Opposite Functions of Histamine H$_1$ and H$_2$ Receptors and H$_3$ Receptor in Substantia Nigra Pars Reticulata

Fu-Wen Zhou, Jian-Jun Xu, Yu Zhao, Mark S. LeDoux, and Fu-Ming Zhou. Opposite functions of histamine H$_1$ and H$_2$ receptors and H$_3$ receptor in substantia nigra pars reticulata. J Neurophysiol 96: 1581–1591, 2006. First published May 31, 2006; doi:10.1152/jn.00148.2006. The substantia nigra pars reticulata (SNr) is a key basal ganglia output nucleus. Inhibitory outputs from SNr are encoded in spike frequency and pattern of the inhibitory SNr projection neurons. SNr output intensity and pattern are often abnormal in movement disorders of basal ganglia origin. In Parkinson’s disease, histamine innervation and histamine H$_3$ receptor expression in SNr may be increased. However, the functional consequences of these alterations are not known. In this study, whole cell patch-clamp recordings were used to elucidate the function of different histamine receptors in SNr. Histamine increased SNr inhibitory projection neuron firing frequency and thus inhibitory output. This effect was mediated by activation of histamine H$_1$ and H$_2$ receptors that induced inward currents and depolarization. In contrast, histamine H$_3$ receptor activation hyperpolarized and inhibited SNr inhibitory projection neurons, thus decreasing the intensity of basal ganglia output. By the hyperpolarization, H$_3$ receptor activation increased the irregularity of the interspike intervals or changed the pattern of SNr inhibitory neuron firing. H$_3$ receptor–mediated effects were normally dominated by those mediated by H$_1$ and H$_2$ receptors. Furthermore, endogenously released histamine provided a tonic, H$_1$ and H$_2$ receptor–mediated excitation that helped keep SNr inhibitory projection neurons sufficiently depolarized and spiking regularly. These results suggest that H$_1$ and H$_2$ receptors and H$_3$ receptor exert opposite effects on SNr inhibitory projection neurons. Functional balance of these different histamine receptors may contribute to the proper intensity and pattern of basal ganglia output and, as a consequence, exert important effects on motor control.

METHODS

Patch clamp

Wild-type, 16- to 25-day-old male and female C57BL/6J mice were used. Animal handling and use followed National Institutes of Health guidelines.
guidelines. These mice were kept at the animal facility of the University of Tennessee Health Science Center in Memphis. They had free access to food and water. The room light was on 7:00 AM to 7:00 PM and off for the night. Under deep halothane anesthesia, mice were decapitated and their brains were quickly dissected out. Coronal midbrain slices (300 μm thickness) containing the midrostral part of substantia nigra were prepared according to well-established procedures (Bonci and Malenka 1999; Richards et al. 1997). Coronal sections were chosen to maximally sever afferent fibers such that SNr neurons can be studied in relative isolation. The cutting solution contains (in mM): 220 sucrose, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 0.5 CaCl2, 7 MgCl2, and 20 d-glucose. The slices were then transferred to a holding chamber containing the normal extracellular solution (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 2.5 CaCl2, 1.3 MgCl2, and 20 d-glucose. The solution was continuously bubbled with 95% O2-5%CO2 to supply oxygen and keep pH at 7.4. Recordings were made at 30°C under visual guidance of a video microscope (Olympus BX51WI) equipped with Nomarski optics and ×30 water immersion lens. Relatively large (the longest dimension of the soma was about 25 μm) oval or spindle-shaped SNr neurons were chosen for recording. These characteristics are typical of rodent SNr GABA projection neurons (Grofova et al. 1982; Juraska et al. 1977). This selection was biased against smaller neurons that are potential interneurons.

Conventional whole cell patch-clamp techniques were used (Zhou and Hablitz 1999). Patch electrodes had resistances of 2–3 MΩ when filled with an internal solution containing (in mM): 130 KCl, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, and 4 Na-phosphocreatine. pH was adjusted to 7.3 with NaOH. Axopatch 200B and Multiclamp 700B amplifiers, pClamp 9.2 software, and Digidata 1322A interface (Axon Instruments) were used to acquire and analyze data. Signals were digitized at 5–20 kHz and analyzed off-line. The Mini Analysis Program (Synamptosoft, Fort Lee, NJ) was also used to analyze spontaneous events. Recordings with access resistance increase of >15% were rejected. Whole cell conductance was measured by 100-ms voltage pulses, from −70 to −80 mV.

**Histology**

Neurobiotin (0.2%) was dissolved in the pipette solution before each experiment and allowed to passively diffuse into neurons from the recording electrode (Zhou and Hablitz 1996). After electrophysiological recordings, brain slices were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) for 2 h at 4°C. As shown in Fig. 1A, SNr is a fairly large structure ventral to substantia nigra pars compacta (SNc) and dorsal to the cerebral peduncle (Paxinos and Franklin 2001). SNr also has a low cell density compared with the densely packed cells in SNc. SNr is populated largely by two types of neurons: the majority GABA projection neurons and the minority dopamine (DA) projection neurons (Fallon and Loughlin 1995; Tepper et al. 1995). Both cell types are relatively large and often oval-shaped neurons and cannot be distinguished based on their appearances (Deniau et al. 1982; Grofova et al. 1982; Juraska et al. 1977; Nelson et al. 1996). However, they have very different electrophysiological characteristics (Diana and Tep- per 2002; Ibanez-Sandoval et al. 2006; Lacey et al. 1989; Richards et al. 1997; Shen and Johnson 1997; Yung et al. 1991).

In our present sample, the presumed SNr GABA projection neurons (SNr GABA neurons hereafter) spiked spontaneously at 10.8 ± 0.5 Hz (n = 58; Fig. 1A, A2, A3, and B). These action potentials had a duration at the base of 0.96 ± 0.03 ms (n = 58, Fig. 1B). These GABA neurons had a very weak, hyperpolarization-activated cation current or Ih current. In contrast, the presumed DA neurons spiked spontaneously at 1.5 ± 0.2 Hz (n = 27; Fig. 1B). DA neuron action potentials had a base duration of 2.53 ± 0.07 ms (n = 27). DA neurons displayed a prominent Ih current. As shown in the scatter plot of Fig. 1B, these electrophysiological properties clearly separate SNr GABA neurons and DA neurons into two non-overlapping groups. Therefore SNr GABA neurons and DA neurons can be reliably identified by their electrophysiological properties. This report focuses on SNr GABA projection neurons. DA neurons were excluded from results presented below.

All chemicals including d-2-amino-5-phosphonopentanoic acid (d-AP5), 6-cyano-7-nitroquinolinic acid (CNQX), and bicuculline (BIC) were purchased from Sigma–Aldrich (St. Louis, MO) or Tocris Cookson (Ballwin, MO). d-AP5, CNQX, and BIC were present when examining action potential firing, depolarization, and inward current to prevent the complications from synaptic activity. All values were expressed as means ± SE. Statistical comparisons were performed using paired t-test or Kolmogorov–Smirnov (K-S) test (to compare the distributions of two sets of events such as spontaneous synaptic currents). P < 0.05 is significant.

**RESULTS**

Identification of substantia nigra pars reticulata GABA projection neurons

Substantia nigra pars reticulata (SNr) can be easily identified. As shown in Fig. 1A, SNr is a fairly large structure ventral to substantia nigra pars compacta (SNc) and dorsal to the cerebral peduncle (Paxinos and Franklin 2001). SNr also has a low cell density compared with the densely packed cells in SNc. SNr is populated largely by two types of neurons: the majority GABA projection neurons and the minority dopamine (DA) projection neurons (Fallon and Loughlin 1995; Tepper et al. 1995). Both cell types are relatively large and often oval-shaped neurons and cannot be distinguished based on their appearances (Deniau et al. 1982; Grofova et al. 1982; Juraska et al. 1977; Nelson et al. 1996). However, they have very different electrophysiological characteristics (Diana and Tepper 2002; Ibanez-Sandoval et al. 2006; Lacey et al. 1989; Richards et al. 1997; Shen and Johnson 1997; Yung et al. 1991).

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Also, SNr GABA projection neurons did not show any significant slow afterhyperpolarization (sAHP) after a train of high-frequency spikes evoked from their natural membrane potentials by injecting depolarizing current pulses (Fig. 1C). To remove any potential interference from the spontaneous spikes, hyperpolarizing holding currents were applied to bring the membrane potential to −65 to −70 mV, such that the SNr neurons completely ceased to fire spontaneous action potentials. Under this condition, there was still no significant sAHP after a train of spikes evoked by depolarizing current pulses (Fig. 1D).

Histamine excites SNr GABA projection neurons by inducing depolarization and inward current

High-frequency spike firing encodes the output from SNr GABA projection neurons (Hikosaka et al. 2000). Therefore
our first goal was to investigate whether histamine affected action potential firing in well-identified and synaptically isolated SNr neurons. In the presence of 20 μM d-AP5, 10 μM CNQX, and 10 μM BIC to remove complications of synaptic activity, bath application of histamine (10 μM) reliably increased the firing rate of SNr neurons by 37.2 ± 3.5%, from 10.4 ± 0.8 to 14.1 ± 1.0 Hz (n = 11, P < 0.001, Fig. 2, A and C). This effect was fully reversed after prolonged wash. Histamine did not affect spike amplitude (67.8 ± 3.4 mV under control vs. 67.4 ± 3.6 mV during histamine, n = 11) and base duration (0.97 ± 0.07 vs. 0.98 ± 0.08 ms). The fast AHP (fAHP, 20.1 ± 2.1 vs. 19.9 ± 2.4 mV) and medium AHP (mAHP, 10.6 ± 1.5 vs. 10.6 ± 1.7 mV) were also not affected (Fig. 2B). These results indicate that histamine was not affecting voltage-gated Na+ and K+ channels or Ca2+-activated K+ channels that are responsible for spike generation and repolarization.

The increased spike firing was accompanied by a small depolarization and a small increase in whole cell conductance. However, these two modest changes were difficult to monitor when the neuron was spiking at high frequency. Thus we blocked action potentials with 0.5 μM tetrodotoxin (TTX), a specific voltage-gated Na+ channel blocker. In the presence of TTX, membrane potential was stable in SNr neurons (Fig. 2D).

Under this condition, bath application of 10 μM histamine caused a slowly developing depolarization of 3.8 ± 0.2 mV, from baseline membrane potential of −50.6 ± 1.1 to −46.8 ± 1.1 mV (n = 7, P < 0.001, Fig. 2D). This depolarization is likely the primary mechanism underlying histamine’s enhancement of SNr neuron firing.

The depolarization was associated with an increase in whole cell conductance, indicating that histamine was opening ion channels in SNr neurons. To test this idea, SNR neurons were voltage clamped at −70 mV. At this holding potential, bath application of histamine (10 μM) induced an inward current (39.6 ± 4.0 pA, n = 10), increasing the holding current from −164.9 ± 20.9 to −204.5 ± 23.3 pA (Fig. 2E). The majority of the relatively large baseline holding current arose from a tonic inward current (Atherton and Bevan 2005). Whole cell conductance, monitored with 10-mV voltage pulses, was also significantly increased from 5.32 ± 0.46 nS under control to 7.21 ± 0.75 nS (n = 19, P < 0.01) during histamine application, suggesting an opening of ion channels. Voltage ramp experiments revealed that histamine increased the whole cell current. The current was linear between −90 and +20 mV with no signs of voltage-dependent activation or inactivation and reversed its polarity at −42.8 ± 2.1 mV (n = 8, Fig. 3A). These results indicate that histamine was enhancing a tonic, voltage-independent current.

Consistent with the above results that histamine increased SNR neuron firing and reports that SNR neurons innervate each other by their relatively sparse intranigral axonal collaterals, in addition to receiving other GABA inputs (Celada et al. 1999; Deniau et al. 1982; Mailly et al. 2003), histamine enhanced spontaneous inhibitory postsynaptic currents (sIPSCs) in SNR neurons. BIC (10 μM)-sensitive sIPSCs were recorded at a holding potential of −70 mV in the presence of d-AP5 (20 μM) and CNQX (10 μM) to block glutamate-mediated synaptic current. Under these conditions, bath application of 10 μM histamine increased the sIPSC frequency by 38.4 ± 5.6% Hz (P < 0.001, n = 10) and sIPSC amplitude by 31.3 ± 7.7% (P < 0.001) (Fig. 4, A–D). On the other hand, histamine induced a minimal, 5% decrease in the frequency of miniature IPSCs (mIPSCs) recorded in 0.5 μM TTX with no change in amplitude. These results indicate that the predominant histamine effect was at the cell body area of SNR neurons and the histamine effect on GABA terminals in SNR was minor.

These results indicate that histamine can directly excite SNR neurons. In other brain areas, H1 and H2 receptors are often

**FIG. 1.** Identification of substantia nigra pars reticulata (SNR) γ-aminobutyric acid (GABA) projection neurons. A1: Nissl-stained coronal mouse brain section showing that SNR, as marked by the red circle, is a distinct, relatively large, and uniquely located brain structure. Arrowhead points to the dense cell band of SNc. d, dorsal; m, medial. A2: example of frequency of SNR neurons that were chosen for recording. This neuron fired fast spikes and therefore was a presumed GABA projection neuron. Roughly 85% of the SNR neurons chosen this way were fast-spiking neurons or presumed GABA projection neurons and the rest were typical of dopamine (DA) neurons. A3: photograph showing the neuron in A2 stained with neurobiotin. Scale bar in A2 applies to A3. Gross morphology is typically seen of SNR GABA projection neurons, although it is not distinctly different from SNR DA neurons. B: scatterplot showing that SNR DA and GABA neurons can be unequivocally distinguished based on their spontaneous action potential duration and frequency. GABA neurons formed a cluster with high frequency and short duration, whereas DA neurons formed another cluster with low frequency and long duration. Note that these 2 clusters do not overlap. Inset: example action potentials from a DA neuron and a GABA neuron, respectively. Scale bar: 2 ms and 20 mV. C: at their natural membrane potential, SNR GABA projection neurons lack the slow afterhyperpolarization (sAHP). D: injection of −100-pA hyperpolarizing current brought the membrane potential to −70 mV and the SNR neuron ceased to fire spontaneous action potentials. In the absence of the interference of spontaneous spikes, there was still no significant sAHP after a train of spikes evoked by a pulse of depolarizing current.
Histamine $H_1$ receptor activation excites SNr GABA projection neurons

No selective $H_1$ receptor agonists are commercially available. Therefore we studied the potential effects of $H_1$ receptor activation by first blocking $H_2$ receptor with 5 $\mu$M ranitidine or tiotidine and $H_3$ receptor with 100 nM clobenpropit (van der Goot and Timmerman 2000). As will be discussed in later sections, these $H_2$ and $H_3$ receptor antagonists at the concentrations used here completely blocked $H_2$ and $H_3$ receptor agonist–induced effects, indicating that these $H_2$ and $H_3$ receptor antagonists were able to fully inhibit $H_2$ and $H_3$ receptors.

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Consequently, after incubation with these H2 and H3 receptor antagonists, only H1 receptor can still respond to histamine. Under these conditions, bath application of 10 μM histamine increased the firing rate of SNr neurons by 19.6 ± 2.6%, from 11.1 ± 1.5 to 13.4 ± 2.0 Hz (n = 10, P < 0.01, Fig. 5, A and B). This enhancement was blocked by 2 μM trans-triprolidine, a specific H1 antagonist (van der Goot and Timmerman 2000), further confirming that H1 receptor activation was responsible for this histamine-induced excitation of SNr neurons. Also, under these conditions, histamine did not significantly affect action potential shape or the fAHP or mAHP, suggesting that the main effect of H1 receptor activation was not that of affecting voltage-gated Na+ and K+ channels and Ca2+-activated K+ channels in SNr neurons.

To further study how H1 receptor activation increased SNr neuron firing, action potentials were blocked with 0.5 μM TTX such that a stable membrane potential was established and small changes in membrane potential can be reliably detected. After blocking H2 and H3 receptors with 5 μM ranitidine and 100 nM clobenpropit, 10 μM histamine caused a slowly developing depolarization of 2.0 ± 0.3 mV, from a baseline membrane potential −50.2 ± 0.9 to −48.2 ± 1.1 mV (n = 5, P < 0.001, Fig. 5C). Similarly, when voltage clamped at −70 mV, bath application of 10 μM histamine in the presence of ranitidine and clobenpropit induced an inward current of 23.7 ± 4.1 pA (n = 7, Fig. 5D). Whole cell conductance was increased from 5.59 ± 0.43 nS under control to 7.17 ± 0.85 nS during H1 receptor activation (n = 8, P < 0.05). All these effects were blocked by 2 μM H1 antagonist trans-triprolidine, indicating that H1 receptor activation was inducing the depolarization and inward currents. Voltage ramp experiments showed that H1 receptor activation increased the whole cell current and this current was linear between −90 and −20 mV without any voltage dependency and reversed its polarity at −41.3 ± 1.7 mV (n = 8, Fig. 3B). These results indicate that H1 receptor activation was enhancing a tonic current in SNr.

Consistent with the excitatory effect of H1 receptor activation, histamine (10 μM), in the presence of ranitidine (5 μM) and clobenpropit (100 nM) to block both H2 and H3 receptors, increased sIPSCs frequency by 15.8 ± 4.6% (n = 5, P < 0.05) and amplitude by 16.6 ± 5.1% (P < 0.01) (Fig. 4, C and D). However, when action potentials were blocked with 0.5 μM TTX, mIPSCs were not significantly altered by H1 receptor activation, indicating a lack of functional H1 receptors on GABA axon terminals innervating SNr neurons.

Histamine H2 receptor activation enhances SNr GABA projection neurons

To examine the potential involvement of H2 receptor in histamine-induced excitation of SNr neurons, we used the highly selective H2 receptor agonist amthamine (van der Goot and Timmerman 2000). After establishing a stable baseline recording, bath application of 10 μM amthamine had a clearly significant excitatory effect on SNr neurons (Fig. 6, A–D). The spontaneous action potential firing rate was increased by 33.0 ± 8.8%, from 11.5 ± 1.9 Hz in control to 15.3 ± 2.5 Hz during the treatment of amthamine (n = 7, P < 0.01, Fig. 6, A and B). This effect was blocked by a selective H2 receptor antagonist ranitidine at 5 μM (Fig. 6B). Clearly, H2 receptor activation has excitatory effects on SNr neurons.

Next, we investigated the mechanisms by which H2 receptor activation enhanced SNr neuron firing. Because amthamine did...
Whole cell conductance was increased from 5.39 ± 0.6 mV, from baseline membrane potential of 49.4 ± 1.4 to 45.5 ± 1.4 mV (n = 6, P < 0.01, Fig. 6C). When the neurons were voltage clamped at −70 mV, 10 μM amphetamine induced an inward current of 38.8 ± 4.1 pA (n = 7, P < 0.01, Fig. 6D). Whole cell conductance was increased from 5.39 ± 0.49 nS under control conditions to 7.58 ± 0.81 nS during 10 μM amphetamine (n = 12, P < 0.01). Voltage ramp experiments revealed that amphetamine increased the whole cell current and the amphetamine induced a linear current between −90 and −20 mV with a reversal potential of 42.2 ± 2.4 mV (n = 6, Fig. 3C). These results indicate that H₂ receptor activation was enhancing a tonic current.

Consistent with the excitatory effect of H₂ receptor activation, bath application of 10 μM specific agonist amphetamine (10 μM) increased sIPSC frequency and amplitude by 28.1 ± 8.7% and 24.2 ± 5.1%, respectively (P < 0.01, Fig. 4, C and D). However, mIPSCs recorded in the presence of 0.5 μM TTX were not significantly increased by amphetamine treatment, indicating a lack of functional H₂ receptors on GABA axon terminals innervating SNr neurons.

Histamine H₂ receptor activation inhibits SNr GABA projection neurons

H₃ receptor is known to be an inhibitory autoreceptor on histamine neurons (Brown et al. 2001). Modest levels of H₃ receptor are expressed in SNr (Pillot et al. 2002; Ryu et al. 1995; Vizuete et al. 1997). We hypothesize that H₂ receptor activation may induce a mild, direct inhibition of SNr neurons. To test this hypothesis, we did the following experiments using strategies similar to those for H₂ receptor.

First, we examined the effects of an H₃ receptor agonist, imetit (van der Goot and Timmerman 2000). If H₃ receptor activation produces inhibitory effects, then imetit should inhibit SNr neurons. Indeed, bath application of 100 nM imetit significantly decreased SNr neuron firing rate by 15.6 ± 3.7% (n = 11, P < 0.05, Fig. 7, A and B). This inhibitory effect was recovered after prolonged wash (Fig. 7B). Furthermore, imetit-induced inhibition of SNr GABA neuron firing was completely blocked by a selective H₂ receptor antagonist clobenpropit (100 nM). Histamine (10 μM) induced similar effects in the presence of 2 μM H₂ blocker trans-triprolidine and 5 μM H₂ receptor blocker ranitidine (n = 5). These findings suggested that H₃ receptor activation mildly inhibited SNr neurons. H₃ receptor activation by imetit also did not significantly affect the action potential shape of fAHP or mAHP in SNr GABA neurons.

Next, we blocked spikes with 0.5 μM TTX to stabilize the membrane potential such that the imetit-induced small hyperpolarization can be characterized. After blocking action potentials, membrane potential was stable. Bath application of 100 nM imetit induced a slowly developing hyperpolarization of −2.7 ± 0.5 mV; from baseline membrane potential −49.7 ± 1.5 to −52.4 ± 1.2 mV (n = 7, P < 0.01, Fig. 7E). Similarly, when SNr neurons were voltage clamped at −70 mV, bath application of 100 nM imetit caused an outward current of 26.6 ± 3.0 pA (n = 12, Fig. 7F). The hyperpolarization and outward current were associated with a decrease in whole conductance from 5.68 ± 0.48 nS under control to 4.71 ± 0.38 nS (n = 11, P < 0.01), indicating a closing of ion channels. Voltage ramp experiments revealed that imetit reduced the whole cell current and the imetit-induced current was linear between −90 and −20 mV without any voltage dependency and reversed its polarity at −41.7 ± 1.4 mV (n = 6, Fig. 3D). These results indicate that H₃ receptor activation was inhibiting a tonic current.

Histamine H₂ receptor activation increases the irregularity of SNr GABA projection neuron firing

During imetit treatment, the decrease in firing frequency or increase in interspike interval (ISI) was also accompanied by an increase in irregularity in ISI. To quantify this irregularity,
Histamine H3 receptor activation diminishes inhibitory synaptic inputs to SNr GABA projection neurons by presynaptic mechanisms

As expected from H3 receptor’s hyperpolarizing effect on SNr neurons, bath application of H3 receptor agonist imetit (100 nM) slightly but significantly decreased sIPSCs. The sIPSC frequency was reduced by 17.9 ± 2.5% (n = 5, P < 0.05) and the amplitude by 9.8 ± 3.6% (P < 0.05) (Fig. 4, C and D). These results indicate that a fraction of action potential–dependent sIPSCs disappeared during imetit activation of H3 receptor.

We also hypothesized that H3 receptor may act as an inhibitory presynaptic receptor on GABA terminals. To test this idea, mIPSCs were recorded in SNr neurons in the presence of 0.5 μM TTX to block action potentials. Bath application of 100 nM imetit decreased the frequency of mIPSCs from 7.0 ± 1.7 Hz under control to 6.1 ± 1.5 Hz during imetit application (P < 0.001; n = 5, Fig. 9B). This effect was almost fully recovered after washing out imetit (Fig. 9B). mIPSC amplitude was not significantly affected (49.1 ± 10.4 pA in control and 48.3 ± 11.1 pA in imetit treatment) (P > 0.05, Fig. 9C). Imetit inhibition of mIPSCs was prevented by a selective H3 receptor antagonist clobenpropit (100 nM, n = 3). These results indicate that H3 receptor may inhibit GABA vesicle release from axon terminals synapsing onto SNr neurons.

Endogenous histamine release induces a tonic excitation in SNr GABA projection neurons

Like other neurotransmitters, histamine may be released spontaneously from histamine terminals and induce a low level, tonic activation of histamine receptors and exert a tonic influence on SNr neurons. Consequently, blocking histamine receptors may have detectable effects in SNr neurons. We did the following experiments to test this idea.

After establishing stable baseline recording of spontaneous action potential firing, H1, H2, and H3 antagonists (2 μM trans-triprolidine, 5 μM ranitidine, and 100 nM clobenpropit) were individually tested; however, none of the antagonists induced statistically significant change in SNr neuron firing. This is not surprising because even the large doses of exogenous histamine agonists induced only mild effects as described earlier. Because both H1 and H2 receptors are excitatory, we reasoned that combined application of H1 and H2 receptor antagonists might induce detectable effects. Indeed, a combined application of 2 μM trans-triprolidine (H1 receptor antagonist) and 5 μM ranitidine (H2 receptor antagonist) induced a small hyperpolarization of 1.3 ± 0.3 mV (n = 6) and significantly decreased SNr neuron firing frequency by 9.2 ± 3%. The sIPSC frequency was reduced by 17.9 ± 2.5% (n = 5, P < 0.05) and the amplitude by 9.8 ± 3.6% (P < 0.05) (Fig. 4, C and D). These results indicate that a fraction of action potential–dependent sIPSCs disappeared during imetit activation of H3 receptor.

We also hypothesized that H3 receptor may act as an inhibitory presynaptic receptor on GABA terminals. To test this idea, mIPSCs were recorded in SNr neurons in the presence of 0.5 μM TTX to block action potentials. Bath application of 100 nM imetit decreased the frequency of mIPSCs from 7.0 ± 1.7 Hz under control to 6.1 ± 1.5 Hz during imetit application (P < 0.001; n = 5, Fig. 9B). This effect was almost fully recovered after washing out imetit (Fig. 9B). mIPSC amplitude was not significantly affected (49.1 ± 10.4 pA in control and 48.3 ± 11.1 pA in imetit treatment) (P > 0.05, Fig. 9C). Imetit inhibition of mIPSCs was prevented by a selective H3 receptor antagonist clobenpropit (100 nM, n = 3). These results indicate that H3 receptor may inhibit GABA vesicle release from axon terminals synapsing onto SNr neurons.

Histamine H3 receptor activation diminishes inhibitory synaptic inputs to SNr GABA projection neurons by presynaptic mechanisms

As expected from H3 receptor’s hyperpolarizing effect on SNr neurons, bath application of H3 receptor agonist imetit (100 nM) slightly but significantly decreased sIPSCs. The sIPSC frequency was reduced by 17.9 ± 2.5% (n = 5, P < 0.05) and the amplitude by 9.8 ± 3.6% (P < 0.05) (Fig. 4, C and D). These results indicate that a fraction of action potential–dependent sIPSCs disappeared during imetit activation of H3 receptor.

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H<sub>1</sub> and H<sub>2</sub> receptor activation increases SNr GABA projection neuron firing

We found that activation of H<sub>1</sub> receptors induced an inward current and increased spike firing in SNr neurons (Figs. 3 and 5). These effects were accompanied by increased whole cell conductance, indicating an opening of unknown type(s) of ion channels. In the cortex, hippocampus, septum, striatum, and thalamus, activation of H<sub>1</sub> receptors increases neuronal excitability by blocking a leak K<sup>+</sup> conductance or decreasing whole cell conductance (Bell et al. 2000; Gorelova and Reiner 1996; McCormick and Williamson 1991).

Activation of H<sub>2</sub> receptors also induced an inward current and enhanced spike firing in SNr neurons (Figs. 3 and 6). These effects were also accompanied by increased whole cell conductance, indicating opening of ion channels. In thalamocortical neurons, H<sub>2</sub> receptor activation enhanced the hyperpolarization-activated I<sub>h</sub> current (McCormick and Williamson 1991). However, I<sub>h</sub> is very small in SNr neurons. Furthermore, this I<sub>h</sub> usually starts to activate when the membrane potential is more negative than −60 mV. In contrast, the histamine-induced current was linear between −90 and −20 mV with no signs of voltage-dependent activation or inactivation (Fig. 3), indicating that I<sub>h</sub> is not likely histamine’s target conductance in SNr neurons. Also, neither H<sub>1</sub> nor H<sub>2</sub> receptor activation affected the Ca<sup>2+</sup>-activated K<sup>+</sup> channel–mediated fAHP and mAHP in SNr neurons. H<sub>2</sub> receptor activation inhibits sAHP in hippocampal and cortical neurons (Haas and Konnerth 1983; McCormick and Williamson 1989; Yanovsky and Haas 1998), but SNr GABA neurons lack sAHP (Fig. 1, C and D). Clearly, H<sub>1</sub> receptor and H<sub>3</sub> receptor in SNr neurons are likely coupled to different effectors or ion channels compared with those in other brain areas.

An extracellular recording study reported that exogenous histamine slightly increased the firing rate of SNr neurons by H<sub>1</sub> receptor activation and that H<sub>2</sub> receptor was not involved, although H<sub>2</sub> receptor’s effects were not studied (Koroktova et al. 2002). Apparently, these authors missed some important histamine effects in SNr.

H<sub>3</sub> receptor activation increases SNr projection neuron spiking irregularity

Our data clearly demonstrate that activation of H<sub>3</sub> receptor induced a small hyperpolarization or a small outward current and consequently an inhibition of SNr neuron firing (Fig. 7). Whole cell conductance was decreased, indicating a closing of an unknown type ion channel. These results suggest that H<sub>3</sub> receptor is on the somatodendritic area of SNr neurons. In addition, H<sub>3</sub> receptor activation also slightly reduced the frequency of mIPSCs in SNr neurons, indicating a low level of H<sub>3</sub> receptor expression on GABA axon terminals synapsing onto SNr neurons. This is consistent with histochemical studies (Pillot et al. 2002; Vizuete et al. 1997) and also with previous findings that H<sub>1</sub> receptor serves as an inhibitory presynaptic receptor (Arrang et al. 1983; Brown and Haas 1999; Jang et al. 2001; Threlfell et al. 2004; for review see Haas and Panula 2003).

Importantly, the small hyperpolarization induced by H<sub>3</sub> receptor activation was able to alter the pattern of SNr neuron firing, making the firing of these neurons more irregular (Fig. 7, A–D). Apparently, under normal conditions SNr neurons are depolarized sufficiently and can spike reliably and regularly. When hyperpolarized by H<sub>3</sub> receptor activation or direct negative current injection (Fig. 8), these neurons are no longer sufficiently depolarized, such that they reach action potential threshold less reliably and consequently spike more irregularly.

The molecular identities of the ion channel(s) affected by H<sub>3</sub> receptor and also those affected by H<sub>1</sub> and H<sub>2</sub> receptors are not known. Our results show that histamine did not affect the usual suspects such as the leak K<sup>+</sup> conductance and AHPs in SNr GABA neurons. Instead, our observations suggest that histamine may modulate a tonically active, Na<sup>+</sup>-dependent inward current that was critical to the spontaneous firing of SNr GABA neurons (Atherton and Bevan 2005). Specifically, H<sub>1</sub> and H<sub>2</sub> receptors appeared to upregulate this tonic inward current, whereas H<sub>3</sub> receptor seemed to downregulate it. How-
ever, a definitive answer requires cloning of the channel conducting the tonic Na\(^+\)-dependent inward current (for an example of histamine regulation of molecularly identified K\(^+\) channel see Atzori et al. 2000).

**Tonic activation of histamine receptors by endogenous histamine**

We found that blockade of H\(_1\) and H\(_2\) receptors induced a small hyperpolarization, decreased firing frequency, and increased the firing irregularity in SNr neurons (Fig. 10). These results indicate that spontaneously released endogenous histamine may induce a low-level, tonic activation of histamine receptors and influence SNr neuron activity. This is not surprising because other neurotransmitters such as acetylcholine, glutamate, GABA, dopamine, and serotonin are known to be released spontaneously from axon terminals (Colquhoun and Sakmann 1998; Katz 1969; Zhou et al. 2005). Even though in vivo confirmation will be required, the tonic activation of H\(_1\) and H\(_2\) receptors arising from endogenous histamine release may help to keep SNr neurons sufficiently depolarized and spiking reliably. Furthermore, the results on endogenous histamine are consistent with those obtained with exogenous histamine ligands, suggesting that the observations and conclusions made in this study are physiologically relevant. It should also be pointed out that the potential constitutive activity of H\(_1\) and H\(_2\) receptors may also contribute to the tonic H\(_1\) and H\(_3\) receptor activity detected here (Bakker et al. 2000; Smit et al. 1996).

**Functional implications**

Our present study indicates that the direct effects of histamine on SNr GABA projection neurons are a mild, H\(_1\) and H\(_2\) receptor-mediated excitation and a weak, H\(_3\) receptor-mediated inhibition. Functional balance of these different histamine receptors may alter the intensity and pattern of SNr GABA neuron activity. Consequently, basal ganglia output and movement control may also be affected. Although the situation is likely to be more complex in vivo because histamine may also affect afferents to SNr neurons (Brown et al. 2001; Hass and Panula 2003; Threlfell et al. 2004), the direct histamine effects on SNr neurons described herein are likely to be important. Indeed, selective activation of H\(_3\) receptors by injection of an H\(_3\) receptor agonist into SNr has been shown to influence motor behavior in rats (Garcia-Ramirez et al. 2004). In addition, a recent study found that systemic administration of an H\(_3\) receptor agonist worsened parkinsonian symptoms in a primate model of Parkinson’s disease (Gomez-Ramirez et al. 2006). In aggregate, these findings indicate that H\(_3\) receptors may regulate the activity of SNr GABA projection neurons and basal ganglia output. In Parkinson’s disease, histamine levels in the substantia nigra (both SNc and SNr) were substantially increased (Anichtchik et al. 2000; Rinne et al. 2002). Nigral H\(_3\) receptor expression was also increased in patients with Parkinson’s disease (Anichtchik et al. 2001) and a rodent model of the disease (Ryu et al. 1994). Because H\(_3\) activation causes hyperpolarization, decreases firing rates, and increases the irregularity of spike firing, abnormally high levels of histamine innervation and H\(_3\) receptor expression in SNr in parkinsonian brain may adversely alter the intensity and pattern of the basal ganglia output and consequently contribute to multiple aspects of movement disorders of basal ganglia origin.

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**DISCLOSURE**

The authors declare no conflict of interest.

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