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Effects of maternal exposure to the galactagogue Sulpiride on reproductive parameters in female rats

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HIGHLIGHTS

- Lactational exposure to SUL did not affect the development of female offspring.
- No effect was detected on estrous cycle and on estradiol plasmatic level.
- Changes in sexual behavior of these animals were found in adulthood.
- Lactational exposure leads to implications that might be detected only in adulthood.

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ABSTRACT

The antipsychotic Sulpiride has been documented as an effective galactagogue that acts blocking dopamine receptors, increasing prolactin concentrations. However, this drug passes through the milk exposing neonates during postnatal development, which may result in functional and morphological alterations in adult life. Therefore, the aim of this study was to investigate whether maternal exposure to Sulpiride during lactation could impair reproductive development of female offspring. The dams were treated daily by gavage with Sulpiride doses of 2.5 mg/Kg (SUL 2.5 mg group) and 25 mg/Kg (SUL 25 mg group), or distilled water (Control group) throughout the lactation period. During early life, body weight, anogenital distance, and vaginal opening were analyzed on the female offspring. In adulthood, estrous cycle, sexual behavior, estrogen levels as well as the weight of the reproductive organs were evaluated. There were no differences regarding body weight, anogenital distance, puberty onset, frequency and duration of the estrous cycle and estradiol levels on female offspring. Nonetheless, there were changes in sexual behavior. There was an increase in the number of observations in reflex magnitude 0 (absence of lordosis) and reflex magnitude 2 as well as a reduction of reflex magnitude 3 in the rats of SUL 25 mg group in relation to the Control group, suggesting a decrease in sexual receptivity of these animals. These results demonstrate that maternal exposure to Sulpiride can alter reproductive function in female offspring rats.

1. Introduction

Breast milk is extensively recognized as ideal nutritional source for the newborn because it presents all nutrients necessary for growth, development and immune protection as well as an important role in disease prevention [1]. One of the main causes of early abandonment of breastfeeding is due to the inability to produce sufficient quantities of milk to feed the baby in the first days of postpartum [2]. Therefore, mothers have resorted to pharmacotherapy [3], as the galactogogues.

Galactogogues are drugs that help to induce and maintain suitable milk production [4]. The drugs used for this purpose (Metoclopramide, Domperidone, Sulpiride) act by blocking dopamine receptors and neutralizing the inhibitory effect of dopamine on prolactin secretion, resulting in increased serum prolactin levels with consequent increase in milk production [5].

Sulpiride, an antipsychotic of the benzamides group, has been appointed as an effective galactagogue [4,6]. It acts by blocking D2 dopaminergic receptors of stereoselective form [7,8], with the hyperprolactinemia as side effect of this drug [9]. However, no antipsychotic was approved for use during pregnancy and lactation, since these drugs can cause potential adverse effects on the fetus and newborn [8]. Nevertheless, obstetricians and gynecologists have prescribed Sulpiride to women with the aim of promoting lactation [10], because this drug

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rarely produces side effects to the mother, such as thirst, nausea and tiredness [11].

It is known that Sulpiride is transferred in unaltered way to the milk [6,10–13] exposing neonates to the effects of this drug. Furthermore, it was shown that maternal treatment with this drug can block dopamine receptors in the offspring, leading to alterations in prolactin plasmatic concentrations [6]. Increased prolactin levels, induced by antipsychotic drugs, are known to affect reproduction in humans, leading to loss of libido, infertility [14–16] and to other adverse events such as: amenorrhea [17], gynecomastia and galactorrhea [18,19]. Likewise, studies have shown that this condition can cause ovulatory dysfunction as well as anovulation in humans and irregular estrous cycle in animals [20–25]. Authors have also related hyperprolactinemia to hormonal dysfunction. Studies have shown decreased luteinizing hormone (LH) and suppression of LH-RH release in rats [26], even as a significant reduction in plasmatic estradiol concentrations in women by administration of Sulpiride [27]. It was also suggested that prolactin can mediate the sexual behavior, since high concentrations of this hormone may suppress such behavior in adult female rats [28].

Moreover, the dopaminergic blockage during brain development could impair reproductive functions, since dopamine plays an important role in the regulation of reproductive behaviors, such as female sexual behavior [29].

Thus, this study aimed to investigate whether maternal exposure to Sulpiride, during lactation, could compromise the reproductive development of female offspring, whose effects only may be identified at puberty or even in adulthood.

2. Materials and methods

2.1. Animals and treatment

Female and male Wistar rats (85–90 days of age) 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 25 ± 1 °C; 12 h light/dark cycle (lights on at 6:00 a.m.) and had free access to regular lab chow and tap water. 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. Dams were divided into three groups:

- Control group (CTR): dams (n = 22) received daily 0.20 mL of distilled water, by gavage, from post-natal day (PND) 0 to PND 21;
- SUL 2.5 mg/kg group (SUL 2.5 mg): dams (n = 23) received daily 2.5 mg/kg of Sulpiride (Equilid™, Aventis, Brazil) [30], by gavage, from PND 0 to PND 21;
- SUL 25 mg/kg group (SUL 25 mg): dams (n = 23) received daily 25 mg/kg of Sulpiride (Equilid™, Aventis, Brazil) [30], by gavage, from PND 0 to PND 21.

The dams were daily treated at 12:00–2:00 p.m. The drug was dissolved in distilled water immediately prior to the treatment.

The commonly indicated dose for the induction of lactation in humans is 50 mg, two to three times per day [4,10,13,31], which corresponds to approximately 1.7 to 2.5 mg/kg. It is assumed that animals could be more resistant than humans, so it was chosen for this study the dose of 25 mg/kg, which corresponds to ten times higher the recommended dose for humans as galactagogue.

At post-natal day 1 (PND 1), the litter was culled to eight and pups were kept with each mother until weaning (PND 21) in order that all receive equal amounts of milk and preventing litter size effects on offspring development. The litter was the experimental unit (i.e. one female pup per litter was used for each evaluation at each time point). All animals had free access to water and regular lab chow (Nuvital™, Nuvilab, Brazil) and all animal procedures were approved by the UEL Ethics Committee for Animal Research (CEEA 26/11). The experimental design is represented in Fig. 1.

2.2. Female offspring

2.2.1. Parameters analyzed from birth to puberty

Experiment 1. Body weight and anogenital distance

The female pups were weighed on PND 0, 7, 14, 21 and 30 (n = 22 animals/group). The anogenital distance (AGD, distance from the anus to the genital tubercle) was measured by using vernier caliper on PND 0 and PND 21 (n = 21 animals/group). The measurements obtained were normalized through their division by the cube root of body weight. These data were expressed as litter mean.

Experiment 2. Vaginal open

In order to evaluate sexual development, females were observed from PND 30 for vaginal opening. The day of full opening of the vaginal orifice was recorded and the animals were weighed. These data are expressed as litter mean (n = 21–23 animals/group).

2.2.2. Parameters analyzed in adulthood

For the evaluation of females in adulthood, two puppies from each litter were used, one for the estrous cycle evaluation and the other one for the sexual behavior, collection of organs and determination of estradiol plasmatic concentration.

Experiment 1. Estrous cycle

The estrous cycle (n = 20 animals/group) was assessed on the basis of vaginal smears collected every morning over a period of 15 days (PND 75 to PND 90). The material was observed under a light microscope and the estrous cycle phases were classified as diestrus, proestrus, estrous and metaestrus. Proestrus for the prevalence of nucleated epithelial cells. The same was performed to identify an estrous phase, by noting a large amount of cornified epithelial cells. The presence of the three cell types, leukocytes, cornified and nucleated epithelial cells was identified as metaestrus. The estrous cycle duration was calculated as the number of days between one estrous phase to the next.

Experiment 2. Sexual behavior

The female sexual behavior analyses (n = 19–20 animals/group) were performed according to the methodology described by Felicio and Nasello [32]. Sexual behavior was assessed in cycling rats 3 to 4 h after a proestrus smear was observed. Sexually experienced males were used in these tests, which lasted until ten mounts had been observed [33]. Results were expressed as the lordosis quotient (LQ, number of lordosis / ten mounts × 100) [34] as well as the frequency of each reflex magnitude (LM, on a scale of 0 to 3). The classification was as follows: 0 absence of lordosis; 1 the female showed little flex of spine, head and hips slightly elevated from floor; 2 the female showed spinal flex and head raise close of an angle of 30° with the floor; 3 maximum lordosis, with accented spinal flex and the head inclined at an angle of 45° or more relative to the floor [35].

Experiment 3. Collection of organs and determination of estradiol plasmatic concentration.

Females, in estrous phase, were weighed and euthanized by inhalation of ethyl ether. The uteri and ovaries were removed and their weights were determined (n = 18–22 animals/group). Blood samples were collected from the abdominal aorta artery, centrifuged (2500 rpm for 20 min at 2 °C), plasma was obtained and frozen. The estradiol plasmatic concentration (n = 9–10 animals/group) was measured by radioimmunoassay, using the MP Biomedicals® 17β-Estradiol (E2) Double Antibody kit. The minimum detectable dose was 1.2 pg/mL