The role of endogenous GHRH in arginine-, insulin-, clonidine- and L-dopa-induced GH release in normal subjects

Kunihiko Hanew and Atsushi Utsumi
Second Department of Internal Medicine and 1 Department of Psychiatry, Tohoku University School of Medicine, Sendai and 1 Hanew Endocrine Clinic, Sendai, Japan

Abstract
Objective: The role of endogenous GHRH in arginine-, insulin-, clonidine- and L-dopa-induced GH secretion was studied in man using a GHRH antagonist (GHRH-Ant).
Design: Ten healthy adult males were studied for serum GH responses to arginine or insulin singly, or sequentially 120 min after GHRH injection with or without combined administration of GHRH-Ant. Further, GHRH, clonidine or L-dopa were sequentially administered to these subjects 120 min after the GHRH injection.
Results: The combined administration of GHRH-Ant distinctly inhibited the arginine- and insulin-induced GH release. When these four agents were sequentially administered 120 min after GHRH injection, the GH responses to clonidine and L-dopa disappeared completely while clear responses were observed to arginine and insulin administration. These responses to arginine and insulin were also completely inhibited by the combined administration of GHRH-Ant.
Conclusions: These results indicate that clonidine and L-dopa stimulate GH secretion mainly through the release of hypothalamic GHRH, and that arginine- and insulin-induced hypoglycaemia stimulate GH secretion mainly through the inhibition of hypothalamic somatostatin release. However, the presence of endogenous hypothalamic GHRH seems to be essential for the maximal stimulation of GH release induced by arginine and insulin.

Introduction
To evaluate growth hormone (GH) secretory capacity, GH responses to pharmacological stimuli, spontaneous 24 h GH secretory profile, urinary GH, plasma insulin-like growth factor (IGF)-I and IGF-binding protein-3 are studied (1–4). GH-releasing hormone (GHRH), L-dopa, clonidine, arginine, insulin and glucagon are used widely as pharmacological stimuli (1, 3, 4). GH secretion appears to be mainly regulated by hypothalamic GHRH and somatostatin (SS), and it might also be regulated by putative GH-releasing peptide (GHRP), IGF-I and other neuropeptides (5–7). Peripheral GHRH is not necessarily of hypothalamic origin, since circulating GHRH and SS also originate from extra-hypothalamic tissues (3, 8, 9).

The GH secretory mechanisms of these pharmacological stimuli except GHRH are not well established in man. In addition, distinct species-specific differences in GH neuroregulation make the extrapolation of animal data to humans difficult (3, 10).

Synthetic human GHRH antagonist (GHRH-Ant) is a competitive antagonist at the level of the GHRH receptor, and it is therefore possible to evaluate whether hypothalamic GHRH is participating in the GH response induced by those pharmacological stimuli (3).

We have previously reported that clonidine (α2-adrenergic receptor agonist)-induced and L-dopa-induced GH release was significantly inhibited by the combined administration of GHRH-Ant (9). Similarly, Jaffe et al (3) have reported that GH responses to arginine and insulin as well as those to clonidine and L-dopa were clearly inhibited by the combined administration of GHRH-Ant. These results indicate that endogenous GHRH is required for their GH-releasing activity. However, it was reported that clear GH responses were observed when arginine and insulin were sequentially given after GHRH, whereas no GH response was observed when GHRH was given after GHRH (11). These results seems to indicate that arginine and insulin stimulate GH secretion through the inhibition of hypothalamic SS release rather than the stimulation of hypothalamic GHRH (10). Therefore, it is still not clear which hypothalamic hormone is mainly participating in arginine- and insulin-induced GH secretion.
To clarify what is the main mechanism for arginine-, insulin-, clonidine- and L-dopa-induced GH release, we have examined GH responses to these four agents sequentially administered after GHRH injection, and examined using GHRH-Ant whether endogenous hypothalamic GHRH has some role even in the GHRH insensitive state (i.e. after the preceding administration of GHRH) in normal adults.

Materials and methods

Ten healthy and non-obese adult males (age: 24.1 ± 1.5 (s.e.m.), range 20–33 years; body mass index: 21.2 ± 0.4, range 19.4 – 22.7 kg/m²) were studied after obtaining approval from the independent Local Ethics Committee in Sendai and informed consent from every subject. These subjects had no illnesses and were on no medication. After an overnight fast, an indwelling cannula was placed in an antecubital vein at 0830 h. Arginine (0.5 g/kg i.v. for 30 min) or regular insulin (0.05 U/kg i.v) was administered singly at 0 min or sequentially at 120 min after GHRH (GHRH(1–44)NH₂; Sumitomo, Osaka, Japan) injection (100 μg i.v.) with or without concomitant administration of GHRH-Ant (Bachem AG, Bubendorf, Switzerland) (800 μg, from −30 to 120 min on the single study or from 90 min to 240 min on the sequential study). Similarly, GHRH (100 μg i.v.) was repeatedly administered at 0 min and 120 min. Clonidine (150 μg p.o.) and L-dopa (500 mg p.o.) were administered sequentially at 120 min after the GHRH injection (100 μg i.v.).

Blood samples were obtained at 30 min and 0 min before, and 15, 30, 45, 60, 90 and 120 min after insulin administration, and every 30 min until 120 min after arginine infusion. For the combined administration, blood samples were taken at 30 and 0 min before, and 15, 30, 45, 60, 90, 120, 135, 150, 165, 180, 210 and 240 min for GHRH plus insulin or GHRH plus GHRH-Ant plus insulin; at 30 and 0 min before, and 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min (for GHRH plus GHRH-Ant or GHRH plus GHRH-Ant plus arginine), and 270 min (for GHRH plus clonidine or GHRH plus L-dopa).

Plasma GH was measured in duplicate using a commercial IRMA kit (Daiichi, Tokyo, Japan), and the sensitivity was 0.006 μg/l and the intra- and interassay coefficients of variation ranged between 1.4 and 3.2% and between 1.5 and 3.7% respectively. The hormonal responses are expressed as GH increment from the basal (ΔGH) as well as AUC (area under the curve) over the baseline calculated by trapezoidal integration. Data are expressed as means ± S.E.M. and statistical analysis was carried out using ANOVA followed by the Student–Newmann–Keuls test, Fisher’s randomization test or Student’s t-test where appropriate for statistical comparisons between groups. If the data were not normally distributed, they were logarithmically transformed before analysis.

Results

**Plasma GH responses to single administration of arginine or insulin with or without GHRH-Ant infusion**

Plasma GH response (ΔGH) to arginine in the ten normal adults was significantly inhibited by the concomitant administration of GHRH-Ant (arginine vs GHRH-Ant + arginine: 90 min, 10.4 ± 3.1 vs 3.1 ± 0.8 μg/l, P < 0.05; 120 min, 9.7 ± 3.2 vs 1.3 ± 0.4 μg/l, P < 0.05) (Fig. 1a). The mean maximal ΔGH and AUC over the baseline were significantly suppressed by GHRH-Ant (arginine vs GHRH-Ant + arginine: ΔGH, 22.3 ± 3.9 vs 9.5 ± 1.5 μg/l, P < 0.01; AUC, 1404.7 ± 319 vs 271.5 ± 96.0 μg/l, P < 0.01).

Plasma GH response (ΔGH) to insulin in the ten normal adults was also significantly inhibited by the concomitant administration of GHRH-Ant (insulin vs GHRH-Ant + insulin: 45 min, 22.6 ± 5.6 vs 10.6 ± 3.3 μg/l, P < 0.01; 60 min, 27.3 ± 7.1 vs 10.4 ± 3.7 μg/l, P < 0.05) (Fig. 1b). The mean maximal ΔGH and AUC were significantly suppressed.
by GHRH-Ant (insulin vs GHRH-Ant + insulin: ΔGH, 35.5±5.8 vs 13.5±3.8 μg/l, P < 0.01; AUC 2012.7±474.0 vs 467.6±202.9 μg/l, P < 0.01).

**Plasma GH responses to sequential administration of GHRH, arginine or insulin after GHRH injection with or without GHRH-Ant infusion**

Plasma GH response in the ten normal adults was remarkably diminished when GHRH was administered sequentially 120 min after the first GHRH administration (Fig. 2a). The mean maximal ΔGH and AUC after the second GHRH administration were significantly lower than those after the first GHRH administration (1st vs 2nd: ΔGH, 40.6±7.0 vs 13.5±3.8 μg/l, P < 0.005; AUC, 2554.1±343.8 vs 658.0±190.1 μg/l, P < 0.001).

In contrast, a much larger GH response compared with the single administration was observed with the second administration of arginine (vs single arginine test, P = NS; Fig. 2b). Such a GH response was almost completely abolished by the preceding and concomitant administration of GHRH-Ant (GHRH plus arginine vs GHRH plus GHRH-Ant plus arginine: 180 min, 24.2±5.0 vs 7.9±2.8 μg/l, P < 0.05; 210 min, 17.2±4.4 vs 4.4±1.4 μg/l, P < 0.05; 240 min, 6.1±1.8 vs 1.3±0.4 μg/l, P < 0.05) (Fig. 2b). GH showed similar responses to GHRH within 120 min (from 0 to 120 min) in the above two protocols. However, the mean maximal ΔGH and AUC after the sequential administration of arginine were significantly suppressed by combined administration of GHRH-Ant (GHRH + arginine vs GHRH + GHRH-Ant + arginine;...
ΔGH, 29.0±3.8 vs 10.2±2.5 μg/l, P < 0.001; AUC, 1772.1±293.4 vs 633.9±153.0 μg/l, P < 0.005).

After the sequential administration of insulin following the first GHRH injection, a similar GH response to the single administration was observed (vs single insulin test, P = NS) (Fig. 2c). Such a GH response was also substantially inhibited by the preceding and concomitant administration of GHRH-Ant (GHRH plus insulin vs GHRH plus GHRH-Ant plus insulin: 165 min, 19.2±2.6 vs 7.3±1.7 μg/l, P < 0.01; 180 min, 28.9±3.9 vs 8.5±2.2 μg/l, P < 0.01; 210 min, 17.3±5.0 vs 4.9±1.2 μg/l, P < 0.05) (Fig. 2c). GH responses to GHRH within 120 min were similar between the above two protocols. The mean maximal ΔGH and AUC after the sequential administration of insulin were significantly suppressed by combined administration of GHRH-Ant (GHRH + insulin vs GHRH + GHRH-Ant + insulin: ΔGH, 30.7±3.3 vs 9.8±1.8 μg/l, P < 0.001; AUC, 1873.3±269.4 vs 624.5±96.3 μg/l, P < 0.001).

**Plasma GH responses to sequential administration of clonidine or l-dopa after GHRH injection**

Contrary to the cases of arginine and insulin, plasma GH response to clonidine in the ten normal adults almost disappeared after the GHRH injection (GH value at clonidine administration (120 min), 10.1±3.9 μg/l; peak (240 min), 5.3±2.5 μg/l) (Fig. 3a). Similarly, plasma GH response in the ten normal adults disappeared when l-dopa was sequentially administered after the GHRH injection (GH value at l-dopa administration (120 min), 11.4±3.0 μg/l; peak (180 min), 12.1±3.4 μg/l) (Fig. 3b).

**Discussion**

In this study, plasma GH responses to arginine and insulin were significantly inhibited by the combined administration of GHRH-Ant. These results imply that endogenous hypothalamic GHRH is necessary for GH responses to these agents (3). However, we cannot conclude simply that arginine and insulin mainly stimulate GH secretion through the stimulation of hypothalamic GHRH.

Plasma GH response to the second administration of GHRH following the first GHRH administration was markedly reduced (only subtle non-significant increases were observed) as was previously reported (11). However, it has been reported that the sequential administration of arginine and insulin following the first GHRH brought about distinct GH responses which are similar to or greater than those induced by the single administration of each agent (11, 12).

The greater responses compared with the single administration of GHRH were similarly observed when arginine was co-administered with GHRH, or insulin was administered 30 min prior to GHRH injection (4, 13, 14). From these observations and the fact that the GHRH dose used is pharmacological, it is theoretically difficult for arginine and insulin administered after GHRH to cause GH secretion via the hypothalamic GHRH release. Rather it seems that arginine and insulin stimulate GH secretion through non-GHRH mechanism, e.g. through the inhibition of hypothalamic SS release (11) and/or through the release of other putative mediators of GH (ghrelin/GHRP) (15). Regarding ghrelin, a recent study revealed that human hypothalamus and pituitary possess human ghrelin-binding sites (16).

The fact that thyrotropin (TSH) is under tonic inhibitory control by SS (17) and infusion of arginine to normal subjects increases TSH levels as well as GH, whereas ghrelin does not affect TSH secretion (15), suggests a possible inhibitory action of arginine on SS release from the hypothalamus (18). Consistent with these observations, plasma TSH response to thyrotropin-releasing hormone (TRH) after the preceding administration of arginine is significantly enhanced compared with that to TRH alone (13).

As no enhanced GH response occurs when insulin and arginine are administered together to humans (19), similar GH secretory mechanism of these agents in humans is considered.

The GH responses to arginine and insulin after GHRH injection were significantly inhibited by the combined administration of GHRH-Ant. Accordingly it can be inferred that endogenous GHRH, even subtle amounts, are required for arginine- and insulin-induced GH release when these stimuli are exerted indirectly to non-GHRH receptors. In other words, endogenous GHRH plays an important role in GH release induced probably by SS withdrawal.

GHRH promotes GH secretion by stimulating the adenylate cyclase–cAMP system through stimulatory GTP-binding protein, and SS decreases GH secretion by inhibiting the above system through inhibitory GTP-binding protein (20–26).

Probably slight somatotroph stimulation by GHRH and subsequent activation of the adenylate cyclase–protein kinase A system is required for GH stimulation by SS withdrawal induced by arginine and insulin.

Relating to these assumptions, it is reported that GH response to GHRP-6, which binds to the specific GH secretagogue receptor and stimulates GH secretion via the phosphatidylinositol–protein kinase C system (27, 28), is evidently inhibited by the combined administration of GHRH-Ant (29). In addition, GHRP-6 can not induce an adequate GH response in patients with hypothalamic lesions who would have hypothalamic GHRH deficiency (30).

These results also suggest that the endogenous GHRH–cAMP–protein kinase A system is also necessary for GH release induced by the GHRP–6-phosphatidylinositol–protein kinase C system.
Plasma GH responses to clonidine and L-dopa in this study were quite different from those to arginine and insulin, i.e. the responses to the sequential administration of clonidine and L-dopa disappeared almost completely when these agents were administered after the first GHRH injection. These results and the previous observations that the clear GH response induced by clonidine or L-dopa was almost completely inhibited by GHRH-Ant (9) indicate that the above agents probably stimulate GH secretion through the release of hypothalamic GHRH.

Regarding the GH secretory mechanism of clonidine, similar results were reported by Alba-Roth et al. (31), that preceding administration of clonidine lowered the subsequent GH responses to GHRH and vice versa. In contrast, Valcavi et al. (32) suggested that clonidine stimulates GH secretion through the inhibition of SS release, since i.v. administration of clonidine brought a GH response even after the preceding GHRH injection. However, the GH response to sequential injection of clonidine 120 min after the first GHRH injection seems to be not significant by the comparatively wide S.E.M.

Our data on L-dopa are compatible with the previous observation that the preceding administration of L-dopa did not enhance GH response to GHRH (33), but is discordant with the observation that the plasma GH showed a similar response to single administration of L-dopa when L-dopa was sequentially administered 90 min after GHRH injection. The reason for the discrepancy between the latter observation and ours is not clear, although the timing of L-dopa administration was different.

In conclusion, it is considered that arginine and insulin mainly stimulate GH secretion through the inhibition of hypothalamic SS release and clonidine and L-dopa mainly stimulate GH secretion through the release of hypothalamic GHRH. Endogenous hypothalamic GHRH, even subtle amounts, seems to be crucial for the maximal stimulation of arginine- and insulin-induced GH secretion.

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