Cerulein-Induced Acute Pancreatitis in the Rat
Study of Pancreatic Secretion and Plasma VIP and Secretin Levels

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A study was made with different doses of cerulein (2, 4, 10 and 20 µg/kg) administered subcutaneously to rats by four injections at intervals of 1 hr; the aim of this work was to study exocrine pancreatic secretion of the rat under cerulein-induced acute pancreatitis, analyzing enzyme and hydroelectrolyte secretion of pancreatic juice. A further aim was to study the relationship between the dose of cerulein and the plasma levels of peptides controlling hydroelectrolyte secretion of the pancreas, like secretin and vasoactive intestinal peptide (VIP). At the lowest dose schedule, the amounts of total protein and enzymes (amylase and trypsin) in pancreatic juice decreased significantly, plasma amylase increased, and the pancreas became edematous. Higher doses magnified these effects. By contrast, ductular function (flow and HCO\textsubscript{3}⁻) was well preserved in cerulein-treated rats, and this was probably due to the significant increase in plasma levels of immunoreactive secretin whereas VIP levels were unchanged. The secretin released by treatment with cerulein is able to palliate the lack of flow from acinar origin that is affected in the process of acute pancreatitis, being a beneficial response to the cerulein treatment.

KEY WORDS: acute pancreatitis; cerulein; pancreatic secretion; secretin; VIP.

Over many years a great number of experimental studies on acute pancreatitis have been carried out to investigate the pathophysiological mechanisms and potential therapeutic approaches to combat this disease. It has been shown that cholecystokinin (CCK) plays an important role in the pathogenesis of acute pancreatitis (1) and that intravenous administration of large doses of cerulein, an analog of CCK, causes acute interstitial pancreatitis in rats (2, 3).

Recently, it has been shown that subcutaneous or intraperitoneal administration of high doses of cerulein induces acute necrotizing pancreatitis in mice (4) and in rats (5, 6). These have been used as a model to study the mechanisms of pancreatitis and the effects of partially protective agents. Similarly, blockade of the hormone by peptide (7) or nonpeptide antagonists (8) has proved to be effective in the prevention and amelioration of experimental acute pancreatitis in the rat (1) and mouse (7). In this sense, the use of peptide hormones such as secretin has led to contradictory results. In a study recently carried out by us, secretin infusion was found to...
partially restore the secretion of pancreatic enzymes of rats with cerulein-induced acute pancreatitis (5), while in other studies it was not seen to elicit any effect on acute pancreatitis (2).

The aim of the present work was to analyze the effect of different doses of cerulein (2, 4, 10 and 20 μg/kg) administered by four subcutaneous injections at intervals of 1 hr on exocrine pancreatic secretion in the rat, while studying the levels of secretin and vasoactive intestinal polypeptide (VIP) immunoreactives; the later are peptides to which no important role in the processes of acute pancreatitis has been attributed.

MATERIALS AND METHODS

The study was performed on male Wistar rats weighing about 300 g. They were randomly assigned to five treatment groups of four rats each.

Treatments. The animals fasted for 9 hr, with free access to water, and then received four subcutaneous injections of 100 μl of 0.9% NaCl containing 0.2% bovine serum albumin (BSA, Sigma, St. Louis, Missouri) at hourly intervals, without (group 1) or with cerulein (Peninsula, Belmont, California) at doses of 2 μg/kg (group 2), 4 μg/kg (group 3), 10 μg/kg (group 4) or 20 μg/kg (group 5).

Collection of Pancreatic Juice. Animals fasted for an additional period of 9 hr after the first subcutaneous injection (18 hr of continuous fasting) then were anesthetized with sodium pentobarbital (3 mg/100 g body weight, intraperitoneal) for the collection of pancreatic juice. For this, tracheotomy was done, a median laparotomy performed and the pylorus ligated. The bile duct was cannulated at the exit of the liver to deviate bile flow to the exterior and the bile–pancreatic duct was cannulated at its exit to the duodenum to collect pure pancreatic juice. Animals were unconscious during the entire juice collection period and body temperature was maintained at 37 ± 1°C by placing them on a heating pad and monitoring temperature with a rectal thermometer. The juice was collected on ice to preserve the enzyme content in preweighed plastic tubes. The pancreatic juice samples were collected over 60 min, after a 15-min period of equilibration in which pancreatic juice was discarded. Blood samples were obtained at the end of each experiment for measuring plasma amylase and secretin and VIP immunoreactive levels. Finally, the entire pancreas was removed for measuring the percentage of fluid in the pancreas.

Assays. Pancreatic juice flow was estimated by direct weighing on an electronic balance assuming the density of juice to be 1 g/ml. Bicarbonate concentrations were determined as total CO2 in a Natelson microgasometer. Total protein concentrations were determined by the method of Bradford (9). Amylase activity in plasma and in pancreatic juice was determined immediately according to the method of Hickson (10). Trypsin activity was carried out by a modified method of Caldwell and Sparrow (11). Briefly, trypsinogen was activated by incubation of a dilution of pancreatic juice with bovine trypsin type III (Sigma), during 24 hr at 0°C in 0.1 M Tris HCl buffer, pH 7.6, CaCl2 0.05 M (final conditions: 100 μg/ml of pancreatic juice protein and 25 μg/ml of bovine trypsin). Nα-benzoyl-DL-arginine-p-nitroaniline (BAPNA, Sigma) 0.2 mM in Tris HCl buffer was used as substrate; 100 μl of trypsin solution were incubated with 2.4 ml of BAPNA at 37°C during 20 min. The reaction was stopped with 500 μl of 20% acetic acid, and the liberated p-nitroaniline measured at 410 nm. The amount of trypsin in pancreatic juice was determined by means of extrapolation from a standard curve made with several concentrations of bovine trypsin.

Radioimmunoassay of secretin and VIP was done according to a previously described method (12, 13) with some modifications. Briefly, antibodies were raised in rabbits with purified secretin (Karolinska Institutet) or VIP (Sigma) coupled to BSA by the carbodiimide technique (14) and emulsified with Freund's complete adjuvant for the first injection and incomplete adjuvant for booster injections. Iodination of each peptide was performed by the chloramine T method (15) and later purification of the iodinated peptides was achieved by the Chang and Chey technique (16) using a two-step chromatographic separation with G-15 Sephadex followed by C-25 SP Sephadex. Then 100 μl of [125I]secretin or [125I]VIP plus 50 μl of appropriate antibody dilution were mixed with 100 μl of standard solution of rat plasma to reach a final volume of 400 μl with 0.05 M phosphate buffer, pH 7.4, containing 0.3% BSA, 0.1% Tween, 600 UIK Trasylol, and 0.02% sodium azide. At the end of the incubation period (72 hr) separation was carried out by adding a suspension of activated charcoal (Norit GSX, BDH), 8 mg of charcoal/tube for secretin and 2.5 mg/tube for VIP, and T-70 dextran (Pharmacia) at a proportion of 1:10 (charcoal–dextran) and later centrifugation at 4000 rpm for 20 min. The sensitivity of both assays was 1 fmol/ml. Intra- and interassay variations were, respectively, 13% and 18% for secretin and 10% and 14% for VIP. The study of specificity showed a cross-reactivity of 0.012% for VIP with secretin antibody and 0.005% for secretin with VIP antibody. The identity of immunoreactivity determined by RIA has been confirmed by HPLC using a nucleosil 300 C-18 column (Sugelabor). The elution of both secretin and VIP peptides was performed using a gradient of 30–45% acetonitrile containing 1% of trifluoroacetic acid during 20 min. The analysis of immunoreactivity of extracted plasma showed only one peak of immunoreactivity for each peptide appearing at 7 and 14 min for, respectively, VIP and secretin, which coincides with the retention time of porcine VIP and secretin standards (Sigma).

The amount of pancreatic edema was calculated by drying the pancreas at 90°C over 48 hr. The relationship between pancreatic fresh weight and dry weight is expressed as percentage of fluid.

Statistical Analysis. Results are expressed as means ± SEM. Statistical analysis was carried out using the ANOVA test followed by Duncan's test (17). A P value of less than 0.05 was considered to be statistically significant.