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Effects of Pancreatic Polypeptide on the Secretion of Enzymes and Electrolytes by *in Vitro* Preparations of Rat and Cat Pancreas

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Pancreatic polypeptide (PP) is released from the pancreas in response to vagal stimulation. Amongst other effects, PP has been reported to inhibit pancreatic exocrine function. Apart from any potential physiological role, such inhibition could have important consequences for *in vitro* studies of pancreatic function employing acetylcholine as a stimulus. We have therefore tested the effect of bovine PP on two *in vitro* pancreatic preparations: the incubated, uncinate pancreas of young rats and the perfused cat pancreas. In the former, PP (10^{-10} - 10^{-8}M) had little or no effect on enzyme discharge or ^{45}Ca efflux under basal conditions or during stimulation with caerulein, CCK-PZ or acetylcholine. In the perfused cat pancreas, similar concentrations of PP were also without effect on fluid secretion evoked by secretin infusion, or enzyme discharge evoked by CCK-PZ injection or infusion. We conclude that bovine PP has no direct effects on the cellular mechanisms responsible for pancreatic electrolyte secretion or enzyme discharge in the species studied.

Pancreatic polypeptide was discovered independently in the chicken (Kimmel *et al*, 1975) and in cattle (Lin *et al*, 1977) during the purification of insulin. It is a product of specific endocrine cells (F cells) which are located almost entirely within the pancreas (usually distributed in both islets and exocrine tissue), with a small number of cells detectable elsewhere in the gastrointestinal tract (Larsson *et al*, 1976; Adrian *et al*, 1976).

The physiological functions of pancreatic polypeptide remain unknown. However, a number of biological actions have been described including: *stimulation* of basal acid secretion (Lin *et al*, 1977), choledochal tone (Lin & Chance 1974), DNA-synthesis in rat pancreas (Greenberg *et al*, 1977) and growth of developing avian gut (Laurentz & Hazelwood 1979); and *inhibition* of gallbladder contraction (Lin & Chance 1974), glucagon stimulated lipolysis (McCumbee & Hazelwood 1977), pentagastrin-stimulated acid secretion (Lin *et al*, 1977), gastrointestinal motility (Duke *et al*, 1979), somatostatin release from gut and pancreas (Arimura *et al*, 1979) and pancreatic enzyme and electrolyte secretion in dog (Lin *et al*, 1977, Taylor *et al*, 1979), man (Adrian *et al*, 1979) and calves (Davicco *et al*, 1979).

Eating a meal causes a rapid and considerable rise in the concentration of pancreatic poly-

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peptide in the plasma (Adrian *et al*, 1976). It can also be detected in pancreatic juice (Carr-Locke & Track 1979). Its release seems to be largely under vagal, cholinergic control (Schwartz *et al*, 1978; Taylor *et al*, 1978).

Together, these observations suggest that pancreatic polypeptide could have a physiological role in digestion. Irrespective of any such role, the release of pancreatic polypeptide from the pancreas may have important consequences for *in vitro* studies of pancreatic function, in which pancreatic enzyme secretion is often evoked by cholinergic stimuli.

We have therefore studied the effects of bovine pancreatic polypeptide in two *in vitro* preparations of the pancreas stimulated by CCK-PZ, caerulein, acetylcholine and secretin. An abstract of this work has been published (Kim & Case, 1979).

METHODS

All experiments were performed on one of two *in vitro* preparations of the pancreas: the incubated uncinate pancreas of baby rats (Case & Clausen 1973) or the saline-perfused cat pancreas (Case *et al*,1968). Full details of these preparations are given in the references cited.

Incubated rat pancreas

Rats weighing 60-70g were denied food overnight and killed by decapitation. The uncinate pancreas was separated from the duodenum and body of the gland and transferred to a dish containing 0.9% saline at room temperature. It was trimmed of any adherent lymph nodes and stored in a wash-vial containing 0.4 ml of Krebs-Ringer bicarbonate buffer of the following composition (in mmol. l^{-1}): NaCl 120, KCl 4.5, $NaHCO_3$ 25, $MgSO_4$ 1.0, NaH_2PO_4 1.0, $CaCl_2$ 2.5 and glucose 5, supplemented with 0.1% bovine serum albumin. Usually ten glands were used in one experiment. Since 4-5 min elapsed between killing an animal and storage of each gland, the total preparation time for ten glands was 40-50 min. Glands were incubated at all times in screw-top, high-density polyethylene scintillation vials shaken continuously at 80 cycles per minute in a water bath maintained at 37°C. Vials contained 2 ml buffer and were equilibrated by 'top-gassing' with a mixture of 95% O_2 : 5% CO_2 so as to maintain pH at 7.4.

All experiments involved simultaneous measurement of ^{45}Ca efflux and amylase release into the vial. After isolation, all ten glands were loaded for 60 min by incubation in buffer containing ^{45}[Ca] Cl_2 (2 μCi/ml). After loading, glands were briefly washed in normal buffer and then transferred through a series of 10 vials. The first 70 min of ^{45}Ca efflux mainly reflects washout of the isotope from the extracellular space and has been ignored in this study. However, to prevent re-accumulation of significant amounts of ^{45}Ca, glands were transferred through 3 vials during this period (20, 30 and 20 min per vial, respectively). Thereafter they were transferred at 10 min intervals through the remaining 7 vials which either did or did not contain pancreatic polypeptide. After two such 10 min periods (which acted on controls), caerulein, CCK-PZ or acetylcholine were added in 20 μl distilled water to the remaining 5 vials immediately before introducing the gland. At all times, glands were transferred by means of hooks made from fine polyethylene tubing. After removal from the final vial, each gland was blotted gently, weighed and dissolved in Soluene-350 (Packard) at 50°C.

Aliquots of 0.5 ml were removed from each incubation vial and stored at 4°C for subsequent amylase assay. To the remaining 1.5 ml of incubation medium, and to each of the dissolved glands, was added 10 ml of a toluene:triton scintillation fluid.

Perfused cat pancreas

Glands were surgically isolated from nine cats weighing 1.4 to 2.0 kg which had been denied food overnight. Following isolation, each gland was transferred to an organ bath maintained at 37°C. Perfusion fluid was led by means of a roller-pump from a resevoir, through a heat-exchange coil and infused into the gland's arterial supply via the coeliac artery. The effluent was drained to waste through the superior mesenteric vein after occlusion of the portal tract. The perfusion fluid had the same composition as that in which the rat glands were incubated, except that it was not supplemented with albumin, and the concentration of NaCl was 120 $mmol.l^{-1}$.

The protocol of a typical experiment is illustrated in Fig. 4. Pancreatic fluid and electrolyte secretion was stimulated throughout by the continual infusion of submaximal doses of secretin (0.006 to 0.03 C.U. min^{-1}). Pancreatic enzyme secretion was stimulated by injection or infusion of CCK-PZ. Super-imposed on this stimulation with secretin and CCK-PZ, pancreatic polypeptide was infused in a variety of concentrations. Pancreatic juice was collected at 4°C to await assay for amylase. The volume of secretion and output of amylase are expressed in table 1 as a % of the mean of the control responses before and after the test response.

Counting and analyses

^{45}Ca activiy was determined in a liquid scintillation counter (Beckman, LS 230). The efficiency of counting in Soluene-350 was approximately equal to that in the incubation medium. Efflux of ^{45}Ca from the glands is expressed as a rate coefficient (the fraction of ^{45}Ca lost from the gland per min), calculated as described by Clausen (1969).

Amylase activity in the incubation medium and pancreatic juice was measured using the method of Bernfeld (1955), as modified by Case & Clausen (1973).

The significance of difference between means was determined using Student's t test. The level of significance was set at $P < 0.05$.

Materials

All chemicals used were of analytical grade. Bovine serum albumin (Sigma) was dialysed against distilled water for 24 hr before use. Acetylcholine chloride and caerulein were purchased from B.D.H. and Sigma, respectively. Pure, natural CCK-PZ was a gift from Professor V Mutt (Karolinska Institute, Stockholm, Sweden). Synthetic secretin was donated by Professor T Scratcherd (Department of Physiology, Sheffield University, U.K.) from a gift to him by Professor H Beyerman. Bovine pancreatic polypeptide was a gift from Dr T Schwartz (Institute of Medical Biochemistry, Aarhus University, Denmark).

RESULTS

1. Effects of pancreatic polypeptide on incubated rat pancreas

Stimulation of the incubated uncinate pancreas with CCK-PZ at a concentration of 10^{-9}M caused the expected increase in amylase release and ^{45}Ca efflux. Under basal or stimulated conditions, the rate of amylase release and ^{45}Ca efflux were not significantly altered by the presence of bovine pancreatic polypeptide at a concentration of 10^{-8} $mol.l^{-1}$ (Fig. 1).

Stimulation with a higher concentration of CCK-PZ (10^{-8} $mol.l^{-1}$) caused a more marked rise in amylase secretion and ^{45}Ca efflux (Fig. 2). Although the presence of pancreatic polypeptide at various concentrations (10^{-10}, 10^{-8} or 10^{-6} $mol.l^{-1}$) appeared to inhibit amylase secretion in a dose-related manner, there was no significant difference from control at any dose.

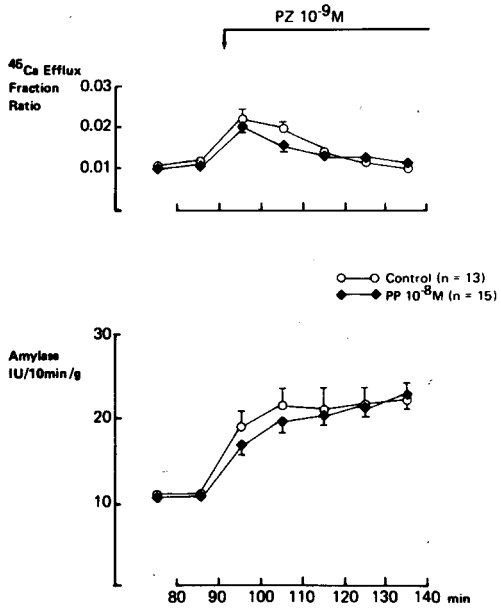

Fig. 1. Effect of bovine pancreatic polypeptide (PP) (10^{-8} mol.l^{-1}) on the release of ^{45}Ca (above) and amylase (below) from the incubated, uncinate pancreas of baby rats, evoked by CCK-PZ (10^{-9} mol.l^{-1}). Glands were loaded *in vitro* for 1 hour with ^{45}Ca and then transferred through a series of vials containing standard incubation medium without (○ n=13) or with(♦ n=15) PP, to which CCK-PZ was added for the duration of the horizontal bars. The error bars represent the S.E.M. There was no significant difference between any points.

Under similar conditions, pancreatic polypeptide (10^{-8} mol.l^{-1})was also without effect against high (10^{-8} mol.l^{-1}) or low (10^{-10} mol.l^{-1}) concentration of caerulein (data not illustrated).

Acetylcholine evoked similar responses to CCK-PZ. At a concentration of 10^{-5} mol.l^{-1} it caused even more marked rises in amylase release and ^{45}Ca efflux than CCK-PZ or caerulein (Fig. 3). At two points (in the case of ^{45}Ca efflux) and one point (in the case of amylase release) pancreatic polypeptide apparently augmented the response to this dose of acetylcholine.

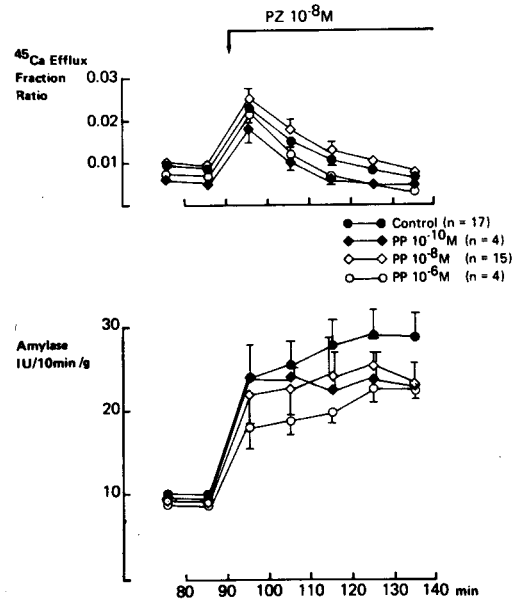

Fig. 2. Effect of bovine pancreatic polypeptide (PP) at concentrations of 10^{-10} mol.l^{-1} (♦), 10^{-8} mol.l^{-1} (◇) and 10^{-6} mol. l^{-1} (○) on the release of ^{45}Ca (above) and amylase (below) evoked by CCK-PZ (10^{-8} mol. l^{-1}). Experimental protocol as for Fig. 1. There was no statistical difference between any points.

Table 1. The effect of bovine pancreatic polypeptide (PP) on secretory response in the perfused cat pancreas

	PP dose (mol/l)	n	Volume (% Control)	Amylase (% Control)
CCK-PZ	10^{-11} moles injection			
	10^{-10}	3	112±7	118±32
	10^{-8}	5	106±8	90±6
CCK-PZ	5×10^{-11} moles injection			
	10^{-8}	2	98	132
CCK-PZ	2×10^{-12} moles/min infusion			
	10^{-10}	2	141	118
	10^{-8}	2	133	97

This table summarises the effect of PP (10^{-10} and 10^{-8} mol.l^{-1}) on nine glands in which electrolyte secretion was sustained by sub-maximal stimulation with secretin (0.006 to 0.03 CU min^{-1}) and amylase release evoked by submaximal injection or infusion of CCK-PZ at the doses indicated. The volume of secretion and output of amylase are expressed on a % of the mean of the central responses before and after exposure to PP.

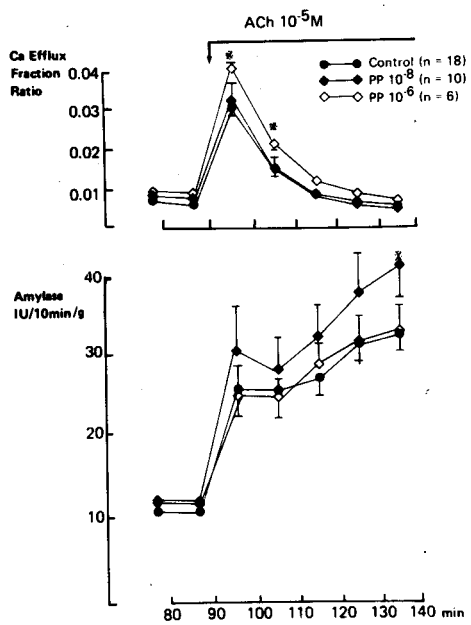

Fig. 3. Effect of bovine pancreatic polypeptide (PP) at concentrations of 10^{-8} mol.l^{-1} (♦) and 10^{-6} mol.l^{-1} (◇) on the release of ^{45}Ca (above) and amylase (below) evoked by a maximal dose of acetylcholine (10^{-5} mol.l^{-1}). Experimental protocol as for Fig. 1. The asterisks indicate those points which are significantly different from control (●).

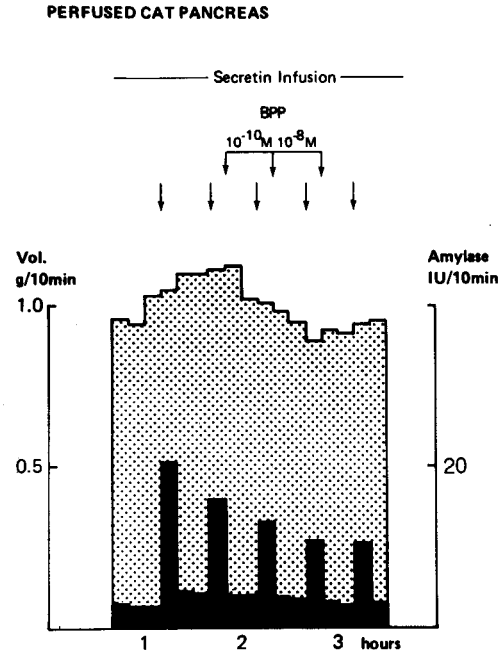

Fig. 4. The effect of bovine pancreatic polypeptide (PP) (10^{-10} and 10^{-8} mol.l^{-1}) on the secretion of electrolytes and amylase from an isolated, perfused preparation of the cat pancreas. Electrolyte secretion was evoked by the continual infusion of synthetic secretion (0.006 C.U. min^{-1}) and amylase release by single injections of 10^{-11} moles of pure, natural CCK-PZ at the times indicated by the arrows. PP was added to the perfusion fluid at the concentrations indicated, for the duration of the horizontal bars (i.e. 30 min at each dose).

2. Effects of pancreatic polypeptide on perfused cat pancreas.

An example of the effects of pancreatic polypeptide (10^{-10} and 10^{-8} mol.l^{-1}) on the perfused cat pancreas stimulated with secretin and CCK-PZ is shown in Fig. 4. The results obtained from 9 such experiments are summarised in Table 1.

Neither concentration of pancreatic polypeptide showed consistent effects on pancreatic electrolyte secretion evoked by secretin or enzyme secretion evoked by CCK-PZ.

DISCUSSION

Despite the accumulation of information about the distribution of pancreatic polypeptide and the regulation of its secretion (see Introduction), little is known of its physiological functions. Among a variety of reputed actions, inhibition of pancreatic enzyme and electrolyte secretion has been observed in dogs, using bovine (Lin *et al*, 1977) or porcine (Taylor *et al*, 1979) pancreatic polypeptide, and in man (Adrian *et al*, 1979) and calves (Davicco *et al*, 1979) using bovine pancreatic polypeptide. However, avian pancreatic polypeptide is without effect on chicken pancreatic secretion (Kimmel *et al*, 1978). Inhibition of canine pancreatic secretion

by porcine pancreatic polypeptide occurs at doses less than those observed in the plasma after a meal, and has therefore been suggested as a potential physiological function of this peptide (Taylor *et al*, 1979).

In the present experiments on *in vitro* preparations of rat and cat pancreas, no inhibitory effects of bovine pancreatic polypeptide were observed on basal amylase release, on stimulated amylase release evoked by CCK-PZ, caerulein or acetylcholine, or on electrolyte secretion evoked by secretin. Accelerated ^{45}Ca efflux, which is also evoked by these latter stimuli, was likewise not reduced. If anything, there was a tendency for pancreatic polypeptide to increase enzyme release and ^{45}Ca efflux from rat pancreas during acetylcholine stimulation.

There are two possible explanations for this lack of effects. Either bovine pancreatic polypeptide is not active in the cat and the rat, or pancreatic polypeptide has no direct effect on pancreatic secretory processes and the inhibitory effects previously observed *in vivo* are indirect and caused by the release of an inhibitor, or by alterations in blood flow etc.

The first possibility seems unlikely in view of the many effects of bovine pancreatic polypeptide in species other than cattle, including effects on isolated rat liver cells (Schwartz *et al*, 1980). We therefore conclude that pancreatic polypeptide has little or no effects on the cellular mechanisms responsible for pancreatic enzyme discharge and electrolyte transport; and further that the release of pancreatic polypeptide from the gland during *in vitro* experiments is unlikely to influence the secretory response to gastrointestinal hormones or cholinergic stimuli. Of course, this does not preclude a direct action of pancreatic polypeptide on some other, perhaps metabolic, aspect of pancreatic function.

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