Effects of histamine H₁-, H₂- and H₃-receptor selective drugs on the mechanical activity of guinea-pig small and large intestine

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1 In this study we have evaluated the possible contribution of acetylcholine release in histamine-induced contractions of guinea-pig large and small intestinal smooth muscle. Moreover, the presence of the histamine receptor types involved in smooth muscle relaxations and inhibition of electrically-induced twitches was studied by use of several selective agents.

2 Histamine-induced contractions appeared to be a pure H₁-receptor-mediated effect. Responses were not attenuated by the presence of 0.1 μ M atropine and were competitively and stereoselectively inhibited by the two enantiomers of chlorpheniramine with pA₂ values of 6.73 $\pm$ 0.08, 7.30 $\pm$ 0.06, 6.93 $\pm$ 0.03 and 7.19 $\pm$ 0.04 for the L-isomer and 8.63 $\pm$ 0.09, 8.85 $\pm$ 0.09, 9.01 $\pm$ 0.16 and 8.98 $\pm$ 0.11 for the D-isomer in the duodenum, jejunum, ileum and colon, respectively.

3 There appeared to be a marked regional difference in sensitivity to histamine. In ileal and jejunal preparations pD₂ values of 6.24 $\pm$ 0.06 (*n* = 22) and 6.37 $\pm$ 0.07 (*n* = 22) were found, whereas the pD₂ values in the duodenum and colon were 5.55 $\pm$ 0.05 (*n* = 36) and 5.68 $\pm$ 0.06 (*n* = 31) respectively.

4 This regional difference in sensitivity to histamine was not due to variations in receptor affinity since pA₂ values for the two enantiomers of chlorpheniramine did not differ markedly among the four tested preparations. Since a similar variation in sensitivity was found for methacholine, it is likely that the signal transfer mechanism in guinea-pig ileum and jejunum is more efficient than in the duodenum and colon.

5 The H₂-agonists dimaprit and impromidine relaxed methacholine-precontracted ($\pm$ 70% of maximum contraction) intestine at high concentrations (pD₂ values of 3.79 $\pm$ 0.03 and 4.44 $\pm$ 0.09 for the jejunum). These relaxations could not be antagonized by 0.1 μ M tiotidine, famotidine or mifentidine and were observed in all parts of the intestine investigated.

6 The dimaprit analogues nordimaprit and homodimaprit (inactive at H₂-receptors) were equipotent in relaxing the methacholine-precontracted smooth muscle. Since several H₂-antagonists were also able to produce relaxations, we do not consider these relaxations to be mediated by a H₂-receptor subtype, but to be due to some nonspecific effects at the high concentrations used.

7 The histamine receptor involved in the inhibition of electrically-induced contractions in the presence of atropine could be classified as an H₃-receptor effect. In all parts of the intestine the H₃-agonist R- α -methylhistamine inhibited the twitches with pD₂ values ranging from 8.10 $\pm$ 0.06 (ileum) to 8.27 $\pm$ 0.03 (colon). This effect was competitively antagonized with the selective H₃-antagonist thioperamine (pA₂ values are 8.09 $\pm$ 0.07, 8.13 $\pm$ 0.05, 8.15 $\pm$ 0.04 and 8.36 $\pm$ 0.04 in duodenum, jejunum, ileum and colon, respectively).

8 The guinea-pig intestine is a suitable preparation for the evaluation of either H₁- or H₃-receptor effects. H₂-receptors, causing smooth muscle relaxation appear not to be present in our preparations. At high concentrations of H₂-receptor agents (agonists and antagonists) relaxations might be observed, due to unknown nonspecific effects.

Introduction

In their original paper, describing the subclassification of histamine receptors into H₁- and H₂-receptors, Ash & Schild (1966) used the *in vitro* contraction of guinea-pig isolated ileum as a test system for H₁-receptor activity. Although the intestinal preparation has become a very valuable tool for the screening of H₁-receptor selective drugs, the original notion of guinea-pig ileum as an H₁-receptor preparation (Ash & Schild, 1966) has been complicated by the outcome of several investigations.

It has been suggested that the contractile effects of histamine on guinea-pig ileum are partly dependent on a tetrodotoxin-sensitive release of acetylcholine (Rubenstein & Cohen, 1985). Moreover, using selective H₂-receptor agents Barker & Ebersole (1982) reported the presence of H₂-receptors on guinea-pig myenteric plexus neurones, mediating the release of acetylcholine. To complicate the situation even more, Bertaccini & Zappia (1981) described the presence of an H₂-receptor subtype on guinea-pig duodenum, mediating relaxations of precontracted muscle. H₂-receptor agonists, like dimaprit, impromidine and clonidine (also an

α_2 -agonist), caused relaxations with a potency lower than that usually found for e.g. the positive chronotropic effect on guinea-pig right atrium (Bertaccini & Zappia, 1981). The relaxations could however not be inhibited by the H₂-antagonists metiamide, cimetidine, ranitidine or oxmetidine, nor by tetrodotoxin, propranolol or phentolamine (Bertaccini & Zappia, 1981). Moreover, Fjalland (1979) also suggested the existence of an H₂-receptor subtype in guinea-pig ileum because histamine was shown to suppress electrically-induced twitches of ileal smooth muscle, although the order of potency of H₂-antagonists in inhibiting this action of histamine did not correlate with the known activity of the compounds at the H₂-receptor of guinea-pig right atrium (Fjalland, 1979). Recently the classification of histamine receptors has been extended (Arrang *et al.*, 1983) and the presence of an H₃-receptor, which is involved in the regulation of neurotransmitter release in both brain and peripheral tissues (Timmerman, 1990), is currently accepted. Since some H₂-antagonists can also act as H₃-antagonists (Schwartz *et al.*, 1986) the findings of Fjalland (1979) were re-evaluated by Trzeciakowski (1987) and from the latter study it was concluded that the observed inhibition of the electrically-induced

twitches was due to an H_3 -receptor-mediated reduction of acetylcholine release (Trzeciakowski, 1987). Previously it has been reported that histamine could also inhibit electrically-induced twitches of guinea-pig ileum in the presence of atropine (Ambache & Zar, 1970; Ambache *et al.*, 1970). Based upon the inhibitory action of burimamide Ambache *et al.* (1973) also suggested the involvement of an H_2 -receptor subtype. However, a pharmacological characterization of this effect in view of the currently accepted histamine receptor sub-classification has not been provided.

In the present study we examined several of the problems outlined above, using highly selective histamine receptor agonists and antagonists. In both small and large intestine of the guinea-pig we investigated the possible indirect contractile effects of histamine (Rubenstein & Cohen, 1985), the existence of an H_2 -receptor subtype, possibly mediating relaxations (Bertaccini & Zappia, 1981), and the inhibition of electrically-induced twitches of the intestinal smooth muscle in the presence of atropine, as originally described by Ambache & Zar (1970).

Methods

Tissue bath studies

Male guinea-pigs (350–450 g) were killed by a blow on the head. Small and large intestine were immediately excised and placed in Krebs-buffer (composition mM: NaCl 117.5, KCl 5.6, $MgSO_4$ 1.18, $CaCl_2$ 2.5, NaH_2PO_4 1.28, $NaHCO_3$ 25 and glucose 5.5). The first 12 cm of the small intestine was taken as duodenum and the middle portion was used as jejunum. The portion of the small intestine that lies 5–15 cm from the ileocaecal valve was considered to be the ileum. The colonic smooth muscle was taken from the ascending colon. Intestinal smooth muscle strips (10 mm long, 2 mm wide) were prepared by cutting the intestine in the longitudinal direction; the mucosa was not removed. Muscle strips were mounted in 20 ml Krebs buffer, continuously gassed with 95% O_2 :5% CO_2 and maintained at 37°C. Contractions were recorded isotonically under 0.4 g tension with a Hugo Sachs Hebel-Messvorsatz (TL-2)/HF-modem (Hugo Sachs Elektronik, Hugstetten, West Germany) connected to a pen-recorder.

After equilibration for at least 45 min, with replacement of fresh Krebs-buffer every 10 min, cumulative dose-response curves for histamine or methacholine were recorded using half-log increments in agonist concentration in the organ bath until stable responses towards the contractile agent used were obtained.

To investigate the effect of the smooth muscle relaxants, smooth muscle strips were contracted to 70–80% of the maximal methacholine or histamine response, using single doses. In some experiments muscle strips were contracted with 40 mM KCl. This concentration of KCl also yielded 70–80% of the maximal methacholine response. The smooth muscle relaxants were subsequently added cumulatively.

In some experiments the muscle strips were mounted between two platinum electrodes. Rectangular-wave electrical pulses (Grass stimulator S-88, Grass Instruments Co., Quincy, U.S.A.) were delivered to the preparations at a frequency of 50 Hz and 0.5 ms duration in 10-pulse trains every 33 s. Muscle strips were stimulated supramaximally (50 V).

Antagonists were usually equilibrated for 5 min after which contractile or relaxant responses were retested; this period of time was usually sufficient to reach equilibrium as shown by Schild slope values that did not deviate from unity. For the antagonists atropine and D-chlorpheniramine, concentrations of 3, 10, 30 and 100 nM were tested, whereas for L-chlorpheniramine, 0.1, 0.3, 1 and 3 μ M concentrations were used. Thioperamide was tested at concentrations of 30, 100 and 300 nM. Antagonistic activity (pA_2) was determined by use of a Schild-plot.

Statistics

All data shown are expressed as mean $\pm$ s.e.mean. EC_{50} values were transformed to logarithmic values (pD_2 values) for calculation of a mean value and statistical comparison. Tissue preparations from at least three different animals were used for each drug treatment. Statistical analysis was carried out with Student's *t* test; *P* values < 0.05 were considered to indicate a significant difference.

Chemicals

Histamine dihydrochloride, procaine hydrochloride, 4-aminopyridine, cimetidine and atropine sulphate were purchased from Sigma Chemical Company Ltd. (St. Louis, U.S.A.). Ranitidine hydrochloride and tetraethylammonium chloride were obtained from Research Biochemicals Incorporated (Natick, U.S.A.). Gifts of famotidine (Merck Sharp & Dohme), tiotidine (Imperial Chemical Industries), mifentidine (De Angeli), thioperamide, R- α -methylhistamine maleate (Dr J.-C. Schwartz, Centre Paul Broca de l'INSERM, Paris), impromidine trihydrochloride (Smith Kline & French), 2-amino-6-dimethylamino-4,5,6,7-tetrahydrobenzo[d]thiazole (SND 861 C12, Boehringer Ingelheim), D- and L-chlorpheniramine (Dr A.J. Beld, Catholic University Nijmegen, The Netherlands) and pinacidil (Leo Pharmaceuticals) are gratefully acknowledged. Dimaprit dihydrobromide, nordimaprit dihydrobromide and homodimaprit dihydrobromide were taken from laboratory stock (Sterk *et al.*, 1984). All other reagents were of analytical grade.

Results

Histamine contracts smooth muscle of guinea-pig large and small intestine in a concentration-dependent way. There appeared to be a significant difference in sensitivity of the various intestinal preparations to histamine (Table 1). Ileal and jejunal smooth muscle have an approximately equal sensitivity to histamine, but are almost 5 fold more sensitive than the duodenal and colonic preparations under the present conditions (Table 1). Similar observations were made for the muscarinic agent methacholine, which was also more potent in contracting ileal and jejunal smooth muscle than duodenal and colonic preparations (Table 1).

The histamine-induced contractions were competitively inhibited by the two chlorpheniramine enantiomers. Schild-slope values did not significantly differ from unity (Table 2). In all tissue preparations the D-isomer of this H_1 -antagonist appeared to be the most potent agent (50–80 fold more active than the L-isomer, Table 2). The contractions elicited by methacholine were competitively antagonized by atropine; Schild slope values did not deviate significantly from unity (Table 2).

Based on the results of the antagonism of methacholine-induced contractions a concentration of 0.1 μ M atropine was chosen for the subsequent experiments, which were undertaken to elucidate a possible indirect contractile mechanism for histamine. As can be seen in Table 1, there was no indication of a contribution of histamine-induced acetylcholine release to the observed contractions of intestinal smooth muscle after histamine application. The pD_2 values for histamine in the parts of guinea-pig intestine examined were not influenced by the presence of 0.1 μ M atropine under our experimental conditions (Table 1). Also the maximal response to histamine was not attenuated in the presence of atropine (Table 1).

To study the presence of an H_2 -receptor subtype mediating relaxation of intestinal smooth muscle, we extended the original study of Bertaccini & Zappia (1981). As can be seen in Figure 1, dimaprit induced relaxation of methacholine-precontracted duodenal muscle strips. However, the potency of dimaprit was rather weak. High concentrations of dimaprit as well as of impromidine were necessary to relax the intesti-

Table 1 Characteristics of histamine- and methacholine-induced contractions of guinea-pig large and small intestinal smooth muscle

	Duodenum	Jejunum	Ileum	Colon
pD ₂ Histamine	5.55 ± 0.05 (36)	6.37 ± 0.07 (22)	6.24 ± 0.06 (22)	5.68 ± 0.06 (31)
pD ₂ Methacholine	6.09 ± 0.12 (13)	6.73 ± 0.09 (7)	6.74 ± 0.10 (8)	6.17 ± 0.05 (12)
pD ₂ Histamine + atropine	5.53 ± 0.14 (8)	6.52 ± 0.14 (4)	6.26 ± 0.03 (6)	5.59 ± 0.12 (8)
% maximal response histamine + atropine	99 ± 3 (8)	99 ± 2 (4)	100 ± 9 (6)	108 ± 5 (8)

The pD₂-values for histamine and methacholine for each part of the intestine are shown. Histamine-induced contractions were also measured in the presence of 0.1 μM atropine. The pD₂ values and maximal responses (expressed as the percentage of the control responses) are shown for each part of the intestine. Data shown are mean ± s.e.mean. Numbers in parentheses are the numbers of independent experiments.

nal smooth muscle completely (Figure 1, Table 3, Table 4). We also tried to antagonize this relaxant effect of dimaprit with the H₂-antagonists tiotidine, mifentidine and famotidine. In concentrations that can be expected to inhibit H₂-receptor responses (0.1 μM) none of these H₂-antagonists affected the dimaprit-induced relaxations of guinea-pig duodenum. To characterize the receptor subtype involved more clearly, two dimaprit analogues, devoid of H₂-receptor activity, were examined. Both nordimaprit and homodimaprit were able to relax the methacholine-precontracted duodenum completely and were equipotent with dimaprit (Figure 1). The pD₂ values for the relaxations of the duodenum were 3.34 ± 0.12 (n = 9),

3.42 ± 0.05 (n = 4) and 3.28 ± 0.11 (n = 4) for dimaprit, homodimaprit and nordimaprit respectively.

These findings were not confined to guinea-pig duodenum. Dimaprit was able to relax methacholine-precontracted muscle strips of guinea-pig ileum as well as jejunum and colon with similar potency (Table 3). Homodimaprit proved to be as effective as dimaprit in this respect (Table 3). Guinea-pig colon was the least sensitive to dimaprit and homodimaprit; contractions were not completely relaxed in this preparation (Table 3). Moreover, dimaprit was also able to relax methacholine-precontracted rat jejunal muscle strips (pD₂ = 3.60 ± 0.09, n = 2).

Table 2 Characteristics of the antagonism of the histamine- and methacholine-induced contractions respectively by the two stereoisomers of chlorpheniramine and atropine in the various parts of the intestine

	Duodenum	Jejunum	Ileum	Colon
pA ₂ L-Chlorpheniramine vs histamine	6.73 ± 0.08 (1.2 ± 0.1) n = 6	7.30 ± 0.06 (1.0 ± 0.1) n = 4	6.93 ± 0.03 (1.1 ± 0.1) n = 6	7.19 ± 0.04 (0.9 ± 0.1) n = 3
pA ₂ D-Chlorpheniramine vs histamine	8.63 ± 0.09 (1.2 ± 0.1) n = 5	8.85 ± 0.09 (1.1 ± 0.2) n = 5	9.01 ± 0.16 (1.2 ± 0.1) n = 6	8.98 ± 0.11 (0.9 ± 0.1) n = 6
pA ₂ Atropine vs methacholine	8.49 ± 0.06 (1.1 ± 0.1) n = 6	8.44 ± 0.09 (1.3 ± 0.1) n = 4	8.72 ± 0.12 (1.3 ± 0.1) n = 6	8.85 ± 0.04 (1.1 ± 0.1) n = 6

pA₂-values and Schild slopes are the mean ± s.e.mean of several independent experiments. Numbers in parentheses are the Schild slopes. n: number of experiments.

Figure 1 Representative recorder tracings of relaxations of guinea-pig duodenum induced by dimaprit, nordimaprit or homodimaprit. The duodenal preparation was precontracted with methacholine 3 μM and relaxations were recorded by cumulative applications of the relaxing agents.

Table 3 pD_2 values and maximal responses of dimaprit and homodimaprit for relaxing methacholine-precontracted muscle strips from guinea-pig duodenum, ileum, jejunum and colon

Preparation	Dimaprit			Homodimaprit		
	pD_2	% relaxation	<i>n</i>	pD_2	% relaxation	<i>n</i>
Duodenum	3.34 ± 0.12	95 ± 4	9	3.43 ± 0.05	109 ± 2	4
Ileum	3.67 ± 0.14	108 ± 10	7	3.83 ± 0.05	104 ± 3	7
Jejunum	3.79 ± 0.03	102 ± 1	62	4.13 ± 0.12	99 ± 4	7
Colon	3.34 ± 0.04	22 ± 3	7	3.38 ± 0.03	48 ± 2	3

Muscle strips of duodenum and colon were precontracted with methacholine $3 \mu\text{M}$, whereas ileal and jejunal strips were stimulated with methacholine $1 \mu\text{M}$. Data shown are mean $\pm$ s.e.mean of at least three independent experiments.

n: number of experiments.

Another dimaprit analogue, SND 861 C12, which comprises the complete structure of dimaprit in a cyclic molecule, behaved as a weak competitive H_2 -antagonist against the positive chronotropic actions of histamine on guinea-pig right atrium ($pA_2 = 5.49 \pm 0.16$, $n = 2$, unpublished data from our laboratory). Nevertheless, despite its H_2 -antagonistic properties this compound was 10 fold more potent than dimaprit in relaxing methacholine-precontracted jejunal smooth muscle strips (Table 4). Several other H_2 -antagonists were also evaluated for their relaxant activity. Cimetidine, tiotidine, mifentidine and famotidine all appeared to be relaxing agents with a potency comparable to dimaprit (Table 4).

Dimaprit not only relaxed methacholine-precontracted intestinal smooth muscle but also relaxed jejunal strips precontracted with $1 \mu\text{M}$ histamine. The pD_2 value for relaxing these contractions was 3.83 ± 0.23 ($n = 5$). This value was not significantly different from the pD_2 values for the relaxation of methacholine-precontracted preparations (Table 4). Yet, if jejunal muscle strips were precontracted with KCl, using a concentration leading to an approximately similar contraction to that obtained with $1 \mu\text{M}$ methacholine, most of the histamine receptor agonists and antagonists were not able to relax the smooth muscle. Only at the highest tested concentrations (usually 3 mM) some small relaxations were sometimes noticed. Also the potassium channel opener, pinacidil, was more potent in relaxing methacholine-precontracted preparations ($pD_2 = 5.12 \pm 0.12$, $n = 10$) compared with 40 mM KCl-precontracted jejunal muscle strips ($pD_2 = 3.93 \pm 0.08$, $n = 11$) (Figure 2). Pinacidil concentration-dependently inhibited the spontaneous activity of the jejunal preparations. In contrast the potassium channel blockers tetraethylammonium (TEA, 0.3 – 10 mM), procaine (0.3 – 10 mM) and 4-aminopyridine (0.1 mM) concentration-dependently increased the amplitude of the basal rhythmic activity. Spontaneous activity induced by 1 mM TEA was, depending on the preparation, completely reduced by 5 – $20 \mu\text{M}$ pinacidil (Figure 3). However, none of the tested compounds affected basal jejunal activity and neither dimaprit (Figure 3) nor tiotidine could inhibit TEA-induced spontaneous activity at a concentration of 1 mM .

Table 4 pD_2 values and maximal responses of several compounds acting at histamine receptors for relaxing methacholine-precontracted muscle strips from guinea-pig jejunum

Drug	pD_2	% relaxation	<i>n</i>
Dimaprit	3.79 ± 0.03	102 ± 1	62
Impromidine	4.44 ± 0.09	95 ± 3	7
SND 861 C12	4.97 ± 0.18	100 ± 1	4
Cimetidine	3.61 ± 0.15	95 ± 3	7
Tiotidine	3.67 ± 0.02	98 ± 2	3
Famotidine	3.53 ± 0.05	93 ± 3	4
Mifentidine	3.97 ± 0.08	102 ± 1	4

Jejunal strips were precontracted with methacholine $1 \mu\text{M}$. Data shown are mean $\pm$ s.e.mean of at least three independent experiments.

n: number of experiments.

Finally, we tried to characterize the reported effects of histamine on electrically-stimulated intestinal preparations in the presence of $0.1 \mu\text{M}$ atropine (Ambache & Zar, 1970; Ambache *et al.*, 1970; 1973). Tetanic stimulation of guinea-pig small

Figure 2 Dose-response curves for the pinacidil-induced relaxations of jejunal smooth muscle. The jejunal preparations were precontracted with $1 \mu\text{M}$ methacholine ($\circ$, $n = 10$) or 40 mM KCl ($\bullet$, $n = 11$). Results are expressed as mean with s.e.mean shown by vertical bars. **Figure 3** Effects of tetraethylammonium (TEA), pinacidil (Pin) and dimaprit (Dim) on the spontaneous motility of guinea-pig jejunum. Basal activity was enhanced by the addition of TEA 1 mM and was inhibited by pinacidil (a and b). Dimaprit did not attenuate the TEA effects (b).

intestine induced an atropine-resistant spasm, which consisted of several components (Figure 4a). Stimulation of colonic muscle strips resulted in a biphasic pattern: first a fast relaxation, which was followed by a rather slowly developing contraction (Figure 4b). In the present study we used the highly selective and potent H₃-receptor agonist R- α -methylhistamine to modulate the observed responses. The H₃-agonist was able to reduce the twitch height of the muscle strips at concentrations of 30 nM; maximal effect was usually noticed at a 0.3 μ M concentration of R- α -methylhistamine (Figures 4 and 5). In the experiments with the colonic muscle strips only the contractile component of the response was modulated by R- α -methylhistamine (Figure 4b). The pD₂ value for R- α -methylhistamine did not differ markedly among the four preparations tested and was approximately 8.15 in all intestinal tissues (Table 5). The observed contractions could be inhibited by 60–70% with R- α -methylhistamine (Figure 5, Table 5).

The inhibitory effects of R- α -methylhistamine were competitively inhibited by the highly selective H₃-receptor antagonist thioperamide (Figure 5 inset). The potency of this compound was similar in all preparations; a pA₂ value of approximately 8.2 was found for all tissues (Table 5). The Schild-slope values did not deviate from unity in the various preparations (Table 5).

Discussion

The stimulatory action of histamine on intestinal smooth muscle has been known since the initial work of Dale & Laidlaw (1910, 1911). The development of the classical antihistamines and the observation that the histamine-induced effects on rat isolated uterus were refractory to inhibition by these drugs led to the hypothesis that histamine acted via at least two distinct receptor populations (Ash & Schild, 1966). After the discovery of burimamide (Black *et al.*, 1972) and related H₂-antagonists, this initial subclassification into H₁- and H₂-receptors was widely accepted.

Recently a new histamine receptor subtype has been described. The histamine H₃-receptor has been shown to affect [³H]-histamine release from preloaded rat brain slices after potassium-depolarization (Arrang *et al.*, 1983) or electrical stimulation (Van der Werf *et al.*, 1987). This new receptor subtype shows remarkably different pharmacological characteristics; highly selective agonists (R- α -methylhistamine) and antagonists (thioperamide) have already been described (Arrang *et al.*, 1987). Due to the development of these selective H₃-receptor agents, investigations on the possible physiological role of the H₃-receptor have been conducted. It is now becoming clear that the H₃-receptor forms a general presynaptic regulatory system, which is not restricted to the brain (Timmerman, 1990).

Although the guinea-pig ileum was the first tissue used as an H₁-receptor preparation (Ash & Schild, 1966), there are now several reports indicating that histamine can act via several histamine receptor subtypes in the intestinal preparation. Histamine contracts both ileal longitudinal and circular smooth muscle layer via activation of H₁-receptors (Barker, 1985; Yamanaka & Kitamura, 1987), but can probably also relax circular muscle via an H₂-receptor-induced

Figure 4 Representative recorder tracings of electrically-stimulated ileal (a) and colonic (b) smooth muscle strips. The arrow indicates the addition of R- α -methylhistamine 1 μ M.

Figure 5 Dose-response curve for the inhibition of electrically induced twitches of guinea-pig colonic muscle strips by the H₃-agonist, R- α -methylhistamine. A dose-response curve for R- α -methylhistamine in the absence (○) and presence of 30 (●), 100 (□) and 300 nM thioperamide (■) is shown. The inhibitory effects could be competitively antagonized by thioperamide. The inset shows the transformation of the data in the presence of thioperamide into a Schild plot. Results are expressed as mean of $n = 7$; s.e.mean shown by vertical bars.

Table 5 Activities of the H₃-receptor agonist R- α -methylhistamine and H₃-receptor antagonist thioperamide on electrically-stimulated muscle strips of guinea-pig large and small intestine

Preparation	<i>R</i> - α -methylhistamine			<i>n</i>	Thioperamide		
	pD ₂	% inhibition			pA ₂	Schild slope	<i>n</i>
Duodenum	8.09 $\pm$ 0.07	69 $\pm$ 4	6	8.09 $\pm$ 0.07	0.9 $\pm$ 0.1	6	
Ileum	8.10 $\pm$ 0.06	65 $\pm$ 3	6	8.15 $\pm$ 0.04	0.9 $\pm$ 0.1	6	
Jejunum	8.18 $\pm$ 0.06	65 $\pm$ 4	7	8.13 $\pm$ 0.05	1.0 $\pm$ 0.1	7	
Colon	8.27 $\pm$ 0.03	57 $\pm$ 4	7	8.36 $\pm$ 0.04	0.9 $\pm$ 0.1	7	

Data shown are mean $\pm$ s.e.mean of several independent experiments.
n: number of experiments.

hyperpolarization of the membrane (Yamanaka & Kitamura, 1987). Besides, H_2 -receptor agonists have been reported to be both contractile and relaxant agents of guinea-pig intestinal longitudinal smooth muscle (Bertaccini & Zappia, 1981; Barker & Ebersole, 1985). Dimaprit has been reported to release acetylcholine from guinea-pig ileum myenteric plexus neurones in a tiotidine-sensitive manner (Barker & Ebersole, 1985), whereas acetylcholine release is also reported to contribute to the histamine-mediated contraction of the longitudinal smooth muscle (Rubenstein & Cohen, 1985).

In the present study such a contribution of acetylcholine could not be found in any of the intestinal preparations tested. Histamine proved to be a potent contractile agent, but the pD_7 value for histamine was not affected by the presence of $0.1 \mu M$ atropine. Based upon the measured pA_2 values for atropine against methacholine-induced responses (8.44–8.85), it can be concluded that in our preparations, $0.1 \mu M$ atropine is a suitable concentration for the study to a contribution of acetylcholine release to the histamine-mediated contractions. Such a contribution is therefore rather unlikely.

Finally, the histamine-induced contractions were competitively and stereoselectively antagonized by the two stereoisomers of the H_1 -receptor antagonist chlorpheniramine. The pA_2 values for the two stereoisomers correspond well with their reported affinity for the H_1 -receptor (Hill & Young, 1980) and do not differ markedly among the different intestinal preparations. These data suggest that the histamine-induced contraction is only due to the activation of H_1 -receptors. Based upon the pA_2 values of the H_1 -antagonists, there appeared to be no marked regional variation in H_1 -receptor affinity. Previously similar findings have been reported by Barker (1985) for guinea-pig ileum and colon. In contrast to the pA_2 values for the H_1 -receptor, a remarkable regional variation in sensitivity to histamine was noticed among the four preparations tested. This difference might be due to differences in H_1 -receptor density or the stimulus transfer mechanism. Since methacholine-induced responses show a similar variation in sensitivity we assume that in contrast to the duodenum and colon, the guinea-pig ileum and jejunum possess an efficient stimulus transfer mechanism leading to a receptor reserve.

As already mentioned, it has been reported that H_2 -receptor agonists can relax duodenal longitudinal smooth muscle (Bertaccini & Zappia, 1981). According to these authors, the relaxations could not be antagonized by a number of H_2 -antagonists such as cimetidine, metiamide, ranitidine and oxmetidine and it was therefore suggested that they were mediated by an H_2 -receptor subtype (Bertaccini & Zappia, 1981). In the present study these relaxations were observed in guinea-pig ileum, jejunum, colon and rat jejunum and several agents acting on histamine receptors were used for a more detailed pharmacological characterization. The recently developed H_2 -receptor antagonists famotidine, mifentidine and tiotidine were all unable to antagonize the dimaprit-induced relaxations. Besides dimaprit, impromidine also relaxed the precontracted intestinal muscle at relatively high concentrations. The length of the alkyl chain in dimaprit is rather essential for H_2 -receptor agonistic activity as both nor-dimaprit and homodimaprit possess hardly any H_2 -receptor activity (Ganellin, 1982). Yet, these two compounds are approximately equipotent with dimaprit in relaxing the guinea-pig duodenum. Moreover, SND 861 C12, which comprises the structure of dimaprit in a cyclic molecule, is the most potent relaxant agent observed in this study. Nevertheless, this compound is devoid of any H_2 -agonistic activity and behaves even as a weak competitive H_2 -receptor antagonist against histamine-induced positive chronotropic effects in guinea-pig right atrium ($pA_2 = 5.49$). Moreover, other H_2 -receptor antagonists also proved to be able to relax intestinal smooth muscle at high concentrations. Based on these results, the presence of an H_2 -receptor subtype, involved in smooth muscle relaxation, seems rather unlikely. Despite their activity at the H_2 -receptor (agonist, antagonist or inactive) all

tested compounds were able to produce relaxations of visceral smooth muscle. Since α_2 -adrenoceptor agonists (clonidine and tolazoline) are also relaxant agents in this preparation (Bertaccini & Zappia, 1981) the observed effects are probably related to a nonspecific effect at the high concentrations used.

In this study it was observed that muscle strips, when precontracted with KCl, were rather resistant to relaxation by agents acting on histamine receptors. This might indicate that the observed relaxations are due to an action at the potassium channels in the plasma membrane. It has been shown that some recently developed smooth muscle relaxants act via the opening of potassium channels (Weir & Weston, 1986). These drugs have been reported to be able to relax vascular pulmonary and also intestinal smooth muscle (Matsui *et al.*, 1986; Weir & Weston, 1986; Buchheit & Bertholet, 1988; Mellemkjaer *et al.*, 1989). The potassium channel opener, pinacidil, bears some structural similarity to some H_2 -receptor antagonists and indeed proved to be more effective in relaxing methacholine-precontracted muscle strips than KCl-precontracted preparations. However, pinacidil also concentration-dependently inhibited jejunal spontaneous motility. This activity is not shared by any of the agents acting on histamine receptors. The inhibition of basal motor activity can probably be related to interference with the potassium channels, since similar findings have been published for another potassium channel opener, cromakalim (Buchheit & Bertholet, 1988). Moreover, in the present study, several potassium channel blockers (tetraethylammonium, 4-aminopyridine, procaine) proved to enhance the intestinal motility. This effect could be inhibited by pinacidil, but was not affected by dimaprit or tiotidine. These data indicate that interference at the level of potassium channels cannot explain the relaxant effects of the agents acting on histamine receptors. The mechanism of action involved therefore remains to be elucidated.

Finally we studied the involvement of the H_3 -receptor in the inhibitory response of histamine on electrically stimulated intestinal smooth muscle strips (Fjalland, 1979; Ambache & Zar, 1970; Ambache *et al.*, 1970; 1973). Based on the inhibitory effects of burimamide, this effect was suggested to be mediated by an H_2 -receptor subtype (Ambache *et al.*, 1973; Fjalland, 1979). Recently Trzeciakowski (1987) showed that under conditions of electrical stimulation, that release acetylcholine, the histamine-induced inhibition could be denoted as an H_3 -receptor effect. However, the myenteric plexus contains a rather heterogeneous neuronal population, which might release various substances (Sternini, 1988). Besides acetylcholine, noradrenaline, 5-hydroxytryptamine, several neuropeptides and excitatory aminoacids are probably also involved in the regulation and modulation of intestinal motility (Sternini, 1988; Nemeth & Gullikson, 1989; Shannon & Sawyer, 1989). Indeed, Ambache & Freeman (1968) observed that tetanic stimulation of guinea-pig ileum produced a contraction due to the release of a non-cholinergic substance(s) from the myenteric plexus. The nature of the contractile substance(s) is not clear yet, but is now under investigation in our laboratory. The contractions after tetanic stimulation (in the presence of $0.1 \mu M$ atropine) were reduced by histamine (Ambache *et al.*, 1970; Ambache & Zar, 1970) via a suggested H_2 -receptor subtype (Ambache *et al.*, 1973). By use of the highly selective H_3 -receptor agonist $R\text{-}\alpha$ -methylhistamine, twitch responses in the presence of $0.1 \mu M$ atropine could be inhibited in all intestinal preparations. These effects are effectively and competitively antagonized by thioperamide, a highly selective H_3 -receptor antagonist. The pA_2 values for this compound correlate well with previous findings (Arrang *et al.*, 1987) and do not differ among the preparations tested, indicating a homogeneous population of H_3 -receptors in the intestinal tract. These receptors appear to modulate the release of acetylcholine (Trzeciakowski, 1987), but can also inhibit the release of other non-cholinergic contractile substances. The H_3 -receptor appears therefore to be a rather general regulatory mechanism in the intestine.

In conclusion, our study indicates that the guinea-pig intestine contracts in response to histamine via a pure H₁-receptor-activated mechanism. In the preparations we used, no contribution of acetylcholine release was observed. At high concentrations of certain histamine agonists and antagonists relaxation of the smooth muscle unrelated to H₂-receptor stimulation occurs via a yet unidentified nonspe-

cific action. Moreover, in all parts of the intestine H₃-receptors appear to be present. Besides acetylcholine, release of unidentified, non-cholinergic substances are also inhibited via H₃-receptor stimulation. The intestinal smooth muscle might therefore be a suitable preparation for the evaluation of either H₁-receptor or H₃-receptor effects.

References

- AMBACHE, N. & FREEMAN, M.A. (1968). Atropine-resistant longitudinal smooth muscle spasm due to excitation of non-cholinergic neurones in Auerbach's plexus. *J. Physiol.*, **199**, 705–727.
- AMBACHE, N. & ZAR, M. ABOO (1970). An inhibitory action of histamine on the guinea-pig ileum. *Br. J. Pharmacol.*, **38**, 229–240.
- AMBACHE, N., VERNEY, J. & ZAR, M. ABOO (1970). Evidence for the release of two atropine-resistant spasmogens from Auerbach's plexus. *J. Physiol.*, **207**, 761–782.
- AMBACHE, N., KILLICK, S.W. & ZAR, M. ABOO (1973). Antagonism by burimamide of inhibition induced by histamine in plexus containing longitudinal smooth muscle preparations from guinea-pig ileum. *Br. J. Pharmacol.*, **48**, 362–363P.
- ARRANG, J.-M., GARBARG, M. & SCHWARTZ, J.-C. (1983). Auto-inhibition of brain histamine release by a novel class (H₃) of histamine receptors. *Nature*, **302**, 831–837.
- ARRANG, J.-M., GARBARG, M., LANCELOT, J.-C., LECOMTE, J.-M., POLLARD, H., ROBBA, M., SCHUNACK, W. & SCHWARTZ, J.-C. (1987). Highly potent and selective ligands for histamine H₃-receptors. *Nature*, **327**, 117–123.
- ASH, A.S.F. & SCHILD, H.O. (1966). Receptors mediating some actions of histamine. *Br. J. Pharmacol. Chemother.*, **27**, 427–439.
- BARKER, L.A. (1985). Regional variation in the sensitivity of longitudinal smooth muscle to histamine at H₁-receptors in guinea-pig ileum and colon. *Br. J. Pharmacol.*, **85**, 377–381.
- BARKER, L.A. & EBERSOLE, B.J. (1982). Histamine H₂-receptors on guinea-pig ileum myenteric plexus neurons mediate the release of contractile agents. *J. Pharmacol. Exp. Ther.*, **221**, 69–75.
- BERTACCINI, G. & ZAPPALÀ, L. (1981). Histamine receptors in the guinea-pig duodenum. *J. Pharmac. Pharmacol.*, **33**, 590–593.
- BLACK, J.W., DUNCAN, W.A.M., DURANT, G.J., GANELLIN, C.R. & PARSONS, M.E. (1972). Definition and antagonism of histamine H₂-receptors. *Nature*, **236**, 385–390.
- BUCHHEIT, K. & BERTHOLET, A. (1988). Inhibition of small intestine motility by cromakalim (BRL 34915). *Eur. J. Pharmacol.*, **154**, 335–337.
- DALE, H.H. & LAIDLAW, P.P. (1910). The physiological action of imidazolyethylamine. *J. Physiol.*, **41**, 318–344.
- DALE, H.H. & LAIDLAW, P.P. (1911). Further observations on the action of beta-imidazolyethylamine. *J. Physiol.*, **43**, 182–195.
- FJALLAND, B. (1979). Evidence for the existence of another type of histamine H₂-receptor in guinea-pig ileum. *J. Pharmac. Pharmacol.*, **31**, 50–51.
- GANELLIN, C.R. (1982). Chemistry and structure-activity relationships of drugs acting at histamine receptors. In *Pharmacology of Histamine Receptors*. ed. Ganellin, C.R. & Parsons, M.E. pp. 10–102. Bristol: John Wright & Sons Ltd.
- HILL, S.J. & YOUNG, J.M. (1980). Histamine H₁-receptors in the brain of the guinea-pig and the rat: differences in ligand binding properties and regional distribution. *Br. J. Pharmacol.*, **68**, 687–696.
- MATSUI, K., OGAWA, Y. & IMAI, S. (1986). Relaxant effects of pinacidil, nicorandil, hydralazine and nifedipine as studied in the porcine coronary artery and guinea-pig taenia coli. *Arch. Int. Pharmacodyn.*, **283**, 124–133.
- MELLEMEKJAER, S., NIELSEN-KUDSK, J.E., NIELSEN, C.B. & SIGGAARD, C. (1989). A comparison of the relaxant effects of pinacidil in guinea-pig trachea, aorta and pulmonary artery. *Eur. J. Pharmacol.*, **167**, 275–280.
- NEMETH, P.R. & GULLIKSON, G.W. (1989). Gastrointestinal motility stimulating drugs and 5-HT receptors on myenteric neurons. *Eur. J. Pharmacol.*, **166**, 387–391.
- RUBENSTEIN, R. & COHEN, S. (1985). Histamine-mediated acetylcholine release in the guinea-pig ileum. *Eur. J. Pharmacol.*, **111**, 245–250.
- SCHWARTZ, J.-C., ARRANG, J.-M. & GARBARG, M. (1986). Three classes of histamine receptors in brain. *Trends Pharmacol. Sci.*, **7**, 24–28.
- SHANNON, H.E. & SAWYER, B.D. (1989). Glutamate receptors of the N-methyl-D-aspartate subtype in the myenteric plexus of the guinea-pig ileum. *J. Pharmacol. Exp. Ther.*, **251**, 518–523.
- STERK, G.J., VAN DER GOOT, H. & TIMMERMAN, H. (1984). Studies on histaminergic compounds, part II—Synthesis and histamine H₂-activity of dimaprit and some of its analogues. *Eur. J. Med. Chem.*, **19**, 545–550.
- STERNINI, S. (1988). Structural and chemical organisation of the myenteric plexus. *Ann. Rev. Physiol.*, **50**, 81–93.
- TIMMERMAN, H. (1990). Histamine H₃ ligands: just pharmacological tools or potential therapeutic agents? *J. Med. Chem.*, **33**, 4–11.
- TRZECIAKOWSKI, J.P. (1987). Inhibition of guinea-pig ileum contractions mediated by a class of histamine receptor resembling the H₃ subtype. *J. Pharmacol. Exp. Ther.*, **243**, 874–880.
- VAN DER WERF, J.F., BAST, A., BIJLOO, G.J., VAN DER VLIET, A. & TIMMERMAN, H. (1987). HA autoreceptor assay with superfused slices of rat brain cortex and electrical stimulation. *Eur. J. Pharmacol.*, **138**, 199–206.
- WEIR, S.W. & WESTON, A.H. (1986). The effect of BRL 34915 and nicorandil on electrical and mechanical activity and on ⁸⁶Rb efflux in rat blood vessels. *Br. J. Pharmacol.*, **88**, 121–128.
- YAMANAKA, K. & KITAMURA, K. (1987). Electrophysiological and mechanical characteristics of histamine receptors in smooth muscle cells of the guinea-pig ileum. *Eur. J. Pharmacol.*, **144**, 29–37.

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