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Research report

Methylphenidate differentially regulates c-*fos* and *fosB* expression in the developing rat striatum

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Abstract

Methylphenidate (MPH, Ritalin) is a psychostimulant drug used in very young children with attention deficit hyperactivity disorder (ADHD). To explore the central effects of MPH, we compared repeated MPH treatments on c-*fos* and *fosB* expression in the striatum of immature and adult rats. Prepubertal (PD25–38) or adult (PD53–66) male rats were treated once daily for: (a) 14 days with either saline or MPH (2 or 10 mg/kg) or (b) 13 days with saline followed by a single dose of MPH (2 or 10 mg/kg) on day 14. To determine long-term effects of MPH, another group of prepubertal rats was allowed a drug-free period of 4 weeks following the initial 14 days of treatment, and received a challenge dose of MPH at adulthood. All rats were sacrificed 2 h post-injection on the final day. Expression of c-*fos* and *fosB* was quantified by densitometric analysis of cFOS and FOSB-immunoreactivity (-ir). We demonstrated that FOSB-ir was increased by a single dose of MPH in the prepubertal and adult striatum, and this effect was further *elevated* by chronic MPH in prepubertal rats, in contrast to the *inhibitory* effect of MPH (2 and 10 mg/kg) on cFOS-ir. In adult rats, repeated MPH down-regulated cFOS-ir only at the higher dose (10 mg/kg), while *fosB* expression remained at levels comparable to acute MPH. The reduction in cFOS-ir observed in prepubertal rats given repeated MPH (10 mg/kg) *persisted* in the adult striatum following MPH challenge at adulthood. Our results suggest that (1) repeated MPH treatment *differentially* regulates c-*fos* and *fosB* expression in the immature and adult brain; (2) MPH-induced changes in gene expression may be enduring, and (3) the immature brain is more sensitive to the stimulant effects of MPH than the adult. Thus, our findings have implications for the long-term use of MPH in ADHD.

Theme: Neural basis of behavior
Topic: Psychopharmacological agents

Keywords: Methylphenidate; Ritalin; Attention deficit hyperactivity disorder; ADHD; Development; c-*fos*; *fosB*; Striatum

1. Introduction

Ritalin (Methylphenidate, MPH) is a drug commonly used to treat children with attention deficit hyperactivity disorder (ADHD). ADHD children are characteristically hyperactive, impulsive, or inattentive, which has negative consequences for their academic and personal lives. These children are at risk for developing mood, anxiety, and drug-abuse disorders as adults [58]. It is estimated that the percentage of children with ADHD ranges from 1 to 20% and that it occurs predominantly in boys [25], though the rate of diagnosis in girls is increasing [48]. There has been an exponential increase in the use of stimulants compared to previous decades. Canada was reported to have the second highest consumption of psychostimulants (mainly Ritalin), out of 10 countries studied, and recent trends estimated a 93% increase in consumption [4]. There is also a disturbing

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trend to prescribe stimulants to younger children, preschoolers as young as 2–3 years, and for longer periods of time (decades) into adulthood [37,38]. Even though MPH is widely used in children, little is known about the neurochemical and behavioral consequences of chronic MPH treatment on the developing brain. There is great concern about the "potential adverse long-term effects of psychostimulants on learning and behavior" [15]. The rationale for concern is compelling, since, in children, the CNS continues its maturation and growth well into the second decade of life [3]. There are also implications associated with treating women, of child-bearing age, with long-term MPH. Prenatal MPH exposure may have adverse effects on the developing fetus, such as premature birth, delayed growth, and withdrawal symptoms in newborns [16].

A precise neurochemical cause remains unknown, but ADHD could be the result of a dopamine deficiency induced by elevated levels of the striatal dopamine transporter (DAT), which have been reported in adults and children with ADHD [14,33,56]. The psychostimulant effects of MPH may involve other neurotransmitter systems such as norepinephrine [34], serotonin [20], and glutamate [21]. MPH could also influence presynaptic dopamine release through action at the vesicular monoamine transporter (VMAT2) [24]. Therapeutic doses of MPH block the DAT and elevate extracellular dopamine levels in the human [57] and rodent brain [54]. Specifically, imaging studies have localized MPH binding to the striatum, suggesting that this is a major site of MPH action in the brain [22,57].

While stimulants are accepted as the treatment of choice, very little is known about the long-term effects of chronic MPH treatment on gene expression in the developing brain. The use of immediate early gene (IEG) expression as a probe to localize the neurochemical effects of psychoactive drugs is a dependable and commonplace approach [27,29,30]. The effect of MPH on central IEGs in the developing brain has attracted little attention. We described a stimulatory effect of acute MPH treatment in the prepubertal mouse [26,47] and rat striatum [11], and similar results were recently published by Brandon and Steiner (2003) [6]. MPH-induced c-*fos* expression was observed in the neonatal, prepubertal, and adult striatum of CD-1 mice, and the distribution pattern of cFOS-ir varied with developmental age [47]. Moreover, the increase in striatal cFOS-ir induced by acute MPH was significantly attenuated with chronic treatment in the prepubertal rat and mouse striatum [11,26]. Thus, our data indicated that repeated MPH treatment, at a clinically relevant dose (2–5 mg/kg), inhibited the normal c-*fos* response to this drug. Whether this attenuation of c-*fos* expression is permanent remains to be determined. In a previous study [42], chronic treatment of immature rats with MPH via the drinking water decreased the number of striatal dopamine transporter binding sites when measured in adults.

In the present study, we investigated the developmental effects of acute and chronic MPH exposure on c-*fos*, as well as another IEG, fosB, in immature and adult rats. The expression of c-*fos* may reflect the short-term neural response to acute drug exposure, whereas expression of fosB reflects neural adaptation to chronic drug treatment [39]. Expression of fosB has been implicated in the neurochemistry underlying the development of drug addiction [39]. We used MPH-induced c-*fos* and *fosB* gene expression, quantified in terms of cFOS and FOSB-ir, as markers to investigate and compare the effects of repeated, daily, MPH treatment in the striatum of both prepubertal and adult male rats. We also determined whether any of the changes observed in the young brain would persist in the adult. We hypothesized that chronic MPH treatment would elevate FOSB-ir in the striatum of prepubertal rats in contrast to the inhibitory effect of MPH on cFOS-ir. We also hypothesized that the immature brain would respond differently to repeated MPH treatment compared to the adult brain. To our knowledge, this is the first comparison of c-*fos* and *fosB* expression following repeated MPH treatment at different stages of development.

2. Materials and methods

2.1. Animals

Litters of male Sprague–Dawley rat pups arrived with their mothers at postnatal day (PD) 15 from Charles River Laboratories (Montreal, Quebec). They were housed on a 14:10 light/dark cycle (lights on from 0700 h to 2100 h), in plastic cages (28 × 12 × 16 cm), with free access to food (Lab Diet 5P00, Prolab RMH 3000) and reverse osmosis drinking water. The Dalhousie University Committee on Laboratory Animals approved all procedures and protocols for handling of laboratory animals. All measures were taken to minimize any possible animal discomfort. Rats were weaned on PD21, housed 2 or 3 per cage, and were weighed every 2 days throughout the experiments to monitor the effect of chronic MPH treatment on body weight.

MPH (Medisca Pharmaceutique, Montreal, Quebec) was dissolved in 0.9% sterile saline, and was administered subcutaneously (0.2 ml) between 10 am and noon (12 pm) each day, so that injections were 24 h apart. There were three independent experiments involving rats of different developmental periods and drug treatment schedules: (1) immature rats treated and sacrificed prepubertally, (2) adult rats treated and sacrificed as adults, and (3) immature rats treated prepubertally and later sacrificed at adulthood following a challenge dose of MPH. To investigate the effects of repeated MPH, prepubertal (PD25), or adult (PD53) male rats were injected (s.c.) once daily for: (a) 14 days with either saline (control group) or MPH (2 or 10 mg/kg), or (b) 13 days with saline followed by a single dose of MPH (2 or 10 mg/kg) on day 14. All rats from the saline control, single, and repeated MPH treatment groups were sacrificed 2 h post-injection on the final day (PD38 for prepubertal, and PD66 for adult rats).