Influence of Blood Glucose Levels on Growth Hormone Secretion in Sheep[1] (35190)

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Blood glucose, a principal source of metabolizable energy in monogastric animals, has been given a role in the regulation of growth hormone (GH) secretion because a rapid fall in blood glucose, inhibition of intracellular glucose utilization, or hypoglycemia are all stimuli for GH release in primates (1–4). In ruminant animals, however, most of the metabolizable energy is derived from short-chain fatty acids produced by the microbial fermentation in the rumen, rather than being derived from glucose. The present studies were conducted to evaluate the significance of blood glucose in regulation of GH secretion in sheep. Plasma GH levels were measured during fasting, after insulin-induced hypoglycemia and after injection of glucose. In addition, disappearance of GH injected intravenously was used to estimate GH secretion rates in sheep.

Materials and Methods. Animals. All the sheep used in these studies were castrated crossbred males, less than 1 year old, weighing 30 to 45 kg, penned individually indoors and fed twice daily.

Fasting. Ten sheep were fed a mixed diet containing 35% ground corn, 10% ground corn cobs, 40% ground alfalfa hay, 6% soybean meal, 8% molasses, and 1% minerals for 3 weeks before the experiment. On the day the experiment was started, the animals were fed at 8:00 a.m. and allowed access to only water for the next 72 hr. Blood samples were collected in heparinized tubes by puncture of the jugular vein.

Insulin and glucose injection. The sheep were fed the diet described above. The jugular vein was catheterized after an overnight fast; and 1 to 2 hr later, blood samples were taken before injecting insulin (U-40 protamine zinc insulin, Eli Lilly and Co., Indianapolis, Indiana, 1.0 U/kg of body wt) or insulin (1.0 U/kg) and glucose (270 mg/kg). Two animals received each injection. After each injection, another series of blood samples was taken over a period of 3 to 4 hr.

Growth hormone injections. This experiment was conducted with sheep fed alfalfa hay. Following the morning feeding, jugular veins in three animals were catheterized; 2 hr later, four blood samples were taken from each animal during a period of 40 min. Ovine GH prepared by the method of Wallace and Ferguson (5), was dissolved in 0.85% NaCl and injected intravenously. The concentration of hormone in the solution injected was measured by radioimmunoassay. The catheter was rinsed several times with 0.85% NaCl, and six additional blood samples were taken from each animal within 60 min.

Chemical methods. Aliquots of whole blood from each sample were immediately frozen and stored at −15° for determination of glucose by methods for the Technicon AutoAnalyzer. Aliquots of plasma were stored at −15° for determination of plasma-free fatty acids (FFA) by the method of Duncombe (6), GH by a solid-phase radioimmunoassay procedure described in detail elsewhere (7), and insulin by modification of a coated-charcoal radioimmunoassay described by Herbert *et al.* (8). Antibodies to bovine insulin, produced in guinea pigs, were incu-

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bated for 5 days at 4° with solutions of standard bovine insulin (Lot No. 62106, Calbiochem, Los Angeles, California, 23.4 U/mg) or the plasma samples being assayed, before addition of ^{125}I-insulin. The tubes were incubated an additional 2 days at 4° before addition of 1.5 ml of a suspension of coated-charcoal [7.5 g Norite A, 0.75 g of dextran-80 and 3 g of bovine serum albumin/100 ml of the acetate-barbital buffer (pH 7.4), defined by Herbert *et al.* (8)]. The tubes were allowed to stand for 2 hr at 4° before centrifuging to sediment the charcoal. The radioactivity bound to charcoal was measured in an automatic gamma counter.

Ovine GH, prepared by the method of Wallace and Ferguson (5), was purified by gel filtration on Sephadex G-100 and used as the standard in the immunoassay. The purified hormone showed a major component and a minor, more rapidly moving anionic component with vertical acrylamide gel electrophoresis in Tris–glycine buffer (pH 9.3). The hormone was found to have growth-promoting activity in female hypophysectomized rats.

Calculations. Statistical analysis was carried out by standard procedures described by Snedecor (9). During the 40 min after injection of GH, there was a straight-line logarithmic decline of plasma GH concentrations. The method of least squares was used to calculate a regression equation from which the fractional turnover rate (K) was obtained for each animal. The line was extrapolated to zero time to obtain the theoretical concentration of plasma GH after the exogenous GH was completely distributed in body fluids but before any had been lost. The volume of distribution (V_{GH}) was calculated from the equation:

$$\frac{\text{exogenous GH (m}\mu\text{g)}}{\text{plasma GH}_{(\text{zero time})} - \text{basal plasma GH (m}\mu\text{g/ml)}}.$$

Basal plasma GH (BGH) was the mean GH concentration in the four plasma samples obtained before injecting the hormone. Secretion rate of GH (GHSR) was obtained from: $V_{GH} \cdot K \cdot BGH$.

Results. Effect of fasting. The averages of the values obtained from the 10 animals at each sampling time are shown in Fig. 1. No significant changes occurred in plasma GH levels that could be related to fasting. Blood glucose levels remained constant during the first 12 hr and then decreased ($p<.01$) during the next 36 hr. Concentrations of plasma FFA increased ($p< .01$) from 4 to 48 hr after feeding and then remained unchanged during the next 24 hr of observation. Plasma insulin levels were the highest 4 hr after feeding and rapidly declined during the next 8 hr. Seventy-two hr after feeding, the level of plasma insulin had decreased ($p<.01$) to 30% of the level at 4 hr.

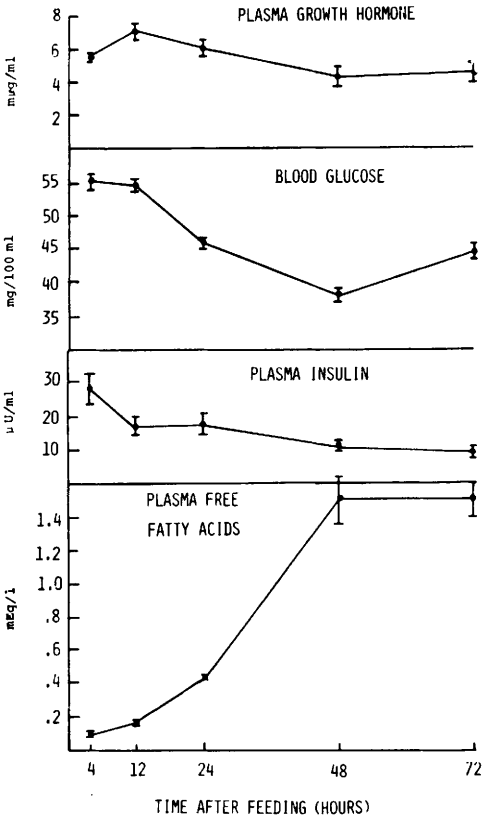

FIG. 1. Plasma GH, blood glucose, plasma FFA, and plasma insulin in 10 fasted sheep. Standard error of the means shown as vertical lines.

Effect of insulin and glucose injections. Typical changes observed in concentrations of blood glucose and plasma GH after intravenous injection of insulin (1.0 U/kg of body wt) in sheep fasted overnight are shown in Fig. 2. After the injection of insulin,

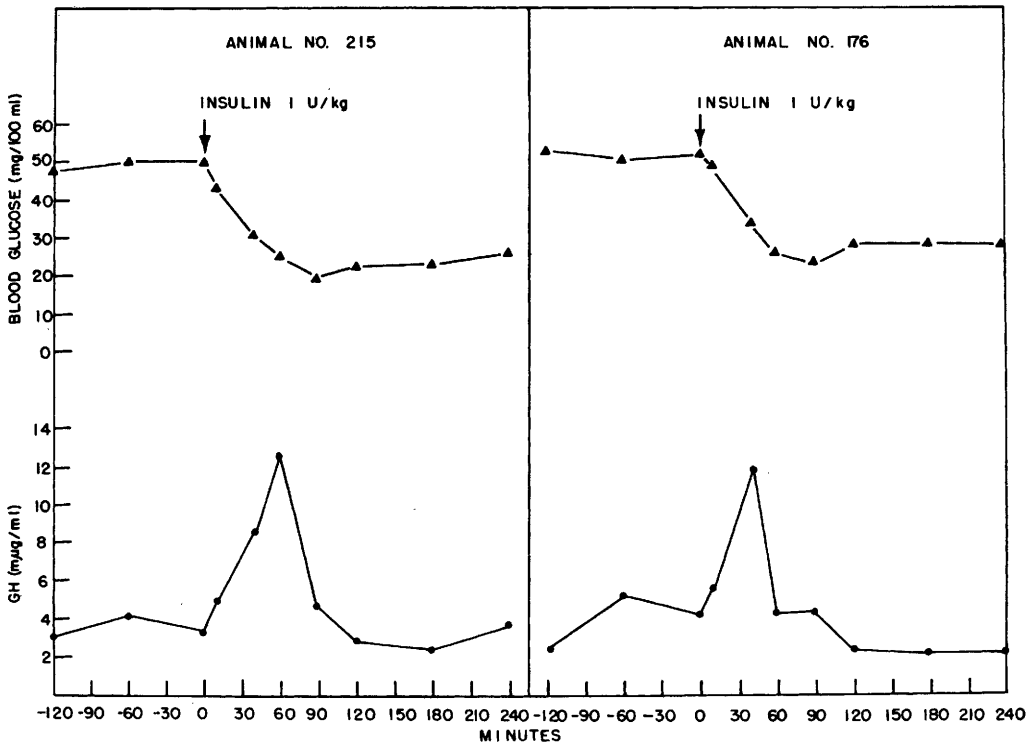

FIG. 2. Blood glucose and plasma GH concentrations after intravenous administration of insulin to sheep following an overnight fast.

blood glucose levels decreased from about 50 to 20 mg/100 ml within 90 min and remained at these low levels during the remainder of the experiment. Plasma GH levels remained near preinjection levels of below 5 mμg/ml for 20 min after administration of insulin and then more than doubled to 12.5 mμg/ml in animal 215 at 60 min and to 11.7 mμg/ml in animal 176 at 40 min. Plasma GH levels then decreased to preinjection levels within 60 min. Blood glucose levels remained low throughout this 60-min period.

The effects of injecting glucose along with insulin on blood glucose and plasma GH levels are shown in Fig. 3. Levels of plasma GH were increased at 40 min even though blood glucose had not yet returned to preinjection concentrations. After blood glucose had declined to a constant level of about 25 mg/100 ml, plasma GH had returned to levels observed before injecting glucose and insulin.

Growth hormone secretion rates. A summary of the data obtained on the disappearance of intravenously administered GH is shown in Table I. The half-life of GH in plasma ranged from 8.6 to 14.7 min in the three animals. The average distribution volume of GH was 5.0% of body weight. Secretion rates necessary to maintain basal plasma GH levels were calculated to be 0.54 to 0.76 μg GH/kg of body weight/hr.

Discussion. A low blood glucose level *per se* was not a stimulus to GH release in sheep because only basal levels of the hormone were observed during prolonged insulin-induced hypoglycemia and during fasting. It seemed that a stimulus for GH secretion was a rapid decrease in blood glucose concentrations, since plasma GH levels increased in the animals injected with insulin when blood glucose concentrations were decreasing. In the animals injected with glucose and insulin, the increase in plasma GH concentration occurred when blood glucose was actually at decreasing hyperglycemic levels (Fig. 3) for the sheep (70 to 85 mg/100 ml), which also indicates that a falling level of blood glucose

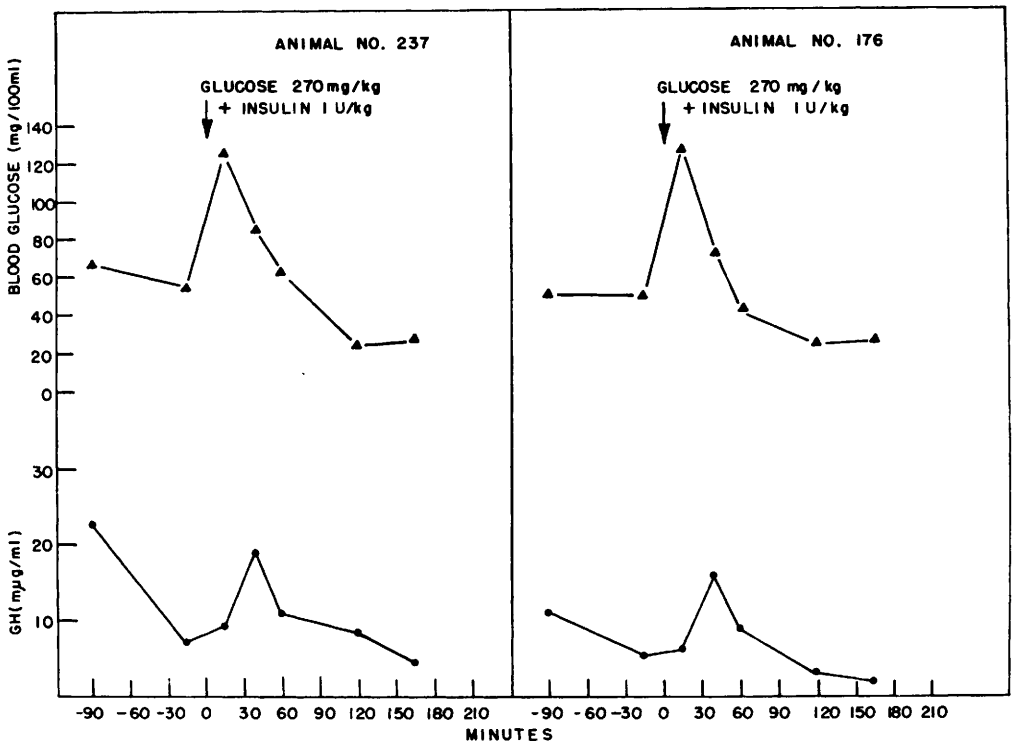

FIG. 3. Blood glucose and plasma GH concentrations in fasted sheep following intravenous injection of glucose and insulin.

rather than hypoglycemia was the stimulus for GH secretion. The large dose of insulin used in these studies was required to induce a decrease in the blood glucose levels to at least 50% of initial concentrations, since sheep are insensitive to the hypoglycemic effects of insulin (10). The changes in plasma GH levels in sheep after injection of insulin were small in contrast to the change in plasma GH levels produced in primates by hypoglycemia (1–4). Hertelendy *et al.* (11) have reported a study with sheep in which intravenous injection of glucose did not depress plasma levels of GH; but rather, when glucose levels were declining towards normal, an increase in plasma GH levels was observed.

Werrbach *et al.* (12) have suggested that the alpha and beta adrenergic receptors in the hypothalamic nuclei of primates regulate GH release in response to glucose. There is no evidence of glucose sensitive receptors in the hypothalamus of ruminants (13); and likewise, there is no evidence that levels of blood glucose have any effect on appetite (14) as suggested for nonruminant animals (15, 16).

Fasting of sheep resulted in increased plas-

TABLE I. Growth Hormone (GH) Secretion Rate and Its Components in Sheep.

Animal no.	Body wt (kg)	GH injected (mg)	Basal GH (mμg/ml)	Turnover of GH pool (times/hr)	Half-life (min)	GH vol of distribution (% body wt)	GH secretion	
							Per hr (μg)	Per hr/kg (μg)
184	44	0.672	3.5	3.58	11.6	4.6	25.5	0.58
115	45	0.336	3.9	2.81	14.7	6.9	34.5	0.76
167	36	0.672	3.2	4.83	8.6	3.5	19.5	0.54

ma FFA concentrations, which indicated that energy was mobilized from adipose tissue. Based upon the GH levels observed in the fasting animals, changes in GH secretion alone probably could not have accounted for the increased mobilization of fatty acids; however, a decrease in plasma insulin during fasting might have been involved. Machlin *et al.* (17) proposed, from a study with pigs, that a decrease in concentrations of insulin in proportion to GH might be a hormonal mechanism that could be responsible for mobilization of FFA. In this study with sheep, the ratios of insulin to GH in plasma were 5.1, 2.5, and 2.0 at 4, 48, and 72 hr after feeding, respectively. The largest increase in plasma FFA (Fig. 1) coincided with the largest decrease in the ratio of insulin to GH in plasma. It seems that changes in plasma levels of insulin might be more important than GH levels in regulation of fatty acid mobilization in ruminants.

The average half-life of GH disappearance from circulation was 11.6 min in sheep, which is less than the 23 to 38 min for human GH in adult humans (18) and the 20 to 30 min for porcine GH in pigs (17). Yousef *et al.* (19) have reported a half-life of 22.5 min for bovine GH in cattle.

Summary. Concentrations of plasma GH, as estimated by radioimmunoassay, did not change significantly in sheep during the 72 hr after feeding; however, the concentrations of blood glucose and plasma insulin decreased and levels of plasma FFA increased markedly. Furthermore, insulin-induced hypoglycemia was not a stimulus for GH secretion. Rapidly falling blood glucose levels from normal or hyperglycemic levels stimulated an increase in plasma GH concentration of short duration. Growth hormone disappeared from plasma with a half-life of 8.6 to 14.7 min. Growth hormone was distributed in a volume equal to 5.0% of body weight. The average GH secretion in 40-kg sheep was estimated to be 0.7 μg/kg/hr.

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