Inhibition of secretin stimulated pancreatic secretion by pancreatic polypeptide

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SUMMARY The effect of PP on secretin-stimulated pancreatic secretion was assessed in five healthy subjects. During an intravenous infusion of BPP at a dose which produced plasma levels similar to those seen after meals in healthy young adults the volume and bicarbonate content of duodenal juice was reduced by 25% ($P < 0.05$) and 24% ($P < 0.05$) respectively, while protein and bilirubin concentrations were more markedly reduced by 68% ($P < 0.0005$) and 67% ($P < 0.0005$) respectively. PP, thus, may be an important inhibitory factor in the control of bilirubin and pancreatic enzyme secretion in man.

Pancreatic polypeptide (PP) is a recently characterised 36 amino acid peptide from the endocrine pancreas discovered during the investigation of minor impurities in insulin preparations (Lin and Chance 1972). PP circulates in plasma and the postprandial rise is of the same order of magnitude as that of insulin (Adrian *et al.*, 1976; Floyd *et al.*, 1976; Schwartz *et al.*, 1976). Studies have shown that the mechanism of PP release shows many similarities to that of pancreatic enzymes (Schwartz *et al.*, 1976; Adrian *et al.*, 1977).

The physiological role of PP is unknown but among its many biological actions bovine pancreatic polypeptide (BPP) causes marked effects on the exocrine pancreas (Lin *et al.*, 1977). When infused into dogs against a background secretin infusion, BPP at doses of 1-10 $\mu\text{g/kg/h}$ inhibited protein secretion markedly and was accompanied by a biphasic response in water and bicarbonate secretion, first stimulating and then inhibiting the flow of alkaline juice. No comparable studies have been reported in man and in order to determine whether PP could be of any importance in the control of pancreatic and biliary secretion in man the effect of BPP during secretin stimulation has been observed in healthy subjects.

Methods

Five healthy volunteers aged between 24-54 years (mean 40 years) were studied. Fully informed consent

was obtained in each case. During each PP infusion pulse rate, blood pressure, body temperature, and ECG were monitored and blood was taken for routine haematological and biochemical tests (blood urea, electrolytes, liver function tests, calcium, and phosphate). No objective or subjective side-effects were noted and all screening measurements remained unchanged.

After a 12 hour fast the subjects were intubated under fluoroscopic control: a double lumen tube was positioned in the second part of the duodenum and a nasogastric tube in the antrum. Gastric and duodenal juice was aspirated continuously and the latter kept on ice and divided into 10 minute samples. After the resting duodenal juice had been removed, two 10 minute basal samples were collected. Secretin (GIH 0.15 CU/kg/h) was infused for three hours into an antecubital vein using a syringe ram pump *via* an indwelling intravenous catheter. During the middle hour pure natural BPP was added to the infusate at 200 pmol/kg/h in human serum albumin (200 $\mu\text{mol/l}$) (Blood Products Laboratory, Lister Institute, Elstree, Herts). At the end of the three hour secretin infusion each subject received a standard 20 minute infusion of secretin (Boots, 2 Crick Harper Raper units/kg/h) and pancreozymin (Boots, 2 Crick Harper Raper units/kg/h) to ensure that the pancreas was secreting normally.

Blood samples were taken every 10 minutes throughout the entire experiment from a second catheter placed in an antecubital vein of the opposite arm to the infusion catheter. The rise of plasma PP and the concentration in the infusate were

measured using a radioimmunoassay, as previously described (Adrian *et al.*, 1976) but using standards prepared from pure BPP and antibody to BPP at a dilution of 1.6×10^6 . The assay could detect changes of 5 pmol BPP per litre plasma with 95% confidence. Plasma secretin was measured using a specific and sensitive radioimmunoassay (Häcki *et al.*, 1977).

Duodenal juice bicarbonate concentration was measured by the addition of hydrochloric acid, boiling, and back titration with 0.1M sodium hydroxide. Protein concentration was measured by the method of Lowry *et al.* (1951), trypsin by the method of Haverback *et al.* (1963), and bilirubin by the method of Malloy and Evelyn (1937).

Results

The mean basal plasma secretin level was 0.7 ± 0.2 pmol/l and rose to 9.3 ± 1.1 pmol/l during the secretin infusion, and was unaffected by the PP infusion. The mean basal plasma PP concentration was 22.5 ± 3.6 pmol/l and was unchanged during the secretin infusion (23.8 ± 2.9 at 60 minutes). The mean dose of PP as measured in the infusion fluid was 202 ± 13 pmol/kg/h and the mean plasma PP concentration at plateau was 711 ± 69 pmol/l. The mean volume and bicarbonate output of the recovered duodenal juice is shown in Fig. 1 and the mean bilirubin, protein, and trypsin output

Fig. 1 Effect of BPP on water and bicarbonate output during secretin stimulation. PP is added to secretin during the middle hour of a three hour infusion.

Fig. 2 Effect of BPP on total protein, trypsin, and bilirubin output during the middle hour of a three hour secretin infusion.

are shown in Fig. 2. Because of the skewed nature of this data it was normalised by expressing the half hourly output of each parameter as a percentage of the 30 minute period immediately before the PP period for each individual (Table 1). Statistical analysis was then carried out using paired *t* tests; the results are summarised in Table 2. The bicarbonate concentration of duodenal juice reached a plateau by the fourth 10 minute period of the secretin infusion and remained steady at approximately 140 mmol/l thereafter. The change in bicarbonate output during the PP infusion therefore reflects the change in the volume of duodenal juice. Enzyme output measured both by specific tryptic activity and also by total protein concentration was significantly reduced during the PP infusion and did not return to the pre-PP levels when the PP was stopped. Bilirubin output was reduced markedly and juice collected during the second half of the PP infusion was always colourless. In three subjects pigment did not return until 40-60 minutes after the end of the PP period and in the other two subjects pigment was seen in the post PP hour. The response to the infusion of Boots' pancreozymin and secretin at the end of the experiment was substantial for this stimulus. In all subjects in the second 10 minute period mean volume had risen to 46 ± 5 ml, bi-

Table 1 *Change in output during and after PP infusion expressed as percent of pre-PP infusion 30 minute period (mean $\pm$ SEM)*

Percent output	Volume	Bicarbonate	Protein	Trypsin	Bilirubin
During first half hour of PP infusion	86 ± 6	86 ± 7	59 ± 6	59 ± 7	56 ± 14
<i>P</i> <	NS	NS	0.005	0.005	0.05
During second half hour of PP infusion	75 ± 6	76 ± 7	32 ± 7	41 ± 6	34 ± 12
<i>P</i> <	0.02	0.05	0.001	0.001	0.01
During first half hour recovery period	105 ± 9	105 ± 13	61 ± 9	68 ± 13	41 ± 14
<i>P</i> <	NS	NS	0.01	NS	0.02
During second half hour recovery period	92 ± 9	95 ± 11	59 ± 8	66 ± 14	52 ± 17
<i>P</i> <	NS	NS	0.01	NS	NS

carbonate output to 5.73 ± 0.86 mmol/10 min, protein to 266 ± 116 mg/10 min, trypsin to 358 ± 122 units/10 min, and bilirubin to 4.2 ± 1.3 pmol/10 min.

Table 2 *Percentage change of duodenal juice output in individual subjects during PP infusion*

Subject	(i)	(ii)	(iii)	(iv)	(v)	Mean $\pm$ SEM
Volume						
A	100	100	100	100	100	86 ± 6
B	78	82	73	109	88	75 ± 6
C	59	67	82	95	72	105 ± 9
D	108	82	85	123	126	92 ± 9
Bicarbonate						
A	100	100	100	100	100	86 ± 7
B	79	75	80	115	79	76 ± 7
C	65	62	87	96	68	105 ± 13
D	96	73	86	139	130	95 ± 11
Protein						
A	100	100	100	100	100	59 ± 6
B	64	41	79	54	55	32 ± 7
C	26	32	60	20	24	61 ± 9
D	54	67	77	30	75	59 ± 8
Trypsin						
A	100	100	100	100	100	59 ± 7
B	77	76	50	43	47	41 ± 6
C	46	59	47	25	30	68 ± 13
D	94	71	96	27	52	66 ± 14
Bilirubin						
A	100	100	100	100	100	56 ± 14
B	9	82	53	51	86	34 ± 12
C	9	38	79	16	26	41 ± 14
D	19	37	93	18	38	52 ± 17
	30	36	109	11	74	

Percent output: A = during first half-hour of PP infusion; B = during second half-hour of PP infusion; C = during first half-hour recovery period; D = during second half-hour recovery period.

Discussion

The mean plasma PP concentration achieved during BPP infusions was approximately twice the mean concentration seen half an hour after lunch in healthy young adult subjects and may therefore be considered to be just within the physiological range. Species differences need to be considered as there are three amino acid sequence differences between BPP and HPP (Chance, 1974). This gives rise to slight but detectable differences in affinity between the antibody to one species and the heterologous antigen. At present, however, there is insufficient HPP available to undertake infusion studies.

BPP had a marked inhibitory action on pancreatic secretion reducing volume, enzyme activity, and total protein concentration. On the other hand, PP appeared to have no effect on bicarbonate concentration. In the dog, total water and bicarbonate responses to BPP were reported to parallel each other, as in this study in man, but occasionally a biphasic response, with augmentation followed by inhibition, was observed (Lin *et al.*, 1977). This may reflect either a dose or a species difference. The slow recovery of protein secretion after inhibition by PP was also observed in the dog studies. Similarly, the protein concentration did not return to pre-PP levels until three-quarters to one hour after PP infusion ceased (Lin *et al.*, 1977). As in our experiments the infusion was not continued after three hours, we were unable to ascertain when a full return to pre-PP levels occurred. During the PP infusions reported here the duodenal juice became colourless and the bilirubin content was dramatically reduced, perhaps reflecting increased gall bladder storage of bile, an effect also observed in the dog (Lin *et al.*, 1977). However, a change in biliary secretion rate cannot be excluded by this study.

PP has been found in considerable quantities in the alimentary system of all mammals examined and is also present in birds. It circulates in high concentration and rises rapidly after food. Nothing, however, is so far known of its physiological role and the experiments reported here are the first attempt to examine this in man. It is clear that PP can significantly influence pancreatic and biliary function. However, it is remarkable that a peptide

which rises in the blood after food appears to inhibit pancreatic enzyme and bilirubin output. This is exactly opposite to the actions of cholecystokinin-pancreozymin. Further studies to explain this anomalous finding and to illuminate the possible role of PP in disorders of digestion are now required.

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