Jejunal bypass stimulation of pancreatic growth and cholecystokinin secretion in rats: importance of luminal nutrients

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SUMMARY The effect of jejunal bypass on pancreatic growth and plasma cholecystokinin (CCK) was investigated in rats. Rats underwent bypass of jejunum or sham operation. Rats with jejunal bypass were further divided into three groups; one group received a continuous infusion of a partially hydrolysed liquid diet (Vital) into the bypassed jejunum; a second group received the nutrient solution mixed with trypsin and infused into the bypassed jejunum; the third bypass group did not receive infusion of nutrient or trypsin into the jejunum. Jejunal bypass alone did not significantly stimulate pancreatic growth or DNA content at one or two weeks postoperative. Infusion of nutrient solution into the bypassed jejunum stimulated pancreatic growth and DNA content, with maximal increases of 185% and 181% for pancreatic weight and DNA content, respectively, at two weeks. This coincided with significant increases in postabsorptive plasma CCK concentrations. Infusion of pancreatic proteases into the bypassed jejunum partially reversed the effects of nutrient infusion. These results suggest that exclusion of bile-pancreatic juice or pancreatic proteases from the jejunum does not lead to maximal release of CCK unless the jejunum receives luminal nutrients. It is proposed that CCK release from rat jejunum occurs spontaneously in the absence of pancreatic proteases, and that luminal nutrients in bypassed jejunum increase plasma CCK and stimulate pancreatic growth by maintaining synthesis of CCK.

Exclusion of bile-pancreatic juice (BPJ) from the proximal small intestine stimulates pancreatic secretion and growth,¹ and increases plasma cholecystokinin (CCK) in the rat.^{2,3} These phenomena are attributed to loss of feedback inhibition of CCK release by luminal pancreatic proteases.^{4,5} Transposition of the duodenum to lie between the jejunum and ileum also stimulates pancreatic growth in the rat.⁶ We suggested that this was caused by the consequent diversion of BPJ from the jejunum to the ileum, resulting in loss of protease induced inhibition of CCK release from the jejunum.¹ The results of this latter study⁶ indicated that the jejunum may be the major site of feedback control of CCK release in the proximal small intestine in the rat, because in that model BPJ was diverted from the jejunum, but not

from the duodenum. This line of reasoning led us to conclude that jejunal bypass should cause increased CCK release and pancreatic hypertrophy, since jejunal bypass excludes BPJ from the jejunum. Jejunal bypass also excludes gastric juice and food from the jejunum, and this might be expected to affect CCK release since CCK release in dogs and humans is stimulated by products of protein and fat digestion.⁷ Removal of pancreatic protease activity in the rat, however, appears to result in spontaneous release of CCK. This is based on results of experiments showing that diversion of pancreatic juice or inhibition of pancreatic proteases stimulates pancreatic secretion and CCK release in fasted rats,^{8,9} and stimulates pancreatic secretion in rats with pyloric ligation¹⁰ – that is, such stimulation occurs in the absence of luminal nutrients or gastric juice. Therefore, we hypothesised that jejunal bypass would result in spontaneous release of CCK from the bypassed segment, stimulating pancreatic growth.

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Fig. 1 Surgical anatomy. SC = sham-operated controls; JB = jejunal bypass; JB+N = jejunal bypass with nutrient (Vital) infusion into bypassed segment; JB+NT = jejunal bypass with nutrients and trypsin (pancreas protease concentrate) infused into bypassed segment. Cannulae in groups SC and JB deliver nutrient solution into duodenum, equivalent to volume infused into jejunum in groups JB+N and JB+NT.

Methods

SURGERY

Jejunal bypass or sham operation was performed in male Wistar rats (Charles River) weighing 300–400 g (mean = 330 g). Rats were anaesthetised with Na pentobarbital (Nembutal). The jejunum was transected at two sites, approximately 1 cm and 50 cm distal to the ligament of Treitz, and the proximal end was sutured closed. The distal end was anastomosed (end-to-side) to the colon. Gut continuity was reestablished by duodenal-ileal anastomosis (end-to-end). Sham operated controls (SC) underwent transection and reconnection of the jejunum. Silastic cannulae for intestinal infusion (0.024 × 0.036 in, ID × OD) were inserted into the proximal end of the bypassed jejunum, or into the duodenum, depending on the group (Figure). The cannulae were brought out subcutaneously to exit at the back. After surgery, rats were kept in Bollman-type restraint cages for the duration of the study (1 or 2 weeks). In preliminary studies, sham-operated controls (n = 5) or jejunal bypass (n = 6) rats were prepared without infusion cannulae and were kept unrestrained in individual wire bottom cages for one week.

FEEDING AND TISSUE COLLECTION

All rats were fed commercial rat chow (Tek Lad, Madison, WI) *ad libitum* before and after surgery.

Rats kept in restraint cages were infused *via* intestinal cannulae with a partially hydrolysed, defined formula diet (Vital, Ross Laboratories, Columbus, OH), containing 4.11% fat (medium chain triglycerides), 70% carbohydrate (hydrolysed corn starch), and nitrogen equivalent to 15.8% protein (partially hydrolysed whey, meat and soy protein). It was diluted with water (79 g to 300 ml) to contain 1 cal/ml, and was infused at 2 ml/h, 24 h/day throughout the study and up to the moment of sacrifice. Chow was also available at all times, and rats were not fasted before being killed.

Rats in preliminary experiments (unrestrained) were divided into two groups, jejunal bypass and sham-operated controls, and were analysed only for pancreas weight at one week postoperative. In the second set of experiments, rats were divided into three groups, sham-operated controls (SC), jejunal bypass (JB), and jejunal bypass plus liquid nutrient (Vital) infusion (JB+N), into the bypassed jejunal segment. These animals were killed after one week. To ensure equivalent nutrient intake among the groups, Vital was also infused in the other two groups (SC and JB), but into the duodenum as indicated in the Figure. These rats were analysed for pancreas weight, DNA and protein content. In the third set of experiments, rats were set up as in the second set plus an additional group in which Vital was mixed with crude pancreatic protease extract and infused into the bypassed jejunum. The protease extract (Trypsin 1:300, Sigma Chemical Co, St. Louis, MO) was mixed at 25 mg/ml of Vital, which resulted in a final concentration equivalent in activity to 1 mg/ml and 1.24 mg/ml of purified bovine trypsin and chymotrypsin, respectively. In addition to pancreatic measurements, plasma CCK was analysed in this third set of experiments.

Rats were killed between 9 am and 11 am one or two weeks after surgery. Trunk blood was collected in plastic heparin treated centrifuge tubes. Plasma was quickly separated by centrifugation for 20 min at 10 000 g and 4 °C and held at –70 °C. The pancreas was removed and freed of fat and lymph nodes, frozen and lyophilised to constant weight.

DNA AND PROTEIN DETERMINATIONS

Pancreata were analysed for DNA and protein. To assay DNA and protein the lyophilised pancreas was homogenised in 10 ml 0.6 N perchloric acid. A 3 ml sample of this homogenate was incubated for two hours at 4 °C. From this sample, DNA and protein were determined. DNA was extracted as described by Mainz *et al.*¹¹ and determined by the diphenylamine method with calf thymus DNA as standard.¹² The pellet after DNA extraction was dissolved in 4 ml 0.3 N KOH, heated at 100 °C for 20 minutes and

cooled at room temperature. Protein was determined by the method of Lowry *et al*¹³ with bovine serum albumin as standard.

CHOLECYSTOKININ ASSAY

Plasma CCK was measured by bioassay as previously described.⁹ In brief, CCK was extracted from rat plasma by adsorption onto octadecylsilylsilica (Sep-Pak) cartridges (Waters Associates, Milford, Mass.) and eluted with 1 ml ethanol and 1% trifluoroacetic acid (4:1, v/v) into incubation vials and dried under N₂. Plasma cholecystokinin in these extracts was quantified by its ability to stimulate amylase release from isolated pancreatic acini, prepared by collagenase digestion of whole rat pancreas.¹⁴ Values were compared with a standard curve of CCK-8 and results were expressed as CCK-8 equivalents (pM). The assay was sensitive to CCK concentrations as low as 1 pM and with the ability to concentrate plasma up to sixfold (by adsorbing 6 ml plasma onto a single Sep-Pak and eluting it in 1 ml) plasma CCK levels as low as 0.2 pM could be detected.

STATISTICAL ANALYSES

Results were analysed by Student's *t* test or one way

analysis of variance (ANOVA) and the Neuman-Keuls multiple comparison test, where appropriate. *p* < 0.05 was considered significant.

Results

In the preliminary experiments, there was a small, non-significant difference between groups in pancreatic dry weights (66.5 ± 4.4 v 74.2 ± 3.4 mg/100 g body weight, control v jejunal bypass animals, respectively). Final body weights were 369 ± 17 g and 319 ± 16 g for control and jejunal bypass, respectively.

Results of the second and third sets of experiments are presented in Tables 1 and 2, respectively. After one week, jejunal bypass alone (JB) did not significantly affect any pancreatic parameters (Table 1). Infusion of nutrients into the bypassed jejunum (JB + N) stimulated significant increases in pancreatic weight and protein content compared to bypass alone or sham operated controls (SC). DNA content was increased by 31%, but this was not significant by ANOVA. After two weeks, jejunal bypass alone stimulated small increases in pancreatic weight, DNA and protein content (Table 2), but these changes were not statistically significant. Infusion of nutrient

Table 1. Effect of jejunal bypass on pancreatic growth after one week

	SC† (n = 5)	JB (n = 5)	JB + N n = 7)
Body weight (BW), g.			
Initial	344 ± 14	350 ± 7.8	353 ± 8.0
Final	318 ± 6.4	310 ± 8.8	325 ± 9.0
Pancreas dry weight, mg/100 g BW†	100 ± 5.5	96.2 ± 5.6	140 ± 9.0*
Protein, mg/pancreas/100 g BW	58.0 ± 4.7	54.4 ± 4.3	73.1 ± 5.3*
DNA, mg/pancreas/100 g BW	1.74 ± 0.11	1.75 ± 0.14	2.27 ± 0.24

* Significantly different from other two groups (*p* < 0.05); † SC = Sham operated controls; JB = Jejunal bypass; JB + N = Jejunal bypass with nutrients infused into jejunum, ‡ Final body weight.

Table 2. Effect of jejunal bypass on pancreatic growth and plasma cholecystokinin levels after two weeks

	SC (n = 5)	JB (n = 10)	JB + N (n = 13)	JB + NT (n = 5)
Body weight (BW), g				
Initial	318 ± 19	315 ± 6.1	325 ± 4.5	323 ± 6.7
Final	358 ± 2.1	353 ± 7.8	338 ± 3.6	318 ± 8.2
Pancreas dry weight, mg/100g BW	94.5 ± 3.3*	113 ± 6.5 ^{ab}	175 ± 7.4 ^c	127 ± 10.8 ^b
Protein, mg/ pancreas/100 g BW	48.9 ± 3.7*	57.5 ± 5.7*	81.3 ± 5.5 ^b	60.5 ± 7.7*
DNA, mg/pancreas/100 g BW	1.59 ± 0.08*	1.67 ± 0.10*	2.87 ± 0.12 ^b	2.31 ± 0.19 ^c
Plasma CCK, pM	0.60 ± 0.09*	2.38 ± 0.48 ^b	5.51 ± 0.33 ^c	2.24 ± 0.23 ^b

Values not sharing a common superscript are significantly different from each other (*p* < 0.05). SC = Sham operated controls; JB = Jejunal bypass; JB + N = Jejunal bypass with nutrients infused into jejunum, JB + NT = Same as JB + N but with crude trypsin added to nutrients.

solution into the bypassed segment (JB + N) stimulated large and significant increases in all pancreatic parameters. Addition of pancreatic protease extract (JB + NT) tended to reverse the pancreatic changes, but pancreatic weight and DNA were still significantly increased compared to sham operated controls. Plasma CCK was significantly increased by jejunal bypass alone (JB) and was further increased by nutrient infusion into the bypassed segment (JB + N). Pancreatic protease extract (JB + NT) significantly reduced plasma CCK compared to nutrient infusion without extract, but values were still much higher than sham operated controls.

Discussion

These experiments were carried out to test the hypothesis that absence of pancreatic proteases in the isolated proximal small intestine would stimulate pancreatic growth due to spontaneous release of CCK. This hypothesis was based on the observation that removal or inhibition of pancreatic proteases stimulated pancreatic secretion in fasted rats^{8,9} and in fasted rats with pyloric ligation,¹⁰ indicating that no background stimulus, such as amino acids, fatty acids, or gastric juice in the intestine, was required to release CCK. The results of our preliminary experiments did not support this view, because exclusion of BPJ from the bypassed jejunum did not stimulate pancreatic growth. This suggested that absence of nutrients in the bypassed intestine might be important. Nutrient infusion into the bypassed jejunum significantly increased pancreatic weight and protein content after one week, and markedly increased all pancreatic parameters after two weeks. Nutrient infusion into the bypassed jejunum also greatly increased plasma CCK. Addition of pancreatic protease to the nutrient solution entering the bypassed loop significantly reduced these increases but did not completely reverse them.

It was hypothesised that replacement of luminal protease activity in the bypassed jejunum could completely reverse changes in pancreas parameters and plasma CCK, resulting in values not different from sham fed controls. We presume that this did not occur because of failure to reestablish normal levels of pancreatic protease activity in the bypassed jejunum. The rate of infusion of trypsin and chymotrypsin into the jejunal loop, in terms of activity equivalent to purified enzymes, was 2 mg/h and 2.48 mg/h, respectively, which is roughly equivalent to the normal rate of pancreatic secretion of these enzymes under basal conditions.⁸ The pancreatic proteases were, however, infused into the bypassed jejunum in absence of bile, and we have shown that normal intestinal proteolytic activity cannot be maintained in absence of bile acids.¹⁵

Jejunal bypass alone caused small increases in all three pancreatic parameters at two weeks. Although not statistically significant, these increases are consistent with significantly increased plasma CCK in this group. These results suggest that jejunal bypass alone, in spite of absence of luminal nutrients, still tends to increase pancreatic growth, and that this is caused by moderate increases in secretion of CCK. Plasma CCK concentrations were very low in controls infused intraduodenally with Vital (group SC), and are similar to those shown in rats after feeding hydrolysed protein.¹⁶

These results indicate that nutrients in the lumen are involved in feedback regulation of plasma CCK by pancreatic proteases. One interpretation of these results could be that removal of the suppressive effect of pancreatic proteases results in CCK release, but only if a positive stimulus for CCK release is also present – for example, products of protein or fat digestion. This would seem reasonable based on the well established role for luminal amino acids and fatty acids in CCK release in dogs and man.⁷ As discussed above, however, stimulation of rat pancreatic secretion or CCK release occurs simply by removing pancreatic protease activities from the proximal intestine, in absence of luminal nutrients. Furthermore, neither protein hydrolysates nor fatty acids stimulate CCK release in the rat,¹⁶ in contrast with dogs and man. We suggest, therefore, that the effect of luminal nutrients in these studies may not be on CCK release, but rather on CCK synthesis. Buchan *et al.*¹⁷ have shown that parenteral nutrition results in a 50 % decrease in CCK-containing cells in the rat intestine. This finding indicates that luminal nutrients may be necessary for maintaining normal levels of CCK synthesis in the small intestine. We propose, therefore, that the role of luminal nutrients in our results is to maintain normal CCK synthetic activity and that the release of CCK occurs spontaneously in the absence of the suppressive effects of pancreatic proteases.

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