Rapid Publications

Cysteamine Blocks Somatostatin Secretion Without Altering the Course of Insulin or Glucagon Release

A New Model for the Study of Islet Function

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SUMMARY

Cysteamine (300 mg/kg) administered subcutaneously depletes pancreatic somatostatin to 36% of control levels, but does not alter pancreatic insulin or glucagon content. Although perfusion of pancreata from normal animals with glucose (300 mg/dl) markedly stimulated somatostatin release, pancreata from cysteamine-treated animals failed to secrete somatostatin in response to glucose. Cysteamine treatment was without effect on insulin and glucagon release under the conditions tested. The isolated perfused pancreas from the cysteamine-treated rat provides a model for further investigations into regulation of islet hormone release in the absence of stimulated somatostatin release.

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Szabo and Reichlin[1] reported that cysteamine (β-mercaptoethylamine) markedly depletes somatostatin in the gastric and duodenal mucosa, hypothalamus, pancreas, and plasma. There was no effect on the gut-brain peptide VIP. These observations led to the hypothesis that cysteamine was selective in its effect on somatostatin-containing cells. Subsequently, Sagar et al.[2–4] studied the effect of cysteamine treatment on somatostatin depletion in various areas of the brain and spinal cord. Recently the same group has demonstrated that cysteamine is also a potent and specific depletor of pituitary prolactin but is without effect on the content of TSH, GH, LH, or FSH in the anterior pituitary. A further contribution to the selectivity of syteamine was made by Palkovits et al.[5] who demonstrated that LH-RH, vasopressin, enkephalin, VIP, and CCK levels in the rat brain were unaffected by cysteamine.

To further characterize the effect of cysteamine in the pancreas, we measured somatostatin, insulin, and glucagon in the pancreata from cysteamine-treated rats. In addition, we studied the effect of glucose on somatostatin, insulin, and glucagon release from the isolated perfused pancreas from cysteamine-treated rats.

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MATERIALS AND METHODS

Cysteamine (Sigma, St. Louis, Missouri) was prepared as an aqueous 6% solution and neutralized with NaOH before administration subcutaneously as a dose of 300 mg/kg body wt to 200–250-g male Sprague-Dawley rats. Control animals were placebo injected. For the depletion studies, the animals were killed 24 h later by decapitation. The pancreas was extracted within 48 h with acid alcohol (75% ethanol, 0.18 N HCl). Somatostatin, insulin, and glucagon were measured by radioimmunoassay as previously described.[6–8] Cysteamine at concentrations 10-fold in excess to those used in this study was without effect on the radioimmunoassays. For the perfused pancreas studies the rats were treated with administered cysteamine as above and used for the perfusion study 24 h later.

The perfusion procedure used was a modification[9] of that of Loubatiere.[10] Pancreata isolated from pentobarbital-anesthetized, fasted, 150–250-g male Sprague-Dawley rats were maintained at 37°C in a water-jacketed perfusion chamber. The medium, consisting of Krebs-bicarbonate balanced salt solution containing 2.5 g/L bovine serum albumin (Armour Pharmaceutical, Kankakee, Illinois), 0.1 g/L soybean trypsin inhibitor (Sigma, type 1-S), 38 g/L dextran (Sigma), and glucose as required, was perfused into the pancreas with a Gilson Minipuls-2 pump at a rate of 1.3 ml/min through the cannulated celiac trunk. Effluent exiting from the organ via the severed portal vein was collected as 2.6-ml fractions every 2 min. The perfusion medium was maintained at 37°C and gassed with O_2/CO_2 (95%/5%). Statistics were carried out using Student's unpaired *t* test.

RESULTS

Hormone depletion studies. When cysteamine (300 mg/kg) was administered to rats and pancreatic hormone content determined 24 h later, somatostatin content was markedly decreased, but no effect was detected on insulin or

TABLE 1
Pancreatic hormone content (± SEM) in cysteamine-treated (300 mg/kg) and control animals

	Insulin (U/g pancreas)	Glucagon (μg/g pancreas)	Somatostatin (μg/g pancreas)
Cysteamine (N = 10)	1.69 ± 0.17	8.4 ± 0.67	0.91 ± 0.10
Control (N = 5)	1.43 ± 0.30	10.0 ± 2.9	2.52 ± 0.82
P	NS	NS	<0.01

glucagon content (Table 1). The somatostatin content of the pancreas was significantly reduced ($P < 0.01$) from 2.5 ± 0.82 μg/g to 0.91 ± 0.10 μg/g wet weight pancreas, 36% of the control level. Control pancreatic insulin was 1.43 ± 0.30 μU/g wet weight of pancreas and was not significantly different from the pancreas from the cysteamine-treated group, which was 1.69 ± 0.17 μU/g wet weight pancreas. Likewise, glucagon was not significantly altered by cysteamine treatment where control levels were 10 ± 2.9 μg/g wet weight of pancreas, and cysteamine treatment resulted in 8.4 ± 0.67 μg/g wet weight of pancreas.

Pancreatic perfusion studies. Once it was determined that cysteamine treatment of rats resulted in selective depletion of islet somatostatin without apparent effect on islet insulin or glucagon, it was of interest to determine whether depletion of somatostatin resulted also in the inability of delta-cells to secrete somatostatin. To test this, isolated rat pancreata were perfused for 20 min with 50 mg/dl glucose before onset of sample collection. Samples were then collected for 10 min before perfusing the pancreata for 40 min with 300 mg/dl glucose and then for the final 10 min with 50 mg/dl glucose.

Glucose-stimulated insulin release was of the typical biphasic pattern (Figure 1). In the control pancreata mean glucose-stimulated insulin release was significantly ($P < 0.005$) increased from 7 ± 4 μU/ml in 50 mg/dl glucose to 510 ± 38 μU/ml in 300 mg/dl glucose. In the pancreata from cysteamine-treated animals, mean glucose-stimulated insulin release significantly ($P < 0.005$) increased from 5 ± 4 μU/ml in 50 mg/dl glucose to 486 ± 38 μU/ml in 300 mg/dl glucose. There was not a significant difference in the release of insulin from the control pancreata and insulin release from the pancreata from cysteamine-treated rats.

Glucose-stimulated somatostatin release from the control pancreata was of the typical biphasic pattern (Figure 1). In the control pancreata mean glucose-stimulated somatostatin release significantly ($P < 0.005$) increased from 20.6 ±8.1 in 50 mg/dl glucose to 294 ± 70 in 300 mg/dl glucose. In the pancreata from cysteamine-treated animals, glucose (300 mg/dl) had no effect on somatostatin secretion. The glucose-stimulated somatostatin release from cysteamine-treated animals was significantly ($P < 0.005$) different from that of the control animals. Basal glucagon release from the control pancreata was 1302 ± 430 pg/ml and was significantly depressed ($P < 0.05$) to 420 ± 195 pg/ml in the presence of 300 mg/dl glucose (Figure 1). Basal glucagon release from pancreata of cysteamine-treated animals was 591 ± 128 pg/ml and was significantly depressed ($P < 0.10$) to 398 ± 140 pg/ml in the presence of 300 mg/dl glucose. There was no significant difference in glucagon release between the control and cysteamine-treated pancreata.

DISCUSSION

Szabo and Reichlin[1] hypothesized that cysteamine was selective in its ability to deplete pancreatic somatostatin, but did not determine whether cysteamine had an effect on the pancreatic content of insulin or glucagon. In this study the effect of cysteamine treatment on the pancreatic content of somatostatin, insulin, and glucagon was determined. The results confirm Szabo and Reichlin's hypothesis in that cysteamine depletes pancreatic somatostatin without significantly altering the pancreatic insulin and glucagon content. In addition, it was determined that cysteamine treatment renders the delta-cell unresponsive to glucose but is without a detectable effect on the alpha- and beta-cells. The fact that either the presence or absence of somatostatin release is without effect on glucose-stimulated insulin release or glucose inhibition of glucagon release provides further evidence for our previous contention that glucose-stimulated somatostatin release does not regulate glucose-stimulated insulin release or glucose inhibition of glucagon release via local or within islet pathways.[9,11,12]

Until now there has not been an adequate experimental model for studying islet function in the absence of the putative paracrine influence[13] of somatostatin. The isolated perfused pancreas from the cysteamine-treated rat provides such a model for further investigations into the function of endogenously released islet somatostatin in the rat pancreas.

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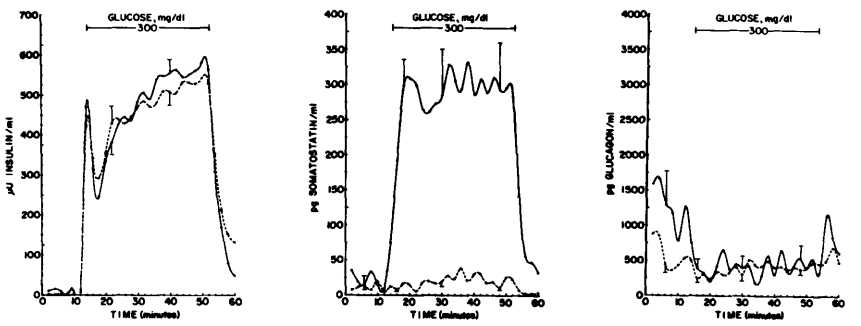

FIGURE 1. The effect of 50 and 300 mg/dl glucose on insulin, somatostatin, and glucagon release from isolated perfused rat pancreata from cysteamine-treated (----; N = 8) and control (——; N = 8) pancreata.

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