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Histamine H_3 receptor activation inhibits dopamine synthesis but not release or uptake in rat nucleus accumbens

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ABSTRACT

We studied the effect of activating histamine H_3 receptors (H_3Rs) on rat nucleus accumbens (rNAcc) dopaminergic transmission by analyzing [^{3}H]-dopamine uptake by synaptosomes, and dopamine synthesis and depolarization-evoked [^{3}H]-dopamine release in slices. The uptake of [^{3}H]-dopamine by rNAcc synaptosomes was not affected by the H_3R agonist RAMH (10^{-10}–10^{-6} M). In rNAcc slices perfusion with RAMH (1 μM) had no significant effect on [^{3}H]-dopamine release evoked by depolarization with 30 mM K^+ (91.4 ± 4.5% of controls). The blockade of dopamine D_2 autoreceptors with sulpiride (1 μM) enhanced K^+-evoked [^{3}H]-dopamine release (168.8 ± 15.5% of controls), but under this condition RAMH (1 μM) also failed to affect [^{3}H]-dopamine release. Dopamine synthesis was evaluated in rNAcc slices incubated with the L-dihydroxyphenylalanine (DOPA) decarboxylase inhibitor NSD-1015 (1 mM). Forskolin-induced DOPA accumulation (220.1 ± 10.4% of controls) was significantly reduced by RAMH (41.1 ± 6.5% and 43.5 ± 9.1% inhibition at 100 nM and 1 μM, respectively), and this effect was prevented by the H_3R antagonist ciproxifan (10 μM). DOPA accumulation induced by preventing cAMP degradation with IBMX (iso-butyl-methylxantine, 1 mM) or by activating receptors for the vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase-activating peptide (PACAP) with PACAP-27 (1 μM) was reduced (IBMX) or prevented (PACAP-27) by RAMH (100 nM). In contrast, DOPA accumulation induced by 8-Bromo-cAMP (1 mM) was not affected by RAMH (100 nM). These results indicate that in rNAcc H_3Rs do not modulate dopamine uptake or release, but regulate dopamine synthesis by inhibiting cAMP formation and thus PKA activation.

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1. Introduction

The nucleus accumbens (NAcc) plays a key role in the neural systems responsible for translating motivation derived from limbic regions to goal-directed behaviors (Sesack and Grace, 2010). At least 95% of NAcc neuronal cells are GABAergic medium spiny neurons (MSNs) with the rest of the neuronal population represented by GABAergic and cholinergic interneurons. NAcc MSNs expressing dopamine D_1-like receptors innervate the ventral tegmental area (VTA) via the so-called direct pathway whereas in the indirect pathway MSNs expressing D_2-like receptors (D_2Rs) project to GABAergic neurons located in the ventral pallidum that in turn send projections to the VTA (Russo and Nestler, 2013). The NAcc also sends afferents to hypothalamus, brainstem, globus pallidus and *substantia nigra pars reticulata* (SNr). In turn the latter two nuclei innervate the mediodorsal and other thalamic divisions, thus completing cortico-NAcc-pallidal/SNr-thalamocortical loops (Sesack and Grace, 2010).

The NAcc is innervated by excitatory glutamatergic afferents from cerebral cortex, amygdala, hippocampus and thalamus, and inhibitory GABAergic afferents from ventral pallidum (Humphries and Prescott, 2010; Sesack and Grace, 2010; Russo and Nestler,

Abbreviations: D_2R, dopamine D_2 receptor; DOPA, L-dihydroxyphenylalanine; GABA, γ-aminobutyric acid; H_3R, histamine H_3 receptor; MSNs, medium spiny neurons; RAMH, (R)(-)-α-methylhistamine; rNAcc, rat nucleus accumbens; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

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2013). The Nacc also receives modulatory afferents from the brain stem, including dopaminergic projections from the VTA, serotonergic fibers from the dorsal raphe nucleus, a small noradrenergic projection from the *locus coeruleus* and the nucleus *tractus solitarii*, and sparse projections from the pedunculopontine tegmentum, the parabrachial nucleus and the periaqueductal gray (Beckstead et al., 1979; Sesack and Grace, 2010).

Dopaminergic axons innervating the NAcc release their neurotransmitter in response to reward-related stimuli, and alterations in accumbal dopaminergic synaptic transmission have been implicated in the acquisition, maintenance and relapse of addiction (Koob and Volkow, 2010; Sesack and Grace, 2010).

In the mammalian brain histamine acts as a neuromodulator through the activation of three (H_1, H_2 and H_3) of the four G protein-coupled receptors cloned so far (Panula and Nuutinen, 2013). The rat NAcc (rNAcc) receives histaminergic innervation (Inagaki et al., 1988) and possesses a high density of both histamine H_3 receptors (H_3Rs) and the corresponding mRNA (Pillot et al., 2002). H_3Rs are primarily expressed on nerve terminals, where they regulate the synthesis and release of histamine as well as the release of other neuroactive substances (for reviews see Feuerstein, 2008; Osorio-Espinoza et al., 2010; Panula and Nuutinen, 2013). Dopaminergic neurons located in *sustantia nigra pars compacta* (SNc) and that innervate the striatum (Ikemoto, 2007) express H_3R mRNA (Pillot et al., 2002), and in mouse striatal slices H_3R activation inhibited depolarization-evoked [^{3}H]-dopamine release (Schlicker et al., 1993). However, the virtual absence of H_3R mRNA in the rat VTA reported by Pillot et al. (2002) did not support a similar action in the rNAcc.

The rNAcc derives its dopaminergic innervation not only from the VTA but also from the dorsal tier of the SNc and the A8 (retrorubral) cell group (Hasue and Shammah-Lagnado, 2002; Bjorklund and Dunnett, 2007). Moreover, recent data by González-Sepúlveda et al., (2013) using an enhanced probe showed that VTA dopaminergic neurons co-express mRNAs coding for the H_3R and tyrosine hydroxylase (TH), the limiting enzyme in dopamine synthesis, as well as immunoreactivity for the corresponding proteins. This evidence led us to examine the effect of H_3R activation on rNAcc dopaminergic transmission by analyzing [^{3}H]-dopamine uptake by synaptosomes and [^{3}H]-dopamine release and dopamine synthesis in slices.

A preliminary account of this work was presented in the abstract form to the European Histamine Research Society (Aquino-Miranda et al., 2014).

2. Materials and methods

Rats (males, Wistar strain, 250–300 g, bred in the Cinvestav facilities) were used throughout the experiments. All procedures were in accordance to the guidelines for the care and use of laboratory animals issued by the National Institutes of Health (NIH Publications No. 8023, revised 1978) and the Mexican Council for Animal Care, as well as approved by the Cinvestav Animal Care Committee. All efforts were made to minimize animal suffering and to use only as many animals were required for proper statistical analysis.

2.1. Preparation of rNAcc slices

Animals were decapitated, the brain was quickly removed from the skull, immersed in ice-cold Krebs-Henseleit (KH) solution and coronal slices (300 μm thick) were obtained with a vibratome (World Precision Instruments, Sarasota, FL, USA). The NAcc was carefully dissected from the slices, with special care to avoid the adjacent striatum which contains high levels of H_3Rs. The composition of the KH solution was (mM): NaCl, 116; KCl 3, $MgSO_4$, 1; KH_2PO_4, 1; $NaHCO_3$, 25; D-glucose, 11; pH 7.4 after saturation with O_2/CO_2 (95:5% v:v). In order to reduce excitoxicity, $CaCl_2$ was not added to this solution.

2.2. Synaptosome preparation

Synaptosomes were prepared using a modification of the method of Gray and Whittaker (1962). Briefly, NAcc slices from 5 rats were placed in 5 ml 0.32 M sucrose/5 mM Hepes (pH 7.4 with NaOH) and homogenized using 10 strokes of a hand-held homogenizer. The homogenate was brought up to 15 ml with 0.32 M sucrose/5 mM Hepes and centrifuged at 1,000 × *g* (10 min, 4 °C). The supernatant was collected, adjusted to 20 ml with 0.32 M sucrose/5 mM Hepes and centrifuged at 20,000 × *g* (20 min, 4 °C). The pellet was resuspended in 7 ml 0.32 M sucrose/5 mM Hepes, layered onto 20 ml 0.8 M sucrose/5 mM Hepes and centrifuged at 20,000 × *g* for 20 min (4 °C). The pellet (synaptosomes) was used for binding or uptake experiments as described below.

2.3. Binding of N-α-[methyl-^{3}H]-histamine ([^{3}H]-NMHA) to synaptosomal membranes

2.3.1. Obtention of membranes

The synaptosomal pellet was resuspended in 20 ml of lysis solution (10 mM Tris–HCl, 1 mM EGTA, pH 7.4) and incubated for 20 min at 4 °C. The suspension was then centrifuged (32,000 × *g*, 20 min, 4 °C) and the resulting pellet (synaptosomal membranes) was resuspended in incubation buffer (50 mM Tris–HCl, 5 mM $MgCl_2$, pH 7.4).

2.3.2. [^{3}H]-NMHA binding assay

For saturation analysis membranes (~20 μg protein aliquots; BCA assay) were incubated in 100 μl incubation buffer containing [^{3}H]-NMHA (0.01–10 nM), whereas for inhibition experiments incubations contained [^{3}H]-NMHA (3 nM) and increasing concentrations (10^{-11}–10^{-6} M) of H_3R ligands. Equilibration was for 60 min at 30 °C and terminated by filtration through Whatman GF/B glass fiber paper, pre-soaked in 0.3% polyethylenimine. Nonspecific binding was determined as that insensitive to 10 μM histamine and accounted for ~30% of total binding. Filters were soaked in 3 ml scintillator and the tritium content was determined by scintillation counting.

Saturation binding data were fitted to a hyperbola by non-linear regression with GraphPad Prism 5 (Graph Pad Software, San Diego, CA, USA). Inhibition curves were fitted to a logistic (Hill) equation and values for inhibition constants (*K*i) were calculated according to the equation (Cheng and Prusoff, 1973): $Ki = IC_{50}/1 + \{[D]/K_d\}$, where [D] is the concentration of [^{3}H]-NMHA present in the assay and K_d the mean value for the equilibrium dissociation constant estimated from the saturation analysis (1.32 nM, see Results).

2.4. Uptake of [^{3}H]-dopamine by rNAcc synaptosomes

Synaptosomes were resuspended in Krebs-Ringer-Hepes (KRH) buffer and aliquoted (140 μl) into plastic tubes. Drugs under test were added in a 10-μl volume and equilibrated for 10 min at 37 °C before the addition of [^{3}H]-dopamine in a 50-μl volume to yield 50 nM as the final concentration. After 30 min at 37 °C, incubations were filtered through Whatman GF/B glass fiber paper, pre-soaked for 2 h in 0.3% polyethylenimine. Filters were washed 3 times with ice-cold KRH buffer, soaked in 3 ml scintillator and the tritium content was determined by scintillation counting. Nonspecific uptake was determined in samples incubated at 4 °C or in the presence of 1 μM GBR-12909. The composition of the KRH buffer was