Action of pancreatic polypeptide on rat pancreatic secretion: in vivo and in vitro

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LOUIE, DEXTER S., JOHN A. WILLIAMS, AND CHUNG OWYANG. *Action of pancreatic polypeptide on rat pancreatic secretion: in vivo and in vitro.* Am. J. Physiol. 249 (Gastrointest. Liver Physiol. 12): G489–G495, 1985.—The biological activity of bovine pancreatic polypeptide (BPP) on rat exocrine pancreatic secretion was compared in vivo and in vitro. In anesthetized rats prepared with a bile-pancreatic duct cannula, BPP inhibited cholecystokinin (CCK)-stimulated (10 IDU·kg^{-1}·h^{-1}) protein secretion in a dose-related manner ($P < 0.001$). CCK, from 5–20 IDU·kg^{-1}·h^{-1}, did not alter the degree of inhibition by BPP at 40 μg·kg^{-1}·h^{-1}, suggesting a nonsurmountable inhibition. Analogues of BPP, including rat pancreatic polypeptide, neuropeptide Y, peptide YY, and the C-terminal hexapeptide of PP, also inhibited CCK-stimulated protein secretion. To determine whether BPP acts directly on acinar cells to suppress enzyme secretion, in vitro studies were performed. BPP and its analogues did not suppress octapeptide of CCK (CCK-8)-stimulated amylase release from either isolated rat pancreatic acini or preparations of pancreatic lobules. Specific binding of ^{125}I-BPP to pancreatic acini was also not observed. From our data we conclude that BPP acts to inhibit pancreatic enzyme secretion in the rat in a noncompetitive manner. Absence of an effect by BPP or its analogues in vitro coupled with an absence of ^{125}I-BPP binding to acini suggest that the inhibitory action of PP on exocrine pancreatic function is mediated by indirect mechanisms.

bovine and rat pancreatic polypeptide; neuropeptide Y; peptide YY; pancreatic polypeptide-6

PANCREATIC POLYPEPTIDE is a 36-amino acid, straight-chain polypeptide derived primarily from the pancreas (14) and known to be localized in the islets and in cells scattered among the acini of the exocrine pancreas. Subsequently, pancreatic polypeptide-like immunoreactivity has also been found in the brain (18) and enteric nerves (22), but this is now thought to be neuropeptide Y (21). Pancreatic polypeptide is released postprandially with most of the release mediated by vagal reflexes initiated by the presence of food in the intestine. In both humans and dogs, infusion of bovine pancreatic polypeptide (BPP) inhibited secretin- and cholecystokinin-stimulated pancreatic secretion (1, 2, 9, 10, 13, 17). The inhibitory action was observed with infusion rates of BPP that produced plasma levels similar to those observed postprandially. This observation suggests that pancreatic polypeptide may be important in the physiological regulation of pancreatic function. However, the cellular locus of its inhibitory effect is unknown and specific PP receptors on the pancreatic acinar cells have not been demonstrated.

In this study we established the biological activity of BPP on pancreatic secretion in vivo in the rat and investigate if pancreatic acinar cells possess PP receptors by studying the binding of BPP and the biological effect of BPP and other structurally related peptides on isolated rat pancreatic acini and pancreatic lobules.

MATERIALS AND METHODS

Materials

The following were purchased: soybean trypsin inhibitor (type I-S), bacitracin, and N^2,O^2-dibutyryl guanosine 3′,5′-monophosphate (Bt_2cGMP) from Sigma Chemical, St. Louis, MO; chromatographically purified collagenase from Worthington Biochemicals, Freehold, NJ; minimal Eagle's medium amino acid supplement from Grand Island Biological, Grand Island, NY; *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) from Calbiochem-Behring, La Jolla, CA; bovine serum albumin (fraction V) from Miles Laboratories, Elkhart, IN; cholecystokinin octapeptide (CCK-8) from Squibb, New Brunswick, NJ; cholecystokinin (CCK-33) and Sephadex G-50 superfine from Pharmacia, Piscataway, NJ; rat pancreatic polypeptide (RPP), neuropeptide Y (NPY), peptide YY (PYY), and the C-terminal hexapeptide amide of pancreatic polypeptide (PP-6) from Peninsula Laboratories, Belmont, CA; and carrier-free Na^{125}I from New England Nuclear, Boston, MA.

BPP was a gift from Dr. Ronald Chance of the Lilly Research Laboratories, Indianapolis, IN.

In Vivo Studies

Animal preparation. Male, fed, Sprague-Dawley rats, weighing between 250 and 350 g, were anesthetized with a mixture of xylazine and ketamine (13 and 87 mg/kg body wt, respectively, im), and a midline incision was made to expose the abdomen. A polyethylene cannula (Clay-Adams PE-100) with a tapered tip was inserted into the common bile-pancreatic duct at the sphincter of Oddi. A second cannula was placed into the duodenum, slightly above the sphincter of Oddi, to permit infusion

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of pancreaticobiliary juice. To infuse hormones, a cannula (PE-10) was introduced into the external jugular vein. Both incisions were covered with moist cotton gauze. Body temperature was monitored and maintained between 37 and 38°C with a heating pad.

Pancreatic secretion studies. Combined bile-pancreatic secretions were collected in small vials for 10-min periods. The volume was measured, and an aliquot was taken and diluted with distilled water for protein determination. The remainder of the undiluted bile-pancreatic juice was pumped back into the rat via the duodenal cannula during the next collection period at the rate of secretion of the preceding collection period.

After a basal period of 50 min, CCK-33 at 10 Ivy dog units (IDU)·kg^{-1}·h^{-1} or CCK-8 at 1 μg·kg^{-1}·h^{-1} was infused for 150 min. During the middle 50 min, BPP was added to the infusion in doses of 0, 5, 20, 40, or 80 μg·kg^{-1}·h^{-1}. The effect of peptides with structural similarities to BPP (C-terminal hexapeptide of PP, peptide YY, and neuropeptide Y) on CCK-33-stimulated enzyme secretion was tested in a similar fashion. All peptides were dissolved in 1% BSA-saline solution and infused at 1.24 ml/h. Collection of bile-pancreatic juice was stopped 50 min after termination of CCK infusion.

To further evaluate the inhibition of CCK-33-induced bile-pancreatic protein output caused by BPP, we examined the relation between the degree of inhibition induced by a fixed dose of BPP (40 μg·kg^{-1}·h^{-1}) and different doses of CCK-33 (5, 10, 15, and 20 IDU·kg^{-1}·h^{-1}).

Changes in protein output attributed to biliary volume and biliary protein secretion were assessed by cannulating the bile duct prior to its entrance into the pancreas. Collections were made under both basal and CCK-33-stimulated conditions with and without BPP and analyzed for protein.

The amount of bile-pancreatic juice protein that is attributed to bile was determined on randomly selected experiments. After the last collection of bile-pancreatic juice, pure bile juice was collected from the bile duct before its entry into the pancreas. Protein content was measured. To assess any interference of bile on protein determination, known amounts of BSA, α-amylase, or trypsin were added to bile juice and the protein content measured.

Dispersed Pancreatic Acini Studies

Preparation of pancreatic acini. Isolated rat pancreatic acini were prepared by enzymatic digestion of pancreases from male Sprague-Dawley rats as previously described (24). Briefly, Krebs-Henseleit-bicarbonate (KHB) buffer containing minimum Eagle's medium amino acid supplement, 0.1 mg/ml purified collagenase, 2 mg/ml BSA, and 0.1 mg/ml soybean trypsin inhibitor was injected into the interstitium of the pancreas with a 27-gauge needle. The tissue was incubated under 95% O_2-5% CO_2 at 37°C with shaking (120 oscillations/min) for 10 min, the medium replaced with fresh identical medium, and the incubation continued for another 40 min. Acini were released by mechanical disruption by passing tissue through pipette orifices of decreasing size. Acini were harvested by filtering through 150-μm nylon cloth followed by centrifugation through 4% BSA. Acini were then resuspended in HEPES-buffered Ringer solution (HR). HR was similar to KHB, but contained 10 mM HEPES as buffer and 0.5% BSA and was gassed with 100% O_2.

Biological activity of acini. Two-milliliter aliquots of acinar suspension were distributed into 25-ml polycarbonate Erlenmeyer flasks. The basal and CCK-8-stimulated release of amylase, in the absence and presence of BPP, was measured as previously described (24). In brief, at the beginning of the incubation period, 1 ml of acinar suspension was centrifuged at 10,000 rpm for 15 s in an Eppendorf microcentrifuge, and the medium and pellet were analyzed for amylase. Amylase in the cell pellet of this *time 0* sample was used as a measure of initial acinar amylase content. After incubating for 30 min at 37°C in a shaking water bath (60 oscillations/min) in the presence of varying concentrations of CCK-8 (0–3,000 pM) and various amounts of BPP, PP-6, NPY, or PYY, 1-ml aliquots of acini suspension were centrifuged as described above and the supernatant was assayed for amylase. Amylase release was determined by subtracting the *time 0* medium value from the amount in the medium after incubation and dividing by the *time 0* acinar amylase content.

Binding studies with ^{125}I-BPP. Bovine pancreatic polypeptide was labeled with ^{125}I using a modification of the chloramine-T method reported by Freychet et al. (7). Briefly, 35 μl 0.3 M phosphate buffer (pH 7.4) were added to 2 μg of BPP in 10 μl of HCl-H_2O (pH 2.5). One microcurie of ^{125}I in 2 μl of 0.1 M NaOH was added, followed by 0.4 μg of chloramine-T in 10 μl of phosphate buffer, and gently swirled for 60 s. Sodium metabisulfite (0.2 μg) was added and gently shaken for 30 s. One hundred microliters of 2.5% BSA were added and the mixture transferred to a 1 x 50 cm Sephadex G-50 column. The column was eluted with barbital saline Merthiolate (0.1 M) buffer and 1.0-ml fractions collected. The tracer was checked for its immunoreactivity by binding to BPP antiserum (R. Chance, Lilly Research Laboratories). The immunoactive ^{125}I-BPP eluted in a single peak off the Sephadex column. The specific activity was determined by chromatoelectrophoresis and was in the range of 100–110 mCi/mg. Tracer was used within 2 wk of iodination.

Measurement of peptide binding was performed on isolated acini (0.1–0.2 mg acinar prot/ml) suspended in HR containing 0.5 mg bacitracin per milliliter and incubated in tissue culture flasks. Radioiodinated BPP (60–70 pM) was added to the suspension and incubated in a shaking water bath (60 oscillations/min) at 37°C. At specific times, 1 ml of the suspension was removed, centrifuged at 300 *g* for 2 min at 4°C, and washed twice with ice-cold 0.9% saline. The radioactivity of the washed pellets and an aliquot of the incubation medium (to determine total counts) was measured in a gamma scintillation counter. Nonspecific binding was measured by incubating acini with labeled BPP and an excess of unlabeled BPP (2.4 μM).

Degradation of labeled hormones in the medium was measured by taking supernatant from the above centrifuged acini and adding trichloroacetic acid (TCA) to a final concentration of 10%. The TCA solution was centrifuged (4°C) for 10 min at 1,300 *g*. The radioactivity in the precipitate was compared with the total radioactivity.

Pancreatic Lobules Studies

Pancreatic lobules were prepared by the method of Scheele and Palade (20). Briefly, the pancreas was removed, and HR buffer solution was injected into the interstitium. Lobules were removed with forceps and scissors. Lobules were preincubated in groups of three in 2 ml of HR in 25-ml polycarbonate flasks for 30 min at 37°C with shaking (90 oscillations/min). The medium was removed and replaced with 2 ml of fresh medium. Lobules were incubated with varying concentrations of CCK-8 and BPP. After 30 min, the medium was removed and the lobules homogenized with a Teflon-glass homogenizer in 2 ml of HR buffer. Homogenates were diluted with HR buffer, and amylase content of diluted homogenates and mediums was determined.

Assays

Pancreaticobiliary juice protein was measured spectrophotometrically at 280 nm with BSA as standard. The spectrophotometric method was validated using the assay method of Bradford (4). Both assays were used to measure bile protein and the protein in bile with known amounts of protein added. Acinar protein was measured by the method of Bradford. Amylase activity was determined by the method of Jung (11), with Procion yellow starch as substrate.

Calculations and Statistics

The bile-pancreatic protein output in response to CCK-33 plus or minus BPP over the course of the experiment was calculated as the percent change from basal output. Inhibition of protein secretion by NPY, PYY, PP-6, and varying doses of BPP or the response to a fixed dose of BPP against varying amounts of CCK-33 was calculated as the average protein output during the last three collections when CCK-33 was infused with BPP or its analogues divided by the average protein output during the last three collection periods of CCK-33 infusion prior to infusion of one of the PP analogues. Amylase output from isolated pancreatic acini was initially calculated as percent release of the total amylase content of acini at the beginning of incubation. Maximal amylase release ranged from 18 to 25% of total amylase content. These values were normalized by subtracting the basal secretion and expressing the results as a percentage of the maximal observed secretory response in each experiment. In experiments with pancreatic lobules, amylase secretion (amylase in medium) was expressed as the percentage of total amylase content (amylase in medium plus amylase in lobules).

Data were statistically analyzed using Student's *t* test or analysis of variance, with Tukey's high significant difference contrasts used to compare group means at the $P < 0.05$ level.

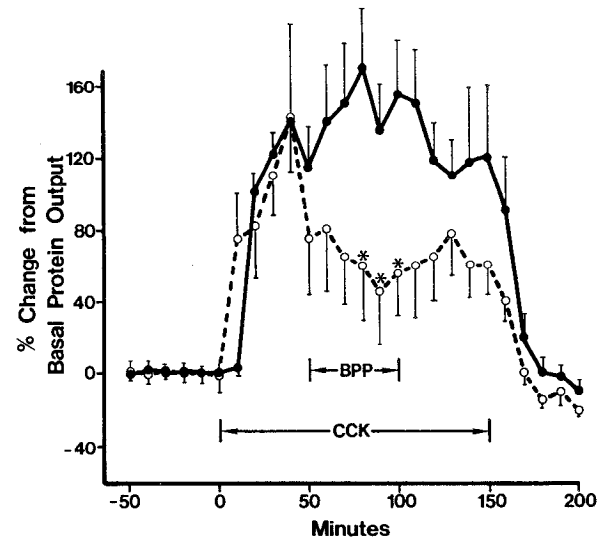

FIG. 1. Rate of bile-pancreatic protein secretion in rats intravenously infused with cholecystokinin (10 Ivy Dog Units·kg^{-1}·h^{-1}), followed by 0 (●——●) or 40 μg (○- - -○) bovine pancreatic polypeptide (BBP) per kilogram per hour for 50 min. Pancreaticobilary juice was collected at 10-min intervals, sampled for analysis, and continuously returned to intestine during entire experiment. Values are means ± SE of percent increase over basal output in 6 rats for each group. *$P <$ 0.05.

RESULTS

In Vivo Studies

The mean (±SE) bile-pancreatic protein output during the basal collection period was 52 ± 9.7 mg/h, with 93.1 ± 4.63% ($n = 15$) of this protein output being biliary protein. CCK-33 (10 IDU·kg^{-1}·h^{-1}) induced a rapid and large increase in protein output, which was sustained over the entire infusion period (Fig. 1). CCK-33-induced pancreatic bile-protein output averaged 117 ± 15.4 mg/h. In separate experiments we showed that biliary protein did not increase with CCK-33 stimulation. Therefore, the increase in protein output in the bile-pancreatic juice following CCK-33 infusion largely reflected protein from the pancreatic source. Bile-pancreatic flow rate averaged 0.28 ml/10 min under basal conditions and averaged 0.32 ml/10 min during CCK-33-infusion. Bile did not interfere with protein measurements using either the Bradford method or spectrophotometrically at 280 nm. Green et al. (8) have measured total protein, chymotrypsin, trypsin, and amylase and found them to be secreted in parallel. When amylase was measured in selected experiments, its pattern of release paralleled the secretory response of protein. Administration of BPP (40 μg·kg^{-1}·h^{-1}) inhibited CCK-induced protein output during the entire BPP infusion period. The onset of the inhibitory action of BPP was rapid, occurring over the first 10-min collection period but not statistically different from controls until 30 min after initiation of BPP infusion ($P <$ 0.05). After cessation of BPP infusion there was an increase in protein output, which did not return to control levels. After terminating CCK-33 infusion, both

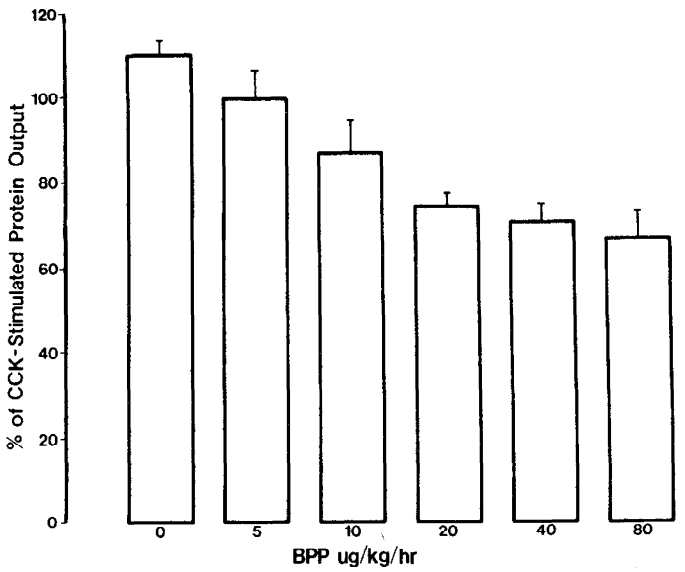

FIG. 2. Effect of varying doses of bovine pancreatic polypeptide (BBP) on CCK-33-stimulated (10 IDU·kg^{-1}·h^{-1}) bile-pancreatic protein output in vivo in rat. Responses are expressed as percentage of response to CCK-33-infusion alone [mean output of 80, 90, and 100 min of CCK-33 infusion (CCK-33 + BPP)/mean output at 30, 40, and 50 min of CCK-33 infusion (CCK-33 alone)] × 100. Values are means ± SE of at least 4 rats. Note: in control rats protein output increases slightly during the CCK-33 + BPP (0 μg·kg^{-1}·h^{-1}) infusion period (see Fig. 1). Thus, calculated response would be greater than 100% for controls.

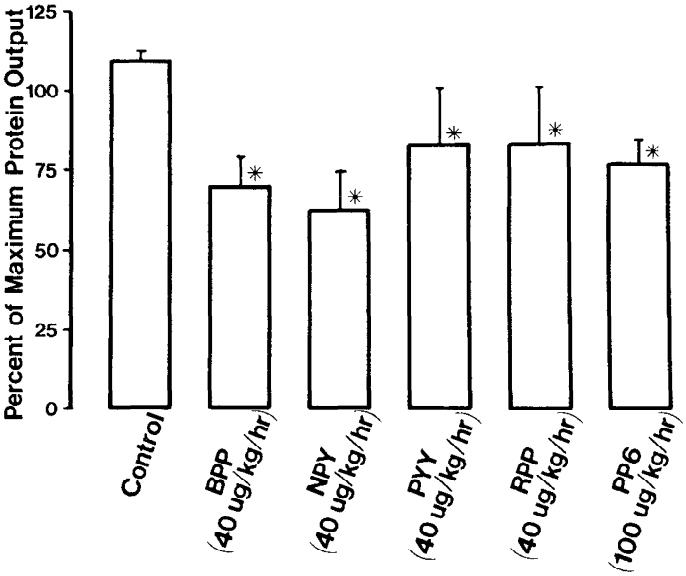

FIG. 3. Effect of bovine pancreatic polypeptide (BPP), neuropeptide Y (NPY), peptide YY (PYY), rat pancreatic polypeptide (RPP), and pancreatic polypeptide-6 (PP-6) on CCK-33-induced (10 IDU·kg^{-1}·h^{-1}) bile-pancreatic protein output in vivo. Responses are expressed as percentage of response to CCK-33 infusion alone as in Fig. 2. Results are means ± SE from at least 3 preparations. *Significantly different from control ($P < 0.05$). Note: in control rats protein output increases slightly during CCK-33 + BPP (0 μg·kg^{-1}·h^{-1}) infusion period (see Fig. 1). Thus, calculated response would be greater than 100% for controls.

groups quickly returned to basal levels. The amount of suppression by BPP of CCK-33 (10 IDU·kg^{-1}·h^{-1})-stimulated protein output was dose related (Fig. 2), and BPP produced a highly significant treatment effect ($F = 7.73$, df 5/28, $P < 0.001$). Statistically significant inhibition of protein output was achieved at 20 μg·kg^{-1}·h^{-1} of BPP and higher doses. BPP also inhibited CCK-8-induced (1 μg·kg^{-1}·h^{-1}) pancreatic protein output in a similar fashion (data not shown). In contrast, BPP had no effect on biliary protein output during CCK-33 or CCK-8 infusion.

To examine the nature of inhibition by BPP, we evaluated the effect of BPP (40 μg·kg^{-1}·h^{-1}) on the bile-pancreatic protein output induced by different amounts of CCK-33. Increasing doses of CCK-33 (5, 10, 15, and 20 IDU·kg^{-1}·h^{-1}) caused a dose-related increase in protein output (from 134 to 386% above basal protein output). The BPP-induced inhibition remained constant at ~80% of CCK-33-induced stimulation. Statistical analysis of this data by analysis of variance did not indicate a significant effect of increasing doses of CCK-33.

Infusion of PP-6 (100 μg·kg^{-1}·h^{-1}) inhibited CCK-33-induced bile-pancreatic protein output by 20%. Infusion of RPP (40 μg·kg^{-1}·h^{-1}), PYY (40 μg·kg^{-1}·h^{-1}), and NPY (40 μg·kg^{-1}·h^{-1}) also produced a similar degree of inhibition of BPP and PP-6 (Fig. 3). All PP analogues significantly reduced CCK-33-stimulated protein output ($P < 0.05$).

Dispersed Pancreatic Acinar Studies

Stimulation of pancreatic acini with various concentrations of CCK-8 for 30 min elicited amylase release in a dose-dependent fashion, with optimal stimulation at 300 pM; higher concentrations of CCK-8 induced a submaximal amylase release (Fig. 4). The addition of BPP at 1.2 μM or 24 nM (not shown) did not alter the response to CCK-8 by acini. Similarly, incubation of acini with PP-6 (12 μM), NPY (0.5 μM), or PYY (0.5 μM) did not inhibit amylase secretion induced by CCK-8 (Fig. 5). In contrast, incubation of acini with CCK-8 and Bt_2cGMP (0.3 mM), a known antagonist to CCK, produced a surmountable inhibition, shifting the dose curve to the right (Fig. 4). BPP also did not alter the dose response to carbachol or the combined stimulation with CCK-8 and vasoactive intestinal peptide (10 nM), nor did RPP (24 nM) alter the dose response to CCK-8 (data not shown).

To determine whether receptors for BPP exist on pancreatic acini, we studied the binding of ^{125}I-labeled BPP. Binding of the tracer ^{125}I-BPP (60 pM) did not differ from nonsaturable binding (plus 2.4 μM BPP), which was 0.25% of total counts in the preparation. The amount of nonsaturable binding remained unchanged over 90 min of incubation. Degradation of ^{125}I-BPP in the binding medium was estimated by measurement of TCA precipitability of label in the supernatant of the acinar suspension and was no greater than 5% of added ^{125}I-BPP after 90 min.

Pancreatic Lobules Studies

Because BPP did not have an inhibitory effect on amylase release from pancreatic acini, nor did BPP bind to acini, the possibility of receptor damage, resulting from collagenase digestion, was raised. We therefore

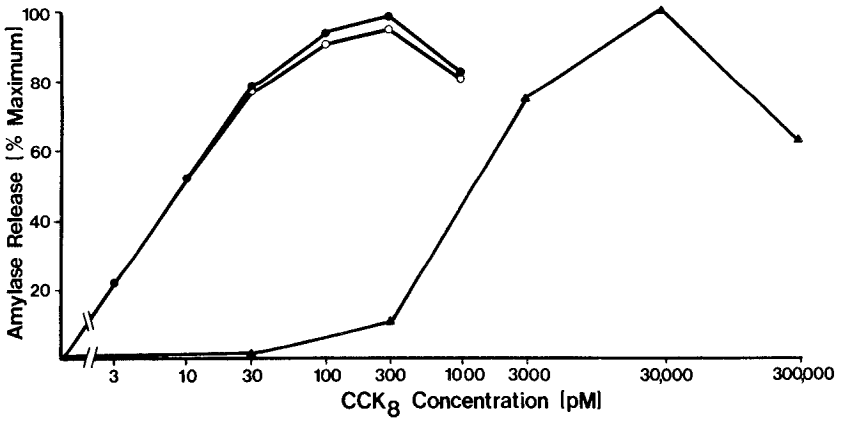

FIG. 4. Comparison of addition of bovine pancreatic polypeptide (BPP) and dibutyryl cGMP (Bt_2cGMP) on CCK-8-stimulated amylase release from isolated rat pancreatic acini. ●——●, CCK-8 alone; ○——○, CCK-8 + BPP at 1.2 μM; ▲——▲, CCK-8 + Bt_2cGMP at 0.3 nM. Values are means from 3 acini preparations. SE was less than 10% of mean for each point.

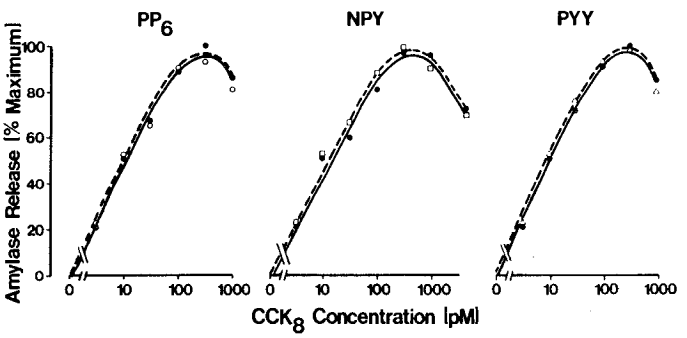

FIG. 5. Comparison of pancreatic polypeptide analogues on CCK-8-stimulated (●——●) amylase release from isolated pancreatic acini. *A*: C-terminal hexapeptide of pancreatic polypeptide (12 μM); *B*: neuropeptide Y (0.5 μM); *C*: peptide YY (0.5 μM). Values are means from 3 acini preparations. SE was less than 10% of mean for each point.

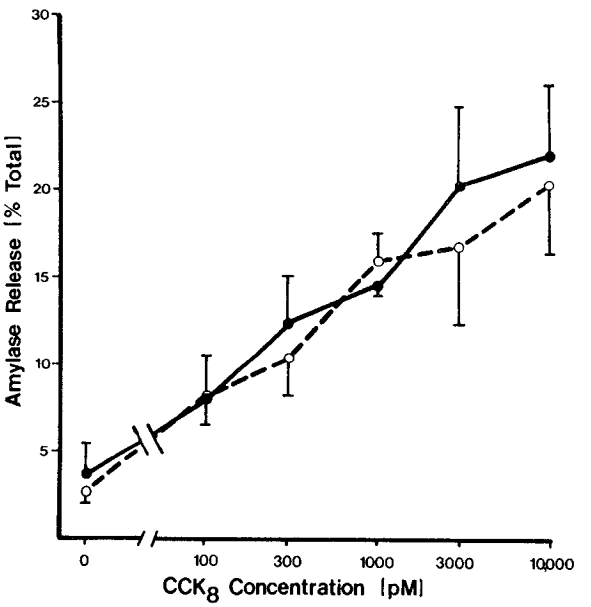

FIG. 6. Effect of bovine pancreatic polypeptide, (1.2 μM, ○- - -○) on CCK-8-stimulated (●——●) amylase release from pancreatic lobules. Results are means ± SE for 3 lobule preparations.

examined the effect of BPP on pancreatic lobules, a preparation which does not require collagenase digestion and contains a heterogenous cell population and nerve endings. Pancreatic lobules secreted amylase in a dose-dependent manner to CCK-8. Addition of BPP (1.2 μM) did not alter amylase release in response to CCK-8 (Fig. 6).

DISCUSSION

Bovine pancreatic polypeptide is a straight-chain polypeptide of about 4,200 daltons whose sequence of 36 amino acid residues is known (5). Bovine, human, canine, porcine, and ovine pancreatic polypeptides differ among one another in only one or two amino acid residues (15). However, the amino acid composition of avian PP differs greatly from mammalian PP (5). In radioimmunoassays antisera to human PP and bovine PP are used, pancreatic extracts from rats cross-react poorly and nonspecifically (6), suggesting that rat PP is structurally different from peptides isolated from higher mammals. Recently, the structure of rat PP has been elucidated. The amino acid sequence differs significantly from PP of higher mammals, although their C-terminal hexapeptides are identical (12). Thus, it was not known whether BPP has biological actions in the rat as shown in dogs (17) and humans (1, 10, 13). Our in vivo studies on anesthetized rats showed that infusion of BPP inhibited CCK-33-stimulated protein output in a dose-dependent fashion. At an infusion rate of 20 $\mu g \cdot kg^{-1} \cdot h^{-1}$ of BPP, maximal inhibition is approached. Further increases in BPP resulted in small increases in inhibition. Maximum inhibition occurred toward the end of the BPP infusion period. In experiments with conscious dogs, Lin et al. (17) achieved peak inhibition (67%) of CCK- plus secretin-stimulated protein output at an infusion rate of 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ of BPP. The higher doses of BPP needed to inhibit protein output in the rat compared with dogs may reflect differences in conscious versus anesthetized animals.

The demonstration of biological activity of BPP in rats in our study is supported by a recent study showing that the C-terminal of rat PP, the biologically active region of the molecule, it immunochemically similar to that of PP extracted from higher mammals (22). This finding may also explain the biological activity of PP-6, PYY, and NPY in the rat, since all these peptides contain the biologically active C-terminal hexapeptide of the PP molecule. Previous studies demonstrate that the C-terminal hexapeptide of PP mimics the action of the whole molecule on gastric secretion, but a higher molar dose is needed (16). The C-terminal tyrosyl amide is also crucial for inhibition of pancreatic secretion. In the dog, Des-C-

Tyr-NH_2-BPP [BPP-(1—35)] had no inhibitory effect on volume, electrolyte, or enzyme secretion (17). The minimum effective C-terminal fragment with full BPP potency is unknown. Infusion of PP-6 in higher doses than BPP inhibited CCK-33-stimulated bile-pancreatic protein secretion. Infusion of PYY and NPY, both 36-amino acid straight-chain polypeptides sharing the same C-terminal hexapeptide as the sequenced mammalian pancreatic polypeptide, also inhibited CCK-33-stimulated bile-pancreatic protein output.

Although BPP has previously been shown to inhibit CCK-33-stimulated pancreatic enzyme secretion in dogs (16) and humans (10), the nature of its antagonistic effect is not known. In this study we have showed that the inhibition of CCK-33-stimulated protein secretion by BPP is a function only of the concentration of BPP and is independent of the concentration of CCK-33. That is, the maximal BPP-induced inhibition of pancreatic protein secretion remains constant and is not surmountable by increasing the concentration of CCK. This observation suggests that BPP is a noncompetitive antagonist of the action of CCK, in contrast to proglumide (23) and Bt_2cGMP (19), which are competitive antagonists of the action of CCK on pancreatic enzyme secretion. This nonsurmountable inhibition by BPP observed in the rat may be species specific.

The absence of an inhibitory effect of BPP, NPY, PYY, or PP-6 on amylase release from isolated rat pancreatic acini, coupled with our failure to demonstrate specific BPP receptors, suggests that the inhibitory action of BPP on pancreatic exocrine secretion is mediated by indirect mechanisms. Failure of ^{125}I-BPP to bind to acinar cells may also be a result of oxidative damage to BPP by chloramine-T. Bonnevie-Nielsen et al. (3) were able to show binding of [^{125}I-Tyr]BPP to rat hepatocytes. The radiolabeled BPP used in our study was freshly prepared, retained full immunoreactivity, and eluted in a single peak off the Sephadex column and thus should be suitable for binding studies. However, we did not perform binding studies in other systems such as rat hepatocytes. Nevertheless, the absence of an inhibitory response to BPP by acini strongly indicates the absence of receptors for PP. Damage to receptors for BPP on acini due to collagenase digestion can be ruled out, since BPP also had no effect on CCK-8-stimulated amylase release from pancreatic lobules, which were not subjected to enzyme digestion. The lack of a direct inhibitory effect by BPP on pancreatic secretion is not unique. Many substances, including somatostatin, Met-enkephalin, epinephrine, and glucagon, work in vivo to suppress pancreatic enzyme secretion but are unable to act directly on the acinar cell (J. A. Williams, unpublished data; T. E. Solomon, personal communication). Somatostatin does exhibit specific binding to purified pancreatic plasma membranes, but the functional significance is not known since it does not have any effect on amylase release from pancreatic acini.

In summary, BPP can inhibit pancreatic exocrine secretions in the rat by noncompetitive antagonism. Inhibitory effects are observed only with in vivo preparations, and there is an absence of biological activity on preparations of pancreatic acini and pancreatic lobules. This suggests the inhibitory action of BPP on exocrine secretion may be mediated by indirect mechanisms. Further investigations are needed to determine whether the action of PP is indirectly mediated by way of long neural pathways, by changes in blood flow, or through release of other hormones.

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