Interaction between glucagon and human corticotropin-releasing hormone or vasopressin on ACTH and cortisol secretion in humans

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Abstract

Objective: It is known that glucagon administration elicits ACTH and cortisol responses in humans, although this effect takes place after intramuscular or subcutaneous but not after the intravenous route of administration. The mechanisms underlying this stimulatory effect on corticotroph secretion are unknown but they are unrelated to glucose variations and stress-mediated actions.

Design and Methods: To throw further light on the stimulatory effect of i.m. glucagon on the pituitary–adrenal axis, using six normal young female volunteers (26–32 years, body mass index 19.7–22.5 kg/m²) we studied the interaction between glucagon (GLU; 0.017 mg/kg i.m.) and human corticotropin-releasing hormone (hCRH; 2.0 μg/kg i.v.) or vasopressin (AVP; 0.17 U/kg i.m.). The interactions between hCRH and AVP on the hypothalamo–pituitary–adrenal (HPA) axis and the GH response to GLU alone or combined with hCRH or AVP were also studied.

Results: GLU i.m. administration elicited a clear increase in ACTH (peak vs baseline, means ± SEM: 11.6 ± 3.3 vs 4.2 ± 0.3 pmol/l, P < 0.05) and cortisol (613.5 ± 65.6 vs 436.9 ± 19.3 nmol/l, P < 0.05) and GH levels (11.6 ± 3.4 vs 3.3 ± 0.7 μg/l, P < 0.05). The ACTH response to GLU (area under the curve: 426.4 ± 80.9 pmol/l per 120 min) was higher than that to AVP (206.3 ± 38.8 pmol/l per 120 min, P < 0.02) and that to hCRH (299.8 ± 39.8 pmol/l per 120 min) although this latter difference did not attain statistical significance. The GLU-induced cortisol response (28 336.9 ± 2430.7 nmol/l per 120 min) was similar to those after hCRH (24 099.2 ± 2075.2 nmol/l per 120 min) and AVP (21 808.7 ± 1948.2 nmol/l per 120 min). GLU and hCRH had an additive effect on ACTH (964.9 ± 106.6 pmol/l per 120 min, P < 0.02) and a less than additive effect on cortisol levels (35 542.5 ± 2720.2 nmol/l per 120 min). Similarly, GLU and AVP had an additive effect on ACTH (825.6 ± 139.6 pmol/l per 120 min, P < 0.02) and an effect less than additive on cortisol levels (33 059.2 ± 1965.3 nmol/l per 120 min). The effects of GLU co-administered with hCRH or AVP were similar to those of the combined administration of hCRH and AVP on ACTH (906.0 ± 152.7 pmol/l per 120 min) and cortisol (34 383.5 ± 1669.2 nmol/l per 120 min) levels. The GH response to GLU was not modified by hCRH or AVP.

Conclusions: These results show that i.m. glucagon administration is a provocative stimulus of ACTH and cortisol secretion, at least as potent as hCRH and AVP. The ACTH-releasing effect of i.m. glucagon is not mediated by selective CRH or AVP stimulation but the possibility that both neurohormones play a role could be hypothesized.

Introduction

It has been known for many years that intramuscular or subcutaneous administration of glucagon induces a clear increase in growth hormone (GH) and cortisol levels in humans (1–8). In fact, glucagon is considered a classical provocative stimulus of GH secretion for the diagnosis of GH deficiency (2–5, 8) and has been proposed by some authors as an alternative to insulin-induced hypoglycemia for the diagnosis of secondary adrenal insufficiency (4, 5, 7).

Several reports have shown that glucagon stimulates somatotroph and corticotroph secretion after intramuscular or subcutaneous but not after acute intravenous administration (9–12), suggesting that glucagon per se is not a GH and an adrenocorticotropic (ACTH) secretagogue. It has been hypothesized that intramuscular glucagon proteolysis could generate a peptidyl...
fragment endowed with GH- and ACTH-releasing activity (10, 11). This could represent a new factor involved in the control of anterior pituitary function.

The mechanisms underlying the GH-releasing effect of glucagon could involve the inhibition of hypothalamic somatostatin release, while a GH-releasing hormone (GHRH)-mediated action or a direct pituitary effect seem to be unlikely (10, 12). On the other hand, a role of glucagon-induced free fatty acids (13), but not glycemic (1, 4, 10, 11, 14) variations have also been suggested.

The mechanisms underlying the stimulatory effect of glucagon on the hypothalamo–pituitary–adrenal (HPA) axis are even less clear. Until now, it has been known that the stimulatory effect on cortisol secretion is dependent on the ACTH response which follows i.m. glucagon administration (4). Moreover, the HPA response to i.m. glucagon is unlikely to be related to glucose variations and stress-mediated actions (1, 4, 10, 11, 14). Moreover, glucagon does not act at the pituitary level (10).

In order to clarify the ACTH-releasing activity of i.m. glucagon in humans we studied its interaction with human corticotropin-releasing hormone (hCRH) and arginine-vasopressin (AVP), two hypophysiotropic neurohormones which play a major role in the neural control of the HPA axis (15). These results were compared with those observed after the combined administration of hCRH and AVP. As CRH has been reported to play an inhibitory role on somatotroph secretion (16–20), we also verified the effect of hCRH or AVP on the glucagon-induced GH rise.

Subjects and methods

Peptides and drugs

Vials containing 1 mg glucagon (GLU) were purchased from Novo Nordisk ( Bagsvaerd, Denmark), vials containing 100 μg lyophilized hCRH were purchased from Ferring (Copenhagen, Denmark) and vials containing 10 IU AVP were purchased from Parke-Davis (New York, USA).

Study design

Six normal young women (age 26–32 years, body mass index 19.7–22.5 kg/m²) were studied in their early follicular phase. The study had been approved by the independent Ethical Committee of the University of Turin and informed consent was obtained from all subjects.

All subjects underwent the following treatments, at least 3 days apart. (a) i.m. GLU (0.017 mg/kg as a bolus at 0 min) followed by i.v. placebo (1.0 ml saline at 90 min); (b) i.m. placebo (at 0 min) followed by i.v. hCRH (2.0 μg/kg as a bolus at 90 min); (c) i.m. placebo (at 0 min) followed by i.m. AVP (0.17 IU/kg as a bolus at 90 min); (d) GLU (at 0 min) + hCRH (at 90 min); (e) GLU (at 0 min) + AVP (at 90 min); (f) hCRH (at 90 min) + AVP (at 90 min) preceded by placebo (at 0 min).

The tests started between 0730 and 0800 h after an overnight fast and 30 min after venous cannulation which was kept patent by slow infusion of isotonic saline. Blood samples for ACTH, cortisol, GH and glucose measurements were taken at 30-min intervals from 0 min to 90 min and at 15-min intervals from 90 to 210 min. All samples from an individual subject were analyzed at the same time.

Plasma ACTH levels were measured in duplicate by immunoradiometric assay (Allegro HS-ACTH; Nichols Institute Diagnostic, San Juan Capistrano, CA, USA). The sensitivity of the assay was 0.22 pmol/l. The inter- and intra-assay coefficients of variation ranged from 6.9 to 8.9% and from 1.1 to 3.0% respectively.

Serum cortisol levels were measured in duplicate by radioimmunoassay (CORT-CTK 125 RIA; Sorin, Saluggia, Italy). The sensitivity of the assay was 11.0 nmol/l. The inter- and intra-assay coefficients of variation ranged from 6.6 to 7.5% and from 3.8 to 6.6% respectively.

Serum GH levels were measured in duplicate by immunoradiometric assay (hGH-CTK IRMA; Sorin, Seluggia, Italy). The sensitivity of the assay was 0.15 μg/l. The inter- and intra-assay coefficients of variation were 2.9–4.5% and 2.4–4.0% respectively.

Plasma glucose levels (nmol/l) were measured by gluco-oxidase colorimetric method (Menarini Diagnostic, Florence, Italy).

Hormonal responses (means ± S.E.M.) are expressed as absolute levels and areas under response curve (AUC; from 90 to 210 min).

The statistical analysis was carried out using a non-parametric ANOVA (Freedman test) and then with the Wilcoxon test.

Results

Administration of GLU i.m. elicited a significant increase in the levels of ACTH (peak vs baseline, at 165 min: 11.6 ± 3.3 vs 4.2 ± 0.3 pmol/l, P < 0.05), cortisol (peak at 180 min: 613.5 ± 65.6 vs 436.9 ± 19.3 nmol/l, P < 0.05) and GH (peak at 150 min: 11.6 ± 3.4 vs 3.3 ± 0.7 μg/l, P < 0.05) (Figs 1 and 2).

The ACTH response to GLU (AUC; 426.4 ± 80.9 pmol/l per 120 min) was higher than that to AVP (206.3 ± 38.8 pmol/l per 120 min, P < 0.02) but not significantly different from that to hCRH (299.8 ± 39.8 pmol/l per 120 min). On the other hand, the GLU-induced cortisol response (28 336.9 ± 24 307.7 nmol/l per 120 min) was similar to those after hCRH (24 099.2 ± 20 752.2 nmol/l per 120 min) and AVP (21 808.7 ± 19 482.2 nmol/l per 120 min) (Figs 1 and 3).

GLU and hCRH had additive effects on ACTH (964.9 ± 106.6 pmol/l per 120 min, P < 0.02) and less than additive effects on cortisol levels (35 542.0 ±
2720.2 nmol/l per 120 min). Similarly, GLU and AVP had additive effects on ACTH (825.6 ± 139.6 pmol/l per 120 min, $P < 0.02$ vs the hormonal responses to the single stimulation) and an effect less than additive on cortisol levels (33 059.2 ± 1965.3 nmol/l per 120 min) (Fig. 3).

The ACTH- and cortisol-releasing effect of the co-administration of GLU and hCRH or AVP overlapped that of the combined administration of hCRH and AVP which showed, however, a synergistic effect on ACTH (906.0 ± 152.7 pmol/l per 120 min, $P < 0.05$ vs the arithmetic sum of the hormonal responses to the single stimulation) and an additive effect on cortisol (34 383.5 ± 1669.2 nmol/l per 120 min, $P < 0.05$ vs the hormonal responses to the single stimulation) levels (Fig. 3).

The GH response to GLU (479.1 ± 115.7 µg/l per 120 min) was not significantly modified by hCRH (377.9 ± 61.7 µg/l per 120 min) or AVP (425.3 ± 157.6 µg/l per 120 min) (Fig. 2).

GLU administration induced biphasic variations in blood glucose levels: a significant increase was recorded at 30 min (5.9 ± 0.2 vs 4.1 ± 0.14 nmol/l, $P < 0.05$) followed by a decrease at 180 min (3.2 ± 0.2 nmol/l,

**Figure 1** Mean ± S.E.M. ACTH and cortisol responses to i.m. glucagon, hCRH or AVP in six normal subjects.

**Figure 2** Mean ± S.E.M. GH responses to i.m. GLU alone or combined with hCRH or AVP in six normal subjects.
The GLU-induced blood glucose variations were not modified by hCRH or AVP co-administration.

**Side-effects**

Mild and transient nausea was recorded in four subjects after the intramuscular administration of GLU. Facial flushing was induced by hCRH in all subjects. Nausea and vasoconstriction was observed in all subjects while contraction of diuresis was recorded in two subjects after AVP administration. Co-administration of the various drugs slightly increased the side-effects observed after single drug administration but neither stopping the testing nor medication were required.

**Discussion**

The results of the present study demonstrated that: (a) the intramuscular administration of glucagon releases ACTH and cortisol to the same extent as hCRH and more than AVP; (b) the co-administration of i.m. glucagon and hCRH or AVP has an additive effect on ACTH release so that these corticotroph responses became similar to that recorded after the synergistical effect of hCRH and AVP; and (c) the GH response which follows the intramuscular administration of glucagon is not modified by hCRH or AVP.

It is well known that i.m. glucagon administration is followed by a clear increase in ACTH and cortisol secretion (4, 5). In fact, testing with glucagon is considered a reliable alternative to insulin-induced hypoglycemia for the diagnosis of secondary adrenal insufficiency (4, 5, 7). Our present data showing that i.m. glucagon alone represents a stimulus at least as potent as hCRH and stronger than AVP further indicates its potency as a provocative stimulus of ACTH and cortisol reserve.

The mechanisms underlying the stimulatory effect of glucagon on the HPA axis are still unknown. It is worthy of note that this stimulatory effect follows the intramuscular or subcutaneous but not the acute intravenous administration of glucagon, suggesting that this hormone per se is not a true ACTH secretagogue (9–12). It has been hypothesized that intramuscular glucagon proteolysis could generate a peptidyl fragment endowed with ACTH-releasing activity (11).

The stimulatory effect of i.m. glucagon on the HPA axis is not likely to be explained by stress-related mechanisms due to unpleasant side-effects, such as nausea and vomiting. In fact, side-effects are generally negligible after intramuscular administration in comparison with those recorded after intravenous injection, which had no effect on the HPA axis (11). Moreover, though catecholamines play a well-known stimulatory
role on the HPA axis (21), glucagon administration induces catecholamine release after both intravenous and intramuscular administration (22, 23), but only the latter is followed by hormonal release (11).

The positive effect of i.m. glucagon on the HPA axis does not seem to be due to metabolic mechanisms. The stimulatory effect of absolute and even relative hypoglycemia on ACTH and cortisol secretion is well known (21, 24). Indeed, glucagon administration is followed by a prompt increase in blood glucose levels and later relative hypoglycemia but these effects overlap after both intravenous and intramuscular administration (10,11); this evidence makes it unlikely that the ACTH and cortisol increase which follow i.m. glucagon administration reflects the response of the HPA axis to relative hypoglycemia.

It is widely accepted that CRH and AVP play the major neurohormonal role in the control of the HPA axis (15, 21). Our aim was to clarify the possibility that the stimulatory effect of i.m. glucagon on corticotroph secretion could be due to a selective mediation by CRH or AVP, which showed the well-known synergistic effect on corticotroph release (15). We found that the administration of either hCRH or AVP with i.m. glucagon had only additive effects on ACTH secretion and this evidence makes the hypothesis that the stimulatory effect of i.m. glucagon is selectively mediated by endogenous CRH or AVP unlikely. However, it is noteworthy that the ACTH response to the combined administration of i.m. glucagon with hCRH or AVP became similar to that recorded as a result of the synergistic effect of hCRH and AVP. Thus, it could be hypothesized that both neurohormones play a role in mediating the corticotroph rise which follows the intramuscular administration of glucagon. On the other hand, the possibility that the HPA response to i.m. glucagon is mediated by unknown mechanisms other than CRH and AVP must be taken into account. It is clear that our study provided only indirect evidence and that animal studies such as those evaluating neurohormonal levels in the portal blood could give more direct information about this hypothesis.

Our present data also confirm the well-known stimulatory effect of i.m. glucagon on GH secretion in humans (1–7) and show that this effect is not modified by co-administration with both CRH and AVP. An inhibition of hypothalamic somatostatin release has been proposed to explain the stimulatory effect of i.m. glucagon on somatotroph secretion (10–13). On the other hand, it has been reported by some (17, 18, 20), but not by other authors (25–27), that hCRH inhibits the GHRIH-induced GH response probably via enhancement of the hypothalamic somatostatin release (28). Our present results do not allow an increase in the understanding of the mechanisms underlying the GH-releasing effect of intramuscular glucagon administration while they make it more unlikely that CRH has a major inhibitory role on somatotroph secretion in humans.

In conclusion, the results of the present study, though indirect, emphasize the stimulatory effect of intramuscular glucagon administration on the HPA axis and indicate that it is not mediated by selective CRH or AVP stimulation.

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