

## Pancreatic secretion evoked by cholecystokinin and non-cholecystokinin-dependent duodenal stimuli via vagal afferent fibres in the rat

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1. We have recently demonstrated that cholecystokinin (CCK) at physiological levels stimulates pancreatic enzyme secretion via gastroduodenal mucosal vagal afferent fibres in the rat. The present study was designed to investigate if non-CCK-mediated pancreatic stimuli which activate duodenal receptors also utilize similar vagal afferent pathways.
2. Intraduodenal administration of maltose (300 mM), hypertonic saline (500 mosmol l<sup>-1</sup>) and mucosal light stroking in anaesthetized rats evoked 70, 57 and 200% increases, respectively, in pancreatic protein secretion with no changes in plasma CCK concentration. Administration of the CCK receptor antagonist L364,718 did not affect pancreatic secretion evoked by these luminal stimuli.
3. Administration of atropine, acute vagotomy and duodenal mucosal application of capsaicin each completely abolished the pancreatic response to these stimuli.
4. Infusion of a subthreshold dose of the octapeptide of CCK (15 pmol (kg body wt)<sup>-1</sup> h<sup>-1</sup>) potentiated the pancreatic response to duodenal infusion of maltose (300 mM) and hypertonic saline (500 mosmol l<sup>-1</sup>).
5. In conscious rats, perivagal application of capsaicin abolished the pancreatic response evoked by physiological doses of CCK and intraduodenal administration of maltose or hypertonic saline, confirming the physiological relevance of the observations in anaesthetized rats.
6. These results suggest that like CCK, non-CCK-mediated luminal stimuli evoke pancreatic enzyme secretion via stimulation of a vagal afferent pathway originating from the duodenal mucosa.

The control of postprandial pancreatic enzyme secretion has been ascribed mainly to the hormone cholecystokinin (CCK) and to vagovagal reflexes that activate cholinergic post-ganglionic neurons in the pancreas (Owyang & Williams, 1991; Solomon, 1994). *In vivo* canine (Konturek, Tasler, Cieszkowski, Szewczyk & Hladij, 1988) and human (Adler *et al.* 1991; Schmidt *et al.* 1991) studies utilizing potent CCK antagonists indicate that CCK accounts for 50–60% of postprandial pancreatic enzyme secretion, whereas vagal cholinergic pathways account for the remainder. Recent studies in the rat have shown that CCK at physiological levels stimulates pancreatic enzyme secretion via a capsaicin-sensitive afferent vagal pathway (Li & Owyang, 1993, 1994b). This renders the separation of neural and hormonal control of pancreatic secretion somewhat artificial.

The duodenum is richly innervated with afferent fibres of vagal and splanchnic origin which convey a large range of physiological information such as chemo-, thermo- and

mechanosignals to the central nervous system (Mei, 1985; Grundy & Scratcherd, 1989). In humans, stimulation of duodenal volume receptors and osmoreceptors elicits a pancreatic enzyme response by a CCK-independent mechanism mediated by cholinergic neurons (Owyang, May & Louie, 1986). In addition, increased firing rates in vagal afferent neurons and central sites have been recorded after gastric or intestinal distension (Davison, 1972; Contrell & Iggo, 1984) and intestinal perfusion with amino acids (Jeanningros, 1982), lipids (Melone, 1986) and carbohydrates (Mei, 1978). The aim of the present study was to investigate the mechanism(s) and site(s) of action of non-CCK-dependent duodenal stimuli of pancreatic secretion using an *in vivo* rat model. We hypothesized that like CCK, non-CCK-dependent duodenal meal-related factors stimulate gastroduodenal mucosal vagal afferent fibres that utilize a common cholinergic pathway to mediate pancreatic secretion. Three types of duodenal stimuli were examined: a disaccharide,

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maltose, which stimulates duodenal chemoreceptors; hypertonic saline, which activates osmoreceptors; and light stroking, which stimulates mucosal mechanoreceptors. We also investigated the functional interaction between CCK and non-CCK-dependent stimuli on pancreatic enzyme secretion.

## METHODS

### Materials

The following were purchased from Sigma: maltose, capsaicin, 2-deoxy-D-glucose and atropine sulphate. Octapeptide of cholecystokinin (CCK-8) was purchased from Peninsula Laboratories, Belmont, CA, USA. Casein was purchased from ICN Biomedicals, Inc., Aurora, OH, USA. L364,718 was a gift from Dr Victor Lotti, Merck Sharp and Dohme, West Point, PA, USA.

### Animal preparation

Male Sprague-Dawley rats weighing between 250 and 300 g were used. After an overnight fast, rats were anaesthetized with a mixture of xylazine and ketamine (13 and 87 mg (kg body wt)<sup>-1</sup>, respectively, i.m.) and a third of the initial dose of anaesthetic was repeated every 90 min to maintain surgical anaesthesia (stage III). One or more polyethylene catheters were placed in the external jugular vein for intravenous infusion with a syringe-drive pump. Through a mid-line incision, a polyethylene cannula (PE-10; Clay-Adams, Becton Dickinson, Sparks, MD, USA) was inserted into the common bile-pancreatic duct at the sphincter of Oddi. To permit the infusion of bile-pancreatic juice, a second cannula was placed into the duodenum slightly above the sphincter of Oddi. The abdominal wound was covered with a saline-moist gauze, and the rats were maintained at 37 °C with a heating pad.

### Pancreatic secretion study

After a 30 min stabilization period, bile-pancreatic secretions were collected for 15 min periods. The volume was measured, and an aliquot taken and diluted with distilled water for protein determination. The remainder of the undiluted bile-pancreatic juice was pumped back into the rat via the duodenal cannula during the next collection period, at the rate of secretion of the preceding collection period.

Bile-pancreatic juice protein was measured spectrophotometrically using the assay method of Bradford (1976). It was confirmed in our previous study (Louie, May, Miller & Owyang, 1986) that the increase in protein output in the bile-pancreatic juice after CCK infusion largely reflected protein from the pancreas. Biliary proteins did not increase with CCK stimulation.

### Duodenal perfusion and mucosal light stroking studies

A 20 cm segment of small intestine, which included the whole duodenum and proximal part of the jejunum, was isolated between two cannulas positioned at 4 cm (PE 60; 76 mm i.d., 1.22 mm o.d.) and 24 cm (PE 190; 1.19 mm i.d., 1.7 mm o.d.) from the pylorus. After a 30 min basal period, NaCl (500 mosmol l<sup>-1</sup>, pH 6.0) or maltose (300 mM, pH 6.0) was given for 30 min at a constant perfusion rate of 3 ml h<sup>-1</sup> by means of a peristaltic pump. Free intestinal drainage was established to avoid an increase in intraluminal pressure. Each test solution was administered separately for 30 min, with a 1 h resting period between experiments to allow pancreatic secretion to return to basal levels.

In a separate study, mechanical stimulation of the intestinal mucosa was performed by gently moving an intraluminal small cannula (Tygon 1/10 in i.d., 3/20 in o.d., laboratory grade tubing; Becton Dickinson) to and fro against the mucosa to mimic the passage of solid particles (Clarke & Davison, 1978; Mei, 1985). Bile-pancreatic juice was collected every 5 min.

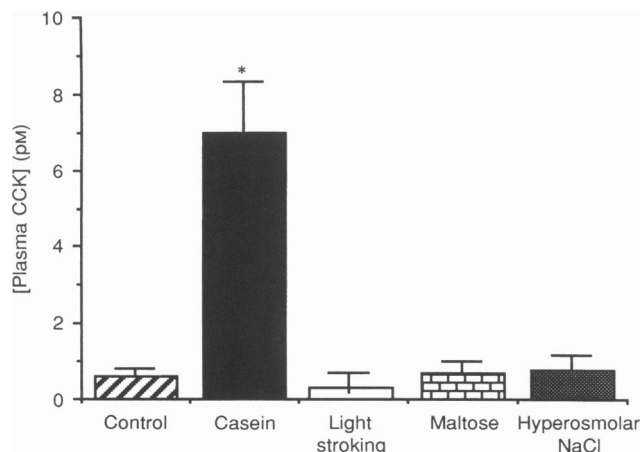
In another separate group of rats, blood samples for CCK measurements were obtained by cardiac puncture at the end of the infusion of each test solution or after each mechanical stimulation. As a positive control, blood samples were obtained 30 min after intraduodenal infusion of 18% casein. Plasma CCK levels were measured by a bioassay method previously reported (Louie *et al.* 1986).

### Effect of CCK receptor antagonist L364,178 on pancreatic secretion evoked by duodenal stimuli

To investigate if pancreatic secretion evoked by maltose, hyperosmolar NaCl or mucosal stroking involved the release of CCK, we examined the effect of the CCK receptor antagonist L364,718. In this study, vehicle (4% DMSO: polysorbate 80) or L364,718 (0.5 mg kg<sup>-1</sup>) was administered intravenously after three 15 min basal periods. L364,718 was dissolved in 1:1 dimethylsulphoxide (DMSO): polysorbate 80. The solution was diluted with 0.9% saline to a final concentration of 4% DMSO: polysorbate 80. Bile-pancreatic juice secreted in response to varying duodenal stimuli was collected 30 min after the administration of L364,718.

### Atropine and acute vagotomy studies

To determine the role of cholinergic pathways in the mediation of pancreatic secretion evoked by the various duodenal stimuli, atropine (50 µg (kg body wt)<sup>-1</sup> h<sup>-1</sup>) was infused 30 min before intraduodenal infusion of maltose or hyperosmolar NaCl solution, and continued throughout the remainder of the experiment. Pancreatic enzyme secretion rates were compared with those obtained without atropine. Similarly, mucosal light stroking studies with and without atropine were performed as described above.



**Figure 1. Basal and stimulated plasma CCK levels**

Duodenal infusion of casein (18%) significantly elevated plasma CCK levels. In contrast, intestinal perfusion of maltose (300 mM), hyperosmolar NaCl solution (500 mosmol l<sup>-1</sup>) or mucosal light stroking did not change basal plasma CCK levels (fasting as a control). Values are means ± S.E.M. for 6 rats in each group. \* *P* < 0.01.

To investigate the role of the vagus, acute vagotomy was performed. Through a mid-line incision of the abdominal wall, the stomach was carefully manipulated to expose the oesophagus. The subdiaphragmatic vagal trunks were exposed half-way between the diaphragm and the gastric cardia. Both anterior and posterior trunks of the vagal nerves were transected. For control experiments, the abdominal vagal nerves were exposed but not cut. Pancreatic protein secretion studies in response to the various duodenal stimuli were then performed as described earlier.

### Mucosal application of capsaicin

Vagal afferent fibres are classified into muscle and mucosal afferents. To investigate if mucosal afferent fibres are responsible for mediating pancreatic secretion evoked by duodenal stimuli, we examined the effects of duodenal mucosal application of capsaicin, which impairs neurotransmission of sensory fibres. After laparotomy, a 20 cm segment of the small intestine, which included the duodenum and proximal jejunum, was temporarily ligated at both ends and filled with 2 ml of capsaicin solution (6 mg ml<sup>-1</sup>, dissolved in 10% Tween 80 in olive oil). After 30 min, the capsaicin solution was removed by needle aspiration. Vehicle treatment with 10% Tween 80 in olive oil was performed in control rats. Intestinal perfusion studies were performed 7 days after this local capsaicin application. Rats were checked for normal eye wiping movement to indicate that the local mucosal treatment with capsaicin had had no systemic effects. Briefly, a drop of capsaicin solution (0.1 mg ml<sup>-1</sup>) dissolved in saline was instilled into one eye according to the method of Jancso (1968). Protective wiping movements with the forepaws indicated the absence of systemic effects due to capsaicin following the mucosal application. When a positive response was observed, the eye was immediately and thoroughly rinsed with

water to keep discomfort to a minimum. The stimulation is mild and brief and there was no evidence of eye inflammation or any continued discomfort to the rats. The procedures were approved by the university committee on use and care of animals.

### Interaction between CCK and non-CCK-dependent stimuli

To assess the potential interaction between CCK and non-CCK-dependent duodenal stimuli, the effects of the combined administration of a subthreshold dose of CCK and duodenal non-CCK pancreatic stimuli were studied in anaesthetized rats. Following 60 min of stabilization, CCK (15 pmol (kg body wt)<sup>-1</sup> h<sup>-1</sup>) was infused for 45 min in a control group of rats. In a separate group of rats, CCK (15 pmol kg<sup>-1</sup> h<sup>-1</sup>) was infused 45 min after the start of intraduodenal perfusion of maltose (300 mM) or hyperosmolar NaCl (500 mosmol l<sup>-1</sup>).

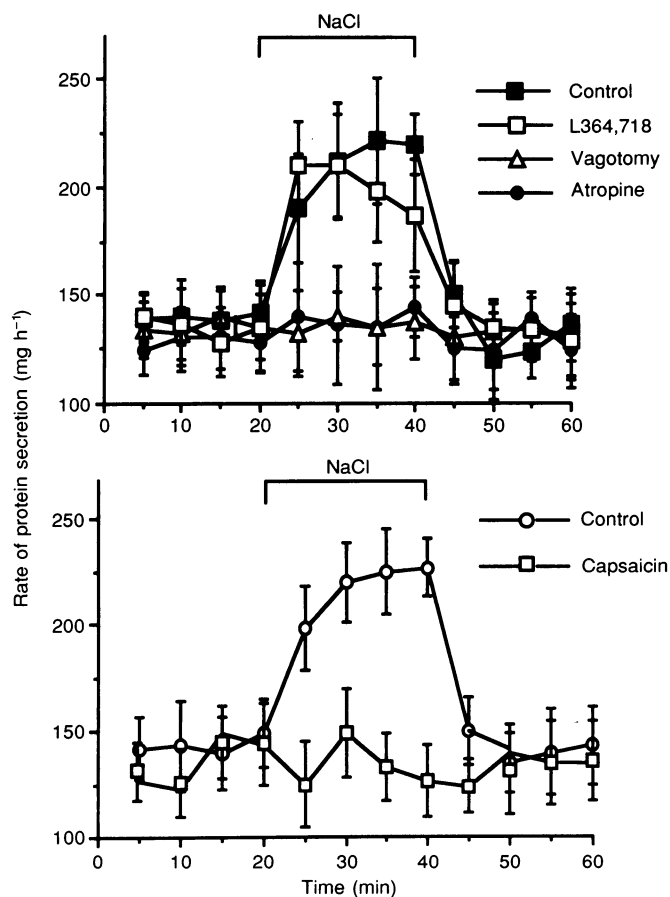
### Pancreatic secretion studies in conscious rats

Physiological control of pancreatic enzyme secretion may differ between anaesthetized and conscious rats. Therefore we performed additional studies to evaluate the effects of perivagal capsaicin treatment on pancreatic secretion evoked by the various duodenal stimuli in a separate group of conscious rats.

Before surgery, atropine (0.5 mg kg<sup>-1</sup>, i.p.) was administered to reduce the acute effects of capsaicin on the cardiovascular and respiratory system. Following anaesthesia with xylazine and ketamine (13 and 87 mg (kg body wt)<sup>-1</sup> i.m., respectively), the abdominal vagal trunk was exposed. A small piece of gauze soaked in 1% capsaicin solution (0.2 ml per rat) was left on the vagal trunk for 30 min. Vehicle alone was applied to control rats. The rats were allowed to recover and a second operation was performed 7 days later for the insertion of bile-pancreatic duct and duodenal

### Figure 2. Pancreatic protein secretion evoked by hyperosmolar NaCl

Duodenal infusion of NaCl solution (500 mosmol l<sup>-1</sup>) at 3 ml h<sup>-1</sup> caused a 57% increase in protein output over basal. This was not affected by the CCK antagonist L364,718 (0.5 mg kg<sup>-1</sup>, i.v.). Administration of atropine (50 µg kg<sup>-1</sup> h<sup>-1</sup>) and acute vagotomy completely abolished the stimulated pancreatic secretion (upper panel). Mucosal application of capsaicin also abolished protein secretion evoked by hyperosmolar NaCl (500 mosmol l<sup>-1</sup>) (lower panel). Values are means ± s.e.m. for 6 rats in each group. In this and the following figures, the brackets indicate the times of infusion of the various solutions.



catheters; following anaesthesia (as above), a polyethylene catheter (PE 10) was inserted through a mid-line incision into the bile-pancreatic duct. A second catheter (PE 50) was placed in the duodenum slightly above the sphincter of Oddi for intestinal perfusion of pancreatic biliary juice. The catheters were brought through the body wall and pulled through a cutaneous tunnel to an exit site between the scapulae. The catheters were connected between experiments to allow the free flow of bile and pancreatic juice into the intestine. One catheter (PE 10) was placed in the external jugular vein for intravenous infusion of 2-DG.

Pancreatic secretion studies were performed 7 days later, after the rats had fully recovered from surgery. Following an overnight fast, rats were lightly restrained in Bollman cages and pancreatic secretion studies in response to maltose (300 mM) and hyperosmolar NaCl solution (500 mosmol l<sup>-1</sup>) were performed as described earlier. For comparison, we also infused a physiological dose of CCK (40 pmol kg<sup>-1</sup> h<sup>-1</sup>) for 60 min. Bile-pancreatic juice was collected every 15 min. At the end of the experiment, the rats were killed by decapitation under anaesthesia.

#### Statistical analysis

Results were expressed as means  $\pm$  s.e.m. The multivariate analysis of variance method was used to evaluate the effects of repeated measurements over time and treatment effect, and the interactions between them. Significance was determined using Student's *t* test and was accepted at the 5% level.

## RESULTS

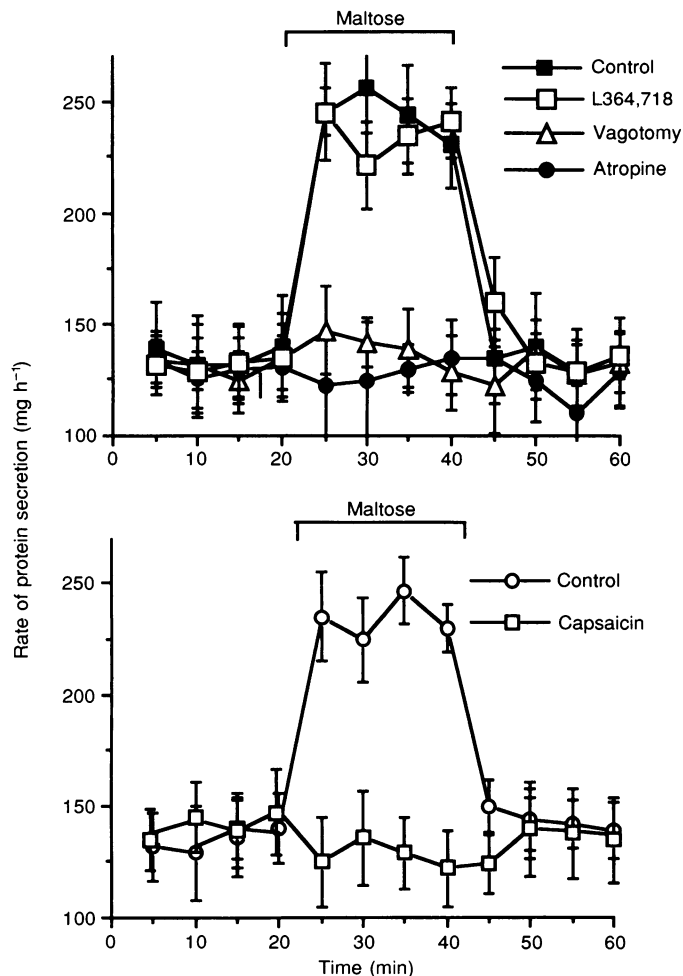
### Plasma CCK levels

Plasma CCK levels under basal conditions were  $0.65 \pm 0.1$  pM. Basal levels were not affected by atropine or vagotomy (CCK levels,  $0.5 \pm 0.1$  pM). Intestinal infusion of 18% casein elevated plasma CCK levels to  $7.0 \pm 1.6$  pM. In contrast, administration of maltose (300 mM), hyperosmolar NaCl solution (500 mosmol l<sup>-1</sup>) and light stroking did not elevate plasma CCK levels (Fig. 1).

### Intraduodenal infusion of hyperosmolar NaCl

Basal pancreatic protein secretion was stable, averaging  $142 \pm 16$  mg h<sup>-1</sup>. Infusion of hyperosmolar NaCl solution (500 mosmol l<sup>-1</sup>) at 3 ml h<sup>-1</sup> increased protein secretion to  $220 \pm 19$  mg h<sup>-1</sup> (Fig. 2), a 57% increase in protein output over basal. As shown in Fig. 2, administration of the CCK receptor antagonist L364,718 did not reduce the pancreatic response to 500 mosmol l<sup>-1</sup> NaCl, which suggests that activation of the duodenal osmoreceptors stimulates pancreatic enzyme secretion by a CCK-independent pathway.

Administration of atropine and acute vagotomy did not affect basal secretion, but abolished the response to hyperosmolar NaCl solution (500 mosmol l<sup>-1</sup>) (Fig. 2). This indicates



**Figure 3. Pancreatic protein secretion evoked by maltose**

Duodenal perfusion of maltose (300 mM) at 3 ml h<sup>-1</sup> caused a 70% increase in protein output over basal. This was not affected by the CCK antagonist L364,718 ( $0.5$  mg kg<sup>-1</sup>, i.v.). Administration of atropine ( $0.5$   $\mu$ g kg<sup>-1</sup> h<sup>-1</sup>) or acute vagotomy completely abolished the stimulated pancreatic secretion (upper panel). Mucosal application of capsaicin also abolished protein secretion evoked by maltose (lower panel). Values are means  $\pm$  s.e.m. for 6 rats in each group.

that the hyperosmolar solution stimulates pancreatic secretion via a vagal cholinergic pathway. As with truncal vagotomy, mucosal application of capsaicin also completely abolished the pancreatic protein response to hyperosmolar NaCl solution ( $500 \text{ mosmol l}^{-1}$ ) (Fig. 2). This suggests that the osmosensitive receptors that mediate pancreatic secretion are located in the duodenal mucosa.

#### Intraduodenal infusion of maltose

Intraduodenal perfusion of maltose at a concentration of 300 mM caused a 70% increase in protein output over basal. The pancreatic response to maltose (300 mM) was not affected by the CCK receptor antagonist L364,718 (Fig. 3). As with the response to hyperosmolar saline, administration of atropine and acute vagotomy abolished the pancreatic responses to the maltose infusion (Fig. 3). In addition, mucosal capsaicin completely abolished the pancreatic responses evoked by maltose (Fig. 3). This suggests that specific nutrients such as maltose activate a capsaicin-sensitive duodenal mucosal vagal afferent pathway to stimulate pancreatic secretion.

#### Mucosal light stroking

Light stroking of the mucosa caused a 3-fold increase in protein output over basal which was not affected by L364,718 (Fig. 4). However, administration of atropine, acute

vagotomy and mucosal application of capsaicin abolished these secretory responses (Fig. 4). This indicates that, as with hyperosmolar saline and maltose, duodenal mucosal mechanical stimulation elicits pancreatic secretion via a capsaicin-sensitive vagal afferent pathway.

#### Interaction between CCK and non-CCK stimuli

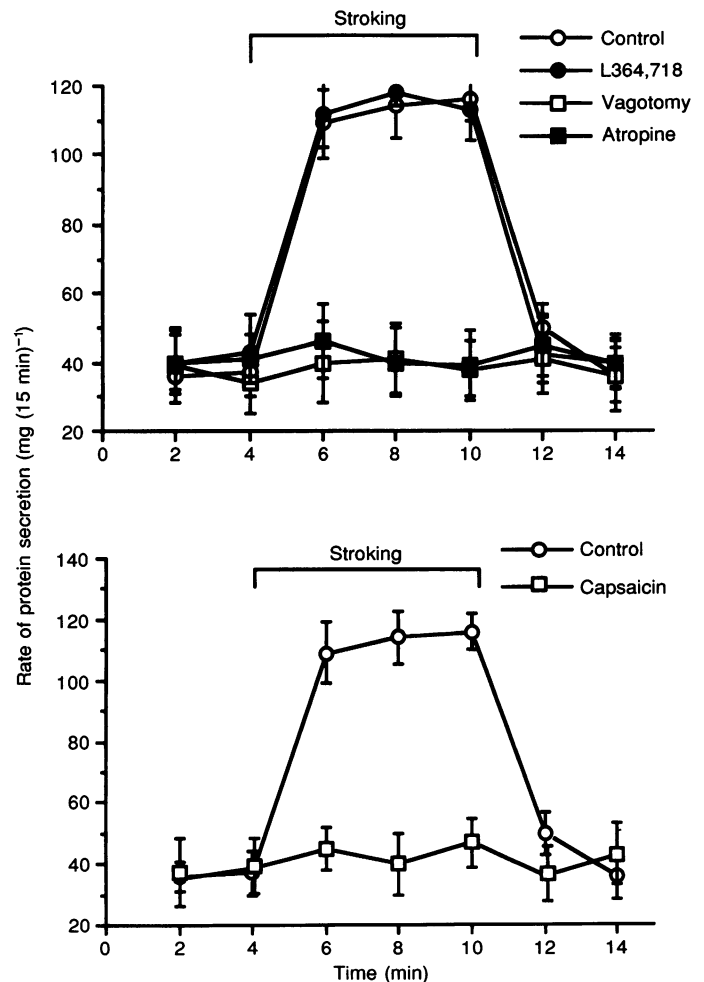
We next investigated the possible effects of interactions between CCK and maltose or hyperosmolar NaCl on pancreatic secretion. CCK infusion at a subthreshold dose ( $15 \text{ pmol kg}^{-1} \text{ h}^{-1}$ ) failed to stimulate pancreatic secretion (Fig. 5). On the other hand, infusion of the same dose of CCK with a background of duodenal infusion of maltose (or hyperosmolar NaCl solution) resulted in a rate of secretion significantly larger than that observed with maltose (or hyperosmolar NaCl) alone (Fig. 5). These observations indicate that CCK potentiates non-CCK-dependent duodenal stimuli to stimulate pancreatic secretion.

#### Effects of capsaicin on pancreatic secretion evoked by CCK and non-CCK stimuli in conscious rats

Conscious rats have relatively high basal pancreatic secretion rates, averaging  $335 \pm 32 \text{ mg h}^{-1}$ . CCK infusion at a dose of  $40 \text{ pmol kg}^{-1} \text{ h}^{-1}$ , which produces a physiological plasma CCK level similar to that observed postprandially (Li & Owyang 1993), evoked a 65% increase

**Figure 4. Pancreatic enzyme secretion evoked by light stroking of duodenal mucosa**

Stroking by gentle movement of a small intraluminal cannula against the mucosa (indicated by the brackets) caused a 3-fold increase in protein output over basal. This was not affected by the CCK antagonist L364,718 ( $0.5 \text{ mg kg}^{-1} \text{ h}^{-1}$ ). However, administration of atropine ( $50 \mu\text{g kg}^{-1} \text{ h}^{-1}$ ) and acute vagotomy completely abolished the stimulated pancreatic secretion (upper panel). Mucosal application of capsaicin also abolished protein secretion evoked by light stroking (lower panel). Values are means  $\pm$  s.e.m. for 6 rats in each group.



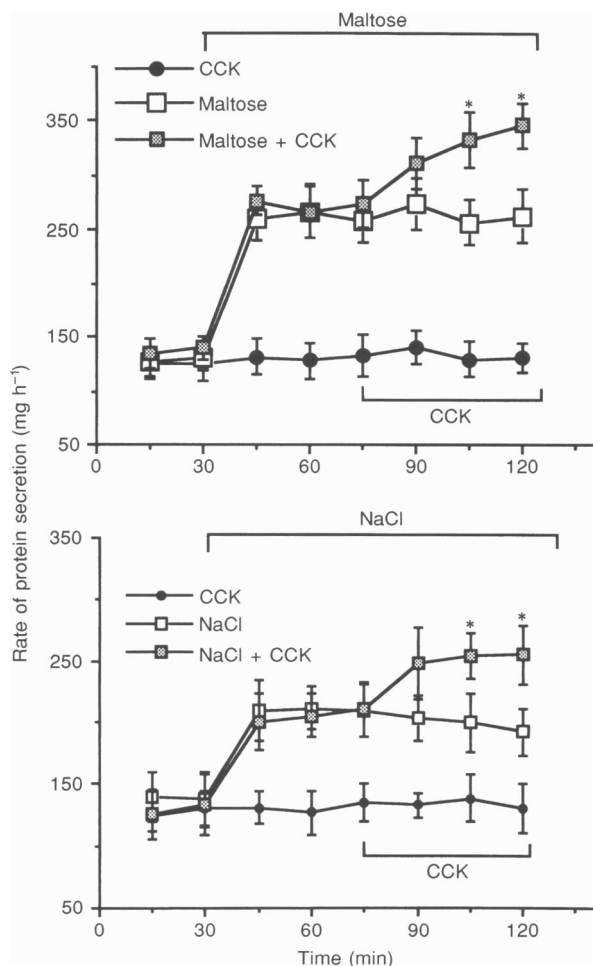
in protein secretion over basal (Fig. 6). Similarly, intestinal maltose (300 mM) and hyperosmolar NaCl (500 mosmol l<sup>-1</sup>) also produced 70 and 65% increases in protein secretion over basal, respectively (Fig. 6). Perivagal capsaicin treatment abolished the pancreatic responses to these stimuli (Fig. 6). These observations further confirm the importance of vagal afferent pathways in mediating the physiological action of CCK and non-CCK pancreatic stimuli.

Capsaicin is a sensory neurotoxin. To rule out a toxic effect on the vagal efferent pathway, we demonstrated that perivagal capsaicin treatment did not affect pancreatic secretion stimulated by intravenous infusion of 2-deoxy-D-glucose (75 mg kg<sup>-1</sup>), a central vagal stimulant (Fig. 6).

## DISCUSSION

Physiological studies in experimental animals and humans indicate that CCK and the vagal cholinergic pathways are the major mediators of postprandial pancreatic enzyme secretion. Until recently, CCK was believed to act directly on the acini to stimulate pancreatic secretion, as CCK receptors are present in abundance on pancreatic acini (Solomon, 1994). Furthermore, dispersed rat pancreatic acini appear to be sensitive to CCK at concentrations as low as 1 pM (Owyang & Williams, 1991). However, *in vivo*

studies in humans (Adler *et al.* 1991; Soudah, Lu & Owyang, 1992) and dogs (Inoue, Fried, Wiener, Zhu, Greeley & Thompson, 1984) have shown that pancreatic secretion stimulated by CCK can be blocked by atropine, which suggests an action via the cholinergic pathways. Recently, using an anaesthetized rat model, we have further shown that the sites of action of CCK in stimulating pancreatic secretion are dose dependent (Li & Owyang, 1993). Doses of CCK that produce physiological plasma CCK levels act via stimulation of the vagal afferent pathway, whereas doses that produce supraphysiological CCK levels act to stimulate intrapancreatic neurons and, to a lesser degree, pancreatic acini. Other studies further demonstrate that endogenous CCK released by the diversion of bile-pancreatic juice or intraduodenal infusion of casein also act via stimulation of a vagal afferent pathway to evoke pancreatic secretion (Li & Owyang, 1994b). These observations indicate that although CCK receptors are present in abundance on pancreatic acini, they do not appear to play a major role in mediating pancreatic secretion. There are other examples whereby *in vitro* observations do not necessarily apply to *in vivo* physiology. For instance, calcitonin gene-related peptide and somatostatin receptors are present on pancreatic acini but do not appear to mediate pancreatic secretion (Esteve *et al.* 1983; Bunnnett, Mulvihill & Debas, 1991).



**Figure 5. Interaction between CCK and non-CCK-dependent pancreatic stimuli**

Subthreshold doses of CCK (15 pmol kg<sup>-1</sup> h<sup>-1</sup>) potentiated the pancreatic responses to intraduodenal administration of maltose (300 mM) (upper panel) and hyperosmolar NaCl (500 mosmol l<sup>-1</sup>) (lower panel). Values are means  $\pm$  s.e.m. for 6 rats in each group. \**P* < 0.01.

Apart from CCK, luminal factors such as osmolarity, distension and non-CCK-releasing nutrients also play a major role in the intestinal phase of pancreatic secretion (Solomon 1994). Studies in dogs (Konturek, Radecki, Biernat & Thor, 1972) and humans (Adler *et al.* 1991) have demonstrated that administration of atropine or vagotomy substantially reduces the pancreatic responses to various intraduodenal stimulants. Furthermore, studies of denervated, transplanted pancreas in dog have shown that there is an impaired responsiveness to nutrients. Subsequent vagotomy or administration of atropine does not further impair the pancreatic responses to nutrient stimuli in this model (Solomon & Grossman, 1979). These observations indicate the importance of vagal innervation in the mediation of pancreatic enzyme secretion. Similar to studies on humans (Owyang, May & Louie, 1986), our current studies in rats show that the stimulation of duodenal osmo- and mechanoreceptors evokes pancreatic enzyme secretion via a CCK-independent cholinergic pathway. Furthermore, we show that vagotomy also abolishes pancreatic secretion stimulated by hyperosmolar saline, maltose and light stroking. These observations support our hypothesis that key duodenal factors stimulate pancreatic secretion via a

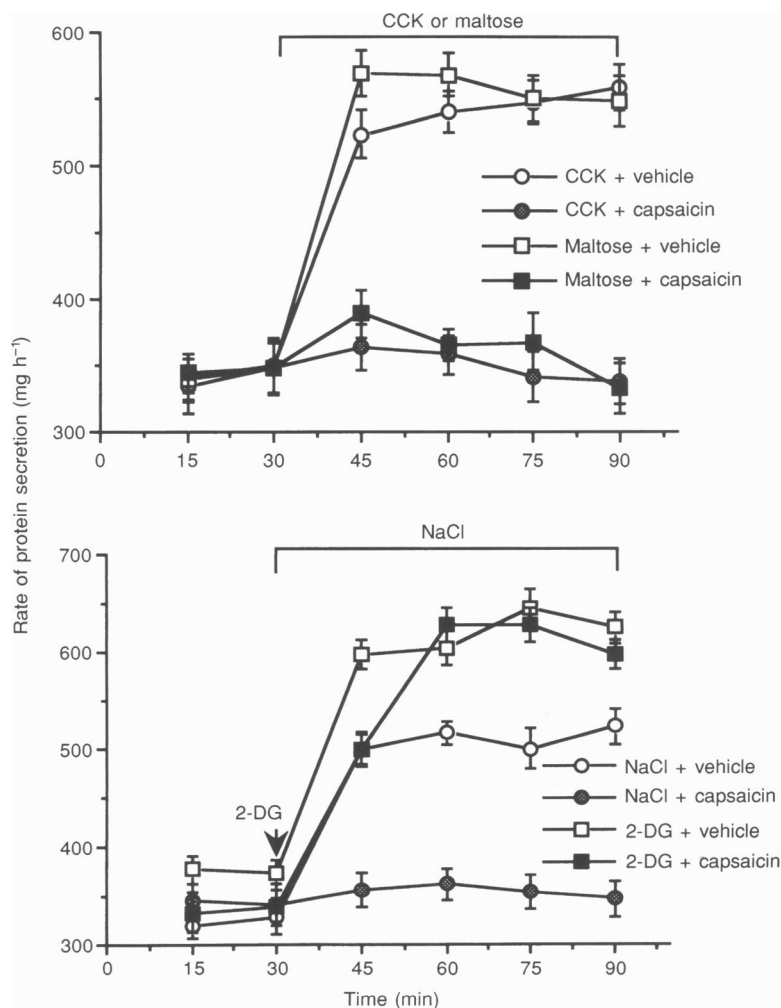
long vagovagal reflex, and argue against the involvement of an enteropancreatic reflex.

The effect of vagotomy on pancreatic secretion remains controversial. Both profound (Konturek, Radecki, Biernat & Thor, 1972; Malagelada, Go & Summerskill, 1974) and no effects (Singer, Solomon & Grossman, 1980) have been reported. These contradictory findings are likely to be related to the use of supraphysiological doses of CCK, excessive duodenal stimulation, and testing at different lengths of time after the performance of the vagotomy. In fact, our preliminary studies in rats have indicated that the responsiveness of the pancreas to CCK (Li & Owyang, 1994a) and duodenal stimuli (authors' unpublished data) returns within 2 weeks of a vagotomy, possibly because of adaptive changes involving the recruitment of a subpopulation of intraduodenal cholinergic neurons which activate the enteropancreatic neural pathway (Li & Owyang, 1994a).

To investigate if duodenal stimuli exert their actions via an afferent vagal pathway, we examined the effects of perivagal and mucosal treatment with the sensory neurotoxin capsaicin. Capsaicin has been widely used as a tool to investigate the role of afferent C fibres in a number of

**Figure 6.** Effect of perivagal application of capsaicin on pancreatic enzyme secretion evoked by exogenous CCK, duodenal infusion of maltose or hyperosmolar NaCl in conscious rats

Perivagal capsaicin treatment abolished pancreatic responses to CCK ( $40 \text{ pmol kg}^{-1} \text{ h}^{-1}$ , i.v.), maltose ( $300 \text{ mM}$ ) (upper panel) or hyperosmolar NaCl solution ( $500 \text{ mosmol l}^{-1}$ ) (lower panel). Pancreatic protein responses to 2-deoxy-D-glucose (2-DG;  $75 \text{ mg kg}^{-1}$ ) stimulation (arrowhead) remained intact in rats that had received perivagal capsaicin treatment (lower panel). Values are means  $\pm$  s.e.m. for 6 rats in each group.



physiological processes (Buck & Burks, 1986). In this study, we applied capsaicin directly to the vagal trunk to avoid affecting the afferent terminals in the peripheral and central nervous system. Perivagal application of capsaicin has been shown to inhibit axonal transport of peptides, including substance P and somatostatin (Gamse, Petsche, Lembeck & Jancso, 1982). Previous studies have demonstrated that perivagal capsaicin treatment lesions the vagal afferent pathway by which CCK increases satiety (South & Ritter, 1988), decreases gastric emptying (Raybould & Tache, 1988) and stimulates pancreatic secretion (Li & Owyang, 1993, 1994b). In this study, we show that perivagal pretreatment with capsaicin also impairs pancreatic responses to duodenal stimuli such as hyperosmolar saline solution and maltose, suggesting that these stimuli evoke pancreatic secretion via vagal afferent pathways. To show that capsaicin treatment does not affect efferent vagal function, we demonstrated that pancreatic responses to 2-deoxy-D-glucose stimulation remained intact in capsaicin-treated rats.

Vagal afferent fibres are traditionally classified into muscle and mucosal afferents according to the locations of their endings in the muscularis or mucosa (Mei 1985; Grundy & Scratcherd, 1989). These muscle and mucosal vagal afferent fibres appear to originate from different cell bodies in the nodose ganglia (Wang, Li & Owyang, 1994). Using retrograde tracer studies, it has been demonstrated that the majority of neurons in the nodose ganglia projecting to the gastroduodenal region supply the myenteric plexus, whereas 10–15% of the neurons project to the mucosa (Wang *et al.* 1994). Potentially, either or both types of afferent fibres may be responsive to duodenal stimuli. To address this question, mucosal capsaicin was used to denervate chemically the sensory fibres in the duodenal mucosa (Takeuchi, Matsumoto, Ueshima & Okabe, 1991; Li & Owyang, 1993). We confirmed that mucosal capsaicin denervates sensory fibres in the duodenal mucosa by performing retrograde tracer studies with the fluorescence dye True Blue injected into the mucosa and showed no detectable fluorescence in the nodose ganglion cells (authors' unpublished data). On the other hand, similar retrograde tracer studies with True Blue injected into the muscle layers showed normal patterns of fluorescence in the nodose ganglia in rats treated with mucosal capsaicin. This suggests that the myenteric plexus was not affected. We found that duodenal application of capsaicin completely abolished pancreatic secretory responses to maltose, hypertonic saline and light stroking, indicating that these duodenal stimuli evoke pancreatic secretion by acting on vagal afferent fibres terminating in the duodenal mucosa. These capsaicin-sensitive afferent nerve fibres have also been shown to participate in the regulation of motility, acid secretion and blood flow (Lundberg, Brodin, Huz & Saria, 1984; Takeuchi *et al.* 1991).

Recent electrophysiological studies using single-fibre techniques and microelectrodes implanted in the nodose ganglia demonstrate the richness of the sensory information

originating from the gastrointestinal tract (Davison, 1972; Mei, 1983). These afferent fibres transmit sensory information relating to the mechanical (degree of distension and contraction) and physicochemical states (osmotic pressure, temperature and quantity of nutrients) of the digestive tract. Furthermore, these sensory fibres may also be responsive to locally released peptides and hormones such as CCK, substance P and 5-HT (Lew & Longhurst, 1986; Davison & Clarke, 1988; Blackshaw & Grundy, 1990).

It is interesting to note that both CCK and non-CCK-dependent duodenal stimuli act via gastroduodenal mucosal vagal afferent fibres to stimulate pancreatic secretion. This observation is supported by recent electrophysiological studies in the ferret, which indicate that the duodenum is endowed with a rich supply of mucosal afferent fibres that are sensitive to luminal stimuli such as hypertonic saline, HCl and light stroking (Blackshaw & Grundy, 1990). In addition, some of these mucosal afferent fibres are also sensitive to CCK (Blackshaw & Grundy, 1990). Potentially, CCK and duodenal stimuli may act on the same or distinct vagal afferent fibres in the mucosa. We showed that infusion of a subthreshold dose of CCK potentiated pancreatic responses to intraduodenal infusion of maltose or hyperosmolar saline. The site(s) of this potentiation is unclear. It is however possible that CCK and duodenal stimuli may act on distinct duodenal mucosal afferent fibres to potentiate pancreatic secretion. Alternatively, the interaction may occur in the efferent limb of the vagal reflex arc. Direct electrophysiological recording of vagal afferent fibres is needed to examine these possibilities.

It is important to point out that physiological control of pancreatic secretion may be different in anaesthetized and conscious rats. Previous studies have reported that in contrast to anaesthetized rats, atropine did not significantly affect the net increase in pancreatic enzyme secretion in response to physiological doses of CCK in conscious rats (O'Rourke, Riedelberg & Solomon, 1991). However, it should be noted that atropine is likely to produce widespread cholinergic blockade in the peripheral and enteric nervous system, resulting in alterations of normal gastrointestinal physiology. The atropine experiments in conscious rats therefore do not necessarily negate the importance of vagal mediation in the action of CCK, since generalized suppression of cholinergic tone may mask the action of CCK on the vagal afferent pathway. To confirm the physiological relevance of our observations made in anaesthetized rats, we evaluated the effects of perivagal capsaicin treatment on pancreatic secretion in conscious rats. The administration of capsaicin directly to the nerve trunk of interest has the advantage of defining the neural pathway and avoids the generalized non-specific neural blockade observed with atropine. Although conscious rats showed a significantly higher level of basal secretion, we demonstrated that perivagal capsaicin treatment completely abolished pancreatic responses to both CCK and non-CCK stimuli such as maltose and hyperosmolar saline.



In conclusion, we have shown that, as in the case of CCK, non-CCK-dependent duodenal stimuli evoke pancreatic enzyme secretion via stimulation of the vagal afferent pathways originating from the duodenal mucosa. These observations indicate that the cholinergic vagal afferent pathways represent the primary targets on which postprandial mediators such as CCK and duodenal stimuli act to stimulate pancreatic secretion. This supports the Pavlovian concept that the neural system is the major regulator of pancreatic secretion.

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