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Characterization of antisecretory and antiulcer activity of CR 2945, a new potent and selective gastrin/CCK_B receptor antagonist

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Abstract

The antigastrinic, antisecretory and antiulcer activities of CR 2945, (*R*)-1-naphthalenepropanoic acid,β-[2-[[2-(8-azaspiro[4.5]dec-8-yl-carbonyl)-4,6-dimethylphenyl] amino]-2-oxoethyl], were investigated in vitro and in vivo in rats and cats. Its activities were compared with those of two gastrin/CCK_B receptor antagonists, L-365,260 (3*R*(+)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-*N*′-(3-methylphenyl)urea and CAM-1028 (4-[[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2-[[[1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)oxy]carbonyl]amino]propyl]amino]-1-phenylethyl]amino-4-oxo-[1*S*-1α,2β[*S*′(*S*′)4α]]-butanoate-*N*-methyl-D-glucamine), of the histamine H_2 receptor antagonist, ranitidine, and the proton pump inhibitor, omeprazole. Cytosolic Ca^{2+} elevation in rabbit parietal cells induced by gastrin (50 nM) was blocked by CR 2945 with an IC_{50} value of 5.9 nM. CAM-1028 and L-365,260 showed similar activity. CR 2945 antagonized pentagastrin-stimulated gastric acid secretion in rats (ED_{50} = 1.3 mg kg^{-1} i.v. and 2.7 mg kg^{-1} i.d.) and cats (1.6 mg kg^{-1} i.v.). CR 2945 was slightly less potent than the reference compounds after i.v. administration, whereas after intraduodenal (i.d.) administration, it was more potent than both ranitidine and omeprazole. In the rat, the gastrin antagonism exhibited by CR 2945 was reversible and competitive, with a pA_2 value of 7.33. CR 2945 had specific antigastrin activity, as it was unable to antagonize the gastric acid secretion stimulated by histamine or carbachol in rats up to the dose of 30 mg kg^{-1}. CR 2945 was about as efficacious as ranitidine against the indomethacin- and ethanol-induced gastric ulcers and the cysteamine-induced duodenal ulcer in rats. On the contrary, L-365,260 was only slightly effective. These results suggest that CR 2945 might be a promising compound for the therapy of acid-related disorders, and that its clinical use could help clarify the therapeutic potential of gastrin/CCK_B receptor antagonists in the gut.

Keywords: CCK_B/gastrin receptor antagonist; CR 2945; CAM-1028; L-365,260; Gastric antisecretory activity; Gastric ulcer; Duodenal ulcer

1. Introduction

The polypeptide hormone, gastrin, is normally released by G cells of the gastric antrum in response to a protein-containing meal (Elwin, 1974; Richardson et al., 1976; Mulholland and Debas, 1988), to gastric distension (Debas et al., 1975) and increases in intragastric pH (Walsh et al., 1975). The principal physiological effects of gastrin are stimulation of gastric acid secretion by parietal cells in the oxyntic mucosa of the stomach and a trophic action on the gastrointestinal mucosa (Sundler et al., 1986; Walsh, 1990). Gastrin enhances gastric acid secretion through two pathways: direct stimulation of the gastrin/CCK_B receptor on the parietal cells (Berglindh et al., 1976) and indirect stimulation mediated by the release of histamine from enterochromaffin-like (ECL) cells (Sandvik and Waldum, 1991; Prinz et al., 1993) by activation of histidine decarboxylase (Håkanson et al., 1994). Parietal cells, ECL cells, somatostatin-secreting cells (D cells) and, in the pyloric antral non-acid secreting mucosa, the gastrin-producing G cells, interact through their messengers to regulate acid secretion (Dockray et al., 1996).

In the last 10 years, some high-affinity gastrin/CCK_B receptor antagonists have been identified. A benzodiazepine derivative, L-365,260, was the first potent non-peptide receptor antagonist of the gastrin/CCK_B receptor synthesised by the Merck group (Lotti and Chang, 1989). L-365,260 showed nanomolar affinity for the gastrin/

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CCK_B receptor, and inhibited gastrin-stimulated gastric acid secretion in mice, rats and guinea pigs, whereas it was less effective in dogs (Lotti and Chang, 1989). These authors also reported that L-365,260 did not affect basal and histamine- or betanechol-induced gastric acid secretion. More recently, Nishida et al. (1992) and Pendley et al. (1995) demonstrated that L-365,260 also inhibited basal gastric acid secretion and histamine- or betanechol-induced gastric acid secretion at doses higher than that active to antagonize pentagastrin-stimulated gastric acid secretion.

The potency of L-365,260 was surpassed with compound YM022 (Nishida et al., 1993) that was about 100 times more active than L-365,260 to inhibit pentagastrin-stimulated gastric acid secretion in the rat, without affecting the histamine- or betanechol-stimulated gastric acid secretion.

An improvement of the selectivity for the CCK_B over CCK_A receptors was obtained with YF476, a 3-methylamino derivative of YM022 (Semple et al., 1997). YF476 inhibited pentagastrin-stimulated gastric acid secretion, showing a potency about 15-fold higher than that of famotidine (Takinami et al., 1997). This compound showed good oral bioavailability.

Hughes et al. (1990) developed a series of tryptophan dipeptoids receptor antagonists of the gastrin/CCK_B receptor. The compounds of this series showed high affinity for the gastrin/CCK_B receptor (Horwell et al., 1991; Woodruff and Hughes, 1991) and inhibited the pentagastrin-stimulated gastric acid secretion with a potency comparable to that of ranitidine (Hayward et al., 1991). These compounds seem to exert a partial agonist effect in stomach and pancreas (Schmassmann et al., 1994).

Böhme et al. (1994) described a novel family of non-peptide gastrin/CCK_B receptor antagonists with a ureidoacetamide structure, of which RP73870 is a member. RP73870 binds with nanomolar affinity to the gastrin/CCK_B receptor. In vivo, RP73870 inhibits basal and pentagastrin-stimulated gastric acid secretion and was as potent as omeprazole and famotidine to prevent experimental ulcers (Pendley et al., 1995).

In 1992, we described the antigastrin activity of CR 2194 (spiroglumide), a non-peptide gastrin/CCK_B receptor antagonist, with a pentanoic acid derivative structure (Revel et al., 1992). This compound showed micromolar affinity for the gastrin/CCK_B receptor. In vivo, CR 2194 demonstrated good antisecretory activity in the rat, dog and cat. In humans, spiroglumide in a phase II randomised, double-blind, placebo-controlled study in healthy volunteers, decreased both basal and meal-stimulated gastric acid secretion (Beltinger et al., 1994). Despite its excellent bioavailability, the relatively low affinity and selectivity for the gastrin/CCK_B receptor precluded further development of spiroglumide as a potential therapeutic tool for peptic ulcer diseases.

More recently, with an objective to improve the potency of CR 2194, we synthesised a new series of non-peptide CCK_B/gastrin receptor antagonists. From this series, CR 2945, an anthranilic acid derivative, showed nanomolar affinity (IC_{50} = 2.3 nM) and excellent selectivity (CCK_A/CCK_B = 9000) for the CCK_B receptor (Makovec and D'Amato, 1997).

We now describe the inhibitory potency of CR 2945 against the cytosolic Ca^{2+} elevation elicited by gastrin in rabbit parietal cells, its gastric antisecretory activity in the 'in situ' perfused rat stomach induced by pentagastrin, histamine or carbachol, its antisecretory activity in chronic gastric fistula rats and cats and its antiulcer activity in several models of gastro-duodenal damage in rats.

The results were compared with those of simultaneous experiments performed with the gastrin/CCK_B receptor antagonists, L-365,260 and CAM-1028, the histamine H_2 receptor antagonist, ranitidine, and the proton pump inhibitor, omeprazole.

2. Materials and methods

All animal procedures used are in accordance with the Italian Agency's policies (Ministero della Sanità, Direzione dei Servizi Veterinari, authorization no. 202/95) about animal experimentation.

2.1. Intracellular Ca^{2+} monitoring in enriched rabbit parietal cells preparation

The in vitro functional response to gastrin was evaluated by measuring the increase of the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in enriched rabbit parietal cells preparations (Letari et al., 1996). The intracellular $[Ca^{2+}]_i$ concentration was stimulated by 50 nM human gastrin I and $[Ca^{2+}]_i$ was monitored with the fluorescent Ca^{2+} indicator FURA-2/AM {1-[2(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methylphenoxy)-ethane-*N*,*N*,*N*′*N*′-tetraacetic acid pentaacetoxymethyl ester}. Antagonists were added 1 min prior to the agonist; controls received as the vehicle either Ca^{2+}-HEPES-buffered saline or dimethyl sulfoxide (for L-365,260). Antagonism studies were performed with a suspension enriched in parietal cells in which gastrin evoked at least a 50% increase of $[Ca^{2+}]_i$ over resting levels.

2.2. Antisecretory activity in the in situ perfused rat stomach

Male Sprague–Dawley rats (Harlan, S. Pietro al Natisone, Italy) weighing 230–260 g were used. After 24-h fasting, the animals were anaesthetized with urethane (6 ml kg^{-1} of 25% solution) and underwent surgery for stomach perfusion as described previously (Ghosh and Schild, 1958), with slight modifications (Lai, 1964). Each rat stomach was perfused with 0.25 mM NaOH, at 37°C, at a rate of 1 ml min^{-1}, for the whole period of the experiment

and the perfusate was collected at 10-min intervals. Acid secretion was measured by titration of 1 ml gastric perfusate to neutrality with NaOH 0.001 N. The acid output was expressed as micromole of acid per unit time (10 min). After a 30-min stabilization period, pentagastrin (30 μg kg^{-1} h^{-1}) or carbachol (30 μg kg^{-1} h^{-1}) or histamine (3 mg kg^{-1} h^{-1}) was infused through the jugular vein at constant rate of 0.95 ml h^{-1}, for 120 min. Antagonists or vehicles were administered i.v. or intraduodenally (i.d.) as a bolus, 60 min after starting stimulant infusion.

In antagonism studies, a cumulative dose–response curve for pentagastrin (0.08–1280 μg kg^{-1} h^{-1}) was made in the presence or absence of increasing doses of CR 2945 (range 0.1–1 mg kg^{-1} h^{-1}) infused during the total period of pentagastrin stimulation.

In separate experiments, during a 6-h pentagastrin infusion (30 μg kg^{-1} h^{-1}), CR 2945 was infused at doses of 0.1–0.3–1 mg kg^{-1} h^{-1}, in the 60–120 min interval, in order to establish the reversibility of its gastrin antagonism.

2.3. Antisecretory activity in chronic gastric fistula rats

Male Sprague–Dawley rats (Harlan, S. Pietro al Natisone, Italy) with 250–280 g body weight were used. Each animal was prepared with a chronic gastric fistula using aseptic surgical methods as previously described (Parè et al., 1977). A recovery period of at least 2 weeks was included before the gastric secretory experiments. After 24-h fasting, the gastric cannula was opened and the stomach was rinsed with saline. A minimum of a 30-min period was allowed for equilibration of the basal secretion. When basal gastric acid secretion was studied, gastric juice was collected in 60-min aliquots for 3 h. CR 2945 or vehicle was administered i.v. as a bolus at the start of the experiment. When pentagastrin-induced gastric acid secretion was to be studied, the animals received s.c. 30 μg kg^{-1} of pentagastrin. Maximal gastric stimulation by pentagastrin was obtained during the first 30 min after administration. CR 2945 or vehicle was administered i.v. as a bolus, 15 min before pentagastrin administration and gastric juice was collected in one aliquot of 30 min after pentagastrin stimulation. Samples of gastric juice were centrifuged at low speed for removal of solids and 1-ml aliquots were titrated to pH 7.0 with 0.01 N NaOH. Total acid output was expressed as micromole of acid per 30 min aliquots.

2.4. Antisecretory activity in chronic gastric fistula cats

Eight male adult hybrid cats (Al Serio, Bergamo, Italy), weighing 3–3.5 kg and provided with a gastric cannula as previously described by Impicciatore et al. (1980), were used. Secretory studies started 1 month after surgery. After 18-h fasting, intravenous catheters were placed in the hind limbs and, after a 30-min equilibration period, pentagastrin was infused at a dose of 4 μg kg^{-1} h^{-1}, for a period of 3 h; CR 2945 was administered i.v. as a bolus 60 min after the start of pentagastrin infusion, at doses of 0.5–1.25–5 mg kg^{-1}. Acid secretion was measured by titration at pH 7.0 of 1-ml aliquots of gastric juice using 0.1 N NaOH. Total acid output was expressed as micromole of acid per unit time (10 min).

2.5. Antiulcer activity in rats

Male Sprague–Dawley rats (Harlan, S. Pietro al Natisone, Italy) with 175–200 g body weight fasted from 15 to 18 h were used for all experiments.

2.5.1. Indomethacin-induced gastric lesions

Ulcers were induced by p.o. administration of 10 mg kg^{-1} of indomethacin, suspended in methylcellulose 0.5%. The animals were killed 4 h after the indomethacin administration; the stomach was excised, opened along the greater curvature; and the mucosa was examined with a 10× magnifying binocular microscope for the presence of necrotic lesions which were counted and quantified according to the following arbitrary scoring: 0 = no mucosal damage, 1 = haemorrhagic petechiae, 2 = haemorrhagic suffusion, 3 = one to five little ulcers, 4 = several little ulcers, 5 = many severe ulcers, 6 = perforated ulcers, 7 = many perforated ulcers. Drugs were administered s.c. 15 min before indomethacin in at least three dose levels to groups of five animals ($n \geq 15$).

2.5.2. Ethanol-induced gastric lesions

Gastric lesions were induced by p.o. administration of 1.5 ml of absolute ethanol (Robert et al., 1979). The animals were killed 1 h later, the stomach was excised, opened along the greater curvature, and the mucosa was examined with a 10× magnifying binocular microscope, for the presence of necrotic lesions. The length (mm) of individual lesions in the corpus was measured, and the sum of the length of all lesions in each stomach was used as the lesion index. Drugs were administered i.v. 15 min before ethanol, in at least three dose levels to groups of five animals ($n \geq 15$).

2.5.3. Cysteamine-induced duodenal ulcers

Duodenal ulcers were induced by two p.o. administrations of 300-mg kg^{-1} doses of cysteamine administered at 4-h intervals (Szabo et al., 1977). The animals were killed 24 h after the initial dose, the stomach and duodenum were excised, opened and duodenal mucosa was examined with a 10× magnifying binocular microscope for the presence of lesions. The area (square mm) of each lesion was calculated by multiplying the two greatest perpendicular axis and the sum of the areas of all duodenal lesions was used as the lesion index. Drugs were administered i.p. 15

min before the first dose of cysteamine and s.c. 1 h before the last dose of cysteamine. Three dose levels of each drug were used for groups of six animals ($n \geq 18$).

2.6. Chemicals

Pentagastrin, histamine, carbachol, ranitidine and urethane were purchased from Sigma (St. Louis, MO, USA). FURA-2/AM was from Calbiochem (La Jolla, CA, USA); omeprazole, CR 2945, (*R*)-1-naphthalenepropanoic acid, β[2- [[2- (8- azaspiro[4.5]dec- 8-ylcarbonyl)-4,6-dimethylphenyl]amino]-2-oxoethyl] sodium salt and L-365,260, (3*R*(+)- *N*-[2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-ben-zodiazepine-3-yl]-*N*′-[3-methylphenyl] urea) were synthesised in the Chemistry Department of Rotta Research Laboratorium (RRL) (Monza, Italy). CAM-1028, (4-[[2-[[3-(1*H*-indol-3-yl)-2-methyl-1-oxo-2[[[1.7.7-trimethylbicyclo[2.2.1]hept-2-yl)oxy]carbonyl]amino]propyl]amino]-1-phenylethyl]amino-4-oxo-[1*S*-1α.2β[*S*′(*S*′)4α]]-butanoate *N*-methyl-D-glucamine), was a gift of Dr. J. Hughes (Parke-Davis Neuroscience Research Centre, Cambridge, UK). CR 2945, CAM-1028 and ranitidine were dissolved in saline, L-365,260 was dissolved in PEG400-EtOH-saline 60:10:30 (v/v), omeprazole in sodium carbonate-saline.

2.7. Calculation and statistical analyses

In $[Ca^{2+}]$ response experiments, concentration–response curves were analyzed with a computerised curve fitting technique (ALLFIT) using the four-parameter logistic equation (DeLean et al., 1978). Results of separate experiments were expressed as IC_{50}, with $P = 0.05$ fiducial limits. Dose–response curves from gastric acid secretion models were analyzed by a least square linear regression analysis. For ethanol and cysteamine gastrointestinal damage models in rats, the gastric acid lesion indexes of treated groups were calculated as percentages in comparison with the respective control groups and dose–response curves were analyzed as described above. The ED_{50} with $P = 0.05$ fiducial limits were calculated. Prevention of indomethacin-induced gastric damage in rats was determined by comparing results in treated animals vs. vehicle controls by using a Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks, followed by a multiple comparison procedure (Dunn's method). Tests for parallelism, linear regression of dose–response curves and relative potencies were performed with a quantitative bioassay procedure according to Sen (1971). Dose–response curve comparisons were made only between doses common to each curve. In the antagonism studies in rats, the pA_2 value with 95% fiducial limits was calculated from the Schild plot (Arunlakshana and Schild, 1959) using a computer statistics package developed by Tallarida and Murray (1981). Data for gastric antisecretory activity in chronic fistula rats were expressed as means ± standard error of the mean (S.E.M), and analyzed by one-way ANOVA. Statistical significance of differences between groups was evaluated with Duncan's test, and the level of significance was taken as $P < 0.05$. Where indicated, Tukey's multiple comparison test was used.

3. Results

3.1. Intracellular Ca^{2+} monitoring in enriched rabbit parietal cells preparation

In enriched rabbit parietal cells preparation, gastrin 0.1 nM–4 μM increased cytosolic Ca^{2+} concentration dependently. A gastrin concentration of 50 nM was used in antagonism experiments. At this concentration, gastrin induced a $[Ca^{2+}]$ increase ranging from 40 to 140% in the various experiments. To normalize and compare the data, we expressed the effect of receptor antagonists as percentage $[Ca^{2+}]_i$ increase, taking the increase induced by 50 nM gastrin alone as 100%.

CR 2945 antagonized the response to gastrin in a dose-dependent manner with an IC_{50} value of 5.9 nM. CAM-1028 and L-365,260 showed similar activity, with IC_{50} of 2.6 and 10.4 nM, respectively (Table 1).

3.2. Antisecretory activity in the in situ perfused rat stomach

The i.v. infusion of pentagastrin stimulated dose-dependently the gastric acid secretion in perfused rat stomach over a dose range of 0.08–40 μg kg^{-1} h^{-1}. An infusion of 30 μg kg^{-1} h^{-1} of pentagastrin induced a submaximal secretory response and this dose was used for antagonism studies. A similar secretory response was elicited by histamine 3 mg kg^{-1} h^{-1} and carbachol 30 μg kg^{-1} h^{-1} (Fig. 1).

CR 2945, i.v., antagonized dose-dependently the gastric acid secretion induced by pentagastrin infusion, with an ED_{50} of 1.3 mg kg^{-1}. The reference compound CCK_B/gastrin receptor antagonist, L-365,260, showed similar activity in the same model, with an ED_{50} of 0.5 mg kg^{-1}. The histamine H_2 receptor antagonist, ranitidine, and the proton pump inhibitor, omeprazole, given i.v.

Table 1
Effect of CCK receptor antagonists on gastrin-induced cytosolic free $[Ca^{2+}]_i$ elevation

Substance	IC_{50} nM (fiducial limits)	*n*
CR 2945	5.9 (3.4–10.2)	3
CAM-1028	2.6 (2.0–3.3)	2
L-365,260	10.4 (9.2–12.4)	2

Data were obtained using rabbit parietal-enriched cell suspensions, loaded with 4 μM FURA-2/AM. Cells were stimulated with 50 nM human gastrin I. Antagonists were added about 1 min prior to the agonist. Data are expressed as $\Delta\%[Ca^{2+}]_i$; n = number of separate experiments; P = 0.05 fiducial limits.

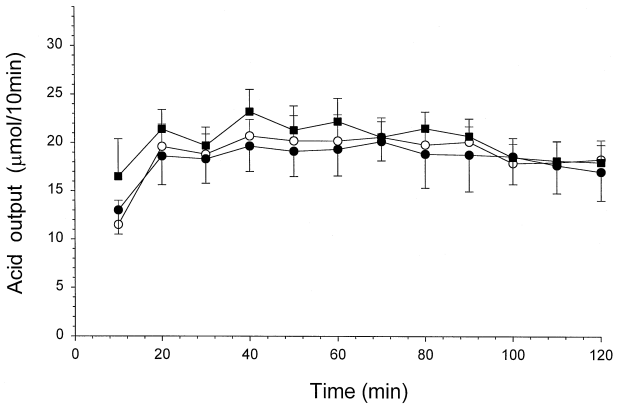

Fig. 1. Gastric acid secretion in the 'in situ' perfused rat stomach stimulated by pentagastrin 30 μg kg^{-1} h^{-1} (○), histamine 3 mg kg^{-1} h^{-1} (●) and carbachol 30 μg kg^{-1} h^{-1} (■), respectively. Data are means ± S.E.M. obtained from four to five experiments. Tukey's multiple comparison test was used. No significant differences between treatment groups were found ($P > 0.05$).

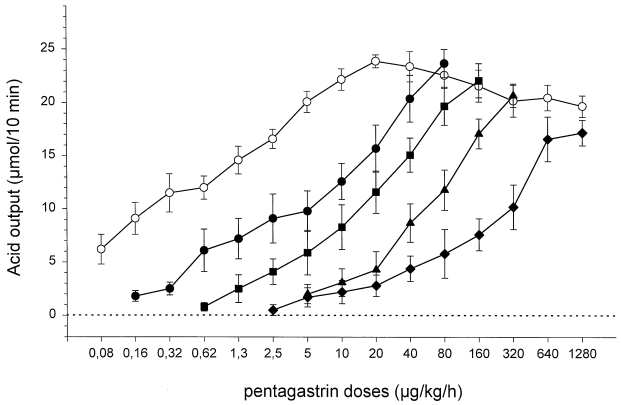

Fig. 2. Inhibition by CR 2945 of pentagastrin-stimulated gastric acid secretion in the 'in situ' perfused rat stomach. Dose–response curves were obtained with pentagastrin alone (○) or in the presence of increasing doses of CR 2945 perfused i.v. (●: 0.1 mg kg^{-1} h^{-1}; ■: 0.3 mg kg^{-1} h^{-1}; ▲: 0.7 mg kg^{-1} h^{-1}; ◆: 1 mg kg^{-1} h^{-1}). Each point indicates the mean ± S.E.M., for $n = 6$ observations ($n = 5$ for 0.1 mg kg^{-1} group).

inhibited dose-relatedly the pentagastrin-induced gastric acid secretion with an ED_{50} of 0.3 mg kg^{-1} and 0.6 mg kg^{-1}, respectively. After i.d. administration, CR 2945, as well as the reference compounds, inhibited the pentagastrin-stimulated acid secretion. The ED_{50} calculated for CR 2945 was 2.7 mg kg^{-1} and this value was two or three times lower than that for omeprazole and ranitidine, respectively. On the contrary, L-365,260 inhibited the pentagastrin-induced acid secretion at doses higher than 10 mg kg^{-1} (Table 2).

Intravenous administration of CR 2945 did not affect histamine- and carbachol-induced gastric acid secretion up to 30 mg kg^{-1}, a dose about 20 times higher than its anti-gastrin ED_{50}. In contrast, ranitidine and omeprazole were about equiactive to inhibit gastric acid secretion induced by both pentagastrin and histamine, whereas omeprazole was much more effective to inhibit the gastric acid secretion induced by carbachol. Also, L-365,260 inhibited the histamine- and carbachol-induced gastric acid secretion, but with an ED_{50} about 4–10 times higher than its anti-gastrin ED_{50} (Table 2).

The antagonistic profile of CR 2945 on pentagastrin-stimulated gastric acid secretion in the in situ perfused rat stomach was evaluated over the dose-range of 0.1–1 mg kg^{-1} (Fig. 2). Pentagastrin produced a dose-related increase of gastric acid secretion; this response was linear between 0.1 and 20 μg kg^{-1} h^{-1}. CR 2945 caused a right shift of the cumulative dose–response curves of pentagastrin, without decreasing the maximum effect. The inhibition was dose-dependent and consistent with a model of competitive antagonism; the Schild plot was linear, with the slope not statistically different by 1. The calculated pA_2 with 95% fiducial limits was 7.33 (5.7–9.3).

The reversibility of CR 2945 antagonistic activity was demonstrated in experiments where CR 2945 was infused for 1 h during a 6-h period of pentagastrin infusion. As Fig. 3 shows, the pentagastrin-induced acid output was decreased during the 60-min infusion of CR 2945 at doses of 0.1–0.3 and 1 mg kg^{-1}, but when the infusion of CR 2945 was stopped, the acid output increased in a dose-related way, to reach its control values 6 h after the start of pentagastrin infusion. Fig. 3 also shows the duration of action of CR 2945. The acid output remained completely inhibited for about 2 h after the infusion of CR 2945 1 mg kg^{-1} was stopped.

Table 2
Effects of CR 2945 and reference antisecretory agents on gastric acid secretion induced by pentagastrin, histamine and carbachol in anaesthetized rats

Substance	Pentagastrin (30 μg kg^{-1} h^{-1})		Histamine (3 mg kg^{-1} h^{-1})	Carbachol (30 μg kg^{-1} h^{-1})
	i.v.	i.d.[a]	i.v.	i.v.
CR 2945	1.3 (0.8–2.3)	2.7 (1.0–7.8)	> 30	> 30
L-365,260	0.5 (0.3–0.9)	> 10	2.2 (0.8–6.2)	5.1 (1.9–14.0)
Ranitidine	0.3 (0.1–0.7)	8.3 (1.0–66.9)	0.6 (0.1–2.6)	13.3 (2.7–66.2)
Omeprazole	0.6 (0.5–0.7)	5.9 (1.2–29.2)	0.4 (0.1–1.4)	1.8 (0.4–7.4)

Data are ED_{50} (mg kg^{-1}) with $P = 0.05$ fiducial limits.
[a] i.d. (intraduodenal).

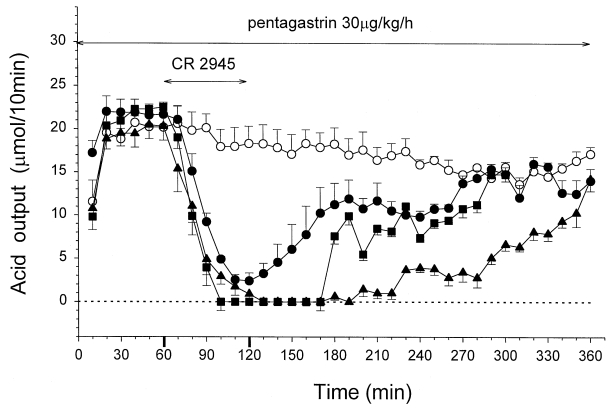

Fig. 3. Reversibility of antagonism of CR 2945 in pentagastrin-stimulated gastric acid secretion in the 'in situ' perfused rat stomach. Pentagastrin alone (○) or in the presence of CR 2945 0.1 mg kg^{-1} h^{-1} (●), 0.3 mg kg^{-1} h^{-1} (■) and 1 mg kg^{-1} h^{-1} (▲). Values are means ± S.E.M. for $n = 5$ replicates.

3.3. Antisecretory activity in chronic gastric fistula rats

3.3.1. Basal secretion

In conscious rats implanted with a chronic gastric fistula, the basal gastric acid secretion measured every 60 min for 3 h remained stable around a mean value of 117 ± 12 μmol of acid. CR 2945 (i.v.), at the doses of 3 and 10 mg kg^{-1}, decreased dose-dependently the basal gastric acid output (Fig. 4). The dose of 10 mg kg^{-1} reduced to about 50% the basal gastric acid secretion for 2 h after CR 2945 administration; this effect was statistically significant, with respect to the control group.

3.3.2. Pentagastrin-stimulated gastric acid secretion

In chronic gastric fistula rats, pentagastrin at the dose of 30 μg kg^{-1} s.c increased gastric acid secretion from 75 ± 9.2 (basal values) to 208 ± 19.8 μmol. CR 2945 in a dose-range of 0.1–10 mg kg^{-1} (i.v.) antagonized in a dose-dependent way the pentagastrin-induced gastric acid secretion (Fig. 5). This effect became statistically significant at the dose of 3 mg kg^{-1}. The dose of 10 mg kg^{-1} antagonized almost completely the stimulator effect of pentagastrin. The calculated ED_{50} was 0.8 (0.1–4.2) mg kg^{-1} with $P = 0.05$ fiducial limits.

3.4. Antisecretory activity in chronic gastric fistula cats

In chronic gastric fistula cats, pentagastrin induced a dose-related increase in gastric acid secretion; the activity

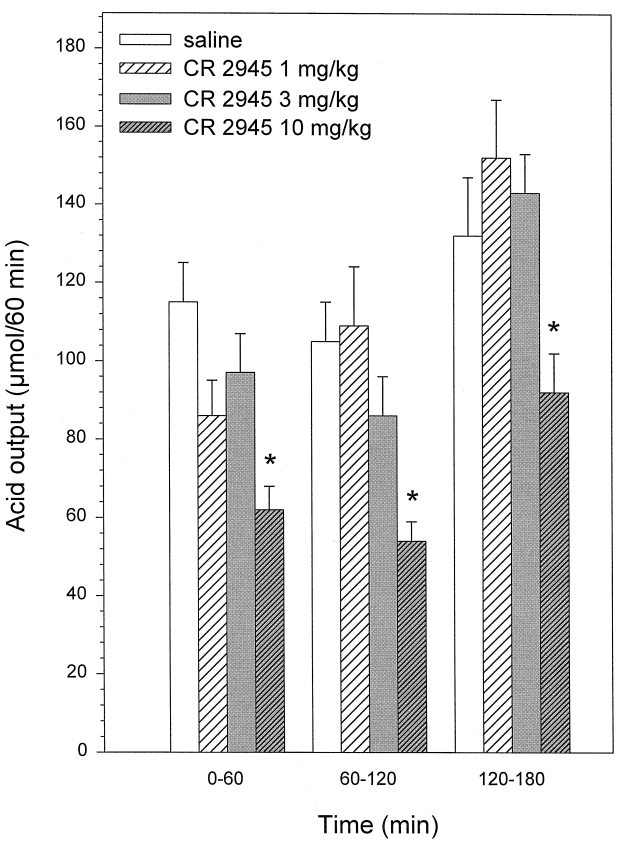

Fig. 4. Effect of CR 2945 on basal gastric acid secretion in chronic gastric fistula rats. Data represent acid output (μmol) in 60-min intervals. CR 2945 was administered i.v. Data are means ± S.E.M. for $n = 8$ replications. $^{*}P < 0.05$ vs. saline-treated group.

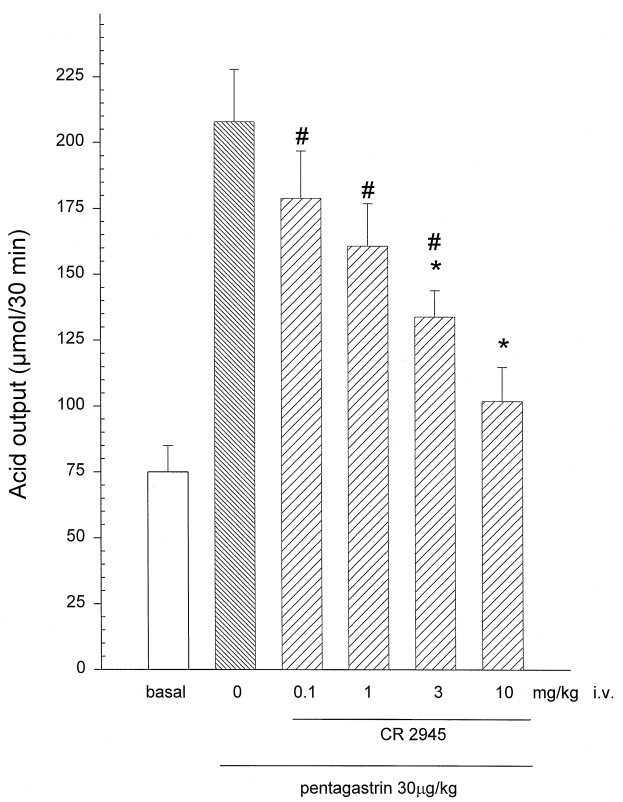

Fig. 5. Effect of CR 2945 on pentagastrin-stimulated gastric acid secretion in chronic gastric fistula rats. Data represent acid output (μmol) in one 30-min aliquot. CR 2945 was administered i.v. Data are means ± S.E.M. for $n = 6$ replications. $^{*}P < 0.05$ vs. pentagastrin alone; $^{\#}P < 0.05$ vs. saline basal.

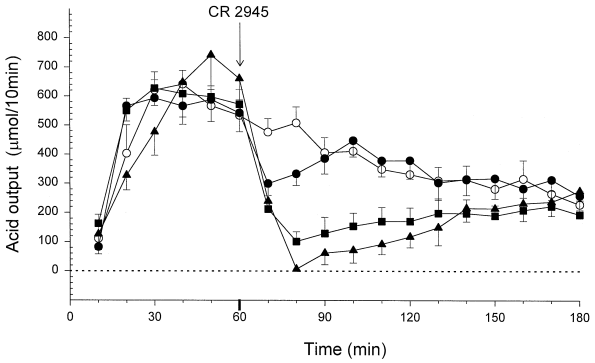

Fig. 6. Inhibition by CR 2945 of 4 μg kg^{-1} h^{-1} infusion of pentagastrin- stimulating gastric acid secretion in chronic gastric fistula cats. Dose–response curves were obtained with pentagastrin alone (○) or in the presence of increasing doses of CR 2945 perfused i.v. (●: 0.5 mg kg^{-1} h^{-1}; ■: 1.25 mg kg^{-1} h^{-1}; ▲: 5 mg kg^{-1} h^{-1}). Each point indicates the mean for $n = 4$ observations.

of CR 2945 was studied against the 50–60% stimulated maximal pentagastrin response produced by 4 μg kg^{-1} h^{-1}. CR 2945 was administered at doses of 0.5–1.25–5 mg kg^{-1} i.v. at the time of maximal response to stimulation (60 min after starting pentagastrin infusion). In this model, CR 2945 induced a dose-dependent decrease of gastric acid secretion (Fig. 6). In the first hour after its administration, CR 2945 had its maximal antagonistic effect and the dose of 5 mg kg^{-1} completely abolished pentagastrin stimulation. The calculated ED_{50} was 1.64 (0.7–3.8) mg kg^{-1} with $P = 0.05$ fiducial limits. In the second hour after administration, the activity of CR 2945 decreased, showing an ED_{50} of 2.95 (1–9) mg kg^{-1} with $P = 0.05$ fiducial limits.

3.5. Antiulcer activity

The antiulcer activity of CR 2945 was evaluated and compared to that of standard antisecretory agents in gastric acid-dependent and gastric acid-independent gastrointestinal damage models in rats. The gastric-acid dependent models included indomethacin-induced gastric lesions and cysteamine-induced duodenal damage. The gastric acid-independent damage models included ethanol-induced gastric lesions.

Table 4
Effects of CR 2945, L-365,260 and ranitidine on gastrointestinal damage induced by ethanol and cysteamine in rats

Substance	Ethanol gastric ulcer	Cysteamine duodenal ulcer
	i.v.	i.p. + s.c.
CR 2945	7.6 (3.3–17.6) ($n = 30$)	8.8 (4.3–18.2) ($n = 36$)
L-365,260	> 10[a] ($n = 15$)	> 10 ($n = 18$)
Ranitidine	10.0 (2.3–43.1) ($n = 15$)	8.5 (2.1–34.0) ($n = 18$)

Data are ED_{50} (mg kg^{-1}) with $P = 0.05$ fiducial limits.
[a] Through s.c. administration.
n = Number of animals/product.

Indomethacin administered p.o. at dose of 10 mg kg^{-1} induced severe gastric damage in the glandular part of the stomach. The overall median ulcer index with interquartile ranges in the control animals from four experiments was 6.0 (4.5–7) ($n = 20$). When given s.c., CR 2945, as well as all standard antisecretory agents tested, prevented dose-relatedly the gastric damage induced by indomethacin. The CR 2945, ranitidine and L-365,260 dose–response curve slopes were determined to be parallel lines. CR 2945 exhibited about the same inhibitory potency as the H_2 receptor antagonist, ranitidine, whereas L-365,260 was 3.3 (1.4–5.3) times ($P < 0.05$) less potent than CR 2945 (Table 3).

Two oral administrations of cysteamine 300 mg kg^{-1} to fasted rats induced in 24 h a near 100% incidence of duodenal ulcers; the mean (± S.E.M.) lesion index (area in square millimeters) in the control animals was 24.6 ± 2.2 ($n = 23$). In this model, CR 2945 administered twice (i.p. and s.c.) reduced in a dose-related way the mucosal damage with an ED_{50} of 8.8 mg kg^{-1}. Ranitidine exhibited about the same efficacy (8.5 mg kg^{-1}), whereas L-365,260 was less effective, as its highest dose tested (10 mg kg^{-1}) reduced the duodenal ulcer area by 30% only (Table 4).

The oral administration of 1.5 ml of absolute ethanol to fasted rats induced severe and extended mucosal lesions mainly in the glandular stomach. In this model, saline-treated animals had much of the mucosa ulcerated. The mean (± S.E.M.) lesion index (length in millimeters) for control animals was 79.2 ± 4.2 ($n = 20$). CR 2945 given i.v. reduced in a dose-range of 1–10 mg kg^{-1} the effect of ethanol. The calculated ED_{50} was 7.6 mg kg^{-1}. Ranitidine,

Table 3
Effect of CR 2945 and reference standards on indomethacin-induced gastric lesions

	CR 2945 ($n = 25$)	Ranitidine ($n = 15$)	L-365,260 ($n = 15$)
Saline	7.0 (4.0–7.0)	6.0 (2.5–6.0)	6.0 (5.75–7.0)
Drug 0.1 mg kg^{-1}	7.0 (5.25–7.0)	–	–
Drug 0.3 mg kg^{-1}	4.0 (1.0–7.0)	–	–
Drug 1 mg kg^{-1}	3.0 (0.75–4.0)	4.0 (2.75–6.0)	5.0 (3.75–5.5)
Drug 3 mg kg^{-1}	2.0 (1.5–3.25)[a]	2.0 (0.0–3.0)	3.0 (2.75–4.25)
Drug 10 mg kg^{-1}	1.0 (0.0–1.5)[a]	1.0 (0.0–3.0)[a]	2.0 (1.75–2.25)[a]

Values represent the median ulcer index and interquartile ranges.
[a] $P < 0.05$ vs. saline (Kruskal–Wallis one-way analysis of variance on ranks; Dunn's test post-hoc analysis).

given i.v., inhibited the gastric damage induced by ethanol with similar potency, whereas L-365,260 administered s.c. up to 10 mg kg^{-1} was ineffective.

4. Discussion

CR 2945 is a novel, potent and selective non-peptide gastrin/CCK_B receptor antagonist shown, in binding studies, to have nanomolar affinity for CCK_B/gastrin receptors in rat brain cortex and excellent selectivity (about 9000-fold) over the CCK_A receptor (Revel et al., 1998). These results were confirmed by functional tests in vitro. In enriched rabbit parietal cells preparations, CR 2945 antagonized the cytosolic Ca^{2+} elevation induced by a submaximal dose of gastrin at nanomolar concentrations, whereas it exhibited only weak (micromolar) antagonistic activity on guinea pig gallbladder contraction induced by sub-maximal concentrations of sulphated CCK-8 (data not shown).

We now explored the antisecretory and antiulcer activity of this compound in several in vivo animal models. The results were compared with those for conventional standard antiulcer agents such as ranitidine and omeprazole and the gastrin/CCK_B receptor antagonists, L-365,260 and CAM-1028.

In anaesthetized rats, CR 2945 given i.v. inhibited pentagastrin-induced gastric acid secretion with an ED_{50} of 1.3 mg kg^{-1}, being slightly less potent than ranitidine, L-365,260 and omeprazole. However, after i.d. administration, this may be considered as very similar to oral administration, the compound was three times more potent than ranitidine and twice as potent as omeprazole. This suggests good bioavailability for CR 2945. L-365,260, however, was inactive up to 10 mg kg^{-1} when given this way.

With the same model of the 'in situ' perfused rat stomach, CR 2945 perfused i.v. shifted to the right the cumulative dose–response curves for pentagastrin without decreasing the maximum effect. This inhibition was consistent with a model of competitive antagonism and the calculated pA_2 was 7.33.

CR 2945 was ineffective to antagonize the gastric acid secretion stimulated by histamine and carbachol at doses more than 10 times higher than those required to antagonize the secretory response to gastrin. On the contrary, as previously reported by Nishida et al. (1992) and Pendley et al. (1995), L-365,260 inhibited histamine- and carbachol-induced gastric acid secretion.

Another important characteristic of CR 2945 is the reversibility of its gastrin receptor antagonism. During pentagastrin infusion to the gastric fistula rats, when the administration of the highest dose of CR 2945 used (1 mg kg^{-1}) was stopped after 60-min infusion, gastric secretion increased in a dose-related way to reach normal values within 4 h.

Basal acid secretion in saline-treated control conscious rats implanted with a chronic gastric fistula was stable for 3 h around a mean value of 120 μmol h^{-1}. CR 2945 at 10 mg kg^{-1} inhibited by about 50% the basal gastric acid secretion at 2 h after i.v. treatment. In the same model of chronic gastric fistula rats, pentagastrin-induced acid secretion was antagonized dose-dependently by CR 2945 in the range 0.1–10 mg kg^{-1} i.v. The calculated ED_{50} for the first 30-min period after the s.c. injection of 30 μg kg^{-1} of pentagastrin was 0.8 mg kg^{-1}. Clearly, lower doses of CR 2945 were able to inhibit pentagastrin-stimulated acid secretion, whereas higher doses also inhibited basal secretion.

In chronic gastric fistula cats stimulated with pentagastrin 4 μg kg^{-1} h^{-1}, CR 2945 had an activity similar to that seen in rats. The calculated ED_{50} was 1.64 mg kg^{-1} and the dose of 5 mg kg^{-1} totally abolished the pentagastrin stimulation in the first hour after i.v. administration.

CR 2945 inhibited the formation of gastric lesions in both gastric acid-dependent and gastric acid-independent damage models in rats.

In the gastric acid-dependent model induced by p.o. administration of 10 mg kg^{-1} of indomethacin, CR 2945 s.c. at the doses of 3 and 10 mg kg^{-1} significantly inhibited the formation of gastric lesions. Its potency was comparable to that of ranitidine and three times greater than that of L-365,260.

The activity of CR 2945 in ethanol-induced gastric lesions, our acid-independent gastric damage model, appeared less. The ED_{50} of CR 2945 was 7.6 mg kg^{-1} on i.v. administration, a value similar to that exhibited by ranitidine (10.0 mg kg^{-1}). On the contrary, L-365,260 administered s.c. up to 10 mg kg^{-1} was ineffective. These data are consistent with the results obtained by other authors, who described gastrin/CCK_B receptor antagonists as hardly protective in this acid-independent model in rats (Pendley et al., 1993; Nishida et al., 1994).

CR 2945 was about as efficacious as ranitidine against cysteamine-induced duodenal ulcer; the calculated ED_{50} values were 8.8 and 8.5 mg kg^{-1}, respectively. L-365,260 was also less effective in this model, as its highest dose (10 mg kg^{-1}) reduced the duodenal ulcer area by 30% only.

In summary, the results reported in this study demonstrated that CR 2945 is a potent gastrin/CCK_B receptor antagonist, with a competitive and reversible profile of gastrin antagonism. Its calculated pA_2 value from 'in vivo' studies was 7.33. CR 2945, administered i.d., was more potent than ranitidine and omeprazole to inhibit the gastric acid secretion stimulated by pentagastrin in rats, whereas L-365,260 was ineffective when given by this route. Doses about three times higher than those able to produce inhibition of pentagastrin-stimulated acid secretion, also inhibited the basal acid secretion in chronic gastric fistula rats.

CR 2945 proved to be as potent as the histamine H_2 receptor antagonist, ranitidine, to give protection from gastrointestinal lesions in three different gastric acid-de-

pendent and -independent damage models in rats. Probably due to its poor bioavailability, L-365,260 was less potent.

5. Conclusions

CR 2945 given i.d. was more potent than ranitidine and omeprazole in inhibiting the gastric acid secretion induced by pentagastrin and had the same efficacy as ranitidine in gastroduodenal ulcer models.

Although gastrin/CCK_B receptor antagonists represent an alternative, novel, therapeutic approach for treatment of peptic ulcer, the results obtained so far are not very encouraging. For instance, the potential therapeutic role of L-365,260 in patients with ulcer disease would appear to be minor, since a 50-mg oral dose of L-365,260 had an antisecretory efficacy equivalent to that of a 5-mg oral dose of famotidine. Furthermore, at the same doses, L-365,260 seems to be ineffective in inhibiting basal acid secretion (Murphy et al., 1993). These results may be explained by the poor bioavailability of L-365,260.

However, CR 2945 is better absorbed than L-365-260 in rats. Furthermore, preliminary bioavailability studies in volunteers have shown that the absorption of CR 2945 in humans is much higher than in rats. In fact, the oral administration of 1 mg kg^{-1} of CR 2945 in humans produced an AUC (0–24 h) of 1200 ng ml^{-1} plasma × h, a value six times higher than that obtained in rats given the same dose but by the i.v. route (unpublished data).

We conclude that CR 2945 might be a promising compound for the therapy of acid-related gut diseases and that its clinical use could help clarify the therapeutic potential of the gastrin/CCK_B receptor antagonists in the gut.

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