Pancreatic Somatostatin is a Mediator of Glucagon Inhibition by Hyperglycemia

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SUMMARY

We have previously shown that a nonimmunoreactive analogue of somatostatin, (D-Ala^5, D-Trp^8)-somatostatin, differentially inhibits pancreatic somatostatin secretion without inhibiting insulin or glucagon secretion. During normoglycemia, suppression of pancreatic somatostatin with this analogue increases glucagon and insulin secretion, suggesting that pancreatic somatostatin tonically inhibits glucagon and insulin secretion by a paracrine mechanism. In our study, we used this analogue to determine whether endogenous pancreatic somatostatin has a role in the inhibition of glucagon secretion by hyperglycemia. The experiments were performed in pentobarbital-anesthetized, laparotomized dogs. To measure the pancreatic output of somatostatin directly, pancreatic venous blood was sampled from the right lobe of the dog pancreas, and the pancreatic blood flow was measured. In the first set of experiments, glucagon secretion was suppressed by a glucose infusion (200 mg/kg bolus and 20 mg · kg^{-1} · min^{-1} i.v.) for 3 h. Plasma glucose rose from 102 ± 6 to 365 ± 34 mg/dl. Pancreatic insulin output increased 10-fold, pancreatic somatostatin output increased from 1.2 ± 0.3 to 3.0 ± 0.8 ng/min, and pancreatic glucagon output was suppressed from 1.4 ± 0.7 to 0.5 ± 0.1 ng/min. After 2 h of glucose infusion, an infusion of the analogue (5.5 μg/min i.v.) reversed both the stimulation of somatostatin and the suppression of glucagon without significantly changing either the plasma glucose level or the pancreatic insulin output. In a second set of experiments, basal somatostatin output was suppressed by the analogue (5.5 μg/min i.v.) for 15 min before the administration of glucose. Pancreatic somatostatin output decreased from 2.6 ± 0.4 to 1.6 ± 0.3 ng/min, and pancreatic glucagon output rose from 1.2 ± 0.6 to 2.9 ± 0.9 ng/min. Glucose was then infused (200 mg/kg bolus and 20 mg · kg^{-1} · min^{-1} i.v.) in combination with the analogue. In response to glucose, somatostatin output rose, and glucagon output was suppressed back toward the original basal levels. Thus, in both sets of experiments, a reciprocal relationship between somatostatin and glucagon secretion was demonstrated regardless of whether the plasma glucose and insulin output changed. These data provide evidence that pancreatic somatostatin is a mediator of the suppression of glucagon during hyperglycemia. *Diabetes* 36:592–96, 1987

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It has been proposed that somatostatin from the δ-cells of the pancreatic islet (1) tonically inhibits pancreatic glucagon and insulin secretion by a paracrine mechanism (1–8). This hypothesis is based partly on the known inhibitory effect of pharmacologic doses of somatostatin on insulin and glucagon secretion in all species (9–11) and the anatomical position of the δ-cells between the outer mantle of α-cells and an inner core of β-cells in the rat islet (1,7,8).

To provide more direct experimental support for this hypothesis, we previously employed an analogue of somatostatin, (D-Ala^5, D-Trp^8)-somatostatin (SS_a), with relative selectivity for inhibiting pancreatic somatostatin compared with insulin or glucagon secretion (2). Infusion of low doses of this analogue into dogs suppressed basal pancreatic somatostatin secretion, and this was associated with a small rise in insulin and a large rise in basal glucagon output (2).

The mechanism by which hyperglycemia inhibits glucagon secretion is unclear. The suggested mechanisms include *1*) a direct inhibitory effect of glucose on the α-cell (12–14), *2*) an inhibitory effect of insulin on the α-cell (15–18), or *3*) indirect mediation by pancreatic somatostatin (19). We have employed SS_a to determine if endogenous pancreatic somatostatin mediates the inhibition of glucagon secretion by hyperglycemia in dogs. This was done by inhibiting pancreatic somatostatin during as well as before glucose-induced suppression of glucagon.

MATERIALS AND METHODS

Animals and surgical preparations. Adult mongrel dogs (weighing 25–35 kg) were fasted for 18 h and then anesthetized with pentobarbital sodium (30-mg/kg i.v. bolus and 0–0.2 mg · kg^{-1} · min^{-1}). Femoral arterial and venous catheters were inserted, and a laparotomy was performed. The superior pancreaticoduodenal vein was cannulated with a Silastic catheter and bypassed via an electromagnetic flowmeter and a sampling port into the portal vein. The small vascular branches between the duodenum and the pancreas were ligated and cut. This preparation allowed the measurement of insulin, glucagon, and somatostatin output from the right lobe of the pancreas without contamination by duodenal venous drainage. Details of the surgical procedures have recently been described (2).

Protocols and assays. In the first group of experiments, we examined the effects of somatostatin suppression on the secretion of glucagon that was already inhibited by hyperglycemia. In seven experiments, glucose was infused as a bolus dose of 200 mg/kg i.v. followed by a continuous infusion of 20 mg · kg^{-1} · min^{-1} for 3 h. After 120 min of the glucose infusion, designed to produce stable glucagon suppression, SS_a was simultaneously infused at 5.5 μg/min i.v. for 30 min.

Blood samples were drawn simultaneously from the pancreatic vein and femoral artery at 15, 5, and 0 min before and at 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 115, and 120 min during the glucose infusion. After beginning the analogue infusion, blood was drawn at 5, 10, 20, and 30 min and at 5, 10, 15, and 30 min after the analogue was discontinued.

In the second group of experiments ($n = 6$), we examined the effect of prior suppression of somatostatin on glucagon inhibition. SS_a was infused (5.5 μg/min i.v.) for 30 min to inhibit somatostatin secretion. After 15 min of analogue infusion, glucose was administered in a bolus of 200 mg/kg followed by an infusion of 20 mg · kg^{-1} · min^{-1} for a total of 45 min. Thus, the analogue infusion was continued simultaneously with the glucose for 15 min, and then glucose alone was infused for 30 min more. Blood samples were drawn simultaneously from the femoral artery and pancreatic vein at 15, 5, and 0 min before the analogue infusion and at 5-min intervals throughout the remainder of the experiment.

Blood samples for insulin (immunoreactive insulin; IRI) and glucose assays were drawn in EDTA, those for glucagon (immunoreactive glucagon; IRG) assay in benzamidine (20) and heparin, and those for somatostatin assay (somatostatin-like immunoreactivity; SLI) in a mixture of anticoagulants and proteolytic enzyme inhibitors as described by DeHaen et al. (21). The radioimmunoassays for IRI, IRG, and SLI have previously been described (2).

Data analysis. Pancreatic hormone output was calculated by subtracting the femoral arterial levels from the pancreatic venous levels and multiplying the difference by the pancreatic venous plasma flow. The data were expressed as means ± SE, and statistical differences were evaluated by the paired *t* test.

RESULTS

With the intravenous infusion of glucose, plasma glucose rose from 102 ± 6 to 365 ± 34 mg/dl after 40 min and remained at a steady level for the duration of the experiment (Fig. 1). Mean pancreatic IRI output from the right lobe of the in situ dog pancreas increased ~10-fold after 40 min of glucose infusion and remained elevated throughout the experiment without significant fluctuation. The analogue (5.5 μg/min) was infused after 120 min of glucose administration and did not significantly alter either the plasma glucose level or the mean insulin output (Fig. 1).

Mean preglucose pancreatic SLI output was 1.2 ± 0.3 ng/min. This increased to 2.2 ± 0.6 ng/min after the first 5 min of glucose infusion ($P < .02$) and remained elevated for the 120 min of the glucose infusion (Fig. 1). Pancreatic IRG output decreased from a basal level of 1.4 ± 0.7 to 0.9 ± 0.4 ng/min after 5 min of glucose infusion and then continued to fall to 0.5 ± 0.1 ng/min ($P < .01$) after 120 min. The analogue, which was infused after 120 min of hyperglycemia, returned the stimulated SLI output toward the original basal levels. This was accompanied by reversal of the glucagon inhibition, with glucagon output returning toward original basal levels. With cessation of the analogue infusion, SLI output again rose, and IRG output was again suppressed to the preinfusion levels (Fig. 1).

In the second series of experiments (Fig. 2) the same dose of SS_a was given 15 min before the initiation of the glucose infusion. After SS_a and glucose were infused simultaneously for 15 min, suppression of SLI was removed by cessation of the SS_a infusion. The infusion of the SS_a alone inhibited mean basal SLI output from 2.6 ± 0.4 to 1.6 ± 0.3 ng/min ($P < .05$) after 15 min of infusion (Fig. 2). Pancreatic vein IRG output rose from 1.2 ± 0.6 to 2.9 ± 0.9 ng/min ($P < .05$), and IRI output rose from 4.0 ± 1.2 to 9.3 ± 3.6 mU/min ($P < .05$). With the addition of the glucose infusion, plasma glucose rose from 124 ± 8 to 365 ± 11 mg/dl, and insulin output increased to 26 ± 8 mU/min. Somatostatin output also increased to 2.3 ± 0.4 ng/min, near the original basal level, whereas glucagon output decreased to 1.2 ± 0.5 ng/min, near its original basal level (Fig. 2). Neither insulin output nor plasma glucose level were significantly altered by discontinuation of the analogue infusion, but SLI output rose to 4.2 ± 0.6 ng/min, and glucagon output was suppressed below basal to 0.5 ± 0.3 ng/min (Fig. 2). Peripheral arterial SLI levels were not altered by the glucose infusion. However, infusion of the SS_a caused a mean decrease of 14 ± 3 pg/ml in peripheral SLI in both sets of experiments.

DISCUSSION

Infusion of SS_a at 5.5 μg/min produced a 30–40% fall in basal pancreatic SLI output (Fig. 2), consistent with our earlier report (2). This decrease of net pancreatic SLI output was caused by a decrease of SLI concentration in the superior pancreatic vein plasma, probably due to direct inhibition of δ-cell secretion. The suppression of pancreatic SLI was associated with a two- to threefold increase of basal glucagon secretion. Although we cannot exclude the possibility that low concentrations of the analogue directly stimulate the α- and β-cells, there is evidence that higher concentrations of the analogue directly suppress these cells (2). Therefore we favor the interpretation that the stimulation of glucagon is indirect via either *1*) suppression of endogenous SLI and release of the α-cell from tonic inhibitory effects of pancreatic somatostatin or *2*) antagonism of endogenous somatostatin action in addition to suppressing

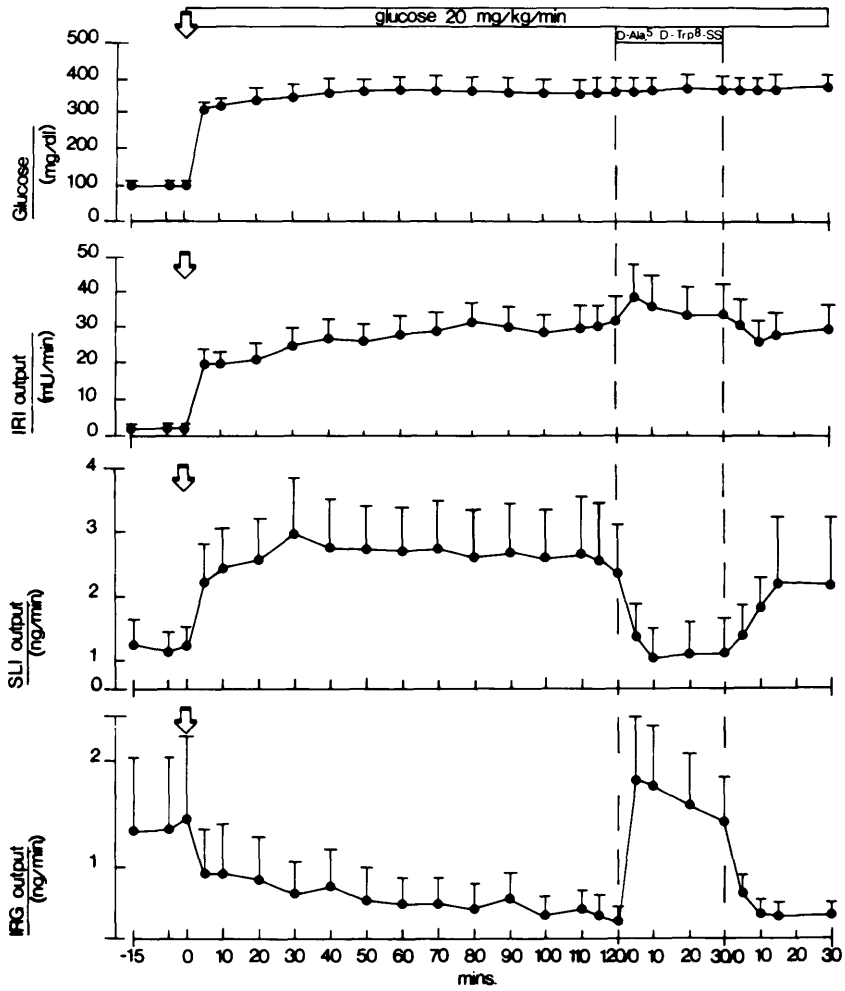

FIG. 1. Effects of glucose infusion (200 mg/kg i.v. followed by 20 mg · kg^{-1} · min^{-1}) and simultaneous infusion of (D-Ala^5, D-Trp^8)-somatostatin (5.5 μg/min i.v.) on plasma glucose and pancreatic output of insulin (IRI), somatostatin (SLI), and glucagon (IRG). n = 7.

SLI release. Either of these explanations would support the hypothesis that pancreatic somatostatin is a paracrine modulator of basal glucagon secretion.

This study was designed to determine the relationship of pancreatic somatostatin to the suppression of glucagon secretion during hyperglycemia. Thus, pancreatic somatostatin increased in response to hyperglycemia at the same time glucagon secretion decreased. Furthermore, suppression of pancreatic somatostatin toward its original basal levels by infusion of the analogue reversed the glucose-induced inhibition of glucagon secretion. Discontinuation of the analogue infusion restored pancreatic somatostatin to its stimulated level and returned glucagon to its inhibited level. A similar pattern was noted in the second series of experiments. Thus, there is a reciprocal relationship between the changes in somatostatin and glucagon secretion induced either by *1*) hyperglycemia alone, *2*) the analogue infusion alone, *3*) the combination of the two, or *4*) the discontinuation of the analogue. This reciprocal relationship is independent of plasma glucose and insulin levels, because it occurs regardless of whether glucose and insulin change. Therefore we conclude that pancreatic somatostatin may be a mediator of glucagon inhibition by hyperglycemia. Note, however, that this conclusion depends on the validity of our assumption that the effect of the analogue on glucagon secretion is mediated indirectly via suppression of pancreatic somatostatin.

In our previous study we noted that suppression of pancreatic somatostatin was associated with a large rise in glucagon secretion but a relatively small rise in insulin secretion (2). Although this may have been due to differential direct suppressive effects of the analogue on the β-cell, compared with the α-cell, an alternative explanation is that pancreatic somatostatin is a more important paracrine mediator of glucagon secretion than insulin secretion. This would be consistent with the microanatomy of the rat islet where the α- and δ-cells seem to be preferentially associated with each other (22), whereas only the β-cells on the periphery of the β-cell core are associated with δ-cells. Moreover, the direction of blood flow within the islet (and thus, presumably, the interstitial fluid drainage) is from the β-cell–rich core to the non–β-cell rim and may limit the penetration of somatostatin into the core of the islet (23). Further evidence from our study that pancreatic somatostatin is not a major physiological mediator of β-cell secretion is that when the analogue was withdrawn in the presence of the high glucose concentration (Fig. 2), the rise in endogenous somatostatin did not inhibit insulin secretion. These data suggest that glucose's powerful stimulation of the β-cell overrides the comparatively minor suppressive effects of pancreatic somatostatin on insulin secretion.

Although our study suggests that pancreatic somatostatin mediates part of the inhibition of glucagon secretion during

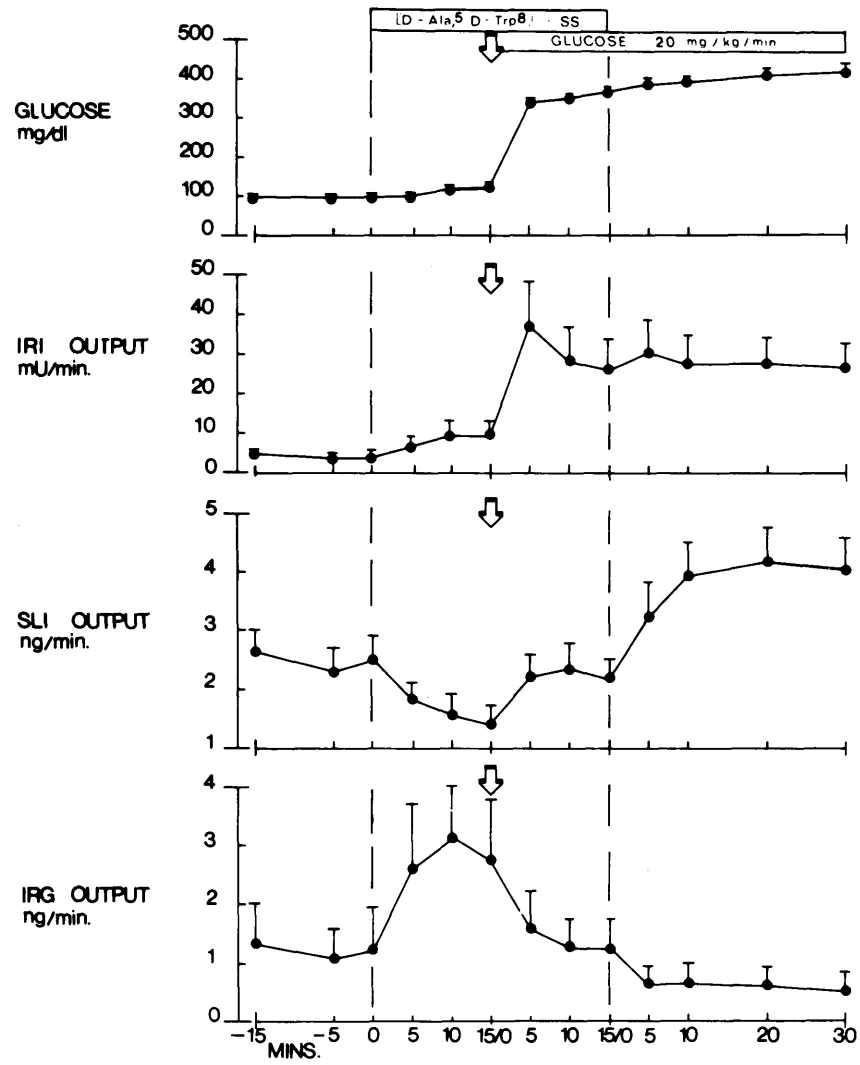

FIG. 2. Effects of glucose infusion (200 mg/kg i.v. followed by 20 mg · kg^{-1} · min^{-1}) and simultaneous infusion of (D-Ala^5, D-Trp^8)-somatostatin (5.5 μg/min i.v.) on plasma glucose and pancreatic output of insulin (IRI), somatostatin (SLI), and glucagon (IRG). n = 6.

hyperglycemia, other mechanisms have been proposed. Recent evidence suggests that at least high concentrations of glucose have a direct inhibitory effect on the α-cell: in isolated rat α-cells, 20 mM glucose inhibited the glucagon secretion stimulated by a mixed amino acid solution (14). In hyperglycemic C-peptide–negative diabetic patients, Asplin et al. (12) have provided evidence that glucagon can be suppressed directly by glucose, whereas in diabetic dogs made euglycemic by phloridzin treatment, glucose infusion inhibited the elevated glucagon levels (13). In contrast, Stagner and Samols (24) have provided evidence that the direct effect of glucose on the α-cell may be to increase glucagon secretion.

It has also been proposed that insulin has a direct inhibitory effect on the α-cell. Infusion of insulin in high doses inhibits glucagon secretion both in vivo and in vitro (15,16,18,19). In normal humans the suppression of glucagon secretion by mild hyperglycemia during a hyperinsulinemic clamp, which suppresses endogenous insulin secretion and β-cell activity, appears to be related to endogenous β-cell activity rather than to the circulating insulin levels (25). Thus, it has been proposed that the β-cell controls the α-cell response to hyperglycemia either by a paracrine mechanism (18,25) or via the microcirculation (17,19).

It is possible that somatostatin mediates the inhibition of glucagon secretion together with insulin. The secretion patterns of the β- and δ-cells are remarkably similar, both during basal oscillatory cycles (26) and in response to numerous stimuli (27–29). High-affinity receptors for somatostatin are present on the isolated α-cell of the rat (30) and are exquisitely sensitive to inhibition by somatostatin (14). Thus our data support the notion that somatostatin acting via a paracrine mechanism is secreted in concert with insulin and is a mediator of the inhibition of glucagon by hyperglycemia.

Sorenson and Elde (31) have disputed this view and have suggested that glucose is the major mediator of glucagon inhibition. They showed in isolated, perfused rat pancreas that when the glucose in the perfusate was raised from hypoglycemic levels, glucagon secretion was inhibited at a glucose level below that at which insulin or somatostatin secretion occurs. However, hypoglycemia stimulates glucagon secretion, and this has been reported to depend on an intrapancreatic adrenergic pathway (32). Thus, inhibition of the glucagon secretion, which had been stimulated by the low glucose levels, may have been due to deactivation of this neural pathway. In contrast, somatostatin and/or insulin may be the predominant regulator of glucagon secretion during hyperglycemia.

In conclusion, the use of an analogue to inhibit somatostatin release is a new approach in determining the potential role of pancreatic somatostatin as a paracrine modulator of glucagon secretion. The data presented here provide evidence that pancreatic somatostatin mediates at least part of the inhibition of glucagon secretion seen during hyperglycemia.

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