Effect of Cadmium and Bombesin on Exocrine Pancreatic Secretions and Plasma Levels of Gastrin and Cholecystokinin (CCK) in Rats

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ABSTRACT

The effects of cadmium and bombesin on exocrine pancreatic secretions and plasma levels of gastrin and cholecystokinin (CCK) were studied in anesthetized rats with pancreatic and gastric fistulas. Rats treated only with saline were used as controls. Both control and cadmium (0.1 mg per kg) treated rats were infused with saline, secretin, and bombesin (BBS). Blood and pancreatic juice samples were collected at regular time intervals. Plasma levels of gastrin and CCK were measured in blood samples by specific radioimmunoassay. Pancreatic juice samples were measured for volume, protein, and trypsin outputs. Compared to saline treated rats, outputs of volume, protein, and trypsin were significantly greater in cadmium treated rats. Plasma levels of gastrin were suppressed with secretin but significantly elevated with BBS. Plasma CCK levels were not different from basal after secretin or BBS in rats treated with either cadmium or saline. The results suggest that the administration of cadmium stimulated exocrine pancreatic secretion by a mechanism that does not involve gastrin or CCK. Bombesin may have a direct influence on the stimulation of exocrine pancreatic secretion in rats.

Introduction

Studies in man and experimental animals have shown that digestive processes are regulated, at least in part, by actions and interactions of gut hormones released postprandially.15,16 In addition, plasma levels of secretin, cholecystokinin (CCK) and pancreatic polypeptide (PP) have been associated with exocrine pancreatic secretions.16,27,30 Bombesin (BBS), a gut peptide isolated from the amphibian skin,31 is known to influence the release of several gastrointestinal hormones (including CCK, gastrin, and PP) and the release of pancreatic exocrine secretion.11,28 Secretin and CCK have been shown to have dose dependent effects on volume, bicarbonate, and protein secretions of pancreatic juice in
man and dogs.\textsuperscript{9,17,20} To our knowledge, the effects of cadmium on the biological actions of these peptides in rats have not been studied.

Cadmium is a naturally occurring environmental toxic trace element and is ingested through various dietary sources\textsuperscript{13,14,22} and through cigarette smoking.\textsuperscript{40} In dogs, intravenous infusions of cadmium alone and cadmium with secretin had no significant effect on outputs of exocrine pancreatic secretions or plasma levels of gastrin, CCK, and PP.\textsuperscript{4} It has been demonstrated in dogs that intravenous administration of cadmium potentiated bombesin stimulated release of plasma levels of CCK and pancreatic polypeptide (PP)\textsuperscript{3} but not gastrin. The current study was conducted in rats to determine the effects of orally administered cadmium on exocrine pancreatic secretions and plasma levels of gastrin and cholecystokinin during infusions of saline (basal), secretin, and bombesin.

**Materials and Methods**

**Animals**

Twenty male Sprague Dawley rats, approximately 400 g of body weight, were used in the study. The animals were divided into two groups of 10 rats each. Ten rats received saline and ten received cadmium. For 14 days daily, the animals were fed either saline or cadmium (0.1 mg per kg body weight) as CdCl\textsubscript{2} solution in a total volume of 0.2 ml via intragastric tube. The dose of cadmium selected was based on the 5 to 10 percent absorption of cadmium by GI tract as shown by us\textsuperscript{5,6} and others.\textsuperscript{22} Based on this estimate, the oral dose of cadmium used in this study should produce an equivalent blood cadmium level which could come from dietary or environmental exposure. After 14 days of continuous treatment, the animals were fasted for 24 hours prior to experimental procedure.

**Surgery**

Rats were anesthetized with a mixture of ketamine-HCl and acepromazine maleate (1:10). The animals were surgically prepared with pancreatic and gastric fistulas by use of the following procedures. An abdominal incision was made to locate the bile duct. The bile duct was cannulated with PE-50 tubing near the liver and bile was diverted to the duodenum. A second PE tubing about 10 cm long was inserted into the bile pancreatic duct and was led out to the exterior and secured through the wall of the abdominal muscle to collect pancreatic juice. In order to avoid the contributions of gastric juice in the stimulation of exocrine pancreatic secretions, pylorus was ligated, and a gastric cannula was inserted and secured to the avascular part of the stomach. The gastric cannula was left open throughout the study period.

**Experimental Protocol**

Saline was continuously infused during and after the surgical procedures were completed. After surgery, secretin (5U per kg-hr)* was infused throughout the experiment. This dose of secretin was found optimal to attain a background volume output in rats, as found in our preliminary experiments.\textsuperscript{38} After 45 min of secretin infusion, BBS (1 µg per kg-hr)\textsuperscript{f} was infused for 45 min. After BBS infusion was stopped, secretin infusion was continued for another 45 min. The experiment was terminated after a final infusion of saline for 15 min.

Pancreatic juice samples were collected continuously for 15 min intervals for measurements of volume, protein, and trypsin. Blood samples were collected before and after infusions of test substances. Blood was kept cold after

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collection, and centrifuged at 4°C; plasma was separated and kept frozen at −20°C for future measurements of immunoreactive gastrin and CCK by specific radioimmunoassays.

**MEASUREMENT**

Volume was measured in the PE tubing with known internal diameters and was converted to microliters. Pancreatic juice was diluted to a fixed volume of 200 μl of saline. Protein concentration in the pancreatic juice was measured by Bradford’s method with bovine plasma albumin as a standard. Total protein outputs were calculated by multiplying the protein concentration with the volume measured during that time interval. Trypsin concentration in pancreatic juice was measured using the method of Erlanger et al employing N-α-benzoyl-DL-arginine-p-nitroanilide (BAPNA) as substrate. Enterokinase (25 μg) was added to 0.1 ml of diluted pancreatic juice and incubated for 30 min before being subjected to trypsin assay. Trypsin outputs were calculated by similar procedures as described under protein measurements.

**Radioimmunoassay**

**Gastrin**

Plasma gastrin was measured by a specific radioimmunoassay using antiserum (UT.55). The gastrin radioimmunoassay was developed utilizing antiserum UT-55 that recognized the C-terminal amino acid part of the gastrin molecule. The antiserum measures all molecular forms of gastrin. Physiologic changes in gastrin levels are detected with this antiserum with accuracy after food and after various stimuli. In previous studies, it has been shown that the antiserum measures gastrin in rat plasma.

**CHOLECYSTOKININ**

Concentrations of CCK in plasma were measured by a specific radioimmunoassay. Validation of the assay has been reported; however, a brief description of the assay procedure is given for clarity. An antibody was generated in New Zealand white rabbits by repeated inoculation with 16 percent pure CCK over a six month period, followed by one injection of 50 μg of 99 percent pure CCK. The CCK variant (CCK-39) was labeled with 125I by the modified chloramine-T method. The labeled hormone was separated from contaminants on a column (0.86 × 16 cm) containing Sephadex G-25 fine and purified further on a 4 cm column containing cellulose CF-11 immediately before being used in the assay. Antibody (1:2,500 initial dilution), sample, and buffer were added to each tube, incubated for 24 hours; labeled CCK in 200 μl (7500 to 8000 cpm) was then added to each tube and incubated for an additional 48 hrs. Bound hormone was separated from free hormone by the addition of goat anti-rabbit gamma globulin and incubation continued overnight at 4°C. The CCK antibody bound 25 to 35 percent of labeled CCK-39 at a final dilution of 1:25,000; more than 80 percent of the tracer was bound with excess antibody.

CCK-33 and CCK-39 were equally potent when measured in radioimmunoassay, whereas CCK fragments and gastrin varied in potency from 0.3 percent to 0.05 percent when compared to

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† Kindly supplied by Professor Viktor Mutt Gastrointestinal Hormone Laboratory, Karolinska Institutet, Stockholm.

‡ Whatman Chemical Corporation, Clifton, NJ.
CCK-33 and CCK-39. Little or no displacement of 

$^{125}$I CCK-39 from combination with antibody was measured with gastrin inhibitory peptide, vasoactive intestinal peptide, secretin, motilin, bombesin, gastrin releasing peptide, and other peptides of pancreatic, gastrointestinal or pituitary origin. Dose response curves of 99 percent pure CCK-33, our reference standard, and rat plasma in serial dilutions were generated in the assay. The slopes of these dose response curves did not differ significantly from each other, suggesting that the antibody measures substances in rat plasma that are immunologically similar to CCK-33 and CCK-39. The assay measures the release of CCK by food, and plasma levels of CCK in rats have been correlated with exocrine pancreatic secretions (unpublished observations).

**Calculations**

Hormone concentrations were calculated as pg per ml and expressed as mean ± standard error of the mean. Students unpaired "t" test was used to test the significance of differences between means. Differences with a p-value of less than 0.05 were considered statistically significant.

**Results**

**Effect of Secretin and BBS on Basal Outputs of Volume in Control and Cadmium Treated Rats**

Volume outputs (µl per 45 min) during the initial saline infusion were 7.90 ± 2.30 and 6.03 ± 0.50 in control and cadmium treated rats, respectively (figure 1). A significant stimulation of volume to 75.60 ± 6.05 for control and 101.90 ± 9.00 for Cd treated rats occurred after infusion of secretin. When BBS was administered during secretin infusion, the volume outputs were enhanced significantly from both saline and secretin phase to 237.40 ± 51.80 for control and 390.00 ± 47.00 for cadmium treated rats. Withdrawal of BBS infusion resulted in volume outputs of 126.50 ± 25.00 for control and 199.40 ± 11.00 for Cd treated rats, levels significantly higher than the pre-BBS phase. During the last infusion with saline, the volume outputs were reduced; however, these levels were found significantly higher than those found during the initial infusion of saline (figure 1).

Pancreatic protein outputs (mg per 45 min) during the initial saline infusion were not different between control and Cd treated rats (figure 2). Secretin infusion resulted in moderate but significant increase in protein outputs to 0.49 ± 0.06 for control and 0.54 ± 0.13 for Cd treated rats. With BBS infusion during secretin, protein outputs were significantly increased to 2.34 ± 0.57 for control and 4.41 ± 0.60 for Cd-treated rats (P < 0.05). Secretin infusion without
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EFFECT OF SECRETIN AND BBS ON PLASMA GI PEPTIDE LEVELS IN CONTROL AND Cd-TREATED RATS

Basal levels of gastrin (pg per ml) during saline were 134.90 ± 23.90 for control and 94.30 ± 20.30 for Cd-treated rats (figure 4). With secretin infusion, gastrin levels were decreased to 72.30 ± 12.90 and 53.30 ± 9.80 for control and Cd-treated rats. The reduction in gastrin levels in control rats during secretin was significant when compared to saline, but they were not different from Cd-treated
rats. Infusion of BBS during secretin resulted in gastrin levels of 135.80 ± 23.70 and 159.00 ± 39.70 for control and Cd rats, values significantly different from the secretin phase. No further changes in gastrin levels were found with or without the continuation of secretin or saline infusion.

Basal levels of CCK (pg per ml) during saline infusion were 145.20 ± 9.00 for control and 132.20 ± 16.00 for Cd-treated rats (data not shown). With secretin, the CCK levels were 101.80 ± 11.00 and 97.20 ± 15.00 for control and Cd rats. With BBS during secretin infusion, the CCK levels were 91.70 ± 15.00 for control and 94.50 ± 6.00 for Cd treated rats. No significant changes in plasma CCK levels were found before and after infusions of either secretin or BBS.

**Discussion**

Gastrointestinal peptides, in general, play a major role in the process of digestion. Release and interactions of GI peptides during the digestive phase after a meal have also been considered important regulating factors for stimulation of exocrine pancreatic secretion. Gastrointestinal tract, in addition to liver and kidney, has been shown to be a primary target site for accumulation of cadmium when cadmium is injected through various route of administration. The effects of chronic cadmium administration on GI peptides and exocrine pancreatic secretions in rats have not been reported; however, acute studies of cadmium from our laboratory indicated that it influenced exocrine pancreatic secretions in dogs. Our major interest in this study was to determine the effect of cadmium on normal biological actions of peptides.

The basal (saline infusion) volume outputs in both control and cadmium treated animals found in this study are not different from each other (figure 1); however, they are low compared to the volume outputs reported in conscious and anesthetized rats. Differences in basal outputs of volume could have been due to the differences in species and the anesthetics that were used in the two studies. Secretin stimulated volume outputs significantly in both saline and cadmium treated rats, and this observation is consistent with the earlier reports by Petersen and Grossman and others. The outputs of volume were substantially enhanced in control rats when bombesin was infused with secretin. In Cd-treated animals, the potentiating effect of bombesin is more pronounced compared to the control group (figure 1). These in vivo data in rats confirm the in
vitro observations of Iwatsuki and Peterson\textsuperscript{25} who showed that BBS acts directly on mouse pancreatic acinar cells to stimulate exocrine secretion. The enhanced response of exocrine pancreatic secretory outputs, as observed in this study, appeared to be the direct effect of BBS on pancreatic acinar and duct cells of rats treated with cadmium.

Protein and trypsin outputs were substantially higher in the Cd group when compared to control group (figures 2 and 3). In a previous study in dogs, found by some of us that cadmium, when infused alone and with secretin, did not increase outputs of exocrine pancreatic secretions or plasma levels of GI peptides.\textsuperscript{4} In this study in rats, it was found that cadmium potentiates the effects of BBS on protein and volume outputs which suggests that cadmium synergizes the effects of bombesin on acinar and duct cells of the pancreas. These results provide evidence that BBS and cadmium act on different receptors of the exocrine secreting cells of the pancreas. Henrikson\textsuperscript{21} and Way\textsuperscript{42} described the potentiation or synergism to occur only when two agonists stimulate different receptors resulting in the activation of the same effector mechanism.

Divalent cations such as Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, and Zn\textsuperscript{2+} have been shown to have direct effect on the release of GI peptides (gastrin, CCK, and PP) and on exocrine pancreatic secretions.\textsuperscript{23} Effects of cadmium on release of GI peptides and stimulation of exocrine pancreatic secretion have not been studied extensively and may be different since this metal exhibited some unique characteristics in regard to its affinity to certain phase reactant proteins.\textsuperscript{8} In addition, Cd\textsuperscript{2+} and Zn\textsuperscript{2+} have been shown to influence the release of Ca\textsuperscript{2+} from its intracytoplasmic stores,\textsuperscript{35} and the released calcium may act as an important mediator for endocrine and exocrine function.\textsuperscript{10,19,29,33,39,43} This may be the mechanism by which cadmium potentiates the effects of BBS on exocrine pancreatic secretion, as found in this study.

Our study indicates that gastrin release is significantly inhibited by secretin, an observation that is consistent with the earlier reports in dogs.\textsuperscript{41} In the current study, BBS stimulated the release of gastrin in rats. The dose of bombesin used and pattern of release of gastrin in this study in rats is similar to the dose of bombesin and the pattern of release of gastrin observed in other species with BBS.\textsuperscript{11,28} Thus, BBS at the dose levels used did not release measurable immunoreactive CCK in rats. This observation is consistent with studies reported earlier by this group.\textsuperscript{38} Exogenous administrations of CCK-8 have been shown to stimulate pancreatic protein outputs by Izzo, Brugge, and Praissant.\textsuperscript{24} gastrin is a weak stimulant for exocrine pancreatic secretion.\textsuperscript{24,32} Cholecystokinin octapeptide (CCK-8), a circulating form of CCK, and gastrin are not detectable by our CCK radioimmunoassay unless present at a very high concentration. Since in this study elevated plasma levels of gastrin were found but not CCK, increased pancreatic enzyme output in both control and cadmium treated rats may be due to either a direct response to BBS, release of forms of CCK that are not detected by our radioimmunoassay, or release of other stimulatory substances. The current study, however, does suggest that in addition to the stimulatory effect of secretin and bombesin, the presence of Cd appears to provide an additional stimulus to gastrin cells of the gut and exocrine cells of the pancreas.

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