Inhibition of Glucagon Secretion by Exogenous Glucagon in the Isolated, Perfused Dog Pancreas

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SUMMARY

To determine if glucagon can inhibit its own secretion, exogenous glucagon was perfused in the isolated canine pancreas at concentrations ranging from 90 to 1050 pg/ml during either stimulation or suppression of endogenous glucagon secretion. When endogenous glucagon secretion was completely suppressed by the addition of 200 mg/dl of glucose to the perfusate, the concentration of glucagon in the venous effluent ranged from 80.8 to 89.9% of the level perfused; these deficits of 10–20% were attributed to uptake and/or degradation of exogenous glucagon by the pancreas. By contrast, when endogenous glucagon secretion was stimulated by perfusing with 10 mM arginine or by glucopenia of 25 mg/dl, there was a significant increase in the glucagon deficit (the efflux glucagon level during glucagon perfusion minus the sum of the pre-perfusion efflux level of endogenous glucagon and the concentration of glucagon perfused); only 60–76% of the expected glucagon concentration was present in the venous efflux. This increase in deficit is assumed to reflect suppression of endogenous glucagon. The glucagon deficit rose progressively with the perfused concentration of glucagon until the deficit reached the approximate level of endogenous glucagon, at which no further increase occurred, suggesting that suppression at that point was complete.

To determine if the glucagon-suppressing action of glucagon was mediated by an increase in glucagon-stimulated insulin or somatostatin secretion, these hormones were measured in the various experiments. Insulin and somatostatin increased significantly when glucagon was perfused with the glucose arginine-containing solution but did not rise significantly when the glucopenic buffer was used, although suppression of endogenous glucagon was similar in each instance. It is concluded that glucagon suppresses glucagon secretion. Evidence that insulin and/or somatostatin mediate this action could not be obtained. DIABETES *31*:512–515, 1982.

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Insulin and somatostatin have been shown to inhibit their own secretion.[1–4] The present study was designed to determine if glucagon also inhibits its own secretion.

MATERIALS AND METHODS

After an overnight fast, male mongrel dogs weighing 23–30 kg were subjected to laparotomy under sodium pentothal anesthesia. The pancreas was isolated and after a 40-min equilibration period perfused according to the method of Iversen and Miles[1] as previously modified.[5] The celiac artery and portal vein were catheterized and the preparation was placed into a tissue floatation bath maintained at 37°C and perfused without recirculation with Krebs-Ringer bicarbonate solution containing 4% dextran T-70 (Pharmacia Fine Chemicals, Piscataway, New Jersey), 0.2% bovine serum albumin, 5 mM each of pyruvate, fumarate and glutamate, and either 200 mg/dl of glucose with or without 10 mM arginine or 25 mg/dl of glucose. This solution was maintained at 37°C and bubbled constantly with a 95% O_2/5% CO_2 mixture. The flow rate was 18 ml/min and the perfusing pressure was maintained between 25 and 40 mm Hg. The pancreatic effluent was collected at 1-min intervals in chilled tubes containing 2 ml EDTA-benzamidine mixture (0.03 M/0.3 M). These were stored at −20°C until assay. Exogenous pork glucagon (Novo Research Institute, Copenhagen, Denmark) in 0.3% bovine serum albumin (Sigma Chemical, St. Louis, Missouri) solution was added to the perfusate through a side-arm syringe. The infusion pump was a Model 942 (Harvard Apparatus Co., Millis, Massachusetts).

Glucagon was assayed as previously described.[6] Somatostatin-like immunoreactivity was determined using a modification[7] of the methods of Arimura et al.[8] and Kronheim et al.[9] Insulin was assayed by a modification of the method of Yalow and Berson.[10]

Statistical analysis was made by Student's *t* test or analysis of variance.

RESULTS

Recovery of exogenous glucagon perfused through the pancreas. To determine how much of the glucagon perfused through the pancreas is removed and/or degraded in transit, exogenous glucagon was perfused at various concentrations under circumstances in which secretion of endogenous glucagon had been completely suppressed by perfusing with a buffer containing 200 mg/dl of glucose. In 28 such experiments, baseline glucagon in the pancreatic venous effluent measured zero (Table 1A). Thus, in this circumstance any difference between the glucagon concentration perfused and that in the venous efflux would be entirely attributable to uptake and/or degradation of exogenous glucagon by the pancreas. The "deficit" of glucagon (concentration perfused minus that measured in the venous effluent) ranged from 17 pg/ml, during perfusion of 90 pg/ml, to 150 pg/ml, during perfusion of 1050 pg/ml (Table 1A). Recovery of glucagon exceeded 80% at every concentration perfused, i.e., less than 20% of the perfused glucagon was lost in transit.

Effect of exogenous glucagon on endogenous glucagon secretion stimulated by arginine. To stimulate A-cell secretion, 10 mM arginine was added to the buffer used in the preceding experiments. The basal glucagon concentration in the venous effluent averaged 127.6 ± 8.0 pg/ml in 26 such experiments. During perfusion of glucagon at concentrations of 90–1050 pg/ml, the total deficit of glucagon [the expected concentration (sum of the baseline level of endogenous glucagon plus the level of exogenous glucagon perfused) minus the actual concentration in the venous effluent] was invariably greater (Table 1B) than the deficit observed in the absence of endogenous glucagon secretion (Table 1A). The venous effluent concentration of glucagon averaged only 60–75.8% of the expected level at all but the lowest perfusion rate. This increase in the glucagon deficit, which is attributed to suppression of endogenous glucagon, rose progressively with each increase in the perfusion concentration of glucagon. However, it did not exceed 172 pg/ml, which is the approximate concentration of endogenous glucagon prior to the start of a glucagon perfusion. The fact that higher perfusion concentrations did not further increase this deficit suggests that it reflects endogenous glucagon secretion and that complete suppression had been attained.

Effect of exogenous glucagon on endogenous glucagon secretion stimulated by glucopenia. To exclude the unlikely possibility that the arginine used to stimulate glucagon secretion had somehow enhanced glucagon uptake and thus led us to an incorrect interpretation of the findings, the foregoing experiments were repeated during stimulation of endogenous glucagon secretion by glucopenia. With a perfusate glucose concentration of 25 mg/dl endogenous glucagon averaged 85.9 ± 13.0 pg/ml in 18 experiments. The glucagon deficits during perfusion of exogenous glucagon (Table 1C) once again were significantly greater ($P < 0.01$) than those observed in the absence of endogenous glucagon secretion (Table 1A) and did not differ significantly from those observed during arginine-stimulated glucagon secretion (Table 1B). The venous efflux concentration during glucagon perfusion ranged from 62.4 to 73.9% of the expected. Once again, the increase in deficit did not significantly exceed the endogenous glucagon level at any point.

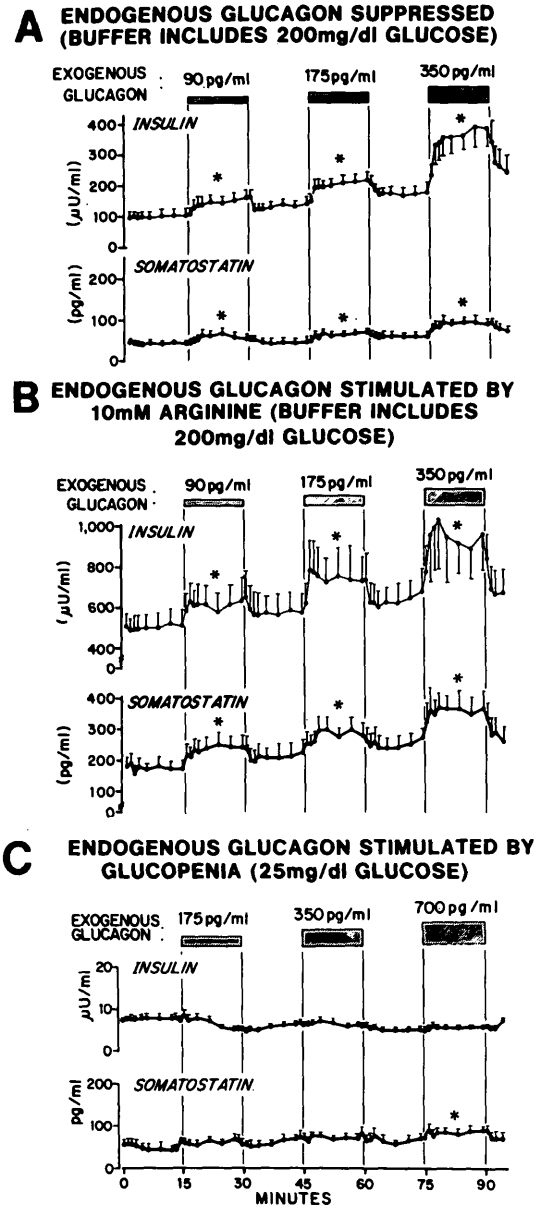
TABLE 1
Actual and expected glucagon levels in the venous effluent of the isolated dog pancreas during perfusion with glucagon during suppression (A) or stimulation (B,C) of endogenous glucagon secretion*

Exogenous glucagon perfused (pg/ml)	Endogenous glucagon in venous effluent before glucagon perfusion (mean* ± SEM) (pg/ml)	Expected glucagon in venous effluent during glucagon perfusions (exogenous + endogenous) (pg/ml)	Actual glucagon in venous effluent during glucagon perfusion (mean* ± SEM) (pg/ml)	Glucagon deficit (expected minus actual) (pg/ml)	Difference of glucagon deficits from the deficits in A (pg/ml)
A. Endogenous glucagon secretion suppressed by 200 mg/dl of glucose in perfusion buffer					
90 (N = 6)	0	90	73 ± 3	17 ± 4	
175 (N = 6)	0	175	161 ± 8	14 ± 6	
350 (N = 6)	0	350	340 ± 14	10 ± 12	
700 (N = 6)	0	700	565 ± 22	135 ± 43	
1050 (N = 4)	0	1050	891 ± 55	159 ± 21	
B. Endogenous glucagon secretion stimulated by 10 mM arginine in perfusion buffer containing 200 mg/dl glucose					
90 (N = 6)	107 ± 17	197	167 ± 17†	30 ± 9	13 ± 9
175 (N = 6)	132 ± 19	307	251 ± 16†	56 ± 5†	42 ± 5
350 (N = 6)	143 ± 22	493	346 ± 16	147 ± 13†	137 ± 13
700 (N = 4)	125 ± 6	825	575 ± 15	250 ± 16†	115 ± 16
1050 (N = 4)	132 ± 17	1182	851 ± 21	331 ± 32‡	172 ± 32
C. Endogenous glucagon secretion stimulated by glucopenia (25 mg/dl)					
175 (N = 6)	74 ± 2	249	185 ± 4‡	64 ± 5†	50 ± 5
350 (N = 6)	85 ± 2	435	342 ± 6	93 ± 16†‡	83 ± 17
700 (N = 6)	99 ± 5	799	542 ± 36	257 ± 22‡	122 ± 22§

* Calculated from the 6 samples between 3 and 15 min after the start of a 15-min perfusion period.
† Differs from corresponding value in A experiments ($P < 0.01$).
‡ Differs from corresponding value in B experiments ($P < 0.05$).
§ Not significantly different from corresponding endogenous glucagon concentration before glucagon perfusion.

Effect of exogenous glucagon on insulin and somatostatin secretion. Glucagon stimulates both insulin[11] and somatostatin secretion,[5] and insulin[12] and somatostatin[13] both inhibit glucagon release. The negative effect of glucagon on glucagon secretion could, therefore, have been mediated by stimulation of one or both of these hormones. To determine if this might be the cause of the suppression of glucagon, insulin and somatostatin were measured in pancreatic venous efflux during all of the foregoing experiments (Figure 1). When 200 mg/dl glucose and 10 mM arginine were present in the perfusate (Figure 1A and B), insulin and somatostatin were both stimulated significantly by all doses of exogenous glucagon ($P < 0.05$), whereas in the experiments employing a perfusate glucose concentration of 25 mg/dl, glucagon failed to elicit a significant sustained rise in either hormone (Figure 1C). Nevertheless, evidence of full suppression of endogenous glucagon was observed both in the presence (Table 1B) and absence (Table 1C) of a significant rise in insulin and somatostatin levels in the pancreatic venous effluent.

FIGURE 1. Changes of insulin and somatostatin concentrations from the isolated dog pancreas during perfusion with exogenous glucagon. Mean ± SEM (N = 6). (A) Perfusate contained 200 mg/dl of glucose. (B) Perfusate contained 200 mg/dl of glucose and 10 mM arginine. (C) Perfusate contained 25 mg/dl of glucose. Statistical comparison between the basal hormone levels and the glucagon-stimulated levels made by calculating the sum of hormone levels during each 15-min and using paired *t* test. *Indicates statistical significance ($P < 0.05$).



DISCUSSION

The findings provide strong evidence in support of an inhibitory action of glucagon on the A-cell similar to that reported previously for insulin and the B-cell[1-3] and somatostatin and the D-cell.[4] The inhibitory effect of glucagon upon A-cell secretion was established by showing that the perfusion of glucagon at various concentrations into the isolated dog pancreas during stimulation of A-cell secretion resulted in a greater deficit in glucagon (secreted endogenous glucagon plus perfused exogenous glucagon) recovered in the venous effluent than could be accounted for by pancreatic uptake and/or destruction of perfused glucagon as observed in the absence of A-cell secretion. Glucagon-induced suppression of endogenous glucagon secretion increased with the amount of glucagon perfused and became complete when glucagon was perfused at a concentration of 350 pg/ml and above, or at about 3–10 times the basal concentration of endogenous glucagon.

The data do not indicate whether or not this inhibition of glucagon secretion occurs via a direct action of glucagon on the A-cells or by stimulating the secretion of insulin[11] and somatostatin,[5] both of which inhibit glucagon secretion.[12,13] In the experiments in which the glucose concentration of the perfusate was reduced to 25 mg/dl, there was no significant glucagon-stimulated increase in insulin and somatostatin secretion (Figure 1C). Nevertheless, suppression of glucagon (Table 1C) was just as great as when arginine was used as the A-cell stimulus (Table 1B) and a prominent glucagon-mediated rise in insulin and somatostatin secretion occurred (Figure 1). The findings offer no clear support for a mediating role of either hormone in the glucagon-glucagon negative feedback, although a local glucagon-induced increase in insulin and/or somatostatin cannot be excluded.

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