Histamine Suppresses A-Type Potassium Current in Myenteric Neurons from Guinea Pig Small Intestine

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ABSTRACT
Perforated patch-clamp methods for recording ionic currents in the whole-cell configuration were used to test the hypothesis that the ionic mechanisms for the excitatory actions of histamine on enteric neurons include suppression of A-type K⁺ current (IA). Histamine and the selective histamine H₂ receptor agonist, dimaprit, reduced the amplitude of IA without affecting the slope factor for IA steady-state inactivation curves. Suppression of IA was restricted to after hyperpolarization-type myenteric neurons that were immunoreactive for calbindin. The selective histamine H₂ receptor antagonist cimetidine suppressed the action of histamine and dimaprit. Elevation of intraneuronal cAMP by forskolin, a membrane-permeant analog of cAMP, and treatment with a phosphodiesterase inhibitor suppressed IA. The results are consistent with the hypothesis that suppression of IA is part of the ionic mechanism responsible for elevation of excitability during both slow synaptic excitation and slow synaptic excitation-like responses evoked by paracrine mediators, such as histamine, in after hyperpolarization-type myenteric neurons.

Enteric immune/inflammatory cells are putative sources of paracrine signals to the enteric nervous system (ENS). Most is known about signaling between mast cells and the neural elements of the local microcircuits of the ENS. Mast cells contain a variety of preformed chemical mediators, including histamine. They are stimulated by antigens to secrete histamine. Antigen stimulation involves receptors for antibodies on the mast cells. When the receptors are occupied by antibodies to a sensitizing antigen, and cross-linking occurs by interaction of the sensitizing antigen with the bound antibody, the mast cells release histamine. Intestinal mast cells proliferate during infection of the intestine with nematode parasites such as Trichinella spiralis and Nippostrongylus brasiliensis. Animal models infected with these parasites as well as food allergy models using hypersensitivity to milk protein have proved informative in studies on mast cell involvement in enteric immunoneural communication (Frieling et al., 1994a,b). In these models, recognition of the antigen by antibodies bound to the sensitized mast cells triggers release of histamine and other mediators. The mediators then become messengers to the ENS, which responds by suppressing other programs in its library and running a program for intestinal behavior adapted for elimination of the antigen from the lumen. The neural program integrates copious mucosal secretion of H₂O and electrolytes with powerful motor propulsion (Wood, 1993, 1998). In this respect, intestinal mast cells are uniquely equipped and situated to recognize agents that threaten whole body integrity and signal the ENS to program an appropriate defensive response.

Several mast cell-derived mediators share common neuropharmacologic actions on electrical and synaptic behavior of the ENS. These include histamine, 5-hydroxytryptamine (Wood and Mayer, 1978), interleukin-1β, interleukin-6 (Xia et al., 1999), leukotrienes (Frieling et al., 1997), prostaglandins (Dekkers et al., 1997), tumor necrosis factor α (Xia et al., 1995), and platelet-activating factor (Xia et al., 1996b). Histamine, which is the focus of this study, is not localized to any extent in enteric neurons and is not considered as a putative neurotransmitter in ENS microcircuits (Panula et al., 1985). Mast cells are the principal source of histamine in the intestine.

Wood and Mayer (1975) reported histamine-evoked excitation of neurons in the myenteric plexus of cat small intestine. Subsequent work in the Guinea pig found that the excitatory effects of histamine mimic slow excitatory postsynaptic potential (sEPSP) in after hyperpolarization (AH)-type enteric neurons (Nemeth et al., 1984; Tamura and Wood, 1992; Frieling et al., 1993). The changes in electrical behavior during the sEPSP include depolarization of the membrane potential, increase in the electrical resistance of the membrane, and enhanced excitability reflected by repetitive spike discharge.

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ABBREVIATIONS: ENS, enteric nervous system; 4-AP, 4-aminopyridine; IA, A-type K⁺ current; AH, after hyperpolarization; IBMX, 3-isobutyl-1-methylxanthine; sEPSP, slow excitatory postsynaptic potential; CPTcAMP, 8-(4-chlorophenylthio)cAMP.
In addition, long-lasting hyperpolarizing afterpotentials of several seconds duration in AH-type neurons are suppressed to permit repetitive spike discharge. Transduction of slow synaptic signals involves activation of adenylate cyclase and second messenger function of cAMP (Palmer et al., 1986, 1987).

Histamine $H_2$ receptors are the mediators of the slow excitatory response to histamine in cell bodies of enteric neurons in the guinea pig (Nemeth et al., 1984; Tamura and Wood, 1992; Frielings et al., 1993). Exposure to histamine elevates levels of cAMP in myenteric ganglia, and this action is also blocked by selective histamine $H_2$ receptor antagonists and mimicked by selective agonists (Xia et al., 1996a).

The ENS neurons that respond to histamine are known to express multiple K$^+$ channels, including a delayed rectifier, an A-type channel, a Ca$^{2+}$-activated channel, and an inward rectifier (Hirst et al., 1985; North and Tokimasa, 1987; Gallician et al., 1989; Zholos et al., 1999). These channels are collectively responsible for setting resting membrane potential and determining action potential frequency and duration (reviewed by Wood, 1989, 1994). Voltage-activated K$^+$ conductance appears to determine the duration of the action potential in AH neurons because the repolarization phase is prolonged by either 4-aminopyridine (4-AP) or tetraethylammonium (Tamura and Wood, 1989). Stimulation of Ca$^{2+}$-activated K$^+$ current in AH neurons accounts for long-lasting hyperpolarizing afterpotentials that lengthen the refractory period and thereby limit the frequency of spike discharge by the somal membrane (North, 1973; Wood and Mayer, 1978). Conversion from low to high excitability in AH neurons during sEPSPs has been suggested to involve suppression of A-current (Wood, 1989, 1994). Nevertheless, the physiological significance of A-type K$^+$ current ($I_A$) in the neurons of the ENS is not well understood.

The aim of this study was to test the hypothesis that the sEPSP-like actions of histamine on enteric neurons include effects on $I_A$. A preliminary report of the results has been published in abstract form (Starodub et al., 1998).

**Materials and Methods**

**Enteric Neural Cultures.** Short-term myenteric neuronal cultures were used for the patch-clamp studies. Initiation of the cultures was based on established methodology for the adult guinea pig (Hanani et al., 1994). The small intestine was removed from male Hartley albino guinea pigs (300–350 g), sectioned into 5-cm-long pieces, and placed over glass rods to facilitate removal by microdissection of the longitudinal muscle together with the adherent myenteric plexus. Myenteric ganglia were enzymatically dissociated from longitudinal muscle-myenteric plexus preparations as described earlier (Xia et al., 1991). Strips of longitudinal muscle with myenteric plexus attached were placed in an enzyme solution composed of 20 mg of collagenase type IA, 15 mg of protease type IX, and 5 mg of deoxyribonuclease I in 15 ml of Krebs' solution. Digestion and dissociation were allowed to proceed for 15 to 25 min at 37°C in a shaker bath. The digested tissue was washed several times with ice-cold Krebs' solution containing 5% antibiotic-antimycotic mixture, and then transferred into polystyrene Petri dishes (15 mm x 100 mm). Dissociated ganglia were collected with suction pipettes under microscopic control (20× magnification, Wild Heerbrugs M4 stereomicroscope; Wild Heerbrugs, Basel, Switzerland). The ganglia, with no visible smooth muscle present, were transferred into medium 199 supplemented with 15% i-glutamate, 10% heat-inactivated fetal calf serum, 33 mM glucose, 1% Penn-Strep solution (10,000 U penicillin and 10 mg/ml streptomycin), and 0.5% gentamycin. The ganglia were then transferred onto 22 x 22 mm coverslips at the bottom of 33-mm plastic Petri dishes. Each dish received 30 to 50 ganglia. About 50% of the ganglia attached to the surface of the coverslip overnight and were used for patch clamp studies the next day.

All animal procedures were done in accordance with the National Institute of Health guide for the care and use of laboratory animals, and were reviewed and approved by The Ohio State University Laboratory Animal Care and Use Committee. All efforts were made to minimize animal suffering and to reduce the numbers of animals used. No in vivo studies were involved.

**Patch-Clamp Methods.** The coverslips with attached ganglia were washed free of culture medium and placed into a custom made recording cell mounted on the stage of an inverted microscope with Hoffmann modulation contrast optics, epi-fluorescence attachments, and a 35-mm camera (Diaphot 300; Nikon, Tokyo, Japan). The perfused patch configuration (Horn and Marty, 1988) was used to record whole cell currents. Preservation of the intraneuronal milieu was accomplished by permeabilizing the membrane with amphoterin B after gigaseal formation. Serial resistances below 5 MΩ were achieved within 5 to 15 min after stable contact between the cell membrane and pipette tip. No swelling of the cells was ever observed during electrophysiological recording.

Pipettes were fabricated from boro silicate glass capillary tubes (7052; World Precision Instruments, Sarasota, FL) on a Flaming/Brown Model P-97 micropipette puller (Sutter Instruments, San Francisco, CA). Tip resistances were ~1 MΩ. An Ag-AgCl reference electrode was connected to the bath through an agar bridge saturated with KCl solution. Ionic currents were recorded and voltage clamp test pulses were applied with an Axopatch 200 amplifier and Labmaster interfaced to a 486 MHz PC computer with pClamp software (Axon Instruments, Foster City, CA). The experiments were done at room temperature (22–25°C). Averaged data are given as the mean ± S.E. Student's paired t test was used for statistical comparison and differences were accepted as significant for P < .05.

The external bathing solution contained: 120 mM choline Cl, 6 mM KCl, 10 mM MgCl$_2$, 20 mM glucose, and 10 mM HEPES, pH adjusted to 7.3 with KOH. The patch pipettes were filled with: 50 mM KCl, 50 mM K$_2$SO$_4$, 40 mM glucose, 10 mM HEPES (pH adjusted to 7.2 with KOH), and 200 μg/ml amphotericin B. Amphotericin B (Sigma, St. Louis, MO) was initially prepared as a stock solution (500×) in dimethyl sulfoxide and dissolved in the pipette solution immediately before the patch clamp experiments.

**Neuronal Identification.** At the end of each electrophysiological experiment, a map illustrating the position of the neurons was sketched. The neurons were injected with Lucifer yellow from a separate pipette and photographed (Zholos et al., 1999). The presence of calbindin, as a marker for AH/Dogiel morphologic type II neurons, was determined with standard immunohistochemical methods, as described previously for our patch clamp studies (Zholos et al., 1999). Coverslips with the attached ganglia were fixed in a solution of 2% formaldehyde, 0.2% picric acid, and 0.1 M sodium phosphate buffer at pH 7.0 overnight at 4°C. The fixative was removed by three washes in dimethyl sulfoxide (10 min) followed by three washes in phosphate-buffered saline (10 min; pH 7.0). The preparations were then incubated overnight at 37°C with anti-calbindin antibody (monoclonal anticalbindin-D28K; Sigma, St. Louis, MO) diluted 1:150. The antibody complex was visualized as insoluble silicate and dissolved in the pipette solution immediately before the patch clamp experiments.

Histamine, 4-AP, cimetidine, the $H_2$O-soluble form of forskolin, 1,9-dideoxyforskolin, Lucifer yellow, 8-(4-chlorophenylthio) cAMP (CPTcAMP), and 3-isobutyl-1-methylxanthine (IBMX) were obtained from Sigma. Dimaprit was obtained from Research Biochemicals International (Natick, MA). All drugs were dissolved in the external bathing solution and were applied by superfusion.
Results

A-type K⁺ Current. $I_A$ was identified by clamping the voltage at $-50$ mV with the neurons in Ca²⁺- and Na⁺-free external bathing solution. Depolarizing voltage steps from $-40$ to $50$ mV evoked an outward current identified in an earlier study as delayed rectifying K⁺ current (Zholos et al., 1999; Fig. 1A). A series of depolarizing steps from $-40$ to $50$ mV starting at the end of hyperpolarizing prepulses to $-110$ mV resulted in a family of transient outward currents ($I_A$) in addition to the delayed rectifier current (Fig. 1B). The transient outward current traces were separated from the delayed rectifier current by subtracting the traces obtained by depolarizing steps from a holding potential of $-50$ mV from traces obtained after a 1-s hyperpolarizing prepulse to $-110$ mV (Fig. 1C). Unmasking in this manner revealed $I_A$ in both calbindin-positive and -negative neurons.

Sensitivity to 4-AP was used to confirm identification of $I_A$. $I_A$ was activated by stepping the membrane potential to $-20$ mV at the end of a 1-s prepulse to $-110$ mV. The current was suppressed significantly ($P < .05$) in six neurons during application of $10$ mM 4-AP (Fig. 1D).

Histamine. $I_A$ was activated at 15-s intervals by stepping to $-20$ mV after hyperpolarizing preconditions pulses. Application of histamine in the bathing solution reversibly suppressed $I_A$ (Fig. 2, A and B). This occurred in 31 of the 52 neurons studied. Calbindin immunoreactivity was present in 27 of the 31 neurons in which histamine suppressed $I_A$. This indicated that only 2 of the 21 neurons, in which histamine did not suppress $I_A$, expressed immunoreactive calbindin. The majority of the neurons, for which histamine suppressed $I_A$, were presumably AH-type neurons based on the presence of calbindin, which is a generally accepted marker for this kind of myenteric neuron (Iyer et al., 1988). Apparently, the calbindin-negative neurons that did not show effects of histamine were S-type myenteric neurons. Histamine is known not to mimic slow synaptic excitation in S-type neurons (Nemeth et al., 1984; Tamura and Wood, 1992).

Suppression of $I_A$ occurred in a concentration-dependent manner over a range from $10$ nM to $1$ μM (Fig. 2). Histamine (10 nM) suppressed $I_A$ by $22 \pm 8\%$ in seven neurons, $100$ nM suppressed $I_A$ by $31 \pm 5\%$ in nine neurons, and $1$ μM suppressed $I_A$ by $35 \pm 4\%$ in 12 neurons. The effect was fully reversible for histamine concentrations less than $100$ nM and was only partially reversible for larger concentrations. The effect of each concentration of histamine developed within the course of 5 min (Fig. 2A).

Steady-state inactivation of $I_A$ was studied by applying 1-s conditioning pulses to potentials between $-120$ and $-60$ mV followed by activating voltage steps to $-20$ mV (Fig. 3A). The normalized amplitudes of $I_A$ were fit with a Boltzmann equation of the form $II/I_{\text{max}} = (1 + \exp (V - Vm/k))^{-1}$, with $I_{\text{max}}$ being the maximal current amplitude, $Vm$ being the half-inactivation voltage, and $k$ being the slope factor. The average value of the steady-state inactivation curve for $I_A$ was $Vm = -86$ mV with an average slope factor of $k = 11.5$ mV. Histamine did not produce any significant shift in the slope factor for the $I_A$ inactivation curve (Fig. 3B).

Dimaprit, the selective histamine H₁ receptor agonist, suppressed $I_A$ in 21 of 35 neurons (Fig. 4, A and B). Calbindin immunoreactivity was present in 19 of the 21 neurons. Application of progressively larger concentrations of dimaprit produced effects on $I_A$ that were similar to histamine. Suppression of $I_A$ occurred in a concentration-dependent manner over a range from $10$ nM to $1$ μM (Fig. 4, A and B). Dimaprit
(10 nM) suppressed $I_A$ by 22 ± 8% in seven neurons, 100 nM suppressed $I_A$ by 31 ± 5% in nine neurons, and 1 μM suppressed $I_A$ by 35 ± 4% in 12 neurons. The time course for maximum suppression was ~4 min and similar to that for histamine. Steady-state inactivation curves for $I_A$ in the presence of progressively larger concentrations of dimaprit are shown in Fig. 4C. Dimaprit did not shift the slope factor for the inactivation curve.

Concentration-response relations for suppression of $I_A$ by histamine and dimaprit were virtually identical (Fig. 5). The EC$_{50}$ value for histamine suppression of the current was 10 nM; the EC$_{50}$ value for dimaprit was 16 nM. Concentration-response curves for each agonist reached a plateau at 1 μM.

Cimetidine, a selective histamine H$_2$ receptor antagonist, blocked suppression of $I_A$ by histamine in 8 of 10 neurons (Fig. 6A). This action of dimaprit was blocked by cimetidine in five of the six neurons studied.

**Elevation of cAMP.** Several lines of evidence suggest that the histamine H$_2$ receptor on myenteric neurons is a metabotropic receptor coupled by G-proteins to adenylate...
cyclase (Wood et al., 1994). Histamine elevates cAMP in dissociated guinea pig myenteric ganglia, and this effect is potentiated by pretreatment with phosphodiesterase inhibitors (Xia et al., 1996a). Intracellular studies with "sharp" microelectrodes found that elevation of cAMP by histamine or forskolin, or exposure to membrane-permeant analogs of cAMP elevated excitability in myenteric neurons and closely mimicked slow synaptic excitation (Nemeth et al., 1984; Palmer et al., 1986). This led us to test, in a preliminary way, whether elevations of cAMP by forskolin, treatment with IBMX, or exposure to the membrane-permeant analog CPT-cAMP had effects on $I_A$.

Effects of forskolin were studied by activating $I_A$ with a voltage step to $-20 \text{ mV}$ after conditioning prepulse to $-110 \text{ mV}$ in eight neurons. Application of the H$_2$O-soluble active isomer of forskolin (10 $\mu$M), but not the inactive isomer, suppressed $I_A$ by 53 $\pm$ 6% in eight calbindin-positive neurons (Fig. 7). Suppression of the current occurred without significant alteration in the slope factor for steady-state inactivation of $I_A$ (Fig. 7B). Effects of CPT-cAMP and IBMX were studied by activating $I_A$ with a voltage step to $-20 \text{ mV}$ from a holding potential of $-80 \text{ mV}$. Application of 500 $\mu$M CPT-cAMP had similar effects in suppressing $I_A$ in 8 of 11 neurons (Fig. 8A), as did application of 5 $\mu$M IBMX (Fig. 8B).

**Discussion**

Histamine is known to be released from enteric mast cells during type I hypersensitivity responses and to have paracrine effects that mimic slow synaptic excitation in myenteric neurons (Wood, 1993, 1998). The mechanism of its action includes suppression of Ca$^{2+}$-activated K$^+$ conductance (Nemeth et al., 1984; Baidan and Wood, 1993) and activation of Cl$^-$ conductance (Starodub and Wood, 1999, 2000). The repetitive spike discharge that occurs during histamine-evoked excitation in AH neurons was postulated to involve repetitive spike discharge that occurs during histamine-evoked excitation in AH neurons was postulated to involve histamine and dimaprit. These results suggest that the action of histamine on $I_A$ is mediated by the H$_2$ receptor subtype.

The potency of histamine in suppression of $I_A$ is equivalent to its potency in the elevation of cAMP levels in myenteric ganglia, which also involves the histamine H$_2$ receptor subtype (Xia et al., 1996). Our finding that elevation of intraneuronal cAMP appeared to mimic the effects of histamine on $I_A$ is consistent with the evidence that the signal transduction mechanism for activation of histamine H$_2$ receptors on AH-type myenteric neurons involves stimulation of adenylyl cyclase and second messenger function of cAMP. Nevertheless, this conclusion is tentative because the results with forskolin, IBMX, and CPT-cAMP are equivocal until studies are done to show that intraneuronal blockade of protein kinase A suppresses the action of histamine. Work of this nature was beyond the scope of this study. On the other hand, it is known that suppression of adenylyl cyclase by adeno-
sine A₁ receptor agonists prevents elevation of cAMP by histamine in myenteric ganglia (Xia et al., 1997).

Suppression of $I_A$ by histamine is expected to have the effect of contributing to the enhanced sEPSP-like excitability that occurs in AH-type neurons in response to histamine. Augmented excitability in response to histamine is reflected by membrane depolarization, repetitive spike discharge, and increased amplitude and lengthening of fast nicotinic EPSPs.

Steady-state activation and inactivation curves for $I_A$ overlap at membrane potentials of −250 mV in calbindin-positive myenteric neurons (A. Starodub and J. Wood, unpublished data). This suggests that $I_A$ might contribute to the resting potential. If so, suppression of the current could contribute to the depolarization and increased input resistance evoked by histamine.

Action potentials that make-up the repetitive firing seen during both slow synaptic excitation and the sEPSP-like action of histamine are preceded by ramp-like prepotentials (Tamura and Wood, 1992; Wood, 1992; Frieling et al., 1993). The prepotentials fall within the lower range of activation voltage for $I_A$. Consequently, suppression of $I_A$ by histamine would be expected to facilitate the rates of rise of the prepotentials to action potential threshold and thereby contribute to increased frequency of repetitive spike discharge.

Some, but not all, AH-type myenteric neurons receive fast excitatory nicotinic synaptic input (e.g., Grafe et al., 1979; Tamura and Wood, 1989; Wood, 1989). These depolarizing responses extend into the range of activation potentials for $I_A$ where it would be expected to truncate the EPSPs. Suppression of $I_A$ would remove some of the braking action of the outward current and thereby lead to augmentation of the EPSP.

Conclusions

Histamine acts at histamine H₂ receptors to suppress $I_A$ in AH-type myenteric neurons in guinea pig intestine. Suppression of $I_A$ is part of the ionic mechanism responsible for elevation of excitability during slow synaptic excitation and sEPSP-like responses evoked by excitatory paracrine mediators, such as histamine.
References


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