Secretin Inhibits Glucagon in the Isolated Perfused Dog Pancreas

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SUMMARY

To investigate the effect of secretin upon islet hormone secretion, highly purified porcine secretin was perfused at 0.0001, 0.001, 0.01, and 0.1 clinical unit (CU)/ml in the isolated normal dog pancreas. The lowest concentration of secretin, a level which is within the physiologic range, significantly suppressed glucagon release and stimulated somatostatin release but was without significant effect on insulin release. At each concentration of secretin tested glucagon was dramatically suppressed and somatostatin was significantly stimulated, both in a dose-dependent manner. B-cell activity was first augmented by the concentration of 0.001 CU/ml (94 pM) of secretin.

These data demonstrate that physiologic levels of secretin suppress pancreatic A-cell activity in the isolated dog pancreas. DIABETES *32*:970–973, October 1983.

In 1972 Santeusanio et al.[1,2] reported that secretin suppressed both basal and stimulated glucagon secretion in conscious dogs. However, subsequent studies in humans,[3,4] pigs,[5,6] rats,[7–10] and the isolated perfused canine pancreas[11] failed to confirm these findings. Consequently, the original claim has been in doubt and secretin is generally considered not to influence glucagon secretion.[3–15]

The present study was designed to reevaluate this issue by determining the effects of both physiologic and pharmacologic concentrations of purified porcine secretin on insulin, glucagon, and somatostatin release in the isolated perfused canine pancreas.

MATERIALS AND METHODS

Male mongrel dogs weighing 18–28 kg and in a healthy state, as evidenced by normal hematocrit and white blood cell count, were used as pancreas donors. After overnight fast, the pancreas was isolated and perfused according to the method of Iversen and Miles[16] as previously modified.[17] In brief, the pancreas with a 10-cm long proximal part of duodenum attached was isolated under pentobarbital anesthesia. During a warm ischemia time lasting less than 30 s, the extracorporeal cannulation of the celiac arterial trunk and portal vein was performed. The preparation was transferred to a tissue floatation bath maintained at 37°C and perfused with Krebs-Ringer bicarbonate solution containing 4% dextran T-70 (Pharmacia Fine Chemicals, Piscataway, New Jersey), 0.2% bovine serum albumin (Sigma Chemical, St. Louis, Missouri), 5 mM each of pyruvate, fumarate, and glutamate, and 5.5 mM glucose with 10 mM arginine. This perfusion medium was maintained at 37°C and pH 7.4 and oxygenated constantly with 95% O_2/5% CO_2 mixture at atmospheric pressure. Flow was maintained at a rate of 17.0–18.0 ml/min by means of a Harvard peristaltic pump (Harvard Apparatus Co., Millis, Massachusetts) with the perfusion pressure between 10 and 20 mm Hg. An equilibration period of 40 min preceded experimental periods of 130 min.

Highly purified porcine GIH secretin (Lot No. 8202551, purchased from Kabi Diagnostica, Studsvik, Sweden) was dissolved in ice-cold isotonic saline containing 0.2% bovine serum albumin (Sigma Chemical) 3–4 h before injection. This was added to the perfusate circulating in the main perfusion circuit through a side-arm syringe by an infusion pump (Model 942 Harvard Apparatus Co.) adjusted to deliver the sample at 1:100 dilution rate. Mixing of infused material in the perfusion circuit was achieved by the slight turbulence produced by the abrupt caliber change of circuit tubings. After a 15-min baseline period, secretin was infused for 15 min at a concentration of 0.0001 CU/ml (29 pg/ml; 9.4 pM), 0.001 CU/ml (290 pg/ml; 94 mM), 0.01 CU/ml (2.9 ng/ml; 940 pM), and 0.1 CU/ml (29 ng/ml; 9.4 nM) in that order, with intervening secretin-free intervals of 15 min.*

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*One microgram of natural porcine secretin is equal to 3.5 CU.[24] The molecular weight of secretin is 3055.

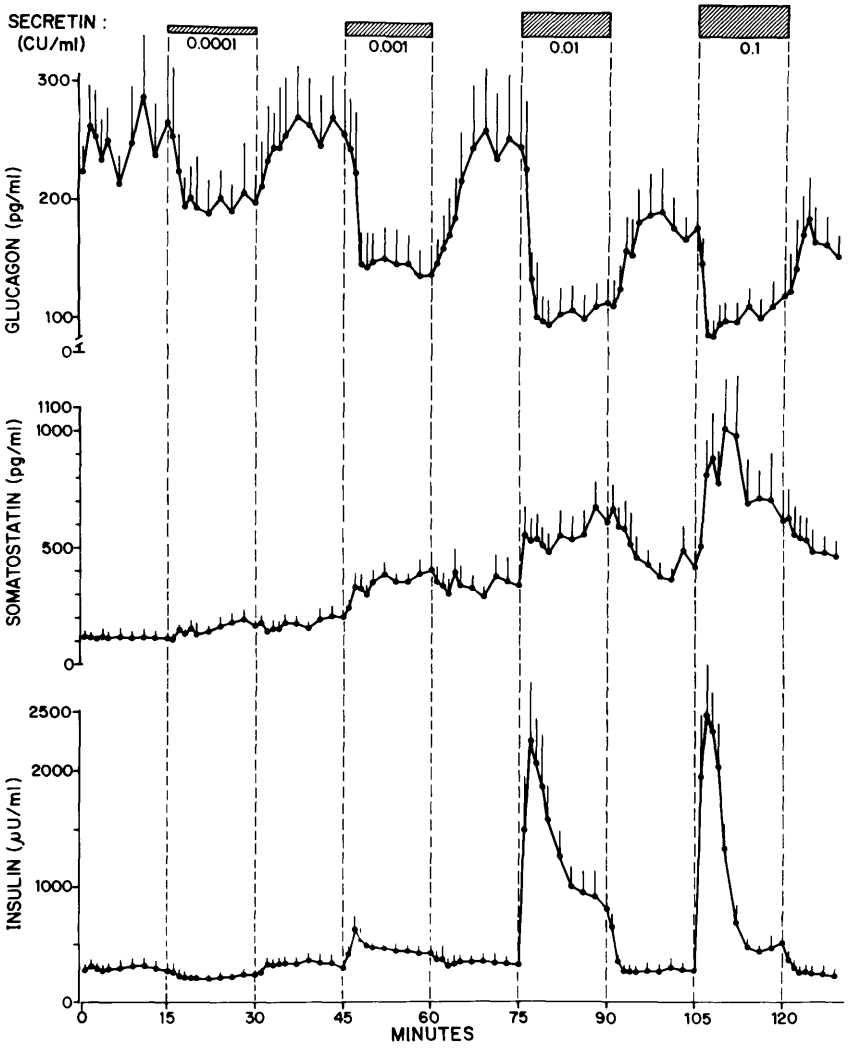

FIGURE 1. Effects of secretin on insulin, glucagon, and somatostatin release from isolated perfused canine pancreas (mean ± SEM; N = 6). Basal perfusion medium contained 5.5 mM glucose and 10 mM arginine. 0.0001, 0.001, 0.01, and 0.1 CU/ml of secretin were infused in the order of increasing dose.

ute aliquots of pancreatic effluent were collected in chilled tubes containing 2 ml EDTA-benzamidine mixture (0.03 M/0.3 M). These were stored at −20°C until assay.

The secretin preparation contained no detectable immunoreactive insulin, glucagon, or somatostatin. The mannitol (Sigma Chemical) and 1-cysteine HCl (Sigma Chemical) added to GIH secretin as a preservative had no effect on islet hormone secretion as determined by two control perfusion experiments conducted under the identical protocol and did not affect the insulin, glucagon, or somatostatin assays.

Glucagon was assayed as previously described.[18] Somatostatin-like immunoreactivity was determined using a modification of the methods of Arimura et al.[19] and Kronheim et al.[20] Insulin was assayed by a modification of the method of Yalow and Berson.[21]

Inhibition or stimulation of glucagon, insulin, and somatostatin secretion was calculated as decrease or increase in their total output from the original baseline secretion of each hormone (t_1–t_{15}) for the last 14 min of each infusion period. Statistical differences were determined by the Student *t*-test with 5% significant level.

Correlation analysis was made by the method of least square and was calculated from the linear levels of glucagon secretion expressed as percentage of mean original basal level and the log 10 of the somatostatin or insulin level indicated as increment from mean original baseline level of each hormone. Original baseline level was defined as the mean level of each hormone during t_1–t_{15}.

RESULTS

Effects of secretin on pancreatic glucagon, insulin, and somatostatin release. The mean glucagon, insulin, and somatostatin levels during the entire experimental time course are indicated in Figure 1 (mean ± SEM; N = 6). At every secretin concentration glucagon release was sharply inhibited, reaching a nadir within 3 min and remaining suppressed until cessation of infusion, whereupon a sharp rebound ensued. This glucagon suppressive effect of secretin occurred at the lowest concentration used (0.0001 CU/ml of secretin) and it increased in dose-dependent fashion (Figure 2). Maximum inhibition was attained at the highest dose of 0.1 CU/ml.

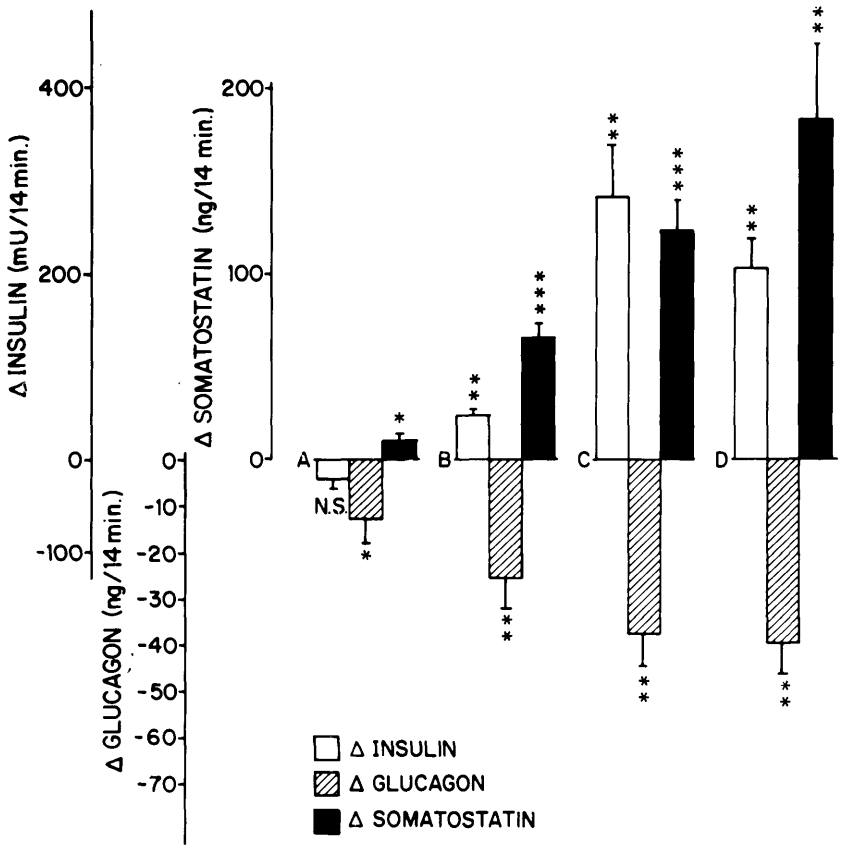

FIGURE 2. Degree of inhibition or stimulation of insulin, glucagon, and somatostatin by perfusion of various concentrations of secretin: (A) secretin 0.0001 CU/ml; (B) secretin 0.001 CU/ml; (C) secretin 0.01 CU/ml; (D) secretin 0.1 CU/ml (mean ± SEM; N = 6). ΔINSULIN, ΔGLUCAGON, ΔSOMATOSTATIN = cumulative IRI, IRG, SLI output during secretin infusion − cumulative IRI, IRG, SLI output during original basal period. NS = not significant; *P < 0.05; **P < 0.01; ***P< 0.001.

Somatostatin release was significantly stimulated by the lowest concentration of secretin, but this was very slight. At higher doses the effect was more dramatic and again was clearly dose-dependent (Figure 2).

Significant biphasic stimulation of insulin secretion was first elicited by 0.001 CU/ml of secretin or more. Thus, 0.0001 CU/ml of secretin strongly suppressed glucagon secretion but had only minimal stimulatory activity on somatostatin release and was without significant effect upon B-cells.

The inhibition of glucagon occurred reciprocally with the stimulation of somatostatin release throughout the experiment. During secretin infusion periods (t_{16-30}, t_{46-60}, t_{76-90}, $t_{106\ 120}$) there was a highly significant correlation between glucagon suppression and somatostatin stimulation ($r = 0.78$; $P < 0.001$). During the three higher concentration secretin infusion periods (t_{46-60}, t_{76-90}, $t_{106-120}$), in which glucagon suppression was associated with insulin stimulation, the r was 0.36 ($P < 0.05$).

DISCUSSION

The present study demonstrates that in the isolated perfused canine pancreas, natural porcine secretin infused at concentrations from the physiologic to the pharmacologic range exerts a dramatic dose-dependent suppressive activity on pancreatic glucagon secretion. In normal dogs the ingestion of a meal increases plasma immunoreactive secretin concentration from a mean fasting level of 5.6 ± 0.9 pg/ml to 22–55 pg/ml,[22] so that the minimum dose of secretin employed in the present studies, 0.0001 CU/ml (9.4 pM, 29 pg/ml), was well within the physiologic range.

It is unlikely that hemodynamic changes could account for these results since flow was mechanically maintained at a constant rate. Even during infusion of the highest concentration of secretin, no perfusion pressure change was noted. Even though total pancreatic flow rate was constant, a change in ratio of endocrine to exocrine flow cannot be excluded. However, the fact that the changes in the three hormones measured were neither parallel nor in the same direction is somewhat against this possibility.

The failure of others to demonstrate this effect may be related to differences in experimental conditions and in species. The effect would appear to be most obvious during ongoing stimulation of glucagon; in this study the perfusate contained 10 mM arginine. Previously, without arginine in the perfusate, we too failed to observe glucagon suppression with even higher concentrations of secretin.[23] Thus, the glucagon-suppressing activity may be evident only when glucagon has been stimulated by an amino acid and previous inability to demonstrate such effects in in vitro and in vivo experiments may be, at least in part, the consequence of a low basal level of glucagon secretion.

The suppressive effect of secretin occurs in alloxan-diabetic dogs[1] and must not therefore be insulin-dependent. This conclusion is supported in the present study by the fact that at the lowest concentration of secretin glucagon release was inhibited without an increase in insulin output. Suppression of glucagon was significantly correlated with stimulation of somatostatin, however, but this neither proves nor disproves a cause-effect relationship between the rise in somatostatin and the decline in glucagon.

The physiologic implications, if any, of this glucagon response to secretin are difficult to identify. Perhaps during a protein-fat meal the restraining action of secretin counters the glucagon-stimulating effects of the other gastrointestinal hormones and the amino acids and, by preventing a more prominent glucagon response relative to the insulin response, maintains a more anabolic mixture of the islet hormones.

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