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Inhibition of serotonin reuptake in the prepubertal rat ovary by fluoxetine and effects on ovarian functions

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ABSTRACT

Fluoxetine (FLX), a selective serotonin reuptake inhibitor is an antidepressant in the treatment of mood disorders. Its impact on reproductive processes is incompletely known. The present study analyzed the reproductive effects of FLX in prepubertal female rats. Two experiments were conducted. First (acute administration), 30-day-old female rats were injected intraperitoneally with 5 mg/kg of fluoxetine-hydrochloride, and were terminated 24, 48 or 72 h after the treatment. Second (subchronic administration), FLX was injected on days 30-33 of age, and the animals were terminated the day of first estrus. In acute treatment estradiol concentration increased to 72 h. In subchronic treatment increased serotonin concentration in ovaries and decreased the number of ova shed. An increase in number of atretic follicles and oocyte fragmentation was observed in these animals. The results suggest that FLX acts on the ovary or hypothalamus–pituitary axis resulting in modifications of the follicular development and ovulation.

1. Introduction

5-Hydroxytryptamine (5-HT) is a biogenic amine that participates in the regulation of the hypothalamus–pituitary–ovary axis. This amine is synthesised from the essential amino acid L-tryptophan by the action of tryptophan hydroxylase (TPH) and is metabolized by monoamine oxidase (MAO) to 5-hydroxyindole-3-acetic acid (5HIAA). These enzymes are the rate-limiting steps in the pathways of 5-HT synthesis and metabolism, respectively. 5-HT acts via 5-HT receptors, which are divided into classes 5-HT1 to 5-HT7 and are further subdivided into subtypes that range from A to F [1]. The serotoninergic receptors have an extensive distribution in the body, including the reproductive axis [2–6].

Within the central nervous system (CNS), the major sources of 5-HT are the neurons located in the raphe nuclei, which send serotoninergic fibres to various regions, including the hypothalamus [2]. In the hypothalamus, 5-HT participates in the modulation of gonadotropin-releasing hormone (GnRH) secretion [5]. In turn, GnRH stimulates the secretion of the gonadotropins, follicle-stimulating hormone (FSH) and luteinising hormone (LH), by the pituitary. The binding of 5-HT to the 5-HT2 receptor stimulates the secretion of GnRH and FSH, while LH secretion is modified when the 5-HT1 and 5-HT2 receptors are activated [6].

Additionally, the serotonergic system in the pituitary is associated with gonadotropin secretion. In some mammals, the gonadotrophs exhibit granules formed from 5-HT and FSH or 5-HT and LH [5]. Furthermore, these cells show the capacity for 5-HT reuptake [7].

The potential involvement of 5-HT in the regulation of the reproductive axis is not limited to the regulation of the hypothalamic or pituitary function. 5-HT has been detected in the ovaries, oviducts and uterus [8]. In the ovaries of mice, Amireault and Dube [4] identified a serotoninergic system that includes TPH, MAO, and the serotonin transporter protein, SERT, along with 5-HTD1 [9] and 5-HT7 in the oocytes. In addition, 5-HT2A and 5-HT2B receptors were detected in cumulus cell complex [10].

Several findings in model species suggest an important role of 5-HT in the regulation of ovarian function. 5-HT levels fluctuate during the estrus cycle, with the highest levels occurring during the estrus stage, suggesting that the fluctuations in 5-HT are associated

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with ovulation [11]. In bovine females, 5-HT is associated with the regulation of steroidogenesis. Culturing bovine corpus luteum due to the cells in presence of 5-HT increased progesterone (P4) secretion, perhaps due to the cells expressing serotonin receptor types 5-HT1 and 5-HT2 [12]. According to Moran et al. [13], in the rat 5-HT inhibits estradiol secretion and diminishes ovulation.

Also, during prepubertal development of female rats, 5-HT acts on the hypothalamus-hypophyseal-gonadal axis [13–16], and has been implicated in modulation of the secretion of GnRH, gonadotropins and steroid hormones that regulate the onset of puberty and ovarian functions. This idea is based on the observation that elimination of serotoninergic innervation arising from the dorsal raphe nucleus in 30-day-old prepubertal female rats, delays the timing of puberty, modifies LH release and blocks ovulation on the day of the first vaginal estrus, and increases atresia in preovulatory follicles [17].

Several substances enhance or inhibit serotonergic activity, and are used in the treatment of psychological and physical disorders; among these is FLX, Prozac®, a selective serotonin-reuptake inhibitor (SSRI). The target of FLX is the SERT. Inhibition of SERT results in an increase in the extracellular 5-HT concentration, which increases the activity of the serotoninergic system in various regions of the forebrain, including the hypothalamus [18]. FLX also acts as an antagonist of the 5-HT2 serotonin receptors [19]. FLX is prescribed in the treatment of mood disorders in the general population, including children and adolescents [20–22].

Relatively few studies have investigated the effects of FLX on reproductive functions in females, particularly in mammals. In contrast, there are reports of toxic effects of FLX in the hypothalamic–pituitary–gonadal axis in fish. In these organisms, exposure to FLX decreases egg production and is considered an endocrine disruptor [23].

In adult female rats of the Fischer strain with regular estrus cycles, chronic FLX administration lengthened the estrus cycle [24]. When the treatment was applied to rats of the Sprague Dawley strain, only 40% showed modifications of the estrus cycle [25]. The effect of FLX on the estrus cycle can be related to the modification of gonadal steroid hormone secretion because it has been shown that estrogens regulate changes in the vaginal epithelium during the estrus cycle stage.

Treatment of rats with FLX induces hyperprolactinaemia, anovulation and decreased serum concentrations of P4 and estradiol (E2) [26,27]. These findings suggest that FLX affects the serotonergic system of the hypothalamus, which results in the modification of LH and prolactin (PRL) secretion. Moore et al. [28] observed that female offspring have longer cycles after exposure to FLX *in utero* until weaning, with increased numbers of ovarian follicles and apoptotic ovarian cells. These results show that prolonged ingestion of FLX during pregnancy induces adverse effects on the sexual development of female rats and modifies some reproductive parameters. According to the authors' interpretation of the results, these effects may be related to changes in follicular development.

Based on the knowledge that 5-HT is involved in the modulation of secretion of hormones that regulate the sexual maturation and onset of puberty in female rats, it is possible that the onset of puberty and first ovulation can be vulnerable to changes in the activity of serotoninergic system induced by the administration of the FLX. To our knowledge, no study has been conducted to analyze the effects of administration of FLX throughout the prepubertal period on sexual maturation and ovarian functions in rats. Therefore, the aim of present study was to analyze the effects of FLX administration in onset of puberty and ovarian functions in prepubertal rats. To accomplish this objective and following the paradigm of the hypothalamus–pituitary–gonad axis, we first analyze the effects of FLX on serotoninergic system; then in circulating gonadotropins and sexual hormones; then in the onset of puberty, the ovary structure and function; then 5-HT1D receptor expression. We showed that FLX administration induced an increase in 5-HT in the ovaries. This increase resulted in an alteration of the ovarian structure and a decrease in the number of ova shed. Thus, our study may provide new insight to the knowledge of possible consequences of FLX consumption during sexual maturation at the onset of puberty, on ovarian function and reproduction.

2. Materials and methods

2.1. Animals

All experiments were performed following the guidelines established by the Mexican Law of Animal Protection Guidelines Treatment. The Committee of the Facultad de Estudios Superiores Zaragoza, UNAM, approved the experimental protocols (Letter 03/01/2012).

Four-month-old healthy pregnant rats of the CII-ZV conventional strain, no SPF, from our breeding stock, were housed individually in polycarbonate cages with bedding materials. These animals were maintained under standard room conditions, controlled temperature at 21 °C, humidity between 37% and 40%, with lights on from 05:00 to 19:00 h; with free access to food (Purina chow pellets, México) and water previously purified with filters. CII-ZV inbred strain is derived from Long–Evans and Wistar rats. CII-ZV strain was bred in the biotherium of the Facultad de Estudios Superiores Zaragoza (UNAM, México). This strain was characterized for various reproductive parameters, such as age of vaginal opening, age of first vaginal estrus, length of estrus cycle, litter size at birth, fecundity and litter size at parturition. These parameters are similar among CII-ZV, Long–Evans and Sprague-Dawley [29]. On postnatal day 1, the number and gender of live offspring of each dam was determined and the litter size was culled to 7 pups, comprised of six females and one male. The excess of offspring were donated to other experimental protocols. The pups had free access to their dams and were weaned at 21 days of age after which they had free access to food (Nutri-cubos, Purina S.A., México) and tap water. At postnatal day 30, females were randomly allocated into different experimental groups: Untouched, injected intraperitoneally with saline solution (0.9%) (Vehicle, VH) or with 5 mg/kg of fluoxetine hydrochloride (Sigma–Aldrich, St. Louis, USA), the minimum effective dose for the modification of the SERT protein in the CNS [30–32]. The animals were euthanized by carbon dioxide inhalation followed by being bled between 12:00 and 13:00 h.

At autopsy the pituitary, ovaries and brain were removed and placed in a cold saline solution. After removal from the brain, the anterior and medial hypothalami were sectioned following the parameters of the stereotaxic atlas of Paxinos [33]. The issues were stored at −70 °C until the 5-HT and 5-HIAA concentrations were measured using high-pressure liquid chromatography (HPLC) [34].

2.1.1. Experiment 1: effects of acute administration of FLX on the serotoninergic system in the hypothalamus–pituitary–ovarian axis and serum steroid levels

FLX is rapidly transformed to its major metabolite, norfluoxetine (NFLX), but the retention times for these drugs in the serum and particularly in tissues is long [32]. The prolonged retention extends the effects of the drug on 5-HT reuptake in the tissues. We also observed that variation of 5-HT concentration in ovaries was associated with modifications in steroid secretion [13]. Therefore, we decided to monitor the effects of a single dose of FLX on the levels of 5-HT and its metabolite, 5-hydroxyindole-3-acetic acid (5-HIAA), in the anterior and medial hypothalami, pituitary and ovaries and of progesterone (P4), testosterone (T) and 17β-estradiol (E2) in serum. Groups of rats of 30-days-old were injected