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Pharmacological Profile of New Histamine H_2-Receptor Antagonists Related to Cimetidine, Ranitidine and Lamtidine

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Abstract—New compounds structurally related to cimetidine, ranitidine and lamtidine have been prepared and tested for their histamine H_2-receptor blocking activity on guinea-pig atria, rat perfused stomach and frog isolated gastric mucosa. These derivatives contain as a polar group, a diaminofurazan moiety, a 3-amino-4-methylfurazan or a 3-amino-4-phenylfurazan moiety. Ranitidine and lamtidine analogues display strong H_2-antagonist activity in-vitro (K_B on atria 0·037 μM and 0·0039 μM, respectively) and in-vivo on the lumen-perfused stomach of the anaesthetized rat (ID50 0·13 μmol kg^{-1} and 0·023 μmol kg^{-1} i.v., respectively). However, lamtidine analogues are ineffective in blocking the histamine-induced increase of H^+ output in the frog isolated gastric mucosa. On the basis of the anomalous results in the frog, it is concluded that caution must be exercised in extrapolating information from amphibian to mammalian tissues with regard to the structure and the function of histamine receptors.

Generally, histamine H_2-receptor blockers are composed of a substituted aromatic component and a polar, 'urea equivalent' group connected by a flexible chain. Our efforts to find new H_2-receptor antagonists focused on changing the polar groups of cimetidine, ranitidine and lamtidine† with aminofurazan moieties. As previously reported (Sorba et al 1985), the physicochemical properties of diaminofurazan are similar to those of the classical 'urea equivalent' groups. All compounds were evaluated in-vitro for H_2-receptor and β-adrenoceptor inhibition using the histamine and isoprenaline-stimulated chronotropic response of guinea-pig atria. In addition, these compounds were tested for antimuscarinic and H_1-receptor blocking activity on guinea-pig ileum. Active compounds were then administered intravenously to anaesthetized rats to determine gastric acid antisecretory activity and finally their gastric antisecretory properties were studied on the frog isolated gastric mucosa.

Materials and Methods

Drugs

All compounds and relative models (Fig. 1) were synthesized in the laboratories of the Dept of Drug Science and Technology of the University of Turin.

Guinea-pig atria

The procedure described by Black et al (1972) was followed. Guinea-pigs, 300 to 500 g, were killed by a sharp blow to the head. The atria were rapidly removed and suspended in a temperature-controlled (31 ± 1 °C) organ bath (50 mL) containing oxygenated Krebs-Henseleit

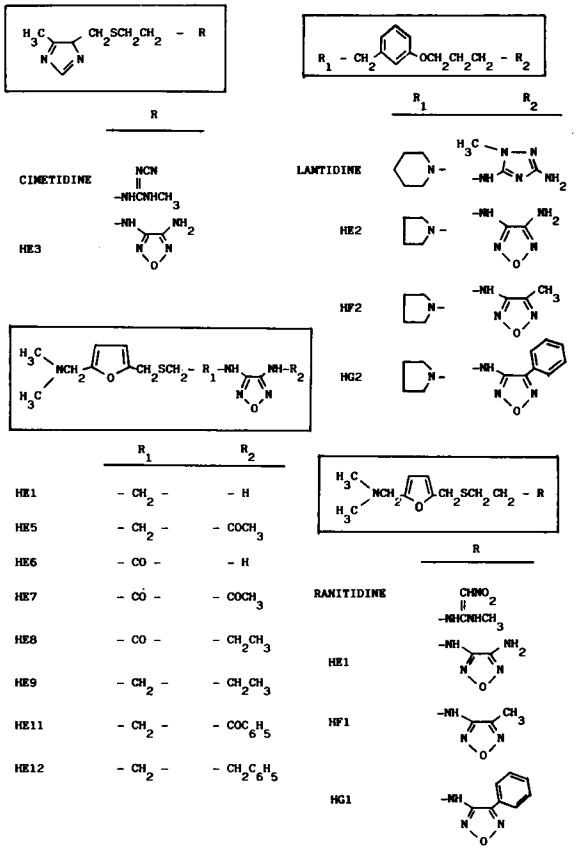

FIG. 1. The figure illustrates cimetidine, ranitidine and lamtidine analogues prepared and tested in-vivo and in-vitro for their histamine H_2-receptor blocking activity. HE series: substituted and unsubstituted diaminofurazan derivatives. HF series: 3-amino-4-methylfurazan derivatives. HG series: 3-amino-4-phenylfurazan derivatives.

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† Lamtidine is the 'lead' compound of a new class of histamine H_2-receptor antagonists containing a dialkylaminoalkylphenoxy moiety. Our compounds are derived from lamtidine by changing the 'urea equivalent' group and by manipulation of the basic substructure.

solution (pH 7·4). The preparations were allowed to stabilize 45–60 min before starting the experiments. The chronotropic response was expressed as a percentage of the maximal change in atrial rate caused by histamine or isoprenaline. Histamine cumulative concentration–effect curves were obtained in the absence and presence of four increasing concentrations of antagonist (time of equilibration 40–50 min). The dissociation constant of the receptor–antagonist complex was calculated by the method described by Arunlakshana & Schild (1959). The parallelism of the regression lines was tested by the method of Snedecor & Cochran (1971). Dose ratios (DR) were calculated as the ratio of histamine concentrations required to produce one-half of maximal stimulation in the presence and absence of antagonist, and the regressions of log (DR − 1) vs log molar concentration of antagonist were calculated by the least squares method. A separate set of experiments was performed in which isoprenaline cumulative concentration–effect curves were obtained in the presence of a high concentration of antagonist (50 μM) to verify the selectivity of these compounds for histamine H_2-receptors.

Guinea-pig ileum

Guinea-pigs, 300–500 g, were used. Segments (2 cm) of the terminal portion of the ileum (5–6 cm from the ileocaecal junction) were removed and suspended in a 30 mL tissue bath in oxygenated Tyrode solution maintained at 37 °C. Contractions were recorded via an isotonic transducer (resting muscle tension 0·5 g) connected to an inkwriting polygraph. The tissue was allowed at least 30–40 min equilibration before a sequential concentration–effect curve for histamine or carbachol was obtained. Antagonists were allowed to equilibrate for 40 min. Ileal responses were expressed as a percentage of the maximum.

Perfused stomach of the anaesthetized rat

The technique described by Ghosh & Schild (1958) was followed with slight modifications. Before an experiment male albino rats (180–200 g) were fasted for 24 h with free access to water. Animals were anaesthetized (ethyl urethane 25%, 0·6 mL i.p. plus 0·6 mL s.c.) and tracheotomized. Following surgery the stomach was continuously perfused via cannulae placed in the oesophagus and in the pyloric antrum with 1·0 mL min^{-1} of a 5·4% glucose solution maintained at 37 °C. The perfusate was collected at 15 min and acid concentration of the samples was measured by titration against 0·01 M NaOH using a Mettler Memotitrator DL 40. Gastric acid secretion was stimulated by means of a histamine infusion (12 mg kg^{-1} h^{-1} i.v.) and antagonists were administered intravenously after the histamine secretion plateau had been reached (40–45 min). The following doses of aminofurazan derivatives were tested: HE1, 30–70–150 μg kg^{-1}: HF1, 0·7–1·5–3·0 mg kg^{-1}; HE2, 5·0–10–30 μg kg^{-1}; HF2, 70–150–300 μg kg^{-1}; HE5, 0·7–1·0–2·0 mg kg^{-1}; HE9, 70–150–300 μg kg^{-1}; HE11, 0·1–0·2–0·4 mg kg^{-1} and HE12, 15–25–50 μg kg^{-1}. Antisecretory activity was expressed in terms of % inhibition. The dose required for 50% inhibition of gastric acid secretion was calculated from the linear regression of log dose vs % inhibition.

Frog isolated gastric mucosa

All experiments were performed on gastric mucosae of the European brown frog, *Rana temporaria*. The animals were decapitated and pithed and the whole stomach removed. The mucosa was stripped by blunt dissection from the serosal muscular layers and mounted between a pair of Plexiglas chambers so that the area exposed to bathing solutions was 1·2 cm^2. The nutrient side was gassed with 95% O_2–5% CO_2 and was bathed with bicarbonate-buffered solution (nutrient solution) containing (mM): Na^+ 102, K^+ 4·0, Ca^{2+} 1·0, Mg^{2+} 0·8, Cl^- 81·0, SO_4^{2-} 0·8, HPO_4^- 1·0, HCO_3^- 25·0 and glucose 10·0, whereas the luminal side was bathed by 8·0 mL of oxygenated (100% O_2) unbuffered solution (secretory solution) containing (mM): Na^+ 102, K^+ 4·0 and Cl^- 106. To obtain homogeneous experimental conditions only winter frogs were used. Experiments were conducted at room temperature (about 20 °C). The H^+ secretory rate was measured by the pH-stat method (Durbin & Heinz 1958). The secretory side was maintained at pH 5·0 by the addition of a 0·005 M NaOH solution. Acid secretion was stimulated by histamine (1·0 μM) and the antagonists were dissolved in the nutrient solution after the histamine secretion plateau had been reached (30–35 min). Antisecretory activity was expressed as % inhibition and the IC50 was calculated from the linear regression of log concentration vs % inhibition. Each four points concentration–effect curve was obtained from two preparations.

Results

Effect on guinea-pig isolated atria

All derivatives did not affect the resting atrial rate (146 ± 4 contractions min^{-1}) during the period of incubation. An exception is represented by the compound HG2 which caused a dose-dependent reduction of the spontaneous contraction frequency in the range 0·5–10 μM. Pretreatment of the atrial tissue with high concentrations (100 μM) of compounds HE3, HE7 and HG1 caused no modification of the histamine dose–response curve, whereas all other compounds produced a parallel rightward shift in the control curve for histamine without affecting the maximal response (197 ± 6 contractions min^{-1}). The Schild regressions of log (DR − 1) vs log molar concentrations of these derivatives were linear with slopes not significantly different from unity. The dissociation constant values and relative 95% confidence limits are reported in Table 1. Compounds HE1 and HE9 are as active as ranitidine whereas HE12 and in particular compound HE2, are more potent H_2-receptor antagonists than ranitidine. In separate experiments, the pretreatment of guinea-pig atria for 40 min with all derivatives (50 μM) did not affect the isoprenaline concentration–response curve.

Effect on guinea-pig isolated ileum

Preincubation of ileal tissue preparations for 40 min with a high concentration (50 μM) of aminofurazan derivatives did not modify histamine and carbachol concentration–effect curves. An exception is represented by compounds HE11 and HE12.

Table 1. 3,4-Diaminofurazan, 3-amino-4-methylfurazan and 3-amino-4-pheylfurazan derivatives: antagonism of histamine-induced positive chronotropic effect on guinea-pig isolated atria. The dissociation constant values (K_B) have been calculated by the method of Arunlakshana & Schild (1959).

Compound	K_B (μM)
Ranitidine	0·076 (0·048–0·12)
Cimetidine	0·52 (0·29–0·93)
HE1	0·037 (0·017–0·079)
HF1	0·64 (0·41–1·02)
HG1	>100
HE2	0·0039 (0·0019–0·0078)
HF2	0·066 (0·033–0·13)
HG2	Negative chronotropic activity
HE3	>100
HE5	0·72 (0·55–0·96)
HE6	>100
HE7	>100
HE8	>100
HE9	0·046 (0·022–0·095)
HE11	0·11 (0·042–0·26)
HE12	0·022 (0·014–0·036)

Effect on gastric acid secretion in-vivo

In our experimental conditions the resting acid output was 12 ± 2·6 μequiv h^{-1}. The basal gastric secretion was not altered by intravenous injection of normal saline. After stimulation with histamine, the H^+ output increased within 40–45 min to 103 ± 8·6 μequiv h^{-1}. In separate experiments, a single dose of saline was injected i.v. when the plateau of maximal secretion was reached: the value of acid output measured 50 min later was not significantly different from that observed before saline administration.

From the results reported in Table 2 it may be noted that the cimetidine analogue HE3 and the 3-amino-4-phenylfurazan derivatives HG1 and HG2 are devoid of anti-secretory activity whereas, with respect to ranitidine, a reduced H_2-blocking activity was found for HF1 and HE5. On the contrary, compounds HE2 and HE12 were, respectively, 10 and 5 times more active than ranitidine as inhibitors of the histamine-stimulated acid secretion. The ID50 values of ranitidine and of the other derivatives are of the same order of magnitude.

Table 2. 3,4-Diaminofurazan, 3-amino-4-methylfurazan and 3-amino-4-phenylfurazan derivatives: antagonism of histamine-stimulated gastric acid secretion in the lumen-perfused stomach of the anaesthetized rat and in the frog isolated gastric mucosa. ID50 and IC50 values have been estimated from the linear regressions of antagonist log dose vs % inhibition.

Compound	Rat gastric acid secretion ID50 μmol kg^{-1} (i.v.)	Frog gastric mucosa IC50 (μM)
Ranitidine	0·30 (0·22–0·46)	0·34 (0·29–0·40)
Cimetidine	1·24 (1·14–1·36)	12·4 (10·3–14·8)
HE1	0·13 (0·11–0·16)	1·18 (1·09–1·29)
HF1	3·08 (2·57–3·70)	51·30 (46·3–56·6)
HG1	inactive	inactive
HE2	0·023 (0·016–0·034)	inactive
HF2	0·315 (0·25–0·40)	inactive
HG2	inactive	inactive
HE3	inactive	inactive
HE5	2·86 (2·61–3·15)	6·76 (6·19–7·38)
HE6	inactive	inactive
HE7	inactive	inactive
HE8	inactive	inactive
HE9	0·29 (0·24–0·36)	1·13 (0·89–1·44)
HE11	0·34 (0·29–0·40)	3·90 (3·50–4·35)
HE12	0·057 (0·05–0·067)	9·37 (7·98–11·0)

Effect on frog isolated gastric mucosa

The stimulation of acid secretion induced by histamine (1·0 μM) produced an increase of H^+ output from the basal value (0·93 ± 0·44 equiv h^{-1} cm^{-2}) to peak level of 3·36 ± 0·74 μequiv h^{-1} cm^{-2}. In control experiments, the high secretory rate induced by histamine was maintained without relevant modifications for 40–50 min. The results reported in Table 2 indicate that both HE1 and HE9 are approximately 3 times less potent than ranitidine in inhibiting gastric acid secretion. HE5, HE11 and HE12 showed a relatively low antisecretory activity (ranitidine is 10–30 times more active) whereas the 3-amino-4-methylfurazan derivative HF1 may be considered a weak antagonist in comparison to the model. Our data also indicate that the stimulation of H^+ output induced by histamine in the frog gastric mucosa is not antagonized by high concentrations (100 μM) of compounds HE2 and HF2.

Discussion

In our experiments, ranitidine and the diaminofurazan derivative HE1 are equipotent as antagonists of the positive chronotropic effect of histamine in isolated guinea-pig atria (Table 1) and of the histamine-stimulated gastric acid secretion in the anaesthetized rat (Table 2). From this finding it follows that the diaminofurazan moiety may be considered a bioisostere of the diaminonitroethene group of ranitidine. A good bioisosteric replacement in one series, however, is not necessarily useful in another. The dramatic loss of H_2-blocking activity obtained with the derivative HE3 supports this observation and confirms that cimetidine is not amenable to a wide variety of structural modifications. On the other hand, the substitution of the polar group of lamtidine, a potent long-acting H_2-receptor antagonist (Brittain et al 1982), generates a competitive blocker, the compound HE2, which is 15–20 times more active than ranitidine in antagonizing the effects of histamine on guinea-pig atria. The pharmacological profiles of lamtidine and HE2 are quite different in-vitro since lamtidine produces an unsurmountable block of histamine H_2-receptors on this preparation (Brittain et al 1982). In-vivo, however, HE2 is approximately 10 times more potent than ranitidine in inhibiting the histamine-stimulated gastric acid output and a similar result has been reported by Brittain et al (1982) for lamtidine in the same preparation.

Our results also indicate that the type of substituent on the furazan ring has a significant effect on activity. The 3-amino-4-methylfurazan analogues HF1 and HF2 are less active than HE1 and HE2 in inhibiting the histamine-stimulated chronotropic response and gastric acid output, while the 3-amino-4-phenylfurazan derivatives HG1 and HG2 are devoid of H_2-receptor blocking activity.

The introduction of the phenyl and methyl moieties, therefore, causes a modification of the antagonist-receptor interaction due either to an excessive increase of the size of the polar group or to the reduction in the molecule of an area capable of binding anionic sites in the H_2-receptor. These suggestions are supported by the results obtained with HE5-HE12 derivatives in which the electronic and steric parameters and the lipophilicity of the 'urea equivalent' group of the ranitidine analogue HE1 have been changed by the introduction of suitable substituents. As shown in Table 2, the size of the polar group does influence the activity and, with the exception of HE9 and HE12, molecular size and antisecretory activity in both rat and frog do seem to parallel each other. The most marked differences are that HE12 is most potent in the rat and least in the frog while for HE9 it is the reverse. Probably an increase in lipophilicity of the polar group may be responsible for the high activity of the benzyl derivative HE12 in the rat but, to confirm this suggestion, additional data are necessary. Furthermore, the introduction of amidic functions which radically change the conformation of the molecule (compounds HE6, HE7 and HE8), produce a complete loss of activity. Finally, of relevant significance are the data concerning the acid antisecretory properties of the lamtidine analogues HE2 and HF2 in the frog (Table 2). As mentioned, these compounds are potent antagonists of histamine both on guinea-pig atria and on rat perfused stomach preparations. In contrast, they are ineffective in blocking the histamine-induced increase of H^+ output in the amphibian isolated gastric mucosa. At present an explanation of this discrepant behaviour of lamtidine analogues in the frog is not apparent. In agreement with Hersey (1981), however, our data suggest that caution must be exercised in extrapolating information from amphibian to mammalian tissues with regard to the structure and the function of histamine receptors.

References

Arunlakshana, O., Schild, H. O. (1959) Br. J. Pharmacol. 14: 48–56

Black, J. W., Duncan, W. A. M., Durant, C. J., Ganellin, C. R., Parsons, E. H. (1972) Nature 236: 385–390

Brittain, R. T., Daly, M. J., Humphray, M. J., Stables, R. (1982) Br. J. Pharmacol. 76: 195P

Durbin, R., Heinz, E. (1958) J. Gen. Physiol. 41: 1035–1041

Ghosh, M. N., Schild, H. O. (1958) Br. J. Pharmacol. 13: 54–61

Hersey, S. J. (1981) Am. J. Physiol. 241G: 93–97

Snedecor, G. W., Cochran, W. G. (1971) in: Statistical Methods. Iowa State University Press, Ames, Iowa, pp 432–436

Sorba, G., Calvino, R., Defilippi, A., Gasco, A. Orsetti, M. (1985) Eur. J. Med. Chem. 20: 571–574