Behavioural Brain Research 247 (2013) 217–226

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Effect of subtype-selective adenosine receptor antagonists on basal or haloperidol-regulated striatal function: Studies of exploratory locomotion and c-Fos immunoreactivity in outbred and A_{2A}R KO mice

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HIGHLIGHTS

- Low doses of theophylline and A2A receptor antagonists induce exploration in mice.
- These pharmacological agents can reverse antipsychotic-induce psychomotor slowing.
- A1 antagonists do not have the same therapeutic and stimulant properties.
- Behavioral responses parallel striatal neural activity as shown by cFos expression.
- A2ARKO mice are resistant to haloperidol-induced reduction of activity and running.

ARTICLE INFO

Article history:
Received 23 January 2013
Received in revised form 20 March 2013
Accepted 23 March 2013
Available online 1 April 2013

Keywords:
Behavioral activation
Psychomotor slowing
Dopamine
Accumbens
Theophylline

ABSTRACT

Behavioral activation is regulated by dopamine (DA) in striatal areas. At low doses, while typical antipsychotic drugs produce psychomotor slowing, psychostimulants promote exploration. Minor stimulants such as caffeine, which act as adenosine receptor antagonists, can also potentiate behavioral activation. Striatal areas are rich in adenosine and DA receptors, and adenosine A_{2A} receptors are mainly expressed in the striatum where they are co-localized with DA D_2 receptors. Adenosine antagonists with different receptor-selectivity profiles were used to study spontaneous or haloperidol-impaired exploration and c-Fos expression in different striatal areas. Because A_{2A} antagonists were expected to be more selective for reversing the effects of the D_2 antagonist haloperidol, A_{2A} receptor knockout (A_{2A}RKO) mice were also assessed. CD1 and A_{2A}RKO male mice were tested in an open field and in a running wheel. Only the A_1/A_{2A} receptor antagonist theophylline (5.0–15.0 mg/kg) and the A_{2A} antagonist MSX-3 (2.0 mg/kg) increased spontaneous locomotion and rearing. Co-administration of theophylline (10.0–15.0 mg/kg), and MSX-3 (1.0–3.0 mg/kg) reversed haloperidol-induced suppression of locomotion. The A_1 antagonist CPT was only marginally effective in reversing the effects of haloperidol. Although adenosine antagonists did not affect c-Fos expression on their own, theophylline and MSX-3, but not CPT, attenuated haloperidol induction of c-Fos expression. A_{2A}RKO mice were resistant to the behavioral effects of haloperidol at intermediate doses (0.1 mg/kg) in the open field and in the running wheel. A_{2A} receptors are important for regulating behavioral activation, and interact with D_2 receptors in striatal areas to regulate neural processes involved in exploratory activity.

1. Introduction

In rodents, locomotor activity is an innate exploratory behavior [1] regulated by a complex cascade of neurochemical interactions involving the basal ganglia and related brain areas. Dopamine (DA) is a key neurotransmitter in the regulation of behavioral activation, and it is well known that dopaminergic mechanisms play

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http://dx.doi.org/10.1016/j.bbr.2013.03.035

an important role in regulating locomotor activity [2]. In particular, nucleus accumbens (Nacb) DA has been clearly implicated in the regulation of many forms of exploratory behavior, including spontaneous, novelty-induced, food-induced, and drug-induced locomotion [3–8]. Administration of DA receptor antagonists, including both D_1 and D_2 selective compounds, decreases a variety of activities [4,9–12]. In contrast, psychostimulant drugs, by potentiating DA transmission, can facilitate behavioral activation [13–16].

Within the last few years, evidence has begun to emerge indicating that brain adenosine plays an important role in regulating the behavioral functions of the basal ganglia [17–19]. Striatal areas that are rich in DA, including neostriatum and Nacb, also have a high concentration of adenosine receptors [17,20]. Several subtypes of adenosine receptors are expressed in the brain, of which the A_1 and A_{2A} adenosine receptor subtypes are most prevalent in the basal ganglia. Moreover, A_{2A} receptors are expressed at very high levels in the striatum and Nacb [21–23], while A_1 receptors are expressed throughout the brain [22,24,25]. Nonselective adenosine receptor antagonists such as caffeine act as minor stimulants that enhance locomotor activity [26–28]. While the locomotor stimulant effects of A_1 antagonists appear to be more variable and may depend upon the selectivity of the particular drug used [27,29–32], pharmacological modulation of adenosine A_{2A} receptors more consistently increases locomotion [28,30,32]. In neostriatum, A_{2A} receptors are reported to interact with DA D_2 receptors that are co-localized with them on enkephalin-positive striatopalllidal neurons [19–21,33–35]. Thus, adenosine A_{2A} antagonists are being intensively studied for their potential antiparkinsonian effects [8,24,36–42].

There is considerable interest in the behavioral actions of drugs that modulate adenosine receptor function for their potential therapeutic effects on energy-related motivational symptoms. Caffeine is commonly consumed by humans to produce activation, providing "energy" and alertness [43,44]. Specific adenosine A_{2A} antagonists are been assessed also for the treatment of anergic symptoms such as psychomotor slowing [12,45–52] and fatigue, which are seen in patients with depression and other disorders. Moreover, DA D_2 antagonists decrease locomotion and interaction with the environment in rodents, and these effects could be also related to the psychomotor slowing that is induced in humans treated with antipsychotic drugs [53].

Because of the interest in identifying novel treatments for energy-related symptoms of many psychological and neurological disorders, it is important to characterize the effects of adenosine antagonists in both human clinical trials and animal models. Most of the preclinical studies of DA/adenosine interactions have been conducted in rats. However, studies of these pharmacological interactions in mice are important not only for establishing generalizations across multiple species [54], but more importantly, because genetic knockout models circumvent the intrinsic limitations of pharmacological agents with partial specificity, and these tools are widely available in mice.

In the present work we comprehensively studied in mice the locomotor stimulating properties of adenosine antagonists with different selectivity profiles for adenosine receptors, and their impact on the locomotor suppression induced by the DA D_2 receptor antagonist haloperidol. Thus, we characterized the activational effects of low doses of the non-selective adenosine antagonist theophylline, which is a metabolite of caffeine with fewer anxiogenic effects [55], as well as the A_1 antagonist CPT and the selective A_{2A} antagonist MSX-3, either alone or in combination with haloperidol. The impact of all these pharmacological manipulations on striatal and Nacb areas was studied using c-Fos expression as a marker of neural activity. Finally, we also determined if adenosine A_{2A}R KO mice are resistant to psychomotor slowing induced by haloperidol using two different paradigms: an open field (OF) and a running wheel (RW).

2. Materials and methods

2.1. Subjects

A total of 226 CD1 adult male mice (n = 7–10 per group) purchased from Harlan-Interfauna Ibérica S.A. (Barcelona, Spain) were 6 weeks old (25–30 g) at the beginning of the study. Male mice lacking the A_{2A} adenosine receptor type (N = 37) and wild-type (WT) (N = 34) littermates weighed 25–30 g at the beginning of the study (Universite Libre de Bruxelles, Brussels, Belgium), and were generated as previously reported [56,57] from a CD1 background. Mice were housed in groups of three or four per cage, with standard laboratory rodent chow and tap water available ad libitum. Subjects were maintained at 22 ± 2 °C with 12-h light/dark cycles (lights on at 13:00 h). All animals were under a protocol approved by the Institutional Animal Care and Use Committee of Universitat Jaume I, and all experimental procedures complied with European Community Council directive (86/609/ECC).

2.2. Pharmacological agents and selection of doses

All drugs were administered intraperitoneally (IP). Theophylline (TOCRIS Bioscience) was dissolved in 0.9% (w/v) saline (pH 7.4). MSX-3 ((*E*)-phosphoric acid mono-[3-[8-[2-(3-methoxphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] ester disodium salt) was synthesized at the laboratory of Dr. Christa Müller at the Pharmazeutisches Institut, Universität Bonn, in Bonn, Germany [58]. MSX-3 (free acid) was dissolved in 0.9% (w/v) saline (pH 7.4). CPT (8-cyclopentyltheophylline) (Sigma Química C.O), was dissolved in 0.9% (w/v) saline (pH 7.4). Haloperidol (Sigma Química C.O), a relatively selective DA D_2 family receptor antagonist, which was selected because it is a widely prescribed antipsychotic drug, was dissolved in a 0.3% tartaric acid solution in water (pH 4.0), which also was used as the vehicle control. Doses of all four drugs were taken from previous mouse and rat studies conducted in our laboratories [38,52].

2.3. Apparatus and testing procedures

OF locomotion. The OF arena consisted of a Plexiglas cylinder with translucent walls (30 cm in diameter and 30 cm high) and an opaque floor divided into four equal quadrants by two intersecting lines. Mice were handled repeatedly before the behavioral test, but were not pre-exposed to the OF. On the test day, treatments were administered acutely IP (haloperidol 50 min, and CPT, MSX-3 and theophylline, 20 min before test started). After these time intervals, animals were placed in the center of the cylinder and immediately observed for 15 min. The behavioral test room was illuminated with a soft light, and external noise was attenuated. Horizontal and vertical locomotion in the OF were simultaneously recorded and registered manually. For horizontal locomotion an activity count was registered each time the animal crossed from one quadrant to another with all four legs. A count of vertical locomotion was registered each time the animal raised its forepaws in the air higher than its back, or rested them on the wall.

RW locomotion. The automated RW consisted of a cage (32 cm × 15 cm × 13 cm) with a wheel (11 cm in diameter) inserted on top. Locomotor activity was registered by an electrical counter connected to the wheel. A completed turn of the wheel was registered as 4 counts. Animals placed in the cage had free access to the wheel. The session lasted 30 min.

c-Fos visualization and quantification. Free floating coronal sections (40 μm) were serially cut using a microtome cryostat (Weymouth, MA, USA). The specific method employed for c-Fos immunostaining and counting has been explained in detail in a previous study [52].

2.4. Statistical analyses

Number of horizontal and vertical locomotion counts was analyzed separately in all the experiments. Experiments 1–6 were analyzed using between-groups ANOVA followed by non-orthogonal planned comparisons using the overall error term [59], comparing vehicle to the other doses in experiments with no haloperidol, and the haloperidol plus vehicle treatment with each of the other treatment conditions (including the vehicle alone group) in the reversal studies. Since the behavior of animals receiving a single injection of vehicle or two separate injections of vehicle were not statistically different, and in order to reduce the total number of animals, only one vehicle group (represented in the graphs as a discontinuous line) was used for experiments A and B (horizontal) and C and D (vertical locomotion). The c-Fos counts in different brain areas were analyzed by a two-way (treatment × brain area) factorial ANOVA. STATISTICA 7 software was used for statistical analysis of the data. All data were expressed as mean ± SEM, and significance was set at $p < 0.05$.