Regional Pancreatic Concentration and In-vitro Secretion of Canine Pancreatic Polypeptide, Insulin, and Glucagon

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SUMMARY

The regional concentrations and in-vitro secretions of canine pancreatic polypeptide (cPP), insulin, and glucagon were studied. CPP is found predominantly in the uncinate process of the dog pancreas, whereas insulin and, more markedly, glucagon predominate in the body and tail of the pancreas. In-vitro secretion studies of pancreatic pieces indicate that dibutyryl cyclic AMP (dcAMP) alone can stimulate cPP release whereas glucose and arginine alone have no effect. Arginine, but not glucose, potentiates this stimulant effect of dcAMP. These studies suggest that the cAMP generating system may play a role in regulation of cPP secretion. DIABETES 27:96-101, February, 1978.

The newly recognized 36-residue mammalian pancreatic polypeptide (PP) described by Lin and Chance[1] is found in discrete endocrine-type cells in the pancreatic islet and/or in surrounding acinar tissue, depending on the species studied.[2-4] Mean plasma PP levels are elevated significantly in maturity- and juvenile-onset diabetic patients.[5] PP is also associated with pancreatic endocrine tumors, although a tumor producing only PP has not been described.[6-8]

The primary physiologic function of PP is unknown. In birds, avian PP (aPP) is a powerful gastric acid stimulant, accelerates hepatic glycogenolysis without altering blood glucose, and lowers plasma glycerol and FFA levels.[9-11] Intravenous infusion of bovine PP (bPP) in dogs affects gut motility and gastric and pancreatic secretions.[1]

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Little is known about the secretion of PP. Determination of serum PP by radioimmunoassay indicates that the concentration of the hormone varies with the prandial state. In man, ingestion of a protein-rich meal induces a brisk, biphasic secretion pattern, comparable to the postprandial release of insulin.[12-15] Likewise, intake of a high-protein meal in the dog results in a dramatic elevation of plasma levels of canine PP (cPP), with a peak response occurring 30 minutes after the meal.[16]

Since data concerning the regional concentration and in-vitro secretion of cPP are not available, the present study was designed to (1) compare the tissue concentration of cPP in three general regions of the dog pancreas, (2) measure in-vitro cPP secretion by pancreatic pieces obtained from the uncinate process and tail of the dog pancreas, and (3) assess the potential stimulatory role of glucose, arginine, and dibutyryl adenosine 3′, 5′-cyclic monophosphoric acid (dcAMP) on cPP release in vitro.

MATERIALS AND METHODS

All experiments were done on pancreatic tissue excised from mongrel dogs (10-20 kg.) fed ad libitum. Dogs were sacrificed with an overdose (50 mg./kg., intravenously) of sodium pentobarbital. The pancreas was quickly removed and placed in a Petri dish containing ice-cold Hanks' buffer (Gibco, Grand Island, NY) and cleaned of extraneous adipose tissue.

Tissue extraction. A block of tissue (2 cm.) was cut from the tip of the uncinate process and tail ends of the pancreas; a similar portion was taken from the body, approximately midway in the gland. The tissues were cut into small pieces (1-2 mm.^{3}), quick-frozen by immersion in liquid nitrogen, and stored at

−70° C. until extracted for total hormone content. After samples from nine dogs were collected, two to three pieces (100-200 mg.) were randomly selected from each region and homogenized in 1.0 ml. of acidified alcohol solution (consisting of 2.1 ml. concentrated H_2SO_4, 80 ml. of 95 per cent ethanol, and 17.9 ml. of distilled water)[17] by a motor-driven glass homogenizing system. The extracted hormones were assayed directly from the acid-alcohol extraction mixture without prior neutralization.

Hormone secretions. Tissues from the uncinate process and tail were placed in a Petri dish containing cold Hanks' buffer, cut with single-edged razor blades into small pieces (4-8 mg.), and rinsed twice with cold buffer. Four pieces were randomly chosen and placed in scintillation vials (Kimble, 20 ml.) containing 2.0 ml. of bicarbonate-buffered incubation media (115 mM NaCl, 5.0 mM KCl, 2.5 mM $CaCl_2$, 24 mM $NaHCO_3$, 1.0 mM $MgCl_2$) that contained glucose (2.8 mM) and bovine plasma albumin (2 gm. per liter, Armour Pharmaceutical, Chicago). The media in the flasks were equilibrated to pH 7.4 with a mixture of oxygen (95 per cent) and carbon dioxide (5 per cent), and the flasks were placed in a Dubnoff metabolic shaker maintained at 37° C. and shaking at 90 cycles per minute. Each experiment in a particular group was done in parallel with pieces of tissue from the same pancreas.

Tissues were preincubated for a total of 60 minutes in low glucose (2.8 mM) prestimulatory media containing no Trasylol. After the first 30 minutes, the prestimulatory media were aspirated and replaced with fresh media. After an additional 30 minutes, the prestimulatory media were replaced with media containing Trasylol (1,000 K.I.U./ml.) plus the various agents being tested and incubated a further 90 minutes. After each change of media, the vials were gassed to maintain a constant pH. At the end of the incubation period, the media were collected on ice and kept frozen (−70° C.) until assayed for pancreatic polypeptide, insulin, and glucagon. The pancreatic pieces were gently blotted and weighed.

Radioimmunoassay Procedures

Insulin. Aliquots of incubation media were assayed for insulin by the alcohol precipitation method of Wright et al.[18] ^{125}I-Insulin (New England Nuclear, Boston), porcine insulin standards (Eli Lilly, Indianapolis), and guinea pig anti-porcine-insulin serum (generously supplied by Dr. Peter Wright, Indianapolis) were used in the assay. The standard curves were performed in the presence of a volume of incubation medium equal to the aliquot of unknown sampled for assay. Separation of the bound from free hormone was accomplished by precipitation with ethanol at a final concentration of 76 per cent.

Glucagon. Aliquots of incubation media were assayed for glucagon by the double-antibody technique of Leichter et al.[19] These assays were kindly performed by the Diabetes and Endocrinology Center Radioimmunoassay Facility at Washington University.

Pancreatic polypeptide. Aliquots of incubation media were assayed for cPP by a heterologous double-antibody technique developed in our laboratory. Highly purified bPP (Lilly lot 615-D63-286) was labeled with iodine-125 (New England Nuclear, Boston) to a specific activity of 300-500 μCi./μg. by the choloramine-T method of Greenwood et al.[20] and purified on a 0.9×30-cm. Sephadex G-50 superfine column. Since an antiserum to cPP is not currently available, all assays were accomplished with Lilly antiserum (lot 615-1054B-19) raised in rabbits against human PP (hPP) as previously described.[2,4] Highly purified hPP (Lilly lot 615-1054B-200) was used for standards. All assay reagents were diluted in a phosphate-saline buffer (0.05 M, pH 7.0) containing normal rabbit serum (12.5 ml. per liter) and merthiolate (0.1 μg. per liter). The final assay volume was 0.4 ml. Human PP standards (0-30 pg./0.1 ml., in triplicate) and unknown samples (0.1 ml., in duplicate) were incubated for 72 hours at 4° C. with diluted anti-hPP serum (0.6 nl./0.1 ml.). A volume (0.1 ml.) of tracer ^{125}I-bPP was then added and the incubation allowed to proceed another 18 hours. Separation of bound from free hormone was accomplished by centrifugation (10 minutes, 4° C.) of the precipitated immunoglobulin formed during a two-hour incubation (4° C.) with goat anti-rabbit gamma globulin serum (10 μl./0.1 ml. buffer). The supernatant was aspirated and the pellet counted for radioactivity. Sensitivity of the assay was 2.0 pg. per tube.

The behavior of purified cPP standards (Lilly lot 615-X53-133-2) in the assay system was found to be identical to hPP standards (figure 1), suggesting a high degree of cross-reactivity between cPP and the anti-hPP serum. Statistical analysis was by Student's *t*-test based on nonpaired comparisons.

RESULTS

Tissue concentrations of cPP, insulin, and glucagon. The concentrations of cPP, insulin, and glucagon in extracts of uncinate process, body, and tail regions of the dog pancreas are shown in table 1. Insulin content

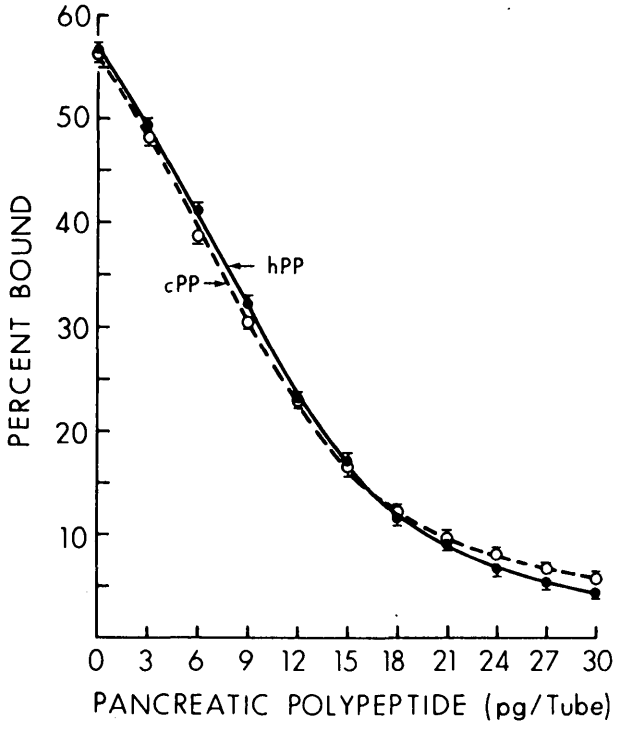

FIG. 1. Cross-reactivity of cPP with hPP antiserum. Standard curves of hPP and cPP were compared under identical assay conditions. Each point represents a mean ± S.E.M. of nine observations.

of uncinate process is about half that of the body and the tail. Likewise, the glucagon content of the uncinate process is substantially less than in the body or tail of the pancreas. In contrast to insulin and glucagon, cPP is found predominantly in the uncinate process of the dog pancreas, while the body and tail regions contain several times less hormone per gram of tissue.

Degradation of ^{125}I-labeled insulin and bPP by incubated pancreatic tissue. To determine the loss of secreted hormones during the incubation period, small pieces of normal dog pancreas were preincubated 60 minutes with two changes of medium followed by a 90-minute incubation in medium (2.0 ml.) containing unlabeled insulin (2.5 mU.) and bPP (100 ng.) along with a trace amount of ^{125}I-labeled hormone. After incubation, a sample (100 μl.) of medium was added to an excess (10×) of antibody (300 μl.) and incubated a further 60 minutes at 4° C. in an effort to bind all remaining immunoreactive hormone. The antibody-antigen complex was precipitated with ethanol (1.6 ml. of 95 per cent) and the precipitate counted for radioactivity. The percentage of the initial radioactive hormone that remained immunologically reactive after 90 minutes of incubation was 83.6 ± 2.1 per cent in the case of insulin and 98.1 ± 1.0 per cent for bPP. These data show that bPP, but not insulin, is relatively stable under the incubation condition described for these studies.

TABLE 1

Hormone concentration in dog pancreas*

	Uncinate Process	Body	Tail
		μg./gm.	
Insulin	69.9 ± 5.3	124.7 ± 12.0	120.9 ± 6.6
Glucagon	0.27 ± 0.05	6.2 ± 0.9	6.8 ± 0.8
Canine PP	118.5 ± 11.3	18.0 ± 2.7	14.1 ± 1.5

*The values represent means ± S.E.M. (n = 9).

CPP release from dog pancreatic tissue. The effects of glucose, arginine, and dcAMP on cPP secretion from statically incubated pieces of dog pancreatic tissues are shown in figure 2. Varying the glucose in the incubation media from low (2.8 mM) to high (27.5 mM) concentration did not alter the rate of cPP released by tissue slices obtained either from the uncinate process or from the tail of the dog pancreas. However, the basal rate of cPP secretion by tissue obtained from the uncinate process was about 10 times greater than the rate of hormone released by tissue obtained from the tail (0.45 ng./mg./90 min. vs. 0.05 ng./mg./90 min., respectively). This difference in the cPP secretory rate between the uncinate process and the tail was observed with each stimulant tested and is in keeping with the tissue concentration of the hormone found in these two regions of the pancreas—the tissue level of cPP in the uncinate process is 118.5 μg./gm. as

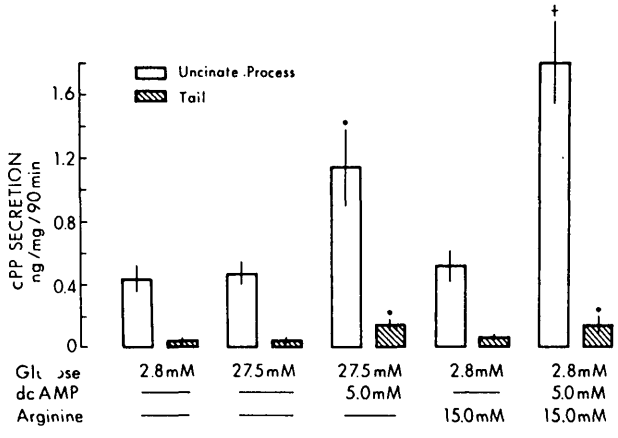

FIG. 2. CPP secretion by dog pancreatic tissue. Pancreatic pieces obtained from the uncinate process and tail region of the dog pancreas were preincubated 60 minutes with two changes of media, then incubated 90 minutes in control media (2.8 mM glucose) or media containing combinations of high glucose (27.5 mM), arginine (15mM), and dcAMP (5.0 mM). Each value represents a mean ± S.E.M. of 18 observations. *p < 0.025 compared with control. †p < 0.001 compared with control.

against a concentration of only 14.1 μg./gm. in the tail (table 1).

Addition of arginine (15 mM) to the media caused a small (13 per cent), nonsignificant increase in the rate of cPP secretion as compared with the control rate of release from tissue incubated in the low glucose (2.8 mM) media. A greater and significant secretory response was observed when tissues were incubated in the presence of dcAMP (5.0 mM) plus high glucose. However, of the agents tested, the combination of dcAMP and arginine provided the best stimulus for cPP secretion, with an increase of about 300 per cent over the control.

A separate series of experiments were accomplished with tissue obtained from the uncinate process to determine whether glucose or arginine is required for the dcAMP stimulatory response (figure 3). In the absence of glucose and arginine, dcAMP significantly increased cPP secretion. Addition of either low or high concentration of glucose did not alter this stimulatory effect of dcAMP. However, when dcAMP was added to the incubation medium in combination with arginine, the rate of cPP secretion was significantly elevated as compared with the rate of release elicited by dcAMP alone. Again, this potentiation by arginine of the dcAMP stimulatory response was not altered by addition of low glucose.

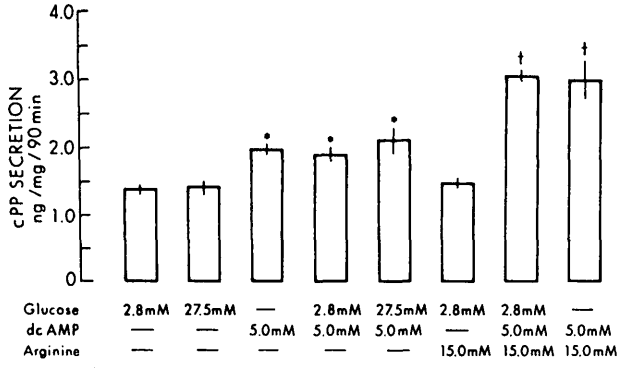

FIG. 3. Effect of dcAMP on cPP secretion. Pancreatic pieces from dog uncinate process were preincubated 60 minutes with two changes of media, then incubated 90 minutes in either control media or in media containing dcAMP (5.0 mM) alone or dcAMP with arginine (15mM) or glucose (2.8 mM, 27.5 mM). Each value represents a mean ± S.E.M. of nine observations. *p < 0.025 compared with control. †p < 0.001 compared with control.

Effect of time of cPP release. Pancreatic tissue slices were incubated 180 minutes either in basal media or in basal media containing dcAMP (5.0 mM) plus arginine (15 mM). Duplicate samples (10 μl.) for assay were taken from the incubation medium of each flask at 15-minute intervals during the first hour and at 30-minute intervals during the remaining two hours of incubation. In both basal and stimulated conditions, cPP was released at a constant rate throughout the 180 minutes of incubation (figure 4). In view of these data, the 90-minute period of incubation previously chosen was judged to be an appropriate time interval to measure cPP secretion.

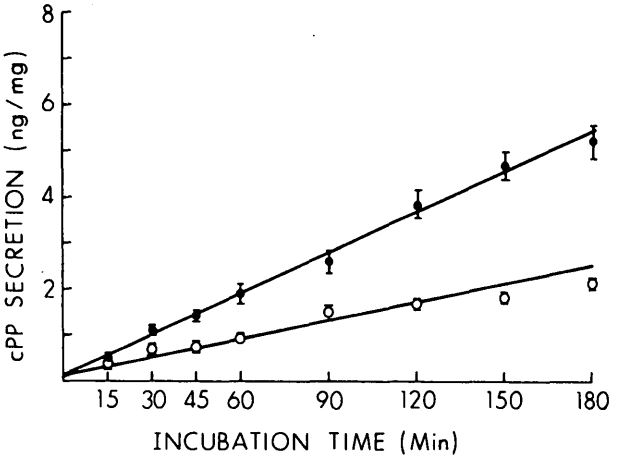

FIG. 4. Time study for the secretion of cPP. Pancreatic tissues from uncinate process were preincubated 60 minutes with two changes of media, then incubated 180 minutes in either basal media (o——o) or basal media containing dcAMP (5.0 mM) plus arginine (15 mM, •——•). Each point represents a mean ± S.E.M. of 18 observations.

Insulin and glucagon secretion from dog pancreatic tissue. Both insulin and glucagon secretion from the incubated pieces of dog pancreas were monitored in an effort to assess the viability of the incubated tissue. The results of insulin secretion are shown on the upper panel of figure 5. Insulin secretion by tissue from the tail of the dog pancreas was elevated fourfold above basal levels when incubated in the presence of high glucose (27.5 mM). Dibutyryl cAMP (5 mM) markedly potentiated this stimulant effect of glucose. Arginine (15 mM) did not alter significantly insulin secretion, even in the presence of dcAMP. A similar pattern of insulin release was observed from pancreatic tissue taken from the uncinate process, but the absolute rate of release was diminished, as would be expected from the decreased tissue concentration of insulin in the uncinate process as compared with the tail region of the dog pancreas (table 1).

Glucagon secretion from these incubated pancreatic pieces was also measured (figure 5, lower panel). Arginine (15 mM) caused a twofold increase in glucagon release from tissue obtained from the tail of the pancreas, and addition of dcAMP increased this arginine response by 100 per cent. While glucose alone did not significantly alter basal glucagon secretion, dcAMP in

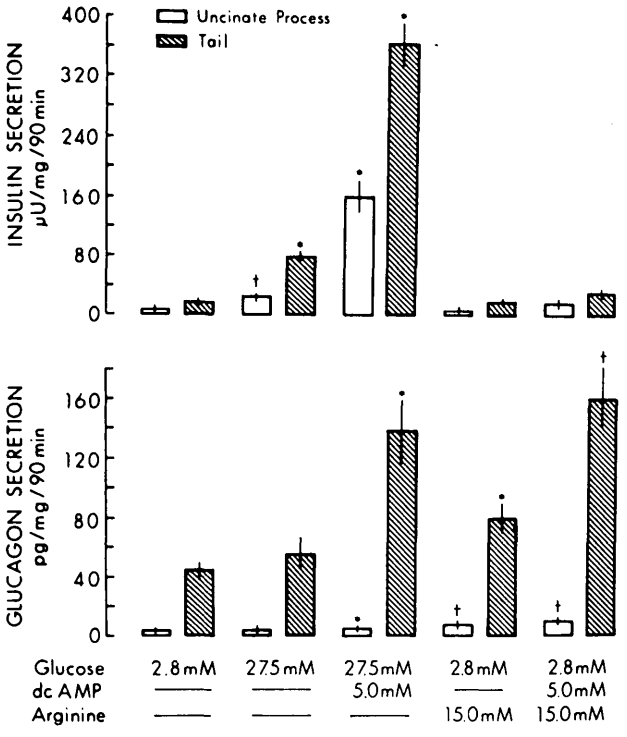

FIG. 5. Insulin (upper panel) and glucagon (lower panel) secretion by dog pancreatic tissue. Pancreatic pieces from the uncinate process and tail regions were preincubated in control media (2.8 mM glucose) for 60 minutes with two changes of media. The tissues were then incubated 90 minutes in either control media or media containing glucose (27.5 mM), arginine (15.0 mM), and dcAMP (5.0 mM). Each value represents a mean ± S.E.M. of 18 observations. *$p <$ 0.001 and †$p <$ 0.005 compared with control.

the presence of high glucose markedly elevated glucagon release. The pattern of glucagon release from the uncinate process was identical to that from the tail, but the rate of release was only 10 per cent of that from the tail. This dramatic decrease in the rate of glucagon release from tissue of the uncinate process corresponds well with the tissue concentration of glucagon in the uncinate process, which is about 5 per cent of the tail (table 1).

While the experimental protocol was designed primarily to test polypeptide secretion, the release of insulin and glucagon in response to the stimuli tested suggests viability of the incubated pancreatic tissues.

DISCUSSION

The present study demonstrates differences in the regional concentration of insulin, glucagon, and cPP (table 1). The insulin content of the uncinate process (69.9 μg./gm.), body (124.7 μg./gm.), and tail (120.9 μg./gm.) reported here agrees with the insulin content (120 μg./gm.) found by Langslow et al. in the total dog pancreas.[21] The low level of glucagon in the uncinate process as compared with the other regions of the dog pancreas is consistent with histologic reports as early as 1955 showing the lack of alpha cells in this region[22,23] and is also consistent with the lack of glucagon activity in bioassays of tissue extracts of the uncinate process.[24]

To our knowledge this is the first report in which the concentration of cPP in different regions of the dog pancreas has been quantitated by radioimmunoassay. Our data show a high concentration of cPP in the uncinate process and are supported by histologic studies that demonstrate by light- and electron-microscopic immunocytochemistry that the frequency of cPP cells is greater in the uncinate process than in the tail of the dog pancreas.[3,4,25] Our data, along with the immunocytochemical studies cited above, are consistent with the hypothesis that cPP is the F-cell hormone in the dog pancreas.

In the present study, cPP is secreted by small pieces of pancreatic tissue in response to dcAMP stimulation alone. This stimulation of cPP secretion by dcAMP suggests a role for cAMP in the release process, a situation common to other hormonal secretory systems.[26-29] Glucose stimulated the release of insulin, and arginine caused glucagon release, but neither stimulant alone was capable of directly eliciting cPP release, at least not in sufficient quantity to be detected with statistical significance in the present static incubation system. However, arginine produced a 13 per cent elevation in cPP secretion as compared with controls, a situation that may represent an initial monophasic release pattern; this possibility remains to be tested in a perifusion system. Interestingly, when arginine was added to the incubation medium in combination with dcAMP, cPP release was enhanced as compared with that from the action of dcAMP alone. At present, no satisfactory explanation can be given to account for this potentiation of dcAMP stimulation, unless arginine is in fact a weak stimulant in its own right, and, in combination with dcAMP, a synergistic response is observed.

Pancreatic pieces were used as the model system to study cPP secretion in this study primarily because immunohistochemical studies show that polypeptide-producing cells in the dog pancreas are found as patches of endocrine cells spread throughout the acinar tissue and only occasionally are they contained within the islet.[3] A potential disadvantage in using the pancreatic pieces technique is the loss of secreted hormone due to release of lytic substances into the incubation medium. It was partly for this reason that the tissues were preincubated 60 minutes and the medium changed twice. With this experimental pro-

tocol and with the addition of Trasylol to the incubation medium, insulin degradation was minimized to about 17 per cent during the 90-minute incubation, as judged by the loss of immunoreactive ^{125}I-labeled insulin. CPP was not susceptible to degradation, with an observed loss in immunoreactivity of less than 2 per cent. Glucagon degradation was not measured. The stability of secreted cPP and the high degree of cross-reactivity of the cPP with antibodies currently available makes the static incubation of dog pancreatic tissue a good system with which to survey potential cPP secretagogues. By discovering the primary stimulus for cPP release, we will be better able to determine the physiologic significance of this newly discovered hormone.

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