Histamine H₂ Receptor Activated Chloride Conductance in Myenteric Neurons From Guinea Pig Small Intestine

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Starodub, Alexander M. and Jackie D. Wood. Histamine H₂ receptor activated chloride conductance in myenteric neurons from guinea pig small intestine. J. Neurophysiol. 83: 1809–1816, 2000. Whole cell perforated patch-clamp methods were used to investigate ionic mechanisms underlying histamine-evoked excitatory responses in small intestinal AH-type myenteric neurons. When physiological concentrations of Na⁺, Ca²⁺, and Cl⁻ were in the bathing medium, application of histamine significantly increased total conductance as determined by stepping to 50 mV from a holding potential of −30 mV. The current reversed at a membrane potential of −30 ± 5 (SE) mV and current-voltage relations exhibited outward rectification. The reversal potential for the histamine-activated current was unchanged by removal of Na⁺ and Ca²⁺ from the bathing medium. Reduction of Cl⁻ from 155 mM to 55 mM suppressed the current when the neurons were in solutions with depleted Na⁺ and Ca²⁺. Current-voltage curves in solutions with reduced Cl⁻ were linear and the reversal potential was changed from −30 ± 5 mV to 7 ± 4 mV. Niflumic acid, but not anthracene-9-carboxylic acid (9-AC) nor 4,4’-diisothiocyanato-stilbene-2,2’-disulfonic acid (DIDS), suppressed the histamine-activated current. A membrane permeable analogue of cAMP evoked currents similar to those activated by histamine. A selective histamine H₂ receptor agonist (dimaprit) mimicked the action of histamine and a selective histamine H₂ receptor antagonist (cimetidine) blocked the conductance increase evoked by histamine. A selective adenosine A₁ receptor agonist (CCPA) reduced the histamine-activated current and a selective adenosine A₂ receptor antagonist (CPT) reversed the inhibitory action. The results suggest that histamine acts at histamine H₂ receptors to increase Cl⁻ conductance in AH-type enteric neurons. Cyclic AMP appears to be a second messenger in the signal transduction process. Results with a selective adenosine A₁ receptor agonist and antagonist add to existing evidence for co-coupling of inhibitory adenosine A₁ receptors and histamine H₂ receptors to adenylyl cyclase in AH-type enteric neurons.

INTRODUCTION

Slow synaptic excitation (sEPSP) is a receptor-mediated, slowly activating depolarization of the membrane potential found in enteric neurons in the guinea pig small and large intestine and in the gastric antrum, but not the gastric corpus. The depolarizing response is associated with increased input resistance and enhanced excitability that is reflected by repetitive action potential discharge lasting for several seconds (Wood and Mayer 1978). Its occurrence in AH-type enteric neurons is associated with suppression of the long-lasting hyperpolarizing afterpotentials that characterize AH-type enteric neurons in their resting state (Grafe et al. 1980). Putative neurotransmitters and paracrine/endoctrine mediators evoke the response through activation of a common ionic mechanism. Substances that mimic the slow excitatory response include acetylcholine, biogenic amines, and peptides (reviewed by Wood 1994). Receptors for the mediators appear to be linked by G-proteins to a common second messenger system. Classic adenylyl cyclase-cAMP signaling is implicated as the mechanism of signal transduction (Palmer et al. 1986). The underlying ionic conductances responsible for generation of the response are not well characterized. Available evidence suggests that conductance changes include suppression of N-type Ca²⁺ channels, a Ca²⁺ leakage current, Ca²⁺-activated K⁺ channels, and an A-type K⁺ current (reviewed by Wood 1994).

Results obtained from neurons in the guinea pig submucous plexus suggest that in addition to suppression of K⁺ conductances, a cation conductance may be increased by the sEPSP mimetics substance P, muscarine, and 5-HT (Shen and Suprenant 1993). Shen and Suprenant (1993) argued against involvement of Cl⁻ current whereas Bertrand and Galligan (1994) suggested that senktide, a selective neurokinin-3 receptor agonist, activated Cl⁻ current coincident with suppression of K⁺ conductance in guinea pig myenteric neurons.

In this study we used perforated patch-clamp recording methods to investigate activation of Cl⁻ conductance as a complementary mechanism in sEPSP-like responses in cultured AH-type myenteric neurons from guinea pig small intestine. Histamine was selected as the sEPSP mimetic for the study based on its importance in enteric neuroimmune communication involving release from enteric mast cells (Wood 1993, 1998). Release of histamine from mast cells in the guinea pig intestine occurs during Type I hypersensitivity reactions to β-lactoglobulin or Trichinella spiralis antigen (Frielings et al. 1990a,b). The released histamine acts as a paracrine mediator at histamine H₂ receptors to evoke sEPSP-like excitation in the enteric neuronal cell soma. Aside from anaphylaxis, several lines of evidence suggest that intestinal mast cells may be degranulated by input from the CNS during psychogenic stress both in animal models and humans (reviewed by Wood et al. 1999a,b).

A preliminary report of the work in the present paper has appeared in abstract form (Starodub and Wood 1999a).

METHODS

Tissue preparation

Methods used to prepare myenteric neuronal cultures in this study were similar to those described in earlier reports (Xia et al. 1991). The myenteric plexus was enzymatically dissociated from strips of longi-
tudinal muscle by incubation in an enzyme solution containing 20 mg collagenase type IA, 15 mg protease type IX, and 5 mg deoxyribo-
nuclease I in 15 ml Krebs solution for 15–25 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 before collecting the dissociated ganglia. Suction pipettes and a stereomicroscope were used to collect single ganglia. The ganglia, with no visible smooth muscle present, were transferred into medium 199 supplemented with 15% l-glutamate, 10% heat-inactivated fetal calf serum, 33 mM glucose, 1% Penn-Strep solution (10,000 units penicillin and 10 mg/ml streptomycin), and 0.5% gentamicyn. They were transferred onto 22 × 22 mm cover slips at the bottom of 33 mm plastic petri dishes and used for patch-clamp studies the next day.

Solutions

The bathing solution was composed of (in mM) 120 NaCl, 6 KCl, 2.5 CaCl$_2$, 1.2 MgCl$_2$, 20 TEACl, 2 CsCl, 0.2 tetrodotoxin, and 10 HEPES (pH adjusted to 7.3 with NaOH), $\omega$-CgTx-MVIIC (300 μM) was used to suppress Ca$^{2+}$ currents. In cation substitution experiments, Cs$^{+}$ and Na$^+$ were replaced by equimolar Mg$^{2+}$ and choline cations respectively. In some experiments, the Cl$^{-}$ concentration in the bathing solution was reduced to 55 mM by substituting 100 mM sodium salt of D-gluconic acid for 100 mM NaCl. Gluconate anion was used because it does not permeate Cl$^{-}$ channels in other preparations (Halm and Frizzel 1992). The patch pipettes were filled with solution composed of (in mM) 50 CsCl, 50 Cs$_2$SO$_4$, 40 glucose, 10 HEPES (pH adjusted to 7.2 with CsOH), and amphotericin B 200 μg/ml. Amphotericin B was initially prepared as a stock solution in DMSO and dissolved in the pipette solution immediately before the experiments.

Agents used and sources were the following: 3-isobutyl-1-methyl-xanthine (IBMX), histamine, cimetidine, antracene-9-carboxylic acid (9-AC), nifluminic acid, 4,4′-diiisothiocyanostibine-2,2′-disulfonic acid (DIDS), 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate (ct-cAMP), N$^{6}$-2′-O-dibutyryladenosine 3′,5′-cyclic monophosphate (db-cAMP), $\omega$-CgTx-MVIIC, and amphotericin B all obtained from Sigma Biochemicals (St. Louis, MO); dimaprit, 2-chloro-N$^{6}$-cyclopentyl-adenosine (CCPA), and 8-cyclopentyl-1,3-dimethylxan-
thine (CPT) were obtained from Research Biochemicals International (Natick, MA).

Patch-clamp experiments

The cover slips 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 equipped with differential interference contrast optics, epifluorescence attachments, and 35-mm camera (Nikon, Diaphot 300, Tokyo). The perforated 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 amphotericin B after gigaseal formation. The intracellular ion concentrations were estimated as described by Bornstein et al. (1994) and Wood (1994) for criteria used in classification of S- and AH-type enteric neurons. However, we restricted detailed analysis of the histamine-activated ionic currents to calbindin-positive neurons because the currents of interest were found mainly in this neuronal population. Histamine-activated current was found in 81 of 183 calbindin-positive neurons and 3 of 47 calbindin-negative neurons. Calbindin is a well recognized chemical code for Dogiel Type II neurons with AH-type electrophysiologic behavior (Iyer et al. 1988). Therefore calbindin-positive cells are referred to as AH-type neurons in this paper.

Voltage-activated K$^{+}$, Na$^+$, and Ca$^{2+}$ currents were effectively suppressed by the experimental conditions designed to suppress Ca$^{2+}$ currents. In cation substitution experiments, Cs$^{+}$ and Na$^+$ were replaced by equimolar Mg$^{2+}$ and choline cations respectively. In some experiments, the Cl$^{-}$ concentration in the bathing solution was reduced to 55 mM by substituting 100 mM sodium salt of D-gluconic acid for 100 mM NaCl. Gluconate anion was used because it does not permeate Cl$^{-}$ channels in other preparations (Halm and Frizzel 1992). The patch pipettes were filled with solution composed of (in mM) 50 CsCl, 50 Cs$_2$SO$_4$, 40 glucose, 10 HEPES (pH adjusted to 7.2 with CsOH), and amphotericin B 200 μg/ml. Amphotericin B was initially prepared as a stock solution in DMSO and dissolved in the pipette solution immediately before the experiments.

RESULTS

Histamine-activated conductance

Both AH- and S-type myenteric neurons were studied [see Bornstein et al. (1994) and Wood (1994) for criteria used in classification of S- and AH-type enteric neurons]. However, we restricted detailed analysis of the histamine-activated ionic currents to calbindin-positive neurons because the currents of interest were found mainly in this neuronal population. Histamine-activated current was found in 81 of 183 calbindin-positive neurons and 3 of 47 calbindin-negative neurons. Calbindin is a well recognized chemical code for Dogiel Type II neurons with AH-type electrophysiologic behavior (Iyer et al. 1988). Therefore calbindin-positive cells are referred to as AH-type neurons in this paper.

Voltage-activated K$^{+}$, Na$^+$, and Ca$^{2+}$ channel conductance was suppressed while residual current was activated by step depolarizations from a holding potential of −30 mV to 50 mV with step depolarizations repeated at 15-s intervals. A: bath application of 1 μM histamine increased the residual current. B: current before (control) and during exposure to 1 μM histamine. C: histamine-activated current determined by digital subtraction of traces in B.

FIG. 1. Histamine-activated current in a single myenteric neuron. Voltage-activated K$^{+}$, Na$^+$, and Ca$^{2+}$ channel conductance was suppressed while residual current was activated by step depolarizations from a holding potential of −30 mV to 50 mV with step depolarizations repeated at 15-s intervals. A: bath application of 1 μM histamine increased the residual current. B: current before (control) and during exposure to 1 μM histamine. C: histamine-activated current determined by digital subtraction of traces in B.
Histamine-activated $\text{Cl}^-$ current

The histamine-activated current reversed sign at $-30 \pm 5$ mV for the 17 neurons studied. The current and corresponding current-voltage ($I$-$V$) relationship are shown in Fig. 2A. The $I$-$V$ curve exhibited outward rectification and reached saturation at potentials more negative than $-90$ mV. With physiological concentrations of $\text{Ca}^{2+}$ and $\text{Na}^+$ present, this histamine-activated conductance could have been interpreted as either nonspecific cation current (Shen and Suprenant 1993), or $\text{Cl}^-$ current (Bertrand and Galligan 1994). To test the hypothesis that the histamine-activated current was a nonspecific cation current, we removed $\text{Ca}^{2+}$ and $\text{Na}^+$ from the bathing solution by substituting $\text{Mg}^{2+}$ and choline, respectively. In $\text{Ca}^{2+}$- and $\text{Na}^+$-free solution, the current at 50 mV was $12.5 \pm 1.9$ pA pF$^{-1}$ in the 11 of 25 neurons that responded to histamine. Figure 2B is an example of the histamine-activated current and corresponding $I$-$V$ relationship. Elimination of cations from the bathing solution did not change the reversal potential or form of the $I$-$V$ curve but did result in reduction in mean amplitude of the current. This suggested that cations were not the charge carriers for the histamine-evoked current. Nevertheless, $\text{Na}^+$ and $\text{Ca}^{2+}$ could have been indirectly affecting the current because the observed reduction in amplitude was similar to that reported for chloride current in cardiac myocytes (Bahinski et al. 1989; Harvey et al. 1990). After finding that the histamine response remained in $\text{Ca}^{2+}$- and $\text{Na}^+$-free solution, we omitted these cations from the bathing medium for the studies designed to test the second hypothesis that the histamine-activated con-

![Graph A](image_url)

**FIG. 2.** Current-voltage relationship for histamine-activated current in a single neuron. Holding potential was $-30$ mV; command steps ranged from $-110$ mV to 50 mV. Histamine-activated current was determined by digital subtraction of background current as in Fig. 1C. A: in a bathing medium with physiological concentration of $\text{Ca}^{2+}$, $\text{Na}^+$, and $\text{Cl}^-$, the reversal potential was $-30 \pm 5$ (SE) mV for 17 neurons. Outward rectification was apparent at negative membrane potentials. Corresponding current traces are shown in the inset. B: current-voltage relationship for histamine-activated current in $\text{Ca}^{2+}$- and $\text{Na}^+$-free bathing medium. Elimination of $\text{Ca}^{2+}$ and $\text{Na}^+$ from the bathing medium did not change the reversal potential for the histamine-activated current. Corresponding current traces are shown in the inset.

do so (Zholos et al. 1999). The residual current after blockade of voltage-activated $\text{K}^+$, $\text{Na}^+$, and $\text{Ca}^{2+}$ currents remained stable throughout the recording periods. The reversal potential for the control current with $\text{Ca}^{2+}$ and $\text{Na}^+$ present in the bathing medium was $-33.7 \pm 2.5$ mV for 56 neurons. The holding potential was established at $-30$ mV to minimize the background current and eliminate low voltage-activated $\text{Ca}^{2+}$ current, for which no specific blocker has been reported (Spedding and Paoletti 1992). High-voltage-activated (HVA) $\text{Ca}^{2+}$ currents were blocked by $\omega$-CgTx-MVIIC (300 $\mu$M) in the bathing solution. This toxin suppressed different HVA $\text{Ca}^{2+}$ currents (P, Q, and N) in our earlier work on myenteric neurons (Starodub and Wood 1999b). Bath application of histamine (1 mM) evoked a current that was stable in the presence of the agonist throughout the course of the experiments (~30 min). Maximal increase in the total current evoked by stepping the membrane potential from $-30$ to 50 mV amounted to a change from $13.2 \pm 1.7$ to $27.4 \pm 5.3$ pA pF$^{-1}$ in the 17 of 30 neurons tested that responded to histamine (Fig. 1, A and B). The current activated by histamine was determined by digital subtraction of the control current from the current recorded in the presence of the agonist (Fig. 1C).

![Graph B](image_url)

**FIG. 3.** Current-voltage relationship for histamine-activated current in $\text{Ca}^{2+}$- and $\text{Na}^+$-free and reduced $\text{Cl}^-$ bathing medium in a single neuron. Reduction of extracellular $\text{Cl}^-$ concentration to match the predicted intracellular concentration (i.e., ~50 mM) converted the $I$-$V$ curve to a linear relationship. Holding potential was $-30$ mV; command steps ranged from $-110$ mV to 50 mV. Reversal potential was $7 \pm 4$ mV for 7 neurons. Corresponding current traces are shown in the inset.
ductance was a Cl\textsuperscript{−} current. For this, we reduced the concentration of Cl\textsuperscript{2−} in the bathing medium from 155 to 55 mM by substituting Cl\textsuperscript{2−} with gluconate, an anion that is virtually impermeable for Cl\textsuperscript{−} channels in other preparations (Halm and Frizzel 1992). Under our experimental conditions (i.e., perforated patches fully permeable to Cl\textsuperscript{−}) the concentration of Cl\textsuperscript{2−} inside the cell was estimated to be ∼50 mM. In Ca\textsuperscript{2+}- and Na\textsuperscript{+}-free and reduced Cl\textsuperscript{2−} solution, the histamine-activated current was lower in amplitude at 50 mV amounting to 0.7 ± 0.3 pA pF\textsuperscript{−1} in 7 of 20 neurons that responded to histamine (Fig. 3). The current activated at −100 mV remained unchanged at 1.9 ± 0.6 pA pF\textsuperscript{−1} in 7 of 20 neurons. The I-V relationship became linear and intersected the abscissa at 7 ± 4 mV in the 7 responding neurons (Fig. 3). These results suggested that Cl\textsuperscript{−} was the charge carrier for the histamine-activated current. The rectification observed with physiological concentrations of extracellular Cl\textsuperscript{−} was apparently caused by unequal Cl\textsuperscript{−} on opposite sides of the membrane, rather than to intrinsic biophysical properties of the chloride channels.

Niflumic acid inhibition

Another test of the hypothesis for Cl\textsuperscript{−} involvement in histamine-mediated responses was whether the current could be blocked by putative Cl\textsuperscript{−} channel blockers. There are no known specific, high-affinity blockers of Cl\textsuperscript{−} currents (Gogelein 1988); nevertheless, several substances are known to inhibit chloride conductance in different systems including 9-AC in skeletal muscle (Palade and Barchi 1977) and epithelial preparations (Welsh 1984), DIDS in rabbit urinary bladder (Hanrahan et al. 1985), and niflumic acid in guinea pig myenteric neurons (Bertrand and Galligan 1994). In view of the experience of others, we tested all three substances on the histamine-activated current. Bath application of 0.4 mM niflumic acid blocked 85 ± 8% of the histamine-activated Cl\textsuperscript{−} conductance in four neurons, *P < 0.05 (Fig. 4, A and B). On the other hand, 9-AC and DIDS failed to have consistent blocking effects. Because it is known that these inhibitors of Cl\textsuperscript{−} conductance can also affect other transport systems (Gogelein 1988), we preapplied each of the inhibitors before histamine application. In the presence of 9-AC (4 mM), the histamine-activated conductance was not changed significantly at 10.4 ± 2.1 pA pF\textsuperscript{−1} in 4 of 10 neurons (P < 0.05). DIDS (0.5 mM) also did not suppress significantly the Cl\textsuperscript{−} conductance. In DIDS, the histamine-activated current was 11.5 ± 1.8 pA pF\textsuperscript{−1} in the 5 of 10 neurons that responded to histamine (P > 0.05). Preapplication of niflumic acid (0.4 mM) significantly suppressed the
action of histamine, with the histamine-activated conductance averaging $3.3 \pm 0.9 \text{ pA pF}^{-1}$ in 2 of 22 neurons that responded to histamine ($P < 0.05$; Fig. 4C).

Membrane permeable cAMP analogues

Because, our laboratory has measured increased levels of cAMP evoked by activation of histamine H₂ receptors in guinea pig small intestinal myenteric neurons (Xia et al. 1996), we tested the hypothesis that activation of cAMP in the signal transduction cascade. Bath application of a membrane permeable analogue of cAMP (0.5 mM ct-cAMP), alone or in combination with the phosphodiesterase inhibitor IBMX (0.4 mM), evoked currents similar to those activated by histamine. In “sharp” microelectrode studies, ct-cAMP or IBMX mimicked the sEPSP-like actions of histamine (Palmer et al. 1986). The amplitude of the current evoked by ct-cAMP was $7.5 \pm 1.4 \text{ pA pF}^{-1}$ in 11 neurons. The reversal potential and form of the cAMP-activated current were similar to that of the histamine-activated current under similar ionic conditions (Figs. 2 and 5).

Histamine receptor subtype

The excitatory actions of histamine and the associated elevation of intraneuronal cAMP in guinea pig myenteric neurons are known to be mediated by histamine H₂ receptors (Wood 1992; Xia et al. 1996). We used dimaprit, a selective histamine H₂ receptor agonist, to test the suggestion that histamine-evoked changes in Cl⁻ conductance were mediated by histamine H₂ receptors. Bath application of dimaprit (1 μM) evoked a conductance change at 50 mV of $10.2 \pm 1.7 \text{ pA pF}^{-1}$ in 11 of 17 neurons (Fig. 6A). Dimaprit (1 μM) also activated the current in a bathing medium with Cl⁻ reduced to 55 mM. A decrease in outward current without change in inward current amplitude was found when Cl⁻ concentration was reduced outside the neurons. As a result, the current recorded with a ramp clamp protocol was virtually linear and reversed sign at $\sim 0 \text{ mV}$ (Fig. 6B). This supported the suggestion that Cl⁻ is a charge carrier for the dimaprit activated current. Moreover, bath application of 100 μM cimetidine, a selective histamine H₂ receptor antagonist, suppressed the histamine-activated Cl⁻ current by 72 ± 11% in five neurons ($P < 0.05$; Fig. 7).

Adenosine A₁ agonist

Inhibition of adenylate cyclase by action of adenosine at adenosine A₁ receptors is known to have an inhibitory action on histamine-evoked excitation of myenteric AH-type neurons and elevation of intraneuronal cAMP (Tamura et al. 1995; Xia et al. 1997). We tested the hypothesis that activation of adenosine A₁ receptors would also suppress histamine-activated Cl⁻ current. Bath application of 1 μM CCPA, a selective A₁ receptor agonist, reduced the histamine-activated current to $19 \pm 6%$ ($P < 0.05$) of control values in seven neurons. Bath application of 10 μM CPT, a selective A₁ adenosine receptor antagonist, reversed the inhibitory effects from a value of $19 \pm 6$ to $41 \pm 7%$ of control current in the four neurons tested ($P < 0.05$; Fig. 8).
DISCUSSION

Histamine-activated current

The results suggest that activation of Cl\(^-\) conductance is part of the excitatory action of histamine on AH-type myenteric neurons. A histamine-evoked current was found in approximately one-half of the calbindin-positive neurons and in only 5% of calbindin-negative neurons. This was consistent with earlier findings in sharp electrode studies which suggested that ~50% of AH neurons are depolarized by histamine (Nemeth et al. 1984; Tamura and Wood 1992). Whether a small group of S-type neurons (i.e., calbindin-negative neurons) responded to histamine was unclear. The proportion of calbindin-negative neurons in our study was generally the same as reported by others. Iyer et al. (1988) reported that up to 20% of guinea pig enteric neurons with AH-type electrophysiologic behavior did not show calbindin immunoreactivity. If it is assumed that many of the calbindin-negative neurons with responses to histamine were AH neurons, then consideration of the possibility that histamine-activated Cl\(^-\) current is restricted to AH-Dogiel type II myenteric neurons is justified. This would be consistent with observations that activation of adenylate cyclase by forskolin and subsequent elevation of intraneuronal cAMP mimic slow synaptic excitation in guinea pig myenteric neurons with AH- but not S-type electrophysiologic behavior (Palmer et al. 1986).

The histamine-evoked current reversed at a holding potential of approximately ~30 mV. This is the theoretical reversal potential for Cl\(^-\) with our experimental protocol. Evidence of outward rectification was apparent on the I-V curves. On the basis of these results, the histamine-activated current could have been interpreted as either a nonspecific cation current or a specific Cl\(^-\) current. Findings that removal of Na\(^+\) and Ca\(^{2+}\) from the bathing medium did not change either the reversal potential or shapes of I-V curves suggested that neither Na\(^+\) nor Ca\(^{2+}\) were charge carriers for the current. On the other hand, reduction of the Cl\(^-\) concentration in the bathing solution to match the intraneuronal Cl\(^-\) concentration significantly reduced the outward current without changing the inward current. This procedure resulted in a linear I-V relationship and shifted the reversal potential from ~0 to 7 ± 4 mV. These observations implicate Cl\(^-\) as the carrier of the histamine-activated conductance. The outwardly rectifying properties of the current most likely reflected asymmetrical concentrations of Cl\(^-\) across the cell membrane rather than being an intrinsic property of the channel itself. These characteristics of the histamine-activated current in enteric neurons are reminiscent of the cAMP-activated Cl\(^-\) conductance found in cardiac cells (Harvey 1996).

Removal of Ca\(^{2+}\) and Na\(^+\) from the bathing medium resulted in decreased amplitude of the chloride current. Although the cation dependence of the Cl\(^-\) current was not studied in detail, we assumed that it was related to the same factors reported for the cAMP-dependent chloride conductance in myocytes (Hume and Harvey 1991). Sodium ions may influence the current by acting at a regulatory site instead of being a charge carrier. Observations that the reversal potential for the current was independent of Na\(^+\) concentration in the bathing medium is consistent with this interpretation. An alternative possibility was that Ca\(^{2+}\) might alter a Ca\(^{2+}\)-dependent conductance on entering the neuron through unblocked “leakage” Ca\(^{2+}\) channels that have been postulated to influence resting Ca\(^{2+}\)- dependent K\(^+\) conductance in AH-type enteric neurons (Wood 1994). This was ruled out by results showing no effects of removal of Ca\(^{2+}\) from the bathing medium.
Suppression of the histamine-activated current by niflumic acid further supports Cl\(^-\) as the ionic carrier. Niflumic acid is known to suppress Ca\(^{2+}\)-, volume-, and cAMP-activated chloride currents in other preparations (Currie et al. 1995; Hughes and Segawa 1993; Korn et al. 1991; White and Aylwin 1990). Neither DIDS nor 9-AC altered the histamine-activated current in the myenteric neurons. This was consistent with the observation of Shen and Suprenant (1993) that currents activated by substance P, muscarine, or serotonin in submucous neurons were resistant to 9-AC.

The results with selective histamine H\(_2\) receptor agonists and antagonists suggest that the histamine H\(_2\) receptor mediates the action of histamine on the Cl\(^-\) channels. Application of membrane permeable cAMP analogues or dimaprit evoked currents that had similar I-V relationships to the histamine-activated current. Like histamine, the actions of both dimaprit and cAMP analogues were independent of cation composition of the bathing medium and were altered by reduction in the extracellular Cl\(^-\) concentration. The results supplement existing evidence that histamine acts at the histamine H\(_2\) receptor subtype on enteric neurons of the guinea pig to elevate intracellular levels of cAMP and trigger the cascade of events leading to sEPSP-like responses (Palmer et al. 1987a; Xia et al. 1996).

Our findings that a selective adenosine A\(_1\) receptor agonist blocked the stimulatory action of histamine on the Cl\(^-\) current is consistent with earlier reports that selective A\(_1\) receptor agonists suppress histaminergic stimulation of cAMP formation in guinea pig myenteric ganglia (Xia et al. 1997). They conform also to an earlier model for the presence of subtypes of inhibitory P\(_1\) purinoreceptors on myenteric neurons (Christofi and Wood 1994).

Our results are inconsistent with the conclusions of Bertrand and Galligan (1995) that elevation of intraneuronal cAMP is not associated with activation of Cl\(^-\) channels in guinea pig myenteric neurons. Bertrand and Galligan based their conclusion on results obtained with single sharp electrode voltage-clamp methods which showed that the reversal potential for forskolin-evoked depolarizing responses approximated the predicted K\(^+\) equilibrium potential. Current-voltage curves for forskolin were reported to be best fit in a majority of their neurons by a one-parameter model suggestive of a pure increase in K\(^+\) conductance. Nevertheless, in 21% of the neurons, analysis with a two-parameter model incorporating changes in both K\(^+\) and Cl\(^-\) conductance produced a significantly better fit for the I-V curves than the one-parameter model.

Two sets of evidence support our conclusion that histamine-induced stimulation of adenylyl cyclase is involved in activation of the histamine-evoked conductance we have interpreted as a Cl\(^-\) current. First, histamine H\(_2\) receptor activation both mimics sEPSP-like responses and elevates cAMP in myenteric ganglia (Nemeth et al. 1986; Xia et al. 1996). Second, activation of adenosine A\(_1\) receptors leads to inhibition of the following: 1) forskolin-evoked sEPSP-like responses (Palmer et al. 1987b); 2) histamine-evoked sEPSP-like responses (Palmer et al. 1987ab); 3) histamine-evoked elevation of cAMP in myenteric ganglia (Xia et al. 1997); and 4) the histamine-evoked increase in conductance we interpret as Cl\(^-\) current.

**Pharmacology of the histamine-activated Cl\(^-\) current**

The slowly activating depolarizing response to histamine H\(_2\) receptor activation in AH neurons is associated with suppression of resting K\(^+\) conductance that is reflected by increased input resistance as determined in sharp microelectrode studies (Nemeth et al. 1984). However, decreased K\(^+\) conductance cannot be the full explanation for the ionic mechanism underlying the depolarization because changes in input resistance are often observed to be biphasic with an increase in input resistance followed by a decrease as membrane depolarization progresses to its peak during the response to histamine. This change suggests that the opening of a second set of conductance channels occurs in concert with closure of K\(^+\) channels as the slowly activating depolarization characteristic of sEPSP-like responses develops.

Our results suggest that the secondary decrease in input resistance, observed with sharp microelectrodes as the membrane potential is progressively depolarized from the resting potential by histaminergic action, could result from activation of Cl\(^-\) conductance. The electrochemical gradient for Cl\(^-\) in enteric neurons is such that activation of Cl\(^-\) conductance leads to depolarization of the membrane potential. The reversal potential for Cl\(^-\) in guinea pig myenteric neurons was estimated by Cherubini and North (1984) to be \(-39\) mV. This predicts that the membrane potential of neurons responding to histamine by opening Cl\(^-\) conductance channels will become depolarized relative to the resting potential. The histamine-activated current could support the depolarized state of the membrane for as long as histamine remained in the surrounding milieu because no inactivation occurred over periods of \(\sim\)30 min. This is reminiscent of findings that the effects of activation of H\(_2\) histamine receptors on AH-type myenteric neurons does not show tachyphylaxis during prolonged exposure to histamine in sharp microelectrode studies (Tamura and Wood 1992). Inward Cl\(^-\) current is much smaller than the outward current in myenteric neurons; nevertheless, a new equilibrium potential will be close to the reversal potential for Cl\(^-\) because background conductance is relatively small in resting AH-type neurons (Baidan et al. 1992). Consequently, activation of the Cl\(^-\) conductance could also be a stabilizing factor that prevents depolarization to higher levels, thereby protecting the neuron from detrimental depolarization.

The histamine-activated current may also contribute to spike repolarization during the action potential in AH-type enteric neurons. With the normal asymmetrical concentrations of Cl\(^-\) across the cell membrane, the outwardly directed histamine-activated Cl\(^-\) conductance is significantly larger than the inward conductance. Therefore under normal physiological conditions, activation of the Cl\(^-\) conductance would be expected to shorten the action potential. Considering that histamine also suppresses A-type K\(^+\) current in myenteric neurons (Starodub et al. 1998), chloride conductance could replace A-current as a significant factor in membrane repolarization during spike discharge. This would be expected to support spike discharge at higher frequencies as occurs during slow synaptic excitation and sEPSP-like responses to paracrine mediators such as histamine.
REFERENCES


