Physiology & Behavior 167 (2016) 118–124

Contents lists available at ScienceDirect

Physiology & Behavior

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

Reproductive parameters of female Wistar rats treated with methylphenidate during development

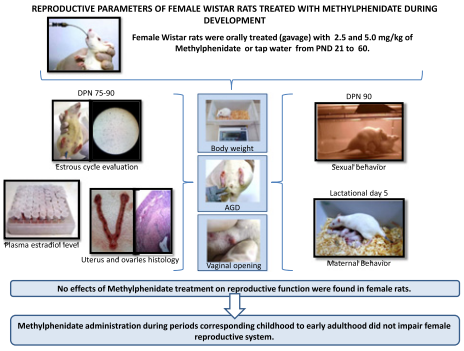
Bruno Garcia Montagnini [a], Kennia Moura Silveira [a], Bruna Caroline Pierone [a], Nathália de Azevedo Camim [a], Janete Aparecida Anselmo-Franci [c], Suzana de Fátima Paccola Mesquita [b], Ana Carolina Inhasz Kiss [d], Daniela Cristina Ceccatto Gerardin [a,*]

[a] Department of Physiological Sciences, State University of Londrina, 86051-980 Londrina, Paraná, Brazil
[b] Department of Biology, State University of Londrina, 86051-980 Londrina, Paraná, Brazil
[c] Department of Morphology, Estomatology and Physiology, University of São Paulo, 14040-900 Ribeirão Preto, São Paulo, Brazil
[d] Department of Physiology, Botucatu Biosciences Institute, São Paulo State University, 18618-970 Botucatu, São Paulo, Brazil

HIGHLIGHTS

- Female rats were treated with MPH from late infancy to early adulthood periods.
- MPH treatment does not affect reproductive function during development.
- No effect was detected in sexual parameters in adult female rats.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 May 2016
Received in revised form 16 August 2016
Accepted 16 August 2016
Available online 26 August 2016

Keywords:
Attention-deficit/hyperactivity disorder
Methylphenidate
Development
Female rat

ABSTRACT

Methylphenidate (MPH), a psychoactive agent that acts mainly by blocking the uptake of dopamine, is the main drug used to treat Attention Deficit Hyperactivity Disorder in children and adolescents. During development, important changes in brain architecture and plasticity occur, these changes, sensitive to exposure to stimulant drugs, are important in the control of GnRH secretion, influencing the release of sex hormones throughout the ovarian cycle. This study investigated the effects of repeated treatment with MPH during development on reproductive parameters of adult female rats. Wistar rats received MPH 2.5 mg/kg, MPH 5.0 mg/kg, or tap water (gavage) from postnatal day (PND) 21 to PND 60. From PND 75, one subgroup of females was selected for evaluation of estrous cycle, estradiol levels, weight of sexual organs, and histomorphological analysis of ovary follicles and uterus. In another subgroup, the sexual and maternal behaviors were evaluated at PND 90 and on lactational day 5, respectively. No significant alterations were observed in the MPH groups. This study demonstrated that repeated administration of MPH during the period corresponding to childhood to early adulthood does not interfere in the reproductive function of female rats in adulthood.

Abbreviations: ADHD, attention deficit hyperactivity disorder; MPH, methylphenidate; PND, post-natal day; AGD, anogenital distance; LD, lactational day.

* Corresponding author at: Department of Physiological Sciences, State University of Londrina - UEL, 86051-980 Londrina, Paraná, Brazil.
E-mail address: dcgerardin@uel.br (D.C.C. Gerardin).

http://dx.doi.org/10.1016/j.physbeh.2016.08.017
0031-9384/

1. Introduction

Over the past few decades, methylphenidate (MPH) has become the main psychostimulant drug prescribed to children and adolescents in the treatment of attention deficit hyperactivity disorder (ADHD) [1–4]. This neurodevelopment disorder is characterized by persistent symptoms of inattention, hyperactivity and impulsivity [5], with a worldwide prevalence of 3.4% in children and adolescents [6].

The therapeutic effect of MPH is mainly due to increasing dopamine (DA) signaling, primarily by blockade of the DA reuptake transporter [7,8], although noradrenergic receptors may also contribute to MPH activity [9].

Several studies have shown that the prevalence of ADHD is higher in boys than in girls [10,11]. However, longitudinal studies in school-aged children have indicated an increase in the proportion of girls using MPH [1,2,12], which raises major concerns for public health due to possible persistent neurobehavioral changes [13–15].

In mammalians, important changes in the brain architecture and plasticity occur in the period from immediately before birth until adolescence and these changes could be influenced by exposure to psychotropic stimulant drugs [16–18]. Monoamines, such as dopamine (DA) and noradrenalin (NA) are widely distributed in the brain and have a strong role in the neuroendocrine control of GnRH release in these periods [19–21]. Alterations in GnRH secretion modulated by DA and NA neurons could change the LH secretion profile throughout the ovarian cycle, including the pre-ovulatory LH surge [22–24]. The presence of DA receptors in the ovaries of rodents also suggests that the function of the corpus luteum and/or follicular development may be regulated by catecholaminergic innervation during the estrous cycle [25–27]. In addition, interactions between central monoaminergic systems and steroid hormones in the integration of reproductive behavior are widely reported in the literature [20,21].

In this sense, despite the efficacy of MPH to reduce ADHD symptoms [28,29], little is known about the long-term changes that treatment with MPH could induce in the developing organism, including the reproductive function. Previously published data from our laboratory show that MPH administration during late infancy until early adulthood may impact on the reproductive function of adult male rats [30]. At the same time, there is a lack of studies evaluating the effect of early treatment with MPH and its effects on the reproduction of adult female rats.

Based on these considerations, this study was conducted to evaluate the long-lasting effects on reproductive function in adult female rats submitted to MPH treatment similar to that established for ADHD, from the late infancy period to early adulthood [31].

2. Materials and methods

2.1. Animals and treatment

A total of 10 male and 20 female Wistar rats (85–90 days) from the colony of the State University of Londrina (UEL) were used as parental generation. The animals were kept in a controlled environment at a temperature of 21 ± 2 °C; 12 h light/dark cycle (lights on at 6:00 a.m.) with free access to regular lab chow (Nuvital™, Paraná, Brazil) and water. The rats were mated (2 females and 1 male per cage) and gestational day 0 was determined if there were sperm and estrous phase cells in vaginal smears. On post-natal day (PND) 4, litters were culled to 8 pups keeping 4 males and 4 females wherever possible. Female pups were weaned on PND 21 and divided into three groups.

- Control group (CTR): animals were gavaged with tap water daily, from PND 21 to PND 60 ($n = 28$);
- MPH 2.5 mg group (MPH 2.5): animals were gavaged with 2.5 mg/kg of MPH (Ritalin™, Novartis) daily, from PND 21 to PND 60 ($n = 20$) [30];
- MPH 5.0 mg group (MPH 5.0): animals were gavaged with 5.0 mg/kg of MPH (Ritalin™, Novartis) daily, from PND 21 to PND 60 ($n = 22$) [30].

To avoid sibling effects, no littermates were used for the same group. Rats were treated daily at 4–6 p.m. The drug was dissolved in tap water immediately prior to the treatment.

In rodents, the puberty period corresponds to the age window between postnatal days 28 (female)/35 (male) and 50 [31]. At puberty, the hypothalamus receives neuronal input from many brain centers for GnRH release, these changes are particularly important in females, maintaining the regularity of the estrous cycle [32], in addition to structuring relevant hypothalamic areas for reproductive behaviors [33]. In this way, the females were treated from PND 21 to PND 60, since the treatment with MPH in humans, although beginning during childhood, can persist into adulthood [34], and in order to cover the critical developmental stages of the reproductive systems.

The effective dose range in children is 0.3–1.0 mg/kg MPH [35]. Applying the $BW^{3/4}$ scaling [36], the equivalent dose in rats would be 1.7–5.5 mg/kg. The highest dose used in this study (5.0 mg/kg) would be equivalent to a clinically relevant dose in humans and higher doses were not tested since it is already described in the literature that oral administration of 5.0 mg/kg does not compromise weight gain [37]. Methylphenidate chloride is expressed as the formula weight for Ritalin™ (10 mg, a 50:50 racemic mixture of the D-threo-and L-threo-methylphenidate enantiomers). The oral gavage method was chosen in order to provide the same administration route used in humans.

All animal procedures were approved by the UEL Ethics Committee for Animal Research (CEUA 16381.2012.45). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments. The experimental protocol is diagramed in Fig. 1.

2.2. Parameters analyzed during development (PND 21–60)

2.2.1. Body weight

Body weight was measured daily during the treatment period as well as observation of signs of toxicity (e.g. lacrimation, piloerection, unusual respiratory pattern and tremors).

2.2.2. Physical sexual development

The anogenital distance (AGD, distance from the anus to the genital tubercle) was measured using a vernier caliper on PND 21, 28 and 35. The measurements obtained were normalized through their division by the cube root of bodyweight [38]. From PND 30, the vaginal opening was verified daily and considered as an indicator of the onset of sexual maturity.

2.3. Parameters analyzed in adulthood (from PND 75)

For the evaluation of female reproductive development, each group (CTR, MPH 2.5 and MPH 5.0) was divided into 2 subgroups ($n = 8$–14/subgroup): one group for estrous cyclicity, estradiol level, sexual organ weight and histomorphological analysis of the ovary follicles and uterus and the other for sexual and maternal behavior evaluation.

2.3.1. Estrous cycle evaluation

The normal length of the estrous cycle of the rodent strain used in this study ranges from 4 to 5 days [39]. Beginning at PND 75, vaginal smears were obtained daily, always at the same time in the morning, over a period of 15 days. The material was observed under a light microscope and the estrous cycle phases were classified as proestrus, estrus, metaestrus and diestrus. Proestrus was defined by smears possessing the prevalence of nucleated epithelial cells and no leucocytes. Estrus