Insulin hypoglycemia and growth hormone secretion in sheep: a paradox revisited

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Jaffe, Craig A., Bryan W. Huffman, and Roberta Demott-Friberg. Insulin hypoglycemia and growth hormone secretion in sheep: a paradox revisited. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E253–E258, 1999.—Although insulin-induced hypoglycemia is a potent stimulus for growth hormone (GH) secretion in humans, hypoglycemia was reported to suppress GH in sheep. We investigated whether GH suppression in sheep during insulin hypoglycemia resulted from the dose of insulin administered or the fed state of the animal. Saline or insulin (0.05, 0.2, 1.0, or 5.0 U/kg) intravenous boluses were administered to eight fasted ewes in a crossover experiment. In another experiment, four sheep were fed 2 h before intravenous administrations of either 0.2 or 5 U/kg of insulin. All doses of insulin resulted in comparable hypoglycemia, although the duration of hypoglycemia increased directly with insulin dose. Hypoglycemia in fasted animals stimulated GH secretion. The GH rise above baseline was inversely related to the insulin dose, and the insulin doses of 1 and 5 U/kg resulted in late suppression of GH below baseline concentrations. Insulin administration to fed animals caused an identical degree of hypoglycemia but no increase in GH. Insulin-inhypoglycemia stimulates GH secretion in sheep in a manner similar to humans, and the response is dependent on both fed state and insulin dose.

hypothalamus; pituitary; neuroendocrine; somatostatin; glucose

AN ACCURATE UNDERSTANDING of the roles played by the hypothalamic peptides GH-releasing hormone (GHRH) and somatostatin (SRIH) in the generation of pulsatile GH secretion in humans is uncertain. Systemic levels of these peptides do not reflect hypothalamic secretion of the peptides (26, 24), and collection of pituitary-portal blood in humans is not practical. In addition, an appropriate animal model is lacking. In an ideal model, neuroendocrine control of GH secretion would closely match that found in humans, and pituitary-portal blood sampling for direct measurements of GHRH and SRIH concentrations would be possible.

Although rodents are used extensively in the study of GH regulation, they have several shortcomings. Pituitary-portal sampling can be performed in rats, but this procedure necessarily results in hypophysectomy (25). In addition, portal sampling must be performed in anesthetized animals and this can alter GH secretion (25). Moreover, the neuroendocrine regulation of GH in rats is clearly different from that of humans because fasting and hypoglycemia potently stimulate GH release in humans (15, 16) but suppress GH in rats (29, 30). Mice are too small for hypophysial-portal blood collections, and hypoglycemia was reported to have no effect on GH in this species (28).

In contrast, pituitary-portal blood collections can be performed in sheep. Whether GH neuroendocrine regulatory mechanisms in humans and sheep are identical is uncertain. However, similarities between the species suggest that these mechanisms are very similar. Many pharmacological or physiological GH releasers in humans, including clonidine, L-dopa, arginine, cholinesterase inhibitors, and fasting, also stimulate GH secretion in sheep (8, 18, 19). Limited data from pituitary-portal sampling during administration of clonidine (19) or arginine (18) demonstrate that GHRH is the stimulus for GH release in both of these cases. This agrees with our data with a competitive GHRH-receptor antagonist in humans (16) and strengthens the hypothesis that sheep are an appropriate model.

Other data suggest that sheep might not be an appropriate model for human GH regulation. Although several early studies suggested that hypoglycemia stimulated GH in sheep (14, 27, 32), more recent data concluded that it suppressed GH release in these animals (8, 12). The latter data were obtained with the very high dose of 5 U/kg of insulin. In contrast, 0.1 U/kg of insulin reliably results in hypoglycemia and GH secretion in humans (16). High levels of insulin can cross-react at the insulin-like growth factor I (IGF-I) receptor (6) and administration of recombinant IGF-I suppresses GH secretion in sheep (10). It is therefore possible that GH suppression during hypoglycemia was an artifact of the dose of insulin administered to the animals. In addition, acute feeding at the time of study might influence these results (7, 33). To investigate whether insulin dose or feeding state could account for this discordance in GH responses to insulin tolerance testing (ITT), we administered graded doses of insulin to fasting and fed sheep.

MATERIALS AND METHODS

The study was approved by the University of Michigan Unit for Laboratory Animal Medicine. Eight 1- to 3-yr-old ovariectomized ewes of primarily Suffolk breeding were studied. The animals were meal fed hay ad libitum at 0800 daily and then fasted with free access to water for 48 h before each experiment. Each animal was studied on five separate occasions. They received either saline (n = 8) or regular insulin (Humulin R; Eli Lilly; Indianapolis, IN) in an 0.05 (n = 6), 0.2 (n = 8), 1.0 (n = 8), or 5.0 (n = 8) U/kg intravenous bolus on each occasion. The order of study was randomized. A second experiment was performed in which four animals were either

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continued on the 48-h fast or fed hay 2 h before the administration of 0.2 (n = 2) or 5 (n = 2) U/kg of insulin intravenously. Experiments were performed at 1-wk intervals.

Blood was collected through indwelling 16-gauge intravenous catheters that were placed in external jugular veins and filled with heparinized saline 1 day before each study. On the day of each experiment, the intravenous catheters were connected to long intravenous lines, and handling of the animals was minimized. Jugular blood was sampled for 1 h before and then for 3 h after the insulin bolus. Three-milliliter blood samples were collected every 20 min into tubes containing heparin (GH) and heparin plus 7.5 mg of sodium fluoride (glucose). The tubes were kept in an ice bath until they were centrifuged. Plasma samples were stored at −20°C until assayed.

GH was assayed in duplicate by RIA with methods similar to the method previously described (11). Ovine GH antiserum and ovine GH were obtained from the National Hormone and Pituitary Program. Antiserum GH-2 was used at a final concentration of 1/200,000, and ovine GH I-4 was used for iodination and standard. The assay was performed at room temperature with 100 μl of plasma in a total volume of 800 μl. Antibody bound tracer was precipitated by the second antibody method. All GH determinants for an individual animal were performed in a single assay, and mean within-assay coefficient of variation (CV) was 7%. The least detectable concentration of GH was 0.07 ng/tube, and the 50% inhibition point on the standard curve was 1.24 ng/tube. Plasma glucose was measured in duplicate with a Beckman glucose analyzer, and CV for this measurement was <5% over the entire measured range.

Baseline GH concentration was defined as the GH concentration at the time of insulin injection. The absolute rise in GH (ΔGH) was defined as the maximum GH concentration over the first 40 min after insulin administration minus baseline GH. The effect of insulin dose on GH was analyzed by repeated-measures ANOVA, and significant differences were identified post hoc by the Tukey-Kramer test. In addition, a separate repeated-measures ANOVA was performed to determine if there was a time effect with each dose. For the latter analysis, GH concentration data were grouped into the first three time points (−60–0, 20–40, and 60–180 min), and mean GH concentration for each time point was calculated. Post hoc testing for these analyses was performed by contrasts. Baseline glucose and GH concentrations were compared between fasting and fed days by two-tailed Student’s t-tests. Homogeneity of variance was achieved by logarithmically transforming mean GH and GH before the analyses. Data are presented as means ± SE. P < 0.05 was considered statistically significant.

RESULTS

Figure 1 shows means ± SE of plasma glucose concentrations for the eight sheep during administration of saline or insulin. Mean fasting glucose after a 48-h fast was 43 mg/dl, and there was no difference in baseline glucose during any of the 5 study days. Plasma glucose was stable after administration of saline. All of the insulin doses caused significant hypoglycemia, and mean glucose nadirs were similar across insulin doses (19 ± 3, 16 ± 1, 16 ± 1, and 16 ± 2 mg/dl for 0.05, 0.2, 1, and 5 U/kg, respectively). In contrast to the effect on glucose nadir, there was a difference in recovery from hypoglycemia, with normalization of plasma glucose by 3 h after the intravenous insulin dose of 0.05 U/kg, partial normalization after administration of 0.2 U/kg, and persistent hypoglycemia with the two largest insulin doses.

GH responses to saline or insulin are shown in Fig. 2, and GH responses during these treatments are shown in Fig. 3. Saline had no effect on GH secretion. By ANOVA, there was a dose effect (F = 5.18, P = 0.005) such that the GH responses to the largest doses of insulin, 1 and 5 U/kg, were indistinguishable from saline treatment. The GH responses to the lower doses of insulin were significantly higher than control. There was a trend toward a decreasing GH response with increasing dose (r = 0.26, P = 0.16), and the relationship became highly significant when a single outlier was excluded (r = 0.57, P = 0.002). The GH responses between the lowest (0.05 U/kg) and highest (5 U/kg) doses were significantly different.

In Fig. 2, the large standard errors observed at the end of the study with 0.05 U/kg of insulin demonstrate that spontaneous pulsatile GH secretion returned within this time period. In contrast, there was late suppression of GH concentrations with the three larger insulin doses. Within 3 h of the insulin injection, mean plasma GH concentration returned to baseline level for the 0.2 U/kg dose but remained suppressed when 1 or 5 U/kg were administered.

A time course for the GH responses is shown in Fig. 4. Plasma GH concentrations were grouped into three time points: preinsulin, acute response, and postresponse. GH concentrations across the three time points were constant during saline treatment. With the two smaller doses, 0.05 and 0.2 U/kg, there was a significant acute GH response, but GH fell to pretreatment levels by the third time point. After administration of the two larger doses of insulin, mean GH also increased during the acute period; however, the postresponses...
were actually lower than the pretreatment GH baseline.

The effect of acute feeding on baseline glucose and on the glucose responses to the ITT is shown in Fig. 5 (top). Baseline plasma glucose concentrations were higher in the fed animals compared with the fasted animals (50.2 ± 2.1 vs. 42 ± 2.1 mg/dl; P = 0.01), but similar levels of hypoglycemia were reached in both fed and fasted sheep. One fed animal had a loss of consciousness at a glucose concentration of 16 mg/dl after administration of insulin (5 U/kg). Administration of intravenous dextrose to this animal resulted in immediate recovery. As was observed in the fasted animals, euglycemia was restored within 3 h after treatment with insulin (0.2 U/kg), whereas hypoglycemia was

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Fig. 2. Means ± SE of plasma growth hormone (GH) concentrations in response to administration of an iv bolus of 0.05 U/kg (A), 0.2 U/kg (B), 1 U/kg (C), and 5 U/kg (D) of insulin. Insulin or saline was administered at time 0. ○, insulin; ●, saline.

Fig. 3. Rise in plasma GH concentration over baseline values (ΔGH) during insulin tolerance testing (ITT). Baseline was defined as GH concentration at time of insulin administration. Effect of insulin dose on plasma GH was analyzed by repeated-measures ANOVA. GH responses after administration of 2 smallest, but not 2 largest, doses of insulin were greater than GH response to saline (*P < 0.05). GH responses to lowest (0.05 U/kg) and largest (5 U/kg) insulin doses were significantly different (†P < 0.05).

Fig. 4. Effect of insulin dose on time course of GH response to ITT. GH concentrations were grouped into 3 time points: preinsulin (Pre), 20 to 40 min post insulin (Response), and after 40 min (Post). Repeated-measures ANOVA across time with subsequent contrast testing was performed for each dose. *P = 0.01; **P < 0.005.
more prolonged after the larger dose. Figure 5 (bottom) shows the effect of insulin-hypoglycemia on GH in the four fed animals. Baseline GH concentrations were similar in fed and fasted sheep \((P = 0.35)\). There was no acute increase in GH during hypoglycemia in fed animals receiving either dose. In contrast, GH began to fall 20–40 min after insulin administration and remained suppressed until the completion of the experiment.

**DISCUSSION**

The present study demonstrates that ITT stimulates GH release in fasted sheep. Moreover, we have shown that the GH response is both inversely related to the dose of insulin used and abolished by feeding. These data confirm early reports that hypoglycemia stimulates GH \((14, 27, 32)\) and a more recent report that demonstrates GH suppression during hypoglycemia \((12)\).

Our data suggest several explanations for these discordant effects of hypoglycemia on GH in sheep. It is likely that the different responses derive, at least in part, from the dose of insulin administered. In the study demonstrating GH suppression during hypoglycemia \((12)\), sheep were administered insulin \((5 \text{ U/kg})\). The rationale for the very large insulin dose was the presence of insulin resistance in sheep and the observation that 5 U/kg gave better corticotropin-releasing hormone and cortisol responses than did 1 U/kg \((9)\). Although glucose clamp studies have documented some degree of insulin resistance in sheep \((3)\), we achieved identical glucose nadirs over the insulin dose range of 0.05–5 U/kg. The different doses did not affect the degree of glucose suppression but did determine the duration of hypoglycemia. The smaller doses resulted in a transient hypoglycemia, similar to that observed in humans treated with 0.1 U/kg of insulin \((16)\). In contrast, animals administered 1 or 5 U/kg of insulin were still profoundly hypoglycemic 3 h after treatment.

It is likely that the large insulin doses \((1 \text{ and } 5 \text{ U/kg})\) used in this and in a previous study \((12)\) directly suppressed pituitary GH secretion. This possibility is supported by data from Yamashita and Melmed \((34)\) demonstrating that insulin suppresses GH release from somatotrophs in vitro. Whether this negative feedback is mediated through the insulin receptor or through the IGF-I receptor is uncertain. Peripheral administration of IGF-I suppressed GH secretion in humans \((2)\) and in sheep \((10)\). Although insulin weakly cross-reacts with IGF-I receptors \((6)\), the larger doses could have a significant IGF-I effect.

An indirect mechanism of GH suppression by the large doses of insulin is also possible. Rat \((1)\) and human studies \((2)\) suggest that at least part of the negative feedback on GH by IGF-I is mediated through an increase in hypothalamic SRIH secretion. Moreover, pituitary-portal SRIH concentrations in sheep acutely increased after the systemic administration of the pharmacological dose of 5 U/kg of insulin \((12)\). Therefore, either a direct pituitary effect or stimulation of hypothalamic SRIH could account for the inverse relationship between insulin dose and the GH response. It is also possible that both mechanisms are important.

Although it was not directly investigated in this study, it is of interest to speculate on the neuroendocrine mechanisms through which lower doses of insulin released GH. We have previously shown that endogenous GHRH is essential in humans for the GH responses during ITT \((16)\) and after GH-releasing peptide-6 administration \((23)\). It is likely that both of these interventions acutely release hypothalamic GHRH. We postulate that the lower doses of insulin also stimulate GHRH and possibly other secretagogues in sheep. The higher insulin doses, either directly or through an increase in hypothalamic SRIH, block the effect of hypoglycemia on these GH secretagogues. This hypothesis is consistent with data demonstrating that a high dose of insulin did not release GHRH in sheep \((12)\). An accurate understanding of the neuroendocrine mechanisms involved in these responses will require insulin dose-response studies with concomitant measurements of pituitary-portal GHRH and SRIH concentrations.

These inhibitory effects of insulin on neuroendocrine systems are not unique to sheep, and they likely play a role in GH regulation in humans. Similar to our observation of an inverse relationship between insulin

![Figure 5. Effect of feeding on glucose (top) and GH (bottom) responses to an ITT. 0.2 U/kg \((0.2A; 0.2B) n = 2\) or 5 U/kg \([5A; 5B (n = 2)]\) of insulin were given as an iv bolus at time 0 to sheep that had been fed hay 2 h earlier. None of the animals had an increase in GH after insulin administration. Late GH concentrations were low. *Sheep 5B was administered iv glucose after 140 min because of loss of consciousness resulting from neuroglycopenia.](http://ajpendo.physiology.org/DownloadedFrom http://ajpendo.physiology.org/ AJR)
dose and the GH response, Diamond et al. (5) demonstrated that raising the magnitude of hyperinsulinemia suppressed the GH response during hypoglycemia clamping. These feedback mechanisms are also operative in patients with severe insulin resistance syndromes who have very high systemic insulin levels and low serum GH and IGF-I concentrations (6). Negative feedback on GH secretion by insulin may not be limited to pathological causes of hyperinsulinemia. Euglycemic insulin clamping at postprandial insulin levels diminished the GH response to GHRH in humans (17). It is therefore conceivable that high insulin levels contribute to low serum GH concentrations found in obesity.

A second possible explanation for the inconsistent reports on the effect of hypoglycemia in sheep is the fed state of the animal. Sheep were fasted overnight in two earlier studies reporting an increase in GH during ITT (27, 32). In a third study demonstrating a GH response to hypoglycemia, the feeding state was not specified (14). Although the limited number of animals studied in our investigation does not allow us to draw firm conclusions, our data strongly suggest that feeding eliminates the GH response to hypoglycemia. Suppression of both spontaneous GH secretion and the GH response to GHRH in sheep (7, 33) and in humans (4) supports this observation.

Feeding could alter GH secretion through changes in endogenous insulin secretion or through other metabolic, endocrine, or neuronal pathways. Data from goats suggest that this inhibition is a result of mechanical distension of the rumen (31). In addition, a regulatory role for short-chain fatty acids is likely because intraruminal (20) or systemic (21) administrations of physiological amounts of short-chain fatty acids block GHRH-induced GH secretion. This feeding-mediated control of GH might be under neuronal regulation because pretreatment with a cholinergic blocker eliminates the GH suppression that follows feeding or intraruminal volatile fatty acid infusion (22). Whatever mechanism is involved, the fed state of the animal is an important variable that must be accounted for in experimental design.

Although a nonglucose-mediated stimulus could be the stimulus for GH release in sheep, most of the data suggest that hypoglycemia per se releases GH. Hertelendy and Kipnis (14) suggested that the rise in GH during ITT was a result of a fall in nonesterified fatty acids. In fact, acute suppression of nonesterified fatty acids stimulates GH release independent of plasma glucose (14). If hypoglycemia was the mediator, 2-deoxy-

Whereas hypoglycemia stimulates GH in humans (16), suppresses it in rats (29), and has no effect in mice (28), the effect in sheep has been less certain. Our studies conclusively demonstrate that insulin-hypoglycemia is a stimulus for GH secretion in sheep. This response is dependent on the animals being studied while they are fasting, and the magnitude of the GH rise is inversely related to the dose of insulin used. Hypoglycemia resulting from small doses of insulin results in early GH release. In contrast, large doses of insulin do not stimulate release but do cause a delayed suppression in GH. These data support the use of sheep as an appropriate model for the study of GH secretion in humans.

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