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Evaluation of a new and potent cholecystokinin antagonist on motor responses of the guinea-pig intestine

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1 The potency and selectivity of D,L-4-(3,4-dichloro-benzoyl-amino)-5-(dipentyl-amino)-5-oxo-pentanoic acid (CR 1409) as a cholecystokinin (CCK) antagonist was investigated on motor responses of the longitudinal and circular muscles of the guinea-pig isolated ileum. CR 1409 was further used to examine whether nerve-mediated motor responses to electrical field stimulation or distension of the gut wall may involve the release of CCK-like peptides

2 CR 1409 (0.06–2.1 μM) antagonized longitudinal muscle responses to ceruletide (caerulein, a CCK-related decapeptide) in a concentration dependent and competitive manner (pA_2 7.77); responses to CCK-octapeptide (CCK-8) were antagonized with a similar potency. Contractions of the circular muscle evoked by ceruletide were also blocked by CR 1409 (0.2–0.4 μM).

3 Longitudinal muscle contractions in response to dimethylphenylpiperazinium, bethanechol, histamine, substance P, or 5-hydroxytryptamine (5-HT), and circular muscle contractions evoked by acetylcholine, 5-HT, substance P, or substance K were not altered by CR 1409 (0.4 μM). Longitudinal muscle contractions induced by electrical field stimulation (with pulses delivered at 0.05 and 1 Hz in the absence, and at 5 Hz in the presence of atropine) were not or only slightly reduced by CR 1409 (0.4 μM). Longitudinal contractions due to activation of extrinsic nerves by capsaicin remained unaltered in the presence of CR 1409 (0.4 μM).

4 Reflex contractions of the circular muscle, induced by balloon distension and recorded orally to the site of distension, and peristaltic activity elicited by intraluminal infusion of Tyrode solution remained unaffected by CR 1409 (0.4 μM).

5 These findings indicate that CR 1409 is a potent and selective antagonist of CCK-like peptides in the guinea-pig ileum. The results do not provide any evidence that CCK-like peptides, released from extrinsic or intrinsic neurones, are involved in nerve-mediated contractions of intestinal muscle and in the peristaltic reflex.

Introduction

In the gastrointestinal tract, cholecystokinin (CCK)-like peptides have been localized not only in endocrine cells (Polak *et al.*, 1975; Buffa *et al.*, 1976) but also in the enteric nervous system (Larsson & Rehfeld, 1979; Schultzberg *et al.*, 1980; Hutchison *et al.*, 1981; Furness *et al.*, 1982; Keast *et al.*, 1984; Leander *et al.*, 1984). In the guinea-pig ileum, these peptides evoke contraction of the longitudinal and circular muscles by stimulating cholinergic and substance P-containing enteric nerves (Bennett, 1965; Hedner & Rorsman, 1968; Vizi *et al.*, 1973; Holzer *et al.*, 1980; Hutchison & Dockray, 1981; Barthó *et al.*, 1983; Holzer, 1984). These effects are probably responsible for the potent stimulant action of CCK-octapeptide (CCK-8) and the related peptide, ceruletide (caerulein)/, on the peristaltic reflex (Frigo *et al.*, 1971; Mantovani & Vizi, 1974; Chijikwa & Davison, 1974; Barthó *et al.*, 1982a).

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CCK-containing neurones may in fact be involved in intestinal peristalsis since proglumide, a glutaramic acid derivative with weak CCK-antagonistic properties, has been found to inhibit distension-induced contractions of the ileal circular muscle (Davison & Najafi-Farashah, 1982). In keeping with this, peristalsis is associated with a release of CCK-like material from the guinea-pig small intestine (Donnerer *et al.*, 1985).

A new derivative of glutaramic acid, D,L-4-(3, 4-dichloro-benzoyl-amino)-5-(dipentyl-amino)-5-oxo-pentanoic acid (CR 1409) has been found to be over 1,000 times more potent than the parent compound, proglumide, in antagonizing CCK-8 on the guinea-pig gall bladder and on guinea-pig, mouse, and rat pancreatic acini (Makovec *et al.*, 1985; 1986; Jensen *et al.*, 1986: Niederau *et al.*, 1986). In the present study, the potency and selectivity of this new CCK antagonist on motor responses of the longitudinal and circular muscles of the guinea-pig isolated ileum were evaluated. In addition, CR 1409 was used to examine whether CCK-like peptides might play a mediator role in nerve-mediated contractions induced by electrical field stimulation or distension of the gut wall. A preliminary account of these findings has been communicated to the British Pharmacological Society (Barthó & Lippe, 1986).

Methods

Guinea-pigs of either sex and weighing 250 to 350 g were killed by a blow to the head and bled out. Segments of middle ileum were excised, rinsed with and incubated in Tyrode solution of the following composition (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.6. The solution was gassed with O_2. Experiments were carried out in appropriate organ baths at a temperature of 37°C. Details for the individual arrangements were as follows.

Longitudinal muscle contractions

Segments of whole ileum, 1.5 to 2 cm in length, were suspended in a vertical position under a tension of 5 mN. Contractions were recorded isotonically using a minimum-friction lever system (Hugo Sachs Elektronik, Freiburg, FRG), connected to a compensographic recorder. Unless stated otherwise, ceruletide or CCK-8 were administered in a cumulative fashion (with a contact time of 1 min for each concentration), at 15 min intervals. Concentration-response curves were constructed for these substances in the absence and in the presence of various concentrations of CR 1409. After reproducible control concentration-response curves had been obtained, CR 1409 was added 5 min before the next recording of a concentration-response curve. This contact time was chosen on the basis of preliminary experiments in which the time-course of the inhibitory action of CR 1409 (0.1 μM) on half-maximal contractions evoked by ceruletide was investigated. These experiments showed that the action of CR 1409 did not differ when the contact time was varied between 5 and 15 min ($n = 4$).

CR 1409 was also tested against a number of non-CCK agonists. Dimethylphenylpiperazinium (10 μM) was repeatedly administered for periods of 1 min at 15 min intervals. Substance P was repeatedly tested at half-maximally effective concentrations (1–2 nM, contact time 40 s) at 10 min intervals. 5-Hydroxytryptamine (5-HT) concentration-response curves were generated by applying single doses at 5 min intervals, whereas histamine and bethanechol were added to the bath in a cumulative manner (40 s contact time in all cases), with intervals of 10 min between the recording of two concentration-response curves. Capsaicin (0.33 or 3.3 μM for 3 min) was administered to each segment only once, because the tissue is very readily and irreversibly desensitized to this drug (see Szolcsányi & Barthó, 1978). In all these experiments, the contact time of CR 1409 was 5 min.

The effect of CR 1409 (contact time 5 min) on electrically induced contractions was also examined. Electrical field stimulation was applied through a pair of longitudinal platinum wire electrodes, 7 mm apart. Rectangular impulses of supramaximal voltage (30V) and of 0.1 ms width were delivered in three patterns. Firstly, cholinergic 'twitch' contractions were evoked by single impulses (Paton, 1955) at a frequency of 0.05 Hz. Secondly, cholinergic contractions were induced by trains of stimuli delivered at 1 Hz for 10 min. This stimulus was applied only once to each segment. Thirdly, non-cholinergic contractions were evoked by trains of stimuli consisting of 200 impulses, which were delivered at 5 Hz at 10 min intervals while atropine (1 μM) was present in the organ bath (Ambache & Freeman, 1968). All three types of response were abolished by tetrodotoxin (0.5 μM, $n = 3$ for each type).

Circular muscle contractions

Contractions of the circular muscle were recorded as described previously (Holzer *et al.*, 1980; Costa *et al.*, 1985). Segments of ileum, 3–4 cm in length, were pulled over a stainless steel rod of 1 mm diameter and placed into an organ bath in a horizontal position. The mechanical activity was recorded by means of a frog heart clip attached to the mesentery and connected to an isotonic lever under a tension of 5 mN.

For studying the ascending reflex excitation of the circular muscle (Costa *et al.*, 1985), ileal segments of approximately 8 cm length were used. An inflatable balloon, made of latex rubber, was introduced into the

lumen of the segments. The balloon was connected to a syringe by a thin tubing and the system was filled with Tyrode solution. Inflation of the balloon to a diameter of 7.5 mm provided the stimulus for the ascending reflex contraction which was recorded 1.5 to 2 cm orally to the site of distension. Distension stimuli were applied for a period of 5 s at 5 min intervals.

Peristaltic reflex

The method used for studying peristalsis evoked by elevated intraluminal pressure was a combination of those described by Bülbring *et al.* (1958) and Holzer & Lembeck (1979). Ileal segments of approximately 8 cm length were secured horizontally in an organ bath containing Tyrode solution at 37°C. The segments were fixed to inflow and outflow cannulae by their oral and aboral ends, respectively. In order to provide an intraluminal pressure stimulus, prewarmed Tyrode solution was continuously infused through the inflow cannula at a flow rate of 1 ml min^{-1}. The fluid expelled by the peristaltic waves had to pass a mercury valve which opened at a pressure of 4.5 mbar. Aboral intraluminal pressure and a pressure signal proportional to the volume of fluid propelled were recorded from a side arm of the outflow cannula and from a vertical glass tube in which the outflow was collected, respectively (see Holzer & Lembeck, 1979). While the volume of fluid that passed the gut was obviously constant (approximately 1 ml min^{-1}) during longer periods, the volume recording provided useful information as to the efficiency of individual peristaltic waves. Drugs were administered at the same final concentration both to the organ bath and to the solution infused into the lumen.

Analysis of results

In general, data are given as means ± s.e.mean. The number of observations (n) refers to the number of preparations taken from at least 4 different animals. Student's t tests for paired or unpaired data were used for assessing significant differences between means. Longitudinal contractions induced by drugs or electrical field stimulation were expressed as a percentage of the maximal contraction in response to histamine (5 μM). The pA_2 value for CR 1409 was calculated according to the method of Arunlakshana & Schild (1959) against ceruletide as an agonist. For this purpose, dose-ratios for the effect of ceruletide and CCK-8 in the presence of various concentrations of CR 1409 were determined at the level of the half-maximal response. Only concentrations of CR 1409 (0.06–2.1 μM) giving rise to an average dose-ratio of at least 3 (4.1–81.1, respectively) were used (van den Brink, 1977). In most experiments 3 different concentrations of CR 1409 were tested in each preparation. Confidence limits of the pA_2 value were calculated according to Tallarida *et al.* (1979). Whether or not the slope of the Schild plot regression line was significantly different from unity was evaluated according to Sachs (1982).

Drugs

The following drugs were used: acetylcholine chloride and tetrodotoxin (Sigma, München, FRG); atropine sulphate and capsaicin (Merck, Darmstadt, FRG); bethanechol chloride (Schuchardt,München, FRG); CR 1409-Na and proglumide-Na (Rotta Research Laboratories); cholecystokinin octapeptide and substance K (CRB; Cambridge); dimethylphenylpiperazinium iodide (Aldrich, Steinheim, FRG); ceruletide (also called caerulein; Farmitalia, Freiburg, FRG); hexamethonium chloride (Fluka, Buchs, Switzerland); histamine dihydrochloride (Serva, Heidelberg, FRG); 5-hydroxytryptamine creatinine sulphate (Calbiochem, San Diego, Cal., USA); substance P (Peninsula, Belmont, Cal., USA).

A stock solution of CR 1409 (1 mM) was made by dissolving the drug in a 119 mM $NaHCO_3$ solution on the day of the experiment; dilutions were made with Tyrode solution. Peptides were dissolved and diluted in 0.01 N acetic acid, and capsaicin was dissolved in pure ethanol (3.3 mM). At the final concentrations in the bath, these solvents had no effect on the intestinal responses investigated in this study. All other drugs were dissolved in distilled water and diluted with Tyrode solution free of glucose.

Results

Contractions of ileum longitudinal muscle due to CCK-like peptides

CR 1409 antagonized the contractile effect of ceruletide and caused parallel shifts of the ceruletide concentration-response curves to the right (Figure 1). The extent of the shifts depended on the concentration of CR 1409. Analysis of the data with the method of Arunlakshana & Schild (1959) yielded a straight line (Figure 2) the slope of which (−0.94) was not significantly ($P > 0.1$) different from unity. The pA_2 value for CR 1409 derived from the Schild plot was 7.77 (95% confidence limits: 7.72–7.83).

The contractile response to CCK-8 was inhibited by CR 1409 (0.2 μM) to a similar degree as that to ceruletide: the dose-ratio for CCK-8 was 11.8 ± 1.4 ($n = 10$) compared with a value of 12.8 ± 1.0 ($n = 9$) for ceruletide. Ceruletide also contracted the longitudinal muscle in the presence of atropine (0.5 μM), but the contractions were smaller than in the absence of atropine (see Hutchison & Dockray, 1981). The

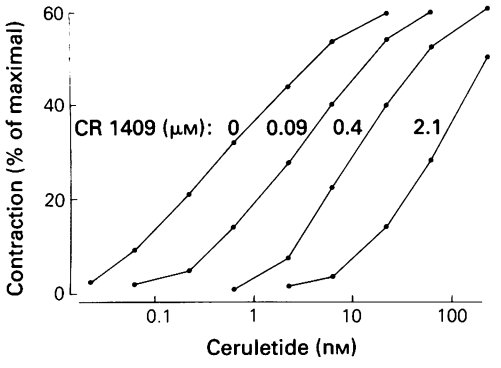

Figure 1 Longitudinal contractions of the guinea-pig isolated ileum. Log concentration-response curves for ceruletide recorded in the absence and presence of various concentrations of CR 1409 as indicated. The contractions are expressed as a percentage of the maximal response to histamine (5 μM). The graph represents the results obtained from one segment of ileum.

atropine-resistant effect of ceruletide was inhibited by CR 1409 (0.2 μM) to a similar extent (dose-ratio: 12.1 ± 1.7, $n = 6$) as that in the absence of atropine.

The action of CR 1409 was rapid in onset (Figure 3) and reversible within 60 min: the sensitivity to ceruletide fully recovered in 3 out of 5 experiments and was nearly completely restored in the other 2 experiments 60 min after CR 1409 (0.2 μM, present for 10 min) had been washed out.

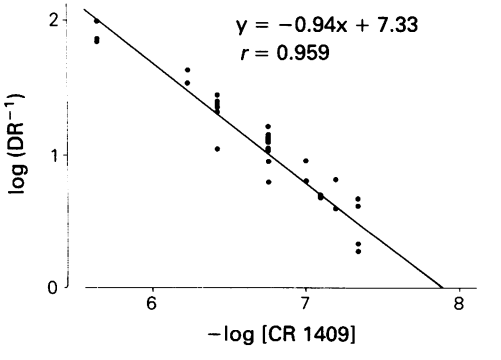

Figure 2 Schild plot of the antagonistic action of CR 1409 on ceruletide-induced contractions of the guinea-pig ileum longitudinal muscle. Abscissa scale: negative log of the concentration of CR 1409 in M. Ordinate scale: log (DR − 1) where DR is the dose-ratio of ceruletide. Regression analysis of the data yielded a straight line which is defined by the formula on the right; r denotes the correlation coefficient.

Contractions of ileum longitudinal muscle due to electrical field stimulation or drugs not related to cholecystokinin

In these experiments CR 1409 was tested at a concentration of 0.4 μM. Contractions evoked by the ganglionic stimulant dimethylphenylpiperazinium (DMPP; 10 μM) were not affected by CR 1409, whereas cholinergic 'twitch' responses due to single electrical pulses delivered at 0.05 Hz were slightly (approximately 3%) but significantly reduced in the presence of the CCK antagonist (Figure 4). Tonic contractions of the ileum due to continuous stimulation at 1 Hz were not changed by CR 1409 (Figure 3). All three types of response (i.e. due to DMPP, 0.05 Hz and 1 Hz field stimulation) were strongly suppressed

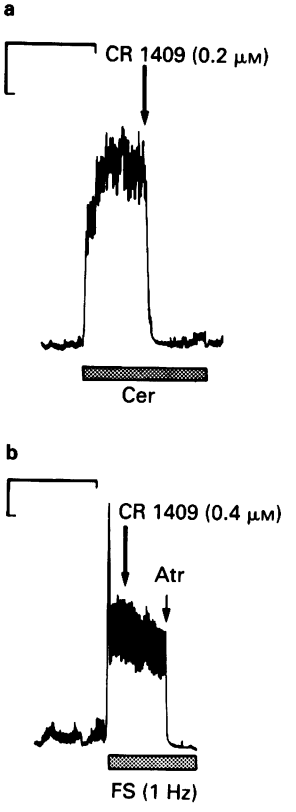

Figure 3 Longitudinal contractions of the guinea-pig isolated ileum evoked by ceruletide (Cer, 0.6 nM, a) or electrical field stimulation at 1 Hz (FS, b). The addition of CR 1409 and atropine (Atr, 1 μM) to the organ bath is indicated by arrows. Calibrations, vertical: 10% of the maximal contraction in response to histamine (5 μM); horizontal: 10 min.

by atropine (0.5 μM; $n = 3$) and abolished by tetrodotoxin (1 μM; $n = 3$). Concentration-response curves for histamine, bethanechol (10 nM–5 μM each) or 5-hydroxytryptamine (5-HT) (0.25 nM–0.25 μM) were not altered by CR 1409 ($n = 3$–5). CR 1409 was also without effect on the contractile responses to capsaicin (0.33 and 3.3 μM, Figure 4). Contractions evoked by field stimulation at 5 Hz in the presence of atropine (1 μM) remained unaffected in the presence of CR 1409 (Figure 4). CR 1409 was also without effect on half-maximal contractions in response to substance P (1 to 2 nM, $n = 5$).

In a separate group of experiments, the specificity of proglumide as a CCK-antagonist was tested. Proglumide (1 mM) shifted the concentration-response curve for ceruletide to the right but also depressed the maximal contractile response to ceruletide by 24 ± 4% ($n = 7$, $P < 0.01$). The apparent dose-ratio for the effect of ceruletide in the presence of 1 mM proglumide was 10 ± 3 ($n = 7$). Proglumide (1 mM) also inhibited cholinergic twitch responses due to electrical field stimulation by 36 ± 7% ($n = 5$, $P < 0.05$) and reduced the contractile responses of DMPP (10 μM) by 30 ± 3% ($n = 6$, $P < 0.01$).

Contractions of ileum circular muscle

Contractile responses of the circular muscle in response to a variety of spasmogens consisted of phasic contractions superimposed on a variable tonic contraction (Figure 5; Holzer *et al.*, 1980). CR 1409 was tested against circular muscle contractions induced by approximately ten fold threshold concentrations of ceruletide, acetylcholine, substance P, substance K, or 5-HT. Exposure to these agonists for periods of 45–60 s at 5 min intervals resulted in fairly reproducible contractile responses. CR 1409, administered at a concentration of 0.2 μM 1 min before ceruletide, abolished the effect of ceruletide (0.18–0.6 nM) in 6 (Figure 5) and partially inhibited it in 3 out of 9 segments tested. At a concentration of 0.4 μM, CR 1409 abolished the response to ceruletide in all cases ($n = 15$). The CCK antagonist (0.4–2.1 μM) did not affect contractions caused by acetylcholine (0.6–1.8 μM, $n = 6$; Figure 5), substance P (0.7–21 nM, $n = 6$), or 5-HT (0.17–1.7 μM, $n = 4$).

Enteric reflexes

Local distension of the gut wall for a period of 5 s resulted in a single brief contraction of the circular muscle on the oral side of the stimulus. The amplitude of this contraction amounted to 95 ± 1% ($n = 10$) of the maximal contraction induced by acetylcholine (6 μM). CR 1409 (0.4 μM) did not alter the ascending reflex contraction as responses in the presence of the CCK antagonist reached 99 ± 3% ($n = 5$) of the control values. Atropine (1 μM) reduced the amplitude of the reflex contraction by 58 ± 8% ($n = 7$). However, CR 1409 (0.4 μM) also failed to change the

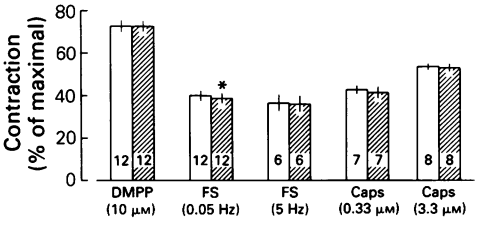

Figure 4 Longitudinal contractions of the guinea-pig ileum evoked by dimethylphenylpiperazinium (DMPP), electrical field stimulation (FS) with single pulses delivered at a frequency of 0.05 Hz, electrical field stimulation with trains of 200 impulses delivered at 5 Hz in the presence of atropine (1 μM), or capsaicin (Caps). The open columns denote the responses before, and the hatched columns those after the administration of CR 1409 (0.4 μM, contact time 5 min) to the organ bath. Note that, since capsaicin was applied only once to each segment, the responses to capsaicin recorded in the absence and presence of CR 1409 are from different segments. The contractions are expressed as a percentage of the maximal contraction to histamine (5 μM). Each column represents the mean with vertical lines showing s.e.mean; n is indicated by number in each column. *$P < 0.01$ (Student's t test for paired data).

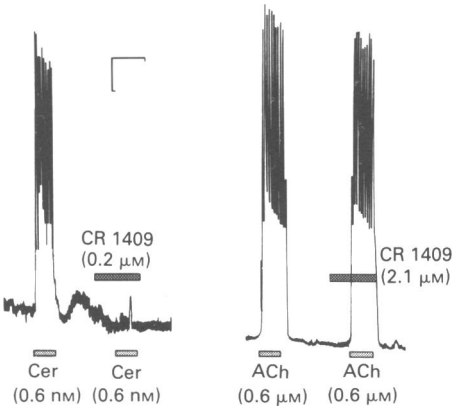

Figure 5 Contractions of the guinea-pig ileum circular muscle in response to ceruletide (Cer) or acetylcholine (ACh) before and after the administration of CR 1409 to the organ bath. Calibrations, vertical: 10% of the maximal response to 6 μM acetylcholine (which corresponded to a virtually maximal obstruction of the lumen of the ileal segment); horizontal: 2 min.

atropine-resistant component of the reflex contraction as responses in the presence of CR 1409 amounted to 102 ± 7% ($n = 7$) of the control responses.

Slow continuous infusion of oxygenated Tyrode solution into the gut lumen gave rise to regular peristaltic waves the frequency of which remained constant within periods of 20–30 min. The presence of CR 1409 (0.4 μM) for 10–15 min in both the organ bath and the solution infused into the gut lumen failed to cause any apparent change in peristalsis ($n = 6$, Figure 6). Raising the concentration of the CCK antagonist to 2.1 μM was without effect ($n = 3$). On the other hand, atropine (0.1–0.5 μM, $n = 3$) strongly inhibited or abolished, while hexamethonium (110 μM, $n = 8$) or tetrodotoxin (0.5 μM, $n = 3$) always abolished peristaltic activity. Within 3 min following their application, these drugs elevated the threshold intraluminal pressure necessary to evoke the emptying phase of the peristaltic reflex, and within 10–15 min their inhibitory effect on peristalsis was fully developed (Figure 6).

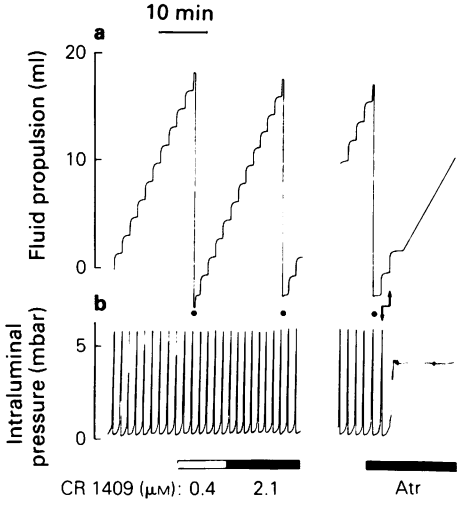

Figure 6 Peristaltic activity of a segment of the guinea-pig ileum evoked by intraluminal infusion of Tyrode solution at a rate of 1 ml min^{-1}. (b) Intraluminal pressure recorded at the aboral end of the segment. (a) Volume of fluid propelled by the peristaltic waves. Dots indicate emptying of the vertical glass tube in which the propelled fluid was collected. The parallaxis between the upper and lower recording is denoted by a double arrow. Shown are the effects of CR 1409 and atropine (Atr, 0.1 μM) which were administered to the organ bath and to the intraluminal infusion medium. The time interval between the left and right recording was 30 min during which CR 1409 was removed from the system by overflow. Atropine caused a complete cessation of peristalsis which resulted in a passive flow of fluid through the gut lumen.

Discussion

The present findings indicate that CR 1409 is a potent antagonist of CCK-related peptides (ceruletide and CCK-8) in the longitudinal and circular muscles of the guinea-pig isolated ileum. Quantitative evaluation of the antagonism in the longitudinal muscle by the method of Arunlakshana & Schild (1959) suggests that CR 1409 interferes with ceruletide in a competitive manner. This is in keeping with previous observations on the guinea-pig gall bladder (Makovec *et al.*, 1985) and rat pancreas (Niederau *et al.*, 1986) where CCK peptides are competitively inhibited by CR 1409. Also the potency of CR 1409 as an antagonist of ceruletide in the guinea-pig ileum ($pA_2 = 7.77$) is quite similar to the potency of this compound as an antagonist of CCK-8 in the guinea-pig gall bladder ($pA_2 = 7.19$; Makovec *et al.*, 1985) and to the potency of CR 1409 in inhibiting the action and binding of CCK peptides in the rat and mouse pancreas (50% inhibitory concentration ~ 0.1 μM; Makovec *et al.*, 1986; Niederau *et al.*, 1986). In contrast, CCK receptors in the mouse cerebral cortex seem to be over 1000 times less sensitive to CR 1409 than CCK receptors in the periphery (Makovec *et al.*, 1986; Niederau *et al.*, 1986). All these observations reinforce the view that CCK receptors outside the central nervous system comprise probably only one class of receptors which are characterized by a high affinity for glutaramic acid derivatives such as CR 1409 (Makovec *et al.*, 1986; Niederau *et al.*, 1986). In this context, it is worth noting that the CCK receptors in the gall bladder and the pancreatic acini seem to be located on non-neuronal cells (Yau *et al.*, 1973; Williams, 1982), whereas those in the guinea-pig ileum are located on enteric neurones (see Introduction). It would appear, therefore, that peripheral CCK receptors share a similar sensitivity to CR 1409 which is independent of their location, an assumption that has also been raised by Dockray (1982) with regard to dibutyryl cyclic guanosine monophosphate.

The specificity of CR 1409 as a CCK antagonist was proved by the finding that contractions of the longitudinal and circular muscles of the guinea-pig ileum, induced by a variety of non-CCK agonists, were not affected. This is in line with previous observations on acetylcholine-induced contractions of the rabbit isolated jejunum (Makovec *et al.*, 1985). Contractions of the guinea-pig ileum in response to CCK-related peptides are due to activation of cholinergic and non-cholinergic enteric neurones (see Introduction). CR 1409 antagonized both the response to ceruletide recorded in the absence of atropine and that recorded in the presence of atropine, the latter being probably mediated by release of endogenous tachykinins (see Barthó & Holzer, 1985). The effect of substance P was, however, not inhibited by CR 1409. Thus, the selec-

tivity of CR 1409 as a CCK antagonist appears to be superior to that of dibutyryl cyclic guanosine monophosphate, another CCK antagonist, which has been found to inhibit responses of the guinea-pig ileum to substance P (Hutchison & Dockray, 1980). The possibility that the inhibitory effect of CR 1409 on the effects of CCK agonists in the guinea-pig ileum might result from an unspecific neurosuppressive action is unlikely for a number of reasons. Nerve mediated responses of the longitudinal muscle to DMPP and 5-HT (Brownlee & Johnson, 1963) and of the circular muscle to 5-HT and substance P (Holzer *et al.*, 1980; Costa *et al.*, 1985) were not influenced by CR 1409 at a concentration which inhibited the response to ceruletide. Likewise, cholinergic and non-cholinergic longitudinal contractions induced by electrical field stimulation at 1 and 5 Hz, respectively (the latter in the presence of atropine), remained unchanged by CR 1409. Cholinergic twitches caused by electrical stimulation at 0.05 Hz were slightly (by 3%) and yet significantly reduced in the presence of CR 1409 but it would be unsafe to draw any conclusion from this minute effect. Taken together, these observations underline the high specificity of CR 1409 as a CCK antagonist in the guinea-pig ileum. As a corollary, they also imply that endogenous CCK-like peptides do not play a mediator role in these effects.

While field stimulation, DMPP, 5-HT, and CCK-like peptides contract the longitudinal muscle of the guinea-pig isolated ileum by stimulating intrinsic neurones of the myenteric plexus, capsaicin causes contraction via activating extrinsic, most likely afferent, neurones which reach the intestine through mesenteric nerves (Szolcsányi & Barthó, 1978; Barthó & Holzer, 1985). Although some of the immunoreactivity may in fact represent a calcitonin gene-related peptide-like material (Ju *et al.*, 1986), primary afferent neurones are known to contain immunoreactive CCK, and a CCK-antiserum has been found to diminish mesenteric vasodilatation produced by intra-arterial administration of capsaicin to the anaesthetized dog (Rózsa *et al.*, 1985). In the guinea-pig ileum, contractions caused by stimulation of mesenteric nerves in the presence of guanethidine are abolished by prolonged exposure to capsaicin. These contractions seem, therefore, to result from the release of the same mediators from afferent nerve endings in the intestinal wall as those released by capsaicin (Szolcsányi & Barthó, 1978). One of the mediators that is released is substance P, which in turn activates intrinsic cholinergic neurones (Barthó *et al.*, 1982b; Chahl, 1982). The possibility that CCK-like peptides may play a mediator role in longitudinal contractions brought about by extrinsic nerve stimulation has, however, been ruled out by the observations that the contractile effect of capsaicin is not affected by CR 1409, and that capsaicin-sensitive contractions induced by mesenteric nerve stimulation are not altered by dibutyryl cyclic guanosine monophosphate (Hutchison & Dockray, 1980).

From the present results it would also appear that CCK-like peptides are not involved in the ascending reflex excitation and in the peristaltic reflex evoked by distension of the gut wall. This conclusion which is based on the observations with CR 1409 is in contrast with that of Davison & Najafi-Farashah (1982), who found that the peristaltic reflex was blocked by a concentration of proglumide (1 mM) that was claimed specifically to antagonize CCK-like peptides. Under the present experimental conditions, however, this concentration of proglumide proved to be non-specific for CCK, since nerve-mediated contractions in response to electrical stimulation and DMPP were also depressed. While these discrepancies are not fully understood it seems clear that proglumide is only a weak antagonist, consequently high concentrations have to be used, and there is only a small margin between the specific and non-specific actions of this antagonist.

In conclusion, the present findings do not support the view that CCK-related substances released from enteric nerves or endocrine cells participate in the regulation of propulsive motility in the guinea-pig isolated ileum. Nevertheless, CCK-related peptides are very potent in enhancing intestinal motor activity and it is, therefore, quite possible that *in vivo* CCK-like peptides released from endocrine cells might modulate peristalsis by a para- or endocrine action.

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