CLINICAL STUDY

Comparisons among old and new provocative tests of GH secretion in 178 normal adults

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Abstract

Classical provocative stimuli of GH secretion such as insulin-induced hypoglycaemia, arginine, clonidine, glucagon and levodopa have been widely used in clinical practice for approximately 30 years. On the other hand, in the last 10 years new potent stimuli of GH secretion have been proposed, but an extensive comparison with the classical ones has rarely been performed, at least in adults.

In order to compare the GH-releasing activity of old and new provocative stimuli of GH secretion, and to define the normative values of the GH response, in 178 normal adults (95 males, 83 females; age range: 20–50 years, all within $\pm 15\%$ of their ideal body weight), we studied the GH response to: insulin-induced hypoglycaemia (ITT, 0.1 IU/kg i.v.), arginine (ARG, 0.5 g/kg i.v.), clonidine (CLO, 300 $\mu\text{g/kg}$ p.o.), glucagon (GLU, 1 mg i.m.), pyridostigmine (PD, 120 mg p.o.), galanin (GAL, 80 pmol/kg per min), GH-releasing hormone (GHRH, 1 $\mu\text{g/kg}$ i.v.), GHRH+ARG, GHRH+PD, hexarelin, a GH-releasing protein (HEX, 2 $\mu\text{g/kg}$ i.v.) and GHRH+HEX (0.25 $\mu\text{g/kg}$ i.v.). The mean ($\pm$ S.E.M.) peak GH response to ITT (21.8 ± 2.8 , range: 3.0–84.0 $\mu\text{g/l}$) was similar to those to ARG (18.0 ± 1.6 , range: 2.9–39.5 $\mu\text{g/l}$) or GLU (20.5 ± 2.2 , range: 10.6–36.9 $\mu\text{g/l}$) which, in turn, were higher ($P < 0.001$) than those to CLO (8.2 ± 1.6 , range: 0.3–21.5 $\mu\text{g/l}$), PD (9.6 ± 1.1 , range: 2.2–33.0 $\mu\text{g/l}$) and GAL (9.3 ± 1.1 , range: 3.9–18.3 $\mu\text{g/l}$). The GH response to GHRH (19.1 ± 1.5 , range: 2.7–55.0 $\mu\text{g/l}$) was similar to those after ITT, ARG or GLU but clearly lower than those after GHRH+ARG (65.9 ± 5.5 , range: 13.8–171.0 $\mu\text{g/l}$) and GHRH+PD (50.2 ± 4.6 , range: 17.7–134.5 $\mu\text{g/l}$) which, in turn, were similar. The GH response to HEX (55.3 ± 5.5 , range: 13.9–163.5 $\mu\text{g/l}$) was similar to those after GHRH+ARG and GHRH+PD but lower ($P < 0.001$) than that after GHRH+HEX (86.0 ± 4.3 , range: 49.0–125.0 $\mu\text{g/l}$) which was the most potent stimulus of GH secretion. In this adult population the third centile limits of peak GH response to various stimuli were the following: ITT: 5.3; ARG: 2.9; CLO: 1.5; GLU: 7.6; PD: 2.2; GAL: 4.0; GHRH: 5.0; GHRH+ARG: 17.8; GHRH+PD: 17.9; HEX: 21.6; GHRH+HEX: 57.1.

These results confirm that, among classical provocative tests of GH secretion, ITT followed by ARG and GLU are the most potent ones and possess clear limits of normality. GHRH+ARG or PD and HEX are strong stimuli of GH secretion which, however, is maximally stimulated by a combination of GHRH and a low dose of HEX. It is recommended that each test is used with appropriate cut-off limits.

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Introduction

Classical provocative stimuli of growth hormone (GH) secretion such as insulin-induced hypoglycaemia (ITT), arginine (ARG), clonidine (CLO), glucagon (GLU) and levodopa have been widely used in clinical practice for approximately 30 years (1–8) and the diagnosis of GH deficiency (GHD) always includes an evaluation of the GH response to provocative testing (9). However, while a normal GH response to provocative testing does not definitively rule out GH insufficiency in childhood, severe GHD in adults must be demonstrated by provocative tests only (9–13).

Among classical provocative tests, ITT has been considered to be the golden standard test for a number of years, though ARG and GLU are also widely used for the diagnosis of GHD both in childhood and, much more, in adulthood (9–11, 13–18).

On the other hand, in the last 10 years new potent stimuli of GH secretion have been proposed. Among them are testing with GH-releasing hormone (GHRH) combined with substances such as pyridostigmine (PD), a cholinergic agonist, and ARG, which markedly potentiate its GH-releasing activity via inhibition of hypothalamic somatostatin release (12, 16, 19–21). The diagnostic reliability of these tests has been already

demonstrated (12, 16, 19, 20, 22, 23). More recently, it has been shown that synthetic peptidyl and non-peptidyl GH secretagogues possess strong and reproducible GH-releasing activity, particularly in humans (24–27). They act via specific receptors at the pituitary and, mainly, at the hypothalamic level, via mechanisms which, at least partially, are different from those of GHRH (24–28). In fact, GH secretagogues, even at a very low dose, generally show true synergistical interaction with GHRH (29–31). The GH response to this stimulus appears to be one of the most marked ever recorded in humans (24–31).

An extensive comparison between classical and new provocative stimuli of somatotroph secretion in normal adults is still lacking. Thus, the aim of our study was to compare the GH-releasing activity of old and new provocative stimuli of GH secretion and to define normative values of the GH response.

Subjects and methods

One hundred and seventy-eight normal adults (95 males (M), 83 females (F); age range: 20–50 years, all within $\pm 15\%$ of their ideal body weight) were studied. The study had been approved by a local, independent ethical committee and all subjects had given their informed consent to participating in the study.

The GH response to the following tests was studied.

(a) ITT (0.1 IU/kg i.v.; $n = 37$; 14 M, age: 20–42 years and 23 F, age: 21–48 years); (b) ARG (0.5 g/kg i.v.; $n = 37$; 15 M, age: 23–49 years and 22 F, age: 24–50 years); (c) CLO (300 μg p.o.; $n = 22$; 10 M, age: 25–45 years and 12 F, age: 20–50 years); (d) GLU (1 mg i.m.; $n = 23$; 10 M, age: 21–45 years and 13 F, age: 29–35 years); (e) PD (120 mg p.o.; $n = 32$; 20 M, age: 21–38 years and 12 F, age: 25–36 years); (f) galanin (GAL; 80 pmol/kg per min i.v.; $n = 16$; 6 M, age: 20–50 years and 10 F, age: 21–48 years); (g) GHRH (1 μg /kg i.v.; $n = 57$; 36 M, age: 28–45 years and 21 F, age: 20–48 years); (h) GHRH+ARG ($n = 48$; 20 M, age: 22–40 years and 28 F, age: 22–35 years); (i) GHRH+PD ($n = 38$; 14 M, age: 20–50 years and 24 F, age: 23–45 years); (l) hexarelin (HEX; 2 μg /kg i.v.; $n = 29$; 11 M, age: 24–46 years and 18 F, age: 22–45 years); (m) HEX (0.25 μg /kg i.v.)+GHRH ($n = 31$; 20 M, age: 22–50 years and 11 F, age: 22–48 years). Forty subjects underwent at least two different provocative tests (15 M, 25 F, age range: 22–40 years). Specifically, fourteen subjects underwent testing with PD and GHRH+ARG, nine subjects underwent testing with HEX and GHRH+ARG, eight subjects underwent testing with GHRH+ARG and GHRH+HEX, six subjects underwent testing with ARG alone and GHRH+ARG and three subjects underwent testing with ARG alone and HEX.

All these tests have been performed in the last 2 years and approximately 40% of them were carried out as part of other study protocols. The tests were

performed in the morning starting at 0830–0900 h, after overnight fasting and 30 min after an indwelling catheter had been placed in an antecubital vein of the forearm kept patent by slow infusion of isotonic saline. Blood samples were taken at baseline and then as follows: (a) every 15 min up to 90–120 min after i.v. ITT, ARG, GAL, GHRH, GHRH+ARG, GHRH+PD, HEX and HEX+GHRH test; (b) every 30 min up to 180 min after oral CLO and PD; (c) every 30 min up to 90 min and then every 15 min up to 210 min after i.m. GLU. Serum GH levels ($\mu\text{g/l}$) were assayed in duplicate at each time-point by IRMA (HGH-CTK, Sorin, Italy). The International Reference Preparation was 80/505 (1 $\mu\text{g/l} = 2$ IU). All samples from an individual subject were analysed together. The sensitivity of the method was 0.15 $\mu\text{g/l}$. The inter- and intra-assay coefficients of variation were 5.1–7.5% and 2.6–5.4% respectively, at GH levels of 2.9–42.4 and 2.8–41.2 $\mu\text{g/l}$ respectively.

The results (means $\pm$ S.E.M.) are expressed as maximal peak GH responses. For each test we report the third and the first centile limits of the normal peak GH response in order to clarify which response below normal limits should be considered as low or severely low in patients suspected of GHD.

Statistical analysis of the data was performed by multiple ANOVA and non-parametric Kruskal–Wallis test.

Results

The mean ($\pm$ S.E.M.) peak GH response to ITT (21.8 ± 2.8 , range: 3.0–84.0 $\mu\text{g/l}$) was similar to those to ARG (18.0 ± 1.6 , range: 2.9–39.5 $\mu\text{g/l}$) or GLU (20.5 ± 2.2 , range: 10.6–36.9 $\mu\text{g/l}$) which, in turn, were higher ($P < 0.001$) than those to CLO (8.2 ± 1.6 , range: 0.3–21.5 $\mu\text{g/l}$), PD (9.6 ± 1.1 , range: 2.2–33.0 $\mu\text{g/l}$) and GAL (9.3 ± 1.1 , range: 3.9–18.3 $\mu\text{g/l}$) (Fig. 1).

The GH response to GHRH (19.1 ± 1.5 , range: 2.7–55.0 $\mu\text{g/l}$) was similar to those after ITT, ARG or GLU but clearly lower than those after GHRH+ARG (65.9 ± 5.5 , range: 13.8–171.0 $\mu\text{g/l}$) and GHRH+PD (50.2 ± 4.6 , range: 17.7–134.5 $\mu\text{g/l}$) which, in turn, were similar (Fig. 1).

The GH response to HEX (55.3 ± 5.5 , range: 13.9–163.5 $\mu\text{g/l}$) was similar to those after GHRH+ARG and GHRH+PD (Fig. 1).

The combined administration of GHRH+HEX elicited the highest GH peak response (86.0 ± 4.3 , range: 49.0–125.0 $\mu\text{g/l}$; $P < 0.001$ vs GHRH+ARG and GHRH+PD) (Fig. 1).

The third centile normative limits of the peak GH response to various stimuli were the following: ITT: 5.3; ARG: 2.9; CLO: 1.5; GLU: 7.6; PD: 2.2; GAL: 4.0; GHRH: 5.0; GHRH+ARG: 17.8; GHRH+PD: 17.9; HEX: 21.6; GHRH+HEX: 57.1 (Table 1).

On the other hand, the first centile normative limits of the peak GH response were the following: ITT: 3.8; ARG: 2.7; CLO: 1.3; GLU: 7.1; PD: 2.1; GAL: 3.9; GHRH: 3.8;

Figure 1 Mean and individual peak GH responses to classical stimuli, GHRH, GHRH+PD, GHRH+ARG, HEX and GHRH+HEX in normal adult subjects.

GHRH+ARG: 15.2 ; GHRH+PD: 17.7 ; HEX: 16.5 ; GHRH+HEX: 51.7 $\mu\text{g/l}$ (Table 1).

The mean GH peaks to ITT, CLO, GLU, PD, GAL, GHRH, GHRH+PD, HEX and GHRH+HEX were similar in both sexes. On the other hand, the GH responses to ARG and GHRH+ARG were significantly higher in women than in men (ARG: 22.1 ± 2.3 vs 15.3 ± 1.3 $\mu\text{g/l}$, $P < 0.03$; GHRH+ARG: 80.3 ± 1.8 vs 64.5 ± 2.1 , $P < 0.01$ respectively).

Side-effects

ITT caused characteristic hypoglycaemic symptoms. ARG and GLU induced vomiting in 2 and 15% of subjects respectively. PD induced transient abdominal pain and/or muscle fasciculation in approximately 40% of the subjects. CLO induced mild somnolence in 35% of subjects, GAL infusion a bitter taste in all subjects, GHRH a transient facial flushing in 40% of subjects,

Table 1 Mean ($\pm$ S.E.M.), range, 3rd and 1st centile limits of the peak GH responses to various provocative stimuli in normal adults.

	Mean ($\pm$ S.E.M.) ($\mu\text{g/l}$)	Range ($\mu\text{g/l}$)	3rd centile ($\mu\text{g/l}$)	1st centile ($\mu\text{g/l}$)
ITT (0.1 IU/kg i.v.; $n = 37$)	21.8 ± 2.8	3.0–84.0	5.3	3.8
ARG (0.5 g/kg i.v.; $n = 37$)	18.0 ± 1.6	2.9–39.5	2.9	2.7
CLO (300 $\mu\text{g/kg}$ p.o.; $n = 22$)	8.2 ± 1.6	0.3–21.5	1.5	1.3
GLU 1 mg i.m.; $n = 23$)	20.5 ± 2.2	10.6–36.9	7.6	7.1
PD (120 mg p.o.; $n = 32$)	9.6 ± 1.1	2.2–33.0	2.2	2.1
GAL (80 pmol/kg per min; $n = 16$)	9.3 ± 1.1	3.9–18.3	4.0	3.9
GHRH (1 $\mu\text{g/kg}$ i.v.; $n = 57$)	19.1 ± 1.5	2.7–55.0	5.0	3.8
GHRH + ARG ($n = 48$)	65.9 ± 5.5	13.8–171.0	17.8	15.2
GHRH + PD ($n = 38$)	50.2 ± 4.6	17.7–134.5	17.9	17.7
HEX (2.0 $\mu\text{g/kg}$; $n = 29$)	55.3 ± 5.5	13.9–163.5	21.6	16.5
GHRH + HEX (HEX, 0.25 $\mu\text{g/kg}$; $n = 31$)	86.0 ± 4.3	49.0–125.0	57.1	51.7

both when given alone and combined with PD, ARG or HEX. HEX alone caused transient facial flushing in 20% of subjects.

Discussion

In the present study, the GH responses to several old and new provocative tests in normal adults were compared in order to define the normative values of somatotroph response. The present results confirm that, among classical provocative tests of GH secretion, ITT followed by ARG and GLU are the most potent ones and possess clear limits of normality. Among non-classical provocative tests, GHRH+ARG or PD and HEX are strong stimuli of GH secretion which, however, is maximally stimulated by the combination of GHRH and low-dose HEX. The remarkable differences in the GH-releasing activities of the various tests leads to impressive differences among normative values of somatotroph responsiveness.

GH secretion in humans can be stimulated by physiological and pharmacological stimuli (9, 32–34). Physical exercise, fasting and sleep are the most recognised physiological stimuli of somatotroph secretion but are generally not used as diagnostic tools because their value is reduced by low potency, the most marked intra-individual variability and/or low compliance (9, 32). Among the notable pharmacological stimuli, the majority of them have been addressed in the present study; a notable exception being L-dopa; however, it has already been demonstrated that its GH-releasing activity in normal adults is clearly lower than that of ITT and is associated with frequent side-effects (4).

Classical and some non-classical stimuli of GH secretion, i.e. ITT, ARG, CLO, PD and GAL, act via central mechanisms involving the stimulation of GHRH-secreting neurones and/or inhibition of hypothalamic somatostatin release (33, 35). GLU releases GH only after intramuscular and subcutaneous but not intravenous administration, via mechanisms still unknown (26, 36).

GHRH, the specific stimulatory neurohormone, stimulates GH synthesis and release from somatotrophs and its action is under the fluctuating inhibitory effect of somatostatin (33, 37). In fact, substances such as ARG and PD which act via the inhibition of hypothalamic somatostatin release (21, 33, 35, 38–40) strongly potentiate the GH-releasing activity of GHRH reducing the intra-individual variability of somatotroph responsiveness to the neurohormone alone (22, 37).

On the other hand, GH-releasing peptides (GHRPs) such as HEX possess strong and reproducible GH-releasing activity in humans and truly synergise with GHRH even when given at low dose (24–28). They act on specific receptors at the pituitary, mainly at the hypothalamic level, probably antagonising somatostatin activity and increasing the activity of GHRH-secreting neurones (24–31).

In agreement with other data in normal adults, our results confirm that ITT is the most potent provocative test of GH secretion and that ARG and GLU are good classical alternatives (7, 10, 11, 13–17, 41, 42).

For each test studied, we have reported the third and the first centile limits of the normal peak GH response, in order to clarify which response below normal limits should be considered as low or severely low in patients suspected of GHD, at least in our laboratory.

Indeed, normative values of the GH response to these stimuli overlap with those reported by other authors (7, 10, 41, 42). In fact, the third and first centile limits of normal response to ITT were 5 and 3 $\mu\text{g/l}$ respectively, the last one being the limit below which severe GHD is demonstrated by ITT (11, 13). Meanwhile, our data confirm that CLO as well as PD and GAL are not good tests with which to evaluate somatotroph response in normal subjects (7, 9, 21, 33, 41, 43).

Our data again confirm that testing with GHRH alone has low value as a provocative test (9, 12, 19, 21, 37). In fact, the mean GH response to GHRH was not significantly higher than that elicited by ITT and showed absent GH responses in some normal adults.

That the somatotroph responsiveness to GHRH is strongly potentiated by PD and ARG is well recognised (18, 20, 21, 29, 39, 40). In fact, testing with GHRH+PD or ARG has already been shown to be one of the most potent stimuli of GH secretion in normal children (16) and the present data confirm this evidence in adults (12, 19, 20, 23). These tests also show good reproducibility (19, 22) and their GH-releasing activity is approximately threefold higher than that of ITT (16, 19, 23 and present study). In fact, they have been already proposed as the best alternatives to ITT for the diagnosis of GHD in adults, provided that appropriate cut-off limits are assumed (9, 13, 18, 19, 21–23).

The strong GH-releasing activity of GHRPs is again confirmed by the present results. In fact HEX alone shows a stimulatory effect of GH secretion as marked as GHRH+PD or ARG. On the other hand, the combined administration of GHRH and HEX, even given at low dose, really represents the most potent stimulus of somatotroph secretion in normal adults, being approximately fourfold more potent than ITT.

In all, this evidence strengthens once more the suggestion that the maximal secretory capacity of the somatotroph cell in normal humans is far greater than that previously thought, and further indicates that normative reference values of GH response must be appropriate to the potency of each stimulus (13, 23). When looking for alternatives to ITT as provocative tests for the diagnosis of GHD, one should not necessarily ask for the most potent test but for a potent and reproducible test without side-effects. Indeed, HEX alone and combined with GHRH as well as GHRH+PD or ARG possess these advantages (29, 31, 44). It is probable that testing including GHRH administration directly explores the pituitary GH-releasable pool, while

testing with a GHRP alone explores more the integrity of hypothalamic mechanisms involved in the control of somatotroph function. The usefulness of this subtle distinction remains to be verified from a clinical point of view.

It must be emphasised that the GH response to various provocative stimuli was generally independent of gender, a notable exception being that to ARG alone or combined with GHRH. The peculiar enhancing effect of oestrogens on the stimulatory effect of ARG on somatotroph secretion had been already shown (45) and our present data agree with this evidence.

In conclusion, this study first compared the GH-releasing activity of old and new provocative tests of GH secretion in adults between 20 and 50 years of age. ITT followed by ARG and GLU are the most potent classical provocative tests but new tests such as GHRH+ARG, GHRH+PD, HEX alone and, above all, low-dose HEX+GHRH are much more potent and show more clear normative values. It is recommended that each test is used with appropriate cut-off limits and we believe that having a high limit of the lowest normal GH response to a provocative test probably allows for better evaluation of insufficient GH responses. However, following the guidelines of the recent GH Research Society consensus (13), it is suggested that normative values are defined in each laboratory employing the same GH assay. In fact, the variability between different assay methods is remarkable and could affect the interpretation of the results (46).

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