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Pharmacological investigation into the effects of histamine and histamine analogues on guinea-pig and rat colon *in vitro*

María-José Aguilar, Francisco J. Morales-Olivas & Elena Rubio[1]

Departamento de Farmacología, Facultad de Medicina, Avda. Blasco Ibáñez, 17, 46010 Valencia, Spain

1 The effects of histamine and specific histamine agonists has been examined on isolated longitudinal colon strips of guinea-pig and rat.

2 Histamine and 2-pyridyl-ethylamine but not 4 methylhistamine produced a concentration-related contractile response in the guinea-pig colon.

3 The H_1-antagonist clemizole antagonized competitively the effect of histamine but the H_2-antagonist ranitidine did not modify the dose-response curve to histamine in the guinea-pig colon.

4 Atropine, hexamethonium, prazosin and propranolol failed to modify the contractile response to histamine.

5 Tone induced with KCl in guinea-pig isolated colon was not modified by histamine agonists even in tissues pretreated with clemizole or ranitidine.

6 Histamine and histamine analogues were without effect on the isolated longitudinal strip of the rat colon.

7 It is concluded that histamine produced dose-dependent contractions of the guinea-pig colon due to direct activation of H_1-histamine receptors. There is no evidence in favour of the existence of H_2-histamine receptors in this preparation. The lack of effect of histamine agonists in rat colon strip argues against the existence of histamine receptors in this preparation.

Introduction

The discovery of selective antagonists for histamine H_2-receptors (Black *et al.*, 1972) has permitted the classification of histamine receptors in many organs. Histamine causes contraction of the intestine in most species, though previous studies have shown a variation in responsiveness and sensitivity of the different regions of the gastrointestinal tract to histamine (Chand & Eyre, 1976; Sakai *et al.*, 1979). It is generally considered to be due to stimulation of histamine H_1-receptors. There is evidence both for and against the occurrence of H_2-receptors (whose stimulation causes relaxation) in the intestinal muscle (Bertaccini, 1982; Parsons, 1982).

On the other hand, the action of histamine has been most thoroughly studied in the small intestine but little is known about the effect of histamine in the large intestine. The purpose of this investigation was therefore, to study the mechanism of action and effect of histamine and two different compounds acting as specific H_1- and H_2-receptor agonists on the guinea-pig and rat colon.

[1]Author for correspondence.

Methods

Adult guinea-pigs weighing 350–450 g and Wistar rats weighing 200–250 g were used. The animals were killed by a blow on the head and exsanguinated. The abdomen was opened to expose the intestine. The distal colon (the part which is immediately adjacent to the caecum) was rapidly removed and immersed in Tyrode solution. Longitudinal muscle strips, about 20 mm × 4 mm, were suspended under a load of 1 g in a 20 ml organ bath containing Tyrode solution. The composition of the Tyrode solution was (mM): NaCl 136, KCl 2.7, $CaCl_2$ 1.4, $MgSO_4$ 0.04, KH_2PO_4 0.4, $NaHCO_3$ 11.9 and glucose 5.6. This solution was aerated with 5% CO_2 in O_2 and was maintained at

32°C in order to prevent spontaneous contractions.

Mechanical activity of the longitudinal muscle strips was recorded by means of an isotonic transducer (Ugo Basile, mod. 7004) and a Ugo Basile (mod. Gemini, 7070) recorder.

The preparations were allowed to equilibrate in Tyrode solution for at least 30 min before any drug was added. At the end of the equilibration period, two different experiments were performed: (i) concentration-response curves to histamine or other agonists were obtained by adding the drugs cumulatively, at increments of one log unit; (ii) a submaximal well-maintained plateau contraction was obtained by adding KCl (37 mM) and then a cumulative concentration-response curve to agonists was performed as described previously.

Antagonists were incubated for 15 min before the agonist was added in the same way as in the control.

In preliminary experiments, the reproducibility of cumulative concentration-effect curves to histamine was determined by generating three curves separated by two, 30 min incubations in Tyrode solution.

In some preparations, acetylcholine (10^{-4} M) was added to the bath at the end of the experiment to test the reactivity of the isolated colon.

Contractile responses to histamine receptor agonists were expressed as a percentage of the maximum response obtained. Effective concentration 50% (EC_{50}) was calculated graphically from a plot of log concentration vs percentage of the maximum response (E_{max}) produced by each agonist in individual experiments.

The calculation of the pA_2 value was made from the dose-ratios for each of the three different concentrations of the antagonist according to the method of Arunlakshana & Schild (1959) using the least square regression analysis to determine the relationship between log (dose-ratio -1) and log of the antagonist concentration (Tallarida *et al.*, 1979). All data are shown as mean ± standard error of the mean (s.e.mean). Statistical analysis of the data was carried out using Student's *t* test at a 5% significance level.

Drugs

The following were used: acetylcholine hydrochloride, atropine sulphate, histamine dihydrochloride, hexamethonium bromide and propranolol hydrochloride (Sigma); 4-methylhistamine dihydrochloride and 2-pyridyl-ethylamine dihydrochloride (Smith, Kline & French); clemizole hydrochloride (Schering España, S.A.); prazosin hydrochloride (Pfizer) and ranitidine hydrochloride (A. Gamir).

All drugs were prepared in Tyrode solution before being added to the bath. Drug concentrations refer to the free base or acid and are expressed as a final bath concentration in M.

Results

Effects of histamine receptor agonists and antagonists in guinea-pig colon

The weaker spontaneous activity of the guinea-pig colon strip declined progressively during a concentration-response curve to agonists (Figure 1a). Histamine (10^{-8} to 10^{-3} M) induced concentration-related contractions of the guinea-pig colon. The contraction is rapid in onset, sustained and slowly reversed by washing; it reached a maximum with 10^{-5} M histamine, higher concentrations producing contractions that were not significantly different. The EC_{50} value determined from concentration-response curves was $3.19 \pm 0.8 \times 10^{-6}$ M. The response was reproducible at 30 min intervals (30 min, $EC_{50} = 3.22 \pm 1.2 \times 10^{-6}$ M; 60 min, $EC_{50} = 3.63 \pm 0.7 \times 10^{-6}$ M, $n = 6$).

The H_1-receptor agonist 2-pyridyl-ethylamine (10^{-8}, 10^{-4} M) like histamine, contracted the colon in a concentration-dependent manner with a maximum response not significantly lower but the EC_{50} ($1.35 \pm 0.4 \times 10^{-5}$ M) was higher than that of histamine (Figure 2) indicating its lower potency. The H_2-receptor agonist 4-methylhistamine failed to contract the guinea-pig isolated colon.

The response to KCl (37 mM) was 52.6 ± 6.2% of the maximum histamine-induced contractions.

The H_1-histamine receptor blocker clemizole, shifted the concentration-response curve for histamine-induced contraction to the right in a dose-dependent manner. Analysis of this displacement by use of the Arulakshana-Schild plot is shown in Figure 3 and yielded a pA_2 value of 10.45 ± 0.44 with a slope not significantly different from unity (0.89 ± 0.07).

Ranitidine at concentrations of 10^{-6} and 10^{-7} M did not produce an effect alone and failed to modify the concentration-response curve to histamine (Table 1). The contractile effect of histamine was also unaffected by atropine (10^{-8} M), hexamethonium (10^{-7} M), propranolol (10^{-7} M) and prazosin (10^{-7} M) (Table 1).

Table 1 EC_{50} value for histamine in the absence and presence of various antagonists in the guinea-pig isolated colon strip

Agonist	*Antagonist* (M)	n	*EC_{50}* (M)
Histamine	—	60	$3.2 \pm 0.8 \times 10^{-6}$
Histamine	Ranitidine 10^{-6}	6	$2.6 \pm 0.7 \times 10^{-6}$
Histamine	Ranitidine 10^{-7}	6	$1.7 \pm 0.6 \times 10^{-6}$
Histamine	Atropine 10^{-8}	5	$2.5 \pm 0.9 \times 10^{-6}$
Histamine	Hexamethonium 10^{-7}	5	$0.9 \pm 0.2 \times 10^{-6}$
Histamine	Propranolol 10^{-7}	5	$4.6 \pm 1.8 \times 10^{-6}$
Histamine	Prazosin 10^{-7}	5	$1.6 \pm 0.7 \times 10^{-6}$

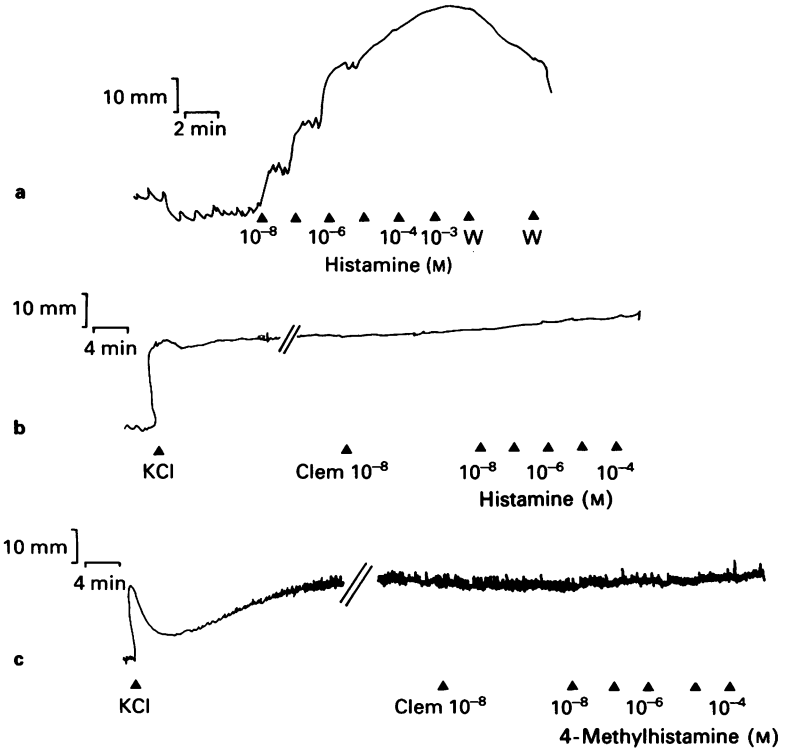

Figure 1 Responses of the guinea-pig colon to histamine and 4-methylhistamine. Histamine produced a contractile response (a). In the presence of H_1-receptor blockade with clemizole (Clem), histamine (b) and 4-methylhistamine (c) did not modify the contraction induced by KCl. W = washout.

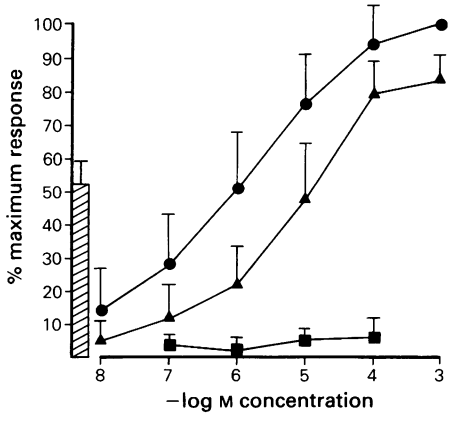

Figure 2 Concentration-response curves to histamine (●), 2-pyridyl-ethylamine (▲) and 4-methylhistamine (■) and contractile response to K^+ 37 mM (histogram) in guinea-pig isolated colon strip.

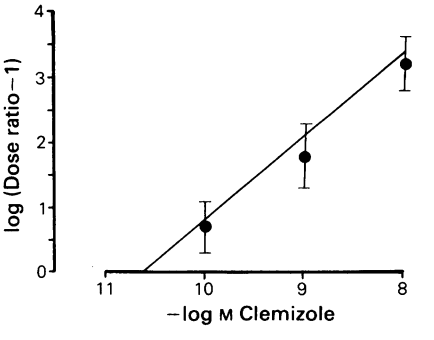

Figure 3 Histamine dose-ratio as a function of clemizole concentration. the intercept on the abcissa scale of the regression line for log (dose ratio − 1) versus the negative log clemizole concentration gives a pA_2 value of 10.45 (each point is the mean of 6 experiments).

Neither histamine (10^{-8}–10^{-4} M), 2-pyridyl-ethylamine (10^{-8}–10^{-4} M) nor 4-methylhistamine (10^{-8}–10^{-4} M) induced any relaxant effect on the contraction produced by KCl (37 mM) in guinea-pig isolated colon. Similarly no response was observed when preparations were pretreated with clemizole (10^{-8} M) (Figure 1b and c).

Effects of histamine receptor agonists and antagonists in rat colon

The longitudinal rat colon strip developed spontaneous contractile activity; when histamine (10^{-10}–10^{-4} M) was tested no response was obtained. Clemizole (10^{-8} M) plus histamine and ranitidine (10^{-6} M) plus histamine also produced no response. In contrast, acetylcholine (10^{-6} M) induced reproducible contractions of the colon which were rapid in onset with an initial peak followed by a progressively declining response (Figure 4).

Like histamine, 2-pyridyl-ethylamine and 4-methylhistamine were without effect on isolated colon when they were used in concentrations up to 10^{-4} M.

Histamine and all histamine-receptor agonists used in concentrations up to 10^{-4} M failed to affect the contraction induced by KCl.

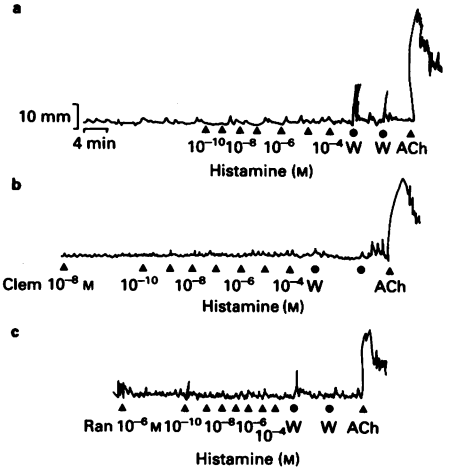

Figure 4 Response to histamine of rat isolated colon: (a) alone, (b) after clemizole (Clem), (c) after ranitidine (Ran). W = washout. ACh = acetylcholine 10^{-6} M.

Discussion

The contractile activity of histamine in the intestinal smooth muscle has long been known (Dale & Laidlaw, 1910) but relatively few studies have been carried out on the effect of histamine on large intestinal smooth muscle (Parsons, 1982). The present results show that histamine produces dose-dependent contractions in guinea-pig isolated longitudinal colon strips. The concentration-response curve to histamine of guinea-pig colon resembles that of guinea-pig ileum (Ariens, 1964) although the latter is more sensitive to histamine than the distal colon. These results agree with those on chicken gut, where regional differences have been found in the sensitivity to histamine of the gastrointestinal tract (Chand & Eyre, 1976). Furthermore as has been noted for smooth muscle of the guinea-pig small intestine, the response to histamine in longitudinal colon strip does not exhibit tachyphylaxis.

The contractile response to histamine in these experiments was antagonized, in a dose-related manner by the H_1-receptor antagonist clemizole. The pA_2 value for clemizole and the slope of the Schild plot strongly suggest the involvement of H_1-histamine receptors in the histamine-induced contractile responses of guinea-pig colon.

To characterize further the receptor subtype mediating contractile responses to histamine, we examined the effect of 2-pyridyl-ethylamine and 4-methylhistamine. 2-Pyridyl-ethylamine, like histamine, produced dose-dependent contractions of the guinea-pig colon but was less potent than histamine; this finding is in agreement with previous results in various tissues (Perpiñá *et al.*, 1980; Goyal & Verma, 1981; Coruzzi & Bertaccini, 1982). The lack of contractile effect of 4-methylhistamine, suggests there are no H_2-receptors mediating contraction in guinea-pig colon. Additional support for this conclusion comes from the results obtained with ranitidine which at two different concentrations did not affect the response to histamine in this preparation. Although Barker & Ebersole (1982) reported a contractile response to dimaprit on the colon of guinea-pig, our results are not in disagreement with these authors since they suggest that the effect of dimaprit was mediated indirectly by release of contractile substances.

Fishlock & Parks (1963) and Bucknell & Whitney (1964) using human colonic circular muscle strips or taenia coli respectively showed that histamine produced both relaxant or contractile responses. Thus, we examined the effects of histamine and two selective histamine receptor agonists in guinea-pig colon strips contracted by KCl.

The absence of relaxant activity to all compounds tested, even after treatment with clemizole or ranitidine, indicated that there are no relaxant histamine receptors in longitudinal strips of guinea-pig colon.

These results further support the view that histamine H_2-receptors play no role in the mechanical response of guinea-pig colon to histamine.

The possibility that acetylcholine mediates part of the response to histamine, as suggested in other parts

of the guinea-pig intestine (Ambache, 1946, 1949; Barker & Ebersole, 1982) was examined in this study by using acetylcholine antagonists. Neither atropine nor hexamethonium modified the contractile effect induced by histamine. Our results suggest that there was no cholinergic involvement in the action of histamine on guinea-pig longitudinal colon strips.

Similarly, Brownlee & Harry (1963) showed that the effect of histamine in longitudinal muscle of guinea-pig ileum was direct although it was mediated partially by acetylcholine in circular muscle.

Moreover, a recent study by Rubinstein & Cohen (1985) showed that the acetylcholine-mediated contractile response to histamine of the guinea-pig ileum was only observed when the solution used contained a critical concentration of calcium (Krebs solution, for example) but it was not observed when Tyrode solution was used. In the same way, no acetylcholine-mediated effect of histamine was observable in our experiments.

On the other hand, histamine has been shown to cause the release of catecholamines in different tissues (Cortijo *et al.*, 1984; Blaber & Fryer, 1985) including intestinal muscle (Everett & Mann, 1967). The contractile response to histamine found in this study was not modified by either propranolol or prazosin. These results agree with a similar observation in the human isolated colon and argue against the involvement of catecholamine-release in the effect of histamine in the guinea-pig longitudinal colon strips.

The rat intestine is not normally used to test histamine and studies using specific H_1 and H_2 agonists and antagonists, to our knowledge, have not been reported. The present study shows that neither histamine, 2-pyridyl-ethylamine nor 4-methylhistamine produced a significant effect in rat colon strips. These histamine-receptor agonists were also ineffective after the blockade of H_1- and H_2-receptors; findings that are in agreement with the lack of effect of histamine on the rat ileum described by Sakai *et al.* (1979). These results may be due to the absence, or paucity, of both types of histamine receptors in the rat colon.

In summary, this study shows that the effect of histamine in the guinea-pig longitudinal colon strip is a dose-dependent contraction due to direct activation of H_1-histamine receptors with no pharmacological evidence to support the existence of H_2-histamine receptors or the possibility of an indirect (acetylcholine-release or catecholamine-release) mechanism. The absence of H_1- and H_2-agonist activity in the rat colon strip argues against the existence of histamine receptors in this preparation.

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