The influence of insulin on circulating ghrelin

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Flanagan, Daniel E., Mark L. Evans, Teresa P. Monsod, Frances Rife, Rubina A. Heptulla, William V. Tamborlane, and Robert S. Sherwin. The influence of insulin on circulating ghrelin. Am J Physiol Endocrinol Metab 284: E313–E316, 2003; 10.1152/ajpendo.00569.2001.—Ghrelin is a novel peptide that acts on the growth hormone (GH) secretagogue receptor in the pituitary and hypothalamus. It may function as a third physiological regulator of GH secretion, along with GH-releasing hormone and somatostatin. In addition to the action of ghrelin on the GH axis, it appears to have a role in the determination of energy homeostasis. Although feeding suppresses ghrelin production and fasting stimulates ghrelin release, the underlying mechanisms controlling this process remain unclear. The purpose of this study was to test the hypotheses, by use of a stepped hyperinsulineemic eu-hyperglycemic glucose clamp, that either hyperinsulinemia or hypoglycemia may influence ghrelin production. Having been stable in the period before the clamp, ghrelin levels rapidly fell in response to insulin infusion during euglycemia (baseline ghrelin 207 ± 12 vs. 169 ± 10 fmol/ml at t = 30 min, P < 0.001). Ghrelin remained suppressed during subsequent periods of hypoglycemia (mean glucose 53 ± 2 mg/dl) and hyperglycemia (mean glucose 163 ± 6 mg/dl). Despite suppression of ghrelin, GH showed a significant rise during hypoglycemia (baseline 4.1 ± 1.3 vs. 28.2 ± 3.9 μg/l at t = 120 min, P < 0.001). Our data suggest that insulin may suppress circulating ghrelin independently of glucose, although glucose may have an additional effect. We conclude that the GH response seen during hypoglycemia is not regulated by circulating ghrelin.

Growth hormone; somatostatin; hypothalamus; hypoglycemia; glucose clamp

Ghrelin is a novel peptide that acts on the growth hormone (GH) secretagogue receptor in the pituitary and hypothalamus, possibly functioning as a third physiological regulator of GH secretion along with GH-releasing hormone (GHRH) and somatostatin. In addition to the action of ghrelin on the GH axis, it appears to have a role in the determination of energy homeostasis (3, 12, 13). Ghrelin acts as an orexigenic hormone, stimulating both neuropeptide Y (NPY) and agouti-related peptide, and thus feeding (14, 21). Although feeding suppresses ghrelin production and fasting stimulates ghrelin release, the underlying mechanisms controlling these processes remain unclear (4, 19). This relationship is the opposite of that seen with leptin (14), which has been shown to be increased by insulin (18). Specifically, the roles that alterations in plasma glucose and insulin have in regulating ghrelin secretion have not been established. To address this issue, we employed a stepped hyperinsulineemic eu-hypo hyperglycemic glucose clamp. This procedure allowed us to examine the ghrelin response to marked variations in circulating concentrations of insulin and glucose in human subjects.

Methods

Eleven young adult volunteers (9 women, 2 men) participated in the study. The age of the subjects was 24 ± 4 yr (range 18–31 yr), and the body mass index was 22.1 ± 2.8 kg/m² (18.4–26.6 kg/m²). All subjects were healthy and taking no medication. They were instructed to maintain their usual diet and activity level. On the day of the study, subjects were admitted at 8:00 AM to the Yale General Clinical Research Center. Before the study, a small Teflon catheter was inserted into an antecubital vein for infusion of insulin and glucose. A second catheter was inserted in a retrograde direction into a wrist vein in the opposite arm for blood sampling. This was kept patent with a slow infusion of isotonic saline. The hand was then placed in a heated box to achieve a temperature of 65°C to obtain arterialized blood through the wrist catheter.

After a baseline period of 1 h, a three-step eu-hyperglycemic glucose clamp was then performed (7). A primed continuous infusion of insulin was administered at a rate of 1 mU·kg⁻¹·min⁻¹. Each step of the study was maintained for 60 min, with a 15-min period of adjustment between steps. Throughout the study, plasma glucose concentrations were monitored every 5 min and used to regulate plasma glucose by the adjustment of a variable infusion of 20% dextrose. Plasma glucose was maintained at 90 mg/dl during the euglycemic phase of the study, at 50 mg/dl during hypoglycemia, and at 160 mg/dl during hyperglycemia. Two samples for measurement of insulin, GH, and ghrelin were taken in the hour preceding the study and repeated during the three steps of the clamp procedure. The glucose and insulin data from these studies are included in another
study that examined the accuracy of glucose sensor measurements (11).

Analytical procedures. All arterialized venous blood samples were immediately centrifuged and frozen at −70°C until analyzed. Glucose levels were determined in duplicate by the glucose oxidase method with a Beckman glucose analyzer (Beckman Instruments, Brea, CA). Plasma insulin (Linco Research, St. Charles, MO) and GH (ICN Pharmaceuticals, Costa Mesa, CA) were measured by use of double-antibody commercially available radioimmunoassays. Human plasma ghrelin was measured with a commercial radioimmunoassay (Phoenix Pharmaceuticals, Belmont, CA) that uses 125I-labeled bioactive ghrelin as a tracer molecule and a polyclonal antibody raised in rabbits against full-length octanoylated human ghrelin (intra-assay coefficient of variation 4%). No cross-reactivity with human secretin, human vasoactive intestinal peptide, human galanin, human GHRH, NPY, or other relevant molecules has been reported (20). Ghrelin concentrations are expressed in femtomoles per milliliter (conversion factor for pg/ml is \(3.372\)).

Statistical analyses. Data are presented as means ± SE. Statistical differences between hormonal responses during baseline, euglycemia, hypoglycemia, and hyperglycemia were analyzed using repeated-measures ANOVA. Paired sample t-tests were used to localize effects found in the initial set of ANOVA. All analyses were performed using SPSS for Windows, version 10.0.0.

RESULTS

Figure 1, A and B, shows the glucose and insulin concentrations during the clamp. During the hour before the clamp, venous plasma glucose remained at ~90 mg/dl and was maintained at this level for the first hour of hyperinsulinemic euglycemia. Plasma glucose was then allowed to fall to a mean level of 53 ± 2 mg/dl and was maintained at this level for the next 60 min. Plasma glucose was then raised to 163 ± 6 mg/dl and maintained at this level for the final hour of the study. The mean fasting insulin level before the clamp was 9.4 ± 0.5 μU/ml and rose at the commencement of the study to 64.0 ± 3.3 μU/ml. This level was maintained for the length of the study; there was no significant change in circulating insulin concentration after the 30-min time point.

Figure 1, C and D, shows the venous plasma ghrelin and GH concentrations over the course of the study. Baseline plasma ghrelin concentration immediately before the insulin infusion was 207 ± 12 fmol/ml for 11 subjects. Plasma ghrelin remained unchanged during the 60-min period before the clamp (\(P = 0.581\)). After commencement of the insulin infusion, mean ghrelin concentration fell rapidly to 169 ± 10 fmol/ml during euglycemia (\(P < 0.001\) for difference from baseline) and remained suppressed for the duration of the study. There was no significant difference in ghrelin levels between euglycemia and hypoglycemia. During the final hour of hyperglycemia, ghrelin fell significantly further (142 ± 13 fmol/ml at 210 min vs. 164 ± 12 fmol/ml at 135 min, \(P = 0.017\)).

Baseline GH concentration before the clamp was 4.1 ± 1.3 μg/l, having been unchanged in the previous...
hour. GH showed no significant change during the period of hyperinsulinemic euglycemia but showed a significant rise after the onset of hypoglycemia; mean GH at $t = 120$ min was $28.2 \pm 3.9 \mu g/l$ ($P < 0.001$ for difference from baseline). GH fell once more during the period of hyperglycemia.

**DISCUSSION**

The recent isolation of the GH secretogogue ghrelin from the fundus of the stomach and the arcuate nucleus of the hypothalamus has furthered our understanding of the control of GH secretion (6, 9, 12). Ghrelin has also been shown to have a physiological role in the control of food intake (10), acting as an orexigenic hormone, probably by stimulating NPY production in contrast to the actions of leptin (14, 21). Thus ghrelin has dual actions to stimulate both food intake and GH secretion, changes that are normally seen in response to insulin-induced hypoglycemia. The current study, however, suggests that these changes in response to hypoglycemia are not mediated by ghrelin. Insulin resulted in a rapid fall in ghrelin concentration; hypoglycemia did not appear to reverse this. By utilizing the glucose clamp procedure, we have demonstrated that hyperinsulinemia is able to suppress circulating ghrelin concentrations in the absence of hypoglycemia in healthy subjects independently of any local gut hormone effects. These observations provide a possible explanation for the recent demonstration that circulating ghrelin levels are decreased in human obesity, a state associated with hyperinsulinemia (20), and increased with weight loss (5).

Our data are consistent with previous work showing that circulating ghrelin is raised in the fasting state and suppressed by food intake in rodents (17). Because that study involved changes in both glucose and insulin, it was impossible to distinguish which of these changes was primarily responsible for the suppression of ghrelin levels. Moreover, the rodent may not be ideal for studies of this sort, as the rat appears to exhibit a paradoxical fall in GH with hypoglycemia (15). In addition, gastric ghrelin mRNA expression has been found to be upregulated by insulin infusion in the rodent model (17).

It is well established that GH release in humans is stimulated by hypoglycemia. The finding of no relationship between GH responses to hypoglycemia and ghrelin secretion supports previous work showing that GHRH and somatostatin are the critical intermediaries in this response. Jaffe et al. (8) have previously shown that the GH response to insulin-induced hypoglycemia can be significantly suppressed by pretreatment with a GHRH antagonist (8). Somatostatin appears to have an additional role in response to hypoglycemia. Continuous infusion of GHRH does not sustain GH secretion, unlike prolonged hypoglycemia, suggesting that hypoglycemia (or insulin) may be suppressing somatostatin (2, 16). An alternative explanation is that ghrelin may be produced locally within the hypothalamus in response to hypoglycemia and there-fore may not be measurable in the circulation (9). The GH response to hypoglycemia may be part of an integrative stress response rather than a mechanism controlling nutritional status. This may be different from the normal fasting situation, when insulin levels are low, perhaps allowing higher tonic circulating ghrelin and thus greater GH secretion. Glucose concentrations in this situation have not fallen to a level that triggers the cascade of counterregulatory responses that are seen in hypoglycemia.

With this study design, we cannot exclude the possibility that hyperglycemia may be having an additional action in further suppressing ghrelin. During the final hour of the study, ghrelin continued to fall, and it is possible that hyperglycemia contributed to this suppression. The degree of suppression of ghrelin during the final hour of the study (hyperinsulinemic hyperglycemia) is similar to that seen by either intravenous or oral glucose or a meal, perhaps supporting the view that the effects may be additive (4, 13). Equally, the continued action of insulin may have been the only factor. Contrary to glucose having a separate role in the finding of no rise in ghrelin during the period of hypoglycemia, making it unlikely that there is a physiological role for hyperglycemia on circulating ghrelin independent of insulin. It should be noted that this study does not involve feeding, and we therefore cannot exclude possible additional incretin effects of locally produced gut hormones. Because the assay that we used measures total ghrelin rather than just the bioactive component, we may be underestimating the relative change in bioactive ghrelin in response to hyperinsulinemia.

It has been repeatedly demonstrated that circulating GH levels are reduced in obese subjects who are insulin resistant and hyperinsulinemic. We have previously reported that such compensatory hyperinsulinemia suppresses IGF-binding protein-1 levels, which in turn may lead to increased bioavailability of free IGF-I and feedback suppression of GH secretion (1). It is intriguing to speculate that insulin-induced suppression of ghrelin may also play a role in the reduction in GH secretion observed in obesity.

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**REFERENCES**


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