Interaction between GRF and somatostatin in regulation of GH secretion

TAMOTSU SHIBASAKI, AKITSUGU MASUDA, TOSHIHIRO IMAKI,
NAOKO YAMAUCHI, HIROSHI DEMURA AND
KAZUO SHIZUME

*Department of Medicine, Institute of Clinical Endocrinology,
Tokyo Women's Medical College, 8-1 Kawada-cho,
Shinjuku-ku, Tokyo 162, Japan*

Growth hormone (GH) secretion from the pituitary is under the dual control of growth hormone-releasing factor (GRF) and somatostatin. In addition to these antagonistic peptides, several other feedback mechanisms are involved in the regulation of GH secretion. For instance, GH itself stimulates somatostatin release from the hypothalamus (1). Insulin-like growth factor-I (IGF-I), which is synthesized in the liver, acts on both the hypothalamus and the pituitary to stimulate somatostatin release (2) and to suppress basal and GRF-induced GH secretion (3), respectively. Furthermore, we have recently found that IGF-I suppresses GRF release from the rat hypothalamic fragments *in vitro* (4).

Plotsky and Vale have recently found that GRF concentration in the hypophyseal portal blood changes in a reverse manner to that of somatostatin in rat (5). Very recently, Aguila and McCann have reported that GRF stimulates somatostatin release from rat hypothalamic fragments (6). These findings suggest a possibility of interaction of GRF to somatostatin in the hypothalamic level. We therefore tried to speculate about the interaction between GRF and somatostatin by examining the effect of GRF administration on plasma GH and TSH

levels in man and the effect of somatostatin on GRF release from rat hypothalamus *in vitro*.

Fig. 1 shows the effect of infusion of 1 mg GRF for 150 min on plasma GH response to subsequent 100 μ g GRF administration in man. Plasma GH levels were gradually elevated after the start of infusion and plateaued in 30 min. One hundred μ g of GRF administered at 150 min induced no significant GH rise. As shown in the upper panel of Fig. 2, a prior bolus injection of 50 μ g GRF increased plasma GH with a peak of 23.9 ± 7.9 ng/ml at 30 min and the plasma GH levels were then gradually lowered during the following 90 min. However, no significant rise of plasma GH levels was found after 100 μ g GRF administration. The same tendency was observed in the administration of 200 μ g GRF 2 hr after the prior injection of 100 μ g GRF as shown in the lower panel. Two hundreds μ g GRF did not induce a significant plasma GH rise.

There are several possibilities to explain the inhibition of GH response to the subsequent GRF by the prior GRF administration. The first possibility is that depletion of releasable GH might occur as the result of GH secretion induced by the prior GRF

Fig. 1. Plasma GH responses to infusion of 1 mg GRF and iv administration of 100 μ g GRF in normal men.

Fig. 2. Plasma GH responses to iv administrations of GRF in normal men.

administration. However, this is unlikely because, as shown in the bottom panel of Fig. 3, a high GH peak was observed in insulin-induced hypoglycemia even after the prior administration of 200 μ g GRF (7). Second, down-regulation of GRF receptors might occur because Vale and his coworkers have reported that number of GRF binding sites of rat anterior pituitary cells decreases after 2 hr of exposure to 0.5 nM GRF (8). Finally, increase in somatostatin release might be induced by the prior GRF administration.

It is difficult to speculate from the peripheral plasma somatostatin levels the manner of somatostatin release from the hypothalamus because most somatostatin in the peripheral blood is thought to originate from the peripheral tissues such as the gut and the pancreas. Several groups have reported the suppressive effect of somatostatin on basal and TRH-induced TSH secretion in man and rat (9, 10, 11, 12). Therefore, we tried to monitor indirectly somatostatin release from the hypothalamus by measuring plasma TSH levels based on the the assumption that somatostatin suppresses TSH secretion.

Fig. 3. Plasma GH responses to 200 µg GRF and insulin-induced hypoglycemia in men.

Fig. 4 shows the effect of GRF administration on plasma GH and TSH levels in man. The intravenous administration of normal saline did not affect the plasma TSH and GH levels as shown in the left upper panel. On the other hand, the iv administration of 50 µg GRF increased plasma GH levels from 1.3 ± 0.9 ng/ml to 17.6 ± 8.3 ng/ml 30 min after the injection, but no significant change in the plasma TSH levels was found during 2 hr after the injection. The administration of 100 µg GRF elevated plasma GH level from 0.9 ± 0.6 ng/ml to 17.6 ± 8.3 ng/ml 30 min after the injection and significantly lowered plasma TSH level from the basal level of 1.9 ± 0.3 µU/ml to 1.6 ± 0.3 µU/ml 120 min after the injection. Furthermore, 200 µg GRF induced elevation of plasma GH level and significantly lowered plasma TSH level from the basal level of 1.9 ± 0.2 µU/ml to 1.5 ± 0.2 µU/ml at 90 min and to 1.3 ± 0.1 µU/ml at 120 min.

These results suggest the intervention of somatostatin in the suppression of plasma TSH level. Three possibilities may be raised to explain the mechanism of somatostatin release after GRF administration. First, GH secreted in response to GRF administration induces somatostatin release from the hypothalamus since it has been reported that GH stimulates somatostatin release in rat (1). If it is the case, the administration of 50 µg GRF also should induce decrease in plasma TSH levels since 50 µg of GRF elevated plasma GH levels to the same extent as that of 100 µg or 200 µg GRF. But, no significant change in plasma TSH levels was found with the administration of 50 µg GRF. Even though no significant change in plasma TSH levels was found, there is still the possibility that somatostatin release is stimulated in response to increase in GH secretion. Because the sensitivity of thyrotroph to somatostatin is lower than that of somatotroph (9), the

Fig. 4. Plasma GH and TSH responses to iv administration of GRF in normal men.

change in plasma TSH levels may not reflect efficiently the change in somatostatin release.

The second possible explanation is that administered GRF itself directly acts on the hypothalamus and stimulates the release of somatostatin since an *in vitro* study has shown that GRF stimulates somatostatin release from rat hypothalamus (6). This possibility is strongly suggested by our result that only the administration of 100 μ g and 200 μ g GRF decreased TSH secretion and that 200 μ g GRF decreased TSH secretion more significantly.

The third possible explanation is that both GH and GRF together may stimulate somatostatin release from the hypothalamus. Since the median eminence is outside blood

brain barrier, GH and administered GRF can reach the median eminence to stimulate somatostatin release.

Fig. 5 shows the change in plasma TSH and GH levels when iv bolus injection of GRF was repeated. The prior 100 μ g GRF injection induced a plasma GH peak of 23.1 ± 10.8 ng/ml at 30 min and a significant suppression of plasma TSH level at 120 min. The following 200 μ g GRF administration induced a low GH peak at 135 min and a significant lowering of plasma TSH level at 240 min. It is therefore suggested that GRF rather than GH has a greater role to stimulate somatostatin release from the finding that the second administration of 200 μ g GRF significantly suppressed TSH level in spite of extremely low

Fig. 5. Changes in plasma GH and TSH levels in iv administration of GRF in normal men.

plasma GH rise induced.

We examined the effect of somatostatin on GRF release from rat hypothalamic fragments *in vitro*. Hypothalamic fragments obtained from male Wistar rats weighing approximately 200 g were incubated in Krebs Ringer bicarbonate buffer supplemented with 0.1% BSA and 0.5% glucose under an atmosphere of 5% CO₂ and 95% O₂ at 37°C for 30 min. After the preincubation, each hypothalamic fragment was incubated with fresh buffer with sample solution for another 30 min. The concentration of immunoreactive GRF in the medium was measured with a specific and sensitive radioimmunoassay. The results of the suppressive effects of somatostatin 14 and somatostatin

28 on GRF release are shown in Table 1. Somatostatin 14 significantly suppressed GRF release from the rat hypothalamic fragments to 87.1%, 86.5% and 83.0% that of the control at concentrations of 10⁻¹⁰, 10⁻⁹ and 10⁻⁸ M, respectively. Somatostatin 28 also significantly decreased GRF release to 87.7%, 82.8% and 76.5% that of the control at concentrations of 10⁻¹⁰, 10⁻⁹ and 10⁻⁸ M, respectively. The concentration of somatostatin in the hypophyseal portal blood was reported to be several hundred pg/ml corresponding to the order of 10⁻¹⁰ M (13). These results, therefore, suggest that somatostatin acts on the hypothalamus to suppress GRF release.

The site of somatostatin action is pre-

Table 1. The effects of SS-14 and SS-28 on GRF release

	somatostatin 14 (M)					
	control	10 ⁻¹²	10 ⁻¹¹	10 ⁻¹⁰	10 ⁻⁹	10 ⁻⁸
IR-GRF (pg/hypo/30 min)	98.0±3.3	100.1±4.6	93.1±3.8	85.4±3.6*	84.8±1.3**	81.3±3.1**
	Somatostatin 28 (M)					
	control	10 ⁻¹²	10 ⁻¹¹	10 ⁻¹⁰	10 ⁻⁹	10 ⁻⁸
IR-GRF (pg/hypo/30 min)	98.0±3.3	97.9±3.1	91.7±3.6	85.9±1.1*	81.1±1.9**	75.0±3.3**

* P<0.01

** P<0.001

sumably the median eminence. However, there may be interactions between GRF neurons and somatostatin neurons in the hypothalamus because somatostatin-positive fibers exist in the arcuate nucleus where GRF neurons are present (14). Our clinical study and the report by Aguila and McCann (6) that GRF stimulates somatostatin release from the rat hypothalamus suggest presence of GRF action on somatostatin release in the hypothalamic level. The median eminence is a possible site where GRF and somatostatin are released into the hypophyseal portal blood because GRF-positive fibers end at the same area as somatostatin-positive fibers end (15). Somatostatin neurons may be innervated by GRF neurons because GRF-positive fibers have been found in the preoptic anterior hypothalamic area where somatostatin neurons are present (16). Somatostatin in the area is considered to be important for the regulation of GH secretion.

Our results suggest that interactions between GRF and somatostatin exist not only at the pituitary level but also at the hypothalamic level in the regulatory mechanism of GH secretion.

References

1. Sheppard MC, Kronheim S, Pimstone BL 1978. Stimulation by growth hormone of somatostatin release from the rat hypothalamus *in vitro*. *Clin Endocrinol* 9, 583.
2. Berelowitz M, Szabo M, Frohman LA, Firestone S, Chu L, Hintz RL 1981 Somatomedin C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science* 212, 1279.
3. Brazeau P, Gillem R, Ling N, Van Wyk J, Humbel R 1982. Inhibition par les somatomédines de la sécrétion de l'hormone de croissance stimulée par le facteur hypothalamique somatocroinine (GRF) ou le peptide de synthèse hpGRF. *CR Acad Sc Paris* (111) 295, 651.
4. Shibasaki T, Yamauchi N, Hotta M, Masuda A, Imaki T, Demura H, Ling N, Shizume K 1986. *In vitro* release of growth hormone-releasing factor from rat hypothalamus: effect of insulin-like growth factor-1. *Regul Peptides* 15, 47.
5. Plotsky PM, Vale W 1985. Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophyseal-portal circulation of the rat. *Science* 230, 461.
6. Aguila MC, McCann SM 1985. The influences of hGRF, CRF, TRH and LHRH on SRIF release from median eminence fragments. *Brain Res* 348, 180.
7. Shibasaki T, Hotta M, Masuda A, Imaki T, Obara N, Demura H, Ling N, Shizume K 1985. Plasma GH responses to GHRH and insulin-induced hypoglycemia in man. *J Clin Endocrinol Metab* 60, 1265.
8. Bilezikjian LM, Seifert H, Vale W 1986. Desensitization to growth hormone-releasing factor (GRF) is associated with down-regulation of GRF-binding sites. *Endocrinology* 118, 2045.
9. Vale W, Rivier C, Brazeau P, Guillemin R 1974. Effects of somatostatin on the secretion of thyrotropin and prolactin. *Endocrinology* 95, 968.
10. Siler TM, Yen SSC, Vale W, Guillemin R 1974. Inhibition by somatostatin on the release of TSH induced in man by thyrotropin-releasing factor. *J. Clin Endocrinol Metab* 38, 742.
11. Ferland L, Labrie F, Jobin M, Arimura A, Schally AV 1976. Physiological role of somatostatin in the control of growth hormone and thyrotropin secretion. *Biochem Biophys Res Commun* 68, 149.
12. Chihara K, Arimura A, Chihara M, Schally AV 1978. Studies on the mechanism of growth hormone and thyrotropin responses to somatostatin antiserum in anesthetized rats. *Endocrinology* 103, 1916.
13. Chihara K, Arimura A, Schally AV 1979. Immunoreactive somatostatin in rat hypophyseal portal blood: effects of anesthetics. *Endocrinology* 104, 1434.
14. Ohtsuka M, Hisano S, Daikoku S 1983. Electronmicroscopic study of somatostatin-containing neurons in rat arcuate nucleus with special reference to neuronal regulation. *Brain Res* 263, 191.
15. Bresson J-L, Clavequin M-C, Fellmann D,

Bugnon C 1984. Ontogeny of the neuroglandular system revealed with hpGRF44 antibodies in human hypothalamus. *Neuroendocrinology* 39, 68.

16. Smith RM, Howe PRC, Oliver JR, Willoughby JO 1984. Growth hormone releasing factor immunoreactivity in rat hypothalamus. *Neuropeptides* 4, 109.