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SOME *in vitro* AND *in vivo* ACTIONS OF THE NEW HISTAMINE H_2-RECEPTOR ANTAGONIST, RANITIDINE

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1 Ranitidine has been investigated as an antagonist of the H_2-receptor-mediated responses to histamine of guinea-pig atrium and rat uterus *in vitro* and as an inhibitor of gastric acid secretion in the rat.

2 Ranitidine competitively antagonized histamine-induced increases in contraction frequency of the guinea-pig isolated right atrium. Ranitidine had a pA_2 of 7.2 and was 7.9 and 4.5 times more potent than metiamide and cimetidine respectively.

3 Ranitidine competitively antagonized histamine-induced relaxations of the rat isolated uterine horn. Ranitidine had a pA_2 of 6.95 and was 3.6 and 5.9 times more potent than metiamide and cimetidine respectively.

4 Ranitidine, even at high concentrations, did not affect responses of the guinea-pig isolated atrium or rat isolated uterus to (−)-isoprenaline. Similarly it was without effect on either histamine or bethanechol-induced contractions of guinea-pig isolated ileum.

5 Ranitidine inhibited histamine- and pentagastrin-induced gastric acid secretion in the perfused stomach preparation of the anaesthetized rat. Ranitidine was 5.2 and 7.0 times more potent on a molar basis than metiamide and cimetidine respectively, as an inhibitor of histamine-induced gastric acid secretion.

6 It is concluded that ranitidine is a potent, competitive and selective antagonist of histamine at H_2-receptor sites and an effective inhibitor of gastric acid secretion *in vivo*.

Introduction

The actions of histamine have been shown to be mediated either through H_1-receptors (Ash & Schild, 1966) or H_2-receptors (Black, Duncan, Durant, Ganellin & Parsons, 1972). The responses of arterial, intestinal and bronchial smooth muscle result predominantly from the stimulation of H_1-receptors and these effects are competitively antagonized by H_1-antagonists such as mepyramine (Loew 1947; Owen 1977). Other actions of histamine such as stimulation of gastric acid secretion and heart muscle are not antagonized by H_1-receptor antagonists but are competitively antagonized by the imidazole derivatives, burimamide (Black *et al.*, 1972), metiamide (Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973) and cimetidine (Brimblecombe, Duncan, Durant, Emmett, Ganellin & Parsons, 1975). Until now, an imidazole moiety has been thought to be a necessary feature for potent H_2-receptor antagonists (Ganellin, Durant & Emmett, 1976). The present paper provides results showing that an amino-alkyl substituted furan, although not containing an imidazole ring, is a potent H_2-receptor antagonist *in vitro* and can inhibit gastric acid secretion *in vivo*.

This substance (Figure 1) is N-{2-[[[5-(dimethylaminomethyl)-2-furanyl]methyl]thio]ethyl}-N^1-methyl-2-nitro-1,1-ethenediamine (AH 19065), approved name, ranitidine. Ranitidine has been compared with metiamide and cimetidine to determine its activity as an H_2-receptor antagonist. A preliminary account of some of these findings has been presented to the British Pharmacological Society (Bradshaw, Brittain, Clitherow, Daly, Jack, Price & Stables, 1979).

Methods

Isolated tissue experiments

Guinea-pig isolated right atrium The spontaneous contraction frequency of a piece of guinea-pig right atrium mounted in a 20 ml bath containing Krebs

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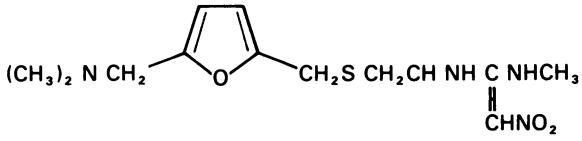

Figure 1 Structure of ranitidine (AH 19065).

solution at 32°C and bubbled with 95% O_2 and 5% CO_2 was recorded continuously. Control cumulative response (increase in atrial rate) curves to histamine (1×10^{-8} to 1×10^{-5} mol/l) were obtained at 60 min intervals. After two constant control curves had been obtained, the test drug was added to the bath and 45 min later a further response curve to histamine was obtained in the presence of the test drug. Response curves were plotted as % of maximum response against log concentration of histamine. Dose-ratios were calculated at the level of the 50% response and the data transformed to an Arunlakshana & Schild (1959) plot. Ranitidine was tested at 3.2×10^{-7}, 9.6×10^{-7} and 3.2×10^{-6} mol/l; metiamide at 1.3×10^{-6}, 4.5×10^{-6} and 1.3×10^{-5} mol/l and cimetidine at 1.2×10^{-6}, 4.0×10^{-6} and 1.2×10^{-5} mol/l. Each dose of antagonist was tested in at least four separate preparations. In other experiments (−)-isoprenaline was used as the agonist instead of histamine and the effects of high concentrations of ranitidine (3.2 and 9.6×10^{-5} mol/l) and cimetidine (1.2×10^{-4} mol/l) examined.

Rat isolated uterine horn A uterine horn removed from a rat in natural oestrus was suspended in a 20 ml bath containing De Jalon's solution at 28°C bubbled with 95% O_2 and 5% CO_2. The uterus was made to contract, by increasing the potassium concentration ten fold, and cumulative dose-response (relaxation of the uterus) curves were obtained to histamine (3×10^{-8} to 3×10^{-5} mol/l). After two control curves the test drug was added to the bath and 30 min later a histamine concentration-response curve was obtained in the presence of the test compound. The doses of ranitidine, metiamide and cimetidine and the method of calculating the results were as described for the atria experiments. Ranitidine (3.2×10^{-5} mol/l) and cimetidine (1.2×10^{-4} mol/l) were also tested against (−)-isoprenaline-induced relaxation of the uterus.

Guinea-pig isolated ileum A section of guinea-pig terminal ileum was mounted in a 20 ml bath containing Krebs solution at 32°C bubbled with 95% O_2 and 5% CO_2. A contraction concentration-response curve was obtained to histamine (10^{-7} to 10^{-3} mol/l) using a 3 min cycle and 15 s contact time. Concentration-response curves were repeated until constant; a further concentration-response curve was obtained 10 min after adding ranitidine or cimetidine to the bath. Equivalent experiments were also carried out with bethanechol (10^{-6} to 10^{-3} mol/l) as agonist. Ranitidine was tested at 9.6×10^{-5} and 3.2×10^{-4} mol/l and cimetidine at 1.2×10^{-4} mol/l and 4.0×10^{-4} mol/l. At least 5 preparations were used for each drug concentration.

In vivo *antisecretory experiments*

Anaesthetized rat perfused stomach preparation The perfused stomach preparation of the rat used was that described by Parsons (1969) as a modification of the Ghosh & Schild (1958) method. Briefly, female Wistar rats (100 to 120 g) were anaesthetized with sodium pentobarbitone (50 mg/kg i.p.). The trachea and both jugular veins were cannulated and the vagus nerves were cut. Two perfusion cannulae were inserted into the stomach to facilitate perfusion of the acid-secreting mucosa at 3 ml/min with 5% dextrose solution at 37°C. The gastric effluent was passed over a microflow pH electrode and the pH recorded via a pH meter on a flat bed recorder.

Once a sub-maximal plateau of acid secretion had been obtained in response to intravenous histamine (100 μg kg^{-1} min^{-1}) or pentagastrin (1 μg kg^{-1} min^{-1}), a dose of antagonist was injected intravenously and the reduction in acid secretion measured. Ranitidine was tested at 0.03 to 1.0 mg/kg, metiamide at 0.3 to 1.0 mg/kg and cimetidine at 0.3 to 3.0 mg/kg. ED_{50} values with 95% confidence limits were calculated for inhibition of gastric acid secretion. At least four rats were used for each dose group.

Drugs

The following drugs were used: bethanechol chloride (Fabrique de Lair), cimetidine (S K & F Ltd), histamine acid phosphate (BDH), (−)-isoprenaline bitartrate dihydrate (Ward Blenkinsop), pentobarbitone sodium (Abbott). Ranitidine hydrochloride was synthesized by Glaxo Group Research Limited. Metiamide was a generous gift from Dr M. E. Parsons (S K & F Ltd). All drug doses are expressed in terms of free base.

Results

Isolated tissue experiments

Guinea-pig isolated right atrium The effect of ranitidine on the response of the guinea-pig isolated right atrium to histamine is shown in Figure 2. Ranitidine produced a dose-related displacement to the right of the histamine concentration-curve without depressing the maximum response, indicating that ranitidine is a

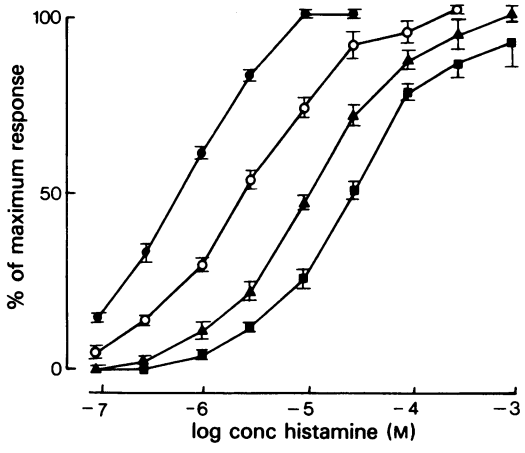

Figure 2 The effect of ranitidine on the histamine-induced contraction frequency of guinea-pig isolated right atria. Response to histamine alone (●) and in the presence of ranitidine 3.2×10^{-7} mol/l (○), 9.5×10^{-7} mol/l (▲) and 3.2×10^{-6} mol/l (■). Each point is the mean from at least 4 preparations; vertical lines show s.e. mean.

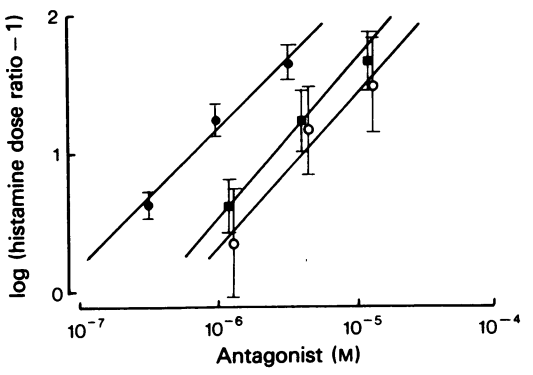

Figure 3 Antagonism of the chronotropic response of the guinea-pig isolated right atrium by ranitidine (●), cimetidine (■) and metiamide (○). Each point is the mean from at least 4 preparations; vertical lines show s.e. mean.

competitive antagonist. Similar results were obtained with metiamide and cimetidine. Arunlakshana & Schild (1959) plots were constructed for each drug as shown in Figure 3. The pA_2 values and slopes derived from these plots are summarized in Table 1. There was a linear regression and the slopes for ranitidine, metiamide and cimetidine did not differ significantly from unity, showing that all three compounds are competitive antagonists of histamine at H_2-receptor sites. Ranitidine was 7.9 and 4.5 times more potent than metiamide and cimetidine respectively.

When (−)-isoprenaline was used as the agonist instead of histamine, ranitidine at 3.2 and 9.6×10^{-5} mol/l produced respective dose-ratios of 1.18 (0.94 to 1.48) and 1.01 (0.81 to 1.24) (geometric mean and 95% confidence limits) and cimetidine at 1.2×10^{-4} mol/l produced an (−)-isoprenaline dose-ratio of 1.17 (0.72 to 1.91). Thus neither ranitidine nor cimetidine significantly affected the response of the guinea-pig atrium to (−)-isoprenaline.

Rat isolated uterine horn The histamine-induced concentration-response curve for relaxation of the rat isolated uterine horn, contracted by high potassium Krebs solution, was displaced to the right in a dose-related manner by ranitidine, metiamide and cimetidine. The three antagonists exhibited competitive antagonism as shown by the Arunlakshana & Schild plots summarized in Table 2 where none of the slopes differ significantly from unity. Ranitidine was 3.6 and 5.9 times more potent than metiamide and cimetidine respectively.

Ranitidine at 3.2×10^{-5} mol/l produced a dose-ratio of 0.78 (0.57 to 1.08) with (−)-isoprenaline as agonist while cimetidine at 1.2×10^{-4} mol/l pro-

Table 1 Antagonism of the effects of histamine on guinea-pig isolated right atrium by ranitidine, metiamide and cimetidine

Drug	*Slope of log (DR − 1) on log concentration regression*	pA_2	n
Ranitidine	0.99 (0.83–1.16)	7.20 (7.01–7.45)	12
Metiamide	1.12 (0.72–1.52)	6.30 (6.02–6.87)	14
Cimetidine	1.05 (0.80–1.29)	6.55 (6.32–6.91)	12

95% confidence interval in parentheses; *n* = number of preparations.

Table 2 Antagonism of the effects of histamine on rat isolated uterine horn by ranitidine, metiamide and cimetidine

Drug	*Slope of log (DR − 1) on log concentration regression*	pA_2	n
Ranitidine	0.93 (0.61–1.24)	6.95 (6.67–7.47)	20
Metiamide	0.93 (0.76–1.09)	6.39 (6.20–6.67)	31
Cimetidine	1.04 (0.80–1.28)	6.18 (6.00–6.44)	12

95% confidence interval in parentheses; n = number of preparations.

Table 3 Effect of high concentrations of ranitidine and cimetidine on histamine and bethanechol-induced contractions of the guinea-pig isolated ileum

Drug	*Concentration (mol/l)*	*Mean dose-ratio* *Histamine*	n	*Bethanechol*	n
Ranitidine	9.6×10^{-5}	0.80 (0.57–1.11)	5	1.14 (0.79–1.65)	5
	3.2×10^{-4}	1.12 (0.89–1.40)	5	1.35 (0.40–4.60)	5
	1.2×10^{-4}	0.90 (0.64–1.27)	6	1.03 (0.54–1.97)	5
Cimetidine	4.0×10^{-4}	1.22 (0.79–1.87)	5	1.41 (0.69–2.86)	7

95% confidence limits in parentheses; n = number of experiments.

Table 4 Effects of ranitidine, metiamide and cimetidine on gastric acid secretion in the perfused stomach of the anaesthetized rat

Secretagogue	*Antagonist dose* (mg/kg i.v.)	*Mean % inhibition of acid secretion ± s.e.* (n) *produced by* *Ranitidine*	*Metiamide*	*Cimetidine*
Histamine	0.03	24.9 ± 6.9 (8)		
	0.10	53.4 ± 5.5 (8)		
	0.30	57.5 ± 9.8 (8)	23.8 ± 9.0 (4)	29.3 ± 7.3 (8)
	0.60		66.5 ± 3.3 (4)	
	1.00	78.5 ± 3.6 (8)	68.8 ± 6.9 (4)	57.6 ± 5.2 (8)
	3.00			79.0 ± 7.0 (8)
Pentagastrin	0.03	27.4 ± 4.9 (4)		
	0.10	37.5 ± 3.3 (4)		19.6 ± 3.6 (5)
	0.30	53.0 ± 3.8 (4)	19.5 ± 4.5 (4)	46.3 ± 0.9 (4)
	1.00		65.3 ± 7.3 (4)	54.0 ± 4.0 (4)
	3.00			93.3 ± 3.2 (4)

(n) = number of experiments.

duced a dose-ratio of 1.65 (0.93 to 2.90). Thus neither ranitidine nor cimetidine significantly affected the response of the rat uterus to (−)-isoprenaline.

Guinea-pig isolated ileum Ranitidine and cimetidine even at high dose levels did not produce a significant displacement of the guinea-pig ileum concentration-response curves to either histamine or bethanechol as shown in Table 3.

In vivo *antisecretory experiments*

Anaesthetized rat perfused stomach preparation Ranitidine produced dose-related inhibition of gastric acid secretion induced by histamine or pentagastrin. Table 4 summarizes the mean inhibitions of acid secretion following ranitidine, metiamide and cimetidine.

Regression lines have been calculated from the data in Table 4 and ED_{50} values calculated. The ED_{50} values in mg/kg (95% confidence limits) were 0.13 (0.08 to 0.22), 0.52 (0.37 to 0.69) and 0.73 (0.45 to 1.06) respectively for ranitidine, metiamide and cimetidine as antagonists of histamine-induced acid secretion. Similarly ED_{50} values of 0.25 (0.15 to 0.77), 0.67 (0.51 to 0.99) and 0.44 (0.30 to 0.64) were obtained respectively for ranitidine, metiamide and cimetidine as antagonists of pentagastrin-induced acid secretion.

Potency ratios have been calculated on a molar basis from these ED_{50} values. Ranitidine was found to be 5.2 and 7.0 times more active than metiamide and cimetidine respectively as an antagonist of histamine-induced gastric acid secretion. Ranitidine was also found to be 3.5 and 2.2 times more active than metiamide and cimetidine as an antagonist of pentagastrin-induced gastric secretion.

Discussion

Ranitidine is a potent competitive antagonist of histamine at H_2-receptor sites in guinea-pig isolated atrium and rat isolated uterine horn. This antagonism is selective since ranitidine does not inhibit the action of histamine at H_1-receptors or of bethanechol at muscarinic receptors in guinea-pig ileum even when tested at concentrations greatly in excess of those required to block H_2-receptors. Similarly ranitidine was without effect on the β-adrenoceptor-mediated response to (−)-isoprenaline of the guinea-pig atrium and rat uterus. Metiamide and cimetidine showed the same profile of action but were approximately 6 and 5 times less active than ranitidine as H_2-receptor antagonists *in vitro*.

Ranitidine when tested in the rat *in vivo*, is a potent antagonist of histamine or pentagastrin-induced gastric secretion. On a molar basis ranitidine was found to be 5 to 7 times more potent than metiamide and cimetidine as an antagonist of histamine-induced gastric acid secretion. These results are in accord with the present *in vitro* studies and previous reports that metiamide and cimetidine possess a similar level of activity (Brimblecombe *et al.*, 1975). Other studies have shown ranitidine to inhibit gastric acid secretion in the dog (Bradshaw *et al.*, 1979; Daly, Humphray & Stables, 1980) and man (Peden, Saunders & Wormsley, 1979).

Until now, published work on histamine H_2-receptors has relied on the use of one or more of three structurally similar H_2-receptor antagonists namely burimamide, metiamide and cimetidine, all of which contain an imidazole ring. Burimamide, the important agent which enabled H_2-receptors to be characterized (Black *et al.*, 1972) was not effective orally and had further disadvantages in releasing catecholamines and blocking α-adrenoceptors (Brimblecombe, Duncan, Owen & Parsons, 1976). Metiamide, the second H_2-receptor antagonist to be developed was ten times more potent than burimamide, orally active and much more selective in its action (Black *et al.*, 1973). Clinical studies showed metiamide to be an effective agent for the treatment of peptic ulcers in man (Wyllie & Hesselbo, 1973), but unfortunately there was a low incidence of reversible agranulocytosis (Forest, Shearman, Spence & Celestin, 1975) which precluded further clinical development. Since this toxicity appeared to be linked with the metiamide molecule this stimulated the development and introduction of cimetidine which incorporates a cyanoguanidine substituent. This compound is effective as an inhibitor of gastric acid secretion in animals and man without producing the toxic renal and haematological effects associated with metiamide (Brimblecombe *et al.*, 1975). Nevertheless, cimetidine produces side effects in a minority of patients, such as increased plasma prolactin levels (Carlson & Ippoliti, 1977) and gynaecomastia (Delle Fave, Tamburrano, De Magistris, Natoli, Santoro, Carratu & Torsoli, 1977) which is probably linked to its anti-androgen action (Leslie & Walker, 1977; Funder & Mercer, 1979).

Ranitidine is a new structural type of H_2-receptor antagonist which does not contain the imidazole ring previously regarded as important for activity at the H_2-receptor. Nor does it contain the thiourea or cyanoguanidine groups present in metiamide and cimetidine respectively. Ranitidine could thus prove to be a useful therapeutic agent and warrants detailed investigation for therapeutic efficacy in patients with peptic ulcers.

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