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Stimulation of formation of adenosine 3′,5′-phosphate by histamine in myenteric ganglia isolated from guinea-pig small intestine

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Abstract

Effects of histamine and related agonists and antagonists on formation of cAMP were determined for enzymatically dissociated ganglia from the myenteric plexus of the guinea-pig small intestine. Formation of cAMP was stimulated by histamine in both dose- and time-dependent manners. The stimulatory action of histamine was suppressed by the histamine H_2 receptor antagonist, cimetidine. The histamine H_1 receptor antagonists, tripelennamine or pyrilamine also suppressed the stimulatory action of histamine, but only at concentrations 3–4 orders higher than required for cimetidine. Formation of cAMP was stimulated dose-dependently by the histamine H_2 receptor agonist, dimaprit. The histamine H_1 receptor agonist, 2-methyl-histamine, also stimulated cAMP production, but required a threshold concentration 4–5 orders higher than dimaprit. We conclude that histamine acts at the histamine H_2 receptor subtype to stimulate adenylate cyclase and the formation of cAMP in myenteric ganglia of the guinea-pig small bowel.

Keywords: Intestine; Ganglion; Enteric nervous system; Myenteric plexus; Histamine; cAMP; Adenylate cyclase

1. Introduction

Histamine is an important signal substance in neuroimmune communication in the intestine. It is stored in intestinal mast cells and released into the extracellular space when the mast cells degranulate in response to antigenic stimulation. When released, histamine becomes a paracrine signal to the enteric nervous system. It signals the enteric nervous system to call-up a specific immune-related neural program of intestinal behavior while simultaneously suppressing the operation of other programs in the nervous system's library. The specific immune-related program is organized to eliminate the threat of a foreign substance or organism to the integrity of the body. The program consists of patterned secretion from the intestinal mucosa in concert with powerful propulsive contractile activity of the musculature that effectively removes the antigenic threat from the lumen of the bowel (Wood, 1991, 1992a,b,1993a,b).

Histamine has two actions on neuronal elements of the enteric microcircuits of the guinea-pig when released from mast cells or applied experimentally. One is at receptors on the neuronal cell bodies of AH/type 2 (after-hyperpolarization/Dogiel morphologic type 2) enteric neurons and consists of long-lasting excitation that mimics slow synaptic excitation (slow EPSP; Nemeth et al., 1984; Frieling et al., 1993). The second is at nicotinic synapses, where it acts at presynaptic inhibitory receptors of the histamine H_3 receptor subtype to suppress synaptic transmission (Tamura et al., 1987; Frieling et al., 1993). These actions are found in the circuitry of both the myenteric and submucous plexuses of the small and large intestine.

Histamine H_2 receptors are the primary mediators of the slow excitatory response to histamine in cell bodies of enteric neurons (Frieling et al., 1993; Nemeth et al., 1984; Tamura and Wood, 1992). The selective histamine H_2 receptor agonist, dimaprit, mimics the excitatory actions of histamine and the H_2 antagonist, cimetidine, blocks them. The hyperexcitability produced by interaction of histamine with the histamine H_2 receptor subtype does not subside with time. All of the slow EPSP-like actions of histamine persist unabated for as long as histamine is present in the bathing media in experiments lasting several hours (Tamura and Wood, 1992).

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Electrophysiological recording from AH/type 2 enteric neurons, that are susceptible to excitation by histamine in the guinea-pig, suggests involvement of adenosine 3′,5′-phosphate (cAMP) in the mechanism of signal transduction for slow synaptic excitation in this group of neurons. Activation of adenylate cyclase by forskolin or application of membrane permeant analogs of cAMP or intracellular injection of cAMP evokes slow EPSP-like responses in the neurons (Frieling et al., 1991; Nemeth et al., 1986; Palmer et al., 1986a; Tack and Wood, 1992), and forskolin elevates cAMP levels in dissociated myenteric ganglia (Xia et al., 1991). Elevation of cAMP in the neurons mimics the slow EPSP-like actions of histamine as well as other slow EPSP mimetics, such as serotonin (Wood and Mayer, 1979), substance P (Katayama and North, 1978), calcitonin gene-related peptide (Palmer et al., 1986b), vasoactive intestinal peptide and bombesin (Zafirov et al., 1985).

Adenosine or selective adenosine A_1 receptor agonists suppress slow EPSPs and the slow EPSP-like actions of both forskolin and histamine, but do not affect the slow EPSP-like effects of intraneuronal injection of cAMP (Palmer et al., 1987a,b; Christofi and Wood, 1993). This suggests that histamine H_2 receptors may be linked to adenylate cyclase in enteric AH/type 2 neurons and that elevation of cAMP may be involved as a second messenger in the process of signal transduction. The aim of the present study was to assess further, the mechanism of signal transduction for histamine in slow excitatory signaling by identifying the effects of histamine and pharmacologically related agents on formation of cAMP in the small intestinal myenteric plexus. An abstract of some of the work has been published (Xia et al., 1992).

2. Materials and methods

Myenteric ganglia were obtained from the small intestine of male albino guinea-pigs (300–400 g) that were killed by stunning and exsanguination. This method of euthanasia was approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee. Enzymatic digestion was used to dissociate the ganglia from the muscle and connective tissue of longitudinal muscle-myenteric plexus preparations. Approximately 600 ganglia were obtained from each animal. Thirty freshly dissociated ganglia were placed in each incubation tube. The ganglia were incubated at 37°C in Krebs solution gassed with 95% O_2/5% CO_2 and buffered at pH 7.4. Histamine and related pharmacological agents were added to the incubation medium for a predetermined time before the experiment was terminated by addition of trichloroacetic acid. The drugs were dissolved in Krebs solution and added as a concentrated stock solution to yield the desired concentration in the incubation tubes. cAMP from each incubation was measured by radioimmunoassay. Specific details of the methods of dissection of longitudinal muscle-myenteric plexus preparations, enzymatic dissociation and harvest of ganglia from the preparations and the assay for cAMP are described in earlier reports (Baidan et al., 1992; Xia et al., 1991).

The cAMP content was normalized to the number of ganglia. An earlier report (Xia et al., 1991) validated expression of cAMP levels in terms of single ganglia rather than normalizing to amounts of protein or DNA. Data are normalized to basal levels in the absence of drugs and expressed as means ± S.E. with each mean representing the number of incubation tubes each of which contained 30 ganglia. Statistical differences among means were determined by one-way analysis of variance with $P < 0.05$ considered significant.

Agents used and sources were: (1) IBMX (3-isobutyl-1-methylxanthine), histamine, pyrilamine, tripelennamine and cimetidine all obtained from Sigma (St. Louis, MO, USA); (2) dimaprit dihydrochloride and 2-methyl-histamine dihydrochloride from Smith Kline and French (Welwyn Garden City, UK).

3. Results

Application of histamine in the incubation medium elevated the level of cAMP in the ganglia. This was unaffected by tetrodotoxin and appeared to be a direct action independent of synaptic influence.

3.1. Time dependence of histamine action

Time dependence of the elevation of cAMP for 0.1 μM histamine was determined over a 30-min period (Fig. 1). Levels of cAMP increased significantly during the first 30 s and thereafter during the first 5 min. The maximum was reached at 5 min, after which the levels progressively declined to about 60% of maximum in the 30-min samples. This established the time of peak stimulation and was the basis for using a 5-min incubation period for the remainder of the study.

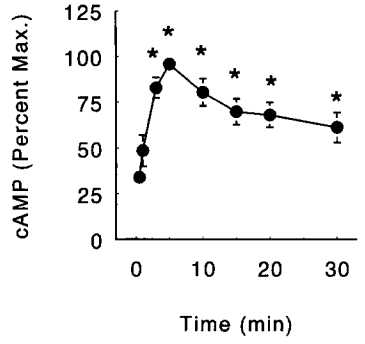

Fig. 1. Time-dependent stimulation of cAMP by 0.1 μM histamine in myenteric ganglia of guinea-pig small intestine. Data are normalized for a basal level of cAMP that was 2.55 ± 0.12 fmol/ganglion after a 5-min incubation and represent means ± S.E. for 8 experiments done in duplicate. * $P < 0.05$ relative to $t = 0$.

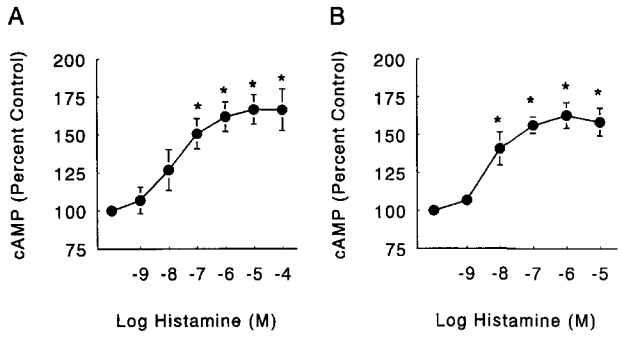

Fig. 2. Dose dependence of stimulation of cAMP formation by histamine in myenteric ganglia of guinea-pig small intestine in the presence (A) and absence of 500 μM IBMX (B). Data are normalized for a basal level of cAMP that was 6.32±1.05 fmol/ganglion for A and 3.33±0.27 fmol/ganglion for B and represent means±S.E. for 9 experiments done in duplicate. * $P < 0.05$ relative to control.

3.2. Histamine dose-response relations

Dose-response relations were determined by incrementing the concentration of histamine in 6 steps over a range of 1.0 nM to 100 μM in the absence of phosphodiesterase inhibition and in the presence of 500 μM IBMX (Fig. 2). The threshold dose in the absence or presence of the phosphodiesterase inhibitor was in the 1-nM range with the EC_{50} occurring between 5 and 20 nM. The maximal response to histamine was unchanged by IBMX.

3.3. Histamine receptor agonists

Application of the selective histamine H_2 receptor agonist, dimaprit, but not the histamine H_1 receptor agonist, 2-methyl-histamine, in the incubation medium elevated the levels of cAMP in the ganglia. Increases in the concentration of dimaprit from 1 nM to 10 μM stimulated cAMP formation dose-dependently with a maximum response similar to that of histamine (Fig. 3). Threshold dose was in the range of 1 nM with an EC_{50} of 10 nM. Application of 2-methyl-histamine in a dose range of 1 nM to 10 μM did not change cAMP production (Fig. 3). However, at a concentration of 100 μM, 2-methyl-histamine did increase the level of cAMP significantly (Fig. 3).

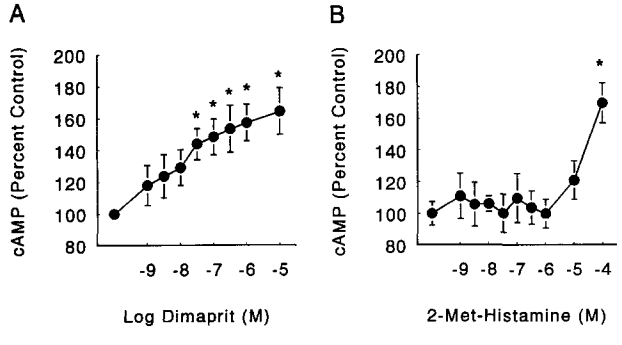

Fig. 3. Dose dependence of stimulation of cAMP formation by dimaprit (A) and 2-methyl-histamine (B) in myenteric ganglia of guinea-pig small intestine. Data are normalized for a basal level of cAMP that was 3.33±0.22 fmol/ganglion for A and 3.34±0.25 fmol/ganglion for B and represent means±S.E. for 8 experiments done in duplicate. * $P <$ 0.05 relative to control.

3.4. Histamine H_2 receptor antagonist, cimetidine

Application of the selective histamine H_2 receptor antagonist, cimetidine, alone in 6 incremented concentrations over a range of 1 nM to 100 μM did not significantly alter the intraganglionic levels of cAMP (Fig. 4). Cimetidine did suppress in dose-dependent manner the stimulation of cAMP formation by histamine. When the intraganglionic levels of cAMP were elevated to 50% above basal by 0.1 μM histamine, cimetidine suppressed the histamine response (Fig. 4). The threshold concentration for this effect was near 10 nM and the EC_{50} between 0.1 and 1 μM. Increasing concentrations of cimetidine in increments of 0.01, 1 and 100 μM resulted in a parallel rightward shift of the histamine dose-response curve (Fig. 4). Analysis of a Schild plot for this data gave a pA_2 value of 7.6.

3.5. Histamine H_1 receptor antagonists, tripelennamine and pyrilamine

Neither of the histamine H_1 receptor antagonists, tripelennamine nor pyrilamine effectively suppressed the stimulating action of histamine on cAMP formation in the ganglia. Application of tripelennamine or pyrilamine alone in 6 incremented concentrations over a range of 1 nM to 10 μM did not significantly alter the intraganglionic levels of cAMP (Fig. 5). The basal levels of cAMP were suppressed significantly when the concentration of tripelen-

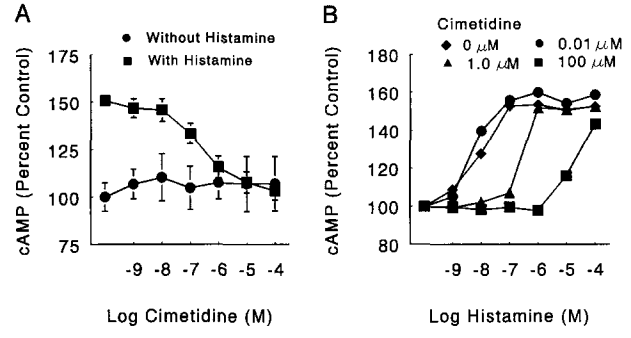

Fig. 4. Suppression by cimetidine of the stimulating action of histamine on cAMP formation in myenteric ganglia of guinea-pig small intestine. (A) Dose-dependent effects of cimetidine on cAMP production in myenteric ganglia of guinea-pig small intestine in the presence and absence of 0.1 μM histamine. (●) cimetidine alone; (■) cimetidine and histamine. Data are normalized for a basal level of cAMP of 3.05±0.23 and represent means±S.E. for 8 experiments done in duplicate. (B) Dose-response curves for histamine in the absence of cimetidine (◆) and in the presence of 0.01 μM cimetidine (●), 1 μM cimetidine (▲) and 100 μM cimetidine (■). Data are normalized for a basal level of cAMP of 2.79±0.16 fmol/ganglion and represent means±S.E. for 9 or 10 experiments done in duplicate.

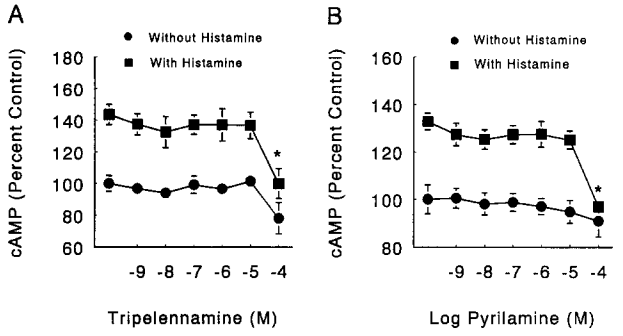

Fig. 5. Dose-dependent effects of tripelennamine or pyrilamine on cAMP production in myenteric ganglia of guinea-pig small intestine in the presence and absence of 0.1 μM histamine. (A) Effects of tripelennamine alone (●); effects of tripelennamine and histamine (■). (B) Effects of pyrilamine alone (●); effects of pyrilamine and histamine (■). Data are normalized for a basal level of cAMP that was 3.14 ± 0.16 fmol/ganglion for A and 3.30 ± 0.20 fmol/ganglion for B and represent means ± S.E. for 8 experiments done in duplicate. * $P < 0.05$ relative to control.

namine, but not pyrilamine, was increased to 100 μM. When the intraganglionic levels of cAMP were elevated above basal by 0.1 μM histamine, neither tripelennamine nor pyrilamine suppressed the histamine response at concentrations of < 10 μM (Fig. 5). Both histamine H_1 receptor antagonists significantly suppressed the histamine response when the antagonist concentration reached 100 μM.

4. Discussion

The stimulating action of histamine on cAMP formation in the myenteric ganglion preparations is consistent with second messenger function in the transduction of the slow EPSP-like actions of histamine seen in electrophysiological studies on myenteric neurons (Frieling et al., 1993; Nemeth et al., 1984; Tamura and Wood, 1992). Results obtained with the selective histamine H_1 and histamine H_2 receptor agonists and antagonists support conclusions from electrophysiological studies that the long-lasting excitatory action of histamine on cell bodies of enteric AH/type 2 neurons is mediated by the histamine H_2 receptor subtype.

All of the results of the present study implicate the histamine H_2 subtype as the receptor responsible for stimulation of cAMP formation in myenteric ganglia. The greater potency of dimaprit relative to 2-methyl-histamine as agonists is consistent with identification of the receptor as the histamine H_2 subtype. Effectiveness of blocking action of cimetidine over pyrilamine or tripelennamine is additional evidence for histamine H_2 receptor involvement. The parallel shifts of the histamine dose-response curves found in the present study with increased concentrations of cimetidine are suggestive of competitive antagonism at the histamine H_2 receptor.

Nemeth et al. (1984), in their electrophysiological studies of histamine action on myenteric neurons of guinea-pig small bowel, found that pyrilamine blocked some of the slow EPSP-like responses to histamine and suggested involvement of histamine H_1 receptors as well as the histamine H_2 receptor subtype. Tamura and Wood (1992) later found that the blocking action of pyrilamine required high concentrations equivalent to those found to suppress cAMP formation by histamine in the present study. The evidence overall suggests that results obtained with pyrilamine and tripelennamine do not identify histamine H_1 receptors on enteric neurons and that histamine H_1 receptors do not contribute to the excitatory actions of histamine on the neurons. This is consistent with findings for the guinea-pig intestine in Ussing chamber studies where the histamine H_2 receptor is responsible for the neurally mediated secretory responses to histamine (Wang and Cooke, 1990) as well as the muscle contractions that are coordinated with the patterned secretory behavior (Cooke et al., 1993).

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