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Histamine H_3 and dopamine D_2 receptor-mediated [^{35}S]GTPγ[S] binding in rat striatum: Evidence for additive effects but lack of interactions

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ABSTRACT

The interactions in the rat striatum between H_3 receptors (H_3Rs) and D_2 receptors (D_2Rs) were investigated with the [^{35}S]GTPγ[S] binding assay. The H_3R agonist (*R*)α-methylhistamine increased [^{35}S]GTPγ[S] binding to striatal membranes with an $EC_{50} = 14 \pm 5$ nM and a maximal effect of $+19 \pm 1\%$. This effect was inhibited by the H_3R antagonist ciproxifan with a $K_i = 1.0 \pm 0.3$ nM. The D_2R agonist quinpirole increased [^{35}S]GTPγ[S] binding to the same membranes with an $EC_{50} = 1.5 \pm 0.5$ μM and a maximal effect of $+28 \pm 2\%$. Its effect was blocked by haloperidol with a $K_i = 0.3 \pm 0.1$ nM. The maximal effects of the H_3R and D_2R agonists were additive ($+46 \pm 3\%$). However, D_2R ligands did not modify the effects of H_3R ligands and vice versa. Ciproxifan behaved as an H_3R inverse agonist and decreased [^{35}S]GTPγ[S] binding. Haloperidol had no effect and did not change the inverse agonist effect of ciproxifan. Administrations for 10 days of ciproxifan (1.5 mg/kg/day) or haloperidol (0.5 mg/kg/day) did not change the effects of quinpirole and (*R*)α-methylhistamine, respectively. These data suggest that striatal H_3Rs and D_2Rs do not interact through their coupling to G-proteins. However, a hyperactivity of histaminergic and dopaminergic neurons being observed in schizophrenia, the additive activations of H_3Rs and D_2Rs suggest that they cooperate to generate some schizophrenic symptoms. Such a postsynaptic mechanism may underlie the antipsychotic-like effects of H_3R inverse agonists and supports their therapeutic interest, alone or as adjunctive treatment with neuroleptics.

1. Introduction

The effects of histamine in the brain are mediated by three histamine receptor subtypes (H_1, H_2 and H_3), which have been defined by means of functional assays followed by design of selective agonists and antagonists and, more recently, cloning of their genes [1,2]. All three belong to the superfamily of G-protein-coupled receptors with seven transmembrane domains. We initially detected and identified the H_3 receptor (H_3R) by traditional pharmacological approaches as an autoreceptor controlling histamine synthesis and release in the rat and human brain [3–5]. The inhibition of adenylate cyclase and activation of phospholipase A_2 mediated by the recombinant receptor in various cell lines [6–9], as well as the sensitivity of responses mediated by H_3Rs in the brain [10,11], indicated that the H_3R is coupled to $G_{i/o}$ proteins.

The inhibition mediated by H_3 autoreceptors is now well established as a major control mechanism for the activity and functions of histaminergic neurons under physiological conditions [4,12]. H_3R antagonists/inverse agonists enhance

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cerebral histamine turnover and release *in vivo*, indicating that autoreceptors are tonically activated [2] and their intensive use during the recent years has considerably clarified the functional roles of histaminergic neurons. Behavioural studies using imidazole or non-imidazole H_3R antagonists/inverse agonists have confirmed the role played by endogenous neuronal histamine in modulation of wakefulness [13], cognition and attention [14], food intake [15] and seizures [16].

More recently, several behavioural studies also support antipsychotic-like properties of H_3R antagonists/inverse agonists [17]. The locomotor activation elicited in rodent by dopaminergic agonists and the NMDA-receptor antagonist MK-801 is attenuated by standard and more recent H_3R antagonists/inverse agonists [18–21] (see Ligneau et al. 2007). Also stereotypies induced in mice by methamphetamine and apomorphine are significantly decreased by ciproxifan [22] as well as by the non-imidazole inverse agonist BF2.649 [23] (see Ligneau et al. 2007). Consistent with these findings, the effect of methamphetamine on locomotor activity and stereotypic behaviour was less pronounced in H_3R knockout mice [24]. H_3R antagonists/inverse agonists also improve sensorimotor gating deficits, which are cardinal signs of schizophrenia, such as those which occur naturally in DBA/2 mice, or which are induced in Swiss mice by apomorphine, as shown by the increase that they induce in prepulse inhibition of startle and N40 auditory-evoked-response [20,25] (see Ligneau et al. 2007).

The neurochemical mechanisms underlying these antipsychotic-like effects induced by H_3R blockade remain unknown. The enhancement of histamine neuron activity induced by H_3R antagonists/inverse agonists may be involved inasmuch as histidine loads or inhibitors of histamine catabolism have also been reported to reduce methamphetamine-induced stereotypies [26,27]. However, high H_3R densities are present on many perikarya and/or dendrites of intrinsic neurons in the cerebral cortex, striatal complex and limbic areas [28,29].

In the striatum, the dense and homogeneous distribution of H_3Rs in the caudate-putamen and nucleus accumbens as well as double labelling experiments indicate that postsynaptic H_3Rs are coexpressed with D_1 or D_2 receptors [28,29]. H_3R activation inhibits D_1-receptor-mediated cAMP formation in the rat striatum [30] and the expression of H_3Rs is influenced by endogenous activation of D_1 receptors [31]. The existence of putative direct interactions between postsynaptic H_3Rs and D_2Rs remains unclear. Complex and/or controversial neurochemical and behavioural interactions have been reported between H_3R antagonists/inverse agonists and neuroleptics. We recently found that the imidazole derivative ciproxifan potentiated the enkephalin, neurotensin and c-fos expression induced in rat caudate-putamen and nucleus accumbens by haloperidol [29]. In contrast, thioperamide, another imidazole compound, has been found to decrease haloperidol-induced c-fos expression in the rat dorsolateral striatum but not in the nucleus accumbens [32]. Ciproxifan and thioperamide potentiated haloperidol-induced catalepsy in the rat [29,33] but not in the mouse [34]. Although it was totally suppressed by co-administration of (*R*)α-methylhistamine [29], the potentiation of catalepsy induced by ciproxifan in the rat, was suggested to result at least partially from an inhibition of cytochrome P450 enzymes by imidazole derivatives [35] and two non-imidazole H_3R antagonists/inverse agonists tended to attenuate risperidone-induced catalepsy [33].

Consistent with an involvement of histaminergic systems in schizophrenia, the existence of a hyperactivity of histamine neurons has been reported not only in several animal models of the disease [19,21,36], but also in schizophrenic patients [37]. These findings indicate that hyperactivity of dopaminergic transmission is associated with an enhanced activity of histaminergic neurons in the disease. Therefore, an enhanced activation of postsynaptic H_3Rs and D_2Rs present in the striatal complex is expected to occur in schizophrenia. In the present study, we have further investigated the putative interactions between striatal H_3Rs and D_2Rs by using the [^{35}S]GTPγ[S] binding assay applied to rat striatal membranes, a functional test system previously shown to be activated by both receptors [38,39].

2. Methods

2.1. Animals

Male adult Wistar rats (160–200 g, Janvier, France) were housed in a quiet room under a 12-h light/dark cycle (with lights on from 7 p.m.), with food and water available *ad libitum*. Animals were killed by decapitation and the brain removed and dissected out on a refrigerated plate.

2.2. [^{35}S]GTPγ[S] binding assay

Brain tissues from rats were homogenized with a Polytron in ice-cold buffer (Tris–HCl 50 mM, pH 7.4). Homogenates were centrifuged twice at 20,000 × *g* for 10 min and the final pellet was resuspended in 50 volumes of buffer. Membranes (20–50 μg) were pretreated with adenosine deaminase (ADA, 1 U/ml), and incubated for 60 mn at 25 °C with 0.1 nM [^{35}S]GTPγ[S] and, when required, the various drugs tested, in 1 ml of assay buffer (50 mM Tris–HCl, 50 mM NaCl, 5 mM $MgCl_2$, 10 μM GDP, 0.02% BSA, pH 7.4). The non-specific binding was determined using non-labelled GTPγS (10 μM). Incubations were stopped by rapid filtration under vacuum through Whatman GF/B filters. Filters were washed twice with 4 ml ice-cold water and the radioactivity retained on the filters was measured by liquid scintillation spectrometry.

2.3. [^{3}H]histamine binding assay

Brain tissues from rats were homogenized with a Polytron in ice-cold buffer (Na_2HPO_4/KH_2PO_4 50 mM, pH 7.5). Homogenates were centrifuged at 300 × *g* for 5 min. The supernatants were centrifuged at 12,000 × *g* for 30 min and the final pellet was resuspended in binding buffer. Aliquots of the membrane suspension (200–300 μg) were incubated for 60 mn at 25 °C with [^{3}H]histamine (20 nM, 1 ml final volume). The non-specific binding was determined using imetit (1 μM). Incubations were stopped by rapid filtration under vacuum through Whatman GF/B filters. Filters were washed twice with 4 ml ice-cold buffer and the radioactivity retained on the filters was measured by liquid scintillation spectrometry.