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European Journal of Pharmacology 574 (2007) 39–48
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Lack of development of behavioral sensitization to methylphenidate in mice: Correlation with reversible astrocytic activation

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Received 6 March 2007; received in revised form 25 June 2007; accepted 26 June 2007
Available online 13 July 2007

Abstract

Methamphetamine is a powerfully addictive psychostimulant that dramatically affects the mammalian central nervous system. Methylphenidate has been shown to have psychostimulus effects similar to methamphetamine. In the present study, we compared several effects of methylphenidate to those of methamphetamine. The subcutaneous administration of either methamphetamine or methylphenidate increased extracellular dopamine levels in the nucleus accumbens of mice. Interestingly, methamphetamine, but not methylphenidate, also increased the extracellular serotonin levels in this area. Further, repeated treatment with methamphetamine induced the development of sensitization to hyperlocomotion, whereas methylphenidate failed to induce behavioral sensitization. Moreover, *in vitro* treatment with methamphetamine, but not methylphenidate, caused long-lasting astrocytic activation in limbic neuron/glia co-cultures. These findings suggest that, unlike methamphetamine, methylphenidate shows a lack of development of behavioral sensitization to its hyperlocomotion and induces reversible astrocytic activation.

Keywords: Methamphetamine; Methylphenidate; Psychological dependence; Synaptic plasticity; Neurotoxicity

1. Introduction

Amphetamines and substituted amphetamines, including methamphetamine, are widely abused for their stimulant and euphoriant properties (Murray, 1998). Animal and human studies have suggested that these effects are mediated through activation of the mesolimbic dopaminergic system, which innervates the limbic and cortical areas, including the orbitofrontal cortex and anterior cingulate cortex, to form a rewarding circuit (Wise and Hoffman, 1992; McBride et al., 1999; Goldstein and Volkow, 2002; Wise, 2002; Kalivas and Volkow, 2005).

Methylphenidate is commonly prescribed for children who have been diagnosed with attention deficit hyperactivity disorder (ADHD), one of the most common chronic neurobehavioral diseases of childhood (Dalby et al., 1989). As with other substituted amphetamines, methylphenidate may have abuse potential. In fact, it could interact with the same brain pathways activated by drugs of abuse, to produce dopamine overflow in the nucleus accumbens, similar to amphetamines and cocaine (Kuczenski and Segal, 2002; Swanson and Volkow, 2002). It has been reported that therapeutic doses of methylphenidate, similar to cocaine, could block the dopamine transporter (DAT) and elevate extracellular dopamine levels in rodent brain (Carboni et al., 2003). Previous studies in rats have shown that exposure to methylphenidate during adolescence caused behavioral changes that endured into adulthood, including enhanced psychomotor responses to cocaine (Brandon et al., 2001), depressive-like effects, and attenuated locomotor habituation (Carlezon et al., 2003). More recently, Adriani et al. (2006) demonstrated that chronic treatment with methylphenidate in adolescent rats induces the up-regulation of mRNAs of cAMP responsive element-binding protein (CREB) and Homer 1a during adulthood. Therefore, chronic exposure to methylphenidate might be a major public health concern as a

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0014-2999/$ - see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2007.06.062

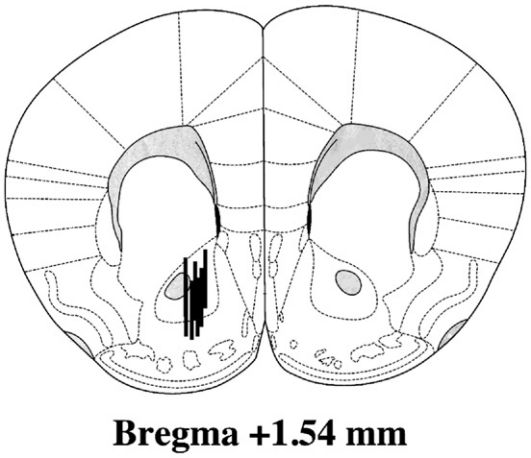

Fig. 1. Localization of microdialysis probes within the nucleus accumbens. Dots represent probe-inserted regions in the mouse brain. The schematic brain sections are from the atlas of Paxinos and Franklin (1997).

result of possible long-term effects in the mammalian central nervous system (CNS). However, very little is known about the differences between the long-term effects of methylphenidate and other addictive substituted amphetamines.

In the present study, we explored the differences between methylphenidate and methamphetamine in the development of behavioral sensitization. We recently reported that astrocytes, which are the most numerous glial cells in the CNS, play an important role in the development of synaptic plasticity induced by methamphetamine (Miyatake et al., 2005; Narita et al., 2005, 2006). Therefore, we also investigated whether *in vitro* treatment with methylphenidate could affect the morphology of astrocytes, compared to methamphetamine.

On the other hand, the serotonin (5-HT) system in the brain is also an important component of the rewarding effect induced by drugs of abuse (Hall et al., 2004; Müller et al., 2007; Trigo et al., in press). In fact, amphetamine and other psychostimulants have also been shown to interact with serotonin transporter and increase extracellular serotonin (5-HT) in the brain (Müller et al., 2007). It has been reported that methamphetamine increases extracellular 5-HT in the nucleus accumbens (Segal and Kuczenski, 1997). Therefore, we investigated the effects of methamphetamine and methylphenidate on the extracellular 5-HT levels in the nucleus accumbens of mice.

2. Materials and methods

The present studies were conducted in accordance with the Guide for Care and Use of Laboratory Animals adopted by the Committee on Care and Use of Laboratory Animals of Hoshi University School of Pharmacy and Pharmaceutical Sciences, which is accredited by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

2.1. Animals

In the present study, we used male C57BL/6J mice and male ICR mice (Tokyo Laboratory Animals Science Co., Ltd, Tokyo, Japan). Animals were housed in a room maintained at 23 ± 1 °C with a 12 h light/dark cycle (light on 8:00 A.M. to 8:00 P.M.). Food and water were available *ad libitum*.

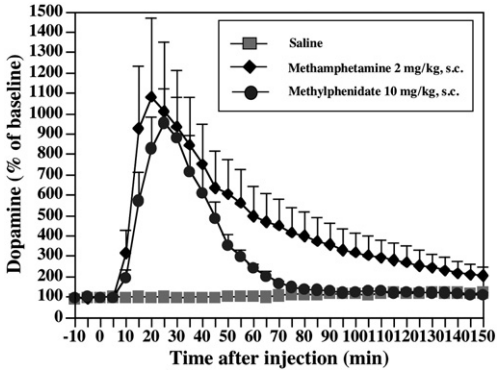

Fig. 2. Effects of methamphetamine and methylphenidate on the extracellular dopamine level in the mouse nucleus accumbens. After baseline fractions were collected, mice were administered methamphetamine (2 mg/kg, s.c.) or methylphenidate (10 mg/kg, s.c.) at time 0 to evoke the release of dopamine. Data are presented as a percentage of the mean basal level with S.E.M. of 5–6 mice. The statistical significance of differences between the groups was assessed with two-way analysis of variance (ANOVA) following by the Bonferroni test. [Saline vs. methamphetamine, $F_{(1, 288)}=5.92$, $P<0.05$; Saline vs. methylphenidate, $F_{(1, 256)}=21.1$, $P<0.01$; Methamphetamine vs. methylphenidate, $F_{(1, 288)}=1.37$, $P=0.27$, not significant].

2.2. Mouse in vivo microdialysis study and quantification of dopamine and 5-hydroxytryptamine (serotonin; 5-HT)

Stereotaxic surgery was performed under sodium pentobarbital (70 mg/kg, i.p.) anesthesia. Male C57BL/6J mice were placed in a stereotaxic apparatus and the skull was exposed. A small hole was then made using a dental drill. A microdialysis probe (D-I-6-01, 1 mm membrane length, Eicom Co., Kyoto, Japan) was implanted into the nucleus accumbens (from

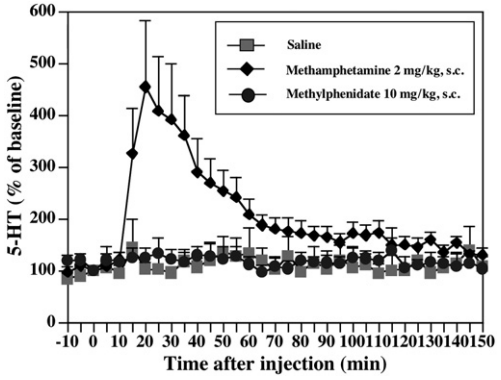

Fig. 3. Effects of methamphetamine and methylphenidate on the extracellular 5-HT level in the mouse nucleus accumbens. After baseline fractions were collected, mice were administered methamphetamine (2 mg/kg, s.c.) or methylphenidate (10 mg/kg, s.c.) at time 0 to evoke the release of 5-HT. Data are presented as a percentage of the mean basal level with S.E.M. of 5–6 mice. The statistical significance of differences between groups was assessed with two-way analysis of variance (ANOVA) following by the Bonferroni test. [Saline vs. methamphetamine, $F_{(1, 288)}=5.89$, $P<0.05$; Saline vs. methylphenidate, $F_{(1, 256)}=0.06$, not significant; Methamphetamine vs. methylphenidate, $F_{(1, 288)}=6.27$, $P<0.05$].