Studies on Growth Hormone Secretion

VII. Effects of Somatostatin on Plasma GH, Insulin, and Glucagon in Sheep

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SUMMARY

To determine whether synthetic somatostatin originally isolated from sheep hypothalamus can inhibit hormone secretion in the same species, we measured plasma levels of GH, insulin, glucagon, and glucose of normal sheep under a variety of experimental conditions in the presence and absence of somatostatin infusion. An oral dose of 2.5 mg./kg. 3,5-dimethylpyrazole increased plasma GH from 10.9 to 376.9 ng. per milliliter, which was suppressed by 50 per cent and 80 per cent with 0.5 and 1 mg. synthetic cyclic somatostatin, respectively. Linear somatostatin (0.5 mg.) was without effect in two animals tested. Propionate (0.5 mmole per kilogram) and arginine (10 gm.) induced a rise in plasma insulin and GH, and glucagon was effectively blocked by cyclic somatostatin (0.5 mg.). Similarly, somatostatin inhibited glucose, and glucagon provoked GH and insulin secretory responses without affecting glucose or FFA levels. Somatostatin had no effect on the disappearance of injected glucagon. Finally, addition of somatostatin to incubation media prevented PGE_1 promoted GH release, and suppressed cyclic AMP accumulation, although to a lesser extent, in sheep anterior pituitary pieces. In view of the large amounts required to suppress stimulated hormone release and the general lack of specificity of somatostatin, it is suggested that this peptide may have a functional role only in the release of hormones of the pituitary, where it could occur in relatively high local concentrations. Its inhibition of extrapituitary hormone secretion may be purely a pharmacologic effect that, nevertheless, suggests an interference with a step common to the secretory process of hormones.
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Somatostatin, a hypothalamic tetradecapeptide that has been isolated on the basis of its inhibitory effect on GH release in cultured rat anterior pituitary cells,[1] has been shown to inhibit GH responses to a wide variety of stimuli in humans and in a number of other mammalian species. It has also been demonstrated that the synthetic peptide inhibits not only GH release but also the secretion of insulin,[2] glucagon,[3,4] TSH,[5] and gastrin.[6]

Previous studies suggested that some of the factors influencing growth hormone (GH) and insulin secretion in sheep and probably other ruminants may differ from those operative in the secretion of these hormones in humans and other monogastric mammals.[7,8] For example, short-chain fatty acids are potent stimulants of insulin secretion in sheep both in vivo and in vitro.[9-12] Fatty acids also appear to play an important role in GH secretion,[13] and there is evidence that rising levels of plasma glucose do not suppress GH release in ruminants.[14]

In view of the fact that somatostatin was isolated from sheep hypothalamic extracts yet very little is known about its effects in sheep,* we examined GH, insulin, glucagon, glucose, and free fatty acid levels in intact sheep under a variety of experimental conditions with and without somatostatin infusion. In addition, the effect of somatostatin in vitro on prostaglandin-stimulated GH release and cyclic AMP accumulation was also measured in sheep anterior pituitary glands.

MATERIALS AND METHODS

Young, castrated male sheep (eight to twelve months old) weighing between 25 and 35 kg. were used in these studies. They had free access to Purina lamb chow and water unless otherwise indicated. On the mornings of the experiments, the animals were strapped into specially designed animal holders that

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immobilized them without causing undue stress. They were kept under these conditions throughout the experimental period. A 14-gauge intravenous catheter was introduced into a jugular vein for the infusion of the test substances, and another catheter of the same size was inserted into the contralateral jugular vein for the withdrawal of blood samples. At least one hour was allowed between the introduction of the intravenous catheters and the commencement of the blood collection. The test compounds were dissolved or diluted in sterile physiologic saline and infused by means of a Harvard infusion pump or a pediatric infusion set at a rate of 0.5 or 1 ml. per minute. The catheters were kept open by a slow intravenous drip of sterile saline. The amount of the compounds infused and the periods of infusion are given in the appropriate figures. Blood samples (4 ml.) were withdrawn and transferred into tubes containing EDTA as an anticoagulant and Trasylol (FBA Pharmaceuticals, New York). Blood samples were chilled in ice immediately and then centrifuged for ten minutes. Plasma was aliquoted to avoid repeated freezing and thawing and was frozen on dry ice.

Fresh sheep pituitary glands were obtained from a nearby abattoir within a few minutes after slaughter and transported to the laboratory in ice-cold saline. The anterior lobe was separated and cut into 10-15-mg. pieces. Two such tissue fragments were incubated in 1 ml. Krebs-Ringer bicarbonate buffer containing 1 mg. per milliliter glucose (KRBG) in a Dubnoff metabolic shaker at 37° under a humidified atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. Double-antibody radioimmunoassay procedures were used for the determination of growth hormone and insulin,[10,15] as well as glucagon[16] and cyclic AMP.[17] Plasma glucose was measured by the glucose oxidase method,[18] free fatty acids by the procedure of Itaya-Ui,[19] and protein according to Lowry et al.[20] Three batches of somatostatin were used. The first one was the synthetic cyclic compound obtained from Wyeth Laboratories through the courtesy of Dr. Sarantakis; the second batch was the linear somatostatin donated by Dr. Arimura, and in the latter studies the cyclic compound was purchased from Bachem Laboratories, California. 3,5-Dimethylpyrazole was received from Eastman Kodak, phentolamine (Regitine HCl) from Ciba-Geigy, and crystalline glucagon from Eli Lilly Co. All other reagents were obtained from Sigma Chemical Company.

RESULTS

Effect of Somatostatin on 3,5-Dimethylpyrazole-induced Growth-hormone Release

We have reported previously that a variety of antilipolytic compounds, including pyrazoles, are potent stimulants of growth hormone secretion in fasting sheep.[13] Since 3,5-dimethylpyrazole appeared to be the most consistent stimulus, the ability of somatostatin to block growth-hormone responses to the administration of this compound was first tested. As shown in figure 1, 3,5-dimethylpyrazole when given orally at a dose of 2.5 mg. per kilogram in a volume of 25 ml. of water caused a dramatic rise in plasma growth hormone from a baseline of 10.9 ± 1.3 ng. per milliliter to a mean maximum of 376.9 ± 11.6 ng. per milliliter ninety minutes later. Infusion of somatostatin significantly suppressed GH response in a dose-related fashion. A 50 per cent inhibition was observed with 500 μg. somatostatin while a more significant inhibition, about 80 per cent, was found with 1,000 μg. of somatostatin. It is noteworthy and consistent with some studies in humans and other animal models that growth hormone levels rapidly rebound after somatostatin infusion as if the capacity to respond to the drug were not impaired but the releasing mechanism were inhibited at some point by somatostatin. Neither dimethylpyrazole nor somatostatin affected basal release of insulin in these experiments, and these and glucose levels, which were also unaffected, are not illustrated here.

Infusion of linear somatostatin (500 μg.) failed to suppress GH responses to 3,5-dimethylpyrazole and had no apparent effect on insulin, glucagon, glucose, and FFA levels (table 1).

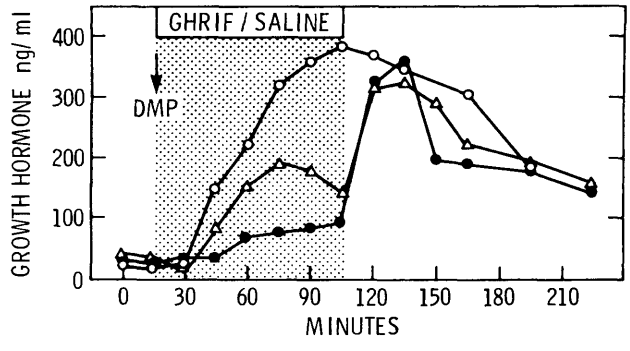

FIG. 1. Inhibition by cyclic somatostatin of 3,5-dimethylpyrazole-induced GH release in sheep. (Δ : 0.5 mg. and ● : 1 mg. somatostatin; O : saline; 2.5 mg./kg. DMP). Each point represents four animals and ± S.E. did not exceed 15 per cent of mean values.

TABLE 1

Effects of 3'5-dimethylpyrazole (2.5 mg./kg. given orally at zero time) and linear somatostatin (500 μg. infused intravenously between zero and ninety minutes) on the plasma levels of GH, insulin, glucagon, and glucose in two sheep (nos. 35 and 36) after a forty-eight-hour fast

Time min.	Growth hormone ng./ml.		Insulin μU./ml.		Glucagon pg./ml.		Glucose mg./100 ml.	
	no. 35	no. 36	no. 35	no.36	no.35	no.36	no. 35	no. 36
−20	13.1	15.3	6.7	9.9	456	423	69	69
0	9.8	11.5	11.3	12.2	411	452	72	64
15	10.8	15.2	9.0	7.5	393	502	69	67
30	120.0	266.0	10.0	9.0	395	494	74	69
45	353.8	350.0	8.0	7.6	378	497	73	58
60	387.3	308.2	7.9	8.7	373	465	72	59
75	367.8	338.4	8.3	6.8	369	447	69	61
90	352.4	327.6	6.7	8.5	385	481	71	59
105	348.4	321.6	8.3	8.5	395	493	71	61
120	414.1	366.8	8.7	9.8	385	492	69	54
150	375.9	250.6	10.0	9.5	432	470	68	55
180	263.0	221.1	13.0	7.2	421	494	73	60

Effect of Arginine and Somatostatin Infusion

Arginine is a known stimulus of growth hormone and insulin release not only in humans and some other monogastric mammals but also in the ruminant.[21] To determine the effect of somatostatin on arginine-provoked hormone secretion, a group of four sheep were given 10 gm. of arginine HCl during a thirty-minute infusion. One week later, the experiment was repeated on the same animals, but this time arginine was administered during a ninety-minute infusion of cyclic somatostatin (500 μg.). The results clearly show that both plasma GH and insulin responses were inhibited by somatostatin infusion (figure 2). Plasma GH levels reached a mean maximum of 100 ng. per milliliter during the control experiment but only 29 ng. per milliliter during somatostatin infusion ($p<0.001$). Similarly, the increase in plasma insulin during somatostatin infusion was suppressed by about 50 per cent ($p<0.01$) below mean maximum responses observed during saline infusion. In addition, the rise in plasma glucagon, which was moderate in comparison to the relative increments in GH and insulin, was suppressed by somatostatin. A moderate hyperglycemia from a mean prearginine level of 70.5 to a mean maximum of 92 mg. per 100 ml. was observed in the control experiment but not during somatostatin infusion.

Effect of Somatostatin on Propionate-induced Hormone Secretion

It is well known that ruminants derive their energy primarily from short-chain fatty acids, such as acetate, propionate, and butyrate, which are formed in the rumen from ingested nutrients by microorganisms. It has also been demonstrated that these short-chain fatty acids, with the exception of acetate, are potent

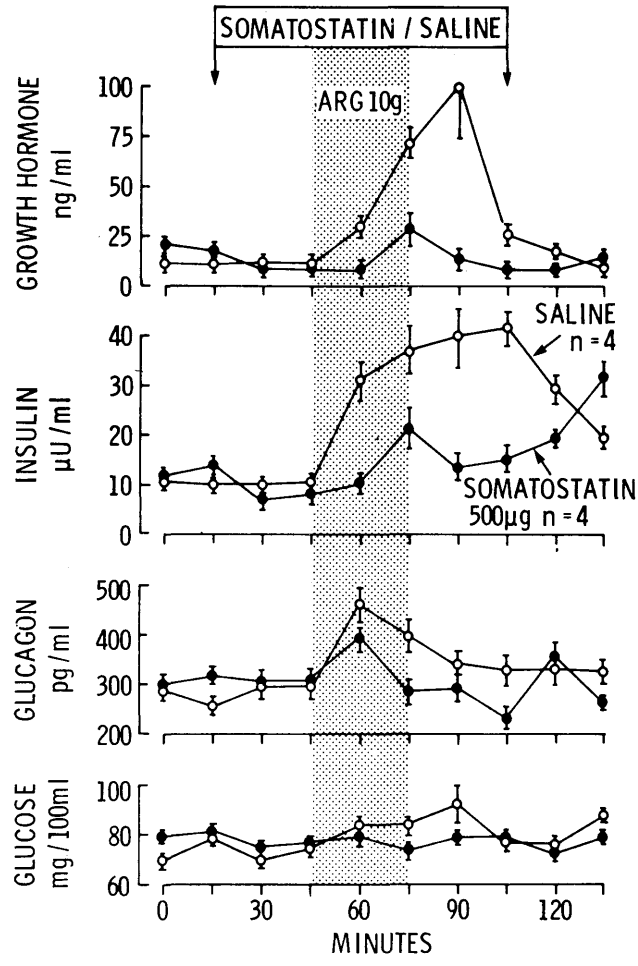

FIG. 2. Effects of cyclic somatostatin on arginine-induced hormone secretion in a group of four wether lambs.

stimulants of insulin secretion in ruminants but not in monogastric mammals.[10] These studies suggested

that short-chain fatty acids may play a role similar to that which glucose plays in the regulation of insulin secretion in humans. It was, therefore, of considerable interest to examine whether somatostatin of ovine origin will block the insulin response to propionate. As illustrated in figure 3, within ten minutes of propionate injection (0.5 mmole per kilogram intravenously), plasma insulin levels rose from a baseline of 18.5 ± 3.2 μU./ml. to 142 ± 25 μU./ml. in the control group, dropping rapidly to preinjection levels during the subsequent thirty minutes. The baseline in the somatostatin group was 10.6 ± 1.8 μU./ml., which fell to 4.4 ± 1.1 μU./ml. fifteen minutes after the beginning of somatostatin infusion and rose to a peak of 11.5 μU./ml. five minutes after propionate injection, followed by a gradual decline to 4.6 ± 0.9 μU./ml. by the end of somatostatin infusion. A moderate rise to 13.4 ± 3.1 μU./ml. was observed after the infusion of somatostatin was stopped. Propionate injection also caused a rise in GH levels from 13.6 ± 2.1 ng./ml. to a peak of 27.8 ± 5.7 ng./ml. thirty minutes after the administration of the short-chain fatty acid. This rise, however, was not observed in sheep receiving somatostatin, although a small increase was noted shortly after the infusion was stopped.

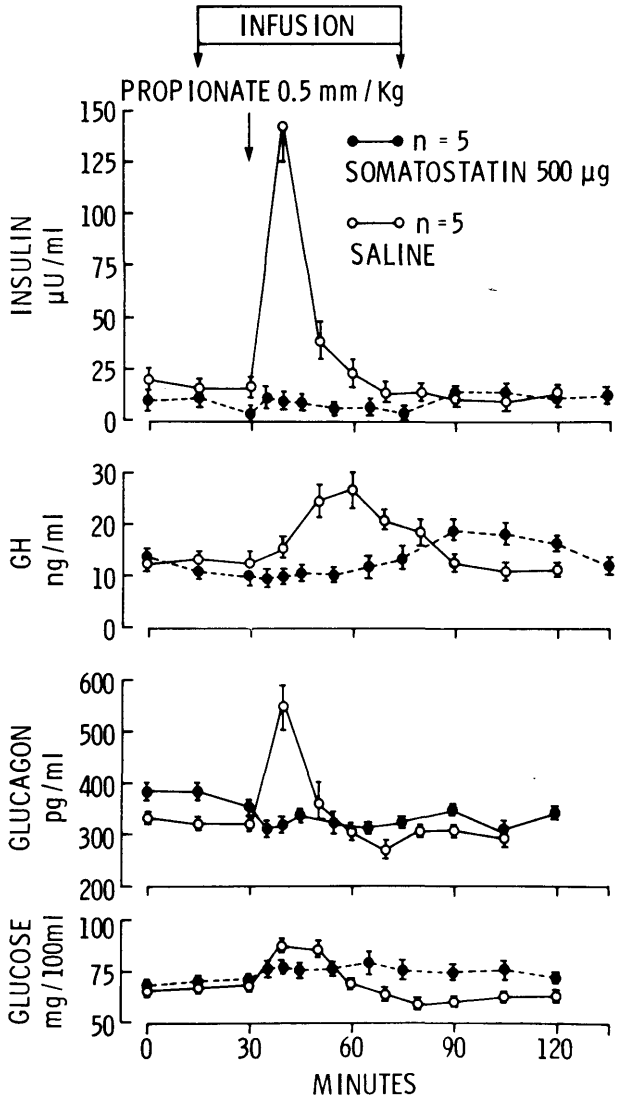

FIG. 3. Inhibition by cyclic somatostatin of insulin, GH, and glucagon release induced by sodium propionate in a group of five sheep.

Confirming previous reports,[22,23] propionate increased baseline glucagon levels from mean of 323 ± 16 pg./ml. to 544 ± 35 pg./ml. observed ten minutes after injection. Before somatostatin infusion, mean baseline values were 386 ± 25 pg./ml., which declined to a low of 310 ± 28 pg./ml. twenty minutes after the beginning of somatostatin administration and despite the injection of propionate. Plasma glucose levels increased from 68 to 86 mg. per 100 ml. in the control experiment but remained virtually constant during somatostatin infusion.

Since the inhibition by somatostatin of the insulin response to propionate administration in this study was very similar to that observed previously during epinephrine infusion,[8] and in view of the fact that epinephrine also blocks GH responses in sheep to a variety of stimuli,[8,13,24] we considered the possibility that the action of somatostatin is mediated by an adrenergic mechanism. We, therefore, injected phentolamine (Regitine), an alpha-adrenergic-receptor blocker, just before the infusion of somatostatin plus phentolamine or saline with phentolamine, followed fifteen minutes later by administration of propionate. Confirming our previous observations,[8] alpha-adrenergic blockade potentiated propionate-stimulated insulin release but somatostatin effectively blocked this as well as GH responses (figure 4). It may be significant to note that the rise in plasma GH was preceded by a precipitous fall in plasma FFA levels from a peak observed shortly after injection of phentolamine. This rise in GH levels during the time of rapidly falling plasma FFA is consistent with our previous studies of the effects of antilipolytic compounds on GH secretion in sheep.[13] Plasma glucose fluctuated within a narrow range throughout the experiment. Thus, the present results do not support a role of an adrenergic mechanism in the action of somatostatin.

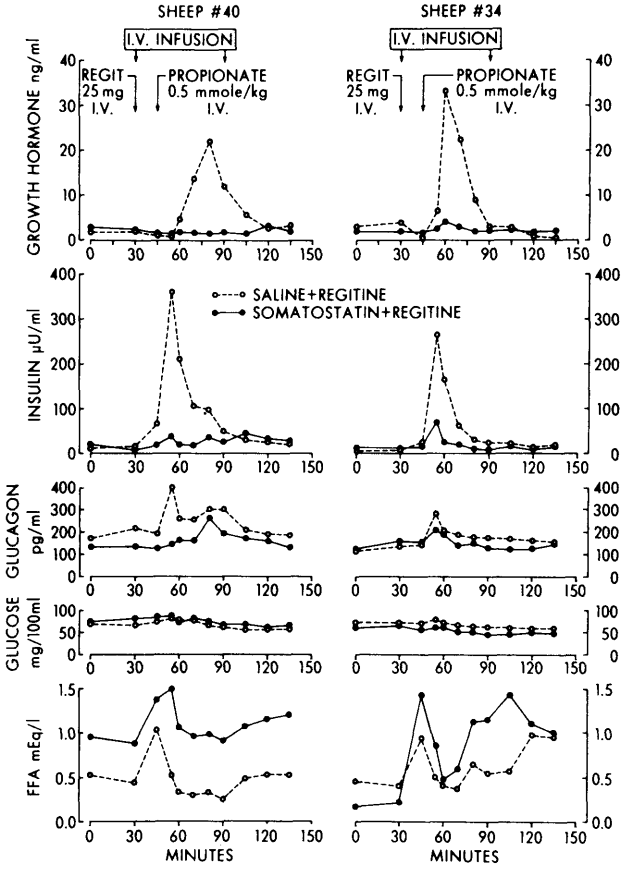

FIG. 4. Effect of somatostatin (500 μg.) in combination with phentolamine (50 mg. Regitine total dose) on propionate-stimulated hormone release in two sheep.

Inhibition of Glucose-stimulated Hormone Release by Somatostatin (Figure 5)

Apart from paradoxic rises of plasma growth-hormone levels often observed in acromegaly and in some other pathologic conditions,[25] it is well established that hyperglycemia suppresses and hypoglycemia or falling blood sugar concentrations raise plasma GH in normal humans. In sheep and other ruminants, only negligible amounts of ingested carbohydrates escape microbial fermentation, and their glucose requirements, which are not dissimilar to those of monogastric mammals, are furnished almost entirely by hepatic gluconeogenesis. It was, therefore, of interest to examine growth hormone responses to intravenously administered glucose alone and in combination with somatostatin. The results clearly demonstrate that intravenous infusion of glucose at a rate of 1 gm. per minute for sixty minutes after a forty-eight-hour fast caused a significant rise not only in plasma insulin from a baseline of 15.6 ± 3.1 μU./ml. to a peak of 248 ± 35 μU./ml. but also of GH from 3.6 ± 0.3 ng./ml. to a mean maximum of 23.8 ± 6.4 ng./ml. ($p<0.001$ and 0.01, respectively). Elevated levels of GH in humans observed after an oral glucose load are associated with falling blood sugar levels, but the temporal relationship between plasma glucose and GH are apparently quite different in sheep. The peak in plasma GH coincided exactly with the peak of plasma glucose. On the other hand, plasma FFA levels fell prior to and during the rise in plasma GH. This and the observations that the GH responses to glucose infusion were absent in fed sheep whose baseline FFA levels were low (results not shown) lend further support to an important role for fatty acids in the regulation of GH secretion in sheep.

Concomitant infusion of somatostatin (500 μg.) and glucose inhibited both GH and insulin responses as long as the peptide was infused. Within fifteen minutes, however, both hormone levels, especially those of GH, rose dramatically. Neither plasma FFA nor glucose levels were affected by somatostatin. It has often been suggested that falling plasma FFA levels after a glucose load are due in part to rising insulin concentrations. However, the almost identical changes in plasma FFA and glucose with and without somatostatin indicate that hyperglycemia per se is primarily responsible for the antilipolytic effect, since most of the rise in plasma insulin was delayed by sixty minutes in the somatostatin-treated animals.

Inhibition of Glucagon-stimulated Hormone Release by Somatostatin (Figure 6)

To examine the effects of hyperglycemia induced by

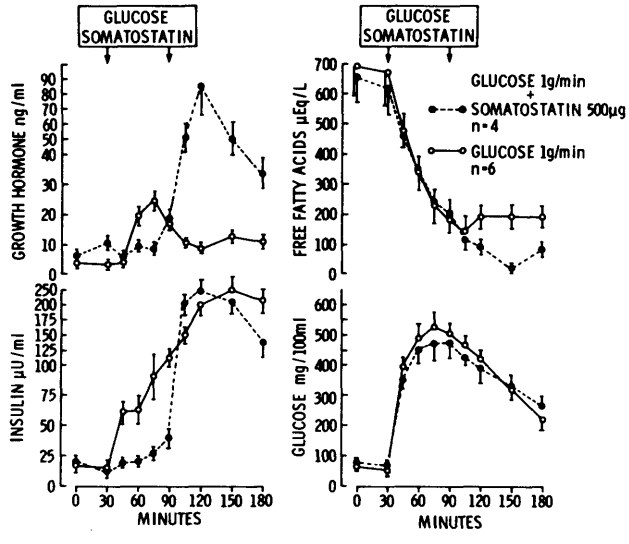

FIG. 5. Effects of glucose and cyclic somatostatin on plasma levels of GH, insulin, FFA, and glucose. Arrows under the boxes indicate period of infusion.

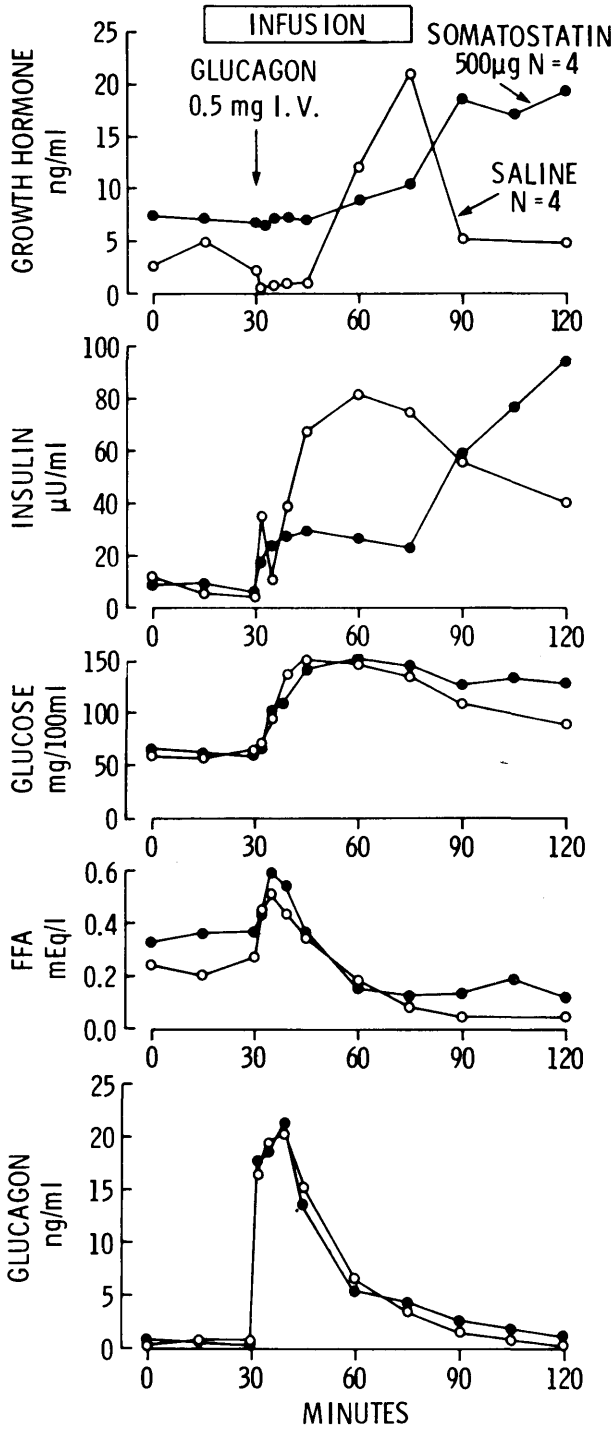

FIG. 6. Inhibition by cyclic somatostatin of glucagon-induced GH and insulin secretion in sheep.

increased endogenous production of glucose, a single intravenous bolus of glucagon (0.5 mg.) was given to sheep during a ninety-minute saline or somatostatin (500 µg.) infusion. There was a typical biphasic insulin response to glucagon in control sheep, the first peak manifesting itself two minutes after injection, returning toward baseline five minutes after injection, with a second, larger and more prolonged rise paralleling the hyperglycemic effect of glucagon. This biphasic pattern was absent when glucagon was given during somatostatin infusion. It appears, however, that the immediate insulin response to glucagon that precedes the rise in plasma glucose may be less effectively blocked by somatostatin (insulin levels rose from 4.5 ± 1.7 to 16.8 ± 4.4 µU./ml. within two minutes of glucagon injection) than by the subsequent insulinotropic effect of glucagon coincident with hyperglycemia. In fact, plasma insulin tended to decline in the presence of somatostatin despite maximal glucose values. Within the next thirty minutes, however, insulin levels rebounded to a peak of 94.2 ± 23.5 µU./ml., which was virtually identical with the mean maximum of the controls (99.5 ± 13.7 µU./ml.). The latter rise, however, occurred forty-five minutes sooner. Plasma GH levels rose from 3.3 ± 1.2 ng./ml. to a peak of 21.5 ± 6.9 ng./ml. in spite of the hyperglycemia but concomitantly with rapidly falling FFA levels. Somatostatin once again inhibited GH responses without affecting plasma glucose and FFA levels.

In Vitro Effects of Somatostatin and PGE$_1$ (Figure 7)

Prostaglandin E$_1$ has been shown to stimulate in vivo GH release in sheep.[26] However, to the best of our knowledge, this observation has not been confirmed in vitro, although it has been demonstrated repeatedly that E prostaglandins stimulate both GH release and cyclic AMP accumulation in pituitary slices of another ruminant, the cow.[27,28] It has also been reported that somatostatin inhibits PGE$_1$-promoted GH release and cyclic AMP accumulation in rat anterior pituitaries.[29,30] We, therefore,

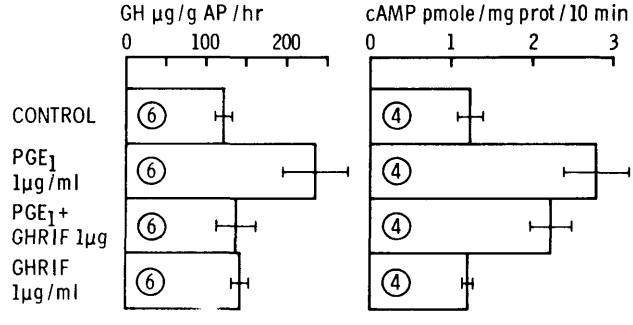

FIG. 7. In vitro effects of somatostatin and prostaglandin E$_1$ on GH release and cyclic AMP accumulation in sheep anterior pituitaries. Figures in circles denote the number of observations per treatment.

examined in this experiment the response of sheep anterior pituitary gland fragments to PGE_1, to somatostatin, and to the combination of both in terms of GH release and cyclic AMP accumulation. The results show that somatostatin (1 μg./ml.) abolished the GH response to PGE_1 (1 μg./ml.), which caused a doubling of the amount of radioimmunoassayable GH in the medium during a sixty-minute incubation. Quantitatively, a similar response to PGE_1 was observed in pituitary cyclic AMP levels during a ten-minute incubation period, which was suppressed by about 25 per cent when pituitary pieces were exposed to the combined treatment of PGE_1 and somatostatin. However, somatostatin alone had no effect on either GH release or cyclic AMP levels.

DISCUSSION

These studies clearly show that cyclic somatostatin originally isolated from sheep hypothalamic extracts inhibits GH, insulin, and glucagon responses of normal sheep to a variety of provocative stimuli. It also effectively blocked in vitro PGE_1-promoted GH release in sheep anterior pituitary tissue. The inefficacy of linear somatostatin at the dose level used in this study (500 μg.) is not readily apparent, for it has been shown in humans and baboons that its potency is similar to that of the oxidized cyclic form. On the other hand, it has been reported that the linear form has half the potency of cyclic somatostatin when both are administered subcutaneously.[31]

The results of our experiments employing arginine and glucagon as stimulants of hormone secretion are consistent with those obtained in human subjects. Furthermore, we have demonstrated that hormone responses to propionate, which has been shown to stimulate insulin and glucagon secretion only in ruminants, were also inhibited by somatostatin infusion.

Another novel aspect of this study was the consistent stimulation of GH release by intravenous infusion of glucose in fasting sheep and its inhibition by somatostatin. (It should be pointed out, however, that the paradoxic rises of GH in acromegalics after glucose administration is also blocked by somatostatin.[32]) The magnitude of GH responses was similar to that observed after propionate or glucagon administration, with a mean maximum of about 25 ng. per milliliter. However, while propionate caused a 26 per cent rise in glucose levels and glucagon a threefold increase, glucose infusion raised plasma glucose from 50 to 500 mg. per 100 ml. It would seem, therefore, that the degree of hyperglycemia is not the sole factor determining the extent of GH responses, although peak GH values generally corresponded to peak glucose levels. Furthermore, somatostatin appeared not to significantly affect basal plasma glucose in sheep, contrary to reports in humans, baboons, and dogs, yet it effectively inhibited hormone responses, including propionate-induced glucagon secretion.

As pointed out elsewhere in this communication, the ruminant under normal nutritional conditions depends to a large extent on hepatic glucose production and release to meet its glucose demand. Since glucagon is both glycogenolytic and gluconeogenic, it may play a central role in regulating glucose homeostasis in ruminants. There is, however, very little known of the secretion, effects, and action of glucagon in these animals. Bassett[23] investigated plasma levels of glucagon by an immunoassay that appeared to be specific for pancreatic glucagon and found that injection of large amounts (2.5 mmoles per kilogram) of short-chain fatty acids (propionate, butyrate, valerate) were followed by rapid and large increases in plasma of both glucagon and insulin as well as glucose concentrations, supporting the suggestion that the hyperglycemic effect of these fatty acids is mediated by glucagon.[33] On the other hand, hyperglycemia induced by exogenous glucose or isoproterenol had little or no effect on plasma glucagon, whereas epinephrine and feeding increased its concentration. Our results confirm the glucagon response to propionate administration and extend to show that arginine is a glucagon secretagogue in sheep as it is in humans. On the other hand, contrary to observations in humans[4] and baboons,[3] somatostatin did not suppress baseline levels of glucagon even when it was infused together with glucose.

Although our data on the effects of glucagon administration are not directly comparable with those published by Bassett,[34] who used a prolonged (two-hour) infusion, the general pattern in terms of marked hyperglycemia and elevation of plasma GH and insulin, and after a short-lived rise, a significant fall in FFA levels is very similar. These observations, together with previous studies, support the suggestion that the glucagon-stimulated GH release is associated with falling plasma FFA values rather than with changes in blood glucose levels. GH responses to glucagon administration have often been reported in humans, but both the time course and the magnitude of this response is different from those observed in sheep. In humans, the GH response manifests itself after a rapid fall in plasma glucose, whereas in sheep

GH levels begin to rise when glucose levels rise and reach their peak at or near the peak of plasma glucose, which remains elevated even ninety minutes postinjection.

The physiologic role of somatostatin in the regulation of hormone secretion remains uncertain. Because of the large amounts required to suppress stimulated hormone secretion, it would seem unlikely that endogenous somatostatin directly affects the endocrine pancreas. (It should be pointed out, however, that none of the agents at the concentration used in this study to provoke the release of GH, insulin, and glucagon represent physiologic stimuli.)

Neither the site of action nor the mechanism by which somatostatin affects hormone secretion is known. In the light of observations that it suppresses cyclic AMP levels and/or increases intracellular concentrations of cyclic GMP in the anterior pituitary,[35] it has been suggested that somatostatin may act by modulating cyclic nucleotide levels.[36] However, before such a mechanism of action can be assigned to somatostatin, a functional role for cyclic nucleotides in hormone secretion should be unequivocally ascertained. In view of the fact that somatostatin inhibits the secretion of a number of hormones and thereby exhibits an unusual lack of specificity, it would seem safe to suggest that it impairs a step common to the secretion of many hormones. Calcium transport would qualify as an obvious step, since the weight of evidence indicates that this ion plays an obligatory role in coupling the stimulus-secretion mechanism.[37]

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