Physiology & Behavior 133 (2014) 122–129

Contents lists available at ScienceDirect

Physiology & Behavior

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

Effects of repeated administration of methylphenidate on reproductive parameters in male rats

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HIGHLIGHTS

- Early MPH treatment induces persistent alterations in reproductive parameters.
- MPH treatment affects sperm morphology and germ cell population.
- MPH treatment does not affect the sexual behavior in male rats.

ARTICLE INFO

Article history:
Received 7 April 2014
Received in revised form 5 May 2014
Accepted 16 May 2014
Available online 24 May 2014

Keywords:
Attention-deficit/hyperactivity disorder
Methylphenidate
Development
Spermatogenesis
Testicle
Sperm morphology

ABSTRACT

Methylphenidate (MPH) is a psychostimulant drug which acts by blocking the dopamine and norepinephrine transporters and is the main drug used to treat attention deficit hyperactivity disorder in children and adolescents. During puberty, changes in neurotransmitter systems (including dopaminergic system) are engaged on the release of gonadal hormones and the development of cephalic structures responsible for reproductive function. This study investigated the effects of repeated treatment with methylphenidate during development on reproductive parameters of adult male rats. Wistar rats received MPH 2.5 mg/kg, MPH 5.0 mg/kg, or distilled water (gavage) from postnatal day (PND) 21 to PND 60. At PND 100, an increase in percentage of abnormal tail morphology sperm in MPH 2.5 and increase in testicular interstitial tissue volume in MPH groups as well as in the number of type A spermatogonia in MPH 5.0 group were observed. This study demonstrated that repeated administration of methylphenidate during periods corresponding childhood to early adulthood interfered on testicular function in rats at adult life.

1. Introduction

The attention deficit hyperactivity disorder (ADHD) is one of the most frequent neuropsychiatric disorders commonly diagnosed in children and adolescents. Its worldwide prevalence is of 5.29% [1] and is defined by persistent symptoms of inattention, hyperactivity and impulsivity [2].

Although ADHD has been established as a disorder acquired during childhood [3], longitudinal follow-up studies reported that ADHD symptoms diagnosed during infancy could persist into adulthood with a margin that exceeds 60% [4–6].

For this psychiatric disease, methylphenidate (MPH) is the main psychostimulant drug prescribed to children and adolescents to treat ADHD [7–9]. It acts by blocking the dopamine (DA) and noradrenaline (NA) transporters [10,11], although it is suggested that the therapeutic effect of MPH is mainly due to the DA transporter blockade [12].

Some studies have evidenced the DA role on the pre-pubertal maturation stages in different vertebrate species [13–15]. It is known that early exposure to MPH could lead to long-lasting alterations in brain DA pathways and natural reward systems [16,17], which are related to sexual behavior performance [18]. In this sense, impairment of sexual behavior performance was demonstrated in adult rats treated with MPH during adolescence [17], and alterations on hormonal profile [19] and spermatogenesis [20,21] were observed shortly after drug discontinuation.

Abbreviations: ADHD, attention deficit hyperactivity disorder; MPH, methylphenidate; DA, dopamine; PND, post-natal day; AGD, anogenital distance; HTF, human tubular fluid.

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http://dx.doi.org/10.1016/j.physbeh.2014.05.016
0031-9384/

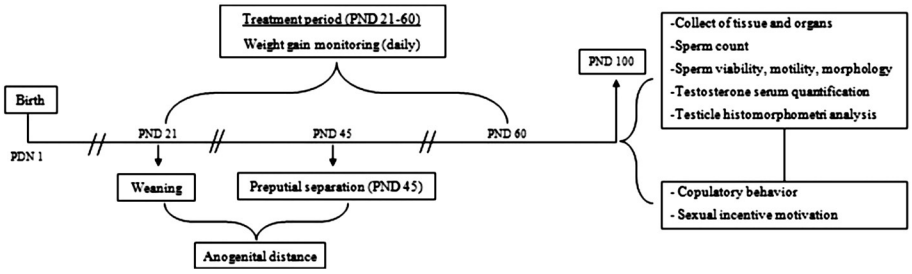

Fig. 1. Diagram of the experimental design. PND: postnatal day.

Based on these considerations, this study was conducted in order to evaluate the enduring effects on reproductive function in adult male rats submitted to similar MPH treatment established for ADHD, from late infancy to early adulthood periods [22].

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. They were kept in a controlled environment with temperature at 21 ± 2 °C; 12 h light/dark cycle (lights on at 6:00 a.m.) and had free access to regular lab chow (Nuvital™, Paraná, Brazil) and tap water. Rats were mated (2 females and 1 male per cage) and gestational day 0 was determined if there were sperm and estrus 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. Male pups were weaned on PND 21 and divided into three groups (26 males/group).

- Control group (CTR): received distilled water daily, by gavage, from PND 21 to PND 60;
- MPH 2.5 mg group (MPH 2.5): received 2.5 mg/kg of MPH (Ritalin™, Novartis) daily, by gavage, from PND 21 to PND 60;
- MPH 5.0 mg group (MPH 5.0): received 5.0 mg/kg of MPH (Ritalin™, Novartis) daily, by gavage, from PND 21 to PND 60.

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

In children, the effective dose range is 0.3–1.0 mg/kg MPH [23]. Applying the $BW^{3/4}$ scaling [24], 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 [7]. 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). The experimental protocol is diagramed in Fig. 1.

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

2.2.1. Body weight

The body weight was measured daily during the treatment period as well as toxicity signs (e.g. lacrimation, piloerection, unusual respiratory pattern and tremors) were observed.

2.2.2. Physical sexual development

The anogenital distance (AGD, distance from the anus to the genital tubercle) was obtained weekly (PND 21, 28, 35, 42) through a vernier caliper until occurrence of preputial separation. AGD was normalized through its division by the cube root of body weight. From PND 45 [25], preputial separation was verified daily and considered as an indicator of the sexual maturity onset.

2.3. Parameters analyzed in adulthood (PND 100)

For the evaluation of male reproductive development, each group (CTR, MPH 2.5 and MPH 5.0) was divided into 2 subgroups (n = 11–15/subgroup): one group for testosterone level, sexual organ weight, sperm parameters and testis histomorphometric analysis and the other one for the sexual behavior evaluation.

2.3.1. Plasmatic testosterone quantification

Male rats were euthanized with diethyl ether and blood samples were collected from the abdominal aorta into syringes containing heparin, always at the same time. Immediately after collection, blood samples were centrifuged (2500 rpm for 20 min at 2 °C) and the plasma was frozen until assayed. Blood plasma testosterone was measured by radioimmunoassay using ImmuChem™ Double Antibody ^{125}I RIA Kit (MP Biomedicals, Orangeburg, NY) according to the manufacturer's instructions. Samples were analyzed in a double assay format, the intra-assay coefficient of variation and the minimum sensitivity of the assay were 10.1% and 0.035 ng/ml respectively.

2.3.2. Collection of tissue and organs

The right testis and epididymis, vas deferens, ventral prostate and seminal vesicle (without the coagulating gland and full of secretion) were removed and their weights (absolute and relative to body weight) were determined. The right testis and epididymis were frozen at −20 °C for sperm counting. The left testis was collected for histomorphometric analysis.

2.3.3. Daily sperm production per testis, sperm number and transit time in the epididymis

Right testis was decapsulated and the caput/corpus and cauda segments from epididymis were separated. Homogenization-resistant testicular spermatids (stage 19 of spermiogenesis) and sperm in the caput/corpus epididymis and cauda epididymis were assessed as

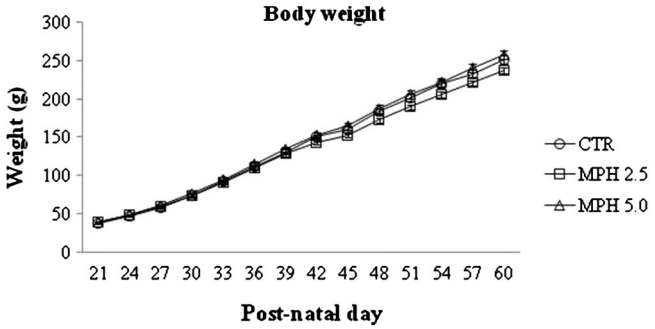

Fig. 2. Body weight of male rats during treatment period. Data are means ± S.E.M. RMANOVA ($p > 0.05$). CTR: distilled water; MPH 2.5: methylphenidate 2.5 mg/kg; MPH 5.0: methylphenidate 5.0 mg/kg.