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ANTAGONISM OF RECEPTORS FOR GASTRIN, CHOLECYSTOKININ AND GRP/BOMBESIN IN POSTPRANDIAL STIMULATION OF EXOCRINE PANCREAS IN DOGS

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Postprandial pancreatic secretion results from the interaction of neural and hormonal factors such as cholecystokinin (CCK), gastrin and gastrin releasing peptide (GRP), but their contribution to the net secretion is not established. Recent description of highly specific and potent hormonal receptor antagonists allows the determination of the physiological role of CCK, gastrin and GRP. In six dogs with chronic pancreatic fistulas, the blockade of CCK receptors by L-364, 718, gastrin receptors by L-365, 260 or GRP/bombesin receptors by nonapeptide RC-3095 failed to affect basal or sham-feeding induced pancreatic secretion indicating that none of these hormonal peptides plays a major role in this secretion. In contrast, the pancreatic response to ordinary feeding (which includes cephalic, gastric and intestinal phases), that was accompanied by a significant increment in plasma CCK and gastrin levels, was strongly inhibited (by over 50%) by L-364, 718 and slightly (by 20—30%) by L-365, 260 but not by RC-3095. Each antagonist was given at a dose that eliminated the secretory response to CCK, gastrin or GRP, respectively. We conclude that specific receptor antagonists are useful tools in assessing the physiological role of gut hormones in the control of pancreatic secretion and that none of the peptides tested appears to be involved in the cephalic phase. However, CCK plays a major role in the postprandial stimulation of pancreatic secretion.

Key words: receptors, pancreas, hormones, peptides, protein

INTRODUCTION

The physiological stimulation of pancreatic secretion such as occurs after feeding, is thought to be mediated by nerves and gut hormones including cholecystokinin (CCK), gastrin and gastrin-releasing peptide (GRP) (1). These mediators are also well recognized as direct stimulants of pancreatic secretion in vitro from the isolated pancreatic acini or acinar cells (2). The relative

contribution of these mediators to the postprandial pancreatic secretion has not been determined.

The assessment of the hormonal contribution has been made possible by the availability of specific radioimmunoassays of the gut hormones involved in the stimulation of exocrine pancreas and by the recent description of highly specific receptor antagonists for CCK (3, 4), gastrin (15, 6) and GRP/bombesin (7). These antagonists permit the measurement of pancreatic secretion in the absence of the biological effects of circulating CCK, gastrin or GRP/bombesin.

This study was designed to characterise the effects of specific hormonal receptor antagonists on pancreatic secretory responses to CCK, gastrin and GRP and to determine the contribution of the hormonal peptides to the cephalic-vagal and postprandial stimulation of pancretic secretion in dogs using these selective receptor antagonists.

MATERIAL AND METHODS

Secretory studies were carried out on conscious dogs, weighing 16—18 kg, and prepared with esophageal, gastric and pancreatic fistulas as described previously (8). The studies started 4—5 wk after the last surgery. Food but not water was withheld for about 18 h before each test. In all tests (except with feeding) the gastric fistula was opened, the stomach was rinsed by the irrigation the gastric cannula and gastric contents were drained to the exterior to prevent the gastric acid from entering the duodenum and releasing the gut hormones affecting the pancreatic secretion. The cannula of the pancreatic fistula was opened, rinsed with water, and provided with the hollowed obturator for the collection of pancreatis juice in 15 min aliquits as previously described (8, 9). The volume of the pancretic juice was recorded to the nearest 0.1 ml. The concentrations and outputs of HCO_3 and protein in the pancretic juice were measured in each sample and presented as 15 or 30 min outputs.

Several secretory tests were performed on each dog. In tests with physiological vagal stimulation, the esophagus was obstructed by pulling out the mucosa to divert the swallowed saliva and food to the exterior so that no food particle could enter the stomach. The dogs were offered 500 g of cooked homogenized ground beef for 15 min; the ingested meal fell from the esophagus back into the feeding pan and was repeatedly reconsumed. Gastric and pancreatic collections were made for 60 min before, and 90 min after the sham feeding. CCK-receptor antagonist (L-364, 718) or gastrin-receptor antagonist (L-365, 260) was injected i. v. as a single bolus dose (1 μmol/kg) 30 min before sham-feeding, while GRP/bombesin-receptor blocker (RC-3095) was infused at a constant dose (200 pmol/kg-h) for 30 min before, during and after sham feeding. In tests with ordinary feeding, 500 g of cooked beef liver homogenized with 100 ml water was fed and the pancreatic secretion was monitored during the next 2 h period. CCK-, gastrin- or GRP/bombesin-receptor antagonist was administered 30 min before the feeding as in tests with sham-feeding. In control tests, sham feeding or ordinary feeding alone with vehicle instead of receptor antagonist was used.

In tests with exogenous hormonal stimulation, synthetic CCK-8 (Squibb Institute for Medical Research, Princeton, N. J.), gastrin heptadecapeptide (G-17) (a gift from prof. R. A. Gregory, Liverpool UK) or GRP (a gift of Prof. N. Yanaihara, Shizuoka, Japan) was infused i. v. at a constant dose to induce the pancreatic secretion similar to that obtained in response to ordinary feeding. For a comparison, the maximal pancreatic protein secretion was induced by i. v. infusion

of CCK-8 at a dose (250 pmol/kg-h) that in our experience produced maximal stimulation. The collection of the pancreatic secretion in those tests was made in similar way to that in sham-feeding experiments.

In tests involving sham feeding, ordinary feeding and infusion of exogenous CCK-8, gastrin or GRP, the blood samples were taken from the peripheral vein at 15—30 min intervals for radioimmunoassay of plasma gastrin (9) and CCK as described previously (10, 11).

In all tests involving CCK-8, gastrin and GRP, a solution of 1% canine serum albumin was used to dissolve these peptides to prevent their degradation and adsorption to the plastic tubes during i. v. infusion.

The antagonists for CCK- and gastrin-receptors (L-364, 718 and L-365, 260 were kindly provided by Dr. P. S. Anderson, Merck Sharp and Dohme Research Labs., West Point, PA, USA). The pseudopeptide GRP/bombesin receptor antagonist (D-Trp6, Leu13ψCH2NHLeu14) bombesin (6—14) was synthesized by one of us (R-Z. C.) in New Orleans Laboratories using solid phase methods on a benzydrylamine resin and cleaved from the resin by HF treatment. After purification of crude product by HPLC, this antagonist showed a purity greater than 95%. It inhibited bombesin binding in 3T3 cells at an IC50 = 5.88 nM and was designated with a laboratory code number RC-3095. It showed a similar potency in suppressing the bombesin-induced amylase secretion from the dispersed pancreatic acini to that of (D-Phe6-Leu13ψCH2 NH Leu 14) bombesin-(6-14) originally synthesized by Coy et al. (7).

Statistics

All data are presented as mean ($\pm$ SEM). For statistical evaluation, one-way analyses of variance for repeated measures and (when appropriate) Student's test were used. Differences were considered significant if $P < 0.05$.

RESULTS

Effects of CCK-, gastrin- and GRP-antagonists on pancreatic responses to exogenous CCK, gastrin and GRP in dogs.

Figs 1—3 show the pancreatic protein responses to CCK-8, gastrin or GRP infused i. v. at a dose producing the rate of pancreatic protein secretion similar to that induced by ordinary meat feeding in these dogs. The most effective stimulant of protein secretion in these experiments was CCK, which was about 4 times and 10 times more potent than GRP and gastrin, respectively. It is of interest that the increase in pancreatic secretion caused by CCK and GRP resulted in similar increment in plasma CCK level. Plasma immunoreactive gastrin level caused by GRP infusion reached about 30% of that achieved by infusion of exogenous gastrin, producing a similar rate of pancreatic secretion (Table 1).

Administration of L-364, 718 (1 μmol/kg i. v.), L-365, 260 (1 μmol/kg i. v.) or RC-3095 (200 pmol/kg-h i. v.) did not affect significantly the basal pancreatic secretion. L-364, 718 completely abolished the protein response to CCK and reduced the responses to GRP and gastrin by about 70% and 50, respectively. L-365,260 completely abolished the response to gastrin and
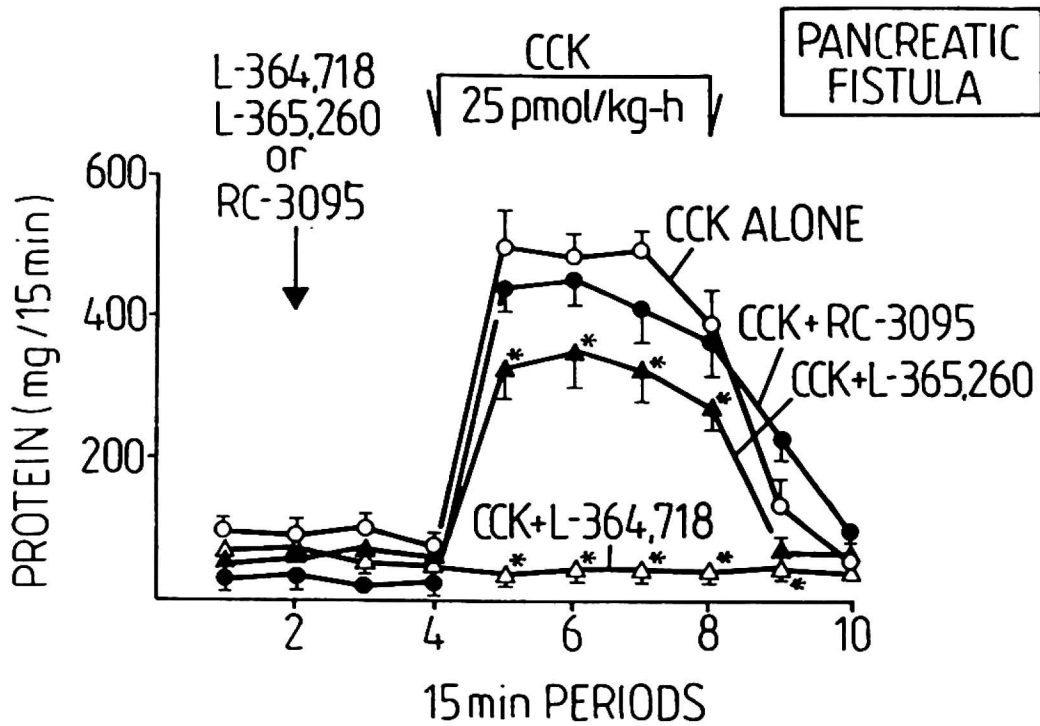
Fig. 1. Effects of L-364,718 (1μmol/kg), L-365,260 (1μmol/kg) or RC-3095 (200 pmol/kg/h) on pancreatic protein secretion under basal conditions and response to i.v. infusion of CCK in a constant dose (25 pmol/kg-h). Means ±SEM of 6 tests on 6 dogs. Asterisk indicates significant (P<0.05) decrease below the value obtained with CCK alone.

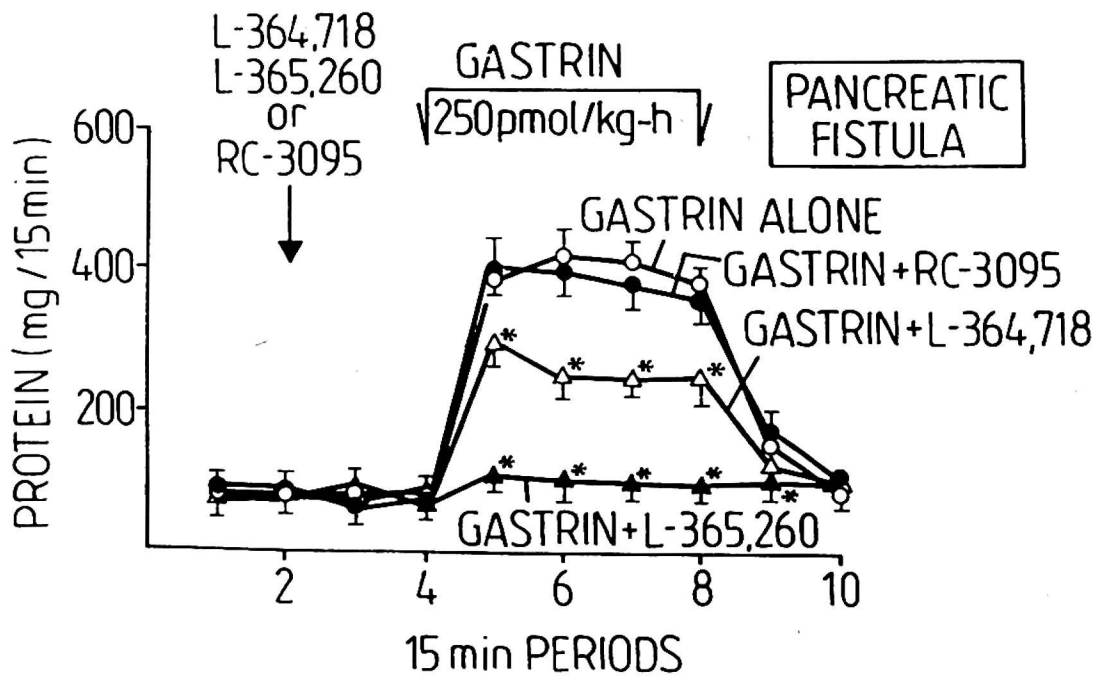*Fig. 2.* Effects of L-364,718 (1μmol/kg), L-365,260 (1μmol/kg) or RC-3095 (200 pmol/kg/h) on pancreatic protein secretion under basal conditions and in response to i.v. infusion of gastrin in a constant dose (250 pmol/kg/h). Means ± SEM of 6 tests on 6 dogs. Asterisk indicates significant (P<0.05) decrease below the value obtained with gastrin alone.
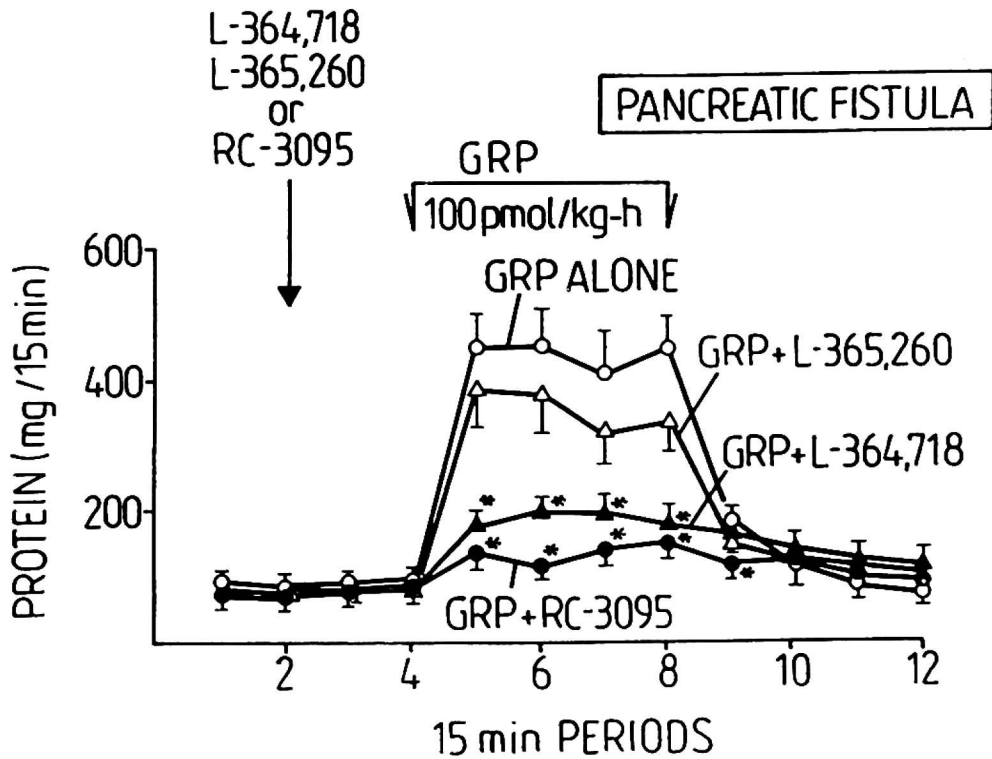
Fig. 3. Effects of L-364,718 (1μmol/kg), L-365,260 (1μmol/kg) or RC-3095 (200 pmol/kg/h) on pancreatic protein secretion under basal state and in response to i.v. infusion of GRP in a constant dose (100 pmol/kg/h). Means ±SEM of 6 tests on 6 dogs. Asterisk indicates significant (P<0.05) decrease below the value obtained with GRP alone.

reduced by about 30% the response to CCK. It tended to decrease the response to GRP but this was significant only at one study period. Infusion of RC-3095 completely eliminated the secretory response to GRP and reduced plasma hormonal (gastrin and CCK) responses to this peptide but failed to affect those induced by CCK or gastrin.

Effects CCK-, gastrin- or GRP-receptor antagonists on pancereatic secretory responses to sham-feeding and ordinary feeding in dogs.

Sham feeding carried out for a standard 15 min period resulted in an immediate and prolonged stimulation of pancreatic protein secretion. There was also a small but significant increase in the volume flow and bicarbonate output but these results have been omitted for the sake of clarity. The protein output reached a peak during the period of sham feeding and then steadily declined during the rest of the experiment. The peak protein output in sham-fed dogs reached about 30% of maximal response (1050 ±180 mg/15 min) to CCK (250 pmol/kg-h) in these animals. The secretory output was significantly increased throughout the examination period (90 min) but tended to decline, falling at the end of experiment to about 50% of the initial peak value. Plasma gastrin was also significantly elevated over basal values during

Table 1. Plasma gastrin and CCK concentrations in tests with infusion of exogenous peptides as in Figs 1—3 and after sham feeding and ordinary meat feeding as in Figs 4 and 5. Means ± SEM of 6 tests on 6 dogs. Values are hormone concentrations in the blood samples taken under basal conditions at the begining of experiment and 60—90 min after the start of peptide infusion. In tests with sham feeding and ordinary feeding, the values represent plasma hormones in blood samples taken, respectively, 15 min and 60 min after the start of these procedures.

	PLASMA GASTRIN (pM)	PLASMA CCK (pM)
BASAL	18 ± 4	0.8 ± 0.2
INFUSION OF CCK (25 pmol/kg-h)	26 ± 4	9.1 ± 1.6*
INFUSION OF GASTRIN (250 pmol/kg-h)	334 ± 28*	1.5 ± 0.4
INFUSION OF GRP (100 pmol/kg-h)	83 ± 14*	7.6 ± 1.8*
INFUSION OF GRP + RC-3095 (200 pmol/kg-g)	38 ± 12**	3.2 ± 1.4**
SHAM FEEDING	31 ± 4*	1.5 ± 0.8
SHAM FEEDING + L-364,718 (1 μmol/kg)	37 ± 6*	2.4 ± 0.7
SHAM FEEDING + L-265,260 (1 μmol/kg)	26 ± 4*	2.3 ± 0.7
SHAM FEEDING + RC-3095 (200 pmol/kg-h)	26 ± 4*	1.4 ± 0.4
MEAT FEEDING	78 ± 12*	5.9 ± 1.2*
MEAT FEEDING + L-364,718 (1 μmol/kg)	120 ± 18**	7.9 ± 1.8*
MEAT FEEDING + L-365,260 (1 μmol/kg)	85 ± 17*	6.4 ± 1.6*
MEAT FEEDING + RC-3095 (200 pmol/kg-h)	64 ± 14*	5.8 ± 1.4*

* Significant ($P < 0.05$) increase above the basal value.

** Significant ($P < 0.05$) change as compared to control value obtained with infusion of GRP, sham feeding or feeding.

the initial postprandial period, but then declined to basal value (*Table 1*). Plasma CCK levels were not significantly affected by sham feeding. Pretreatment with L-364, 718, L-365, 260 or RC-3095 at the doses that completely eliminated the pancreatic secretory responses to CCK, gastrin or GRP, respectively, did not affect significantly the pancreatic protein response to sham feeding (*Fig. 4*). Plasma gastrin and CCK levels were also unaffected by the administration of specific antagonists of CCK-, gastrin- or GRP/bombesin-receptors (*Table 1*).

The pancreatic protein response to ordinary feeding was about twice as high as that to sham feding but similar to that obtained by infusion of CCK
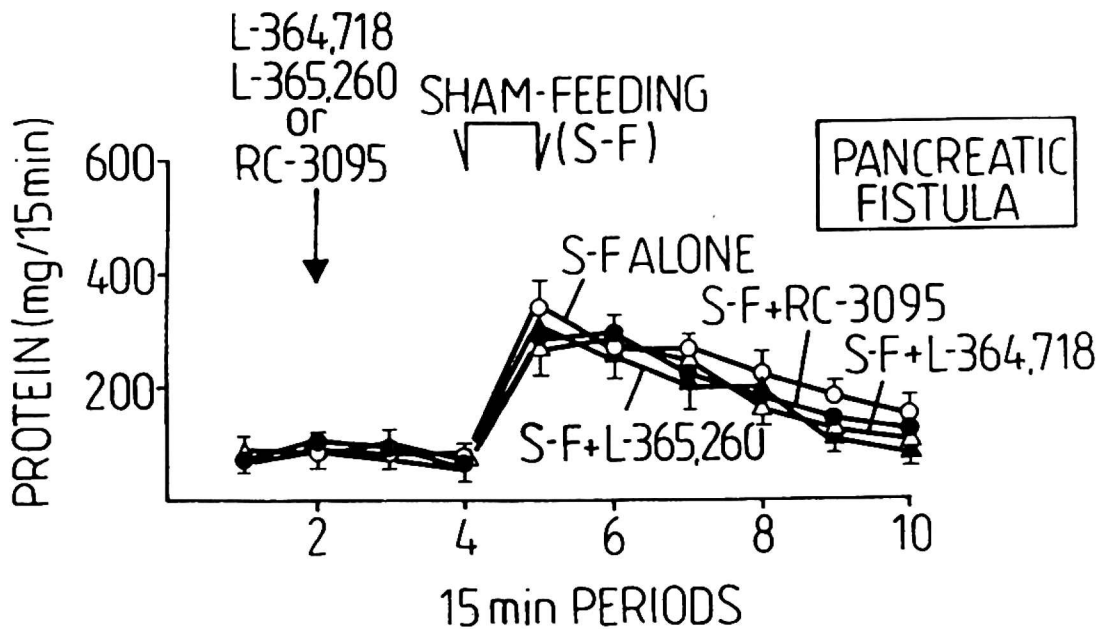
Fig. 4. Effects of L-364, 718, L-365, 260 or RC-3095 on basal and sham-feeding stimulated pancreatic protein secretion. Mean ± SEM of 6 tests on 6 dogs.
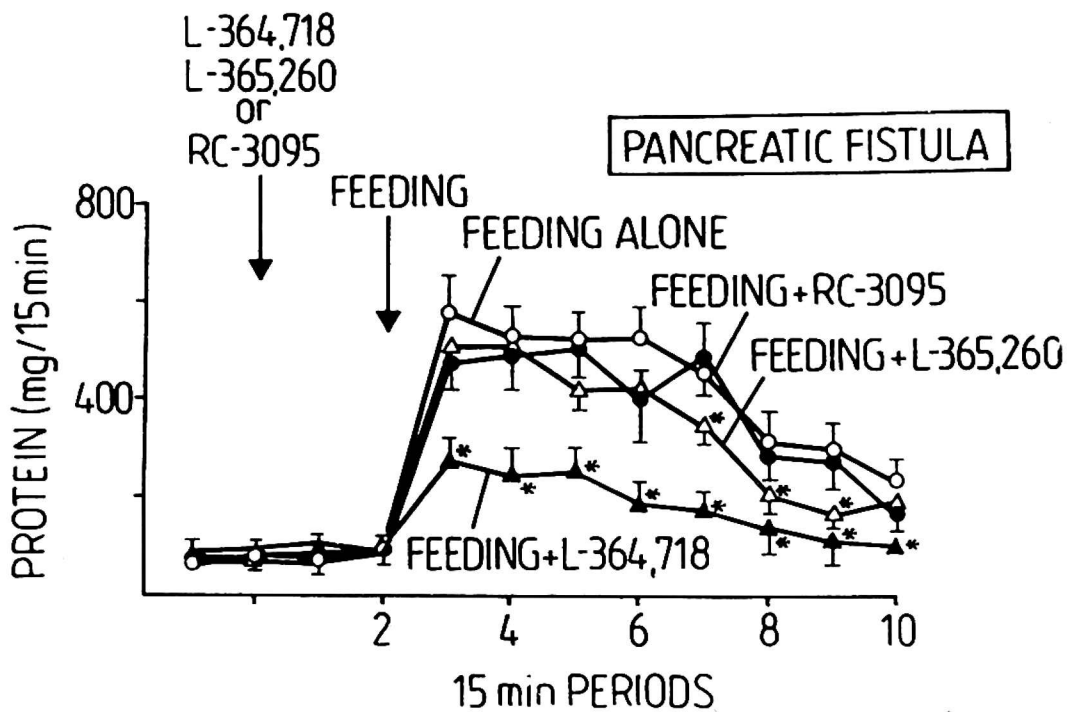

Fig. 5. Effects of L-364, 718, L-365,260 or RC-3095 on basal and meal stimulated pancreatic protein secretion. Mean ±SEM of 6 tests on 6 dogs. Asterisk indicates significant decrease below the value obtained with meal alone.

(25 pmol/kg-h), gastrin (250 pmol/kg-h) or GRP (100 pmol/kg-h). The secretory response started immediately after feeding and was relatively well sustained for 1.5 h but then tended to decline to about 50% at the end of 2 h experiment. The postprandial pancreatic secretion was accompanied by a significant increment in plasma CCK and gastrin (*Table 1*). These increments in plasma hormones were observed throughout the postprandial period. The postprandial plasma CCK levels were similar to those attained after infusion

of GRP, but were somewhat lower than those obtained with infusion of exogenous CCK. The pretreatment with L-364,718 reduced the postprandial protein response by 55—65% and this reduction was significant throughout the postprandial observation periods. Administration of L-365, 260 resulted in a small (20—30%) but significant reduction in pancreatic secretion in the late postprandial period but RC-3095 did not affect significantly the protein response to meat feeding. Plasma gastrin was significantly elevated after administration of L-364,718 but unchanged by L-365,260 or RC-3095. Plasma CCK levels were unaffected by any of the receptor antagonists used (*Table 1*).

DISCUSSION

This study demonstrates that sham feeding induces a marked stimulation of pancreatic protein secretion, that cannot be affected by specific antagonists of receptors for CCK, gastrin or GRP/bombesin. The pancreatic response to ordinary feeding can be strongly reduced (by over 50%) using specfic CCK-receptor atagonist (L-364,718) and slightly (by about 20%) by gastrin-receptor antagonist (L-365,260) but not by GRP/bombesin-receptor antagonist (RC-3095). These results suggest that none of the three peptides tested plays a major role in the mechanism of cephalic-vagal excitation of exocrine pancreas, but that CCK contributes to the major portion of the postprandial stimulation of pancreatic protein secretion.

Although plasma gastrin concentrations were found in this and previous studies (4, 9) to increase the response to the cephalic vagal excitation and to ordinary feeding, these increments do not appear to be sufficient to stimulate exocrine pancreas because neither removal of the endogenous source of this hormone (antral mucosectomy) nor infusion of exogenous gastrin to mimic the postprandial increment in plasma hormone (11), caused any significant alteration in the pancreatic secretion. The failure of the blockade of gastrin receptors by L-365,260 to affect the pancreatic secretion observed in this study also rules against any major role of gastrin in the postprandial stimulation of exocrine pancreas. It is of interest that following the blockade of CCK receptors by L-364,718, a marked increase in plasma gastrin was observed and this could be attributed to the removal of the inhibition of the G-cells by somatostatin whose release from the D-cells appears to be controlled by CCK (12).

Electrical vagal stimulation in anesthetized dogs was reported to stimulate the release of CCK (13), but, as shown in this report, the physiological stimulation by sham feeding did not appear to induce any significant release of CCK. This is indicated by the lack of changes in the plasma level of CCK after sham feeding and the fact that the blockade of CCK receptors by L-364,718

did not cause any alterations in the pancreatic response to cephalic-vagal stimulation. Thus, it is reasonable to conclude that endogenous CCK, like gastrin, is not the major factor in the cephalic phase of exocrine pancreas but probably direct vagal excitation is involved in this stimulation.

The above conclusion does not apply to the pancreatic secretory response to ordinary feeding which involves the combination of cephalic, gastric and intestinal phases and which produces much stronger stimulation of exocrine pancreas and greater increments in plasma levels of both CCK and gastrin. Blocking of CCK-receptors by specific antagonist (L-364,718) reduced by more than 50% the postprandial pancreatic protein secretion. It should be emphasized that various peptides of the CCK/gastrin family may interact with both CCK and gastrin receptors in the pancreatic acinar cells and it may be difficult to determine what effect on the cells is due to the occupation of which receptors (6). With the use of L-364,718 which has higher selectivity for CCK receptors and L-365,260 which shows a greater selectivity for gastrin receptors, the involvement of CCK and gastrin in various physiological processes such as cephalic phase and gastro-intestinal phase (ordinary feeding) can be clearly differentiated.

Although bombesin-like immunoreactivity was detected in the gut (14, 15) and the pancreas (16), little is known about the physiological significance of bombesin-like peptide in the regulation of exocrine pancreas. Bombesin is known to liberate gastrin (17) and CCK (18, 19) and to stimulate exocrine pancreas (20), at least in part, through the release of these gut hormones. Indeed, the blockade of CCK and gastrin receptors by specific antagonists significantly reduced in our study the pancreatic response to exogenous GRP which is a mammalian counterpart of the amphibian bombesin (21). It is of interest that complete elimination of the secretory effects of GRP could be achieved only by using highly specific and competitive receptor antagonist (RC-3095) similar to the pseudopeptide GRP/bombesin blocker that has been recently developed by Coy et al. (7). This antagonist was found in our study to eliminate the pancreatic secretion and to reduce the plasma gastrin and CCK responses to GRP *in vivo*. We thought that this antagonist might be a useful tool for the evaluation of the physiological role of GRP in the stimulation of exocrine pancreas *in vivo*. Recently, Holst et al (22) reported that similar GRP/bombesin receptor antagonism in anesthetized pigs competitively abolished the gastrin release and motor response to vagal stimulation. Our results show, however, that a highly specific and potent GRP/bombesin receptor antagonist used at a dose that completely eliminated the pancreatic response to exogenous GRP, failed to affect significantly the cephalic-vagal stimulation of exocrine pancreas or the accompanying plasma gastrin release in dogs. Similarly the GRP/bombesin antagonism did not affect the postprandial stimulation of exocrine pancreas and related increments in

plasma gastrin or CCK. This suggests that GRP/bombesin peptides do not play a major role in the physiological regulation of exocrine pancreatic secretion in dogs.

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