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Functional comparisons of gastrin/cholecystokinin receptors in isolated preparations of gastric mucosa and ileum

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1 The gastrin cholecystokinin (CCK) receptors mediating stimulation of acid secretion in rat isolated gastric mucosa (RGM) and contraction in guinea-pig isolated ileum longitudinal muscle-myenteric plexus (GPI) have been characterized by use of peptide agonists and the non-peptide antagonists, lorglumide, devazepide and L-365,260.

2 In RGM, gastrin peptides (sulphated gastrin heptadecapeptide (G-17), non-sulphated (ns) G-17 and pentagastrin) were potent agonists of acid secretion (EC_{50} values of 4.3, 16 and 27 nM respectively). Sulphated CCK octapeptide (CCK-8) was also a potent agonist, ($EC_{50} = 0.9$ nM), but was less efficacious, producing a lower maximal response. In contrast, in GPI, CCK-8 was a potent full agonist ($EC_{50} = 1.4$ nM) and was more than 1000 times more potent than the gastrin peptides in producing a sustained contractile response.

3 In GPI, CCK-8 (0.1 to 100 nM) produced sustained contractile responses, whilst CCK-4 (3 to 1000 nM) produced transient responses. These responses had different sensitivities to atropine (1 μM), suggesting that more than one receptor may mediate contraction in this tissue.

4 In RGM, L-365,260 was the most potent antagonist of pentagastrin-stimulated acid secretion ($pA_2 = 7.6$). This functional affinity estimate was similar to that for L-365,260 as an antagonist of excitatory responses in rat ventromedial hypothalamic slices (Kemp *et al.*, 1989) but differed from binding affinity estimates in guinea-pig cortex and gastric glands (Freidinger, 1989).

5 In GPI, devazepide, L-365,260 and lorglumide yielded different affinity estimates when compared against CCK-8 and CCK-4 or pentagastrin respectively. These studies were consistent with the view that the sustained response produced by CCK-8 was mediated by CCK_A receptors and the transient response produced by CCK-4 and pentagastrin was mediated by CCK_B receptors.

6 Affinity estimates for L-365,260 and lorglumide against CCK-4 or pentagastrin in GPI were significantly different from corresponding estimates against pentagastrin in RGM. These studies are consistent with the view that gastrin/CCK_B receptors in GPI may differ from those in RGM.

Keywords: Gastrin/CCK receptor subtypes; rat gastric mucosa; guinea-pig ileum; devazepide; L-365,260; lorglumide; pentagastrin; CCK-8; CCK-4

Introduction

Gastrin and cholecystokinin (CCK) share a high degree of structural homology and have identical pentapeptide sequences at their biologically relevant carboxyl terminal (Rehfeld, 1981). In the gastrointestinal tract, gastrin and CCK have distinct physiological roles. Gastrin stimulates gastric acid secretion and gastrointestinal mucosal cell growth, whilst CCK stimulates pancreatic amylase secretion, gallbladder contraction and modifies gastrointestinal motility (Walsh, 1987).

The different physiological actions of gastrin and CCK suggest that this peptide family mediates its effects via more than one receptor. Binding studies based on the affinities of gastrin/CCK peptides in pancreas and cortex have suggested the existence of two receptor types (Innis & Snyder, 1980); according to the nomenclature proposed by Moran *et al.* (1986), these are termed CCK_A and CCK_B receptors. CCK_A receptors have been demonstrated in pancreas (Innis & Snyder, 1980), gastrointestinal tissue and in discrete regions of the central nervous system (CNS) (Hill *et al.*, 1987). Functional studies of CCK_A receptors demonstrated marked differences in agonist activity, with sulphated CCK octapeptide (CCK-8) being more than 1000 times more potent than other gastrin/CCK peptides, including non-sulphated (ns) CCK-8 (Jensen *et al.*, 1982). Selective antagonists of CCK_A receptors have been identified and include devazepide (Chang & Lotti, 1986) and lorglumide (Makovec *et al.*, 1985).

CCK_B receptors are more widely distributed in the CNS (Innis & Snyder, 1980; Hill *et al.*, 1987) and recent studies in small cell lung cancer cell lines suggest that CCK_B receptors may also be located peripherally (Lin *et al.*, 1990). Comparisons of agonist potency ratios differ for CCK_A and CCK_B receptors with CCK-8 being less than 10 times more potent than other gastrin/CCK peptides at CCK_B receptors (Bohme *et al.*, 1988; Boden & Hill, 1988; Lin *et al.*, 1990). The benzodiazepine, L-365,260 (Lotti & Chang, 1989) and more recently, the dipeptoid PD134308 (Hughes *et al.*, 1990) and the pyrazolidinone LY26684 (Howbert *et al.*, 1991) have been described as potent and selective CCK_B receptor antagonists. Functional studies of acid secretion in dog parietal cells (Soll *et al.*, 1980) and smooth muscle contraction in guinea-pig dispersed fundus (Bitar *et al.*, 1982; Menozzi *et al.*, 1989) provide evidence for the existence of a third subtype, the gastrin receptor. In these studies, gastrin heptadecapeptide (G-17) and CCK-8 are equipotent, but in contrast to studies of CCK_B receptors (Bohme *et al.*, 1988; Boden & Hill, 1988; Lin *et al.*, 1900), CCK-4 is at least 300 times weaker than CCK-8 (Bitar *et al.*, 1982; Menozzi *et al.*, 1989). Binding studies using guinea-pig dispersed gastric glands yield affinity estimates for antagonists similar to those obtained for CCK_B receptor binding (Bock *et al.*, 1989) and this has led to the conclusion that gastrin and CCK_B receptors do not differ (Woodruff & Hughes, 1991).

Gastrin/CCK receptor classification has been decided lar-

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gely on the results of binding studies using homogenates of pancreas (CCK_A receptors), cerebral cortex (CCK_B receptors) and dispersed gastric glands (gastrin/CCK_B receptors) (Bock *et al.*, 1989; Hughes *et al.*, 1990). The purpose of the present study was to evaluate the activity of gastrin/CCK agonists and antagonists by use of functional responses in isolated preparations. The preparations used were chosen to reflect some of the actions of gastrin and CCK in the gastrointestinal tract. These were rat isolated gastric mucosa (RGM), where gastrin stimulates acid secretion and the longitudinal muscle-myenteric plexus preparation of guinea-pig isolated ileum (GPI) where CCK-8 induces contraction (Yau *et al.*, 1974).

Some of this work has been presented at meetings of the British Pharmacological Society (Spraggs & Patel, 1989; 1990). The results obtained in these studies with gastrin/CCK agonists and antagonists are consistent with the view that both CCK_A and CCK_B receptors mediate contraction in GPI. In addition, comparisons of antagonist affinities suggest that gastrin/CCK_B receptors in GPI may differ from those in RGM.

Methods

Rat isolated gastric mucosa

Acid secretion was measured from rat isolated gastric mucosa (RGM) prepared as described by Reeves & Stables (1985). Sprague-Dawley rats weighing 90–110 g were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). Following laparotomy, the stomach was freed from connective tissue and the rumen was removed. The muscle layers overlying the fundic region of the stomach were separated from the mucosa by injection of saline (0.9% NaCl). The muscle layer was then cut and peeled back to expose the mucosa. A perfusion chamber (area = 1.54 cm^2) was placed in the stomach and the exposed mucosa was tied over the chamber. The chamber with attached mucosa was then removed and placed in a 20 ml organ bath which contained Krebs-Henseleit solution at 37°C gassed with 95% O_2 and 5% CO_2 (serosal bathing solution). The mucosal surface which faced into the perfusion chamber was superfused by means of a peristaltic pump (Watson Marlow Ltd) at a rate of 0.5 ml min^{-1} with an unbuffered solution of similar ionic composition at 37°C gassed with 100% CO_2 (mucosal bathing solution). The mucosal effluent was passed over a combination pH electrode (Russell) and pH recorded via a pH meter (Radiometer). All acid output values were converted from pH to nmol min^{-1} of hydrogen ion. The ionic compositions (mM) of the solutions were:- serosal: NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 0.6, KH_2PO_4 1.2, $CaCl_2$ 1.3 and glucose 11.1. Indomethacin has been shown previously to enhance the responsiveness to secretagogues of the RGM (Reeves & Stables, 1985) and consequently was included in all experiments at a concentration of 3 μM. The composition of the mucosal solution was (mM) NaCl 144.5, KCl 4.7, $MgSO_4$ 0.6, $CaCl_2$ 1.3 and glucose 11.1

Responses to pentagastrin and other secretagogues were obtained by cumulative addition. In each preparation, a standard concentration-response curve to pentagastrin (1 to 300 nM) was constructed. For agonist studies this was followed by a concentration-response curve to another gastrin/CCK peptide. For antagonist studies this was followed by incubation of antagonist for 30 min, before a second concentration-response curve to pentagastrin was obtained. Preliminary experiments established that concentration-response curves to pentagastrin were repeatable, with no significant change in EC_{50} or maximal response.

Guinea-pig isolated ileum longitudinal muscle-myenteric plexus (GPI)

Male Dunkin-Hartley guinea-pigs weighing approximately 250 g were killed by cervical dislocation and 10 cm of terminal ileum was rapidly removed and placed in Krebs-Henseleit solution. A 3 cm length of ileum was placed over a glass rod and the longitudinal muscle with adherent myenteric plexus was removed with a cotton tip. This muscle strip was attached to an isometric force transducer and bathed in Krebs-Henseleit solution (serosal, see above). The tissue was washed, and adjusted to a resting tension of 0.8 g. Responses to sulphated cholecystokinin octapeptide (CCK-8), cholecystokinin tetrapeptide (CCK-4), pentagastrin and dimethylphenylpiperazinium (DMPP) were obtained by sequential addition with a contact time of 1 min and a cycle time of 5 min. Preliminary experiments established that concentration-response curves to these agonists could be reproduced. Therefore studies to investigate the effects of agonists and antagonists employed a protocol identical to that used in RGM.

Analysis of data

Responses in these preparations were normalised by expressing the data as a percentage of the maximum response in the control curve. EC_{50} values were determined graphically from individual experiments and are expressed as geometric mean with 95% confidence limits. For agonists, potency ratios (APR) were determined from the ratio of EC_{50} values for test and standard agonist (pentagastrin or CCK-8) in individual experiments. For antagonist studies, equieffective concentration-ratios (CR) were determined from EC_{50} values in the absence and following incubation of antagonist for 30 min. These concentration ratios were subjected to Schild analysis by linear regression to determine pA_2 and Schild slope values for antagonists.

Compounds

The various gastrin/CCK peptide fragments used in these studies were purchased from Peninsula Laboratories Ltd, Bachem Inc or Research Plus Inc. Pentagastrin was dissolved in dimethylsulphoxide (DMSO) at a concentration of 0.01 M. The other peptides were dissolved in 0.05 M NH_4HCO_3 at either 0.01 or 0.001 M. Aliquots of peptides were stored at −20°C and discarded after use.

The non-peptidic antagonists lorglumide, devazepide and L-365,260 ((R)-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N'-(3-methylphenyl)-urea) were synthesised by the Chemistry Division, Glaxo Group Research Ltd. These compounds were dissolved in DMSO at concentrations of 0.1 M or less and subsequently diluted in Krebs-Henseleit solution or DMSO. The bath concentration of DMSO in all experiments did not exceed 1% and did not affect the responses to agonists in RGM or GPI. Histamine, dimethylphenylpiperazinium (DMPP), atropine and tetrodotoxin (TTX) were obtained from Sigma Chemical Co. and dissolved in saline. The peptidase inhibitors, bestatin and phosphoramidon, were obtained from Peninsula Laboratories Ltd and dissolved in saline. All solutions were added to organ baths in volumes of 1% or less.

Results

Agonist studies

Rat isolated gastric mucosa Rat isolated gastric mucosa (RGM) spontaneously secreted acid and reached a stable basal acid secretion rate of approximately 50 nmol H^+ min^{-1} after 90 min.

Following a priming concentration of pentagastrin (100

nM), subsequent concentration-response curves were reproducible with first and second curves producing EC_{50} values of 27 (15–49) and 21 (9–46) nM and maxima of 133 ± 34 and 136 ± 29 nmol H^+ min^{-1} ($n = 4$) respectively. The effects of gastrin/CCK peptides on acid secretion were determined in the presence of the peptidase inhibitors phosphoramidon (1 μM) and bestatin (100 μM) (Stephens-Smith *et al.*, 1988). These inhibitors did not modify responses to pentagastrin, producing a CR of 1.7 (0.9–3.3) and maximum responses of 137 ± 12 and 125 ± 8 nmol H^+ min^{-1} ($n = 4$) before and after peptidase inhibition. Figure 1 shows the effects of gastrin/CCK peptides on acid secretion and these data are summarized in Table 1. The human gastrins (G-17 and non-sulphated (ns) G-17) and pentagastrin were potent stimulants of acid secretion, whilst nsCCK-8 was weaker. CCK-8 had threshold effects similar to the gastrins but was a partial agonist with a maximum response of 29 ± 13% ($n = 4$) at a concentration of 10 nM. In addition, CCK-8 (100 nM) antagonized subsequent responses to pentagastrin at concentrations up to 1 μM (data not shown). CCK-4 was a weak partial agonist, with a maximum response similar to that observed with CCK-8.

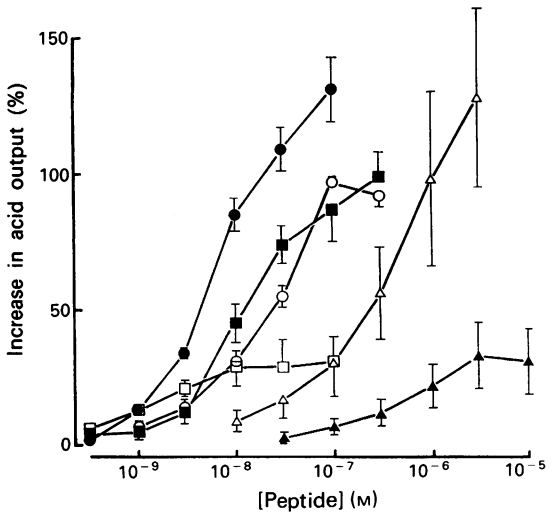

Figure 1 Effects of gastrin/CCK peptides on acid secretion in rat isolated gastric mucosa. Increases in acid secretion are expressed as percentages of the maximal response to pentagastrin in each tissue. For clarity, only one of the concentration-response curves for pentagastrin is shown and is representative of the others in this series of experiments. Values are mean (vertical bars show s.e.mean), $n = 4$ for the following peptides; G-17 (●), nsG-17 (■), pentagastrin (○), CCK-8 (□), nsCCK-8 (△) and CCK-4 (▲). For abbreviations, see text.

Table 1 Potencies of gastrin/CCK peptides in rat gastric mucosa (RGM) and guinea-pig ileum (GPI)

Agonist	*Rat gastric mucosa*	*Guinea-pig ileum*
CCK-8	0.9 (0.2–5)*	1.4 (0.9–2.1)
nsCCK-8	384 (34–4337)	>2000
CCK-4	613 (280–1341)*	31200 (17100–56900)
G-17	4.3 (3.6–5.1)	1170
nsG-17	16 (5–34)	>10000
Pentagastrin	27 (15–49)	>10000

Values are EC_{50} (in nM) expressed as geometric mean with 95% confidence limits ($n > 4$).
In RGM, some peptides produced maxima that were clearly smaller than the standard agonist (pentagastrin) and are denoted by *. In these cases the EC_{50} value is determined from individual 50% responses for that peptide.
In GPI, some agonists produced a biphasic contractile response (see Figure 2). The EC_{50} values for all the peptides in GPI were determined from the sustained component of the contractile response.
For abbreviations, see text.

Guinea-pig isolated ileum At all concentrations tested, CCK-8 produced a biphasic contractile response consisting of a transient 'spike' increase in tension followed by a sustained contraction which was maintained until washout of the compound (Figure 2). In contrast the contractile response profile to CCK-4 differed from that observed for CCK-8 (Figure 2). CCK-4 (3–1000 nM) stimulated a monophasic contraction which was not sustained, unlike the biphasic contractile response of CCK-8. At higher concentrations of CCK-4 (> 10 μM) a biphasic contraction, similar to that produced by CCK-8 was observed. The mechanism of responses to both of these agonists were investigated further by use of tetrodotoxin (TTX) and atropine. CCK-8 (0.1–100 nM) produced sustained contractions of GPI with an EC_{50} value of 1.4 (0.9–2.1) nM and a maximum increase in tension of 1.28 ± 0.06 g at 100 nM ($n = 6$). These responses were reproducible with a second CCK-8 curve producing an EC_{50} value of 1.9 (1.4–2.6) nM and a maximal increase in tension of 1.45 ± 0.10 g at 100 nM. Tetrodotoxin (300 nM) inhibited the sustained response to CCK-8 by 93%, whilst atropine (1 and 10 μM) reduced this response by only 50% (Figure 3a). These results suggested that CCK-8 induced a sustained contraction indirectly, via a neuronal mechanism, which was partly mediated by release of acetylcholine acting at muscarinic receptors. CCK-4 (1 nM–3 μM) produced transient concentration related increases in tension with an EC_{50} of 49 (40–61) nM and a maximal response of 0.67 ± 0.07 g. Concentration-response curves for CCK-4 were reproducible in individual tissues (CR = 1.29, E_{max} for curve 2 = 0.65 ± 0.05g). Both TTX (300 nM) and atropine (1 μM) abolished responses to CCK-4 (Figure 3b) suggesting that neuronally released acetylcholine was the sole mediator of the effect of CCK-4 in GPI. Since this mechanism differed from that for CCK-8, it was possible that the gastrin/CCK receptor responsible for the contractile effect of CCK-4 was different and this was investigated further with the antagonists lorglumide, devazepide and L-365,260 (see later).

CCK-8, nsCCK-8, CCK-4 and the gastrin peptides were tested as agonists in GPI by measuring their effects on the sustained portion of the response in the presence of the peptidase inhibitors phosphoramidon (1 μM) and bestatin (100 μM). Preliminary studies in 6 preparations showed that these inhibitors did not modify concentration-response curves to CCK-8, (CR = 1.1 (0.6–1.8) $n = 6$). A striking difference in agonist potencies was observed, with CCK-8 being more than 1000 times more potent than the other agonists (Figure 4, Table 1).

Antagonist studies

Rat isolated gastric mucosa The effects of gastrin/CCK receptor antagonists on pentagastrin stimulated acid secre-

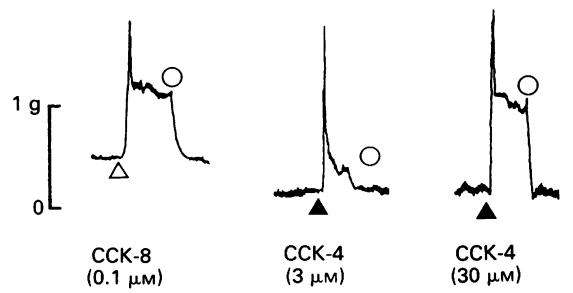

Figure 2 Tracings of representative responses to CCK-8 (10 nM) and CCK-4 (3 μM and 30 μM) in guinea-pig ileum. Peptides were added at time points (△ and ▲) and were removed from the bath following a 60 s contact time (o). CCK-8 produced a biphasic contraction with a sustained phase that was maintained until washout. In contrast, CCK-4 (3 μM) produced only a 'spike' contraction which returned to basal levels before washout. Higher concentrations of CCK-4 (30 μM) produced a response that resembled CCK-8.

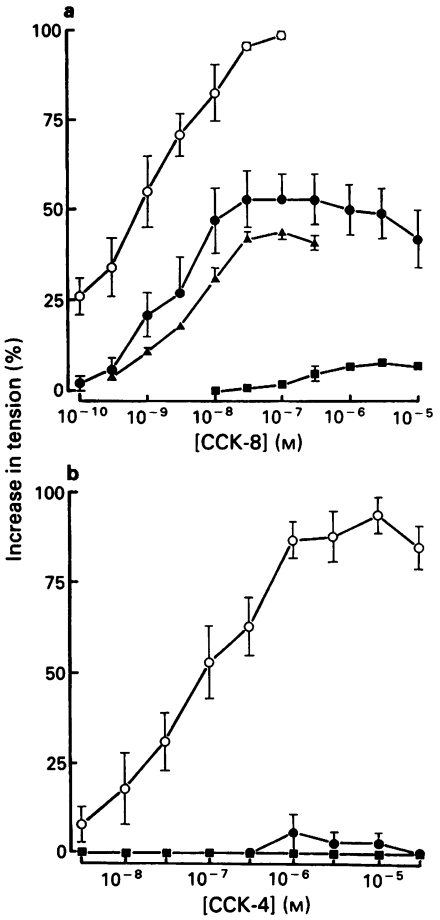

Figure 3 Effects of tetrodotoxin and atropine on contractile responses to CCK-8 sustained response (a) and CCK-4 transient response (b) in guinea-pig ileum. The format of these graphs is similar to Figure 3. Values are mean (vertical bars shown s.e.mean), $n = 4$ for the following treatments; CCK-8 or CCK-4 alone (o,). CCK-8 or CCK-4 in the presence of tetrodotoxin (300 nM, ■) or atropine (1 μM, ● and 10 μM, ▲). For abbreviations, see text.

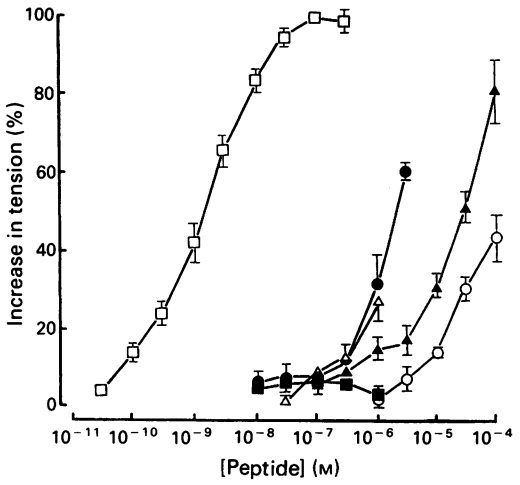

Figure 4 Contractile responses to gastrin/CCK peptides in guinea-pig isolated ileum. Sustained increases in tension were expressed as percentages of the maximal response to CCK-8 in each tissue. For clarity, only one of the concentration-response curves for CCK-8 is shown and is representative of others in this series of experiments. Values are mean (vertical bars show s.e.mean), $n = 4$ to 6, for the following peptides: CCK-8 (□), CCK-4 (▲), G-17 (●), nsCCK-8 (△), nsG-17 (■) and pentagastrin (○). For abbreviations, see text.

tion in RGM are summarised in Table 2. Lorglumide (10–100 μM) produced concentration-related rightward displacements of pentagastrin responses (Figure 5a). At a concentration of 10 μM, lorglumide increased the maximal response to pentagastrin (154 ± 28%, $n = 4$), but at the higher concentration of 100 μM a reduction in the maximum response was observed. Schild analysis yielded a slope value which was significantly greater than unity (Table 2). The steep Schild slopes encountered with this antagonist in RGM did not appear to be due to loss of antagonist from the biophase by enzymic degradation since repeating these experiments in the presence of phosphoramidon (1 μM) and bestatin (100 μM) and in silane-coated organ baths did not modify the antagonist profile or potency of lorglumide (data not shown).

The benzodiazepine analogue, L-365,260 (0.1–3 μM) was the most potent antagonist of pentagastrin encountered in these studies (Figure 5b) and, unlike lorglumide, was a competitive antagonist with a Schild slope value not significantly different from unity (Table 2). In contrast, the apparent affinity of the CCK_A receptor antagonist devazepide was approximately 50 times less potent than L-365,260 (Figure 5c, Table 2).

Secretory responses to pentagastrin in RGM were inhibited by ranitidine (0.1–10 μM) in an insurmountable manner (data not shown), suggesting that these responses were indirect, mediated via the release of histamine. Therefore, the selectivity of gastrin receptor antagonists in RGM was investigated with histamine used as a secretagogue. Histamine (1–300 μM) stimulated large increases in acid secretion

Table 2 Estimates of apparent affinity for antagonists in rat gastric mucosa (RGM) and guinea-pig ileum (GPI)

		Antagonist affinities[a]		
Preparation	*Agonist*	*Devazepide*	*L-365,260*	*Lorglumide*
RGM	Pentagastrin	5.77 ± 0.30	7.62 ± 0.10	5.47 ± 0.21
			(1.11 ± 0.11)	(1.39 ± 0.28*)
GPI	CCK-8	10.40 ± 0.30	7.72 ± 0.33	7.50 ± 0.13
	(sustained)	(0.94 ± 0.13)	(1.14 ± 0.24)	(1.50 ± 0.13*)
GPI[b]	CCK-4	<7.3	8.35 ± 0.26	6.47 ± 0.20
	(transient)		(1.17 ± 0.22)	(1.16 ± 0.21)
GPI	Pentagastrin	<7	8.84 ± 0.24	6.39 ± 0.08
	(transient)		(1.29 ± 0.21)	(1.71 ± 0.15*)

[a]pA_2 and Schild slope values (in parentheses), shown as mean ± s.e.mean, were derived from linear regression of the respective Schild plots, $n = 12$ to 15. Slope values marked * were significantly greater than unity. The values without slope values are pK_B estimates derived from a single concentration using the Gaddum equation ($n = 4$ to 12).
[b]Except for devazepide itself, these experiments were performed in the presence of devazepide (100 nM).

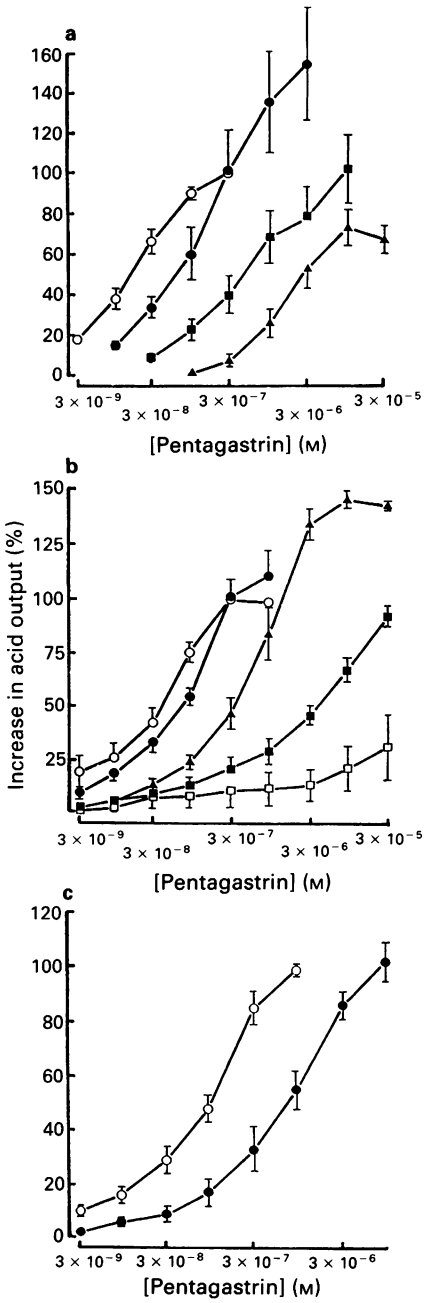

Figure 5 Effects of gastrin/CCK receptor antagonists as inhibitors of pentagastrin-stimulated acid secretion in rat isolated gastric mucosa. The format of these graphs is similar to Figure 1. Values are mean (vertical bars s.e.mean), $n = 4$. In (a) are shown the effects of lorglumide at 10 μM (●), 30 μM (■) and 100 μM (▲); (b) shows the effects of L-365,260 at 0.01 μM (●), 0.1 μM (▲), 1 μM (■) and 10 μM (□); (c) shows the effect of devazepide at 10 μM (●). Representative examples of the pentagastrin concentration – response curves from each of these series of experiments are shown (○).

with an EC_{50} value of 29 (20–44) μM and a maximum of 355 ± 83 nmol H^+ min^{-1} ($n = 4$). These responses to histamine could be repeated in single tissues with no significant change in EC_{50} (CR = 1.4 (0.5–3.8)) or maximum. Lorglumide (100 μM) produced a small displacement of the histamine responses (concentration-ratio = 8) and some depression of maximum ($E_{max} = 68 \pm 17\%$, $n = 4$) which may account for its insurmountable antagonist activity when pentagastrin was used as agonist. A high concentration of L-365,260 (10 μM) produced a nine fold displacement and a reduction in the maximum response to histamine ($E_{max} = 58 \pm 12\%$, $n = 4$).

Guinea-pig isolated ileum The effects of the gastrin/CCK antagonists against CCK-8 induced sustained contractions in GPI are summarized in Table 2. Lorglumide produced concentration-related displacements in concentration-response curves for CCK-8 with a pA_2 value of 7.50 and a Schild slope value of 1.50 which was significantly greater than unity (Figure 6a, Table 2). Devazepide (0.3–30 nM) was extremely potent and competitive antagonist of CCK-8 in GPI (Figure 6c, Table 2). L-365,260 (0.1–3 μM) was also a competitive antagonist of CCK-8, but was approximately 300 times less potent than devazepide (Figure 6d, Table 2).

In contrast to its activity against CCK-8 in GPI, the CCK_A receptor antagonist, devazepide (100 nM) was a weak inhibitor of CCK-4-induced transient contractions (Table 2, Figure 7b). Preliminary experiments demonstrated that concentrations of CCK-4 greater than 30 μM produced a res-

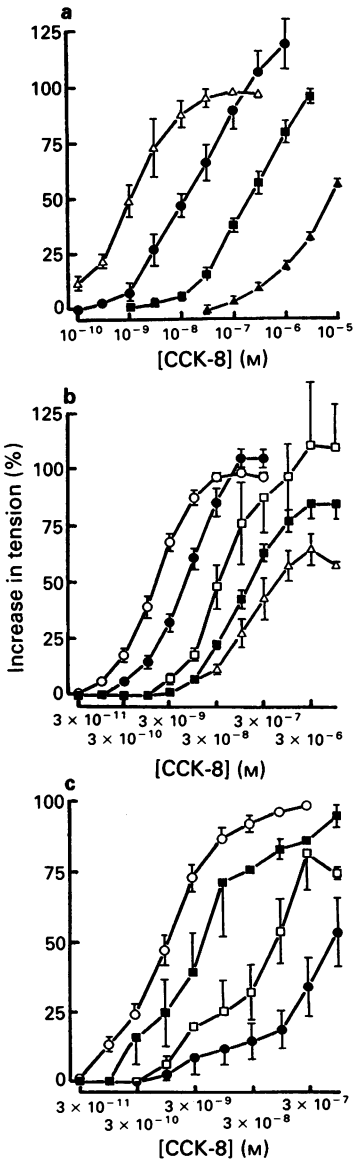

Figure 6 Effects of gastrin/CCK receptor antagonists as inhibitors of CCK-8-induced sustained contractions in guinea-pig isolated ileum. The format of these graphs is similar to Figure 3. Values are mean (vertical bars show s.e.mean), $n = 4$ to 6. In (a) is shown the effects of lorglumide at 0.1 μM (●), 1 μM (■) and 10 μM (▲); (b) shows the effects of L-365,260 at 0.1 μM (●), 0.3 μM (□), 1 μM (■) and 10 μM (△); (c) shows the effects of devazepide at 0.3 nM (■), 3 nM (□) and 30 nM (●). Representative examples of control CCK-8 concentration-response curves are shown for each series of experiments (○, △).

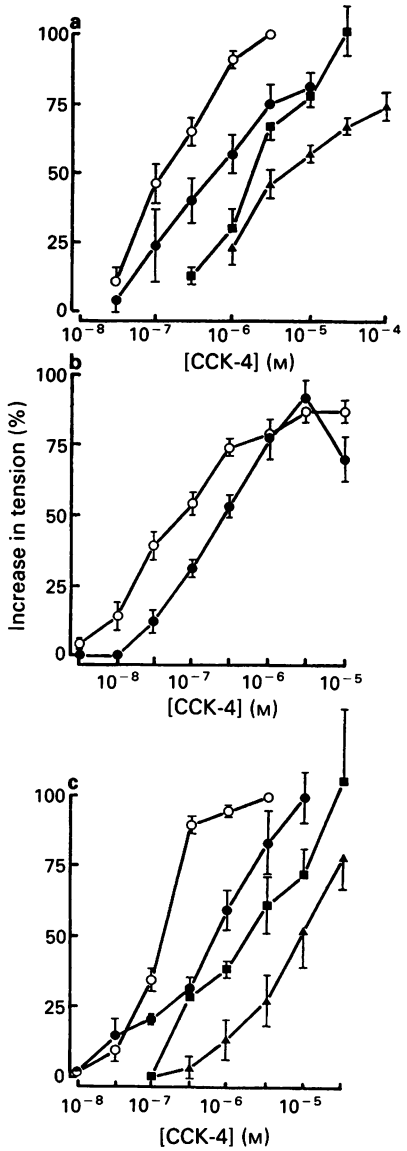

Figure 7 Effects of lorglumide, devazepide and L-365,260 as inhibitors of CCK-4-induced contraction in guinea-pig isolated ileum. The format of these graphs is similar to Figure 3. Values are mean (vertical bars show s.e.mean), $n = 3$ in (a), $n = 12$ in (b) and $n = 5$ in (c). In (a) is shown the effects of lorglumide at 1 μM (●), 3 μM (■) and 10 μM (▲); (b) shows the effects of devazepide (0.1 nM, ●); (c) shows the effects of L-365,260 at 30 nM (●) 100 nM (■) and 300 nM (▲). Experiments with lorglumide and L-365,260 were performed in the presence of devazepide (100 nM) to exclude interference from CCK_A-mediated contractions. Control CCK-4 curves are shown (○).

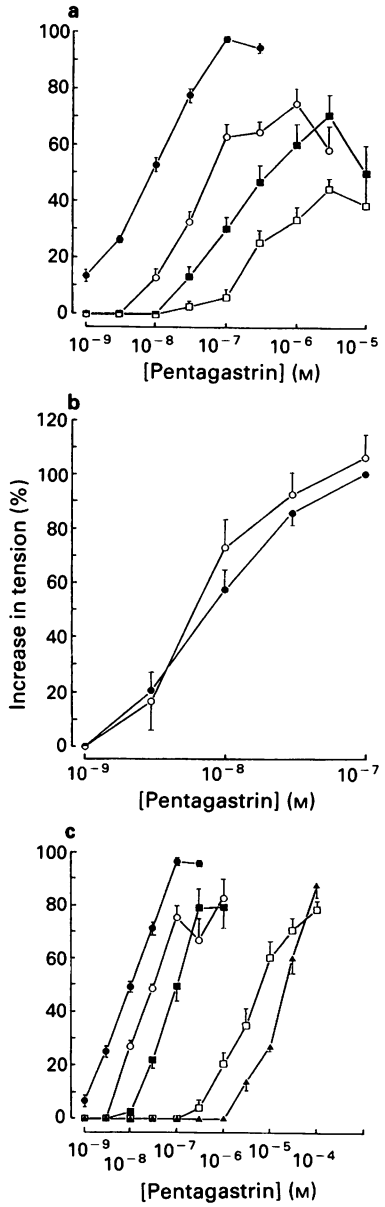

Figure 8 Effects of lorglumide, devazepide and L-365,260 as inhibitors of pentagastrin-induced transient contraction in guinea-pig isolated ileum. The format of these graphs is similar to Figure 3. Values are mean (vertical bars show s.e.mean), $n = 4$. In (a) is shown the effects of lorglumide at 1 μM (○), 3 μM (■) and 10 μM (□); (b) shows the effects of devazepide at 0.1 μM (○); (c) shows the effects of L-365,260 at 3 nM (○), 10 nM (■), 30 nM (□) and 100 nM (▲). Control pentagastrin curves are shown (●).

ponse profile that resembled CCK-8 (Figure 2). Therefore the potential for interference of CCK_A receptor-mediated effects on the transient responses to CCK-4 was excluded by inclusion of devazepide (100 nM) in the bathing medium. In the presence of devazepide, L-365,260 (30–300 nM) was a potent antagonist of CCK-4 (Figure 7c, Table 2) and Schild analysis of these data yielded a pA_2 value of 8.35 and a Schild slope of 1.17 which was not significantly different from unity. Under these conditions lorglumide was also a competitive antagonist of CCK-4 (Figure 7a, Table 2) with a pA_2 value of 6.47 and a Schild slope of 1.16.

In contrast to CCK-4, pentagastrin (1 nM to 100 μM) produced only transient contractile responses, the sustained response being absent throughout the concentration-range. Therefore antagonist affinities of devazepide, L-365,260 and lorglumide (without pretreatment of devazepide) were also determined in GPI against pentagastrin as agonist (Figure 8, Table 2). In these studies, affinity estimates not significantly different from those using CCK-4 (in the presence of devazepide, 100 nM) were obtained.

The specificity of antagonists in GPI was determined by use of the nicotinic agonist, DMPP. Contractile responses to DMPP were inhibited by TTX and atropine by greater than 80% (data not shown) suggesting that the majority of the effect of DMPP was mediated via neuronal release of acetylcholine. At a high concentration of 100 μM, lorglumide abolished the contractile effects of DMPP (data not shown). This non-selective action may account for the increasing antagonist activity and maximal depression seen with lorglumide as an antagonist of CCK-8 (Table 2). Devazepide and L-365,260 appeared selective antagonists of CCK-8 and CCK-4 responses in GPI since they did not modify responses to DMPP at a concentration 300 times their respective pA_2 values (data not shown).

Discussion

Gastrin/CCK receptors have largely been characterized by the affinities of agonists and antagonists in binding studies using parietal cells, gastric glands, and homogenates of brain cortex or pancreas (Makovec *et al.*, 1986; Bock *et al.*, 1989; Hughes *et al.*, 1990). However, different estimates of receptor binding affinity for these antagonists have been observed in other studies (Hill & Woodruff, 1989; Huang *et al.*, 1989). In the present studies we have attempted to characterize gastrin/CCK receptors by determining the apparent affinities of gastrin/CCK receptor antagonists in functional studies using isolated preparations of rat gastric mucosa (RGM) and guinea-pig ileum longitudinal muscle myenteric plexus (GPI).

Prior to investigating the actions of antagonists in these preparations, information on the gastrin/CCK receptors that may mediate the responses was gained by comparing the agonist potencies of a range of peptides from the gastrin/CCK family and these studies are summarized in Table 1. In RGM, CCK-8 and CCK-4 were partial agonists, with a lower maximal response than the gastrin peptides. The differences in maximal responses encountered in RGM make direct comparisons of agonist potency-ratios for gastrin/CCK peptides in other tissues difficult and such comparisons should be interpreted with caution. With this caveat, the difference in potency in RGM between CCK-8 and CCK-4 (680 fold, Table 1) suggests that this response resembled those mediated by gastrin-like receptors (Soll *et al.*, 1981; Bitar *et al.*, 1982; Menozzi *et al.*, 1989) more than those mediated by CCK_B receptors, where CCK-8 is less than 10 times more potent than CCK-4.

The rank order of potency for agonists obtained in GPI differed from RGM, with sustained contractions to CCK-8 being greater than 1000 times more potent than the other peptides. The rank order of agonist potency for this response in GPI resembled that described for stimulation of amylase release in guinea-pig dispersed pancreatic acini (Jensen *et al.*, 1980; 1982) and is generally consistent with the sustained contraction in GPI being mediated by CCK_A receptors.

Non-peptidic antagonists with reported affinity for gastrin/CCK receptors (Makovec *et al.*, 1985; Bock *et al.*, 1988) were used to characterize further the actions of this peptide family in RGM and GPI (Table 2). In GPI, where sustained contractile responses to CCK-8 were measured, devazepide was the most potent antagonist with an apparent affinity estimate similar to that reported previously in this preparation (Chang & Lotti, 1986). L-365,260 was also a competitive antagonist, but was approximately 300 times less potent than devazepide. These effects were consistent with the view that CCK-8-induced sustained contractions of GPI are mediated by CCK_A receptors. The pA_2 value for lorglumide further supported this view (Makovec *et al.*, 1985).

Although CCK-4 was approximately 3000 times less potent than CCK-8 in producing a sustained contraction in GPI, the tetrapeptide was only approximately 50 times less potent than CCK-8 in producing a transient contraction. This latter relative potency in GPI was not consistent with an action solely at CCK_A receptors (Jensen *et al.*, 1982) and the effects of CCK-4 were investigated further. The present studies confirmed previous observations that the responses to CCK-8 in GPI were complex and mediated only in part by neuronal acetylcholine release (Hutchison & Dockray, 1981; Chang *et al.*, 1984). In contrast, transient responses to CCK-4 appeared to be mediated solely by neuronal acetylcholine release, possibly suggesting activity at a different receptor. When CCK-4 or pentagastrin were used as agonists instead of CCK-8 and transient contractions measured, lorglumide, devazepide and L-365,260 produced different antagonist affinity estimates. The most striking difference was a loss of potency of more than 3000 fold when devazepide was used as an antagonist against CCK-4 and pentagastrin. This suggested that the contractile effects of CCK-4 and pentagastrin were not mediated by CCK_A receptors and were more consistent with activity at gastrin/CCK_B receptors (Chang & Lotti, 1986; Freidinger, 1989). Similar observations have recently been made by others (D.G. Trist, personal communication; Lucaites *et al.*, 1991).

In RGM, L-365,260 was the most potent antagonist of pentagastrin-stimulated acid secretion. In agreement with previous studies (Black *et al.*, 1985) pentagastrin-stimulated responses were sensitive to blockade by histamine H_2 receptor antagonists suggesting that pentagastrin stimulates acid secretion indirectly, via release of histamine. In the present studies, L-365,260 was a less potent antagonist of histamine-induced acid secretion, possessing a 100 fold selectivity for gastrin over histamine H_2 receptors. L-365,260 was approximately 50 times less potent in RGM than would have been predicted by its binding affinity in guinea-pig gastric glands and at CCK_B receptors in guinea-pig cortex (Bock *et al.*, 1989). Furthermore, the affinity estimates for L-365,260 and lorglumide versus pentagastrin in RGM were significantly different from corresponding estimates against pentagastrin in GPI ($P<0.05$ by no overlap of confidence limits) consistent with the view that responses in RGM are mediated by a receptor different from the gastrin/CCK_B receptor type mediating transient contraction in GPI.

Agonist potency-ratios have supported the view that gastrin and CCK_B receptors are distinct (e.g. Bohme *et al.*, 1988; Menozzi *et al.*, 1989). However, binding studies comparing affinity estimates of gastrin/CCK antagonists in guinea-pig cortex and gastric glands have been unable to disclose differences in affinity in these two preparations (Bock *et al.*, 1989; Freidinger, 1989; Hughes *et al.*, 1990). This is clearly different from the present studies where affinity estimates for L-365,260 and lorglumide in RGM and GPI (using CCK-4 and pentagastrin as agonists) differ significantly. Differences in apparent affinity of antagonists in RGM and GPI may be due to species differences in the gastrin/CCK_B receptor. For example, affinity estimates for L-365,260 derived from gastrin/CCK_B binding studies in rat spinal cord (Hill & Woodruff, 1989) and functional studies in rat ventromedial hypothalamus (Kemp *et al.*, 1989) correspond to the present value for L-365,260 in RGM. In addition, the present studies demonstrate that L-365,260 has a 50 fold lower affinity for gastrin/CCK_B receptors than is apparent from estimates in guinea-pig gastric gland binding studies (Bock *et al.*, 1989). However, it is possible that binding studies in gastric glands do not reflect interaction with receptors controlling acid secretion. In this context, recent studies have suggested that CCK may interact with more than one receptor type in gastric glands, one of which resembles the CCK_B receptor (Praissman & Brand, 1990). Corresponding functional studies of acid secretion in guinea-pig gastric mucosa are required for comparison with binding studies from this species to help resolve this issue.

The present studies using gastrin/CCK agonists and antagonists in functional isolated preparations are consistent with the view that the actions of this peptide family may be mediated by three receptor subtypes. Furthermore, the present studies suggest it may be possible to distinguish gastrin/CCK_B receptors in preparations from different species. Isolated preparations with which to study functional responses mediated by gastrin/CCK_B receptors are not widely available and additional information in other systems is now required to confirm the view that these receptor subtypes differ.

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References

BITAR, K.N., SAFFOURI, B. & MAKHLOUF, G.M. (1982). Cholinergic and peptidergic receptors on isolated human antral smooth muscle cells. *Gastroenterology*, **82**, 832–837.

BLACK, J.W., LEFF, P. & SHANKLEY, N.P. (1985). Pharmacological analysis of the pentagastrin-tiotidine interaction in the mouse isolated stomach. *Br. J. Pharmacol.*, **86**, 589–599.

BOCK, M.G., DIPARDO, R.M., EVANS, B.E., RITTLE, K.E., WHITTER, W.L., VEBER, D.F., ANDERSON, P.S. & FREIDINGER, R.M. (1989). Benzodiazepine gastrin and brain cholecystokinin receptor ligands: L-365,260. *J. Med. Chem.*, **89**, 13–16.

BODEN, P. & HILL, R.G. (1988). Effects of cholecystokinin and related peptides on neuronal activity in the ventromedial nucleus of the rat hypothalamus. *Br. J. Pharmacol.*, **94**, 246–252.

BOHME, G.A., STUTZMANN, J.M. & BLANCHARD, J.C. (1988). Excitatory effects of cholecystokinin in rat hippocampus. Pharmacological response compatable with 'central' or B-type CCK receptors. *Brain Res.*, **451**, 309–318.

CHANG, R.S.L. & LOTTI, V.J. (1986). Biochemical and pharmacological characterisation of an extremely potent and selective nonpeptide cholecystokinin antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 4923–4926.

CHANG, R.S.L., LOTTI, V.J. & CHEN, T.B. (1984). Further evidence that substance P partly mediates the action of cholecystokinin octapeptide on the guinea-pig ileum but not gallbladder: studies with a substance P antagonist. *Neurosci. Lett.*, **46**, 71–75.

FREIDINGER, R.M. (1989). Development of selective non-peptide CCK_A and CCK_B/gastrin receptor antagonists. In *The Neuropeptide Cholecystokinin*. ed. Hughes, J., Dockray, G.J. & Woodruff, G.N. pp. 123–132. Ellis Horwood.

HILL, D.R., CAMPBELL, N.J., SHAW, T.M. & WOODRUFF, G.N. (1987). Autoradiographic localisation and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective non-peptide CCK antagonists. *J. Neurosci.*, **7**, 2967–2976.

HILL, D.R. & WOODRUFF, G.N. (1989). Differentiation of central CCK receptor binding sites using the non-peptide antagonists MK-329 and L-365,260. *Br. J. Pharmacol.*, **98**, 692P.

HOWBERT, J.J., LOBB, K.L., BROWN, R.F., REEL, J.K., MENDELSOHN, L.G., MASON, N.R., MAHONEY, D.F. & BURNS, R.F. (1991). Evidence that gastrin receptors on AR4-2J cells are distinct from CCK_B receptors using novel pyrazolidinone CCK antagonists. *Soc. Neurosci Abst.*, **16**, 82.

HUANG, S.C., ZHANG, L., CHIANG, H.C.V., WANK, S.A., MATON, P.N., GARDNER, J.D. & JENSEN, R.T. (1989). Benzodiazepine analogues L-365,260 and L-364,718 as gastrin and pancreatic CCK receptor antagonists. *Am. J. Physiol.*, **257**, G169–G174.

HUGHES, J., BODEN, P., COSTALL, B., DOMENEY, A., KELLY, E., HORWELL, D.C., HUNTER, J.C., PINNOCK, R.D. & WOODRUFF, G.N. (1990). Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 6728–6732.

HUTCHISON, J.B. & DOCKRAY, G.J. (1981). Evidence that the action of cholecystokinin octapeptide on the guinea-pig ileum longitudinal muscle is mediated in part by substance P release from the myenteric plexus. *Eur. J. Pharmacol.*, **69**, 87–93.

INNIS, R.B. & SNYDER, S.H. (1980). Distinct cholecystokinin receptors in brain and pancreas. *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 6917–6921.

JENSEN, R.T., LEMP, G.F. & GARDNER, J.D. (1980). Interaction of cholecystokinin with specific membrane receptors on pancreatic acin cells. *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 2079–2083.

JENSEN, R.T., LEMP, G.F. & GARDNER, J.D. (1982) Interactions of COOH-terminal fragments of cholecystokinin with receptors on dispersed acini from guinea-pig pancreas. *J. Biol. Chem.*, **257**, 5554–5559.

KEMP, J.A., MARSHALL, G.R. & WOODRUFF, G.N. (1989). Antagonism of CCK-induced excitation of rat ventromedial hypothalamic neurones by a new selective CCK_B receptor antagonist. *Br. J. Pharmacol.*, **98**, 630P.

LIN, C.W., HOLLIDAY, M.W., BARRETT, R.W., WOLFRAM, C.A.W., MILLER, T.R., WITTE, D., KERWIN, J.F., WAGENAAR, F. & NADZAN, A.M. (1989). Distinct requirements for activation at CCK_A and CCK_B/gastrin receptors: studies with a C-terminal hydrazide analogue of CCK tetrapeptide (30–33). *Mol. Pharmacol.*, **36**, 881–886.

LOTTI, V.J. & CHANG, R.S.L. (1989). A new and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L-365,260. *Eur. J. Pharmacol.*, **162**, 273–280.

LUCAITES, V.L., MENDELSOHN, L.G., MASON, N.R. & COHEN, M.L. (1991). CCK-8, CCK-4 and gastrin-induced contraction of guinea-pig ileum: evidence for differential release of acetylcholine and substance P by CCK_A and CCK_B receptors. *J. Pharmacol. Exp. Ther.*, **256**, 695–703.

MAKOVEC, F., CHISTE, R., BANI, M., REVEL, L., SETNIKAR, I. & ROVATI, A.L. (1985). New glutaramic acid derivatives with potent competitive and specific cholecystokinin-antagonistic activity. *Arzneim-Forsch.*, **35**, 1048–1050.

MENOZZI, D., GARDNER, J.D., JENSEN, R.T. & MATON, P.N. (1989). Properties of receptors for gastrin and CCK on gastric smooth muscle cells. *Am. J. Physiol.*, **257**, G73–G79.

MORAN, T.H., ROBINSON, P.H., GOLDRICH, M.S. & MCHUGH, P.R. (1986). Two brain cholecystokinin receptors: implications for behavioural actions. *Brain Res.*, **362**, 175–179.

PRAISSMAN, M. & BRAND, D.L. (1990). Evidence for two different CCK binding sites in human gastric oxyntic mucosa. *Gastroenterology*, **98**, A518.

REEVES, J.J. & STABLES, R. (1985). Effects of indomethacin, piroxicam and selected prostanoids on gastric acid secretion by the rat isolated gastric mucosa. *Br. J. Pharmacol.*, **86**, 677–684.

REHFELD, J.F. (1981). Four basic characteristics of the gastrin-cholecystokinin system. *Am. J. Physiol.*, **240**, G255–G266.

SOLL, A.H., AMIRIAN, D.A., THOMAS, L.P., REEDY, T.J. & ELASHOFF, J.D. (1984). Gastrin receptors on isolated canine parietal cells. *J. Clin. Invest.*, **73**, 1434–1447.

STEPHENS-SMITH, M.L., IRELAND, S.J. & JORDAN, C.C. (1988). Influence of peptidase inhibitors on responses to neurokinin receptor agonists in the guinea-pig trachea. *Reg. Pept.*, **22**, 177.

SPRAGGS, C.F. & PATEL, M. (1989). Comparison of the effects of gastrin/cholecystokinin-related peptides in rat gastric mucosa and guinea-pig ileum. *Br. J. Pharmacol.*, **98**, 661P.

SPRAGGS, C.F. & PATEL, M. (1990). Functional comparisons of L-365,260 and other gastrin/CCK receptor antagonists in gastric mucosa and ileum *in vitro*. *Br. J. Pharmacol.*, **100**, 360P.

WALSH, J.H. (1987). *Gastrointestinal hormones. Physiology of the Gastrointestinal Tract*. Second edition, ed. Johnson, L.R. pp. 182–190. New York: Raven Press.

WOODRUFF, G.N. & HUGHES, J. (1991). Cholecystokinin antagonists. *Annu. Rev. Pharmacol Toxicol.*, **31**, 469–501.

YAU, W.M., MAKHLOUF, G.M., EDWARDS, L.E. & FARRAR, J.T. (1974). The action of cholecystokinin and related peptides on guinea-pig small intestine. *Can. J. Physiol. Pharmacol.*, **52**, 298–303.

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