CLINICAL STUDY

Acute hyperglycemia and activation of the β -adrenergic system exhibit synergistic inhibitory actions on growth hormone (GH) releasing hormone-induced GH release

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Abstract

Objective: Acute hyperglycemia stimulates somatostatin (SRIH) release by the hypothalamus which, in turn, suppresses growth hormone (GH) secretion from the anterior pituitary gland. Although it has been suggested that the cholinergic pathway mediates glucose-induced SRIH release, other regulatory systems have not been examined. Therefore, we investigated whether blocking or activating the β -adrenergic pathway alters glucose-mediated inhibition of GH release.

Design and methods: One set of experiments was performed with a β -adrenergic antagonist, propranolol, and the other set with a β -adrenergic agonist, isoproterenol. Each set of experiments was performed in ten healthy subjects and consisted of four tests. Test 1, a 100 μ g GHRH bolus i.v. at 0 min; test 2, 100 g glucose orally at –30 min, followed by a 100 μ g GHRH bolus at 0 min; test 3, after a 100 μ g GHRH bolus i.v. at 0 min, a continuous infusion of propranolol (0.2 mg/kg) or isoproterenol (0.012 μ g/kg) was administered between 0 and 120 min; test 4, after a 100 g glucose oral load at –30 min, and a 100 μ g GHRH bolus i.v. at 0 min, a continuous infusion of propranolol (0.2 mg/kg) or isoproterenol (0.012 μ g/kg) was administered between 0 and 120 min. Blood was drawn every 10 min from –30 min to 120 min to measure GH and glucose concentrations.

Results: Pretreatment with glucose significantly suppressed GHRH-induced GH secretion. Propranolol infusion significantly increased the GHRH-induced GH secretion, but it did not block glucose-induced suppression of GH secretion. Isoproterenol infusion alone significantly suppressed GHRH-induced GH secretion and augmented the inhibitory action of glucose on GH release.

Conclusion: This study demonstrates that glucose-induced suppression of GHRH-stimulated GH release is independent of β -adrenergic tone. Since previous data supports a role for SRIH in both glucose and β -adrenergic suppression of GH release, the current results suggest that subsets of SRIH neurons are differentially responsive to these external cues. Therefore, a combined glucose and isoproterenol test may provide a useful assessment of hypothalamic somatostatinergic activity.

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Introduction

Acute hyperglycemia suppresses basal growth hormone (GH) secretion (1, 2) and the GH response to GH releasing hormone (GHRH) in normal subjects (3–5). The latter effect is reversed by the administration of pyridostigmine (6), a cholinesterase inhibitor, which is known to suppress hypothalamic somatostatin (SRIH) release (7–12). These studies suggest that the cholinergic pathway may mediate acute hyperglycemia-induced SRIH release. However, the SRIH-containing nerve fibers receive a variety of neural inputs from the principal ascending monoaminergic system (13, 14). Thus, the possibility that other monoaminergic pathways

are involved in the glucose-induced release of SRIH remains to be investigated.

Among the possible monoaminergic pathways, the β -adrenergic pathway deserves consideration as a candidate in this context. Isoproterenol, a β -adrenergic receptor agonist, suppresses GHRH-induced GH release *in vivo* but not *in vitro* (15). Since pretreatment of rats with SRIH antiserum prevents the inhibitory action of isoproterenol on GHRH-induced GH release (16, 17), it is believed that activation of the central β -adrenergic pathway enhances hypothalamic SRIH release. Such a pathway is supported by the observations that propranolol, a β -adrenergic receptor antagonist, enhances GH release induced by hypoglycemic stress (18),

exercise (19), glucagon (20) and GHRH (21, 22), and also inhibits SRIH release (21, 23, 24) in humans.

Therefore, we asked ourselves whether the β -adrenergic-sensitive SRIH neurons are the same SRIH neurons that are activated by hyperglycemia. To answer this question we investigated the effects of a β -adrenergic receptor antagonist and β -adrenergic receptor agonist on glucose-mediated suppression of the GHRH-induced GH release.

Subjects and methods

Subjects

Twenty healthy young men, aged 20 to 26 years, were enrolled. Ten of them were assigned to experiment 1 and the other ten to experiment 2. Experiments were performed after receiving informed consent and approval from the ethical committee at Kyunghee University Hospital. No subject had received medication during the 2-week period prior to the study and none were obese. Their mean body weight was 66.7 ± 3.9 kg and their mean body mass index was 21.7 ± 0.8 kg/m². None of them had a family history of diabetes mellitus or obesity.

Endocrine tests

The study consisted of two sets of experiments. Each experiment consisted of four tests, which were conducted as described below, in a random order and on four separate occasions, at least 1 week apart. The subjects were fasted from midnight until the end of each test. At 0900 h, an indwelling venous cannula was placed into a forearm vein 1 h prior to the collection of the first blood sample at -30 min and kept open with a slow infusion of 0.9% normal saline. Each subject remained comfortably seated in a chair throughout the test period; no one was allowed to sleep. One set of experiments was performed with the β -adrenergic antagonist, propranolol, and the other set with the β -adrenergic agonist, isoproterenol. Each set of experiments was performed in ten healthy subjects and consisted of four tests. Test 1, a 100 μ g GHRH (Bachem, Torrance, CA, USA) bolus was given at 0 min; test 2, 100 g glucose, dissolved in 300 ml water was given orally at -30 min and a 100 μ g GHRH bolus was given at 0 min; test 3, after a 100 μ g GHRH bolus at 0 min, propranolol (0.2 mg/kg; Inderal, ICI, London, UK) or isoproterenol (0.012 μ g/kg; Isuprel, Sanofi Winthrop, New York, USA) was continuously administered between 0 and 120 min; test 4, after a 100 g glucose oral load at -30 min, and a 100 μ g GHRH bolus at 0 min, propranolol (0.2 mg/kg) or isoproterenol (0.012 μ g/kg) was continuously administered between 0 and 120 min. Blood was drawn every 10 min from -30 min to 120 min to measure GH and glucose concentrations.

Assays

Plasma was separated immediately and frozen at -70°C until the assays were performed. Plasma glucose was measured using the glucose oxidase method on an autoanalyzer. Plasma GH concentration was determined by a commercial immunoradiometric assay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The sensitivity was 0.02 μ g/l, the intra-assay variation was 3.3% and the interassay variation was 5.1%.

Statistics

Data are expressed as means $\pm$ s.e.m. Statistical comparisons of the glucose levels and GH peak values were made using one-way repeated measures analysis of variance (ANOVA). Post-ANOVA comparisons were made using Tukey's multiple comparison tests. The entire secretory response to GHRH was assessed by the calculation of the area under the curve (AUC) by the trapezoidal integration. A simple two-factor ANOVA comparison without repeated measures was used for the statistical comparison of the AUC. All statistical analyses were performed using statistical software (GraphPad Prism, GraphPad Software Inc., San Diego, CA, USA). Significance was defined as $P < 0.05$.

Results

Experiment 1: the use of a β -adrenergic receptor antagonist

Figure 1 (A and B) shows glucose and GH responses to GHRH (test 1), GHRH and glucose (test 2), GHRH and propranolol (test 3), and GHRH, glucose and propranolol (test 4). GHRH significantly increased GH secretion with a peak of 7.8 ± 3.0 μ g/l at 50 min in test 1, but did not change the plasma glucose concentration. In test 2, the glucose pretreatment induced acute hyperglycemia with the peak of 7.9 ± 0.4 mmol/l at 20 min and significantly suppressed the peak level of GHRH-induced GH level (5.0 ± 1.1 μ g/l vs 7.8 ± 3.0 μ g/l, $P < 0.05$). In test 3, propranolol infusion significantly increased the GHRH-induced GH levels between 30 and 120 min, with a peak of 18.9 ± 4.1 μ g/l at 50 min, compared with those in test 1 ($P < 0.05$), without affecting the plasma glucose concentration. However, in test 4 the propranolol infusion failed to reverse the suppression of GHRH-induced GH secretion by glucose. The mean of the GH peaks in test 4 did not differ significantly from that in test 2 (5.4 ± 1.1 μ g/l vs 5.0 ± 1.1 μ g/l). Figure 1(C) shows changes in the mean AUC by the administration of GHRH (674.2 ± 281.2 μ g $\times$ l⁻¹ $\times$ 120 min⁻¹), GHRH and glucose (371.9 ± 71.6 μ g $\times$ l⁻¹ $\times$ 120 min⁻¹), GHRH and propranolol (1596.0 ± 377.0 μ g $\times$ l⁻¹ $\times$ 120 min⁻¹), and GHRH,

Figure 1 Effect of propranolol (Pro) on the suppression by glucose (Glu) of GHRH-induced GH secretion. (A) Changes in plasma glucose levels. (B) Changes in plasma GH levels. (C) Mean GH areas under the curve (AUC). The data represent means $\pm$ S.E.M. Statistical comparisons of the glucose levels and GH peak values were made using one-way repeated measures ANOVA and Tukey's multiple comparison tests. A simple two-factor ANOVA comparison without repeated measures was used for the statistical analysis of the AUC. Significance was defined as $P < 0.05$. * $P < 0.01$ vs test 1 and 3; † $P < 0.01$ vs test 3; ‡ $P < 0.05$ vs test 1.

glucose and propranolol ($539.7 \pm 99.0 \mu\text{g} \times \text{l}^{-1} \times 120 \text{ min}^{-1}$). Statistical comparisons of the AUC revealed the same results as those of the peak GH values. During the infusion of propranolol, no specific adverse reaction or change in blood pressure was observed.

Experiment 2: the use of β -adrenergic receptor agonist

Figure 2 (A and B) shows glucose and GH responses to GHRH (test 1), GHRH and glucose (test 2), GHRH and isoproterenol (test 3), and GHRH, glucose and isoproterenol (test 4). Administration of GHRH induced a significant increase in the GH levels, which peaked at $25.2 \pm 2.3 \mu\text{g/l}$ after 50 min in test 1, while it did not change the plasma glucose concentration. The percentage increase in GH levels in this experiment was 2.5-fold greater than that in experiment 1. It should be noted that the subjects assigned to experiment 1 and experiment 2 were different. Considerable variabilities in GH responsiveness to GHRH among individual subjects in normal men (25, 26) and acromegaly (27) have been reported. Therefore, different GH responses to GHRH seen in this study were considered within the normal range. Acute hyperglycemia significantly suppressed the peak GHRH-induced GH secretion ($14.3 \pm 1.3 \mu\text{g/l}$ vs $25.2 \pm 2.3 \mu\text{g/l}$, $P < 0.01$) as in experiment 1 (Fig. 2). Continuous infusion of isoproterenol also significantly suppressed GHRH-induced GH levels, with a peak of $9.5 \pm 0.9 \mu\text{g/l}$ at 40 min, compared with those in test 1 ($P < 0.01$), without affecting the plasma glucose concentration. However, GH levels did not differ significantly from those observed after the glucose pretreatment in test 2. When glucose and isoproterenol were used together in test 4, the GHRH-induced GH levels were significantly lower than those of the glucose only pretreatment. GH levels did not differ significantly from those observed when only isoproterenol was infused (Fig. 2). Figure 2(C) shows changes in the mean AUC by the administration of GHRH ($1963.0 \pm 647.2 \mu\text{g} \times \text{l}^{-1} \times 120 \text{ min}^{-1}$), GHRH and glucose ($775.7 \pm 420.0 \mu\text{g} \times \text{l}^{-1} \times 120 \text{ min}^{-1}$), GHRH and isoproterenol ($507.1 \pm 168.7 \mu\text{g} \times \text{l}^{-1} \times 120 \text{ min}^{-1}$), and GHRH, glucose and isoproterenol ($204.0 \pm 38.0 \mu\text{g} \times \text{l}^{-1} \times 120 \text{ min}^{-1}$). Statistical analysis of the AUC showed the same results as those of the peak GH values. During the infusion of isoproterenol, mild facial flushing, palpitation and fine tremor were noticed without a significant increase in blood pressure in the majority of subjects, but this was tolerated by all the subjects.

Discussion

Acute hyperglycemia exerts its action on GH secretion through specific hypothalamic structures, but the precise mechanism of its action is unknown. In the

Figure 2 Effect of isoproterenol (Iso) on the suppression by glucose (Glu) of GHRH-induced GH secretion. (A) Changes in plasma glucose levels. (B) Changes in plasma GH levels. (C) Mean GH areas under the curve (AUC). The data represent means $\pm$ S.E.M.. Statistical comparisons of the glucose levels and GH peak values were made using one-way repeated measures ANOVA and Tukey's multiple comparison tests. A simple two-factor ANOVA comparison without repeated measures was used for the statistical analysis of the AUC. Significance was defined as $P < 0.05$. * $P < 0.01$ vs test 1 and 3; † $P < 0.01$ vs test 1; ‡ $P < 0.05$ vs test 2.

human, acute hyperglycemia blocks GHRH-induced GH release, which excludes the possibility that glucose may act by inhibiting endogenous GHRH release (3, 4, 28). These reports suggested that glucose might act in a manner similar to free fatty acids (29), that is, by directly inhibiting pituitary somatotropes or by inducing hypothalamic SRIH release, or by a combination of both mechanisms. In favor of glucose-mediated induction of SRIH tone, Penalva *et al.* (6) demonstrated that pyridostigmine, a cholinesterase inhibitor, causes a reversal of the glucose-induced suppression of GHRH-induced GH secretion, and that hyperglycemia is unable to reduce the potentiating effect of pyridostigmine on GH secretion elicited by GHRH. Since acetylcholine suppresses hypothalamic SRIH release (30–33), they suggested that acute hyperglycemia suppresses GH release from the anterior pituitary gland by cholinergic stimulation of hypothalamic SRIH release.

Although strong evidence exists for a primary role of a central cholinergic signaling pathway in glucose-induced inhibition of GH release, other monoaminergic pathways may also be involved because the SRIH-containing nerve fibers, located primarily in the anterior hypothalamic periventricular nucleus, receive a variety of neural inputs from the principal ascending monoaminergic system (13, 14). In fact, an earlier study (34) demonstrated that propranolol, a β -adrenergic antagonist, augments the GH response by inhibiting SRIH release from dispersed rat hypothalamic cells. Pretreatment of animals with SRIH antiserum prevented the inhibitory action of isoproterenol on GHRH-induced GH release. Since Krieg *et al.* (16, 17) demonstrated that isoproterenol could not suppress the GHRH-induced GH release of dispersed pituitary cells, these studies suggest that the β -adrenergic pathway stimulates hypothalamic SRIH release.

In the present study we sought to determine if the SRIH neurons modulated by the glucose and catecholaminergic stimulation may be one and the same. To this end we examined the effects of glucose on GHRH-stimulated GH release in combination with either a β -adrenergic antagonist or an agonist. Pretreatment with propranolol did not block the suppressive effect of glucose on GHRH-induced GH secretion. These findings demonstrate that glucose-induced suppression of GH release is not mediated by the β -adrenergic pathway. In that the degree of suppression induced by glucose and isoproterenol was comparable while the combined effects were significantly higher than that achieved by glucose and isoproterenol alone, these observations suggest that SRIH neurons mediated by hyperglycemia and catecholamines exist as functionally distinct populations.

In conclusion, the results of this study combined with the work of others indicate that the inhibition of the GH response to GHRH by oral glucose administration is mediated by an increase in hypothalamic

somatostatinergic activity independent of changes in β -adrenergic tone. In that experimental evidence exists that both the activation of the central β -adrenergic system and hyperglycemia suppress GH release by stimulating SRIH release, it can be inferred that there are distinct subpopulations of SRIH neurons that respond differentially to these external cues. Therefore, a combined glucose and isoproterenol test may provide a useful assessment of hypothalamic somatostatinergic activity.

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