Adverse effects of methylphenidate on the reproductive axis of adolescent female rats

Shilla Chatterjee-Chakrabarty, Ph.D.,[a] *Brian T. Miller, Ph.D.,*[b] *Thomas J. Collins, Ph.D.,*[a] *and Manubai Nagamani, M.D.*[a]

[a] Department of Obstetrics and Gynecology and [b] Department of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, Texas

Objective: To examine the effects of chronic methylphenidate use on the reproductive axis of adolescent female rats.
Design: Controlled prospective study.
Setting: University research laboratory.
Animal(s): Twenty prepubertal female Sprague Dawley rats.
Intervention(s): Subcutaneous implantation of drug-filled Alzet minipumps (Durect Corporation, Cupertino, CA) for infusing methylphenidate (450 μg/d, treated) or physiological saline (control) for 4 weeks. Estrous cyclicity was checked from 3 weeks of pump implantation till the termination of the experiments. Animals were killed after 4 weeks of treatment.
Main Outcome Measure(s): Estrous cyclicity, pituitary and peripheral FSH and LH, serum estrogen and progesterone, ovarian histology, and immunocytochemistry for localizing growth differentiation factor-9 and activin receptors-I.
Result(s): Compared with the control group, the treated animals exhibited the following: [1] poor vaginal opening and erratic estrous cyclicity; [2] undeveloped, disrupted, or prematurely luteinized ovarian follicles; [3] absence of growth differentiation factor-9 and of activin receptors I and IB in the oocyte; and [4] high levels of LH in the pituitary.
Conclusion(s): Chronic methylphenidate administration during adolescence perturbs pubertal onset, adversely affects maturation of the female reproductive axis by retarding pituitary LH release, and adversely affects ovarian folliculogenesis. These novel findings may have significant clinical implications in evaluating the effects of methylphenidate abuse on adolescent health. (Fertil Steril® 2005;84(Suppl 2):1131–8. ©2005 by American Society for Reproductive Medicine.)

Key Words: Methylphenidate, adolescent female rats, reproductive axis

Methylphenidate (MPH) is a schedule II psychotropic drug primarily prescribed to treat attention deficit hyperactivity disorder in children (1). As in many other antipsychotic medications, MPH activates parts of the brain stem and the frontal lobe that either produce or use dopamine for neurological functions through blockade of dopamine 2 receptors. As a result, extracellular dopamine levels are increased in the brain, and by lessening the firing of so-called background neurons that are not associated with the specific task performance, MPH allows the brain to transmit a clearer signal and filter out distraction, thereby enhancing the patient's ability to focus on a given task (2). This property of MPH has made it a popular performance-enhancing drug among healthy individuals. Animal and human studies have shown, however, that the stimulus effects produced by MPH are similar to those of cocaine (3); over time, MPH has proven to have high abuse liabilities (4, 5).

Although the bioavailability of MPH is believed to be low because of extensive first-pass metabolism, studies with prepubertal rats show that drug uptake is high in the brain, with the maximal concentration occurring in the striatum (6). The density of dopamine transporters in the striatum is significantly reduced for a long time, even after termination of treatment (7, 8). These findings clearly suggest that MPH has short-term and long-term effects. This is a special cause for concern because MPH abuse is most common and is rapidly rising among 11- to 19-year-old young adolescents (4) who take this drug for its stimulant effects and/or for appetite suppression, wakefulness, increased focus and attentiveness, and euphoria (9). According to a report from the American Association of Poison Control Centers Toxic Exposure Surveillance System, the most common symptoms of MPH abuse in adolescents are tachycardia, agitation and irritability, and hypertension (10, 11). Little is known, however, about the long-term effects of this drug on the reproductive axis.

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Reprint requests: Shilla Chatterjee-Chakrabarty, Ph.D., Department of Obstetrics & Gynecology, University of Texas Medical Branch, 301 University Blvd., Galveston, Texas 77555-1062 (FAX: 409-747-1669; E-mail: schakrab@utmb.edu).

The primary objective of our study was to examine the effects of MPH on the female reproductive axis, which undergoes many dynamic changes during puberty.

MATERIALS AND METHODS

Animals

Fourteen-day-old female Sprague Dawley pups were purchased along with their lactating mothers from Harlan Sprague Dawley (Houston, TX). The animals were housed at the animal care facilities at the University of Texas Medical Branch (Galveston, TX) under uniform conditions of light (14:10 hour light-dark cycle, lights on at 6 AM) and temperature. Upon weaning (day 21), the pups were separated from their mothers and were caged separately for 7 days before starting any treatment. Experiments were performed according to the *Guide for Care and Use of Laboratory Animals* and approved by the University of Texas Medical Branch Animal Care and Use Committee.

Treatment

Twenty prepubertal female rats (28 days old) were divided into two groups of 10 animals each. Animals in the treated group were administered MPH (450 μg/d), whereas those in the control group received normal physiological saline. Treatment was continued for 4 weeks to allow the rats to reach pubertal age (56 days old at the end of treatment). Treatment was administered through Alzet Osmotic pumps (Durect Corporation, Cupertino, CA) to provide continuous subcutaneous delivery (0.25 μL/h for 28 days) of the test substances. Pumps filled with saline or MPH were aseptically implanted under the skin of the experimental animals under halothane anesthesia. The dose of MPH administered was based on body surface area (in milligrams per square meter) and was extrapolated from pharmacologic doses given to children older than 6 years or to young adolescents, assuming 100% bioavailability. The animals were allowed to recover from anesthesia before being returned to their cages. The entire surgical procedure took an average of 10 minutes per animal.

Vaginal Opening and Estrous Cycle Determination

To determine pubertal development in the experimental animals, the age at vaginal opening and establishment of estrous cyclicity was monitored in control and treated animals. Vaginal smears were performed for 6 days after vaginal opening was observed. A miniature spatula was used to remove epithelial cells from the vaginal wall. Cells were placed on a slide with a drop of saline and examined under a light microscope to determine the stage of the estrous cycle.

The histologic characteristics of the unstained cells at proestrus (round, nucleated cells), estrus (cornified cells), metestrus (round nucleated cells, cornified cells, and leukocytes), and diestrus (predominance of leucocytes) were easily identifiable. The shape, color, moistness, and degree of swelling of the vagina at proestrus (gaping and moist reddish-pink), estrus (gaping, but lighter pink and less moist), metestrus (pale and dry with edematous dorsal lip), and diestrus (small opening) also were recorded daily to monitor progression of the estrous cycle. Vaginal smears again were performed on control and treated rats for 8 days (age 48–56 days) before termination of the experiments.

Blood and Tissue Collection

At the end of the experimental period, the animals were asphyxiated in a CO_2 chamber and decapitated. Trunk blood was collected in sterile tubes devoid of anticoagulants, allowed to settle for 5 minutes at room temperature, and processed by centrifuging at 750 $\times$ *g* for 10 minutes to separate the serum, which then was stored at -70°C for hormonal assays. Pituitaries were quickly removed and snap frozen in liquid nitrogen for hormonal assays. Ovaries were fixed in 10% neutralized formaldehyde for histologic and immunohistochemical studies.

Hormone Assays

Luteinizing hormone was assayed in aliquots of serum and homogenate supernatants of the pituitary using the National Institute of Diabetes and Digestion and Kidney Diseases (NIDDK)-rat radioimmunoassay kits according to our established procedure (12). Briefly, duplicate aliquots of 100 μL of serum and 100 μL of diluted (1/100–1/1,000) pituitary supernatants were incubated at 4°C with LH antisera. Twenty-four hours later, approximately 20,000 cpm of labeled LH was added to each tube and allowed to react overnight. The precipitating antibody then was added. After incubating overnight, the tubes were processed by centrifuge at 1,000 $\times$ *g* for 15 minutes, and the radioactivity of the pellets was measured in a gamma counter. The hormonal content of the samples, as estimated from the standard curves, was expressed as nanograms of NIDDK-rLH-RP-1 per milliliter. The final working solution of 1:45,000 NIDDK-anti-rLH-26 resulted in assay sensitivities, using the above incubation conditions, of 0.12 ng of LH per assay tube. The intra-assay and interassay coefficients of variation were 7% and 9%, respectively.

Follicle-stimulating hormone concentrations in serum and pituitary samples were kindly measured for us by the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core. Anti-rat FSH (NIDDK) diluted to a final concentration of 1:446,000 was used as the primary antibody for the radioimmunoassay. The hormonal content of the samples, as estimated from the standard curves, was expressed as nanograms of NIDDK-rFSH-RP-2 per milliliter. The assay had a sensitivity of 1.5 ng/mL and <0.5% cross-reactivity with other pituitary hormones. The average intra-assay and interassay coefficients of variation were 8.7% and 14.5%, respectively. All of the samples were assayed in a single assay.