hormone by luteinizing hormone releasing hormone in active acromegaly. *J. clin. Endocr.*, **37**, 160-162.
 - 19) Shibasaki, T., Shizume, K., Masuda, A., Nakahara, M., Hizuka, N., Miyakawa, M., Takano, K., Demura, H., Wakabayashi, I. & Ling, N. (1984) Plasma growth hormone responses to growth hormone-releasing factor in acromegalic patients. *J. clin. Endocr.*, **58**, 215-217.
 - 20) Thorner, M.O., Rivier, J., Borges, J.L., Vance, M.L., Bloom, S.R., Rogol, A.D., Cronin, M.J., Kaiser, D.L., Evans, W.S., Webster, J.D., MacLeod, R.M. & Vale, W. (1983) Human pancreatic growth hormone-releasing factor selectively stimulates growth hormone secretion in man. *Lancet*, **1**, 24-28.
 - 21) Webb, C.B., Thominet, J.L. & Frohman, L.A. (1983) Ectopic growth hormone releasing factor stimulates growth hormone release from human somatotroph adenomas in vitro. *J. clin. Endocr.*, **56**, 417-419.
-