Rats is clearly different from that of humans because (25). Moreover, the neuroendocrine regulation of GH in anesthetized animals and this can alter GH secretion. In addition, portal sampling must be performed in the procedure necessarily results in hypophysectomy (25). GH regulation, they have several shortcomings. Pituatory-SRH concentrations would be possible.

AN ACCURATE UNDERSTANDING of the roles played by the hypothalamic peptides GH-releasing hormone (GHRH) and somatostatin (SRH) 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 SRH 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 hypophyseal-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) but suppress GH in rats (29, 30).

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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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
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 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 and 5 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...
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 distention 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. Hertlendy 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-d-glucose (2-DG) should also stimulate GH release. Although Funston et al. (13) did not observe GH stimulation after systemic 2-DG treatment, the dose used, 100 mg/kg, might have been inadequate because peripheral administration of 150 mg/kg of 2-DG did reliably stimulate GH release (27). In addition, concomitant administration of glucose and insulin blocked the GH response in 9 out of 10 animals (27), again suggesting that hypoglycemia is the primary releaser of GH.

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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