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Differential control of dopamine ascending pathways by serotonin$_{2B}$ receptor antagonists: New opportunities for the treatment of schizophrenia

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ABSTRACT

Recent studies suggest that the central serotonin$_{2B}$ receptor (5-HT$_{2B}$R) could be an interesting pharmacological target for treating neuropsychiatric disorders related to dopamine (DA) dysfunction, such as schizophrenia. Thus, the present study was aimed at characterizing the role of 5-HT$_{2B}$Rs in the control of ascending DA pathway activity. Using neurochemical, electrophysiological and behavioral approaches, we assessed the effects of two selective 5-HT$_{2B}$R antagonists, RS 127445 and LY 266097, on *in vivo* DA outflow in DA-innervated regions, on mesencephalic DA neuronal firing, as well as in behavioral tests predictive of antipsychotic efficacy and tolerability, such as phencyclidine (PCP)-induced deficit in novel object recognition (NOR) test, PCP-induced hyperlocomotion and catalepsy. Both RS 127445 (0.16 mg/kg, i.p.) and LY 266097 (0.63 mg/kg, i.p.) increased DA outflow in the medial prefrontal cortex (mPFC). RS 127445, devoid of effect in the striatum, decreased DA outflow in the nucleus accumbens, and potentiated haloperidol (0.1 mg/kg, s.c.)-induced increase in mPFC DA outflow. Also, RS 127445 decreased the firing rate of DA neurons in the ventral tegmental area, but had no effect in the substantia nigra pars compacta. Finally, both RS 127445 and LY 266097 reversed PCP-induced deficit in NOR test, and reduced PCP-induced hyperlocomotion, without inducing catalepsy. These results demonstrate that 5-HT$_{2B}$Rs exert a differential control on DA pathway activity, and suggest that 5-HT$_{2B}$R antagonists could represent a new class of drugs for improved treatment of schizophrenia, with an ideal profile of effects expected to alleviate cognitive and positive symptoms, without eliciting extrapyramidal symptoms.

1. Introduction

Schizophrenia is a major neuropsychiatric disorder characterized by three main groups of symptoms: positive (i.e. hallucinations, delusions), negative (i.e. social interaction deficits, blunted affect) and cognitive (i.e. working and reference memory deficits, executive function impairments, decreased vigilance) (Meltzer, 2013; Newman-Tancredi and Kleven, 2011). This multimodal symptomatology is classically related to an imbalance in central dopamine (DA) neurotransmission: positive symptoms would result from DA hyperfunction in the nucleus accumbens (NAc), whereas negative and cognitive symptoms would involve DA hypofunction in the frontal cortex (Newman-Tancredi and Kleven, 2011; Svensson, 2000). The pharmacological treatment of schizophrenia relies on the use of DA-D$_2$ receptor antagonists classified as typical and atypical antipsychotic drugs (APDs; Meltzer and Massey, 2011). Typical APDs, such as haloperidol and chlorpromazine, while effective in controlling positive symptoms, exhibit a

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marked propensity to induce extrapyramidal side effects (EPS), related to altered striatal DA activity (Schapira et al., 2006). Conversely, atypical APDs, of which clozapine is the prototype, display limited propensity to induce EPS along with a wider therapeutic spectrum covering positive, and to some extent, negative and cognitive symptoms (Meltzer and Massey, 2011).

It is well established that the therapeutic benefit of atypical APDs is related to their multi-target properties towards various neurotransmitter systems, involving, in particular, direct or indirect effects on various serotonin receptors (5-HTRs), especially 5-HT_{2A}Rs and 5-HT_{1A}Rs (Meltzer and Massey, 2011; Newman-Tancredi and Kleven, 2011). Interestingly, several atypical APDs display antagonist properties towards the central 5-HT_{2B}R (Abbas et al., 2009; Kiss et al., 2010; Shahid et al., 2009; Shapiro et al., 2003), which has been recently shown to participate in the control of DA neuron activity (Auclair et al., 2010; Devroye et al., 2015; Doly et al., 2008, 2009). Also, microdialysis studies in anesthetized rats, showing that 5-HT_{2B}R blockade decreases DA outflow in the NAc but has no effect in the striatum, suggested that 5-HT_{2B}Rs could contribute to the therapeutic benefit of atypical APDs, by achieving a differential control of subcortical DA pathway activity (Auclair et al., 2010). However, the functional significance of this interaction, as well as the possible role of 5-HT_{2B}Rs in the control of DA outflow in the medial prefrontal cortex (mPFC), remain unknown to date.

Thus, the present study, encompassing neurochemical, electrophysiological and behavioral approaches, aimed at assessing the functional role of 5-HT_{2B}Rs in the control of DA ascending pathways, by using two selective, potent and brain-penetrant 5-HT_{2B}R antagonists, RS 127445 and LY 266097 (Audia et al., 1996; Bonhaus et al., 1999). First, using intracerebral microdialysis in freely moving rats, we investigated the effect of 5-HT_{2B}R blockade on basal DA outflow in the NAc, the striatum and the mPFC. Second, using single unit extracellular recordings, we examined the influence of 5-HT_{2B}R blockade on the basal firing rate of DA neurons located in the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc). Third, the effects of 5-HT_{2B}R antagonists were assessed in behavioral models classically used to predict APDs ability to induce EPS (catalepsy test), and to alleviate positive [phencyclidine (PCP)-induced hyperlocomotion] and cognitive [PCP-induced deficit in novel object recognition (NOR)] symptoms of schizophrenia (Newman-Tancredi and Kleven, 2011). Finally, to provide a deeper insight into the pro-cognitive potential of 5-HT_{2B}R antagonists, the effect of RS 127445 on haloperidol-induced DA outflow in the mPFC was also assessed.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (IFFA CREDO, Lyon, France) weighing 280–350 g were used. Animals, housed in individual plastic cages were kept at constant room temperature (21 ± 2 °C) and relative humidity (60%) with a 12 h light/dark cycle (dark from 20:00 h) and had free access to water and food. For electrophysiological experiments, rats were housed two per cage and kept under standard laboratory conditions as above. Animals were acclimated to the housing conditions for at least one week prior to the start of the experiments. All experiments were conducted during the light phase of the light-dark cycle. Animals use procedures conformed to the International European Ethical Standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

The following compounds were used: the 5-HT_{2B}R antagonists RS 127445.HCl (2-amino-4-(4-fluoronaphth-1-yl)-6-isopropylpyrimidine hydrochloride) and LY 266097.HCl (1-[(2-Chloro-3,4-dimethoxyphenyl) methyl]-2,3,4,9-tetrahydro-6-methyl-1*H*-pyrido[3,4-*b*] indole hydrochloride), the atypical antipsychotic clozapine (8-Chloro-11-(4-methyl-1-piperazinyl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine), purchased from R&D Systems (Abingdon, UK); the non-competitive *N*-methyl-D-aspartate (NMDA)-R antagonist phencyclidine (PCP)·HCl (1-(1-Phenylcyclohexyl)piperidine hydrochloride), purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France), and the DA-D_2R antagonist haloperidol (4-[4-(*p*-chlorophenyl)-4hydroxypiperidino]-4′-fluorobutyrophenone) as the commercially available solution (Haldol 5 mg/ml, Janssen Pharmaceutica, Bersee, Belgium). All other chemicals and reagents were the purest commercially available (VWR, Strasbourg, France; Sigma-Aldrich).

2.3. Pharmacological treatments

RS 127445 was dissolved in a 0.3% Tween 80 distilled water solution or in a 20% hydroxypropyl-β-cyclodextrin distilled water solution for electrophysiological experiments; when administered locally (see Supplementary Fig. S1), it was first dissolved in a 0.3% Tween 80 distilled water solution to obtain a 500 μM concentration, and then further diluted to the required concentration with artificial cerebrospinal fluid just before use. LY 266097, PCP and clozapine were dissolved in distilled water. Haloperidol was diluted in distilled water. All drugs were administered intraperitoneally (i.p.), except haloperidol which was injected subcutaneously (s.c.).

In microdialysis experiments, when assessing DA outflow, dose-response studies were performed in the mPFC with increasing doses of RS 127445 (0.08–0.16 mg/kg) or LY 266097 (0.16–0.63 mg/kg). In the NAc and the striatum, the effect of a single dose of RS 127445 (0.16 mg/kg) was assessed. In an additional experiment, 0.16 mg/kg RS 127445 was administered 15 min before 0.1 mg/kg haloperidol. When assessing serotonin (5-HT) outflow in the mPFC (see Supplementary Fig. S2), the effect of a single dose of RS 127445 (0.16 mg/kg) or LY 266097 (0.63 mg/kg) was tested. Finally, in reverse microdialysis experiments (see Supplementary Fig. S1), RS 127445 was perfused, during the entire experimental period (120 min), *via* the dialysis probe into the NAc and the mPFC at increasing concentrations (0.1–1 μM). In electrophysiological experiments, 0.16 mg/kg RS 127445 was administered 5 min after vehicle injection. In catalepsy experiments, animals were treated with 0.16 mg/kg RS 127445, 0.63 mg/kg LY 266097 or 1 mg/kg haloperidol. In locomotor activity experiments, 0.16 mg/kg RS 127445 or 0.63 mg/kg LY 266097 were administered respectively 15 or 30 min before 5 mg/kg PCP. In NOR experiments, rats were treated with 2 mg/kg PCP twice daily for 7 days. On the testing day, after a 7-day washout period, RS 127445 was administered at 0.16 mg/kg, LY 266097 at 0.63 mg/kg and clozapine at 1 mg/kg.

Doses and pretreatment administration time of 5-HT_{2B}R antagonists were chosen according to previous studies reporting their efficacy to modulate DA outflow and DA-dependent behaviors (Auclair et al., 2010; Devroye et al., 2015). The doses of haloperidol were selected on the basis of previous studies reporting their ability to block central DA-D_2Rs in the rat brain (Lucas et al., 1997) and to induce an increase in mPFC DA outflow or catalepsy (Li et al., 2005; Lucas et al., 2000). The doses and injection procedures (acute or subchronic administration) of PCP were selected according to previous studies reporting its ability to induce hyperlocomotion or a strong deficit in the NOR test (Adams and Moghaddam, 1998; Horiguchi and Meltzer, 2012; Schlumberger et al., 2010). Finally,