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Exogenous Cysteamine Increases Basal Pancreatic Exocrine Secretion in the Rat

To determine whether exocrine pancreatic secretion is regulated by endogenous somatostatin, somatostatin deficiency was induced by cysteamine. Rats were subcutaneously administered a single dose of cysteamine (30 mg/100 g body weight) 12 hr before experiment. Anesthetized rats were prepared with cannulation into bile duct, pancreatic duct, duodenum, and jugular vein and pancreatic juice was collected. For in vitro study, isolated pancreata of rats, pretreated with cysteamine, were perfused with an intraarterial infusion of Krebs-Henseleit solution (37°C) at 1.2 mL/min, and pancreatic juice was collected in 15-min samples. In vivo experiment of the rat, the mean basal pancreatic secretions, including volume, bicarbonate, and protein output were significantly increased from 18.4±0.5 μL/30 min, 0.58±0.05 μEq/30 min, and 214.0±26.1 μg/30 min to 51.6±3.7 μL/30 min, 1.52±0.11 μEq/30 min, and 569.8±128.9 μg/30 min, respectively ($p<0.05$). In the isolated perfused pancreas, cysteamine also resulted in a significant increase in basal pancreatic secretion ($p<0.05$). Simultaneous intraarterial infusion of octreotide (10 pmol/hr) to isolated pancreata partially reversed the effect of cysteamine on basal pancreatic secretion. These findings suggest that endogenous somatostatin play an important role on the regulation of basal pancreatic exocrine secretion.

Key Words : *Pancreatic function tests; Somatostatin; Cysteamine; Rats*

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INTRODUCTION

Somatostatin, a cyclic tetradecapeptide, is widely distributed throughout the gastrointestinal tract and the pancreas (1, 2). Recently, somatostatin receptor has been biochemically characterized on the membrane of pancreatic acini, suggesting that somatostatin modulates the exocrine pancreas (3, 4). Many studies have shown that exogenous somatostatin administration inhibits either basal or stimulated enzyme release from the exocrine pancreas, whereas its effect on water and bicarbonate secretion is less well defined (5). Moreover, it has not been fully understood whether endogenous somatostatin influences the regulation of unstimulated pancreatic exocrine function (5-7).

Cysteamine (β-mercaptoethylamine) administration induces a depletion of somatostatin concentration from various tissues, including the pancreas (8, 9). Thus, cysteamine treatment appears to be a valuable experimental model to study the regulatory role of endogenous somatostatin on the pancreatic endocrine or exocrine function (10, 11).

This study was undertaken to examine the effects of endogenous somatostatin on basal pancreatic exocrine secretion in vivo by using anesthetized rats and isolated perfused pancreas from rats pretreated with cysteamine in vitro.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing 200-250 g, were not fed for 24 hr before surgery but had free access to tap water. Cysteamine-HCl (Sigma Chem. Co., St. Louis, MO, U.S.A.) dissolved in distilled water was administered subcutaneously 30 mg/100 g body weight 12 hr before surgery. Under anesthesia with an intraperitoneal injection of 25% urethane (Sigma Chem. Co.) in a dose of 0.35 mL/100 g body weight, the rats were prepared as follows.

Surgery

For in vivo study, rats were prepared with cannulation of

the jugular vein with a polyethylene tube (PE-50, 0.58 mm ID, 0.97 mm OD, Becton-Dickinson, Parsippany, NJ, U.S.A.). The tube was kept patent by infusing 0.15 M NaCl solution at a flow rate of 1.56 mL/hr with a Harvard infusion pump (Harvard apparatus, South Natick, MA, U.S.A.). A polyethylene tube (PE-10, 0.28 mm ID, 0.61 mm OD, Becton-Dickinson) was inserted into the pancreatic duct at the junction between the duct and duodenal wall for collection of pancreatic juice. Another polyethylene tube (PE-10) was inserted into the bile duct at the level of proximal to the pancreas and bile was diverted exteriorly. A plastic tube (3.0 mm ID, 4.0 mm OD) was inserted via the proximal stomach into the proximal duodenum, 5 mm abroad to the pylorus, and the pylorus was ligated with 3-0 silk. The tube was also fixed on the wall of the stomach by a purse string suture. The abdominal wound was covered with a saline-moistened gauze, and body temperature was maintained at 36-37℃ with a heating pad throughout the experiment.

For in vitro study, isolated rat pancreata were prepared according to the method described by Penhos et al. (12) with minor modifications. Briefly, the abdominal aorta was carefully dissected after laparotomy and cannulated with a PE-50 tubing just above the celiac artery, while the aorta below the superior mesenteric artery was tightly ligated. The portal vein was also cannulated with a Tygon microbore tube (ID 1.27 mm, OD 2.28 mm, Fisher Scientific, Pittsburgh, PA, U.S.A.). Thus the vascular perfusion inlets were celiac and mesenteric arteries, and the outlet was the portal vein. The blood vessels supplying the stomach, liver, and spleen were ligated and severed. Similarly, blood vessels between the pancreas and adjacent tissues, such as colon, were carefully dissected and ligated. After insertion of a plastic tube (ID 1.8 mm, OD 2.2 mm) into the proximal duodenum via the stomach, the pylorus was ligated, and the stomach was removed. The proximal jejunum was also cannulated with a plastic tube (ID 2.6 mm, OD 3.2 mm) near the ligament of Treitz, and all intestine distal to the tube was removed. For the collection of pancreatic juice, PE-10 tubing was placed into the common bile duct near the duodenum, and the hepatic end of the common bile duct was ligated.

The isolated pancreas, including the duodenum, was placed in a temperature-controlled chamber at 37℃, which was continuously supplied with Krebs-Henseleit solution (pH 7.4, 304 mosmol) containing 0.1% bovine serum albumin (Sigma Chem. Co.) and 3% dextran T-70 (Sigma Chem. Co.). The rate of vascular flow of the perfusate was kept constant at 1.2 mL/min by a multistaltic pump (Buchler instruments, Lenexa, KS, U.S.A.) and the chamber was continuously saturated with 95% O_2 containing 5% CO_2 via a tube oxygenator. The organ chamber was also constantly supplied with fresh Krebs-Henseleit solution at a flow rate of 0.35 mL/min using different size of tubes.

Experimental design

Effect of cysteamine on basal pancreatic exocrine secretion in vivo and in vitro experiment

To evaluate the effect of endogenous somatostatin on basal pancreatic secretion in vivo, basal pancreatic juice was collected for 1 hr in 10 rats pretreated with cysteamine and in 10 rats which served as a control group.

To observe the effect of endogenous somatostatin on basal pancreatic secretion in the isolated perfused pancreas, basal pancreatic juice was collected for 1 hour in 7 pancreata pretreated with cysteamine and in 7 pancreata which served as the control group.

Effect of exogenous somatostatin on basal pancreatic secretion in pancreata pretreated with cysteamine

In vitro perfusion study, an additional 7 isolated pancreata of rats pretreated with cysteamine were intra-arterially administrated with a somatostatin analogue, octreotide (Sandostatin, Sandoz Inc., Hanover, NJ, U.S.A.) and the effect of octreotide on the basal pancreatic exocrine secretion was studied. Octreotide was infused at a calculated rate which provide a concentration of 10 pmol/hr via a side-arm injection using an infusion pump (426-2000, Buchler instruments) for 10 minutes after an equilibration period of 30 min. The pancreatic juice was gathered from four subsequent 15-min periods.

Measurements of volume, bicarbonate and protein of pancreatic juice

Pancreatic juice was collected continuously in 15-min intervals into a glass VWR micropipets (Drummond scientific Co., San Francisco, CA, U.S.A.) which had a capacity of 3.85 μL/10 mm tube length. The length of the juice in the micropipets was measured with a ruler to calculate volume. Protein concentrations in the pancreatic fluid were determined by the method of Lowry et al. (13) and bicarbonate concentrations by the carbon dioxide analyzer (model 965, Corning, Medifield, MA, U.S.A.).

The results were expressed as bicarbonate output and protein output in microequivalent and microgram per 30 min, respectively.

Statistics

All results were expressed graphically as means $\pm$SEM. The percentage increase was expressed as an increase in over basal values of the results of each individual experimental. Statistical analysis of the data was made by Student's t test. Statistical tests were considered significant when $p<0.05$.

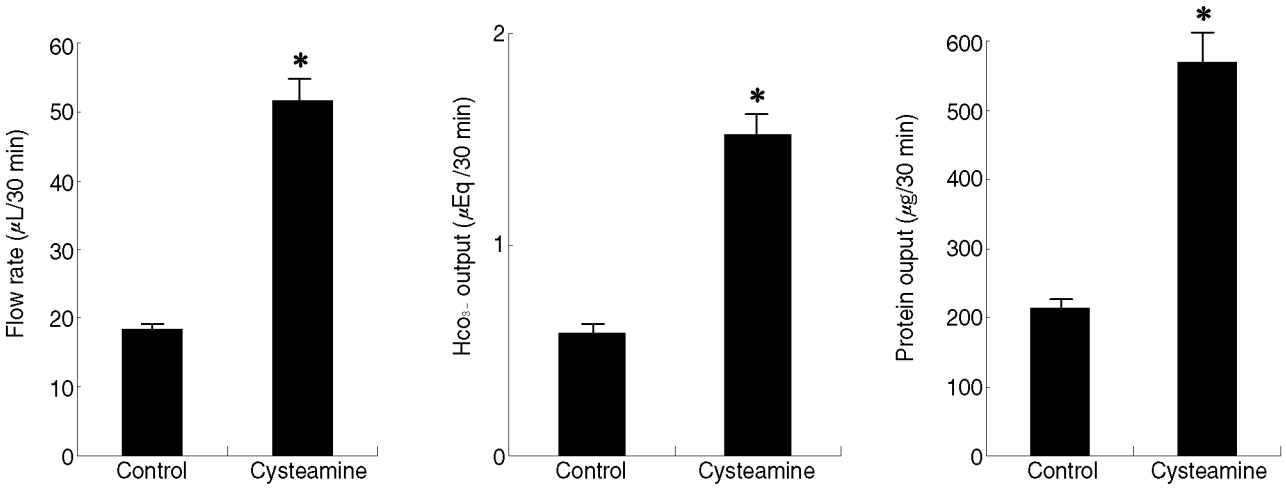

Fig. 1. Effect of cysteamine on basal pancreatic secretion including volume, bicarbonate, and protein output in vivo. Values are expressed as mean±SEM of 10 anesthetized rats. *Significant difference vs. control value ($p<0.05$).

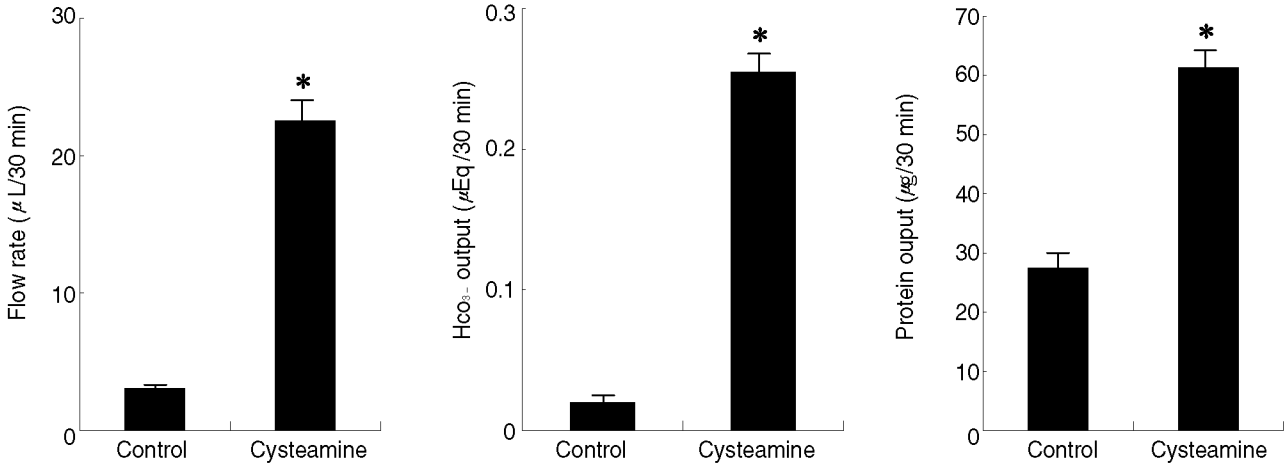

Fig. 2. Effect of cysteamine on basal pancreatic secretion including volume, bicarbonate, and protein output in the isolated perfused pancreas. Values are expressed as mean±SEM of 7 pancreata. *Significant difference vs. control value ($p<0.05$).

RESULTS

Effect of cysteamine on basal pancreatic exocrine secretion in vivo

As shown in Fig. 1, the mean basal pancreatic secretion including volume, bicarbonate, and protein output in 7 rats was 18.4±0.5 μL/30 min, 0.58±0.05 μEq/30 min, and 214.0±26.1 μg/30 min, respectively and that in 7 rats pretreated with cysteamine was 51.6±3.7 μL/30 min, 1.52 ±0.11 μEq/30 min, and 569.8±128.9 μg/30 min, respectively. Thus, cysteamine resulted in a significant increase in basal pancreatic secretion ($p<0.05$).

Effect of cysteamine on basal pancreatic exocrine secretion in the isolated perfused pancreas

In 7 isolated perfused pancreata, the mean basal values of pancreatic juice including volume, bicarbonate, and protein output were 3.1±0.5 μL/30 min, 0.020±0.005 μEq/30 min, and 27.5±2.8 μg/30 min, respectively (Fig. 2) and those in 7 pancreata pretreated with cysteamine were 22.6 ±4.1 μL/30 min, 0.255±0.040 μEq/30 min, and 61.3± 10.7 μg/30 min, respectively. Thus cysteamine resulted in a significant increase in the basal pancreatic secretion ($p<0.05$).

Effect of exogenous octreotide on pancreatic exocrine secretion in the isolated perfused pancreas pretreated with cysteamine

In 7 isolated perfused pancreata pretreated with cysteamine, intraarterial administration of exogenous somatostatin analogue, octreotide produced a significant inhibition

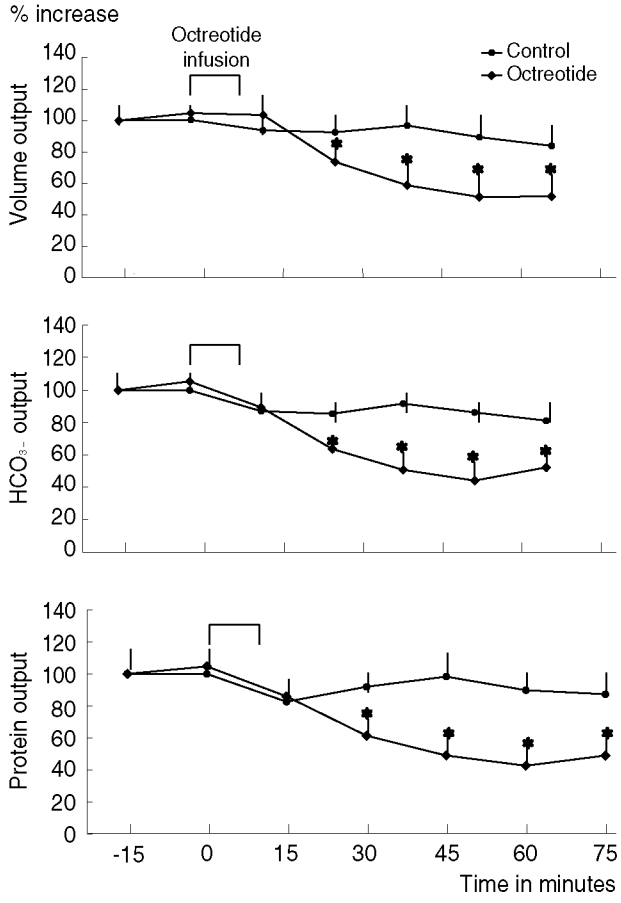

Fig. 3. Effect of exogenous somatostatin analogue, octreotide (10 pmol/hr, 10 min) on basal pancreatic secretion in the isolated perfused pancreas of the rat pretreated with cysteamine (n=7). Each value represents a mean±SEM of % increase over basal values. *p≤0.05-0.01.

of basal pancreatic secretion including volume, bicarbonate, and protein by 54%, 51%, and 50%, respectively (Fig. 3). Thus, intraarterial octreotide alone partially reversed the effect of cysteamine on basal pancreatic secretion, but not completely reversed it.

DISCUSSION

Cysteamine is a thiol compound clinically used in the treatment of acetaminophen poisoning and cystinosis (14). Szabo and Reichlin (15) observed that cysteamine markedly reduced somatostatin in the hypothalamus, gastrointestinal tract, and pancreas. Sorenson et al. (10) demonstrated that cysteamine reduced pancreatic somatostatin without significantly altering the pancreatic insulin or glucagon content and release. These studies indicate the possibility that the cysteamine-treated rat may serve as a model for evaluating the effect of endogenous somatostatin on exocrine pancreatic secretion (10, 11).

The mechanism of action of cysteamine on somatostatin has not been completely elucidated. It has been suggested that cysteamine might act by accelerating the intracellular degradation of somatostatin, thereby inducing a true depletion of somatostatin-14 like immunoreactivity (3) or more likely, through a chemical modification of the disulfide bond, which would render the molecule immunologically and biologically inactive (8, 14). The time course of cysteamine induced somatostatin depletion is of great interest in regard to the use of cysteamine as a tool to investigate the effect of endogenous somatostatin (15). Kanatsuka et al. (6) have reported that somatostatin content in the pancreatic islets was markedly decreased 60 min after oral administration of cysteamine and reduced to less than 20% of control values at 18 hr after cysteamine treatment. In addition, other groups observed that the effect of cysteamine on somatostatin depletion was rapid and at least partly reversible within 24 hr (1, 8, 16, 17). Thus our experiment was performed 12 hr after the administration of cysteamine.

In the present study, we investigated the effect of endogenous somatostatin on basal pancreatic exocrine secretion in vivo and in vitro experiments of the rat pretreated with cysteamine. Our data from both in vivo and in isolated perfused pancreas of the rat pretreated with cysteamine clearly showed that basal pancreatic exocrine secretions, including volume, bicarbonate, and protein were significantly increased. Since cysteamine does not affect the pancreatic exocrine secretion directly, the increase in pancreatic secretion seems likely to be mediated by suppression of endogenous somatostatin. Thus, the present study demonstrates that endogenous somatostatin is an inhibitor of basal pancreatic exocrine secretion in vivo and in the isolated perfused pancreas. This is in agreement with the observations of previous studies (18-20), which demonstrated that endogenous or exogenous somatostatin inhibited pancreatic exocrine secretion in the rat.

We also studied the effect of exogenous somatostatin on basal pancreatic exocrine secretion in vitro experiment of rats pretreated with cysteamine. Under these experimental conditions, increased basal pancreatic exocrine secretion was significantly depressed by the intraarterial infusion of a somatostatin analogue, octreotide at a dose of 10 pmol/hr. Similar findings have been observed in rats by Guan et al. (20) and in dog by Susini et al. (21). Exogenous somatostatin failed to suppress the pancreatic secretion completely, and only reduced pancreatic exocrine secretion by 50%. It is difficult to explain why exogenous somatostatin caused incomplete inhibition of pancreatic exocrine secretion. Because exogenous infusion of somatostatin at a dose of 10 pmol/hr significantly inhibited the pancreatic secretion (22), our infusion concentration of somatostatin seemed to be sufficient to inhibit exocrine pancreatic secretion. In addition,

atropine simultaneously infused with somatostatin did not cause a further inhibition of pancreatic exocrine secretion including volume, bicarbonate, and protein output (data not shown). This implies strongly that there is another factor(s) involved in the stimulatory effect of cysteamine on basal pancreatic exocrine secretion.

The inhibitory effects of somatostatin on pancreatic exocrine secretion could be somatostatin-induced alterations in endogenous hormone levels rather than as a direct effect of somatostatin on the exocrine pancreas (1, 2, 23). Abucham and Reichlin (23) observed that cysteamine increased the rate of amylase secretion by cholecystokinin (CCK) release from the duodenum in the rat. And CCK inhibition was mediated by somatostatin secreted locally. On the other hand, Müller et al. (1) and Schönfeld et al. (2) reported that the inhibitory effects of endogenous somatostatin on pancreatic exocrine secretion were mediated by somatostatin-induced inhibition of endogenous glucagon or insulin release. Others (8, 17, 24) also observed that insulin release after prior cysteamine administration was stimulated. Consequently, these data suggested that cysteamine-induced intrapancreatic somatostatin modulated the local islet hormone levels enough to influence the secretion of exocrine pancreas. Thus, the effect of cysteamine on pancreatic islet hormone, including insulin, glucagon, and pancreatic polypeptide must be re-evaluated to clarify the role of endogenous somatostatin on the regulation of basal pancreatic exocrine secretion in the rat pretreated with cysteamine. In conclusion, endogenous somatostatin is suggested to play a significant role in the regulation of basal pancreatic exocrine secretion.

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