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Pancreatic polypeptide reduces appetite and food intake in humans

R.L. BATTERHAM, C.W. LE ROUX, M.A. COHEN, A.J. PARK, S.M. ELLIS, M. PATTERSON, G.S. FROST, M.A. GHATEI AND S.R. BLOOM

Department of Metabolic Medicine, Imperial College Faculty of Medicine at Hammersmith Campus, Du Cane Rd, London W12 0NN, UK

ABSTRACT: Pancreatic polypeptide (PP) is a gut hormone released from the pancreas in response to ingestion of food. Plasma PP has been shown to be reduced in conditions associated with increased food intake and elevated in anorexia nervosa. In addition peripheral administration of PP has been shown to decrease food intake in rodents. These findings suggest that PP may act as a circulating factor that regulates food intake. Therefore we investigated the effect of intravenous infusion of PP (10 pmol/kg/min) on appetite and food intake in a randomised double-blind placebo-controlled crossover study in ten healthy volunteers. Infusion of PP reduced appetite and decreased the energy intake at a buffet lunch two hours post-infusion by 21.8 ± 5.7% ($P < 0.01$). More importantly the inhibition of food intake was sustained, such that energy intake, as assessed by food diaries, was significantly reduced both the evening of the study and the following morning. Overall PP infusion reduced cumulative 24-hour energy intake by 25.3 ± 5.8%. In conclusion our data demonstrates that PP causes a sustained decrease in both appetite and food intake.

Introduction

Circulating hormones and nutrients convey information about nutritional status to the brain pathways that regulate appetite and food intake. Physiological signals that regulate appetite have been broadly divided into long-term and short-term; long-term regulators, such as leptin, are thought to signal body energy stores and regulate body weight over weeks and months, whereas short-term regulators signal food intake and regulate appetite on a day to day basis (1). Recently, two peptide hormones, ghrelin (2) and peptide YY (PYY) (3), which are released from the digestive tract, have been linked to the short-term regulation of food intake. PYY and PP are structurally related peptides and PP is thought to have arisen by gene duplication of the PYY gene (4). In response to ingestion of food PP is released from PP-cells of the pancreatic islets in proportion to the calories ingested and levels remain elevated for up to 6 hours postprandially (5). Low levels of PP have been found in obese subjects (6,7) and high levels in patients with anorexia nervosa (8,9). Furthermore peripheral administration of PP to rodents has been shown to reduce food intake (10). These findings suggest that PP may play a role in the regulation of appetite. We therefore investigated the effects of PP infusion on appetite and food intake in man.

Methods

Subjects: Ten non-obese volunteers (six females and four males), aged 18-30 years (mean ± SEM, 25.1 ± 1.0) with body mass index 18.7-23.4 (21.1 ± 0.6) kg/m^2 were recruited. Subjects gave written informed consent for the studies and ethical approval was obtained from the Hammersmith Hospital Research Ethics Committee (2002/6274). The study was carried out in accordance with the principles of the Declaration of Helsinki. Criteria for exclusion included smoking, substance abuse, pregnancy, medication (except for the oral contraceptive pill), medical or psychiatric illness and any abnormalities detected on physical examination, electrocardiogram or screening blood tests (full blood count, electrolytes, fasting glucose and liver function tests). Subjects were screened by a dietician, which included assessment of their eating behaviour using the Dutch Eating Behaviour Questionnaire (11) and the Eating Attitudes Test questionnaire (12). They also completed a 3-day diet diary to assess their usual eating habits prior to acceptance into the study. Food preferences were assessed at screening using a 9 point hedonic scale to ensure that food offered at the buffet lunch was acceptable.

Protocol: The study was performed in a randomised double-blind placebo-controlled crossover manner with each subject studied on two occasions one week apart. All female subjects were studied during the follicular phase of their menstrual cycle. The subjects' food intake for the 48 hours prior to each study day was standardised and during this period they completed food diaries to confirm compliance. In addition they consumed an identical meal between 19:00 and 20:00 on the night prior to each study. Subjects refrained from alcohol and

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strenuous exercise for the 24 hours before and after each study day. Subjects fasted from 20:00 the night prior to the study and drank only water. They arrived at 08:30 on each study day. Cannulae were inserted into veins in both forearms; one for collection of blood and the other for the infusion of PP or saline. Following venous cannulation subjects relaxed for 30 minutes before the onset of the study protocol. All time cues were removed from the study room so that subjects were unaware of the time. Throughout the study subjects were encouraged to relax by watching videos. Blood was collected every 30 minutes into heparin-coated tubes (LIP Ltd, UK) containing 5,000 kallikrein inhibitor units (0.2 ml) of aprotonin (Bayer, Haywards Heath, UK). Plasma was separated immediately by centrifugation at 4 C and then stored at -70 C until analysed. Basal samples were taken at -30 and 0 minutes (t_{-30} and t_0). Subjects were infused for 90 minutes (t_0 to t_{90}) with either saline or PP (10 pmol/kg/min for 90 minutes). Two hours (t_{210}) after the termination of the infusion, subjects were offered a buffet lunch in excess, such that all appetites could be satisfied (Figure 1). Food and water were quantified pre- and postprandially and energy intake calculated.

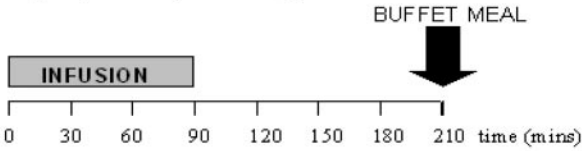

Figure 1: Protocol for the investigation of the effect of saline or PP (10 pmol/kg/min) on appetite and food intake.

Hunger and nausea ratings were made on 100 mm visual analogue scores (VAS) with the text expressing the most positive and the negative rating at each end (13). Subjects remained in the study room until t_{360}. They continued to complete VAS until 09:00 and their food diary until 13:00 the following day to allow continued assessment of appetite and food intake. Food diaries were analysed by a dietician, blinded to the study and energy intake was calculated with the aid of Dietplan (Forestfield Software Ltd, West Sussex, UK).

Materials: Human PP was obtained from Bachem (Merseyside, UK). The limulus amoebocyte lysate assay test for pyrogen was negative and the peptide was sterile on culture. PP was dissolved in 0.9% saline (Bayer, Haywards Heath, UK.) containing Haemaccel (Beacon, Kent, UK) (5% by volume) to reduce adsorption to the syringe and tubing.

Assays: All samples were assayed in one assay eliminating the effects of inter-assay variation. Plasma PP (5), PYY (14), insulin and glucagon-like-peptide 1 (GLP-1) (15) were measured in duplicate using established in-house radioimmunoassay. Plasma ghrelin and leptin were measured in duplicate using the Phoenix Pharmaceutical (Belmont, CA, USA) assay kit and the Linco Research (Missouri, USA) assay kits respectively.

Statistical analysis: Plasma hormone concentrations are expressed as mean ± SEM. Comparisons of food consumption between PP and saline groups were by paired t-test. VAS were compared using Wilcoxin signed-rank test. P-values less than 0.05 were regarded as significant.

Results

Infusion of PP resulted in an increase in plasma PP from a mean basal of 15.5 ± 1.4 pmol/l to 258.5 ± 21.4 pmol/l (Figure 1). After termination of the infusion PP levels returned to baseline by t_{210}.

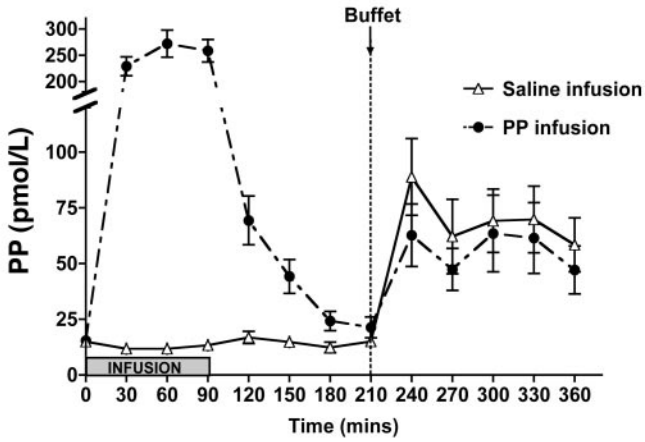

Figure 2. Plasma PP levels during infusion of saline (open triangles) or PP infusion (filled circles), buffet lunch at t_{210}. n = 10.

Postprandially plasma PP levels increased on both study days reaching a peak at t_{240} (PP levels t_{240}; saline infusion day = 88.9 ± 17.2 pmol/l, PP infusion day = 62.7 ± 16.7 pmol/l). PP plasma levels remained elevated at t_{360}.

Infusion of PP caused a significant decrease in hunger as assessed by VAS (Figure 2) which persisted once the infusion had terminated (Figure 2). Hunger scores fell postprandially in both groups. However, on the day of PP infusion subjects continued to feel less hungry, despite consuming fewer calories at the buffet lunch, for up to 9 hours post-infusion. There was no effect on VAS for nausea (data not shown).

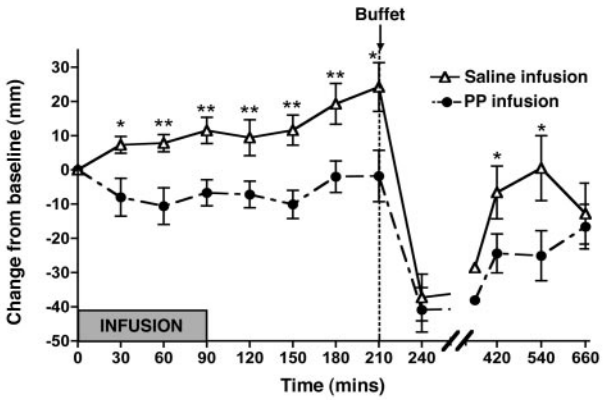

Figure 3. Appetite scores (relative scale). Visual analogue scales for perceived hunger during and after infusions. Buffet lunch offered at t_{210} (dotted line). The results are presented as change from baseline scores and are the mean (± SEM) for all 10 subjects * P<0.05; ** P< 0.01.

PP infusion reduced energy intake during the buffet lunch offered 2 hours post-infusion by 21.8 ± 5.7% compared with saline (Figure 3a). Analysis of subjects' food diaries revealed that PP significantly reduced energy intake in the 12-hour period after the buffet lunch (17.1 ± 7.6%) compared with saline control (Figure 3b). Furthermore, energy intake during the 12 to 24-hour period after the buffet was reduced 33.4 ± 15.4% compared with saline (Figure 3c). Overall PP infusion significantly reduced cumulative 24-hour energy intake by 25.3 ± 5.8% (Figure 3d). There was no change in the proportion of calories obtained from carbohydrate, fat or protein and no effect on fluid intake (data not shown).

There was no significant effect of PP infusion on plasma levels of insulin, ghrelin, PYY, GLP-1 or leptin (Table 1).

Discussion

PP has been shown to decrease food intake in rodents and transgenic mice over-expressing PP have reduced body weight and adiposity. We now demonstrate that PP infusion reduces both appetite and food intake in normal humans. More importantly the inhibition of food intake was sustained, such that energy intake as assessed by food diaries was significantly reduced both the evening of the study and the following morning. The PP concentrations achieved during the PP infusion were three times higher than the postprandial levels produced in the same subjects by the buffet lunch and this may contribute to the sustained inhibition of food intake that we observed.

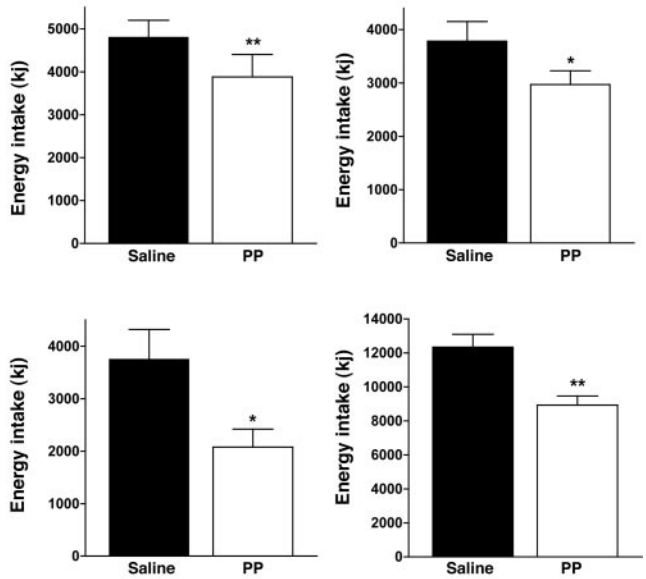

Figure 4. Mean energy intake from a) buffet lunch, b) 12-hr period post-buffet, c) 12 to 24-hr period post-buffet and d) total cumulative 24-hr, n = 10, * P<0.05; ** P < 0.01.

Interestingly children with Prader-Willi syndrome, which is characterised by marked hyperphagia and obesity, have low levels of PP. Moreover infusion of bovine PP to these children reduced food intake (16).

The mechanisms by which PP reduces food intake have not been established. PP is thought to act via the neuropeptide Y4 receptor, which was initially called the PP receptor due to its high affinity for PP. Y4 receptors are expressed within the brainstem and hypothalamus, key brain areas involved in the regulation of appetite and accessible to the peripheral circulation (17). However, the identity of the neuronal populations which express the Y4 receptors and the mechanisms underlying the anorectic actions of PP remains to be determined. There are conflicting reports of the effect of PP on gastric emptying with both increased (18) and decreased effects reported (19). However, we have previously shown that infusion of bovine PP, which differs from human PP by only 2 amino acids, had no effect on gastric emptying (20). Furthermore, in this study the decrease in hunger scores preceded any food intake, suggesting that this was not due to delayed gastric emptying.

PP infusion had no significant effect on plasma concentrations of ghrelin, PYY, GLP-1, leptin or insulin suggesting that its anorectic effect are independent of changes in these hormones.

	Saline infusion			PP infusion		
	Time (mins)			Time (mins)		
	0	90	210	0	90	210
PP (pmol/L)	15.0 ± 1.5	13.4 ± 1.8	15.1 ± 1.4	15.5 ± 1.4	258.5 ± 21.4***	21.4 ± 4.7
PYY (pmol/L)	17.6 ± 1.6	18.1 ± 1.4	16.0 ± 1.5	16.9 ± 1.6	17.7 ± 2.2	16.0 ± 1.1
Insulin (pmol/L)	41.9 ± 4.1	40.4 ± 3.7	31.5 ± 2.2	44.4 ± 3.8	38.8 ± 4.2	32.8 ± 2.5
GLP-1 (pmol/L)	12.7 ± 1.6	10.6 ± 0.9	11.3 ± 1.4	14.5 ± 2.1	11.1 ± 0.8	10.3 ± 0.9
Ghrelin (pmol/L)	164.9 ± 32.0	160 ± 31.6	182 ± 35.4	161.7 ± 33.7	163.2 ± 34.6	188.3 ± 36.6
Leptin (nmol/L)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1

Table 1: Mean (± SEM) plasma hormone for all 10 subjects at baseline t_0, end of infusion t_{90} and prior to meal t_{210}, *** P< 0.001

In response to food ingestion plasma levels of cholecystokinin (CCK), GLP-1, PYY and PP increase within 15 minutes. Postprandially the plasma levels of CCK and GLP-1 return to baseline within a couple of hours. However, PYY and PP remain elevated for up to 6 hours postprandially suggesting that PYY and PP may regulate meal to meal intervals.

In conclusion we have shown that PP reduces appetite and decreases cumulative 24-hour energy intake by 25%, without adverse effects. Further studies are now required to establish both the mechanism of action of PP and its potential as a treatment for obesity.

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