ABSTRACT

The role of histamine H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub>-receptors was studied on neural transmission in ascending excitatory pathways of the guinea pig ileum. A two-compartment (oral and anal compartments) bath was used: ascending neural pathways were activated by electrical stimulation in the anal compartment and the resulting contraction of the circular muscle in the oral compartment was recorded. Drugs were applied in the anal compartment and each agonist was evaluated in the presence of the antagonists of the other two receptors. In the presence of cimetidine (10 μM) and thioperamide (1 μM), histamine (0.03–3 μM) depolarized the nerve-mediated contractions (5–70% inhibition, P < .05–.01). The inhibitory effect of histamine was antagonized by mepyramine. At the higher concentrations (10 and 30 μM), histamine elicited contractions of the circular muscle in the oral compartment, and these were abolished by mepyramine (1 μM) and tetrodotoxin (0.6 μM). The H<sub>2</sub> agonists dimaprit (30 and 100 μM) and amphamine (0.1–300 μM) produced small contractions of the circular muscle in the oral compartment. These contractile responses were abolished by tetrodotoxin (0.6 μM) and cimetidine (10 μM). The H<sub>3</sub> agonist R-α-methylhistamine (0.001–1 μM) inhibited (2–58%, P < .05) the nerve-mediated contractions. This inhibitory effect was antagonized by the H<sub>3</sub> antagonist thioperamide. These results indicate that 1) histamine, acting at H<sub>1</sub> receptors, at lower concentrations depresses synaptic transmission, although at higher concentrations activates the enteric excitatory ascending pathway; 2) activation of H<sub>2</sub> receptors by H<sub>2</sub> agonists stimulates the enteric excitatory ascending pathways and 3) activation of H<sub>3</sub> receptors inhibits synaptic transmission.

Histamine is widely distributed within mammalian tissues in both neural and nonneural compartments and there is evidence that in the central nervous system, it has a role as a primary transmitter or neuromodulator (Schwartz et al., 1991). However, histamine is contained in mast cells and basophils in the wall of the intestine (Burks, 1994) and there is evidence that when it is released during hypersensitivity reactions to allergens, it may mediate neural events (Wood, 1992).

Three classes of histamine receptors, H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub> have been identified in vertebrates (Hill, 1990) and all three are present in the guinea pig small intestine (Leurs et al., 1991; Bertaccini and Coruzzi, 1995). Histamine contracts the longitudinal muscle in the guinea pig small intestine. These contractions are mediated by the H<sub>1</sub> receptor subtype, because they can be antagonized by mepyramine (Zavecz and Yellin, 1982; Trzeciakowski, 1987) and are likely to be due to a direct action on the smooth muscle.

There is ample evidence that histamine stimulates the enteric neurons. A tetrodotoxin-sensitive release of acetylcholine via H<sub>2</sub> receptors and of tachykinins has been demonstrated (Barker and Ebersole, 1982; Rubinstein and Cohen, 1985). In addition H<sub>2</sub> agonists have been shown to increase the amplitude of electrically evoked contractions of the longitudinal muscle (Zavecz and Yellin, 1982). Thus it is likely that histamine activates the longitudinal muscle excitatory motor neurons. Histamine also produces a tetrodotoxin-sensitive contraction of the circular muscle in the guinea pig small intestine indicating that it stimulates excitatory motorneurons to the circular muscle (Harry, 1963). Histamine, acting via H<sub>1</sub>- and H<sub>2</sub>-receptors produces slow depolarization of guinea pig myenteric neurons that is associated with increase in the input resistance, augmented excitability and repetitive spike discharge (Nemeth et al., 1984; Tamura and Wood, 1992).

Histamine also produces inhibitory effects in the guinea pig small intestine inhibiting atropine-resistant contractions of the longitudinal muscle elicited by electrical stimulation, in the presence of H<sub>1</sub> and H<sub>2</sub> receptor antagonists (Ambache and Aboo Zar, 1970). Thus receptors other than H<sub>1</sub> and H<sub>2</sub> appear to be involved in this inhibitory effect. As histamine

Received for publication March 12, 1998.

This work was supported by CNR (Rome, Italy).

ABBREVIATIONS: NO, nitric oxide; L-NMMA, N<sub>ω</sub>-monomethyl-L-arginine; DMSO, dimethyl sulfoxide.
in these conditions does not inhibit the contraction produced by exogenous bradykinin (Ambache and Aboo Zar, 1970) is unlikely to inhibit directly the muscle. Thus the inhibitory action is likely to be via enteric neurons. A third class of histamine receptors has been identified. Histamine H3 receptors were originally identified as inhibitory autoreceptors on histamine-containing nerve terminals in rat cerebral cortex (Arrang et al., 1983), but have since been shown to inhibit the release of a variety of neurotransmitters in both central (Schlicker et al., 1988) and peripheral tissues (Ishikawa and Sterelakis, 1987). Hew et al. (1990) have demonstrated the presence of binding sites for H3 ligands in the guinea pig small intestine. In this preparation the H3 receptor agonists act presynaptically or prejunctionally to inhibit cholinergic and non-cholinergic excitatory transmission (Trzeciakowski, 1987; Tamura et al., 1988; Taylor and Kilpatrick, 1992; Bertaccini and Coruzzi, 1995). In addition histamine has a pre synaptic inhibitory action on nicotinic synaptic transmission in the myenteric plexus (Tamura et al., 1988).

Thus there is ample evidence that histamine has multiple sites of action on enteric neurons. To establish the action of histamine on specific enteric neural pathways and the nature of the receptors involved, we have used a preparation of guinea pig small intestine in which ascending nerve pathways can be stimulated electrically to activate synaptically excitatory motor neurons to the circular muscle and the action of drugs on enteric nerve pathway can be studied without interfering with the recording of the smooth muscle contractions (Izzo et al., 1997b). A preliminary account of this work was presented at the XXVIII National Congress of the Italian Pharmacological Society (Izzo et al., 1997a).

Materials and Methods

Male guinea pigs weighing between 250 and 350 g were used. The animals were killed by being stunned and bled via the carotid arteries. Segments (4–6 cm) of ileum were removed and the content of intestine flushed. The segments were placed horizontally in a bath filled with warm (37°C) oxygenated Krebs’ solution (composition in mM: NaCl 119, KCl 4.7, KH2PO4 1.2, NaHCO3 25, MgSO4 1.5, CaCl2 2.5 and glucose 11) and set up as described previously (Izzo et al., 1997b). The mechanical recording of the circular muscle at the oral end was recorded isotonically (load 0.5 g) with a transducer connected to a “Gemini” recording apparatus (Ugo Basile, Comerio VA, Italy). The enteric nerve pathways were activated by electrical field stimulation (10 Hz for 2 sec, 45 mA, 0.5-msec pulse duration) via a pair of platinum electrodes placed around the intestine in the anal compartment, 30 mm from the partition. The distance between the stimulating electrodes and the recording of the circular muscle was 35 mm. To study neural transmission, drugs were applied in the anal compartment. Stable and reproducible contractions were obtained with stimulation every 2.5 min and expressed as percentage of lumen occlusive contraction produced by 10 μM carbachol applied to the recording site. Previous studies have shown that responses evoked by electrical stimulation are abolished by either tetrodotoxin or hexamethonium (Izzo et al., 1997b), added in the anal compartment.

The effect of H1, H2 and H3 histamine agonists (histamine for H1 receptors, dimaprit and amphetamine for H2 receptors and R-α-methylhistamine for H3 receptors) on neural transmission was evaluated by adding the drugs to the anal compartment. Cumulative concentration-effects curves (0.03–5 μM histamine, 0.1–300 μM dimaprit, 0.1–300 μM amphetamine and 0.001–1 μM R-α-methylhistamine, contact time 5 min for each concentration) were constructed. To determine antagonistic activity (pA2), histamine and R-α-methylhistamine were tested 20 min after mepyramine (5, 10, 30 and 100 nM) and thioperamide (30, 100 and 300 nM) respectively. To ensure that histamine only acted on H1 receptors, the H2 receptor antagonist cimetidine (10 μM) and the H3 antagonist thioperamide (1 μM) were present in the anal bath; the effect of dimaprit and amphetamine was observed in the presence of mepyramine (1 μM) and thioperamide (1 μM) and the effect of R-α-methylhistamine was observed in the presence of mepyramine (1 μM) and cimetidine (10 μM). The concentration of antagonists used were selected from previous work (Trzeciakowski, 1987; Hew et al., 1990; Poli et al., 1994). In some experiments the effect of histamine was observed 20 min after L-NNMA (100 μM), naloxone (1 μM) or phenoltamine (1 μM) although the contractile effect of histamine and dimaprit (or amphetamine) was studied 20 min after hexamethonium (100 μM). These concentrations were shown effective in previous papers (Waterman et al., 1992; Yuan et al., 1995; Izzo et al., 1997b). In another set of experiments, to block all synaptic transmission without blocking the action potentials in neurons, the anal compartment was perfused with Krebs with no Ca++ which was replaced by 12 mM Mg++ (Tonini and Costa, 1990).

Statistical analysis. Results are given as mean ± S.E.M. Competitive antagonism was quantified as the ratio of equi-active molar concentrations. These values were estimated at the level of the half-maximal response. Antagonist activity (pA2 value) was estimated with the Schild analysis of these data (Aranlakshana and Schild, 1959). Statistical analysis was carried out with analysis of variance or Student’s t test for paired data. P <.05 was considered significant.

Drugs. Drugs used were: histamine hydrochloride, dimaprit hydrochloride, R-α-methylhistamine hydrochloride, mepyramine hydrochloride, cimetidine, thioperamide hydrochloride, tetrodotoxin (all purchased from RBI, Milan, Italy), carbachol chloride, hexamethonium bromide, naloxone hydrochloride and L-NNMA acetate, phenoltamine hydrochloride (Sigma, Milan, Italy) and amphetamine dihydrobromide (Tocris Cookson, Bristol, UK). The drugs were dissolved in distilled water with the exception of cimetidine which was dissolved in DMSO. All drugs were added in volumes less than 0.1% of the bath volume. DMSO had no effect on the responses studied.

Results

Contractile effect of H1, H2 and H3 agonists. Histamine (10 and 30 μM, but not lower), applied to the anal compartment, with cimetidine and thioperamide in the bath, produced contractions of the circular smooth muscle in the oral compartment with a latency of 15 to 20 sec; the contractile effect of histamine consisted of a series of 5 to 10 phasic contractions superimposed to a small tonic component (fig. 1). The amplitude of the contraction produced by 30 μM histamine was greater than that of 10 μM (table 1). Mepyramine (1 μM) and tetrodotoxin (0.6 μM) or hexamethonium (100 μM) applied to the anal compartment, abolished the histamine-induced contractions (fig. 1; table 1). Replacing the Krebs’ solution in the anal compartment with a Ca++ free (12 mM Mg++ Krebs’ solution) almost abolished the contractile effect of histamine 30 μM (fig. 1).

The H2 agonist dimaprit (30 μM), applied to the anal compartment, in the presence of mepyramine and thioperamide, evoked a small multiphasic contractile response (fig. 1). The initial phasic contraction reached maximum in 15 to 30 sec. The latter phasic contractions were variable and consisted of a series of three to six phasic contractions. A greater concentration of dimaprit (100 μM), produced smaller contractions and 300 μM dimaprit did not produce any contraction. Amphetamine, another H2 agonist, also produced a contractile response but at lower concentrations.
TABLE 1

<table>
<thead>
<tr>
<th>Drugs (µM)</th>
<th>Contraction (%)</th>
<th>n\textsuperscript{e}</th>
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<tbody>
<tr>
<td>Histamine 10</td>
<td>13 ± 4</td>
<td>12</td>
</tr>
<tr>
<td>Histamine 30</td>
<td>17 ± 3</td>
<td>11</td>
</tr>
<tr>
<td>Histamine 30 + mepyramine 1</td>
<td>0 ± 6</td>
<td></td>
</tr>
<tr>
<td>Histamine 30 + tetrodotoxin 0.6</td>
<td>0 ± 4</td>
<td></td>
</tr>
<tr>
<td>Histamine 30 + hexamethonium 100</td>
<td>2 ± 3</td>
<td>4</td>
</tr>
<tr>
<td>Dimaprit 30</td>
<td>5 ± 2</td>
<td>16</td>
</tr>
<tr>
<td>Dimaprit 30 + cimetidine 10</td>
<td>0 ± 6</td>
<td></td>
</tr>
<tr>
<td>Dimaprit 30 + tetrodotoxin 0.6</td>
<td>0 ± 4</td>
<td></td>
</tr>
<tr>
<td>Dimaprit 30 + hexamethonium 100</td>
<td>0 ± 4</td>
<td></td>
</tr>
<tr>
<td>Amthamine 10</td>
<td>5 ± 4</td>
<td>6</td>
</tr>
<tr>
<td>Amthamine 30</td>
<td>5 ± 3</td>
<td>6</td>
</tr>
<tr>
<td>Amthamine 100</td>
<td>8 ± 4</td>
<td>6</td>
</tr>
<tr>
<td>Amthamine 300</td>
<td>8 ± 3</td>
<td>6</td>
</tr>
<tr>
<td>Amthamine 300 + cimetidine 10</td>
<td>0 ± 6</td>
<td></td>
</tr>
<tr>
<td>Amthamine 300 + tetrodotoxin 0.6</td>
<td>0 ± 4</td>
<td></td>
</tr>
<tr>
<td>Amthamine 300 + hexamethonium 100</td>
<td>0 ± 4</td>
<td></td>
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</tbody>
</table>

\textsuperscript{e} Indicates the number of experiments.

The effect of histamine was evaluated in the presence of cimetidine (10 µM) and thioperamide (1 µM), while the effect of the H\textsubscript{2} agonists dimaprit and amthamine was evaluated in the presence of mepyramine (1 µM) and thioperamide (1 µM).

Effect of \textit{H}\textsubscript{1}, \textit{H}\textsubscript{2}, and \textit{H}\textsubscript{3} agonists and antagonists on the ascending excitatory pathways activated by electrical stimulation. Electrical field stimulation in the anal compartment elicited a single phasic contraction of the circular smooth muscle in the oral compartment that lasted about 2 to 3 sec. These contractions were 28 ± 4% of the contraction produced by 10 µM carbachol (n = 92).

Histamine (0.03–3 µM), in the presence of cimetidine and thioperamide, produced a concentration-dependent inhibition of the nerve mediated contraction when applied to the anal compartment (fig. 2). Significant inhibition was achieved with the 1 to 3 µM concentrations (P < .05 at 1 µM and P < .01 at 3 µM). The effect of histamine was competitively prevented by mepyramine (fig. 2) with a pA\textsubscript{2} of 9.14 ± 0.10 (Schild slope: 0.60 ± 0.03). The inhibitory effect of histamine was not modified by L-NMMA (100 µM), naloxone (1 µM) or phentolamine (1 µM) added in the anal compartment before histamine (fig. 3). L-NMMA, naloxone or phentolamine did not modify the nerve-mediated contractions (% variation of control response: +12 ± 6%, +7 ± 10%, +5 ± 4%, n = 6 for each drug tested, P > .2).

The \textit{H}\textsubscript{2} agonist dimaprit (0.1–300 µM), in the presence of mepyramine and thioperamide, produced a significant (P < .01) inhibition of the nerve mediated contraction only at higher concentration (300 µM, fig. 4). This effect was not modified by the \textit{H}\textsubscript{3} antagonist cimetidine (10 µM, fig. 4). By contrast, amthamine (0.1–300 µM), another \textit{H}\textsubscript{2} agonist, was without effect on the nerve mediated contractions (fig. 4).

The potent \textit{H}\textsubscript{3} receptor agonist R-\alpha-methylhistamine (0.001–1 µM) reduced, in a concentration-dependent manner, the nerve mediated contraction when applied to the anal...
compartment (fig. 5). Significant inhibition (P < 0.05) was achieved with the 0.1 to 1 \( \mu M \) concentrations. The inhibitory effect of R-\( \alpha \)-methylhistamine was competitively inhibited by thioperamide, with a \( pA_2 \) of 8.12 ± 0.23 (Schild slope: 0.96 ± 0.19).

When the stimulating electrode was placed 2 mm just anal to the partition, thus bypassing synaptic transmission along the chain of ascending interneurons (Izzo et al., 1997b), histamine, dimaprit or R-\( \alpha \)-methylhistamine had no significant effect, even at the highest concentrations used (fig. 6).

Addition to the anal compartment of any of the receptor antagonists mepyramine (1 \( \mu M \)) cimetidine (10 \( \mu M \)) or thioperamide (1 \( \mu M \)), failed to modify the ascending nerve mediated contractions (% inhibition: mepyramine 0 ± 7, cimetidine 9 ± 8, thioperamide 1 ± 7).

Discussion
Our study shows that activation of histamine receptors modifies transmission in the ascending excitatory pathways.
to the circular muscle in the guinea pig small intestine. These pathways involve at least three neurons (Izzo et al., 1997b). A final excitatory motor neuron to the circular muscle is located in myenteric ganglia in the oral compartment. The motor neurons receive excitatory nicotinic synaptic inputs from the only class of ascending interneurons that form an excitatory chain of neurons (Tonini and Costa, 1990; Brookes et al., 1991a, 1991b; Izzo et al., 1997b). These neurons and their projections have been identified by tracing experiments (Brookes et al., 1990, 1991b). The electrical stimulation in the anal compartment activates this chain of ascending interneurons thus involving at least two neural synapses in the pathway. Both stimulatory or inhibitory effects of histamine agonists were observed, depending of the concentrations used and the type of receptor involved.

Activation of H1 and H2 receptors, by high concentrations of histamine, dimaprit or amphetamine, added to the anal compartment, produced a contractile response of the circular muscle in the oral compartment. Blockade of nerve activity by tetrodotoxin abolished these contractile responses indicating that they are nerve mediated. Blockade of all synaptic transmission in the anal compartment, by replacing calcium with magnesium, significantly reduced the contractile responses to both agonists. Histamine has been shown to depolarize myenteric neurons via both H1 and H2 receptors (Nemeth et al., 1984; Tamura and Wood, 1992) with consequent release of acetylcholine (Rubinstein and Cohen, 1985) and to elicit synaptic potentials in myenteric neurons (Nemeth et al., 1984; Tamura and Wood, 1992). H2-receptor agonists have been shown to enhance the release of acetylcholine from the enteric nerves (Barker and Erbesole, 1982). We have shown that hexamethonium, a nicotinic receptor antagonist, strongly reduced the contractile effect of both H2 agonists, suggesting that these drugs may act mainly by releasing acetylcholine from the presynaptic nerve terminals of ascending interneurons in the chain.

Histamine acting on H1 receptors, dimaprit or R-α-methylhistamine depressed, in a concentration-dependent manner, the ascending excitatory synaptic transmission. Histamine is unlikely to inhibit directly the myenteric neurons as no postsynaptic hyperpolarizing effects have been observed on myenteric neurons (Tamura et al., 1988). In addition in our experiments when the axons of ascending interneurons were stimulated just anal to the partition, thus bypassing synaptic transmission (Izzo et al., 1997b), histamine, dimaprit or R-α-methylhistamine had no effect on the contractile response. Thus, the inhibitory effects of histamine are most likely to be due to inhibition of synaptic transmission along the ascending chain of interneurons.

The inhibitory action of histamine was competitively antagonized by mepyramine and thus is mediated by H1 receptors. The pA2 value for mepyramine correlates well with previous findings (Barker, 1985). There is no evidence in intestinal preparations for presynaptic inhibition mediated by H1 receptors. Thus it is most likely that activation of these receptors releases endogenous substances from presynaptic nerve endings within the myenteric ganglia, which in turn inhibit synaptic transmission. A number of endogenous substances released by histamine may be involved in this inhibition. Opioids are known to inhibit ascending synaptic transmission (Tonini et al., 1992), are contained in enteric neurons (Costa et al., 1996) and are released during peristalsis (Waterman et al., 1992). NO is synthetised in inhibitory motor neurons and descending interneurons in the guinea pig small intestine (Costa et al., 1992). NO inhibits synaptic transmission in myenteric ganglia in the guinea pig ileum (Yuan et al., 1995) and histamine has been shown to release NO in several tissues (Garrison, 1990). Noradrenaline, released from postganglionic sympathetic neurons, acts presynaptically to inhibit acetylcholine release (Wood, 1987). However it is unlikely that histamine inhibits synaptic transmission in the ascending excitatory pathways by releasing NO, opioids or noradrenaline as the NO synthase inhibitor L-NMMA, the opioid antagonist naxalone or the alpha-adrenoceptor antagonist phentolamine failed to modify the inhibitory effect of histamine.

The inhibitory action of dimaprit does not appear to be mediated by H2 receptors as it was not antagonized by cimetidine. Bertaccini and Zappia (1981) showed that dimaprit-induced relaxation of guinea pig duodenum that was not counteracted by cimetidine and other H2 antagonists. In the same way, Leurs et al. (1991) also showed that the dimaprit-induced relaxation of the guinea pig ileum, jejunum and colon was unaffected by a number of H2 antagonists, such as famotidine, mifentidine and tixidione.

The potent and selective H1 receptor agonist R-α-methylhistamine produced a concentration-dependent inhibition of the neural contraction when applied in the anal compartment. This effect was competitively antagonized by the H2 antagonist thioperamide, indicating that was mediated by H3 histamine receptors. The pA2 value for thioperamide is in keeping with previous studies on guinea pig intestine (Leurs et al., 1991; Taylor and Kilpatrick, 1992). The most likely site and mechanism of action of H3 agonists is by presynaptic inhibition along the chain of ascending excitatory interneurons. Presynaptic inhibition mediated by H3-receptors is widespread in central and peripheral nervous system (Schwartz et al., 1991). There is ample evidence for H3 receptors on longitudinal muscle enteric motorneurons to mediate inhibition of transmitter release (Bertaccini and Coruzzi, 1995; Leurs et al., 1991; Taylor and Kilpatrick, 1992; Trzeckiakowski, 1987). Poli et al. (1994) showed that histamine acts on these neurons in the duodenum by decreasing Ca2+ entry via the N-type Ca2+ channels in the nerve terminals. It is possible that the inhibitory effect of R-α-methylhistamine on synaptic transmission demonstrated in our work is due to a similar mechanism as N-type Ca2+ channels are involved in the ascending excitatory pathways (Izzo et al., 1997b). The discrepancy with the findings of Poli and Pozzoli (1997) who have shown that R-α-methylhistamine did not modify either the ascending excitatory reflex elicited by caudal distention or peristaltic movements in the isolated guinea pig ileum remains to be explained.

The failure of the histamine receptor antagonist, mepyramine, cimetidine and thioperamide, to affect transmission along the intestine indicates that endogenous histamine is not involved in neural transmission along the ascending excitatory pathways activated by electrical stimulation. There is no evidence for the presence of histamine in enteric neurons in the guinea pig intestine although histidine decarboxylase, the enzyme involved in the synthesis of histamine and its mRNA are present in enteric neurons (Ekblad et al., 1985). If histamine plays a functional role in intestinal mo-
ility is more likely to originate from nonneural cells in pathophysiological conditions (Wood, 1992).

The presence of inhibitory and excitatory histamine receptors on different populations of enteric neurons acting at different sites suggests that caution must be exerted in the interpretation of the net effects of pharmacological studies on enteric motor functions. Using histamine receptors agonists.

Acknowledgments

The authors thank Bao Nan Chen for her help in the references. This work was supported by CNR, Murst and Enrico and Enrica Sovena Foundation (Roma).

References


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