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CHOLECYSTOKININ RECEPTOR ANTAGONISM BY PEPTIDERGIC AND NON-PEPTIDERGIC AGENTS IN RAT PANCREAS

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SUMMARY

1. Graded doses of bombesin infused I.V. into conscious rats with chronic pancreatic fistulae induced a dose-dependent stimulation of protein secretion, similar to that obtained with caerulein. This stimulation does not appear to be mediated by cholecystokinin (CCK) receptors because peptidergic (CR-1409) and non-peptidergic (L-364718) CCK antagonists failed to affect protein secretion at a dose range which caused almost complete suppression of caerulein-induced pancreatic secretion.

2. Studies *in vitro* on isolated rat pancreatic acini revealed that caerulein, pentagastrin and bombesin all showed the same efficacy in their ability to stimulate amylase release. In contrast, CCK antagonists competitively inhibited amylase release induced by caerulein and pentagastrin but not by bombesin or urecholine, indicating that the latter two agents act directly on acinar cells via receptors which are separate from those involved in stimulation induced by caerulein and pentagastrin.

3. DNA synthesis, measured by the incorporation of [^{3}H]thymidine into DNA, was significantly stimulated by caerulein, soybean trypsin inhibitor (FOY 305), pentagastrin and by bombesin in a dose-dependent manner. CCK receptor antagonists prevented stimulation of DNA synthesis induced by caerulein, FOY 305 and pentagastrin but not by bombesin.

4. This study indicates that bombesin strongly stimulates pancreatic enzyme secretion, with an efficacy similar to that of caerulein, and also exerts a potent growth-promoting action on the pancreas, both effects appearing to be mediated by mechanisms independent of the CCK receptors.

INTRODUCTION

Cholecystokinin (CCK) is generally recognized as the major hormonal stimulant of pancreatic enzyme secretion (Harper & Raper, 1943) and of tissue growth (Folsch, Winckler & Wormsley, 1978; Peterson, Solomon & Grossman, 1978). The release of CCK has been shown to be increased in dogs by bombesin (Feuder, Curtis, Rayford & Thompson, 1976) and in rats by trypsin inhibitors (Brand & Morgan, 1981; Liddle, Goldfine & Williams, 1984), both of which are known to be potent stimulants of pancreatic secretion (Khayambashi & Lyman, 1968; Feuder *et al.* 1976; Konturek,

Krol & Tasler, 1976; Brand & Morgan, 1981; Liddle *et al.* 1984) and tissue growth (Lhoste, Aprahamian, Pousse, Hoeltzel & Stock-Damge, 1985; Görke, Printz, Koop, Rausch, Richter, Arnold & Adler, 1986; Lee, Newman, Praissman, Cooney & Lebenthal, 1986; Lehy, Puccio, Charriot & Labeille, 1986; Folsch, Cantor, Willms, Schafmayer, Becker & Creutzfeldt, 1987).

The possible role of CCK in modulating pancreatic secretion and growth has been implicated after the recent use of highly specific and potent CCK receptor antagonists which permit measurement of pancreatic secretion in the absence of any physiological effects of CCK (Chang, Lotti, Monagham, Birnbaum, Stapley, Goetz, Albers-Schoenberg, Patchett, Liesch, Hensens & Springer, 1985; Jensen, Zhon, Murphy, Jones, Setnikar, Rovati & Gardner, 1986; Makovec, Christe, Bani, Revel, Setnikar & Rovati, 1986; Lotti, Pendleton, Gould, Hanson, Chang & Clineschmidt, 1987; Pendleton, Bendesky, Schaffer, Nolan, Gould & Clineschmidt, 1987).

This study was undertaken to determine the contribution of exogenous and endogenous CCK to pancreatic secretion and growth in rats using the most potent available CCK receptor antagonists.

METHODS

Secretory studies

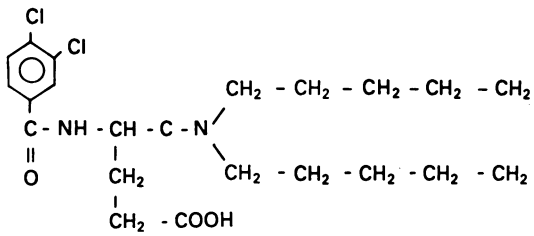
Secretory studies were carried out *in vivo* in conscious rats with chronic pancreatic fistulae and *in vitro* using rat pancreatic acini.

Pancreatic fistulae were prepared surgically under ether anaesthesia in Wistar rats, weighing 200–250 g. The abdomen was opened by a mid-line incision and a polyethylene cannula (PE50) implanted in the distal end of the common bile–pancreatic duct at a point just proximal to the entrance to the duodenum and tied in place. A second cannula (PE 100) was inserted through a small incision in the least vascularized portion of the forestomach and placed into the duodenum with its tip in the mid-duodenum. Both cannulae were brought out through a subcutaneous tunnel in the skin on the back where they were secured and connected by a short silicone tube in order to maintain continuous flow of bile and pancreatic juice into the duodenum until the start of experiments. During each experiment, the cannulae were disconnected. The pancreatic cannula was used for collection of bile and pancreatic juice while the duodenal cannula was employed for reinfusing bile and pancreatic juice into the duodenum. For I.V. hormone infusions, an additional catheter was inserted into the external jugular vein.

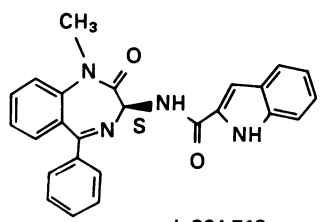
Experiments were started 2 days after recovery from surgery. Combined bile–pancreatic secretion was collected in 30 min aliquots in pre-weighed vials for volume measurements and 50 μl samples from each collection period were taken for protein determination (Konturek *et al.* 1976). The rest was diluted (1:2) with 154 mM-NaCl and returned to the rat via the duodenal cannula. After a resting period of 2 h when only 154 mM-NaCl was infused I.V. (2 ml h^{-1}), secretion was stimulated by the infusion of graded doses of caerulein (12·5-800 pmol kg^{-1} h^{-1}), pentagastrin (300–9600 pmol kg^{-1} h^{-1}) or bombesin (50–1600 pmol kg^{-1} h^{-1}), each dose being infused for 30 min and then doubled. All peptides were dissolved in 154 mM-NaCl containing 0·1% bovine serum albumin (Sigma Co., St Louis, MO, USA) and were infused either alone or in combination with either CR-1409 (10 mg kg^{-1} h^{-1}) or L-364718 (1 mg kg^{-1} h^{-1}). CR-1409 was kindly supplied by Dr L. Rovati, Rotta Research Labs, Milano, Italy and L-364718 was a gift from Dr P. S. Anderson, Merck Sharp and Dohme Research Labs, West Point, PA, USA. The chemical structures of L-364718 and CR-1409 are shown in Fig. 1. The concentrations were selected on the basis of previous studies in which they had been shown to abolish the effects of CCK on target organs (Jensen *et al.* 1986; Makovec *et al.* 1986; Lotti *et al.* 1987; Pendleton *et al.* 1987).

Isolated pancreatic acini were prepared by collagenase digestion of the pancreas obtained from male Wistar rats (150–200 g) fasted overnight. Animals were killed by cervical dislocation and the pancreas was removed and then digested by highly purified collagenase (CLSPA, 540 U mg^{-1}, Cooper Biomedical, Freehold, NJ, USA), according to the method of Amsterdam, Solomon & Jamieson (1978).

Dispersed acini were suspended in an incubation medium (pH 7·4) containing 24·5 mM-HEPES, 98 mM-NaCl, 4·0 mM-KCl, 11·7 mM-KH_2PO_4, 1·0 mM-$MgCl_2$, 0·3 mM-$CaCl_2$, 5·0 mM-glucose, 1 % (w/v) essential and non-essential amino acid mixture (SERVA Feinbiochemica, Heidelberg, FRG), 2·0 mM-glutamine, 0·2 % bovine serum albumin and 0·01 % (w/v) trypsin inhibitor. The incubation solution was saturated with oxygen and maintained at 37 °C in a shaking bath (60 oscillations min^{-1}). Acinar suspensions were incubated in the presence of various concentrations of caerulein,



CR-1409



L-364 718

Fig. 1. Chemical structure of CR-1409 and L-364718.

pentagastrin, bombesin and urecholine alone or in combination with the CCK receptor antagonists, CR-1409 or L-346718. In control experiments, secretagogues alone were added to the incubation medium. After incubation, tubes were centrifuged at 1000 *g* for 5 min and the supernatant separated from the pellet. Amylase content in the supernatant and dissolved pellet were determined separately as described by Bernfeld (1955). Amylase secretion was expressed as the percentage increment over basal value. Incubations were duplicated. Unstimulated amylase release during the entire experimental period was determined and presented as the basal value.

Studies on DNA synthesis in the pancreas

Studies were performed on male Wistar rats weighing 150–200 g. Animals were housed in cages with wire-mesh bottoms in a room with a 12 h light–dark cycle and fasted 24 h before death. Drinking water was available *ad libitum*. All rats received I.P. injections of agonists and/or antagonists at 8 h intervals for 48 h and then were killed by cervical dislocation 8 h after the last injection. The experiments involved using the following agents: (1) CR-1409 (10 mg kg^{-1}) or L-364718 (1 mg kg^{-1}); (2) caerulein alone (0·22 or 3·7 nmol kg^{-1}); (3) caerulein (0·22 nmol kg^{-1}) combined with CR-1409 or L-364718; (4) caerulein (3·7 nmol kg^{-1}) combined with CR-1409 or L-364718; (5) FOY 305 (200 mg kg^{-1} by mouth) alone; (6) FOY 305 combined with CR-1409 or L-364718; (7) bombesin (6·7 nmol kg^{-1}) alone; (8) bombesin combined with CR-1409 or L-364718; (9) pentagastrin (350 nmol kg^{-1}) alone; (10) pentagastrin combined with CR-1409 or L-364718; (11) 154 mM-NaCl in a volume (1 ml) equal to those of the other compounds. Each experimental group included five rats and each study was repeated twice so that $n = 10$ for each series of experiments.

After 2 days of treatment, the animals were killed and the pancreas was carefully dissected free of mesentery and fat, weighed and minced. The rate of DNA synthesis in minced pancreatic tissue from the first study was determined by incubating the tissue at 37 °C for 30 min in incubation medium (GIBCO Lab., Grand Island, NY, USA) containing 2 μCi ml^{-1} [^{3}H]thymidine (19·3 Ci

mmol^{-1}, New England Nuclear, Boston, MA, USA). During incubations, the flasks were gassed continuously with 95% O_2+5% CO_2. The reaction was stopped with 0·4 M-perchloric acid containing carrier thymidine at a concentration of 5 mM. Samples were washed in 0·2 M-perchloric acid and RNA was removed by hydrolysis in 0·3 M-KOH for 60 min at 37 °C. DNA was reprecipitated with 10% perchloric acid. The RNA content of the supernatant was measured using the orcinol reaction (Ceriotti, 1955). After standing on ice for 10 min, the DNA-containing tubes were centrifuged and the supernatant discarded. DNA in the residual pellet was solubilized in 10% perchloric acid (3 ml 100 mg tissue^{-1}) heated to 70 °C for 20 min and then centrifuged (3000 r.p.m. for 5 min). Using calf thymus DNA as a standard, the DNA content of the samples was determined by the procedure of Burton (1956) as modified by Giles & Myers (1975). The incorporation of [^{3}H]thymidine into DNA was determined by counting 0·5 ml DNA-containing supernatant in a Beckman liquid scintillation system (Beckman Instruments, Palo Alto, CA, USA). DNA and RNA contents were expressed as milligrams per total pancreas weight. DNA synthesis was expressed as disintegrations per min [^{3}H]thymidine per microgram DNA.

Statistics

The results are expressed as means (±S.E.M.). Differences between means were evaluated using Student's t test for unpaired data and was considered significant if $P < 0·05$.

RESULTS

Effects of CR-1409 and L-364718 on basal and hormonally stimulated pancreatic protein secretion in conscious rats

In conscious rats with chronic pancreatic fistulae, mean bile–pancreatic protein output at rest was 7·3±2·1 mg (30 min^{-1}). In control experiments in which graded doses of caerulein (12·5–800 pmol kg^{-1} h^{-1}) were infused I.V. there was a dose-dependent increase in protein output which reached a maximum at a dose of 200 pmol kg^{-1} h^{-1} (Fig. 2). Neither CR-1409 (10 mg kg^{-1} h^{-1}) nor L-364718 (1 mg kg^{-1} h^{-1}) affected basal protein secretion but both almost completely abolished the effects of graded doses of caerulein, with the exception of the highest doses (400 and 800 pmol kg^{-1} h^{-1}) which still induced a small, but significant, increase in protein output. This increase only represented 20% of that achieved in tests with caerulein alone.

After infusion of graded doses of bombesin (50–1600 pmol kg^{-1} h^{-1}), pancreatic protein output also showed a dose-dependent increase which reached a maximum at a dose of 200 pmol kg^{-1} h^{-1}) (Fig. 3). This maximal protein response to bombesin was not significantly different from that obtained with caerulein. Administration of CR-1409 (10 mg kg^{-1} h^{-1}) or L-364718 (1 mg kg^{-1} h^{-1}) failed to affect either basal or bombesin-induced pancreatic protein secretion.

Infusion of pentagastrin in graded doses (300–9600 pmol kg^{-1} h^{-1}) caused a small increase in protein output which at the largest doses reached about 35% of the maximum obtained during caerulein infusion. Addition of CR-1409 (10 mg kg^{-1} h^{-1}) or L-364718 (1 mg kg^{-1} h^{-1}) almost completely abolished the protein response to pentagastrin and the rate of secretion fell to a level not significantly ($P < 0·05$) different from basal values. These results have not been included.

Effects of CR-1409 and L-364 718 on amylase release from dispersed pancreatic acini

Incubation of isolated rat pancreatic acini in the presence of various concentrations of caerulein (10^{-15}–10^{-7} M) induced a concentration-dependent stimulation of

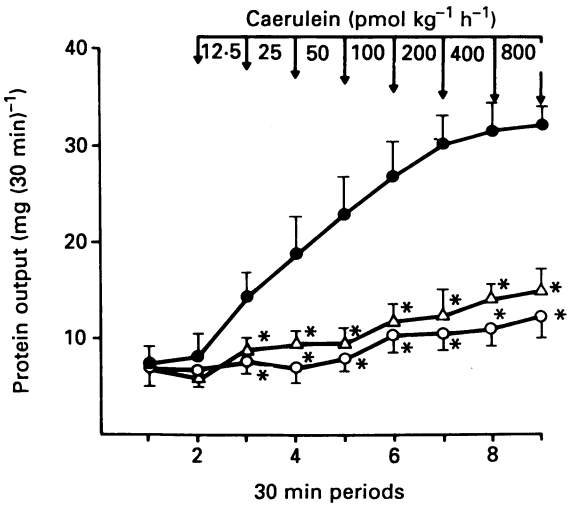


Fig. 2. Effects of graded doses of caerulein infused I.V. either alone or in combination with CR-1409 (10 mg kg^{-1} h^{-1}) or L-364718 (1 mg kg^{-1} h^{-1}), on protein output from pancreatic fistulae in conscious rats. ●, caerulein alone; △, caerulein plus CR-1409; ○, caerulein plus L364718. Means ± S.E.M. of six experiments on six pancreatic fistula rats. * Indicates significant ($P < 0{\cdot}05$) decrease below the control value obtained with caerulein alone.

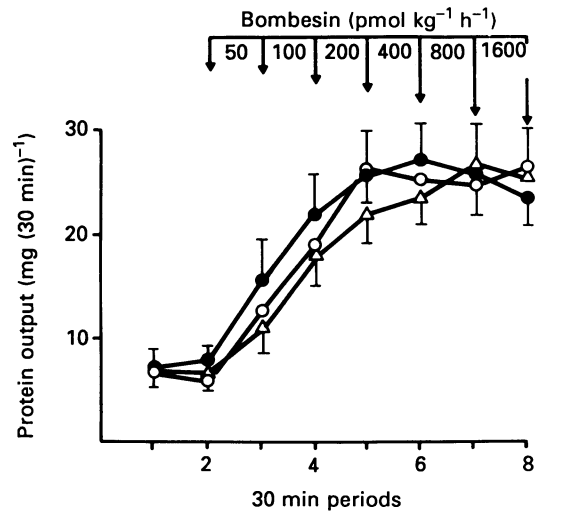


Fig. 3. Effects of graded doses of bombesin infused I.V. either alone or in combination with CR-1409 (10 mg kg^{-1} h^{-1}) or L-364718 (1 mg kg^{-1} h^{-1}). ●, bombesin alone; △, bombesin plus CR-1409; ○, bombesin plus L364718. Means ± S.E.M. of six experiments in six conscious rats with pancreatic fistulae.

amylase secretion, which reached a maximum at 10^{-11} M and then gradually declined with further increase in the caerulein concentrations (Figs 4 and 5). Addition of CR-1409 (10^{-7} or 10^{-6} M) to the incubation medium containing caerulein at various concentrations caused a shift in the amylase dose–response curve to the right without significantly changing the maximal amylase response to caerulein. This maximal

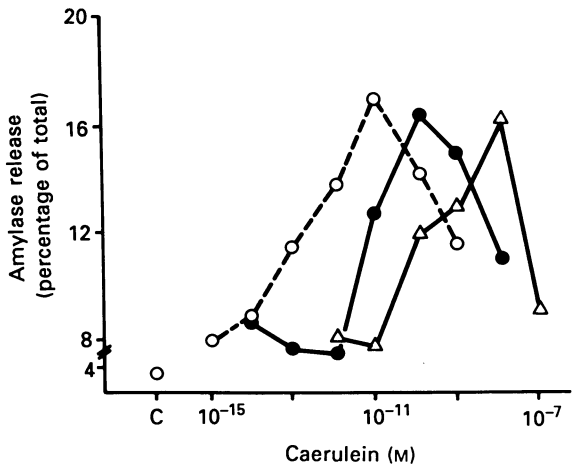


Fig. 4. Amylase release from dispersed rat pancreatic acini in response to various concentrations of caerulein added to the incubation medium, either alone or in combination with CR-1409. ○, caerulein alone; ●, caerulein plus CR-1409 (10^{-7} M); △, caerulein plus CR-1409 (10^{-6} M). Means of six to eight separate tests on acini obtained from six to eight rats. C, control.

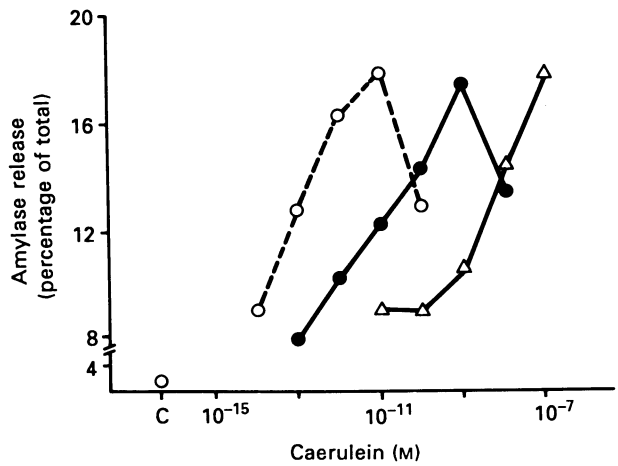


Fig. 5. Amylase release from dispersed rat pancreatic acini in response to various concentrations of caerulein added to the incubation medium, either alone or combined with L-364718. ○, caerulein alone; ●, caerulein plus L364718 (10^{-9} M); △, caerulein plus L364718 (10^{-7} M). Means of six to eight separate tests on acini obtained from six to eight rats. C, control.

response was achieved at concentrations of caerulein of 10^{-10} and 10^{-8} M when the CCK antagonist CR-1409 was used at 10^{-7} and 10^{-6} M, respectively (Fig. 4). Addition of L-364718 to the incubation medium of acini containing various concentrations of caerulein also caused a shift in the amylase dose–response curve to the right without significantly altering the maximal amylase response to this peptide. The maximal amylase response was achieved at 10^{-9} and 10^{-7} M-caerulein when L-364718 was added to the incubation medium at 10^{-9} and 10^{-7} M, respectively (Fig. 5).

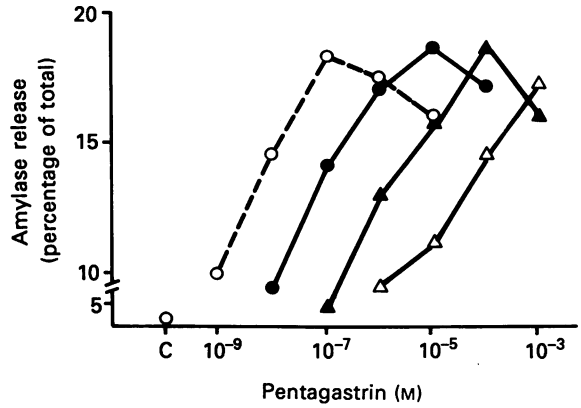


Fig. 6. Pancreatic amylase dose–response curves to pentagastrin alone or in combination with CR-1409. Means of six to ten tests on acini obtained from six to ten rats. ○, pentagastrin alone; ●, pentagastrin plus CR-1409 (10^{-7} M); ▲, pentagastrin plus CR-1409 (10^{-6} M); △, pentagastrin plus CR-1409 (10^{-4} M). C, control.

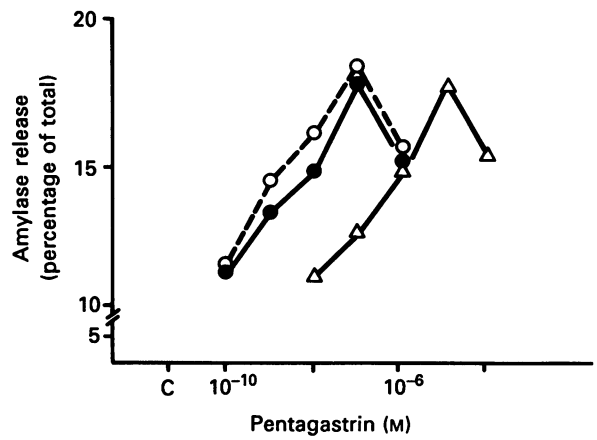


Fig. 7. Pancreatic amylase dose–response curves to various concentrations of pentagastrin alone or combined with L-364718. ○, pentagastrin alone; ●, pentagastrin plus L364718 (10^{-9} M); △, pentagastrin plus L364718 (10^{-7} M). Means of six to ten tests on dispersed pancreatic acini obtained from six to ten rats. C, control.

The dose–response curve for amylase release induced by increasing concentrations of pentagastrin (10^{-9}–10^{-5} M) is shown in Fig. 6. Pentagastrin resulted in a concentration-dependent increase in amylase release which reached a maximal response at 10^{-7} M. There was a shift of the response curve to the right in the presence of various doses of CR-1409; however, the maximal response to pentagastrin remained unaffected, and occurred at 10^{-5}, 10^{-4} and 10^{-3} M when CR-1409 was tested at concentrations of 10^{-7}, 10^{-6} and 10^{-4} M. A similar shift to the right was observed when L-364718 was added to the incubation medium of the acini (Fig. 7).

In contrast to the studies mentioned above, urecholine- and bombesin-induced dose–response curves were unaffected by CR-1409 at a concentration of 10^{-4} M or by L-364718 at 10^{-5} M (Fig. 8).

When added to the incubation medium in gradually increasing concentrations, CR-1409 and L-364718 caused a concentration-dependent inhibition of amylase release stimulated by submaximal concentrations of caerulein (10^{-12} M) or pentagastrin (10^{-8} M) (Fig. 9). The strongest effect was observed at 10^{-4} M-CR-1409 or 10^{-6} M-L-364718, when the amylase release was reduced to the basal value.

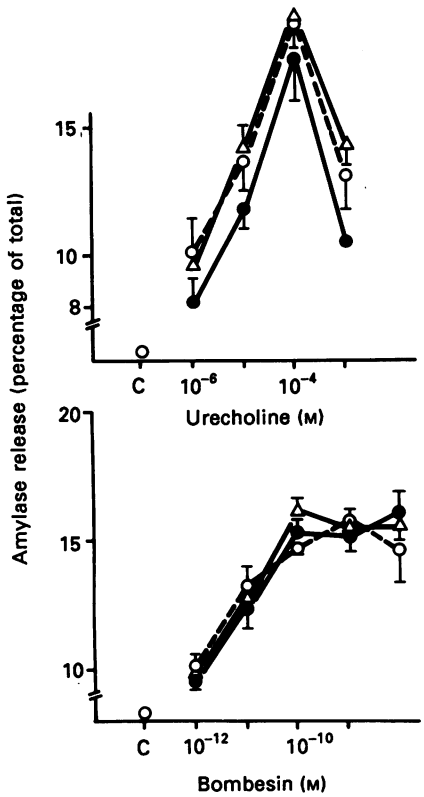


Fig. 8. Pancreatic amylase dose–response curves to urecholine and bombesin added to the incubation medium alone or combined with CR-1409 or L-364718. ○, urecholine or bombesin alone; ●, urecholine or bombesin plus CR-1409 (10^{-4} M); △, urecholine or bombesin plus L-364718 (10^{-5} M). Mean ± S.E.M. of results from eight separate tests. C, control.

The time course of amylase release from unstimulated dispersed acini and from acini incubated in the presence of caerulein at a concentration producing maximal stimulation is shown in Fig. 10. Addition of CR-1409 (10^{-6} M) or L-364718 (10^{-7} M) resulted in a reduction in amylase secretion to a level not different from that of unstimulated acini. No significant difference was observed in the reduction of amylase secretion induced by either CR-1409 or L-364718. Neither bombesin- nor urecholine-stimulated amylase secretion was altered by CR-1409 or L-364718 being added to the incubation medium but these results have not been included for the sake of clarity.

Effects of CR-1409 and L-364718 on DNA synthesis caused by caerulein, pentagastrin, bombesin or FOY 305

Administration of caerulein in either small (0·22 nmol kg^{-1}) or larger (3·7 nmol kg^{-1}) doses for 2 days had no significant effect on pancreatic weight and DNA or RNA content (Table 1). Both small and larger doses of caerulein resulted in a dose-dependent stimulation of DNA synthesis. CR-1409 (10 mg kg^{-1}) or L-364718

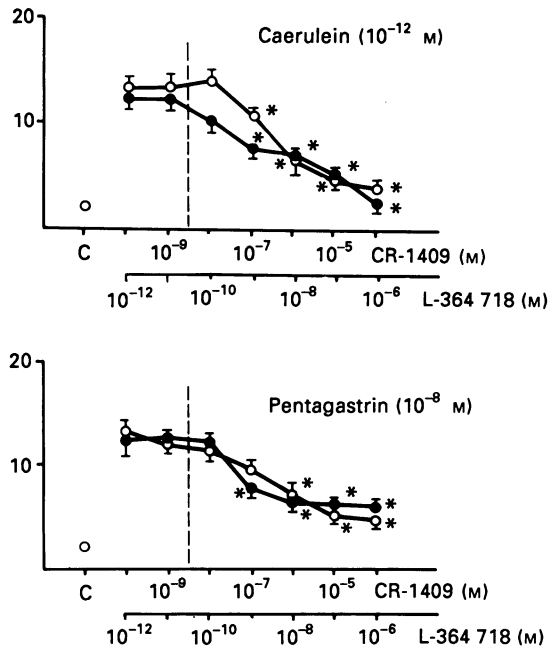


Fig. 9. The effects of addition of gradually increasing concentrations of CR-1409 or L-364718 to the incubation medium of pancreatic acini containing constant concentrations of caerulein or pentagastrin. * Indicates significant ($P < 0·05$) decrease by CR-1409 or L-364718 of amylase release below the value obtained with secretagogue alone. ○, caerulein or pentagastrin plus CR-1409; ●, caerulein or pentagastrin plus L364718. Mean ± S.E.M. of results from ten separate tests. C, control.

(1 mg kg^{-1}) by itself had no effect on the rate of thymidine incorporation into DNA, but when administered with low doses of caerulein, both CCK receptor antagonists completely prevented stimulation of DNA synthesis. When combined with high doses of caerulein, both CR-1409 and L-364718 reduced DNA synthesis significantly but it was still above the control level. There was a significant difference between the increase in DNA synthesis produced solely by high doses of caerulein and by that combined with CR-1409 or L-364718 (Fig. 11).

FOY 305 given by mouth at a dose of 200 mg kg^{-1} day^{-1} resulted in stimulation of DNA synthesis reaching levels similar to that obtained with low doses of caerulein. Both CR-1409 and L-364718 almost completely prevented the increase in the DNA synthesis (Fig. 12).

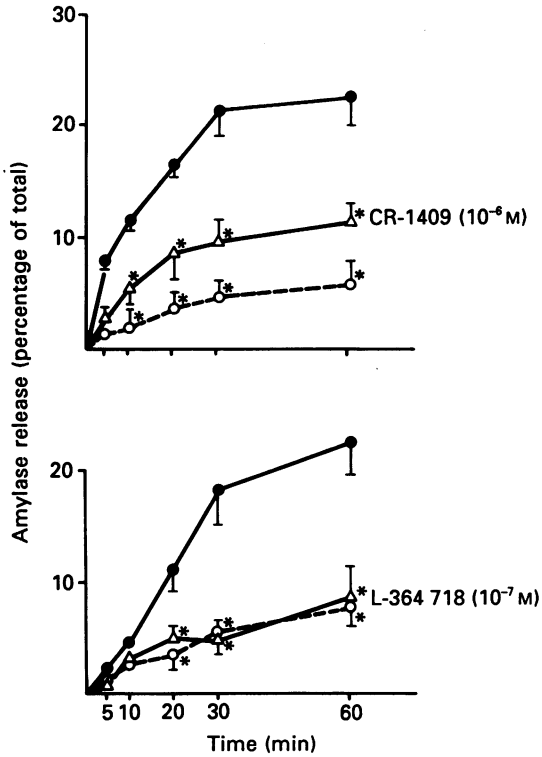


Fig. 10. Time course of amylase release from the dispersed pancreatic acini without any stimulation and following stimulation with caerulein alone or caerulein combined with CR-1409 or L-364718. * Indicates significant ($P < 0.05$) decrease below the value obtained with caerulein alone. ●, caerulein alone (10^{-11} M); △, caerulein (10^{-11} M) plus CR-1409 (10^{-6} M) or L-364718 (10^{-7} M); ○, basal. Means ± S.E.M. of results from eight separate tests.

TABLE 1. Pancreatic weight and total RNA and DNA contents in rats (expressed as mg per total pancreas weight) treated with saline vehicle (154 mM-NaCl), CR-1409 (10 mg kg^{-1}), L-364718 (1 mg kg^{-1}), or their combinations with two levels of caerulein dosage (mean ± S.E.M. of ten rats per group)

	Pancreatic weight (mg)	Total RNA content (mg)	Total DNA content (mg)
Saline	619 ± 42	12·39 ± 0·83	2·72 ± 0·39
CR-1409	609 ± 51	12·27 ± 0·62	2·56 ± 0·31
L-364718	619 ± 31	12·35 ± 0·68	2·55 ± 0·34
Caerulein (0·22 nmol)	658 ± 32	13·31 ± 0·81	3·26 ± 0·30
Caerulein (3·7 nmol)	644 ± 37	12·75 ± 0·83	3·23 ± 0·39
Caerulein (0·22 nmol) + CR-1409	629 ± 37	12·58 ± 0·57	2·70 ± 0·39
Caerulein (0·22 nmol) + L-364718	639 ± 32	12·78 ± 0·49	2·89 ± 0·37
Caerulein (3·7 nmol) + CR-1409	627 ± 29	12·81 ± 0·85	2·72 ± 0·35
Caerulein (3·7 nmol) + L-364718	649 ± 46	12·68 ± 0·54	2·99 ± 0·54

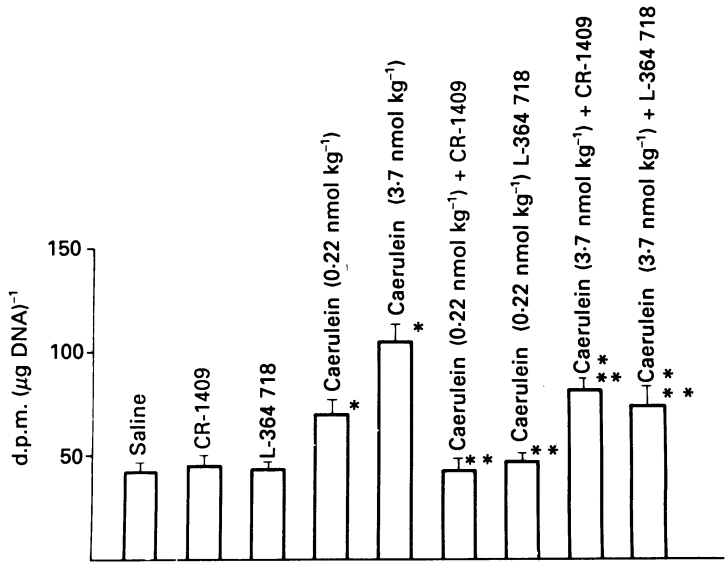


Fig. 11. Stimulation of DNA synthesis (incorporation of [^{3}H]thymidine into DNA) in rats following 48 h injections of saline alone, CR-1409 or L-364718 alone, caerulein in one of two doses alone or combined with CR-1409 or L-364718. Means ± S.E.M. of ten tests on ten rats. * Indicates significant ($P < 0.05$) increase above saline control. ** Indicate significant ($P < 0.05$) decrease below value obtained with caerulein alone either at lower or at higher dose.

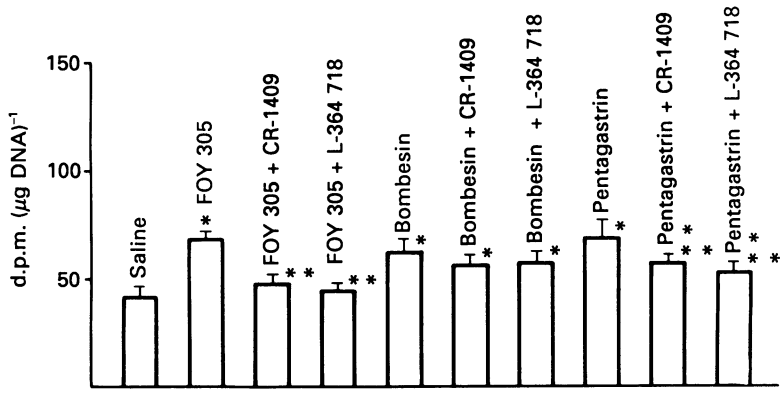


Fig. 12. Stimulation of DNA synthesis (incorporation of [^{3}H]thymidine into DNA) in rats following 48 h injections of saline, oral administration of FOY 305 (200 mg kg^{-1}) alone or combined with injections of CR-1409 or L-364718, bombesin alone or combined with CR-1409 or L-364718 and pentagastrin alone or combined with CR-1409 or L-364718. Means ± S.E.M. of ten tests on ten rats. * Indicates significant ($P < 0.05$) increase above volume obtained with saline control. ** Indicate significant decrease below value with FOY or pentagastrin alone.

Bombesin injected at a dose of 6·2 nmol kg^{-1} caused a significant increase in DNA synthesis which was about 27% higher than the control value. Neither CR-1409 nor L-364718 resulted in any significant alteration in this bombesin-induced elevation in DNA synthesis (Fig. 12).

Pentagastrin (324 nmol kg^{-1}) stimulated DNA synthesis to a similar extent as did

TABLE 2. Pancreatic weight and total RNA and DNA contents in rats (expressed as mg per total pancreas weight) treated with saline vehicle (154 mM-NaCl), FOY 305 (200 mg kg^{-1}), bombesin (6·2 nmol kg^{-1}), pentagastrin (324 nmol kg^{-1}) alone and in combination with CR-1409 (10 mg kg^{-1}) or L-364718 (1 mg kg^{-1}) (mean ± S.E.M. of ten rats per group)

	Pancreatic weight (mg)	Total RNA content (mg)	Total DNA content (mg)
Saline	612 ± 46	12·24 ± 1·02	2·77 ± 0·27
FOY 305	644 ± 31	12·80 ± 0·90	3·09 ± 0·35
FOY 305 + CR-1409	618 ± 42	12·12 ± 0·96	2·90 ± 0·42
FOY 305 + L-364718	629 ± 28	12·44 ± 0·69	2·94 ± 0·20
Bombesin	610 ± 53	12·11 ± 0·51	3·07 ± 0·36
Bombesin + CR-1409	646 ± 58	12·70 ± 0·88	2·60 ± 0·29
Bombesin + L-364718	640 ± 50	12·56 ± 0·69	2·80 ± 0·21
Pentagastrin	661 ± 44	12·87 ± 0·73	3·01 ± 0·33
Pentagastrin + CR-1409	637 ± 38	12·75 ± 0·53	2·87 ± 0·16
Pentagastrin + L-364718	629 ± 43	12·51 ± 0·70	2·94 ± 0·34

FOY 305 and bombesin alone but significantly less than caerulein at the lower dose used. CR-1409 or L-364718 resulted in a small but significant reduction in pentagastrin-induced stimulation in DNA synthesis which remained significantly higher than the basal level, despite administration of CCK receptor antagonists (Fig. 12). Pancreatic weight and RNA and DNA content in rats treated with FOY 305, bombesin or pentagastrin with or without CR-1409 or L-364718 were not significantly different from the saline control (Table 2).

DISCUSSION

This study demonstrates that infusions of bombesin in conscious rats produce a dose-dependent stimulation of pancreatic protein secretion which reaches a maximal rate similar to that achieved with equimolar doses of caerulein. Thus bombesin and caerulein appear to have a similar efficacy and potency in respect of the exocrine pancreas in the rat.

As shown previously (Louie, Williams & Owyang, 1985), basal protein output in bile–pancreatic secretion is mainly of biliary origin, whereas that induced by hormonal stimulation, such as with CCK, is derived principally from the pancreas. Neither CCK receptor antagonist (CR-1409 or L-364718) affected basal or bombesin-induced pancreatic protein secretion but they both abolished that provoked by caerulein, suggesting that neither basal or bombesin-induced pancreatic secretion involves CCK.

Both these CCK antagonists have been found to be highly selective, potent and long-acting agents. CR-1409 (lorglumide) is an amino acid derivative from glutaramic acid and is about 1000 times as active as its prototype, proglumide (Makovec *et al.* 1986; Rovati, Bani, Makovec, Revel & Setnikar, 1987), with a more persistent effect. L-364718 is a benzodiazepine derivative whose CCK antagonism is also highly selective, potent and long-lasting (Lotti *et al.* 1987).

Our results obtained with the above CCK receptor antagonists confirmed previous observations in rats showing that bombesin directly stimulates pancreatic secretion

(Linari, Linari & Lutoslawska, 1977) but contrast with results in dogs (Nealson, Beauchamp, Townsend & Thompson, 1987) where CCK does not appear to have this effect. Furthermore, bombesin has been reported to stimulate amylase release in the rat pancreas *in vitro* (Deschodt-Lankman, Robberecht, De Neef, Lammens & Christophe, 1976; Iwatsuki & Peterson, 1976; Uhleman, Rottman & Gardner, 1979) via specific and separate plasma membrane receptors (Jensen, Moody, Pert, Rivier & Gardner, 1978; Jensen & Gardner, 1981).

We have confirmed that bombesin stimulates amylase release *in vitro* in dispersed rat pancreatic acini in a concentration-dependent manner with a similar efficacy to caerulein although its potency is about 10 times less. Likewise, in this model, pentagastrin caused concentration-dependent stimulation of amylase release but it was far less potent than caerulein. Both CCK receptor antagonists, CR-1409 and L-364718, produced a shift in the amylase dose–response curves for caerulein and pentagastrin to the right. The magnitude of the shift was proportional to the concentration of each antagonist. No significant changes in the maximal response to CCK or pentagastrin were observed at any concentrations of these agents, suggesting that the antagonism was competitive in nature.

Jensen *et al.* (1986) recently reported similar antagonism of the effects of CCK and gastrin using CR-1409 (compound 10) in rat, mouse and guinea-pig pancreatic acini. In the present study we have compared the effectiveness of a peptidergic, proglumide-derived CCK antagonist, CR-1409, with a non-peptidergic antagonist, L-364718, in blocking caerulein-induced pancreatic secretion for the first time. L-364718 was about 10 times more potent than CR-1409 both *in vitro* and *in vivo*. Neither of these CCK receptor antagonists affected bombesin-induced secretion either *in vivo* or *in vitro*. They also failed to affect the amylase response to urecholine from isolated acini.

Our results on the effects of CCK antagonism on caerulein- and bombesin-stimulated pancreatic secretion *in vitro* conflict with those obtained in dogs *in vivo*. We previously observed (Konturek *et al.* 1976) that bombesin and related peptides were equipotent to CCK in their ability to stimulate the secretion of pancreatic enzymes and that their secretory effect could be abolished by antrectomy (to remove the endogenous source of gastrin) plus enterectomy (to eliminate the endogenous source of CCK). We, therefore, concluded that pancreatic secretory stimulation induced by bombesin in dogs largely depends upon the release of gastrin and CCK. This has been further confirmed recently by Nealson *et al.* (1987) who reported that the gastrin/CCK receptor antagonist, proglumide, abolished bombesin-induced pancreatic protein secretion thus reinforcing the notion that bombesin affects pancreatic secretion in dogs primarily through release of gastrin and CCK. While proglumide is a relatively weak and non-specific gastrin/CCK receptor blocker, we found that novel proglumide analogues such as CR-1409 are extremely effective inhibitors of canine pancreatic secretion stimulated by CCK, gastrin and bombesin (Konturek, Tasler, Cieszkowski, Szewczyk & Hladij, 1988).

CCK is now considered to be the major hormonal regulator of pancreatic growth. *in vivo*, CCK exerts both a short-term stimulatory effect on pancreatic protein synthesis (Meldolesi, 1970; Morriset & Webster, 1971) and a long-term action on pancreatic enzyme concentration (Maintz, Black & Webster, 1973; Ihse, Arnesto &

Lanquist, 1976). Exogenous CCK and its natural analogue, caerulein, as employed in this study instead of CCK, are known stimulants of pancreatic growth (Maintz *et al.* 1973; Peterson *et al.* 1978; Dembinski & Johnson, 1980). To prove that the stimulation of pancreatic exocrine growth is a physiological function of CCK, it must be shown that the endogenous hormone has the same effect. Tryptophan plus phenylalanine administered intraduodenally for 5 days has been reported to stimulate pancreatic growth (Scheerman, Chang, Smith & Lyman, 1977). The best evidence that endogenous CCK stimulates pancreatic growth is derived from studies with a soybean trypsin inhibitor, which was reported to increase both pancreatic enzyme secretion (Görke *et al.* 1986; Folsch *et al.* 1987) and tissue growth (Scheerman *et al.* 1977; Görke *et al.* 1986). Our results confirmed that short-term administration of caerulein, pentagastrin or soybean inhibitor (FOY 305) causes significant stimulation of DNA synthesis in the pancreas without affecting the weight of the organs or DNA or RNA content. In the classic sense, growth is an increase in the weight of tissue or an increase in the number of cells in the target tissue, but these phenomena only appear several days after exposure to the growth-stimulating agent. The increase in DNA synthesis which peaks within 24–48 h is considered to be the earliest biochemical indicator of a trophic effect and has been used as a marker for rapid screening of growth-promoting factors (Johnson, 1987). In long-term studies, incorporation of [^{3}H]thymidine into DNA, expressed per microgram of DNA, usually reaches a steady state which may not be different from its control value. In short-term studies, organ weight and RNA and DNA contents are unchanged, while in order to obtain a significant increase in these growth parameters, prolongation of exposure to growth-promoting factors for several weeks is required.

The role of specific CCK receptors in the growth-promoting action of exogenous and endogenous CCK-like hormones is supported by the fact that treatment with the CCK receptor antagonists CR-1409 and L-364718 completely prevented stimulation of DNA synthesis by caerulein, gastrin and FOY 305. Similar prevention of CCK_8-induced stimulation of DNA synthesis was obtained by treatment with proglumide at a dose of 100 mg kg^{-1} but, unlike our study, this agent alone stimulated pancreatic growth and failed to affect the trophic response to high doses of CCK_8 (Yamaguchi, Tabata & Johnson 1985). The difference between our results and those reported by Yamaguchi *et al.* (1985) might be due to the relatively low potency and specificity of proglumide as a CCK receptor antagonist. Most recently it has been shown (Wisner, McLaughlin, Rich, Ozawa & Renner, 1988), that 2 weeks of treatment with FOY 305 results in an increase in pancreatic weight and DNA and RNA tissue contents due to stimulation by endogenous CCK. These effects were completely abolished by L-364718. Thus in studies with CCK and its antagonists our results using DNA synthesis as the biochemical indicator of growth parameters agree with those using organ weight and tissue content of nucleic acid as growth parameters.

This study in rats demonstrates that bombesin is an effective stimulant of pancreatic DNA synthesis but that DNA synthesis is not mediated by CCK/gastrin receptors because blockade of these receptors by peptidergic or non-peptidergic agents did not block the effect of bombesin on DNA synthesis. Previous studies in rats reported that bombesin exerts some trophic effects on the pancreas (Lhoste *et al.* 1985; Lehy *et al.* 1986), but our study with CCK receptor blockers provides evidence

that neither CCK nor gastrin are mediators of bombesin-induced stimulation of tissue growth. It appears, therefore, that bombesin-induced tissue growth, like stimulation of enzyme secretion, results from the direct action of the peptide on the exocrine pancreas and is mediated by separate mechanisms from those involving CCK or gastrin.

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