Experimental Evidence for a Vagally Mediated and Cholecystokinin-independent Enteropancreatic Reflex

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Truncal vagotomy results in diminished pancreatic protein secretion in response to intraduodenal fat. This diminished secretion may be due, at least in part, to interruption of the vagal reflexes between the intestine and the pancreas that work independently of cholecystokinin (CCK). In five dogs with chronic pancreatic fistulas, plasma CCK concentrations and pancreatic protein secretion in response to an intestinal stimulant (intraduodenal oleate) and to two exogenous peptides (bombesin and CCK-33) were compared before and after bilateral truncal vagotomy. Vagotomy decreased integrated protein secretion by about 50% in response to intraduodenal oleate. In contrast, protein output in response to parenteral stimuli increased after vagotomy. Integrated output of CCK in response to intraduodenal oleate or to exogenous bombesin or CCK was not significantly affected by vagotomy, but release of pancreatic polypeptide was decreased significantly in response to all stimuli after truncal vagotomy. These data provide evidence that truncal vagotomy decreases pancreatic protein secretion in response to intestinal stimulants by interrupting enteropancreatic reflexes mediated by the vagus, while maintaining normal (or supranormal) sensitivity of the pancreas to endogenous and exogenous CCK.

PANCREATIC SECRETION of digestive enzymes in response to fatty acids or amino acids within the duodenum decreases greatly after truncal vagotomy (TV).[1-4] We have shown recently that vagotomy produces this effect on pancreatic protein secretion without diminishing the release of cholecystokinin (CCK).[2,3] Others have found that vagotomy does not impair protein secretion in response to infusion of exogenous CCK or its analogues.[1,4] Solomon and Grossman[5] have suggested, therefore, that vagotomy may interrupt reflexes between the intestine and the pancreas, reflexes which appear to be carried through vagal, cholinergic pathways.

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The purpose of this study was to compare the effects of TV on pancreatic protein secretion in response to fat administered intraduodenally with those in response to CCK or bombesin given parenterally. The effects on protein secretion were related to measurements of release of CCK and pancreatic polypeptide (PP), which stimulate and inhibit protein secretion, respectively.

Materials and Methods

Five mongrel dogs (23 ± 2 kg) of both sexes were prepared with a chronic gastric and pancreatic fistula, according to a modification of the Herrera technique,[6] previously described by Llanos and colleagues.[7] The pancreatic fistula permitted collection of pancreatic secretion during study periods, yet allowed the secretions to return to the duodenum between experiments. The fistula also provided direct access to the duodenum for infusion of stimuli. The dogs were allowed 3 weeks to recover from the operation. Food was withheld for 12 hours before each study and the gastric fistula was opened to divert gastric secretions from the duodenum.

Each dog received three different stimuli, administered in random order on separate nonconsecutive days. A bilateral TV was then performed through a left thoracotomy, and the studies were repeated after another recovery period. Each dog thus acted as its own control. The three stimuli were sodium oleate (40 mM), administered as a continuous intraduodenal infusion at 6.8 mmol/hr for 2 hours; bombesin, administered intravenously at 1.0 μg/kg over 1 hour; and highly purified CCK-33 (supplied by Professor V. Mutt, Karolinska Institutet, Stockholm, Sweden), administered intravenously in a dose of 0.5 μg/kg over 1 hour. The hormones

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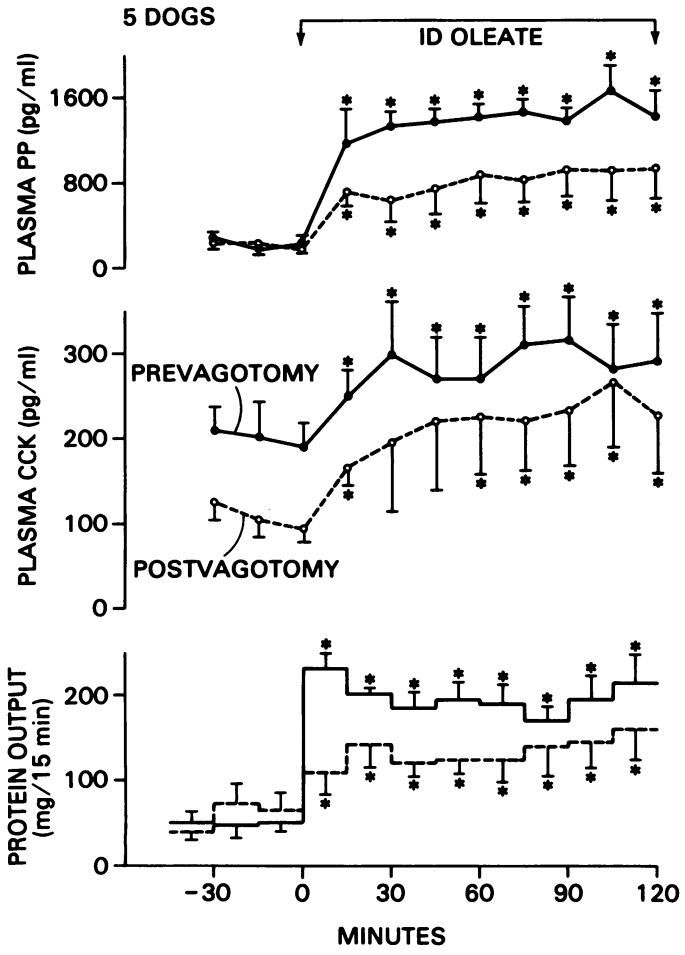

FIG. 1. Plasma concentrations of pancreatic polypeptide (PP) and CCK, and pancreatic output of protein (mean ± SEM), in response to a 2-hour intraduodenal (ID) infusion of oleate (6.8 mmol/hr) in five dogs. * = $p < 0.05$ *versus* basal; solid lines = before vagotomy; dotted lines = after vagotomy.

were prepared in a dilute solution of bovine serum albumin and all tubing and syringes were flushed with the albumin solution to minimize adherence of hormones to glass or plastic surfaces.[8] Intravenous hormones were given through forelimb veins, and blood samples were drawn from indwelling catheters placed in hindlimb veins.

Collection of Samples

Blood was drawn from a peripheral vein and placed in iced glass tubes that contained 100 Kallikrein inhibitory units (KIU) of aprotinin (Novo Research Institute, Bagsvaerd, Denmark) and 15 U of sodium heparin (Organon Diagnostics, West Orange, NJ) per milliliter of whole blood. Plasma was separated by centrifugation at 4 C and stored at −20 C for subsequent radioimmunoassay for CCK and PP.

Pancreatic juice was collected in iced tubes over 15-minute periods. The volume of each sample was recorded and its protein concentration was measured by the method of Lowry and colleagues.[9] Protein output was calculated for each sample.

The samples of blood and pancreatic juice were taken before stimulation (basal period) and at frequent intervals during administration of the stimuli.

Radioimmunoassay for Cholecystokinin and Pancreatic Polypeptide

Concentrations of CCK in plasma samples were measured by a specific radioimmunoassay that was developed in our laboratory. The assay has been described in detail[10,11] and validated extensively.[3,10,12,13] The CCK antiserum, UT-122, has been characterized: it is equally sensitive to the 33- and 39-amino acid forms of CCK, but insensitive to gastrin-17 or gastrin-34 or to the octapeptide of CCK. Intraassay variations were approximately eight per cent for the plasma pools, which contained concentrations of CCK that were within the physiologic range. The sensitivity of the assay is 1.3 fmol CCK/ml and the interassay variation is approximately 14%.

Concentrations of PP in plasma were measured by a specific radioimmunoassay, which employs reagents supplied by Dr. R. E. Chance (Lilly Research Laboratories, Eli Lilly Co., Indianapolis, IN).[14] This assay technique has also been described in detail.[15]

Statistical Analysis

Data were expressed as mean ± one standard error of the mean. Differences in means were tested for significance by the Student's t-test. Differences with a p value of < 0.05 were considered significant. Integrated values were corrected for basal values and calculated as previously described.[16]

Results

Intestinal Stimulation

Before vagotomy, intraduodenal infusion of sodium oleate resulted in prompt stimulation of release of PP and CCK and of pancreatic protein secretion. Significant increases were observed 15 minutes after infusion began, and these increases persisted for the entire 120 minutes of the infusion. After vagotomy, intraduodenal oleate produced a significant release of CCK and PP, measured from 15 to 120 minutes after infusion began, and protein output also increased (Fig. 1). Fasting plasma concentrations of CCK decreased after vagotomy, but the maximum incremental change (from basal to peak concentrations) was not significant (148 ± 27 pg/ml

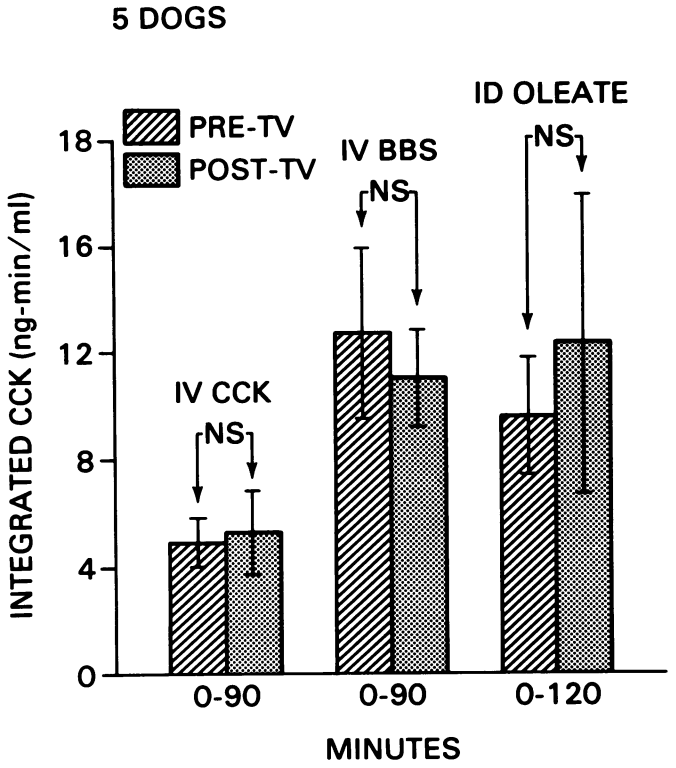

FIG. 2. Integrated release of CCK over 90 minutes in response to intravenous CCK and bombesin, and over 120 minutes in response to intraduodenal oleate. NS = no significant difference before vagotomy and after vagotomy.

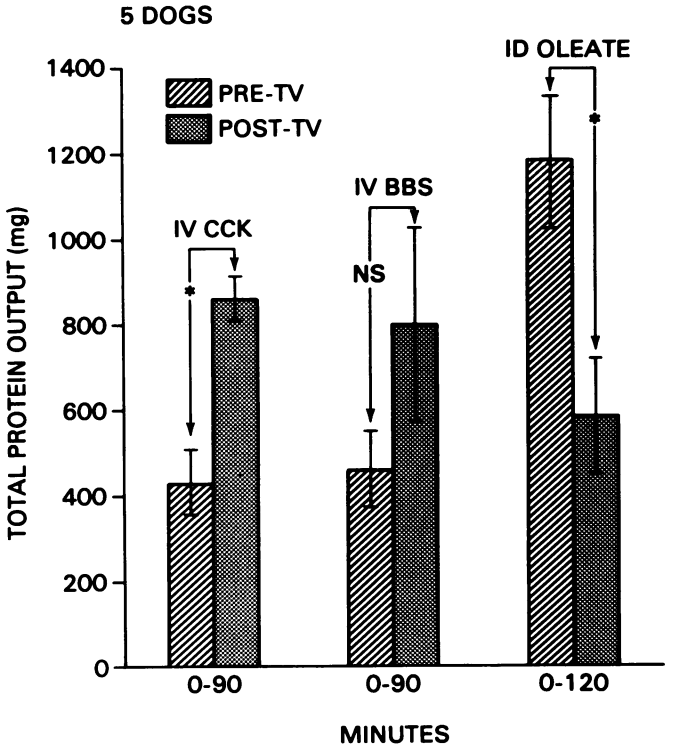

FIG. 3. Total protein output (corrected for basal) over 90 minutes in response to intravenous CCK and bombesin, and over 120 minutes in response to intraduodenal oleate. * = $p < 0.05$ before *versus* after vagotomy; NS = no significant difference.

before vagotomy and 169 ± 57 pg/ml after vagotomy). Furthermore, the differences in *integrated* release of CCK before and after vagotomy were not significant (Fig. 2). In contrast, pancreatic protein output in response to intraduodenal oleate decreased significantly in terms of both peak incremental output (from 222 ± 17 mg/15 min to 130 ± 21 mg/15 min) and integrated output (Fig. 3). The incremental change in PP concentrations decreased by approximately 50% after vagotomy from 1700 ± 240 pg/ml to 887 ± 202 pg/ml). Integrated release of PP also decreased (Fig. 4).

Bombesin

Parenteral bombesin was a potent stimulant for release of CCK and PP and for secretion of pancreatic protein (Fig. 5). Vagotomy had no significant effect on this release of CCK: the incremental rise in CCK concentrations was 313 ± 55 pg/ml before vagotomy and 208 ± 32 pg/ml after vagotomy. The integrated release of CCK was also unaffected by vagotomy (Fig. 2). Protein output (basal to peak) increased after vagotomy from 149 ± 32 mg/15 min to 253 ± 72 mg/15 min, but this difference was not significant, nor were the differences in integrated protein output (Fig. 3). The release of PP was impaired significantly by vagotomy (Fig. 4).

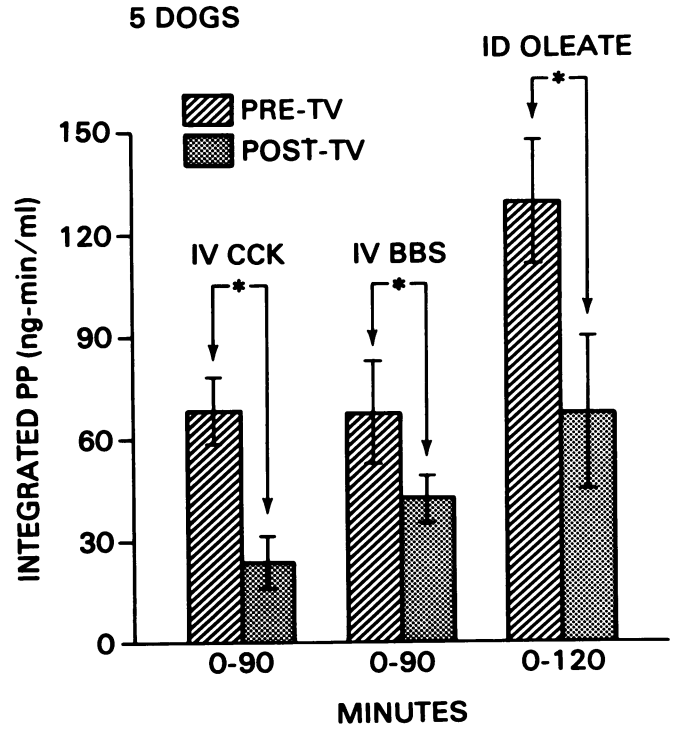

FIG. 4. Integrated release of pancreatic polypeptide (PP) over 90 minutes in response to intravenous CCK and bombesin, and over 120 minutes in response to intraduodenal oleate. * = $p < 0.05$ before *versus* after vagotomy.

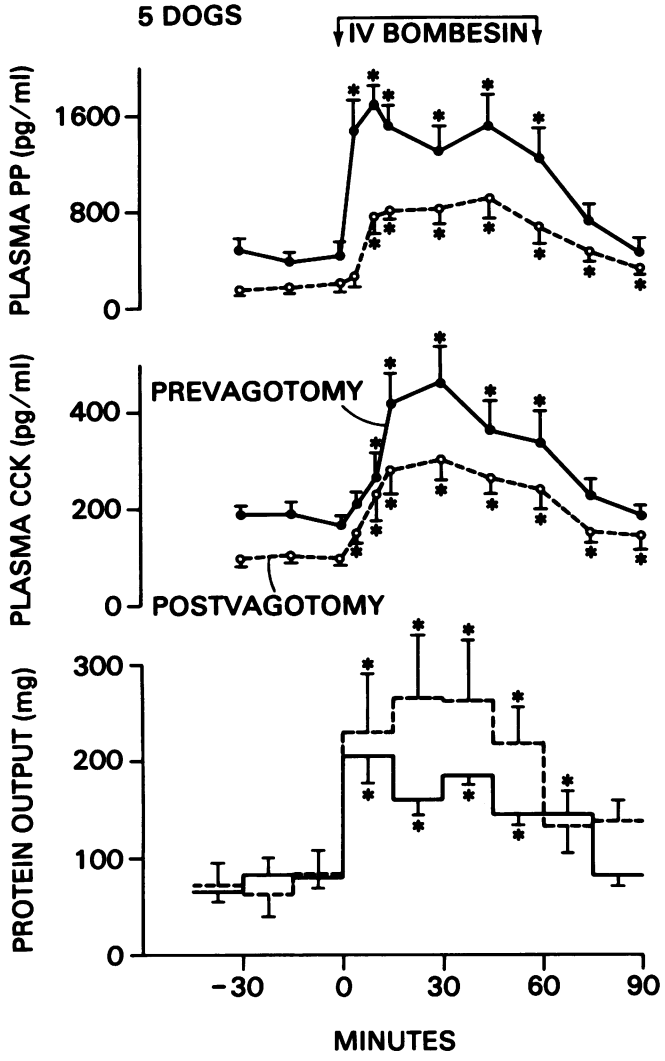

FIG. 5. Plasma concentrations of CCK and pancreatic polypeptide (PP), and pancreatic output of protein (mean ± SEM), in response to a 1-hour infusion of bombesin (1.0 μg/kg) in five dogs. * = $p < 0.05$ *versus* basal; solid lines = before vagotomy; dotted lines = after vagotomy.

Exogenous Cholecystokinin

Infusion of exogenous CCK increased plasma levels of CCK and caused release of PP and secretion of pancreatic protein (Fig. 6). Truncal vagotomy did not affect the change in plasma concentrations of CCK significantly (129 ± 21 pg/ml before vagotomy, 104 ± 23 pg/ml after vagotomy), but the release of pancreatic polypeptide was decreased significantly (Fig. 4). Pancreatic protein output in response to exogenous CCK increased significantly after vagotomy when measured in terms of incremental change (150 ± 22 mg/15 min before vagotomy, 261 ± 22 mg/15 min after vagotomy) or integrated secretion (Fig. 3).

Discussion

This study confirms our earlier findings[3] that truncal vagotomy causes diminished secretion of pancreatic protein in response to intraduodenal fat, but does not impair the release of CCK. Furthermore, vagotomy does not decrease the protein output in response to infusion of CCK (in this study, vagotomy actually increased protein output). Thus, since neither the release nor the action of CCK was affected by vagotomy, the effect of the vagus on the pancreas must not depend on CCK. These results indicate that the pancreas is different from the gastric parietal cell in that it does not require vagal innervation to respond significantly to CCK, whereas

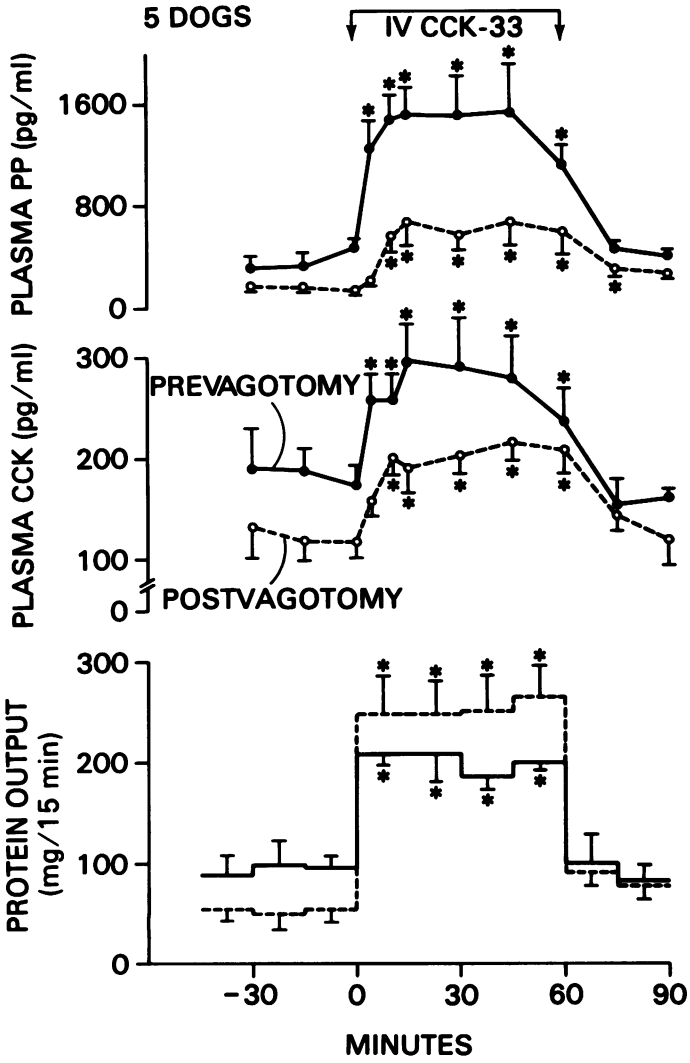

FIG. 6. Plasma concentrations of CCK and pancreatic polypeptide (PP), and pancreatic output of protein (mean ± SEM), in response to a 1-hour infusion of CCK-33 (0.5 mcg/kg) in five dogs. * = $p < 0.05$ *versus* basal; solid lines = before vagotomy; dotted lines = after vagotomy.

the parietal cell does require vagal action to respond fully to gastrin.

Bombesin stimulated the release of endogenous CCK and secretion of pancreatic protein without intestinal stimulation; since neither release of CCK nor output of pancreatic protein decreased after vagotomy, the effects of bombesin on the pancreas and on release of CCK must take place independent of vagal innervation. Our results, therefore, support the concept that vagal stimulation of pancreatic protein secretion is initiated by a stimulus from the luminal surface of the intestine.

Pancreatic protein secretion, in response to intestinal stimulation (by amino acids and fatty acids), most likely results from a combination of direct vagal stimulation by enteropancreatic reflexes and stimulation by endogenous CCK released from the mucosa of the duodenum and jejunum. These two pathways, however, appear to operate independently, since vagotomy abolishes reflex neurogenic stimulation of the pancreas without affecting the release or action of CCK, whereas vagal stimulation causes pancreatic protein secretion without releasing CCK.[17] Infusion of exogenous CCK or endogenous release of CCK by bombesin stimulates protein secretion without vagal innervation. In fact, the role of the vagus during parenteral stimulation may be inhibitory because pancreatic secretion in response to bombesin or CCK increases after vagotomy. The mechanism for this increase in pancreatic secretion may involve PP, since vagotomy brings about a decrease in plasma concentrations of PP, which is an inhibitor of pancreatic protein secretion.

This work complements the studies of Singer and colleagues,[18] who found that atropine or vagotomy depressed pancreatic protein secretion in response to intraduodenal tryptophan, but not to intravenous caerulein (a CCK analogue), when measured from innervated pancreas, but had no effect on secretion from denervated (transplanted) pancreas. Our finding that release of CCK was not affected by vagotomy explains why vagotomy did not affect secretion from the denervated pancreas in response to intraduodenal tryptophan in the earlier studies.

Singer and colleagues[19] have also observed that the latent period of the pancreatic secretory response to intraduodenal stimuli was shorter than that of the response to intraportal injection of CCK. Because this response did not occur after vagotomy or administration of atropine, their findings indicate that it depends on an early, rapid reflex that is mediated by the vagus. Debas and colleagues[1] found that extragastric vagotomy had the same effects on pancreatic secretion as truncal vagotomy. In other studies, insulin hypoglycemia has been shown to stimulate pancreatic enzymes.[20] This response is blocked by truncal vagotomy, but not by highly selective vagotomy. All these reports suggest that the enteropancreatic reflex is a function of the extragastric portions of the vagus.

Summary

Our findings, as well as the findings of others, provide strong evidence for the presence of a stimulatory reflex between the intestine and the pancreas, which is mediated through extragastric, cholinergic vagal fibers. Thus, in response to products of digestion within the proximal intestine, pancreatic protein secretion increases because of stimulation through vagal efferent pathways and through the effects of CCK, which is released endogenously. The release of CCK and its effects on the pancreas appear not to require vagal innervation. Similarly, the effects of stimulation by the vagus do not appear to act through the release of CCK; instead, they seem to stimulate the pancreas independently.[17]

We found that truncal vagotomy produced a significant decrease in protein secretion when stimulation was provided by intraduodenal fat, but no decrease occurred in response to bombesin or CCK. In addition, vagotomy neither diminished the release of endogenous CCK nor affected the response of the pancreas to CCK. We conclude that release of CCK is independent of the vagus and that the vagus stimulates pancreatic protein secretion by enteropancreatic reflexes that are independent of CCK.

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References

1. Debas HT, Konturek SJ, Grossman MI. Effect of extragastric and truncal vagotomy on pancreatic secretion in the dog. Am J Physiol 1975; 228:1172–1177.
2. Fried GM, Ogden WD, Sakamoto T, et al. Truncal vagotomy: effects on plasma concentrations of CCK and pancreatic protein secretion in response to enteral and parenteral stimuli. Gastroenterology 1983; 84:1159.
3. Fried GM, Ogden WD, Greeley G, Thompson JC. Correlation of release and actions of cholecystokinin in dogs before and after vagotomy. Surgery 1983; 93:786–791.
4. Konturek SJ, Becker HD, Thompson JC. Effect of vagotomy on hormones stimulating pancreatic secretion. Arch Surg 1974; 108:704–708.
5. Solomon T, Grossman MI. Vagal control of pancreatic exocrine secretion. *In* Brooks FP, Evers PW, eds. Nerves and the gut. Thorofare, NJ: Charles B. Slack, 1977; 119–132.
6. Herrera F, Kemp DR, Tsukamoto M, et al. A new cannula for

the study of pancreatic function. J Appl Physiol 1968; 25:207–209.
7. Llanos OL, Swierczek J, Teichmann RK, et al. Effect of alcohol on the release of secretin and pancreatic secretion. Surgery 1977; 81:661–667.
8. Miyata M, Rayford PL, Thompson JC. Loss of secretin from plastic infusion systems. N Engl J Med 1979; 300:95.
9. Lowry OH, Rosebrough NJ, Farr RL, Randall RJ. Protein measurement with the Folin reagent. J Biol Chem 1951; 193:265–275.
10. Fried GM, Ogden WD, Swierczek J, et al. Release of cholecystokinin in conscious dogs: correlation with simultaneous measurements of gallbladder pressure and pancreatic protein secretion. Gastroenterology 1983; 85:1113–1119.
11. Inoue K, Yazigi R, Watson LC, et al. Increased release of cholecystokinin after pancreatic duct ligation. Surgery 1982; 91:467–473.
12. Lilja P, Fagan CJ, Wiener I, et al. Infusion of pure cholecystokinin in man. Correlation between plasma concentrations of CCK and gallbladder size. Gastroenterology 1982; 83:256–261.
13. Wiener I, Inoue K, Fagan CJ, et al. Release of cholecystokinin in man: correlation of blood levels with gallbladder contraction. Ann Surg 1981; 194:321–327.
14. Chance RE, Moon NE, Johnson MG. Human pancreatic polypeptide (HPP) and bovine pancreatic polypeptide (BPP). *In* Jaffe BM, Behrman HR, eds. Methods of hormone radioimmunoassay, 2nd ed. New York: Academic Press, 1979: 657–672.
15. Lonovics J, Guzman S, Devitt P, et al. Release of pancreatic polypeptide in humans by infusion of cholecystokinin. Gastroenterology 1980; 79:817–822.
16. Thompson JC, Reeder DD, Bunchman HH, et al. Effect of secretin on circulating gastrin. Ann Surg 1972; 176:384–393.
17. Guzman S, Chayvialle JA, Banks WA, et al. Effect of vagal stimulation on pancreatic secretion and on blood levels of gastrin, cholecystokinin, secretin, vasoactive intestinal peptide, and somatostatin. Surgery 1979; 86:329–336.
18. Singer MV, Solomon TE, Grossman MI. Effect of atropine on secretion from intact and transplanted pancreas in dog. Am J Physiol 1980; 238:G18–G22.
19. Singer MV, Solomon TE, Wood J, Grossman MI. Latency of pancreatic enzyme response to intraduodenal stimulants. Am J Physiol 1980; 238:G23–G29.
20. Smith RB, Edwards JP, Johnston D. Effect of vagotomy on exocrine pancreatic and biliary secretion in man. Am J Surg 1981; 141:40–47.