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Inhibition of dimaprit- and pentagastrin-induced gastric acid secretion in cats by the new histamine H_2 antagonist, CM 57755

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The antisecretory effects of CM 57755, a new histamine-H_2 receptor antagonist, have been compared with those of cimetidine on gastric acid secretion induced by intravenous infusions of dimaprit or pentagastrin into conscious cats with chronically implanted gastric fistulae. Intravenous infusion of CM 57755 induced a parallel shift to the right of the dimaprit dose-response curve. The potency of CM 57755 was comparable with that of cimetidine as shown by similar doses causing a 5-fold displacement to the right of the dimaprit dose-response curve (4·9 μmol kg^{-1} h^{-1} for CM 57755 and 4·7 μmol kg^{-1} h^{-1} for cimetidine). Unlike that with dimaprit, the acid secretion stimulated by increasing doses of pentagastrin was inhibited by CM 57755 with depression of the maximal effect, indicating non-competitive antagonism. In a second series of experiments the time course of the anti-secretory action of intragastrically administered CM 57755 was studied from the gastric acid secretion induced by constant infusion of dimaprit. At equieffective doses, CM 57755 caused more sustained inhibition than cimetidine.

CM 57755, *N*-[2-(5-dimethylaminomethylfuran-2-ylmethylthio)ethyl]-3-pyridinecarboxamide 1-oxide dihydrochloride is a new histamine-H_2 receptor antagonist, which inhibits stimulated gastric acid secretion in different animal species in-vivo and in-vitro (Lavezzo et al 1984).

CM 57755, unlike cimetidine, had no antiandrogenic effects in rats even after prolonged treatment (Lacheretz et al 1984). It did not interact with cytochrome P450 in-vitro (Picard-Fraire et al 1984) and did not interfere with the metabolism of other concurrently administered drugs in the rat (Bianchetti et al 1985). The inhibitory effects of CM 57755 on nocturnal gastric acid secretion in man have also been demonstrated (Wilson et al 1986).

The aim of this work was to compare CM 57755 with cimetidine for their ability to inhibit gastric acid secretion stimulated by pentagastrin or by the selective H_2-receptor agonist dimaprit (Parsons et al 1977) in conscious cats with chronically implanted gastric fistulae.

Materials and methods

Male cats weighing 3·5 to 5 kg with chronically implanted gastric fistulae (Emans 1960) were housed in individual cages, and fasted for 18 h. On the day of the experiment the animals were placed in sling frames and a cannula was inserted in the cephalic vein of a forepaw for the infusion of agonists and antagonists in sterile 0·9% NaCl (saline) (30 ml h^{-1}). The stomach was washed with tepid water through the fistula. Gastric juice was collected in 30 min fractions and the acidity of each fraction was titrated to pH 7 with 0·1 M NaOH with an automatic Mettler titrator. Basal acid secretion under these conditions was negligible compared with the stimulated secretion.

In a first set of experiments, gastric acid secretion was stimulated by intravenous infusion of increasing doses of either dimaprit (0·17 to 87 μmol kg^{-1} h^{-1}) or pentagastrin (0·01 to 120 nmol kg^{-1} h^{-1}), in saline, each dose being infused for 30 min. CM 57755 or cimetidine were dissolved in saline adjusted to pH 7 and added to the infusion fluid. The gastric juice was collected for the last 20 min of infusion of each agonist dose. Mean acid output after maximal stimulation (μequiv/20 min ± s.e.) was 2950 ± 100 with dimaprit and 3200 ± 100 with pentagastrin. The effects of the test substances were compared from the gastric acid secretion curves in the presence and absence of the antagonist.

In the experiment with dimaprit, these curves were used to calculate the ratio of the agonist doses producing the same response (half maximal acid outputs of control) in the presence and absence of the H_2-antagonists (DR). To examine the nature of the antagonism, the log (DR-1) was plotted against the log molar dose of antagonist to obtain Schild plots (Arunlakshana & Schild 1959). The potency comparison between CM 57755 and cimetidine was made on the basis of the doses of antagonists, derived from the plots, causing a 5-fold displacement to the right of the dimaprit dose-response curve. Values of pA_2 were calculated from the same plots (Tallarida & Jacob 1979). In the experiments with pentagastrin the doses of antagonist inhibiting by 50% the maximal effect of pentagastrin (ID50) were extrapolated from the plots of percent maximal reponse vs log molar dose of antagonist.

In a second set of experiments designed to evaluate the duration of the inhibitory effects of the antagonists, gastric acid secretion was stimulated by intravenous infusion of a constant amount of dimaprit (2·7 μmol kg^{-1} h^{-1}). Under these experimental conditions, gastric hypersecretion reached a maximum after 45 min and remained stable for more than 3 h. CM 57755 or cimetidine were administered intragastrically, 15 mg kg^{-1} in aqueous solution (10 ml, pH 7), through a plastic

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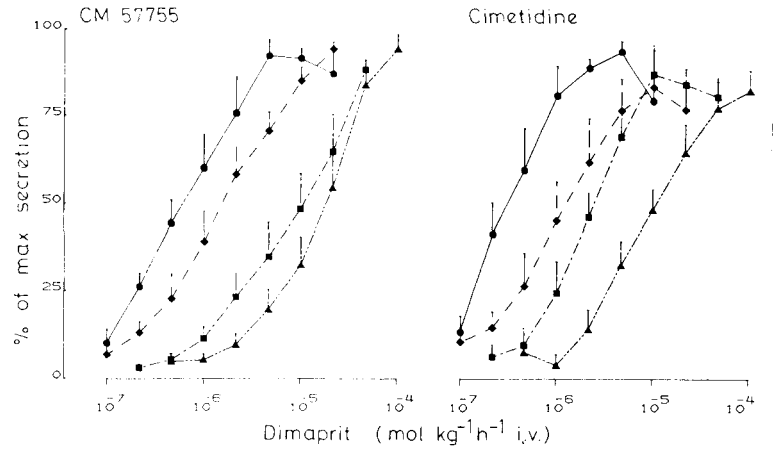

FIG. 1. Dose-response curves to dimaprit (●) and dimaprit plus CM 57755 or cimetidine at 2·5 (◆), 7·5 (■) and 22·5 (▲) μmol kg^{-1} h^{-1} i.v. Each value is the mean of 4 experiments with different cats. Vertical bars represent standard errors of the means. The doses causing a 5-fold displacement to the right of the dimaprit dose-response curve, calculated from the Schild plot, were 4·9 μmol kg^{-1} h^{-1} for CM 57755 and 4·7 for cimetidine. For further details see Methods.

cannula connected to the fistula. The cannula was then rinsed with 10 ml of water. Stimulation with dimaprit was carried out as described above, 1, 2 or 3 h after drug administration. Gastric secretion was measured for 3 consecutive hours after starting dimaprit infusion. Preliminary experiments with phenol red had shown that gastric emptying of the solution containing either CM 57755 or cimetidine was completed in 1 h; thus there was no significant drug loss during collection of gastric juice fractions.

The following drugs and reference compounds were used: CM 57755 (Groupe Sanofi), cimetidine (C.F.M., Milan), dimaprit (Groupe Sanofi), pentagastrin (Gastrodiagnost, Merck).

Results and discussion

Intravenously infused CM 57755, like cimetidine, produced parallel shifts to the right of the dose-response curves of dimaprit-stimulated gastric secretion without any change in maximal response (Fig. 1). The slope of the Schild plot calculated from these curves was not significantly different from one, indicating that, like cimetidine, CM 57755 is a competitive antagonist of dimaprit-induced gastric secretion. CM 57755 and cimetidine inhibited dimaprit with the same potency since a 5-fold displacement to the right of the dimaprit dose-response curve occurred at similar antagonist doses (μmol kg^{-1} h^{-1}) as calculated from the Schild plots (CM 57755: 4·9, cimetidine: 4·7). From the same plots similar apparent pA_2 values were obtained for CM 57755 and cimetidine (5·8 and 5·9, respectively).

The estimation of pA_2 values from the in-vivo experiments is open to question because the conditions of equilibrium are not always satisfied and the actual antagonist concentration at the receptor sites is not known. But in the present experiment, CM 57755 and cimetidine were administered by constant i.v. infusion to obtain equilibrium conditions as required for tentative estimates of in-vivo apparent pA_2 values (Tallarida et al 1979). Even though the actual concentration of CM 57755 at the receptor sites is not known, the apparent pA_2 value for CM 57755 in this experiment was very close to that previously reported in-vitro (5·9) against histamine-stimulated acid secretion in the guinea-pig isolated gastric mucosa (Lavezzo et al 1984), i.e. under experimental conditions used to evaluate the real affinity for the H_2 receptors.

Intravenously infused CM 57755 and cimetidine caused dose-dependent inhibition of gastric acid secretion induced by increasing doses of pentagastrin, with depression of the maximal effect (Fig. 2). This indicates that the antagonism of pentagastrin, unlike that of dimaprit, is not competitive. These results are consistent with the reported non-competitive nature of pentagastrin antagonism by H_2 antagonists in dogs (Daly et al 1981). As illustrated in Fig. 2, either antagonist depressed the maximal effect of pentagastrin with similar potency and the ID50s (μmol kg^{-1} h^{-1}) were, respectively, 6·3 and 12·5 for CM 57755 and cimetidine.

We reported previously that CM 57755 was a long-lasting inhibitor of acid secretion in the cat (Lavezzo et al 1984). In fact, its inhibitory potency against dimaprit-induced hypersecretion declined much less with time than that of cimetidine. In order to confirm the long duration of action of CM 57755, in the present study we compared the time course of the antisecretory activity of CM 57755 and cimetidine by giving the antagonists intragastrically at different times before dimaprit infusion. Equieffective doses of the two antagonists were

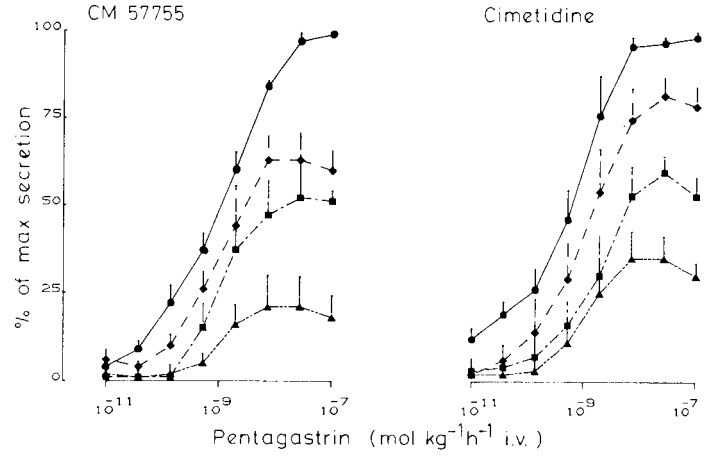

FIG. 2. Dose-response curves to pentagastrin (●), pentagastrin plus CM 57755 at 3·5 (◆), 7·5 (■) and 15 (▲) μmol kg^{-1} h^{-1} i.v. or pentagastrin plus cimetidine at 4 (◆), 8 (■) and 16 (▲) μmol kg^{-1} h^{-1} i.v. Each value is the mean of 4 experiments with different cats. Vertical bars represent standard errors of the means. The calculated doses inhibiting by 50% the maximal effect of pentagastrin were 6·3 μmol kg^{-1} h^{-1} for CM 57755 and 12·5 for cimetidine. For further details see Methods.

Table 1. Time-course of inhibition by intragastric CM 57755 and cimetidine of dimaprit-induced gastric acid secretion in the cat.

				Hourly fraction from the beginning of dimaprit infusion					
				1st		2nd		3rd	
Compound	Dose μmol kg^{-1} i.g.	Pre-treatment time (h)	No. of cats	Acid output μequiv ± s.e.	% Inhib.	Acid output μequiv ± s.e.	% Inhib.	Acid output μequiv ± s.e.	% Inhib.
Control	—	—	11	4500 ± 361	—	6703 ± 516	—	6964 ± 842	—
CM 57755	37	1	5	1101 ± 157**	75	2681 ± 378**	60	3256 ± 384**	53
		2	4	2027 ± 352**	55	3852 ± 658**	42	4504 ± 648**	35
		3	4	2196 ± 533**	51	3875 ± 920**	42	4666 ± 1093**	33
Cimetidine	59	1	5	1121 ± 392**	75	4307 ± 301*	35	5965 ± 434	14
		2	4	2791 ± 301*	38	5773 ± 335	14	6888 ± 319	1
		3	4	3532 ± 670	21	6225 ± 878	7	6783 ± 527	3

Significance of difference vs control: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ } Dunnett's test.

selected in pilot studies beforehand. As shown in Table 1, CM 57755 (37 μmol kg^{-1} = 14 mg kg^{-1} dihydrochloride salt), administered intragastrically 1 h before dimaprit, significantly depressed acid output throughout the 3 h dimaprit infusion, with maximal inhibition (75%) during the 1st hour. In contrast, cimetidine (59 μmol kg^{-1} = 15 mg kg^{-1}) given 1 h before dimaprit substantially depressed the hypersecretion only during the 1st hour (75% inhibition) of dimaprit infusion. The longer-lasting effect of CM 57755 was even more apparent from its marked and persistent inhibition of acid output with pretreatment times of 2 and 3 h (Table 1). With these pretreatment times cimetidine produced little or no inhibition.

In summary, the present results indicate that CM 57755 prevents gastric acid secretion in cats by competitive antagonism at histamine-H_2 receptors, with a potency comparable to that of cimetidine. The previously reported finding that the antisecretory activity of CM 57755 is longer lasting than that of cimetidine has been confirmed (Lavezzo et al 1984).

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Effect of adrenergic drugs on the isolated colon of *Rhesus cynomolgus*

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The effects of adrenaline, noradrenaline, isoprenaline and dopamine were studied on the longitudinal muscle of the ascending and descending colon of the rhesus monkey. All these drugs induced a relaxation of the preparation, dopamine being the less active agonist. The responses seem to be the result of β_2-adrenoceptor stimulation since their inhibition by practolol (β_1) is weaker than their inhibition by propranolol (β_1 and β_2 dopaminergic). There is no evidence for the presence of dopaminergic receptors in this preparation.

The effects of catecholamines on the gastrointestinal tract of many mammalian species are mediated by either α- or β-adrenoceptors (Ahlqvist & Levy 1959; Furchgott 1960; Bowman & Hall 1970). The relative importance of these two types of receptors varies according to the level of the GI tract studied and the animal species. Their stimulation produced essentially inhibitory responses (Ahlqvist & Levy 1959; Ek & Lundgren 1982). However, in some preparations, a contraction can be induced by α-adrenoceptor stimulation (Fontaine et al 1984).

We have previously shown that α- and β-adrenoceptors are present in the longitudinal muscle of the mouse and the dog colon (Fontaine et al 1984; Grivegnée et al 1984). With the availability of tissue from animals used by industry for anatomy and pathology tests, we studied the effect of various sympathomimetic drugs on the ascending and descending colon of the monkey, *Rhesus cynomolgus*.

Materials and methods

Segments of ascending and descending colon which had been removed under pentobarbitone sodium anaesthesia (30 mg kg^{-1}) were excised and immersed in Krebs solution (mM): NaCl 118·1; KCl 4·7; $CaCl_2$ 2·5; KH_2PO_4 1·2; $MgSO_4$ 1·2; glucose 5; $NaHCO_3$ 25, aerated with 95% O_2 and 5% CO_2. The mucosa was then removed and longitudinal strips (0·5 × 5 cm) were each set up in 50 ml organ baths containing Krebs solution at 37 °C gassed with 95% O_2, 5% CO_2 and allowed to equilibrate for at least 60 min.

The load on the tissues was 2 g. Responses were recorded on a kymograph, using an isotonic lever (× 5 magnification). Concentration-response curves were established for adrenaline, noradrenaline, dopamine and isoprenaline and the IC 50 values (concentration needed to develop 50% of the maximal response) determined. As these curves were not very reproducible on the same preparation, the effects of α- and β-adrenoceptor blocking drugs (15 min preincubation period) were studied on responses to the same agonists obtained by repetition of a concentration producing about 75% of the maximal response in the absence of blocking drug.

Drugs used were adrenaline bitartrate (Fluka), dopamine hydrochloride (Winthrop), phentolamine methane sulphonate (Ciba), practolol hydrochloride and propranolol hydrochloride (ICI).

Results

Adrenaline (10 nM to 1 μM), noradrenaline (10 nM to 1 μM), dopamine (1 μM to 100 μM) and isoprenaline (10 nM to 1 μM) all induced relaxations of the longitudinal muscle of the ascending or descending colon. The responses developed slowly and reached their maximum in about 4 min and persisted for more than 10 min. After the agonist had been washed out (after 4 min), a rapid return of the tone of the preparation towards the basal level was observed. The IC50 values for these four agonists are given in Table 1. The results are similar for the descending and the ascending part of the colon, except for dopamine which is more active (IC 50 about ten times higher) for the ascending than the descending part.

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