ELSEVIER

European Journal of Pharmacology 327 (1997) 49–56

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Histamine H_3 receptors do not modulate reflex-evoked peristaltic motility in the isolated guinea-pig ileum

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Received 19 February 1997; accepted 14 March 1997

Abstract

We investigated the role played by histamine H_3 receptors in the control of intestinal peristalsis, using two different in vitro preparations of guinea-pig ileum. (a) Ileal segments were perfused from the oral end, inducing peristaltic movements (emptying waves), due to the activation of intramural reflexes. Such peristaltic motility was measured as changes in the perfusion pressure during the emptying phase and the threshold pressure for triggering the emptying wave was determined. (b) Ileal segments were mounted horizontally and circular muscle contraction evoked by the ascending peristaltic reflex was triggered by caudal distension of the intestinal wall. In perfused ileal segments, specific agonists acting at histamine H_3 receptors, ((*R*)-α-methylhistamine and immepip, 1 nmol–10 μmol/l), did not cause any change in the threshold pressure for triggering the peristaltic wave, or in the rise of the perfusion pressure during the emptying phase. Similarly, circular muscle contractions evoked by caudal distension of the wall were not affected by these histamine H_3 receptor agonists up to 10 μmol/l. In the same conditions, a complete inhibition of peristaltic movements was elicited by agonists acting at α_2-adrenoceptors or adenosine A_1 receptors (compound UK 14,304 and N^6-cyclopentyladenosine, respectively), their effects being prevented by the respective receptor antagonists, idazoxan and 8-cyclopentyl-1,3-dimethyl-xanthine. These data demonstrate that, contrary to α_2-adrenoceptors and adenosine A_1 receptors, histamine H_3 receptors are not primarily involved in the modulation of intramural reflexes that modulate the peristaltic motility of the isolated guinea-pig ileum.

Keywords: Histamine H_3 receptor; Prejunctional receptor; Intramural reflex; Peristalsis; Circular muscle; Ileum, guinea pig

1. Introduction

After the earlier demonstration that histamine H_3 receptors occur at the cholinergic nerve endings of the gut (Trzeciakowski, 1987), it became evident that this prejunctionally located receptor system controls in a negative fashion the release of acetylcholine and of other neurotransmitters from the myenteric plexus (Schwartz et al., 1990; Hew et al., 1990; Coruzzi et al., 1991; Leurs et al., 1991; Poli et al., 1991, 1994). The activation of such receptors blunts the exocytotic release from excitatory neurones innervating the longitudinal muscle of the guinea-pig small intestine (Poli et al., 1991, 1993; Coruzzi et al., 1991; Leurs et al., 1991), with a mechanism resembling that of α_2-adrenoceptors and possibly consistent with an inhibition of N-type Ca^{2+} channels (Poli et al., 1994).

On the basis of these studies, which utilised the electrically evoked contraction of the longitudinal muscle or the release of [^{3}H]choline from the myenteric plexus, it is not clear whether histamine H_3 receptors are really involved in the control of peristalsis as it occurs under physiologic conditions. It is known, in fact, that peristaltic phenomena mainly depend on the contraction–relaxation cycle of the longitudinal together with the circular muscle layer, rather than on the contractile activity of the longitudinal muscle alone (Kosterlitz and Lees, 1964). Hence, the aims of the present study were (1) to investigate the involvement of histamine H_3 receptors in models of intestinal peristalsis, considering the reflex-evoked contractile activity of the circular muscle; (2) to compare the effects mediated by H_3 receptors with those of other prejunctional (presynaptic) receptor systems, such as α_2-adrenoceptors or adenosine A_1 receptors, which are involved in the control of the peristaltic motility by substances occurring in the neuroimmune system (Burks, 1994).

Preliminary data were presented at the European Histamine Research Society meeting, held in Antwerp, May 1996.

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PII S0014-2999(97)00058-7

2. Materials and methods

2.1. General

Male guinea pigs (400–600 g of body weight) were stunned and exsanguinated. Segments of ileum (30 cm) were removed 5–10 cm above the ileo-caecal junction, and kept in Krebs-Henseleit solution of the following composition (mmol/l): NaCl 113.0; KCl 4.7; $MgSO_4 \cdot 7H_2O$ 1.2; $CaCl_2 \cdot 2H_2O$ 2.5; KH_2PO_4 1.2; $NaHCO_3$ 25.0; glucose 11.3, aerated with 5% CO_2 in oxygen (pH 7.4–7.5). The mesentery was removed and the lumen flushed of its content, avoiding excessive stress of the wall before setting up.

2.2. Peristalsis in luminally perfused ileum

A modification of the classical Bülbring's procedure (Bülbring et al., 1958) was essentially followed (Holzer and Maggi, 1994; Costall et al., 1993). Segments of ileum (7–8 cm) were cannulated in both ends and secured horizontally in a 30 ml organ bath, thermostated at 36°C and filled with Krebs' solution of the above described composition, bubbled with CO_2 (5%) in oxygen. The intestinal segment was flushed for 30 min with prewarmed and oxygenated Krebs' solution, infused from the oral end at a constant rate (1 ml/min) by means of a peristaltic pump. Subsequently, the outflow cannula was kept 4 cm above the fluid in the bath, thus generating hydrostatic pressure in the lumen, which opposed the free emptying of the intestinal segment. The outflow cannula was narrow (1 mm internal diameter), ensuing that regurgitation into the system was minimised, without producing a high resistance to the flow of solution within the segment. The final perfusion rate was kept at a level giving an optimum peristaltic motility (usually from 0.8 to 2 ml/min in different preparations). The solution in the organ chamber was replaced with prewarmed and oxygenated fresh solution every 30 min.

The perfusion pressure was continuously monitored at the oral end of the segment by means of a pressure transducer, connected to a side arm of the inflow cannula. The pressure signal was registered on a pen-writing polygraph. The records typically showed two phases of endoluminal pressure changes: a slow preparatory phase, during which the intestinal segment is gradually filled, and a second rapid phase, where endoluminal pressure rises sharply, due to a highly visible contraction of the circular muscle travelling from the oral to the caudal side of the intestine. The second phase occurs once a threshold triggering pressure is reached (Holzer and Maggi, 1994).

Usually, the differentiated pressure (dp/dt) was also recorded, allowing a correct evaluation of the change from the slow to the fast pressure rise and of the threshold triggering pressure (see below).

The drugs were administered into the organ chamber, i.e., to the serosal surface of the ileum. In several experiments, when histamine H_3 receptor agonists and antagonists were studied, the drugs were simultaneously administered into the perfusion fluid and into the organ chamber, to verify whether negative results (see below) were due to an anomalous tissue diffusion or to a true lack of activity.

Preparations showing visible spontaneous contractions due to the activation of ascending excitatory reflexes were discarded.

2.3. Circular muscle contraction evoked by the ascending peristaltic reflex

A classical procedure was essentially followed (Holzer, 1989). Segments of proximal ileum (4–5 cm) were secured horizontally into an isolated organ chamber (10 ml), by means of a stainless steel bar passed through the lumen. The organ bath was filled with the same solution described above and maintained at 36°C. An inflatable latex balloon, connected to a graduated syringe (1 ml) and filled with distilled water, was inserted into the lumen of the caudal end of the intestine. The balloon was inflated for 2 s every 2 min with a constant volume, causing a mechanical distension of the wall, followed by a contraction of the above circular segment (Holzer, 1989; Costa et al., 1985). For each preparation, the threshold volume to elicit reflex contraction was determined (usually 0.08–0.20 ml).

The serosal layer of the intestinal wall was gently pinned 2 cm above the balloon with a frog heart clip, connected to an isotonic transducer by means of a silk thread. Circular muscle contraction evoked by the aboral distension of the wall was then recorded on a pen-writing polygraph (Model 7070, Basile, Milan, Italy).

When non-cholinergic peristalsis was investigated, preparations were treated with atropine (1 μmol/l) and left to incubate for 30 min. Reflex-evoked contractions were then evoked with larger inflation volumes (0.3–0.4 ml), applied for 4 s every 5 min.

2.4. Electrical field stimulation

In some experiments, circular muscle contraction was induced by electrical field stimulation. Preparations were set up as described above for measuring ascending reflexes and checked for their ability to respond to mechanical distension of the wall. A pair of platinum electrodes was positioned 2 mm apart at the caudal end of the segment and square wave pulses were delivered (0.5 ms, 150 mA, every 20 s) by means of an electronic stimulator fitted with a constant current isolator (Lace, Pisa, Italy). Such electrical field stimulation was applied in the absence of any distension of the intestinal wall.

2.5. Evaluation of drug effect

2.5.1. Perfused ileum

The effects of drugs on peristalsis of the perfused ileum were evaluated as modifications of the triggering pressure

(pressure value at which the peristaltic wave is started) and of the maximum rise in the perfusion pressure during peristaltic wave (stroke). The first parameter was measured as a net increase in the perfusion pressure during the slow phase of peristalsis, starting from the minimum pressure value measured at the end of the stroke and expressed in mmHg. The triggering pressure value was determined in two different ways. Usually, the threshold point could be determined by drawing a vertical line from the start dp/dt increasing to the pressure trace, recorded at a chart speed of 10 mm/s. In other experiments, where chart speed was kept very slow (5 mm/min), the stroke was recorded as a sharp increase of the perfusion pressure. On these conditions, the threshold point could be determined directly on the pressure recording.

The ability of drugs to influence the rapid phase of peristalsis was evaluated as modification in the rise of perfusion pressure during stroke, compared with the pre-drug level of response.

2.5.2. Circular muscle contraction evoked by ascending reflex or by electrical field stimulation

Modifications of the circular muscle contraction by drugs were expressed as percent inhibition, with the pre-drug level of twitch response taken as 100.

2.6. Pre-treatment with histamine H_1 and H_2 receptor antagonists

When the effects mediated by histamine H_3 receptors agonists were investigated, experiments were performed in the presence of the histamine H_1 receptor antagonist mepyramine and of the histamine H_2 receptor antagonist famotidine (1 μmol/l of both), to avoid the activation of these receptors by high concentrations of the histamine H_3 receptor agonist, (*R*)-α-methylhistamine (Endou et al., 1994) and by the nonselective agonist, histamine. It was previously shown that mepyramine (Hew et al., 1990) and famotidine (Coruzzi et al., 1991) were devoid of any effect on intestinal histamine H_3 receptors up to 1 μmol/l.

2.7. Statistics

Data were presented as a mean ± S.E.M. of *n* observations. Student's *t*-test for paired or unpaired observations was applied for comparison of two sets of data. *P* values less than 0.05 were considered statistically significant.

pA_2 values for antagonists were estimated using the Gaddum's equation: $pA_2 = -\log[B] + \log[CR - 1]$, where [B] is the antagonist concentration and CR the concentration ratio at the EC_{50} level of the agonist concen-

Table 1
Effects of different receptor agonists on the peristaltic motility of the perfused ileum

Agonist	Concentration of the agonist	Triggering perfusion pressure (mmHg)	Rise in perfusion pressure during stroke (mmHg)	*n*
No drugs		2.1 ± 0.03	18.5 ± 0.21	6
Histamine [a]	10 nmol/l	2.0 ± 0.05	19.4 ± 0.33	6
	0.1 μmol/l	2.1 ± 0.04	19.1 ± 0.21	5
	1 μmol/l	2.2 ± 0.05	18.2 ± 0.27	6
	10 μmol/l	2.2 ± 0.04	17.5 ± 0.18	6
No drugs		1.8 ± 0.03	15.3 ± 0.17	5
(*R*)-α-Methylhistamine [a]	1 nmol/l	1.8 ± 0.03	15.4 ± 0.15	4
	10 nmol/l	1.9 ± 0.04	15.7 ± 0.09	4
	0.1 μmol/l	1.9 ± 0.04	15.9 ± 0.13	5
	1 μmol/l	1.8 ± 0.06	15.4 ± 0.09	5
	10 μmol/l	2.0 ± 0.06	15.3 ± 0.14	4
No drugs		1.7 ± 0.04	16.5 ± 1.10	4
UK 14,304	1 nmol/l	2.3 ± 0.03	12.3 ± 1.09	4
	3 nmol/l	2.9 ± 0.03 [b]	5.0 ± 0.04 [b]	4
	10 nmol/l	3.6 ± 0.03 [b]	0.0 [c,b]	4
No drugs		2.1 ± 0.03	12.5 ± 0.10	4
N^6-Cyclopentyladenosine	1 nmol/l	2.7 ± 0.05	10.5 ± 0.12	5
	3 nmol/l	3.1 ± 0.02 [b]	8.4 ± 0.06 [b]	4
	10 nmol/l	3.9 ± 0.06 [b]	5.2 ± 0.05 [b]	4
	30 nmol/l	4.0 ± 0.04 [b]	0.0 [c,b]	4

Values are the means ± S.E.M. of *n* observations.
[a] Tested in the presence of histamine H_1 and H_2 receptor antagonists (mepyramine and famotidine 1 μmol/l of both).
[b] Significantly different ($P < 0.05$), compared with the respective 'No drugs' value.
[c] Complete absence of peristaltic waves.

tration–response curve, measured in the presence and absence of antagonist.

2.8. Drugs

The following drugs were used: (*R*)-α-methylhistamine dihydrochloride, N^6-cyclopentyladenosine (free base), UK 14,304 (5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinozalinamide, also known as bromoxidine, free base), 8-cyclopentyl-1,3-dipropylxantine and idazoxan hydrochloride were purchased from RBI (Natick, MA, USA); immepip and clobenpropit (dihydrobromide salts) were synthesised by Prof. H. Timmerman (Vrije Universiteit, Amsterdam, Netherlands); yohimbine hydrochloride, mepyramine maleate (pyrilamine), tetrodotoxin (citrate buffer), histamine dihydrochloride, clonidine hydrochloride and hexamethonium chloride were from Sigma (S. Louis, MO, USA); thioperamide maleate (Tocris Cookson, Bristol, UK); famotidine (free base) was a gift of Sigma Tau (Rome, Italy).

The compound UK 14,304 was dissolved in absolute ethanol to obtain 0.1 mol/l stock solutions; all the other compounds were dissolved in distilled water to obtain 10 mmol/l stock solutions. Further dilutions were made in Krebs solution.

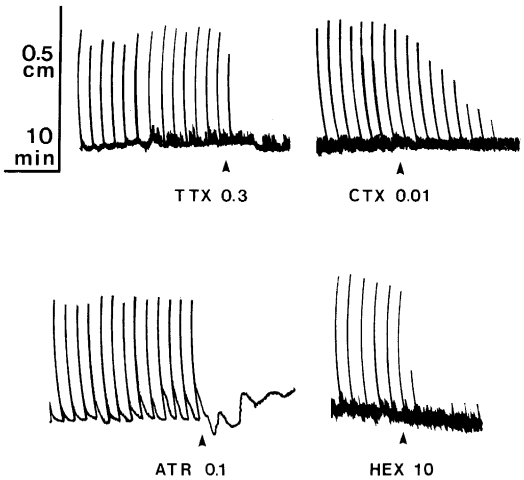

Fig. 2. Inhibitory effects of tetrodotoxin (TTX), ω-conotoxin GVIA (CTX), atropine (ATR) and hexamethonium (HEX) on the cholinergic contractions of the circular muscle, elicited by the ascending peristaltic reflex. Concentrations of drugs are expressed in μmol/l.

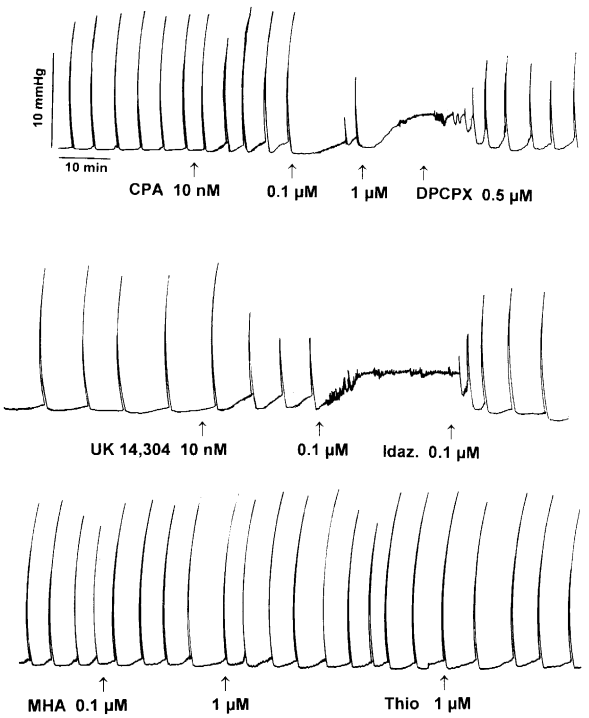

Fig. 1. Original recordings from perfused ileum: effects of different presynaptic receptor agonists and antagonists on the peristaltic motility. Abbreviations: CPA, N^6-cyclopentyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxantine; Idaz., idazoxan; MHA, (*R*)-α-methylhistamine; Thio, thioperamide. Traces refer to three different preparations. Concentrations are expressed in nmol/l (nM) or μmol/l (μM). Experiments with histamine H_3 receptor ligands were performed in the presence of histamine H_1 and H_2 receptor antagonists, mepyramine and famotidine (1 μmol/l of each)

3. Results

3.1. Peristaltic motility in the perfused ileum

The continuous perfusion of the ileum generated peristaltic emptying waves, relatively regular in their amplitude. In the absence of any drug, the rise in perfusion pressure during stroke was 14.5 ± 0.12 mmHg, while the triggering perfusion pressure was 1.8 ± 0.03 mmHg ($n = 37$). No attempt was made to measure frequency, being strongly dependent of the rate of perfusion in the different preparations.

The application of tetrodotoxin (0.3–1 μmol/l), hexamethonium (10–30 μmol/l) or atropine (10 nmol/l) completely prevented the appearance of peristaltic waves, suggesting the involvement of neuronal cholinergic pathways, which contain nicotinic synapses (not shown).

(*R*)-α-Methylhistamine and histamine (both of them in the presence of famotidine and mepyramine 1 μmol/l) did not modify the peristaltic motility up to 10 μmol/l (Fig. 1 and Table 1). Similarly, the histamine H_3 receptor antagonists thioperamide and clobenpropit were ineffective up to 1 and 0.01 μmol/l, respectively (not shown). No differences in the effects of histamine H_3 receptor ligands were observed when these drugs were administered into the perfusion fluid and, simultaneously, into the organ chamber (not shown).

Conversely, a significant rise in the triggering pressure, followed by a complete paralysis of the peristaltic motility

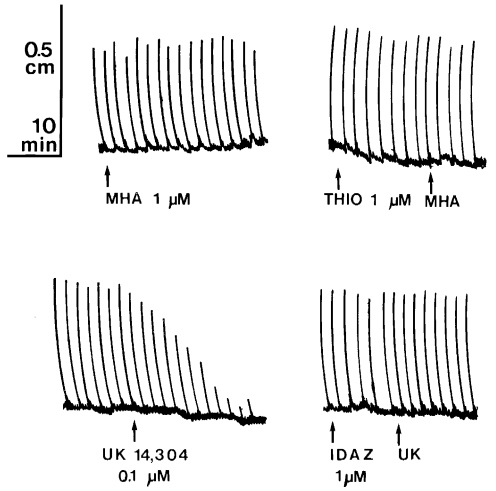

Fig. 3. Effects of (*R*)-α-methylhistamine (MHA) and UK 14,304 (UK), given alone or in the presence of the respective antagonists thioperamide (THIO) and idazoxan (IDAZ), on circular muscle contractions elicited by the ascending peristaltic reflex. Concentration of drugs are expressed in μmol/l (μM).

was seen with agonists acting at α_2-adrenoceptors and adenosine A_1 receptors, like the compound UK 14,304 and N^6-cyclopentyladenosine, respectively (Fig. 1 and Table 1). The effect of each agonist was reversed by the specific receptor antagonists, idazoxan and 8-cyclopentyl-1,3-dipropylxantine, 0.1 and 1 μmol/l, respectively.

Quantitative data relative of the effects of these receptor agonists are presented in Table 1.

3.2. Circular muscle contraction evoked by the ascending peristaltic reflex

The inflation of the balloon (0.08–0.2 ml), inserted into the caudal end of the segment, evoked a phasic contraction of the circular segment above the site of distension. The amplitude of these contractions (0.48 ± 0.05 cm, $n = 31$) was regular for at least 20 cycles. Mechanical responses were almost completely abolished by the neuronal blocker tetrodotoxin, by the ganglion blocker hexamethonium, by the N-type Ca^{2+} channel ω-conotoxin GVIA and by atropine (Fig. 2), suggesting the involvement of intrinsic excitatory nervous circuits, which contain nicotinic synapses and release acetylcholine in a Ca^{2+}-dependent manner.

The application of immepip (not shown) and (*R*)-α-methylhistamine up to 10 μmol/l did not significantly modify either the amplitude (Figs. 3 and 4), or the threshold volume for the activation of mechanical response (not shown). On the contrary, the α_2-adrenoceptor agonist, UK 14,304 (Figs. 3 and 4) and the adenosine A_1 agonist, N^6-cyclopentyladenosine (Fig. 4) caused an almost complete damping of the mechanical response, which was not reversed by raising the inflation volume. The effect of these agonists was antagonised in an apparently competitive manner by the α_2-adrenoceptor and adenosine A_1 receptor antagonists, idazoxan (0.1 μmol/l) and 8-cyclopentyl-1,3-dipropylxantine (1 μmol/l), respectively. These antagonists caused a parallel and surmountable rightward shift of the concentration–response curve of the agonists, as it can be seen in Fig. 4. pA_2 values of idazoxan and 8-cyclopentyl-1,3-dipropylxantine were 8.21 ± 0.14 and 7.97 ± 0.11, respectively.

The selective histamine H_3 receptor antagonist, thioperamide (Arrang et al., 1987), did not significantly affect the concentration–response curve of (*R*)-α-methylhistamine up to 1 μmol/l (Fig. 4).

3.3. Non-cholinergic contractions of the circular muscle

In atropine-treated preparations, non-cholinergic contractile activity could be evoked by larger volumes of

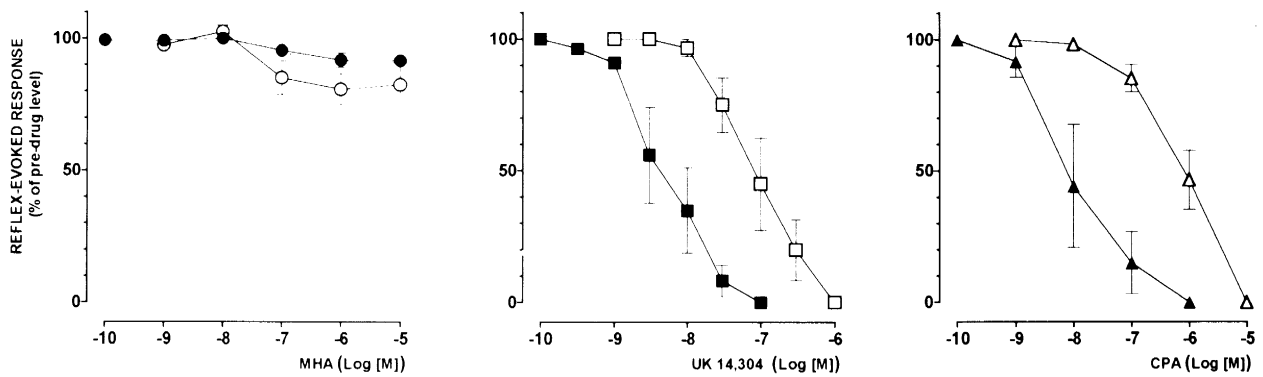

Fig. 4. Effects of (*R*)-α-methylhistamine (MHA), UK 14,304 and N^6-cyclopentyladenosine (CPA) on the cholinergic contractions of the circular muscle, evoked by the ascending peristaltic reflex and antagonism by thioperamide, idazoxan and 8-cyclopentyl-1,3-dipropylxantine. (●) (*R*)-α-methylhistamine control curve; (○) thioperamide 1 μmol/l + (*R*)-α-methylhistamine; (■) UK 14,304 control curve; (□) idazoxan 0.1 μmol/l + UK 14,304; (▲) N^6-cyclopentyladenosine control curve; (△) 8-cyclopentyl-1,3-dipropylxantine 1 μmol/l + N^6-cyclopentyladenosine. Values are the means ± S.E.M. of 3–5 observations. Abscissa: molar concentrations of the agonists; ordinate: percent of tissue response, measured before agonists and taken as 100%.

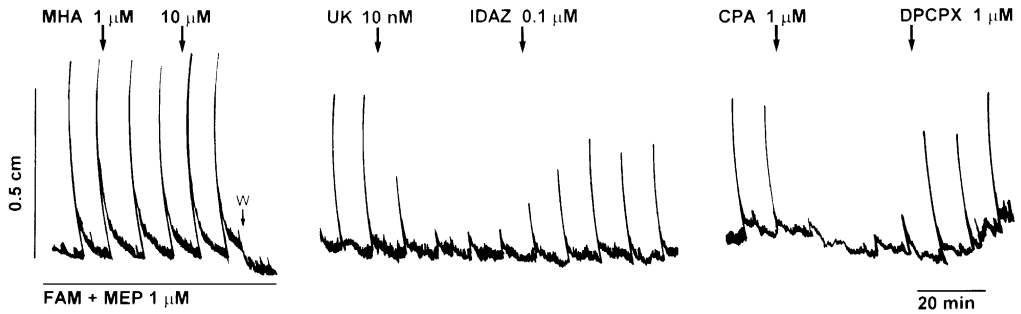

Fig. 5. Non-cholinergic contractions of the circular muscle, evoked by the ascending peristaltic reflex. CPA: N^6-cyclopentyladenosine; DPCPX: 8-cyclopentyl-1,3-dipropylxantine; UK: UK 14,304; IDAZ: idazoxan; MHA: (*R*)-α-methylhistamine; FAM: famotidine; MEP: mepyramine. Experiments were performed in the presence of atropine 1 μmol/l.

inflation (see Section 2). Such activity could be completely prevented by the administration of the α_2-adrenoceptor and adenosine A_1 receptor agonists, UK 14,304 and N^6-cyclopentyladenosine, respectively, but not by (*R*)-α-methylhistamine (Fig. 5), or by other histamine H_3 receptor agonists, such as immepip (not shown). As described above for cholinergic contractions, the inhibitory activity of UK 14,304 and N^6-cyclopentyladenosine was antagonised by idazoxan (0.1 μmol/l) and 8-cyclopentyl-1,3-dipropylxantine (1 μmol/l), respectively (Fig. 5).

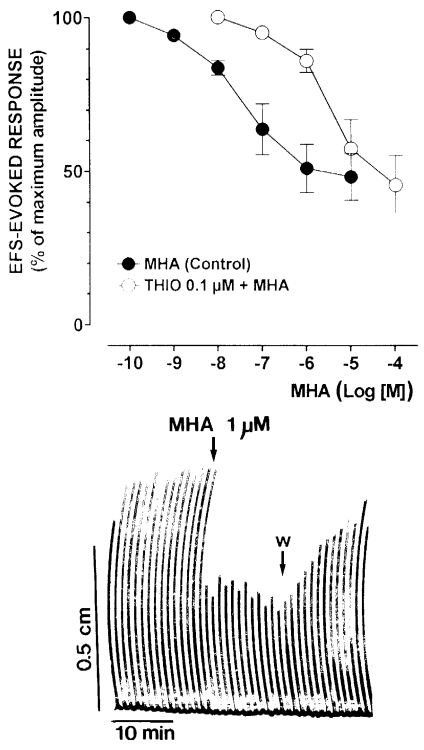

Fig. 6. Electrically evoked responses of the circular muscle of the guinea-pig ileum. Lower panel: original recording of the inhibitory activity of (*R*)-α-methylhistamine (MHA). Concentration expressed in μmol/l (mM). Upper panel: antagonistic activity of thioperamide (THIO) on the inhibitory effect of (*R*)-α-methylhistamine. Values are the mean ± S.E.M. of 4 experiments. Abscissa: molar concentration of (*R*)-α-methylhistamine; ordinate: percent of pre-drug response, taken as 100%.

3.4. Electrical field stimulation-evoked contractions of the circular muscle

When electrical field stimulation was applied to ileal preparations to stimulate circular muscle activity (see Section 2 for details), phasic contractions of regular amplitude (0.64 ± 0.08 cm, $n = 18$) were obtained (Fig. 6, lower panel). Pilot experiments revealed that such contractions were completely prevented by tetrodotoxin (0.3 μmol/l) or atropine (10 nmol/l), but only partially ($32 \pm 5\%$) inhibited by hexamethonium (1 mmol/l) (not shown in figure).

In the presence of mepyramine and famotidine (1 μmol/l), electrically evoked contractions were partially (~ 55%) and concentration-dependently inhibited by immepip (not shown) and by (*R*)-α-methylhistamine, the effects being antagonised by thioperamide (0.1 μmol/l) in a surmountable fashion (Fig. 6, upper panel). The calculated pA_2 value of this antagonist was 8.17 ± 0.05 ($n = 6$). Similarly, the activation of α_2-adrenoceptors and adenosine A_1 receptors by UK 14,304 and N^6-cyclopentyladenosine (1–100 nmol/l), respectively, prevented electrically evoked responses, giving a complete damping of twitch contractions (not shown). The effect of each compound was antagonised by the respective antagonists, idazoxan and 8-cyclopentyl-1,3-dipropylxantine (10–100 nmol/l) (not shown).

4. Discussion

These data apparently rule out a role of histamine H_3 receptors in the control of peristaltic motility of the guinea-pig small intestine. Both the histamine H_3 receptor agonists, (*R*)-α-methylhistamine and immepip (Vollinga et al., 1994), and antagonists, thioperamide and clobenpropit (Arrang et al., 1987; Leurs and Timmerman, 1992), do not modify reflex-evoked muscle contractions in experimental models, which reproduce peristalsis in quasi-physiologic conditions (Holzer, 1989; Costa et al., 1985).

By contrast, the activation of α_2-adrenoceptors and

adenosine A_1 receptors, which are known to participate in the modulation of intestinal peristalsis in physiologic or in pathophysiologic conditions (Burks, 1994; Ruffolo et al., 1993; Christofi and Wood, 1993; Nitahara et al., 1995), induces a complete suppression of peristaltic motility, an effect reversed by the respective antagonists, idazoxan and 8-cyclopentyl-1,3-dipropylxantine. These effects represent a positive control for the modulatory activity of prejunctional receptor systems other than histamine H_3 receptors in the experimental conditions of the present study.

The inability of histamine H_3 receptors to modify the peristaltic motility is surprising, considering that these receptors occur in the guinea-pig small intestine (Trzeciakowski, 1987; Hew et al., 1990; Poli et al., 1991, 1994, 1997; Coruzzi et al., 1991; Leurs et al., 1991) and then could represent the target for exogenous agonists, as well as for endogenous histamine. In fact, histamine is released by parasympathetic nerve stimulation from nerve-supplied mast cells (Bani-Sacchi et al., 1986) and, perhaps, it is taken up and released by enteric neurones (Håkanson et al., 1983; Burks, 1994), even though a direct evidence for a role of histamine as a neurotransmitter at the intestinal level is still lacking. It is evident from negative results with histamine H_3 receptor antagonists that endogenous histamine does not modulate reflex-evoked peristalsis through the activation of histamine H_3 receptors, as mast cells or other histamine-containing cell types are not involved. Obviously, possible effects of endogenous histamine mediated by other histamine receptor subtypes cannot be a priori discarded.

The lack of effect by the activation of histamine H_3 receptors is in evident contrast with results obtained in previous studies, where the electrically evoked longitudinal muscle contraction of the guinea-pig intestine was used as a model to investigate the role of such receptors in the control of the neurotransmission. It was in fact shown that the activation of histamine H_3 receptors by exogenous agonists blunts the neurogenic cholinergic and non-cholinergic contraction of intestinal segments taken from different portions of the intestine (Menkveld and Timmerman, 1990; Leurs et al., 1991; Coruzzi et al., 1991; Taylor and Kilpatrick, 1992) and the release of [^{3}H]choline from the longitudinal muscle-myenteric plexus (Poli et al., 1991). In addition, it was also shown that endogenous histamine, presumably released from stores associated with the myenteric plexus, is able to activate histamine H_3 receptors, producing a modulatory activity of electrical field stimulation-evoked contractions (Coruzzi et al., 1991; Poli et al., 1997) and [^{3}H]choline release (Poli et al., 1991), as revealed by the facilitatory effects of histamine H_3 antagonists, thioperamide and impromidine.

Both distension- and electrically evoked contractions of the gut are due to the activation of post-ganglionic cholinergic or non-cholinergic neurones (Costa et al., 1985; Holzer, 1989; Holzer and Maggi, 1994). Moreover, the release from both kind of neurones is sensitive to ω-conotoxin GVIA, a N-type Ca^{2+}-channel antagonist which is currently employed to investigate the Ca^{2+} dependence of neurosecretory phenomena (Maggi et al., 1988; Allescher et al., 1989; De Luca et al., 1990). Therefore, the inability of histamine H_3 receptors to modulate reflex-evoked responses cannot be the consequence of Ca^{2+}-independent release of neurotransmitters or of the involvement of neurotransmitters from the sensory system as final activators of muscle contractility.

Apparently, electrically evoked (cholinergic) responses and reflex-evoked contractions of the circular layer are due to acetylcholine coming from different pools, differentially susceptible to inhibition by prejunctional histamine H_3 receptors. This difference could be confirmed in segments of ileum, field-stimulated in the absence of distension, where the activation of histamine H_3 receptors by (*R*)-α-methylhistamine or immepip attenuates the cholinergic response of the circular muscle, in a fashion similar to that described for longitudinal muscle (Hew et al., 1990; Leurs et al., 1991). These experiments directly confirm that the pre-treatment of the preparations with mepyramine and famotidine does not affect histamine H_3 receptor-evoked responses, as previously demonstrated in different preparations of the guinea-pig small intestine (Hew et al., 1990; Coruzzi et al., 1991).

The ability of histamine H_3 receptor agonists to influence electrically evoked responses apparently confirms the hypothesis that different pools of neuromediators are mobilised by different stimuli (electrical field stimulation or distension), even though their precise localisation cannot be determined from our experiments. As a consequence, we must also assume that histamine H_3 receptors are heterogeneously distributed on different fibres of the myenteric plexus. Even though such a hypothesis cannot be easily verified, it would confirm the ability of neuromodulators to influence only some components of peristalsis, just as it was shown that endogenous nitric oxide affects the longitudinal muscle contractility, without affecting the circular muscle (Suzuki et al., 1994).

Therefore, a role of histamine H_3 receptors in the regulation of peristalsis cannot be completely excluded, even though the inability to modulate physiological peristalsis (reflex-evoked), but only electrically evoked contractions of the circular muscle, would seem to limit their importance in the global control of intestinal propulsion. This concept must be stressed when considering that the activation of other prejunctional receptors completely suppresses the peristaltic motility in the models used in the present study and in other contexts where longitudinal muscle contractility was investigated (Poli et al., 1994).

In conclusion, the present study confirms that guinea-pig ileum contains histamine H_3 receptors, which modulate electrically evoked responses, but no evidence for a modulation of reflex-evoked responses can be provided. Obviously these data cannot exclude an histamine H_3 receptor-mediated control of intestinal peristalsis at an upper level,

e.g., by histamine H_3 receptors located on extrinsic components of the autonomic nervous system and/or in the central nervous system.

Acknowledgements

We are indebted to D.P. Santicioli and Dr. C.A. Maggi (Menarini Pharmaceuticals, Florence) for their helpful suggestions and technical assistance.

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