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Histamine Excites Neonatal Rat Sympathetic Preganglionic Neurons In Vitro Via Activation of H1 Receptors

Andrew D. Whyment, Andrew M. Blanks, Kevin Lee, Leo P. Renaud, and David Spanswick. Histamine excites neonatal rat sympathetic preganglionic neurons in vitro via activation of H1 receptors. J Neurophysiol 95: 2492–2500, 2006. First published December 14, 2005; doi:10.1152/jn.01135.2004. The role of histamine in regulating excitability of sympathetic preganglionic neurons (SPNs) and the expression of histamine receptor mRNA in SPNs was investigated using whole-cell patch-clamp electrophysiological recording techniques combined with single-cell reverse transcriptase polymerase chain reaction (RT-PCR) in transverse neonatal rat spinal cord slices. Bath application of histamine (100 μM) or the H1 receptor agonist histamine trifluoromethyl toluidide dimaleate (HTMT; 10 μM) induced membrane depolarization associated with a decrease in membrane conductance in the majority (70%) of SPNs tested, via activation of postsynaptic H1 receptors negatively coupled to one or more unidentified K+ conductances. Histamine and HTMT application also induced or increased the amplitude and/or frequency of membrane potential oscillations in electrotonically coupled SPNs. The H2 receptor agonist dimaprit (10 μM) or the H3 receptor agonist dimaprit (10 μM) were without significant effect on the membrane properties of SPNs. Histamine responses were sensitive to the H1 receptor antagonist triprolidine (10 μM) and the nonselective potassium channel blocker barium (1 mM) but were unaffected by the H2 receptor antagonist tiotidine (10 μM) and the H3 receptor antagonist, clovenpropet (5 μM). Single cell RT-PCR revealed mRNA expression for H1 receptors in 75% of SPNs tested, with no expression of mRNA for H2, H3, or H4 receptors. These data represent the first demonstration of H1 receptor expression in SPNs and suggest that histamine acts to regulate excitability of these neurons via a direct postsynaptic effect on H1 receptors.

INTRODUCTION

Histamine (β-imidazolylethylamine) is an endogenous, biogenic amine that is known to mediate numerous physiological processes (Hough 1988; Schwartz et al. 1991; Wada et al. 1991). Histamine is widely distributed throughout the mammalian CNS (Hill 1990; Schwartz et al. 1991; Yamatodani et al. 1991), with histaminergic axons arising almost exclusively from the tuberomammillary nucleus of the posterior hypothalamus, from which efferent fibers project to almost all areas of the brain (Schwartz et al. 1991; Takada et al. 1987; Wada et al. 1991) and spinal cord (Schwartz et al. 1991; Wahlestedt et al. 1985). Extensive pharmacological and molecular analysis has identified at least four subtypes of histamine receptor (H1–H4), see Haas and Panula 2003). Three of the four histamine receptors that have been identified (H1–H3) are prominently expressed in the CNS in specific cellular compartments (Agulló et al. 1990; Inagaki 1989), whereas the fourth (H4) receptor (Nguyen et al. 2001) is detected predominantly in the periphery, for example, in bone marrow and leukocytes (Liu et al. 2001; Oda and Matsumoto 2001; Shin et al. 2002). Consistent with this diffuse distribution, neuronal histamine is capable of inducing a wide variety of cellular effects via its receptors (Mitsuhashi and Payan 1992).

Histamine is indicated as being involved in modulating numerous autonomic functions. For example, in the periphery, histamine excites neurons in the sympathetic superior cervical ganglion (Christian et al. 1989; Snow and Weinreich 1987) and histamine modulates sympathetic postganglionic synaptic transmission via a presynaptic action at H1 and H3 receptors (Christian and Weinreich 1992). Histamine also excites vagal afferent neurons (Higashi et al. 1982; Leal-Cardoso et al. 1993; Undem and Weinreich 1993; Undem et al. 1993), in part by inhibition of intrinsic potassium conductances (Jafri et al. 1997). In addition to this peripheral role, histamine has also been indicated as regulating autonomic function centrally, including activation of the sympathetic nervous system (see Brown et al. 2001; Yasuda et al. 2004). However, to our knowledge, only supraspinal hypothalamic and brain stem sites, antecedent to the origins of the sympathetic outflow in the spinal cord, have been explored. Descending histaminergic inputs to the spinal cord originating in the tuberomammillary nucleus are well documented (Schwartz et al. 1991; Wahlestedt et al. 1985), although the precise neural compartments targeted and the physiological functions regulated at this level are unclear. To investigate the potential role of histamine in regulating autonomic function at the level of the spinal cord, we used whole-cell patch-clamp electrophysiological recording techniques combined with single-cell reverse transcriptase polymerase chain reaction (RT-PCR) to investigate the effects of histamine on SPNs in the intermediolateral cell column. These neurons are the most important final central site for integration of sympathetic autonomic reflexes and the origins of the sympathetic outflow for control of vascular and visceral function (Coote 1988). Here we report for the first time that histamine acts to directly excite SPNs by engaging postsynaptic H1 receptors, negatively coupled to one or more K+ conductances.

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METHODS

Slice preparation

Electrophysiological recordings were made from transverse thora-
colombar spinal cord slices as described previously (Logan et al. 1996; Pickering et al. 1991). Briefly, Wistar Kyoto rats, ages 6–14
days (either sex), were terminally anesthetized using 4% Enflurane in
O2 (Abbott Laboratories, Queensborough, Kent, UK) and decapitated.
The spinal cord was removed and thoracic sections were cut into
300–400 μm thick slices using a Leica VT1000 S (Leica Microsys-
tems UK, Milton Keynes, United Kingdom). Slices were maintained
in artificial cerebrospinal fluid (aCSF) at room temperature for 1 h
after slicing before experimentation was performed. For recording,
individual slices were held between two grids in a custom-built
channel continuously perfused with aCSF at a rate of 4–10 ml min
1, illuminated from below, and viewed under a dissection microscope.
The aCSF was of the following composition (mM): NaCl, 127, KCl,
1.9, KH2PO4, 1.2, CaCl2, 2.4, MgCl2, 1.3, NaHCO3, 26, d-glucose,
10, equilibrated with 95% O2-5% CO2.

Cell identification

SPNs were identified by their characteristic electrophysiological
properties: a long-duration action potential (5–10 ms) with a shoulder
on the repolarization phase, a large-amplitude (18–30 mV) and
prolonged action potential afterhyperpolarization, and the expression
of inwardly rectifying and transient outwardly rectifying conduc-
tances (Logan et al. 1996; Pickering et al. 1991). The neuronal
morphology was also routinely determined retrospectively with luci-
fer yellow (di-potassium salt, 2 mg ml–1, Sigma) or biocytin (5
mM, Sigma) in the patch pipette solution. Methods for visualizing
filled SPNs have been reported in detail previously (see Pickering
et al. 1991 for Lucifer yellow and Spanswick et al. 1998 for biocytin).

Recordings

Whole cell recordings were performed at room temperature (17–
21°C) from neurons in the intermediolateral cell column with an
Axopatch 1D amplifier (Axon Instruments, Foster City, CA.), using
the blind version of the patch-clamp technique (Pickering et al. 1991).
Patch pipettes were pulled from thin-walled borosilicate glass
(GC150-TF10, Clarke Electromedical, Pangbourne, Berkshire, United
Kingdom) and had resistances of between 3 and 8 MΩ when filled
with intracellular solution of the following composition (mM): potas-
sium gluconate, 130, KCl, 10, MgCl2, 2; CaCl2, 1, EGTA-Na, 1,
Hepes, 10, Na2ATP, 2, and Lucifer yellow, 2 (or biocytin, 5), pH
adjusted to 7.4 with KOH, osmolarity adjusted to 310 mosmol
1 with sucrose.

Series resistance compensation of approximately 70–80% was
applied for whole-cell voltage clamp experiments. Access resistance
ranged between 5 and 25 MΩ. Neuronal input resistances were
measured by injecting small, rectangular-wave, hyperpolarizing cur-
rent pulses of constant amplitude (~10 to ~100 pA) and measuring the
mean amplitude of a minimum of five resulting electrotonic potentials,
in control conditions and in the presence of the test compound.
Recordings were monitored on an oscilloscope (Gould 1602, Gould
Instrument Systems), displayed on a chart recorder (Gould, Easygraf
TA240), and stored on digital audio tapes (Biologic, DTR-1205) for
later off-line analysis. In addition, data were filtered at 2–5 kHz, (1
kHz for voltage clamp data), digitized at 2–10 kHz (Digidata 1322,
Axon Instruments) and stored on a PC running pCLAMP 8.2 data
acquisition software. Analysis of electrophysiological data was car-
rried out using Clamptit 8.2 software (Axon Instruments).

Cell harvest and single-cell RT-PCR

The SPN cytoplasm was gently aspirated under visual control into
a patch-clamp recording electrode. The contents of the electrode were
subsequently dissipated into a microtube and reverse transcribed in a
reaction volume of 10 μl containing 1× first-strand buffer, 0.1 M
DTT, 10 mM DNTP, 1.5 U RNAsin (Promega, Southampton, United
Kingdom), 200 U Superscript II reverse transcriptase (Invitrogen,
Paisley, United Kingdom), and 0.5 ng reverse transcription primer
for 60 min at 42°C. Three prime end amplification (TPEA) was
performed (Richardson et al. 2000). Briefly, the RT primer was
composed of an anchored oligo(dT) primer with a specific 5’ heil
sequence: 5’-GACTGGCAGACCCGCGCTGAGAATTACAGCTC
ATGGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-
3’. Second-
strand cDNA synthesis was initiated by incubation of the first-strand
cDNA with 1 ng of a primer consisting of 5’-AAACCTG
CCAGACCCGCGTCAGAGCCTGTAATGCTTTTTTTTTTTTTTTTTTTTT-
AAAGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
3’ (where N represents C, G, T or A) during PCR amplification for 29 cycles, 10 s annealing (50°C),
2.5 min extension (72°C), and 1 min denaturing (94°C). After
the initial round, further amplification was performed by the addition
of 230 ng heil primer consisting of 5’-ACTGCGAGACCCCGGC-
GTTA’-3’, Samples of amplified cDNA were subjected to hot-lid PCR carried out in a total reaction volume of 25 μl.
Reaction components were as follows: 2.5 μl 10 x PCR buffer, 1 μl
25 μM 5’ primer, 1 μl 25 μM 3’ primer, 1 μl 25 mM MgCl2, 0.5 μl
10 mM DNTP, 12.5 μl 2.6 mM Betaine/26% DMSO, 4.25 μl H2O,
0.25 μl Platinum Taq polymerase (Invitrogen, Paisley, United
Kingdom). Amplifications were carried out on a PTC-225 thermal cy-
cler (Tetrad, MJ Research). Following an initial 4 min denaturing
step (95°C), each PCR cycle consisted of 30 s denaturing (94°C), 30 s
annealing (60°C), and 20 s extension (72°C). After the final cycle,
the reaction was held for 5 min at 72°C. The PCR products were then
separated on an ethidium bromide-stained 2% agarose gel and pho-
tographed. Direct sequencing was performed to confirm the identity
of the amplified products. All gene-specific primers are listed in Table 1.

Statistical analyses

Statistical analysis was performed using Excel 2003 (Microsoft)
and Prism 4 (Graphpad), with all values given as means ± SE.
Statistical significance was determined using 2-tailed Mann-Whitney
U tests. P < 0.05 was taken to indicate statistical significance.

Drugs

The following drugs were used: clobenpropit dihydrobromide (10
μM), dimaprit dihydrochloride (10 μM), histamine (100 μM), HTMT
(10 μM), imetit dihydrobromide (100 nM), tiotidine (5 μM), and
trans-triprolidine hydrochloride (triprolidine, 100 μM), all from To-
ris Cookson, and tetrodotoxin (TTX, 500 nM) from Alomone Labo-
atories, Israel.

Tiotidine was prepared as a stock solution using DMSO (Sigma)
and diluted to the required concentration in aCSF immediately prior
to use. Final DMSO concentrations did not exceed 0.1%, and appropriate
vehicle controls were performed, which were without effect. All other
drugs were made as stock solutions in distilled water. The drugs were
administered to the slice by perfusion from 50-ml syringes arranged in
line with the main aCSF reservoir by a series of three-way valves. The
reported agonist final concentrations represent the concentrations
within the perfusion system and do not take into account dilution
within the recording chamber. Antagonists were applied for ≥10 min
prior to the addition of agonists to ensure complete equilibration
within the recording chamber.

RESULTS

Whole cell recordings from 47 neurons, identified as SPNs
on the basis of their characteristic morphology and electrophysio-
logical properties were included in this study. Morphology
was revealed with either Lucifer yellow or biocytin,
revealing somata located within the lateral horn, medially projecting dendrites, and an axon that coursed toward the ventral horn (see Spanswick et al. 1998). The characteristic electrophysiological profile of an SPN included a relatively long duration (5–10 ms) action potential with a distinct shoulder in the repolarizing phase, large amplitude (18–30 mV) afterhyperpolarization, and transient outward rectification observed as a delay in the return to rest of the voltage response following a series of hyperpolarizing current pulses (Spanswick et al. 1998). The neurons had a mean resting membrane potential of −49.9 ± 5.8 mV and a mean resting input resistance of 523 ± 61 MΩ.

The effects of histamine receptor agonists on SPN

Histamine (100 μM, n = 23), the H1 receptor agonist HTMT dimaleate (10 μM, n = 12), the H2 receptor agonist dimaprit dihydrochloride (10 μM, n = 5), or the H3 receptor agonists imetit dihydrobromide (100 nM, n = 4) were bath applied to the slice by superfusion for 15–120 s. Bath application of histamine induced membrane depolarization in 16/23 SPNs tested (69.6%, Fig. 1Aa). The response was characterized by depolarization of the membrane from a mean resting or holding potential of 48.6 ± 1.9 mV to −44.1 ± 2.2 mV, and a mean peak membrane depolarization of 4.5 ± 0.6 mV (n = 16, Fig. 1B). The histamine-induced membrane depolarization was associated with a concurrent increase in neuronal input resistance from a mean of 462 ± 43 MΩ at rest to 540 ± 41 MΩ in the presence of histamine, amounting to a 16.9 ± 3.0% increase (Figs. 2Aa and 5, Aa and Ac). In the remaining seven cells, no significant changes in either membrane potential or neuronal input resistance were observed.

Application of HTMT induced membrane depolarization in 9/12 cells tested (75%, Fig. 1Ab), from a mean resting value of −49.3 ± 2.0 mV to −45.5 ± 2.4 mV, and a mean peak membrane depolarization of 3.9 ± 0.5 mV (n = 9, Fig. 1B). This depolarization was again associated with a significant increase in neuronal input resistance from a resting value of 418 ± 51 MΩ to 468 ± 59 MΩ, amounting to a 12.1 ± 1.5% increase (Fig. 1B). Dimaprit (n = 5) or imetit (n = 4) had no statistically significant effect on either membrane potential or input resistance (Fig. 1B). All responses persisted in the presence of TTX (500 nM; n = 5) and were, for the main part, fully reversible on wash (Fig. 2Ab).

### Table 1. Gene-specific primers used for single-cell RT-PCR

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene Accession Number</th>
<th>Primer Sequence (+), sense; (−), antisense</th>
<th>Product length, bp</th>
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<tbody>
<tr>
<td>H1 receptor</td>
<td>D12800</td>
<td>(+) 5’-GAACGAAAGAGTTCTCTGCT-3’</td>
<td>202</td>
</tr>
<tr>
<td>H2 receptor</td>
<td>S57565</td>
<td>(−) 5’-GTACGCTCTGTGACAGAGT-3’</td>
<td>221</td>
</tr>
<tr>
<td>H3 receptor</td>
<td>AY009371</td>
<td>(+) 5’-GTACGCTCTGTGACAGAGT-3’</td>
<td>135</td>
</tr>
<tr>
<td>H4 receptor</td>
<td>NM131909</td>
<td>(−) 5’-GTACGCTCTGTGACAGAGT-3’</td>
<td>147</td>
</tr>
<tr>
<td>β-actin</td>
<td>V01217</td>
<td>(+) 5’-CAATGTTGACCACTCGCAT-3’</td>
<td>207</td>
</tr>
<tr>
<td>Intron</td>
<td>see Telenius et al. 1992</td>
<td></td>
<td></td>
</tr>
</tbody>
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The effects of histamine receptor antagonists on agonist-induced responses

Application of triprolidine (10 μM, n = 7), a specific antagonist of the H1 receptor, induced a reversible inhibition of the membrane depolarization induced by histamine (Fig. 2Ac). In the presence of triprolidine, the peak amplitude of histamine-induced membrane depolarization was reduced by 86.7 ± 6.7%, from 4.5 ± 0.6 mV to 0.6 ± 0.4 mV in the presence of the H1 receptor antagonist (P < 0.03, Fig. 2B). A similar effect of triprolidine was observed on histamine-induced changes in input resistance. This amounted to an 84.5 ± 17.4% (P < 0.01) reduction in the associated input resistance change induced by histamine in the presence of triprolidine (16.7 ± 2.6 MΩ increase in control conditions compared with a 2.6 ± 1.1 MΩ increase in the presence of the antagonist, P < 0.03, Fig. 2B).

Conversely, application of the specific H2 receptor antagonist, tiotidine (10 μM, n = 4), had no significant effect on either the membrane depolarization or change in neuronal input resistance induced by histamine (Fig. 2B). Likewise, application of clobenpropit (5 μM, n = 5), a specific antagonist of the H3 receptor, had no significant effect on the membrane depolarization or increase in input resistance induced by histamine (Fig. 2Ad and B).

Ionic mechanism underlying the histamine H1 receptor-mediated depolarization

The H1 receptor-mediated depolarization was associated with an increase in neuronal input resistance. Thus experiments were undertaken to elucidate the ionic mechanism underlying the H1 receptor-mediated depolarization. One likely mechanism underpinning membrane depolarization associated with a reduction in input conductance is closure of one or more resting membrane potassium conductances. Thus the actions of the nonselective potassium channel blocker barium (BaCl2) on the histamine-induced membrane depolarization were examined. Bath application of BaCl2 (1 mM, n = 4), a concentration previously demonstrated to be sufficient to block potassium conductance’s contributing to resting membrane potential in SPN (Spanswick and Renaud, unpublished observations), induced a 3 nV membrane potential depolarization and induced action potential discharge in previously silent neurons, therefore mimicking the effects of histamine. On subsequent injection of hyperpolarizing current to hold the neuron below its
firing threshold in the presence of BaCl₂, application of histamine was without significant effect on either membrane potential or input resistance (Fig. 3 Ab). On wash of BaCl₂, subsequent application of histamine induced membrane depolarization (Fig. 3 Ac), suggesting involvement of one or more K⁺ conductances in histamine-induced depolarization.

**FIG. 1.** SPNs express functional postsynaptic H₁ receptors. Aa: samples of a continuous whole-cell current-clamp recording from an SPN with a resting membrane potential of −47 mV, showing histamine-induced depolarization and concomitant increase in neuronal input resistance, indicated by the increase in amplitude of electrotonic potentials (downward deflections of the records) evoked in response to hyperpolarizing rectangular wave current pulses (not shown). Constant negative current was injected to monitor the change in input resistance at the resting membrane potential of the neuron and to negate the effect of any voltage-dependent channels. The start and end of the constant current injection are marked by ▲ and ▼ respectively. Double slashes indicate a break in the trace of 60 s. Ab: samples of a continuous whole-cell current-clamp recording from another SPN showing membrane depolarization and increased action potential firing rate on application of the H₁ receptor specific agonist, HTMT. The response was fully reversible. B: summary histogram illustrating the profile of histamine receptor-mediated responses evoked in SPN. The change in peak membrane potential (open columns) and neuronal input resistance (filled columns) responses to histamine and the specific histamine-receptor agonists are shown. Error bars represent SE. Numbers above individual bars indicate number of cells tested (n).

**FIG. 2.** Histamine-induced membrane responses are blocked by H₁, but not H₂ or H₃ receptor antagonists. Aa: samples of a continuous whole-cell current-clamp recording from an SPN with a resting membrane potential of −44 mV, showing histamine-induced depolarization and concomitant increase in firing rate. Ab: samples of a continuous whole-cell current-clamp recording from the same neuron shown in Aa in the presence of TTX (500 nM). Application of histamine induced membrane depolarization and an increase in input resistance, which was compared before and during the response to histamine at similar membrane potentials, maintained in the presence of histamine by injection of constant negative current. The start and end of the current injection are marked by ▲ and ▼ respectively. Ac: application of the H₁ receptor specific antagonist triprolidine significantly reduced the histamine-induced depolarization and input resistance change. Ad: the H₂ receptor specific antagonist clobenpropit was without effect on the histamine-induced membrane responses. B: summary histogram illustrating the pharmacological profile of histamine receptor-mediated responses evoked in SPNs. The peak membrane potential (open columns) and neuronal input resistance (filled columns) responses to histamine and the specific histamine-receptor antagonists are shown. Error bars represent SE. Numbers above individual bars indicate number of cells tested (n). (* P < 0.01, ** P < 0.03).
To further clarify the ionic mechanism underlying histamine-induced excitation of SPNs, we performed a series of experiments to identify the reversal potential and thus the ions mediating the response. In voltage clamp at a holding potential of \(-60\) mV, application of histamine \((100\ \mu M, 60\ s, n = 4)\) induced a sustained inward current, with a mean peak amplitude of \(18.7 \pm 3.9\) pA (Fig. 4Aa). Voltage ramps from \(-120\) to \(-60\) mV at a rate of \(10\) mVs\(^{-1}\) were applied in control conditions and at the peak of the histamine-induced response to investigate the reversal potential of the histamine-induced current (Fig. 4Ab). Histamine-induced inward currents had a mean reversal potential of \(-93 \pm 5.9\) mV \((n = 4)\), close to the \(K^+\) reversal potential under our recording conditions (Fig. 4Ac).

FIG. 4. Ionic mechanism underlying histamine-induced membrane responses in SPNs. Aa: Whole cell voltage-clamp recording (Vh \(-60\) mV) showing that application of histamine produced a pronounced inward current. Large downward deflections represent current responses to voltage ramps from \(-120\) to \(-60\) mV \((10\) mVs\(^{-1}\)) shown on a faster time base, superimposed in b. Note that the reversal potential for the histamine-induced current is close to the \(K^+\) reversal potential under our recording conditions. Ac: superimposed samples of a continuous whole cell current-clamp recording showing membrane potential responses of an SPN to a series of depolarizing and hyperpolarizing rectangular-wave current pulses before histamine and in the presence of histamine. Bb: plot of the current-voltage (I-V) relationships before \((\circ)\) and in the presence of histamine \((\bigcirc)\), shown in Ba. Again, note the increase in the slope of the I-V relationship in the presence of histamine indicating an increase in neuronal input resistance, and the reversal potential around \(-90\) mV, close to that for potassium ions under our recording conditions.
Histamine has been indicated as being involved in a range of homeostatic mechanisms that ultimately involve the engagement of the sympathetic nervous system (see Brown et al. 2001; Yasuda et al. 2004), including energy expenditure and cardiovascular control. For example, a number of studies have indicated that histamine regulates A-type potassium currents (Starodub and Wood 2000). However, the prolonged transient outwardly rectifying A-like conductance observed in SPNs in the present study was unaffected by histamine (Fig. 4Ac).

Subthreshold oscillations induced by H1 receptor agonists

Bath application of histamine (100 μM) induced biphasic membrane potential oscillations in 5/17 previously quiescent SPNs at the peak of the histamine-induced membrane depolarization (Fig. 5, Aa and Ab). These oscillations, characterized by a fast depolarizing transient followed by a slower hyperpolarization, are a feature of electrotonically coupled SPNs and arise from the passive conduction of action potentials from adjoining neurons through electrotonic synapses (Logan et al. 1996; Nolan et al. 1999). In the remaining 12 neurons, histamine-induced depolarization was not associated with the induction of oscillations, presumably reflecting a lack of electrotonic coupling between these neurons. Application of histamine (100 μM) or HTMT (10 μM) to SPNs discharging spontaneous oscillations in membrane potential induced an increase in both the amplitude and frequency of oscillations in three of five cells tested, (Fig. 5, Ba and Bb), from 9.8 ± 1.2 mV and 0.85 ± 0.13 Hz in control conditions to 17.27 ± 3.4 mV and 2.70 ± 0.37 Hz in the presence of the agonist (n = 3, P < 0.02 and P < 0.01, respectively, for amplitude and frequency).

Single-cell RT-PCR analysis of histamine receptor mRNA expression in SPNs

To further clarify the nature of the histamine receptor(s) expressed by SPN and responsible for the membrane potential depolarization and concurrent increase in neuronal input resistance, we performed single-cell RT-PCR. The cytosolic contents were aspirated from eight neurons, which were subjected to RT-PCR using specific primers for all four histamine receptors, and the housekeeping gene, β-actin (see Table 1).

H1 receptor mRNA expression was detected in six of the eight cells investigated (75%). No detectable levels of mRNA for the remaining three receptors (H2, H3, and H4) were observed in any of the cells tested (Fig. 6Aa and B). Only cells expressing the housekeeping gene β-actin were used in the study, and a negative control was performed, which showed negative expression for all mRNA transcripts tested (Fig. 6Aa).

DISCUSSION

Histamine has been indicated as being involved in a range of homeostatic mechanisms that ultimately involve the engagement of the sympathetic nervous system (see Brown et al. 2001; Yasuda et al. 2004), including energy expenditure and cardiovascular control. For example, a number of studies...
agonist HTMT and was blocked by the potent and selective H1 receptor. This effect was mimicked by the highly selective H1 receptor in addition to the housekeeping gene β-actin mRNA were detected in SPNs. These neurons did not express mRNA for H2, H3, or H4. A negative (-ve) control was performed on the contents of an electrode which was placed next to a cell without seal formation or harvesting of cytoplasmic contents. Molecular weight markers are shown in the lanes on the far left and far right, together with corresponding number of base pairs. (Int, Intronic; -RT, cell contents lacking reverse transcriptase in the PCR amplification process). B: summary histogram illustrating the expression of the mRNA transcripts expressed in SPN. Data from eight cells.

indicate that activation of the central histamine system leads to elevated levels of plasma catecholamines (Akins and Bealer 1993; Knigge et al. 1990; Matzen et al. 1992; Okuma et al. 1997) and changes in blood pressure and heart rate (Gatti and Gertner 1983; Klein and Gertner 1981, 1983; Singewald and Phillips 1996; Tangri et al. 1989). Thus histamine has a role in regulating autonomic function, both peripherally and centrally. However, the only role for histamine in regulating central sympathetic outflow that has been considered to date has been at higher levels in the hypothalamus and brain stem, thus antecedent and supraspinal to SPNs. To our knowledge, there have been no reports of histamine acting directly on the origins of the sympathetic outflow in the spinal cord. Thus the data presented here are the first to indicate a direct role for histamine in the regulation of excitability of SPNs. The major findings are that histamine, through the activation of postsynaptic H1 receptors negatively coupled to one or more K+ conductances, acts to excite two thirds of these neurons.

**SPN express functional postsynaptic H1, but not H2 or H3 receptors**

Bath application of histamine induced membrane depolarization that was associated with an increase in neuronal input resistance in 70% of neurons. Responses persisted in the presence of TTX, indicative of a direct effect on the neuron. This effect was mimicked by the highly selective H1 receptor agonist HTMT and was blocked by the potent and selective H1 receptor antagonist triprolidine, confirming the presence of postsynaptic H1 receptors in the majority of SPNs. This notion was further supported by single-cell RT-PCR data, with mRNA for the H1 receptor being found in 75% of neurons tested. Agonists and antagonists for either H2 or H3 receptors were without effect on SPN. This latter data together with results from single-cell RT-PCR and pharmacological studies yielded no evidence for the expression of H2, H3, or H4 receptors in these neurons. Furthermore, a subpopulation (25%) of SPNs did not express detectable levels of H1 receptor mRNA. Whether this indicates that histaminergic inputs innervate SPN differentially in a target- and function-specific manner, or that H1 receptor expression is subject to modulation in a temporal and/or spatial fashion, remains to be determined.

**Ionic mechanism underlying histamine-induced depolarization in SPNs**

The histamine-induced membrane depolarization and associated increase in neuronal input resistance was barium sensitive and exhibited a reversal potential close to that of K+ ions under our recording conditions, consistent with the effect of histamine being mediated by closure of one or more potassium-selective conductances. Likewise, in voltage clamp, the histamine-induced inward current had a reversal potential, indicating potassium selectivity. Thus the signal transduction mechanism activated through these receptors ultimately leads to closure of one or more resting potassium conductances and increased excitability of SPNs. Similar mechanisms of action of histamine have been reported in peripheral autonomic neurons, where histamine acting via H1 receptors excites vagal afferents by blocking a resting leak potassium conductance and a potassium conductance contributing to afterhyperpolarization (Jafri et al. 1997). Similarly in other central neurons, histamine acting via H1 receptors excites thalamic neurons (McCormick and Williamson 1991), hypothalamic supraoptic neurons (Li and Hatton 1996), striatal cholinergic interneurons (Munakata and Akaike 1994), and cerebral cortical neurons (Reiner and Kamondi 1994). All of these studies on central neurons, as with the SPNs described here, revealed that histamine-induced responses were blocked in the presence of extracellular barium and mediated via inhibition of resting leak potassium conductances. Other potassium conductances targeted by histamine-dependent signaling mechanisms include the M-current (Guo and Schofield 2002) and A-current (Starodub and Wood 2000), although no effect of histamine was observed on the A-like conductance in SPNs in the present study. However, we cannot discount an effect of histamine on nACh receptors in some SPNs in the present study, although this seems unlikely given that we observed no effect of histamine on transient outward rectification in these neurons.

In relation to the signal transduction mechanism, binding of histamine to the H1 G protein-coupled receptor stimulates phospholipase C (PLC) through activation of the G_{q/11} G protein (Leurs et al. 1994). PLC in turn hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to form two second messengers, diacyglycerol (DAG) and inositol triphosphate (IP3). DAG potentiates the activity of PKC, which can block a leak K+ conductance (I_{K(leak)}) (see Brown et al. 2001; Haas and Panula 2003) that contributes to neuronal resting membrane potential. Although a full investigation of the conductance and signal transduction pathway involved was not performed in the present study, it is tempting to speculate that the excitation induced by histamine in SPNs occurs by means of a block of I_{K(leak)} either directly via activation of G_{q/11} or via subsequent activation of DAG and PKC. Further studies are required to clarify this.
Induction of membrane potential oscillations in SPNs

Biphasic membrane potential oscillations were induced by histamine and the histamine H1 receptor agonist HTMT in previously quiescent SPNs, and the frequency of oscillations increased in spontaneously active SPNs. Previous studies in SPNs indicated that such oscillations are the hallmark of electrotonic coupling between these neurons (Logan et al. 1996; Nolan et al. 1999). Thus, these data suggest that histamine acts to regulate excitability of electrotonically coupled SPNs. In cultured supraoptic neurons, H1 receptor activation leads to a significant increase in dye coupling, and has been suggested to reflect an increased number of electrical synapses between neurons (Hatton and Yang 1996, 2001; Yang and Hatton 2002). However, whether this truly reflects such a scenario is questionable, as dye coupling may be increased by changes in the properties of existing electrical synapses rather than the insertion of new channels. Although an extensive study on the effects of histamine on electrical synapses was not performed in this study, the induction of oscillations by histamine appears to be the result of a network-wide depolarization of already-coupled SPNs, and the associated histamine-induced increase in neuronal input resistance underlies the observed increase in the amplitude of oscillations, rather than histamine having a direct action on electrical synapses themselves. A similar mechanism has been proposed for the feeding peptide orexin in SPNs (van den Top et al. 2003).

CONCLUSIONS

Approximately two thirds of SPNs express histamine H1 receptor mRNA and are excited by histamine acting through activation of H2 receptors, leading to the closure of one or more resting leak K+ conductances. Furthermore, histamine-induced depolarization of electrotonically coupled SPN leads to the induction of membrane potential oscillations, indicative of activation of coupled networks of these neurons. Further studies are required to clarify the functional significance of histamine activation of coupled networks of SPNs and their role in regulating sympathetic drive.

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REFERENCES


