Histamine H₃ Receptors Inhibit Serotonin Release in Substantia Nigra Pars Reticulata

Sarah Threlfell,¹ Stephanie J. Cragg,¹ Imre Kalló,²-³ Gergely F. Turi,³ Clive W. Coen,³ and Susan A. Greenfield¹
¹Department of Pharmacology, University of Oxford, Oxford OX1 3QT, United Kingdom, ²School of Biomedical Sciences, King’s College London, London SE1 1UL, United Kingdom, and ³Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest H-1450, Hungary

The substantia nigra pars reticulata (SNr) plays a key role in basal ganglia function. Projections from multiple basal ganglia nuclei converge at the SNr to regulate nigrothalamic output. The SNr is also characterized by abundant amnergic input, including dopaminergic dendrites and axons containing 5-hydroxytryptamine (5-HT) or histamine (HA). The functions of HA in the SNr include motor control via HA H₂ receptors (H₂Rs), although the mechanism remains far from elucidated. In Parkinson’s disease, there is an increase in H₂Rs and the density of HA-immunoreactive axons in the SN.

We explored the role of H₂Rs in the regulation of 5-HT release in SNr using fast-scan cyclic voltammetry at carbon-fiber microelectrodes in rat midbrain slices. Immunohistochemistry identified a similar distribution for histaminergic and serotoninergic processes in the SNr: immunoreactive varicosities were observed in the vicinity of dopaminergic dendrites. Electrically evoked 5-HT release was dependent on extracellular Ca²⁺ and prevented by NaV₁.₈-channel blockade. Extracellular 5-HT concentration was enhanced by inhibition of uptake transporters for 5-HT but not dopamine. Selective H₂R agonists (R)-(−)-α-methyl-histamine or immepip inhibited evoked 5-HT release by up to 60%. This inhibition was prevented by the H₂R antagonist thioperamide but not by the 5-HT₁B receptor antagonist ipsapirone. H₂R inhibition of 5-HT release prevailed in the presence of GABA or glutamate receptor antagonists (ionotropic and metabotropic), suggesting minimal involvement of GABA or glutamate synapses. The potent regulation of 5-HT by H₂Rs reported here not only elucidates HA function in the SNr but also raises the possibility of novel targets for basal ganglia therapies.

Key words: histamine; 5-hydroxytryptamine; basal ganglia; substantia nigra; fast-scan cyclic voltammetry; Parkinson’s disease

Introduction

The substantia nigra pars reticulata (SNr) is a strategic point for integration of inputs from the “direct” and “indirect” pathways of the basal ganglia (Alexander and Crutcher, 1990). It also provides a major output projection from the basal ganglia to the thalamus, where it influences the activity of the thalamocortical relay. Among the inputs to the SNr is a projection from histamine (HA)-containing neurons of the tuberomammillary nucleus (Panula et al., 1989). Although there is limited information on the actions of HA in the SNr, HA has been implicated in the modulation of GABA release at striatonigral synapses (Garcia et al., 1997) and in the local regulation of motor functions (Garcia-Ramirez et al., 2004). In Parkinson’s disease (PD), there is an increase in HA levels and in the varicosity size and density of HA fibers in the SN (Anichchik et al., 2000a). Furthermore, H₂R binding density is elevated in the SN in PD (Ryu et al., 1994; Anichchik et al., 2000b, 2001) and in rats after 6-OHDA lesions (Ryu et al., 1994; Anichchik et al., 2000b, 2001).

Three HA receptors have been identified in the CNS, of which two, H₂ and H₃, are found at high densities in the basal ganglia (Vizuete et al., 1997; Pillot et al., 2002). H₃ binding sites are particularly abundant in the SNr, but there is only low expression of the mRNA for H₂Rs (Pillot et al., 2002). These findings indicate that H₂Rs are principally expressed by afferents to and not local projection neurons within the SN. Regions that express H₂R mRNA and project to the SNr include the striatum, subthalamic nucleus, and raphe nuclei but not the dopaminergic neurons in the substantia nigra pars compacta (SNc)–ventral tegmental area (Pillot et al., 2002).

The H₂R is predominantly a presynaptic receptor that has been shown to inhibit release of HA (Arrang et al., 1983), glutamate (Brown and Reymann, 1996; Brown and Haas, 1999; Molina-Hernandez et al., 2001), GABA (Garcia et al., 1997; Yamamoto et al., 1997; Arias-Montano et al., 2001), norepinephrine (Schlicker et al., 1989, 1992, 1999), dopamine (DA) (Schlicker et al., 1993), acetylcholine (Arrang et al., 1995; Giorgetti et al., 1997; Prast et al., 1999), and 5-hydroxytryptamine (5-HT) in cortex (Schlicker et al., 1988; Fink et al., 1990). The present study was designed to elucidate the role of H₂ receptors (H₂Rs) in the SNr. We initially used immunohistochemical techniques to establish whether there is a comparable distribution of histaminergic and serotoninergic processes in the SNr. We then used fast-scan cyclic voltammetry (FCV) at carbon-fiber microelectrodes (CFMs) to monitor in real time the modulation of electrically evoked 5-HT release in the SNr by H₂R ligands.
Materials and Methods

Tissue preparation for immunohistochemistry. Adult male Wistar rats (150–180 g; Charles River Laboratories, Isaszeg, Hungary) were maintained for 1 week in a light- and temperature-controlled environment (lights on 5:00 A.M. to 7:00 P.M.; 22 ± 1°C) and allowed access to food and water ad libitum. To enhance detection of histamine-containing processes, synthesis of brain histamine was stimulated (Schwartz et al., 1972; Prell et al., 1996) by L-histidine loading (6 mM, i.p.; n = 3) 1 hr before transcardial perfusion with 50 ml of phosphate buffered 4% 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDCDI; Sigma, Poole, UK) under pentobarbital anesthesia (80 mg/kg); the brains were removed and immersed in EDCDI (4 d) followed by 2% paraformaldehyde (PFA) (1 d). For the immunohistochemical detection of serotonergic processes, animals (n = 3) were perfused transcardially with 4% PFA in 0.1 M PBS under pentobarbital anesthesia (80 mg/kg); the brains were removed and postfixed in 2% PFA. All brains were infiltrated in 30% sucrose overnight; midbrain sections (25 μm) were cut in the coronal plane using a freezing microtome (Leica, Vienna, Austria).

Immunohistochemistry. Pretreatment of sections included sequential incubation in 0.5% Triton X-100 (30 min), 0.5% H2O2 (10 min), and 2% normal horse serum (30 min). The procedure used to detect histamine-immunoreactive processes required EDCDI fixation (Panula et al., 1984). Serotonergic processes were identified with antibodies against the serotonin transporter (SERT) in PFA-fixed sections. Tyrosine hydroxylase (TH) immunoreactivity (IR) served to identify dopamine-containing structures following either form of fixation.

For double-label immunohistochemical detection of HA-IR together with TH-IR, sections were initially incubated in rabbit anti-histamine (Panula et al., 1984) (1:12,000) for 72 hr at 4°C. Biotinylated donkey anti-rabbit IgG (1:1000; Jackson Laboratories, Bar Harbor, ME) and the ABC solution (1:1000; Vector Laboratories, Burlingame, CA) were used before amplification (Adams, 1992) with biotinylated tyramide (1:1000; Vector Laboratories). Antibodies were visualized using Ni-DAB as described previously (Kallo et al., 1994, 1998). The TMB+NiDAB method was applied for 48 hr at 4°C and detected by Alexa 488 as a counterstain. 5-HT immunoreactivity (IR) was visualized using Alexa 350 conjugated to goat anti-mouse IgG (1:500, overnight, at 4°C, Molecular Probes).

For double-label immunohistochemical detection of SERT together with TH-IR, sections were initially incubated in rabbit anti-histamine (Panula et al., 1984) (1:12,000) for 72 hr at 4°C. Biotinylated donkey anti-rabbit IgG (1:1000; Jackson Laboratories, Bar Harbor, ME) and the ABC solution (1:1000; Vector Laboratories, Burlingame, CA) were used before amplification (Adams, 1992) with biotinylated tyramide (1:1000); sections were then incubated overnight at 4°C in Alexa 594 streptavidin (1:500; Molecular Probes, Eugene, OR). Subsequently, mouse anti-TH [monoclonal antibody (mAb) 22941, 1:1000; Incstar, Stillwater, MN] was applied for 48 hr at 4°C; this was visualized with Alexa 350 conjugated to goat anti-mouse IgG (1:500, overnight, at 4°C; Molecular Probes).

Electrical stimulation. Concentric bipolar stimulating electrodes (FHC, Bowdoinham, ME) were used to electrically evoke 5-HT release in the SN. The stimulating electrode was positioned on the tissue surface −50–100 μm from the CEM with the aid of a binocular microscope. Stimulus pulses (0.2 msec duration; 0.6 mA) were applied in trains of 20 pulses at 100 Hz at repeat intervals of 5 min; by this time, release is fully reproducible and can be maintained for ≥2 hr. In initial experiments to characterize 5-HT release, the dependence on pulse frequency and number was verified using either trains of 20 pulses at 10–200 Hz in random order or 1–50 pulses at 100 Hz.

Drugs. Drugs were dissolved in water, aqueous alkali [(S)-(-)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (MCPG)], or acid [4-(8-methyl-9H-3-dioxolo[4,5-b] [2,3]benzodiazepin-5-yl)-benzenemine hydrochloride (GYK 52466)] to make stock aliquots at 10000× or 10,000× final concentrations and stored at −20°C until required. Stock solutions were diluted with ACSF to final concentration immediately before use and perfused for >7 min before data were included in drug analysis. t-AP-5, GYK 52466 hydrochloride, tetrodotoxin (TTX), saxofofen, (S)-MCPG, citraloprom hydrobromide, isomaltane hemifumarate, iminepy dihydrobromide, and thioperoxidase maleate were obtained from Tocris Cookson (Bristol, UK). 1-[2-(4-fluorophenyl)methoxy]ethyl-4-(3-phenylpropyl)piperazinae dihydrochloride (GRB 12909) was obtained from Research Biochemicals International (Natick, MA). All other compounds were obtained from Sigma.

Data analysis and statistics. Data were acquired and analyzed using Strathclyde Whole Cell Program (University of Strathclyde, Glasgow, Scotland, UK). Current sampled at the 5-HT oxidation peak potential was measured from the baseline of each voltammogram to provide profiles of [5-HT]o versus time. This procedure minimizes inclusion of contributions from other electroactive and nonelectroactive species to the neurotransmitter oxidation current (Rice and Nicholson, 1989; Venton et al., 2003). Illustrated data represented are means ± SEM (n = number of observations). Three to five animals were used in each experiment. Illustrated voltammograms have background current subtracted. Comparisons for differences in means were assessed for parametric data by one-way ANOVA followed by post hoc multiple comparison t tests (Dunn’s); nonparametric or normalized data were compared by unpaired Mann–Whitney U tests (M-WU) or where appropriate by Kruskal–Wallis variance analysis followed by Dunn’s t tests. Concentration–response
Identification of HA and 5-HT immunoreactivity in SNr

In sections from rats perfused with EDCDI, abundant processes immunoreactive for HA or TH were observed in the SNr (Fig. 1C). After PFA fixation, processes immunoreactive for SERT were found throughout the SNr (Fig. 1D,E). As in the case of HA-IR, SERT-immunoreactive varicosities were found in the vicinity of TH-immunoreactive processes (Fig. 1E). By reference to TH-immunopositive processes, we conclude that within the SNr, there is similarity in the distribution of putative terminal fields containing HA or 5-HT. Analysis of the direct ultrastructural interactions between HA-IR and 5-HT-IR is not currently feasible because of the different methodological conditions required to reveal them.

Electrochemical characterization of 5-HT release in SNr

Electrical stimulation (20 pulses, 100 Hz) in SNr evoked the release, followed by the rapid removal, of an electroactive substance that could be identified as 5-HT. The voltammogram of the released substance was readily identified as 5-HT because of the different methodological conditions required to reveal them.

Ionic and depolarization sensitivity

Evoked release of 5-HT was apparently abolished by inhibition of voltage-gated Na⁺ channels (Naᵥ) by TTX (1 μM) (Fig. 3C).
H3R effects on 5-HT release in SNr

Mean peak evoked [5-HT]o (20 pulses at 100 Hz) was significantly attenuated by bath application of selective H3R agonists (R)-(-)-α-methylhistamine (R-mHA) and immepip (Fig. 4A, B) (Kruskal-Wallis, p < 0.001; n = 14–15). Each drug demonstrated a concentration-dependent inhibition of 5-HT release of up to 49% ± 5% of control by R-mHA (1 μM; n = 14) and to 39% ± 3% by immepip (5 μM; n = 15) with IC50 values of 23 nM (R-mHA; 95% confidence interval range, 13–43 nM; Hill slope, −1.1) and 43 nM, respectively (immepip; 95% confidence interval range, 20–95 nM; Hill slope, −0.73, not significantly different from 1.0). These IC50 values were not significantly different from one another (p > 0.05).

To confirm that the actions of these H3R agonists were attributable to selective agonism at H3Rs, their effects were assessed in the presence of a selective H3R antagonist, thiopermane. Whereas R-mHA (100 nM) reduced mean peak [5-HT]o from 100 ± 2 to 52 ± 4% without thiopermane (Fig. 5A) (M-WU; p < 0.001), R-mHA did not modify mean peak [5-HT]o in the presence of thiopermane (Fig. 5A) (1 μM; 108 ± 13% in thiopermane only vs 94 ± 6% in thiopermane plus R-mHA; n = 19–25; M-WU; p > 0.05). Similarly, immepip (1 μM) reduced mean peak [5-HT]o from 100 ± 3 to 52 ± 4% without thiopermane (Fig. 5B) (M-WU; p < 0.001) but did not modify [5-HT]o in the presence of thiopermane (Fig. 5B) (10 μM; 108 ± 5% in thiopermane only vs 104 ± 6% in thiopermane plus immepip; n = 16–22; M-WU; p > 0.05). In contrast, the effect of R-mHA (1 μM) was not prevented by the presence of a 5-HT1B receptor antagonist, isomaltane (Fig. 5C) (1 μM; 111 ± 11.0% in isomaltane only vs 54 ± 7% in isomaltane plus R-mHA; M-WU; p < 0.001; n = 12–21) at a concentration of isomaltane previously observed to block 5-HT1B agonist effects (Davidson and Stamford, 1996).

Direct or indirect H3R effects?

Studies were undertaken to explore whether H3R-mediated inhibition of 5-HT release might be attributable to H1Rs present on GABAergic or glutamatergic inputs to 5-HT terminals rather than on the 5-HT terminals themselves. The effects of the H3R agonist R-mHA were investigated in the presence of mixtures of either GABA or glutamate receptor antagonists (ionotropic and metabotropic). Despite the application of the GABA receptor antagonists picrotoxin (PTX; GABAA receptor antagonist, 100 μM) and saclofen (GABAB receptor antagonist, 50 μM), R-mHA
immunocytochemistry showed that serotonergic processes are abundant throughout the SNr. Second, the voltammograms resulting from the evoked release indicated that the released substance was identical in its electrochemical profile to the indoleamine 5-HT and not to the catecholamine DA. Third, inhibition of the SERT but not the DAT prolonged the extracellular lifetime and enhanced the peak amplitude of the 5-HT-like signal. These observations indicate that the electrochemical signal was attributable to 5-HT, with negligible contribution from DA, and confirm that the SERT participates in 5-HT, regulation in the SNr as reported previously (Iravani and Kruk, 1997; Bunin et al., 1998).

**H$_3$R control of 5-HT release in the SNr**

In the present study, the evoked release of 5-HT in SNr was potently inhibited by the H$_3$R agonists R-mHA or immepip to levels that were <50% of those seen in controls. This attenuation of 5-HT release was dose dependent, with IC$_{50}$ values for R-mHA and immepip that were consistent with previously reported values for H$_3$R efficacy in guinea pig ileum (Hew et al., 1990) and inhibition of striatal glutamate release (Molina-Hernandez et al., 2001), respectively. The inhibition of 5-HT release in SNr by activation of H$_3$Rs was confirmed further by experiments with the selective H$_3$R antagonist thioperamide. Previous application of thioperamide prevented the inhibition of 5-HT release by R-mHA or immepip. In contrast, the action of these H$_3$R agonists was not modified by antagonism of 5-HT$_1B$ receptors, a 5-HT receptor type that has been shown to participate in presynaptic autoreceptor control of 5-HT release elsewhere in the brain (Barnes and Sharp, 1999). This observation indicates that the actions of the H$_3$ agonists were not attributable to effects on 5-HT autoreceptors.

The potent inhibition of 5-HT signaling by H$_3$Rs identifies a powerful role for these abundant receptors in the SNr (Pillot et al., 2002). Although H$_3$R-dependent regulation of 5-HT signal-
ing has been identified previously in rat cortex (Schlicker et al., 1988; Fink et al., 1990), the current data are the first to identify a comparable mechanism within the basal ganglia.

**H₃R on GABA and glutamate synapses**

To date, there is no anatomical information available regarding the specific identity of the neurons that express H₃Rs in the SNr and the ultrastructural location of these receptors. Thus, the synaptic circuitry by which H₃Rs detected in the SNr by radioligand binding (Pillot et al., 2002) can regulate 5-HT release is unidentified. Because in situ hybridization for mRNA for H₃Rs reveals only low expression within the SN (Pillot et al., 2002), the abundant H₃Rs in SN are unlikely to be expressed or localized on DA neurons in the SNc or on local or projection neurons within the SNr. However, moderate H₃R transcript expression has been identified in the raphe nuclei (Pillot et al., 2002); therefore, it is plausible that the H₃Rs are synthesized by 5-HT neurons and located on 5-HT terminals.

Because a similar level of H₃R mRNA expression has also been identified in the dorsal striatum, subthalamic nucleus, and pedunculopontine nucleus (Pillot et al., 2002), regions that send major non-5-HT projections (e.g., GABAergic or glutamatergic) to the SNr, we evaluated whether such inputs to the SNr might be involved in H₃R-mediated inhibition of 5-HT release. Previous evidence has indicated that H₃Rs in the SNr inhibit GABA release (Garcia et al., 1997; Garcia-Ramirez et al., 2004). The possibility that H₃Rs present on GABAergic or glutamatergic terminals in the SNr contribute to H₃R-mediated regulation of 5-HT release was investigated using receptor antagonists for GABA or glutamate. H₃R-mediated inhibition of 5-HT release was preserved in the presence of GABA–glutamate synaptic block; thus, a role for H₃Rs on GABA or glutamate terminals in this context seems unlikely. Furthermore, because in the absence of H₃R agonists neither GABA nor glutamate antagonists significantly modified [5-HT]₀, the control of 5-HT release in this paradigm does not appear to be under direct control by glutamate or GABA synapses. Whether the inhibitory action of H₃R agonists on GABA release in the SNr (Garcia et al., 1997; Garcia-Ramirez et al., 2004) is mediated via effects on 5-HT release remains to be tested.

The present results raise the possibility that the H₃R-mediated inhibition of 5-HT release is attributable to a direct action on 5-HT terminals via H₃Rs. This regulation of 5-HT release is noteworthy in light of reports indicating little, if any, regulation of 5-HT release in the SNr by 5-HT₁B autoreceptors (Nissbrandt et al., 1992; Iravani and Kruk, 1997); their binding is increased in the SNr of PD patients with L-DOPA-induced dyskinesias (Nicholson and Brotchie, 2002). 5-HT₂C receptors on SNr projection neurons are excitatory (Rick et al., 1995); their binding is increased in the SNr of PD patients with L-DOPA-induced dyskinesias, suggesting a possible compensatory upregulation of receptors in response to decreased endogenous ligand (Fox and Brotchie, 2000). In this context, it should be noted that the selective serotonin reuptake inhibitor, fluoxetine, reduces dyskinesias in PD patients (Durif et al., 1995).

The capacity of HA to regulate 5-HT transmission in the SNr via H₃Rs may provide an attractive nondopaminergic target for improving therapies for PD. Identifying novel approaches to manipulate 5-HT neurotransmission may help to enhance the efficacy of current treatments not only for PD but also for mood disorders such as depression and anxiety, in which serotonergic manipulation is pivotal.

**References**


