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Regional variation in the sensitivity of longitudinal smooth muscle to histamine at H_1-receptors in guinea-pig ileum and colon

L.A. Barker

Department of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, New Orleans, LA, U.S.A.

1 The sensitivity of the distal ileum, proximal colon, medial colon, and distal colon of the guinea-pig to histamine has been evaluated.

2 The rank order of sensitivity was ileum > medial colon > proximal colon ≈ distal colon.

3 The mean $-\log EC_{50}$ values at receptors in the ileum, medial, proximal, and distal colon were 6.74, 6.18, 5.79, and 5.72, respectively.

4 The apparent dissociation constant for the interaction of histamine with receptors in the various regions was determined.

5 The $-\log K_d$ values at receptors in the ileum, proximal colon, medial colon, and distal colon were 4.68, 4.65, 4.62, and 4.44, respectively.

6 The mean apparent $-\log K_d$ values for the antagonism of histamine by mepyramine were 9.0, 9.0, 9.1, and 8.9 for receptors on the ileum, proximal, medial, and distal colon, respectively.

7 The results of these experiments provide no evidence that histamine receptors in the colon are distinguishable from H_1-receptors as characterized on the ileum.

8 The differences in sensitivity to histamine in the various regions of the intestine may be due to differences in the density of H_1-receptors.

Introduction

With the exception of studies on isolated taenia preparations, there have been relatively few investigations on the effects of agents acting on histamine receptors on the contractility of the large intestine. The results of most studies suggest that the responses to histamine are mediated mainly by H_1-receptors (Rees *et al.*, 1950; Cook & Krueger, 1981; Parsons, 1982).

The present study was undertaken to characterize further the histamine receptors in guinea-pig colon and to determine if there are differences in the sensitivity to histamine amongst segments taken from the proximal, medial, or distal colon. In addition, the actions of histamine on segments of the colon were compared to those on the ileum. The results of this study show that there are regional differences in the sensitivity but not in the affinity of histamine for intestinal smooth muscle H_1-receptors. A preliminary account of part of this study has been published (Barker & Lopez, 1984).

Methods

Isolated intestinal preparations

Male and female Hartley albino guinea-pigs, 350–700 g were used. Segments, 3–4 cm, were taken from the proximal, medial, and distal colon and the distal ileum for recording isometric contractions as previously described (Barker & Ebersole, 1982). Segments of the proximal colon were taken approximately 5 cm distal from the caeco-colonic junction; segments of the medial colon were taken approximately 5 cm distal from the midpoint of the colonic flexure; and segments of the distal colon were taken from the most distal region of the colon prior to its entry into the pelvic cavity. Ileal segments were taken between 15 and 30 cm from the ileo-caecal junction. The intestinal contents were removed by flushing with warm Krebs solution and/or gentle squeezing. The segments were

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suspended in a 10 ml jacketed organ bath containing Krebs-Ringer bicarbonate buffer (KRB), pH 7.4, aerated with carbogen and maintained at 37°C. Isometric contractions were recorded with a Grass FT.03 transducer in conjunction with a Grass model 7 Polygraph. The tissues were allowed to equilibrate for 45–60 min before the addition of agonists. During this time the Krebs solution was changed at 15 min intervals. Duplicate concentration-response curves were generated by the sequential variation of agonist concentration; in the initial curve, concentrations were varied in an ascending manner and in a descending manner for the second. The exposure time was 30 s and the interval between doses was 5–10 min.

The spontaneous activity of segments of colonic tissue frequently was very high. When this was the case, either scopolamine (0.1 μM) or tetrodotoxin (0.1 μM) was added to the Krebs solution to render the preparations useful. Because the contractile actions of H_2-agonists are due to agonism at receptors on myenteric plexus neurones (Barker & Ebersole, 1982), the use of either scolopamine or tetrodotoxin precluded a study of dimaprit on colonic tissues.

Determination of concentration-response parameters

The phasic component of the contractile response to histamine (Barker, 1981) was used as the response metameter. Iterative curve fitting was done using the programme 'Allfit' (DeLean *et al.*, 1978) adapted for use on an Apple computer (Teicher, 1983). This programme evaluates experimental date for a fit to a four parameter logistic equation:

$$Y = (a - d)/(1 + (X/c)^b) \times d \quad (1)$$

where X and Y are the dose and measured response respectively; a is the response at zero dose; b is the slope parameter; c is the EC_{50}; and d is the maximum response. The experimental data were fitted with the constraint that a = 0.

Determination of the agonist dissociation constant

The determination of the apparent dissociation constant (K_d) for histamine was based on the method of Furchgott (1966). Irreversible inactivation of a fraction of the active receptor population was achieved by treatment of the tissues with 2 μM dibenamine for 30 min. After treatment with dibenamine, the tissues were extensively washed for 60 min before constructing another set of dose-response curves. The 'Allfit' parameters for curves generated before and after receptor alkylation were used to calculate the apparent K_d by the method of Roberts (1984; personal communication). Tissue efficacy was determined from the equation (Stephenson, 1956):

$$e = (K_d + EC_{50})/EC_{50} \quad (2)$$

where the EC_{50} is that in the absence of an irreversible antagonist.

Drugs

All chemicals used were of reagent grade. Histamine dihydrochloride was bought from Aldrich Chemicals, Milwaukee, WI. Tiotidine was kindly provided by Dr D. McCurdy, Stuart Pharmaceuticals Division of ICI Americas, Willmington, DE. Mepyramine was purchased from Pfaltz and Bauer, Stamford, CT. Dibenamine hydrochloride was obtained from ICN Pharmaceuticals, Plainview, NY. Tetrodotoxin was purchased from Sigma Chemicals, St. Louis, MO. Stock solutions (500 mM) of histamine were prepared in deionized water and neutralized with 5 N NaOH to pH 7 before use to avoid pH artefacts (Hand & Buckner, 1981; Kenakin & Beek, 1982). Working solutions were prepared by serial dilutions of the stock with Krebs solution.

Data analysis

Statistical analyses were done using programmes available on the software package, Biostatistics III (A2 Devices, Almeda, CA). The Applesoft adaptation of 'Allfit' was obtained from BCTIC, Vanderbilt Medical Center, Nashville, TN.

Results

The results of initial experiments on ileal and colonic tissues suggested that the actions of histamine were mediated by H_1-receptors. At EC_{90} concentrations, the effects of histamine were not antagonized by an H_2-receptor antagonist, tiotidine (15 μM); a voltage-dependent Na^+-channel antagonist, tetrodotoxin (0.1 μM); or a muscarinic antagonist, scopolamine (1 μM); but were fully blocked by an H_1-antagonist, mepyramine (1 μM). The concentrations of tiotidine and scopolamine used were about 1000 times their

Table 1 Dose-response parameters for the contractile actions of histamine on different regions of the guinea-pig colon

Region	n	*− log EC_{50}*	*Slope parameter*
Proximal	14	5.79 (5.89–5.69)	1.0 (0.9–1.1)
Medial	14	6.18 (6.35–6.01)	1.0 (0.9–1.1)
Distal	14	5.72 (5.83–5.61)	1.0 (0.9–1.1)

Values are mean and 95% confidence limits. *n* is the number of animals.

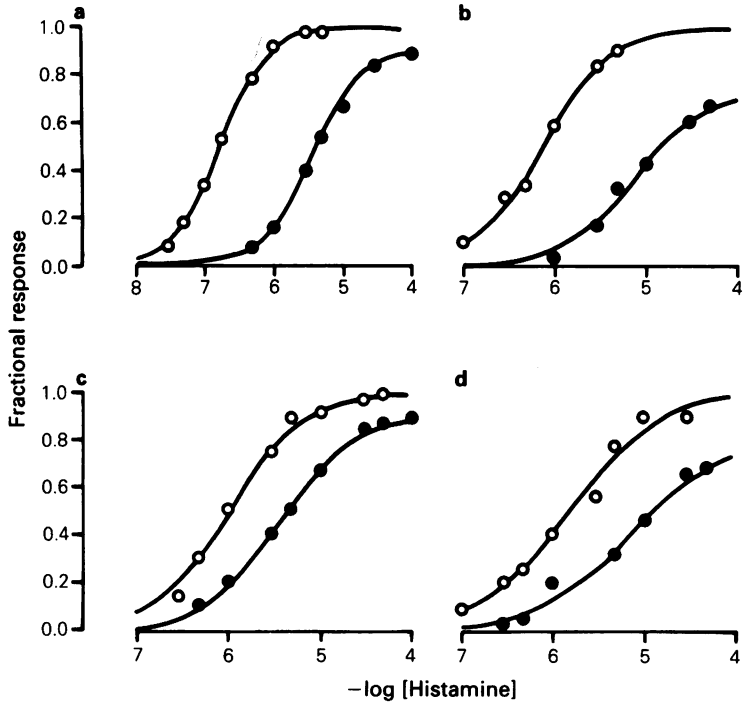

Figure 1 Representative concentration-effect curves for the action of histamine on the isolated ileum (a), proximal colon (b), medial colon (c), and distal colon (d) before (○) and after (●) treatment with 2 μM dibenamine for 30 min. The data points are experimentally observed results and lines are lines of best fit obtained from iterative nonlinear regression analyses for fits to equation 1 (see Methods).

respective K_d values for antagonism at H_2-receptors and muscarinic receptors and the concentration of tetrodotoxin used was shown to be sufficient to block fully the response of the ileum to electrical field stimulation (Barker & Ebersole, 1982). In each case, the antagonist was equilibrated with the tissue for 15 min before the addition of histamine. Under these conditions, scopolamine, tiotidine, and tetrodotoxin totally blocked the actions of an H_2-agonist, dimaprit, on the ileum (Barker & Ebersole, 1982). In separate

Table 2 The effect of receptor alkylation on dose-response parameters for the contractile action of histamine

Intestinal region	n	*Control*		*Post alkylation*		$\frac{E_{max}-2}{E_{max}-1}$
		− log EC_{50}	*Slope parameter*	*− log EC_{50}*	*Slope parameter*	
Ileum	5	6.67 (6.96–6.38)	1.0 (0.8–1.2)	5.30 (5.60–5.00)	1.1 (0.9–1.3)	0.78 (0.68–0.88)
Colon:						
Proximal	5	5.68 (6.09–5.27)	1.1 (0.9–1.3)	5.02 (5.26–4.78)	1.0 (0.9–1.1)	0.68 (0.52–0.84)
Medial	11	5.95 (6.03–5.83)	1.0 (0.9–1.1)	5.36 (5.53–5.19)	1.0 (0.9–1.1)	0.74 (0.64–0.84)
Distal	5	5.73 (6.11–5.35)	1.1 (1.0–1.2)	4.98 (5.23–4.73)	1.0 (0.8–1.2)	0.72 (0.50–0.94)

Values are mean and 95% confidence limits. *n* is the number of animals. $E_{max} - 2/E_{max} - 1$ is the ratio of the maximal response obtained after receptor alkylation to that of the control value.

experiments on relatively quiescent preparations of colonic tissues, it was found that neither scopolamine (0.1 μM, 60 min equilibration) nor tetrodotoxin (0.1 μM, 60 min equilibration) altered the dose-response curves for histamine on segments from any of the three colonic regions; the differences between pre- and post-treatment EC_{50} values or E_{max} values were not significantly different from zero ($P > 0.05$, paired t test, $n = 3$ for each region). In three experiments, the $-\log K_d$ value (see Barlow *et al.*, 1963) was determined for mepyramine at a concentration of 5 nM and an equilibration time of 60 min. The values, mean and 95% confidence limits, were 9.0 (9.3–8.7), 9.0 (9.4–8.6), 9.1 (9.3–8.9), and 8.9 (9.3–8.5) for the ileum, proximal colon, medial colon, and distal colon, respectively. These values are in good agreement with published $-\log K_d$ values for mepyramine inhibition at H_1-receptors (Arunlakshana & Schild, 1959; Barker, 1981; Hill & Young, 1981).

The mean $-\log EC_{50}$ and 95% confidence limits for the action of histamine on the ileum was 6.74 (6.86–6.62; $n = 8$). There were marked animal to animal variations in the EC_{50} value for histamine in a given region of the colon. For example, the EC_{50} value ranged from 0.15 μM to 2 μM in the medial colon. Because of this variation, the differences in sensitivity among the regions are not readily apparent. The distinction is best made on samples obtained from the same animal. In 14 experiments, the contractile activity of histamine was studied on the proximal, medial, and distal colon segments from the same animals. The results of these experiments are summarized in Table 1. The EC_{50} value for histamine on the medial colon was significantly lower than those for the proximal and distal regions ($P < 0.01$, one way analysis of variance and Newman-Keuls Multiple Range Test). The EC_{50} values for the actions of histamine at receptors on proximal and distal regions were not significantly different.

Figure 1 shows the results of single experiments on the effects of receptor alkylation by dibenamine on the contractile activity of histamine. In Table 2, the effects of receptor alkylation on dose-response parameters for histamine are summarized. In Table 3, the apparent K_d values for the interaction of histamine with receptors in the various intestinal regions are given. The apparent K_d values for the interaction of histamine with receptors in the four regions of the intestine were similar; ranging from about 20 μM to about 40 μM. These values are in general agreement with those reported by others: 10 μM (Furchgott, 1966) and 12–20 μM (Chang *et al.*, 1979). The tissue efficacy values calculated from the mean values for the EC_{50} for all experiments and the mean K_d for experiments shown in Table 3 were 121, 14, 30, and 20 for the ileum, proximal colon, medial colon, and distal colon, respectively.

Table 3 Apparent dissociation constant for the interaction of histamine with H_1-receptors in guinea-pig intestine

Region	n	$-\log K_d$
Ileum	5	4.68 (4.90–4.46)
Colon:		
Proximal	5	4.65 (4.95–4.35)
Medial	11	4.62 (4.81–4.43)
Distal	5	4.44 (4.81–4.04)

Values are mean and 95% confidence limits. n is the number of animals.

Discussion

The direct actions of histamine on ileal longitudinal smooth muscle are well established (Parsons, 1982). The contractile actions of histamine on the longitudinal smooth muscle layer of colonic tissues also appear to be direct in that they were not blocked by either scopolamine or tetrodotoxin. In the ileum and the three regions of the colon the actions of histamine were blocked by an H_1-antagonist, but not by an H_2-antagonist, suggesting the involvement of H_1-receptors. However, there were marked differences amongst the regions in their sensitivity to histamine, suggesting the possibility of receptor subtypes or regional differences in receptor density.

The basis for the variation in the sensitivity to the contractile actions of histamine is best explained by regional differences in the density of a homogeneous population of H_1-receptors and/or differences in the stimulus-response coupling within this population. The alternative explanation, that the differences in sensitivity are due to subtypes of histamine H_1-receptors is not supported by the results of this study. The H_1-receptor throughout the intestine appears to be homogeneous with respect to both agonist and antagonist binding sites as shown by the apparent affinities of both histamine and mepyramine. In agreement with Cook & Krueger (1981), it is concluded that H_1-receptors in the colon and the ileum cannot be distingusihed and that the receptor reserve in all regions of the colon is much less than that in the ileum.

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