Neurotoxicology and Teratology 50 (2015) 64–72

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

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

Repeated methylphenidate administration during lactation reduces maternal behavior, induces maternal tolerance, and increases anxiety-like behavior in pups in adulthood

R.A. Ponchio [a], E. Teodorov [a], T.B. Kirsten [b], C.P. Coelho [b], A. Oshiro [a], J.C. Florio [c], M.M. Bernardi [a,b,*]

[a] Centro de Matemática, Computação e Cognição, Universidade Federal do ABC, Av. Dos Estados, 5001, Santo André 09210-971, Brazil
[b] Environmental and Experimental Pathology, Paulista University, Rua Dr. Bacelar, 1212, São Paulo 04026-002, Brazil
[c] Department of Pathology, School of Veterinary Medicine, University of São Paulo, Av. Prof. Dr. Orlando Marques de Paiva, 87, Sao Paulo, SP 05508-270, Brazil

ARTICLE INFO

Article history:
Received 1 March 2015
Received in revised form 2 May 2015
Accepted 18 May 2015
Available online 25 May 2015

Keywords:
Psychostimulant
Maternal care
Motivation
Central nervous system plasticity

ABSTRACT

Methylphenidate (MPD) is a dopamine uptake inhibitor and the most commonly prescribed drug for the treatment of attention-deficit/hyperactivity disorder in children. Several studies have shown that such stimulants as cocaine and amphetamine that are administered during gestation and lactation may disrupt maternal behavior. Also, MPD is used in lactation. Repeated MPD administration can induce either sensitization or tolerance. The aim of the present study was to investigate whether repeated MPD administration alters maternal behavior and promotes tolerance or sensitization in these females. The effects in adult offspring were also examined in models of anxiety. Methylphenidate (5 mg/kg) was administered from lactation day 2 to 4, and maternal pup retrieval behavior was assessed. This treatment was continued until lactation day 7. At weaning, the dams received a challenge dose of MPD, and general activity was evaluated in the open field. Striatal monoamine and metabolite levels were also measured to determine whether this treatment promotes behavioral or biochemical plasticity. The long-term behavioral effects of MPD exposure were evaluated in pups in adulthood. The results showed an increase in the latency to retrieve the first, second, and third pups and a decrease in the number of dams that retrieved all pups. After a challenge dose of MPD, the dams exhibited a decrease in locomotion frequency, an increase in immobility duration in the open field, and a decrease in striatal serotonin levels. In pups, anxiety-like behavior increased in the light/dark box test. These results indicate that repeated MPD administration during early lactation impairs maternal behavior, likely by decreasing maternal motivation. Repeated MPD administration induced maternal tolerance at weaning after a challenge dose of MPD, suggesting the development of central nervous system plasticity. In pups, maternal exposure to MPD during early lactation induced long-term effects and increased anxiety-like behavior in adulthood.

1. Introduction

Methylphenidate (MPD) is a dopamine uptake inhibitor (Schweri et al., 1985) and the most commonly prescribed drug for the treatment of attention-deficit/hyperactivity disorder (ADHD) in children. Children with ADHD often respond favorably to this psychostimulant therapy, contributing to its use by 61% of children with ADHD between the ages of 6 and 13 years (Dalsgaard et al., 2013). Because of the increasing number of ADHD diagnoses, MPD has been more frequently prescribed. Its widespread clinical use increases the risk of the non-medical use of this drug (Teter et al., 2006; Wu et al., 2007). An increasing number of women of childbearing age are also being treated for this condition (Bolea-Alamanac et al., 2013). The use of ADHD medication in pregnancy was associated with different indicators of maternal disadvantage and an increased risk of induced abortion and miscarriage (Haervig et al., 2014; Bolea-Alamanac et al., 2013). Although the default medical position is to interrupt any non-essential pharmacological treatment during pregnancy and lactation, in ADHD this may present a significant risk (Bolea-Alamanac et al., 2013). Although treating ADHD during pregnancy and lactation might have negative effects on the baby, the suspension of treatment or inadequate treatment can also place both the mother and baby at risk (Besag, 2014).

Methylphenidate is a central nervous system stimulant of the phenethylamine and piperidine classes. Chronic exposure to psychostimulants induces behavioral, biochemical, molecular, and morphological adaptations that are linked to central nervous system plasticity (Chao and Nestler, 2004; Nestler, 1994, 2001; Robison and Nestler, 2011). Repeated psychostimulant exposure can also cause behavioral

* Corresponding author at: Universidade Federal do ABC, Av. Dos Estados, 5001, Santo André 09210-971, Brazil.
E-mail address: marthabernardi@ufbc.edu.br (M.M. Bernardi).

http://dx.doi.org/10.1016/j.ntt.2015.05.008
0892-0362/

sensitization or tolerance (Askenasy et al., 2007; Yang et al., 2007a). Methylphenidate also has abuse potential (Swanson and Volkow, 2003; Volkow et al., 2002). Chronic exposure to low to moderate doses of MPD causes behavioral sensitization (Yang et al., 2007a; Gaytan et al., 2000). At higher doses, tolerance develops (Claussen et al., 2014; Chong et al., 2012).

In rodents, repeated MPD treatment during development may also affect reproductive parameters in adult male rats (Montagnini et al., 2014). Several studies have shown that such stimulants as cocaine and amphetamine that are administered during gestation and lactation disrupt maternal behavior (Šlamberová et al., 2005; Kinsley et al., 1994; Piccirillo et al., 1980). Only one study shows that MPD attenuates maternal behavior in mice (Leussis et al., 2008). Methylphenidate has a similar pharmacological profile to cocaine (Chong et al., 2012) and alters synaptic dopamine levels (Swanson and Volkow, 2003). Thus, the mechanisms that underlie the changes in maternal behavior that are induced by MPD may be similar to amphetamine and cocaine by altering dopamine levels within the synapse.

In the present study, we examined the effects of repeated administration of 5.0 mg/kg MPD (orally by gavage) in dams from lactation day (LD) 2 to LD4 on maternal pup retrieval behavior. This treatment was continued until LD7. On the day of weaning, a challenge dose of MPD was administered to investigate whether repeated MPD administration promotes sensitization or tolerance in dams with regard to behavioral and neurochemical parameters. Methylphenidate is a lipophilic drug (Kimko et al., 1999) and excreted in breast milk, thus possibly exposing nursing pups to this drug. However, little information is available on the long-term consequences of MPD exposure in maternal milk on offspring behavior. Therefore, the present study also examined the long-term behavioral effects of postnatal MPD exposure in adult male offspring from dams that were treated with MPD during early lactation.

Methylphenidate was administered by gavage to more closely approximate human dosing conditions (Kuczenski and Segal, 2002). The dose was chosen based on Teo et al. (2002), who showed that this dose increased repetitive sniffing behavior in lactating rats but did not cause hyperactivity. This treatment regimen was previously reported to not induce stereotypy in rats (Crawford et al., 1998; Izenwasser et al., 1999), which can reduce locomotor behavior, an index that was used herein to evaluate whether sensitization or tolerance developed.

2. Methods

2.1. Ethics statement

The present study was performed in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Paulista University (permit no. 256/14 CEP/ICS/UNIP). All efforts were made to minimize suffering. The experiments were performed in accordance with good laboratory practice protocols and quality assurance methods.

2.2. Animals

Male and female Balb-c mice, 6–8 weeks of age from the Department of Pathology, School of Veterinary Medicine, University of São Paulo, were used. After arrival in the laboratory, the animals were randomly selected, individually marked, and housed in groups of five in isolated polypropylene cages (38 × 32 × 16 cm) under controlled temperature (21 ± 2 °C) and humidity (55–60%). Artificial lighting was provided (12 h/12 h light/dark cycle, lights on at 7:00 AM). The animals had free access to Nuvilab rodent chow (Nuvital, São Paulo, Brazil) and filtered water. The experiments began 10 days after the mice arrived in the laboratory to allow them to adapt to the environmental conditions. For mating, one female and one male were housed together for 5 days. The pregnant female mice were then individually housed until pups were born and during the entire lactation period. The day of birth was recorded as postnatal day 1 (PND1). No handling was performed on PND1. On PND2, eight offspring (four males and four females) were randomly selected for the subsequent experiments. No cross-fostering procedures were used. Litters with fewer than eight pups were not used in the experiments. The eight randomly selected pups remained with their dam until weaning on PND21. On PND21, the littermates were separated and co-housed by sex under the same conditions as their parents. The female offspring were used in another experiment.

2.3. Drug

Methylphenidate chloride (Ritalin®, 10 mg, a 50:50 racemic mixture of the D-*threo*- and L-*threo*-methylphenidate enantiomers, Novartis Biociências S.A., São Paulo, Brazil) was dissolved in distilled water and administered orally by gavage at a concentration of 0.5 mg/ml. Distilled water was used as a control solution. In both treatments, the volume that was administered was 0.1 ml/10 g of body weight. Distilled water was used as a control solution. Distilled water was used as a control solution. In both treatments, the volume that was administered was 0.1 ml/10 g of body weight.

2.4. Experimental design

Twelve lactating female mice were divided into two equal groups: experimental and control. The experimental group was orally treated with 5 mg/kg MPD by gavage from LD2 to LD4. The control group received distilled water. In both treatments, the volume that was administered was 0.1 ml/10 g of body weight. On the morning of LD5 (14 h after the last MPD dose), maternal behavior was observed. The treatments were continued from LD5 (after the maternal behavior observations) until LD7. The 5 mg/kg dose (p.o.) was chosen because it has been shown to induce tolerance in male rats (Yang et al., 2007b). Both the control and experimental groups of dams received a challenge dose of MPD (5 mg/kg, p.o.). Two hours after administration, both groups were observed in the open field (between 9:00 AM and 11:00 AM). One hour after the open field observations, these females were decapitated. The striatum was dissected, and neurotransmitter and metabolite levels were measured.

At weaning (LD21), the pups were removed from their dams. At adulthood (PND95-100), the offspring (one male pup per litter) were observed in the open field and light/dark transition test. Fig. 1 shows the experimental design.

2.5. Maternal studies

2.5.1. Maternal behavior

Maternal care is an essential component of offspring survival in all mammals. Many published reports of MPD toxicity involve MPD abuse (Finger et al., 2013; Freese et al., 2012). Few studies have evaluated the effects of MPD administration during the lactation period (Bolea-Alamanac et al., 2013; Leussis et al., 2008) or consequences on offspring development. Therefore, we examined maternal behavior after repeated MPD administration. Maternal behavior was analyzed as previously described (Bernardi et al., 2010) and observed on LD5 ($n = 6$ dams/group) between 9:00 AM and 11:00 AM. Before handling the animals, the presence of pups in the nest with their mother or pups that were only grouped without the presence of their mother (pups in the nest covered by wood shavings) was recorded. The pups were then removed from their dams, placed in another cage, and kept warm. Five minutes after maternal separation, three pups were returned to their mothers' cages and placed on the opposite side of the nest, and maternal behavior was examined. The latencies (in seconds) to sniff the first pup and retrieve the first, second, and third pups were recorded. The number of dams that retrieved all pups and