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Prenatal administration of letrozole reduces SDN and SCN volume and cell number independent of partner preference in the male rat

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HIGHLIGHTS

- Prenatal letrozole produces a subpopulation of males with same-sex preference.
- Prenatal letrozole reduces the volume and cell number in the SDN.
- Prenatal letrozole reduces the volume and cell number in the SCN.
- These reductions were independent of sex preference.

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ABSTRACT

During development, the exposure to testosterone, and its conversion to estradiol by an enzyme complex termed aromatase, appears to be essential in adult male rats for the expression of typical male sexual behavior and female-sex preference. Some hypothalamic areas are the supposed neural bases of sexual preference/orientation; for example, male-oriented rams have a reduced volume of the sexually dimorphic nucleus (oSDN), while in homosexual men this nucleus does not differ from that of heterosexual men. In contrast, homosexual men showed a larger number of vasopressinergic cells in the suprachiasmatic nucleus (SCN). Interestingly, male rats perinatally treated with an aromatase inhibitor, 1,4,6-androstatriene-3,17-dione (ATD), also showed bisexual preference and an increased number of vasopressinergic neurons in the SCN. However, this steroidal aromatase inhibitor has affinity for all three steroid receptors. Recently, we reported that the prenatal administration of the selective aromatase inhibitor, letrozole, produced a subpopulation of males with same-sex preference. The aim of this study was to compare the volume and number of cells of the SDN and SCN (the latter nucleus was immunohistochemically stained for vasopressin) between males treated with letrozole with same-sex preference, males treated with letrozole with female preference and control males with female preference. Results showed that all males prenatally treated with letrozole have a reduced volume and estimated cell number in the SDN and SCN, independent of their partner preference. These results indicate that the changes in these brain areas are not related to sexual preference, but rather to the effects of letrozole. The divergent results may be explained by species differences as well as by the critical windows during which the aromatase inhibitor was administered.

1. Introduction

In humans as well as in a number of animal species, numerous anatomical and functional brain differences have been described in relation to sexual orientation/preference [1–4]. In rodents, the exposure to sex hormones during development alters the typical sex differences in adulthood, such as sexual behavior and sexual preference [5–8]. According to the classical organizational hypothesis [9] subjects that exhibit same-sex preference could have experienced a prenatal hormonal environment with characteristics of the opposite sex. Thus, males with same-sex preference could have been exposed to subthreshold levels of testosterone or estradiol, leading to an incomplete masculinization (defined as an enhancement of male-typical behavior) and/or defeminization (suppression of female-typical responses) [10–13]. Following

Abbreviations: AVP, Arginine vasopressin; SDN, sexual dimorphic nucleus; SCN, suprachiasmatic nucleus; ATD, 1,4,6-androstatriene-3,17-dione.

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this idea, in rodents the perinatal administration of various aromatase inhibitors results, in adult male rats, in same-sex partner-preference [14–17].

In the mammalian brain, one of the best studied areas in relation to sex differences is the sexually dimorphic nucleus of the preoptic area (SDN-POA), which has a larger volume in males than in females [18]. The first description was made for rats, and has since been extended to other species, including ferrets, gerbils, guinea pigs, humans and sheep [19–23], although the homology of the SDN-POA is still under discussion (vide infra). In rodents, the sexual differentiation of SDN-POA depends on the presence of estrogens, metabolites of testosterone, during the perinatal period [24]. Although controversial (vide infra), in adulthood the SDN-POA could play a role in the circuitry that underlies male sexual behavior [25–27]. In sheep, there is a subpopulation of rams that shows spontaneous male preference. These rams display male-oriented precopulatory behaviors and mating attempts exclusively with other rams in a partner preference experiment where they could choose a male or a receptive ewe [28]. Due to their features, these animals are considered one of the best animal models for the study of subjects with same-sex preference. Interestingly, these rams possess an ovine SDN (oSDN) that is smaller than that of female-oriented rams, suggesting that alterations in the volume of the SDN could underlie the same-sex preference [10,22]. The size of this nucleus is under the influence of testosterone during development [29], but is unmodified by castration or testosterone treatment in adulthood [30]. In rats the volume of this nucleus depends on perinatal estrogens derived from testosterone [18,31]. In humans, the SDN or intermediate nucleus (InM) of the preoptic area differed between sexes and, as in all species studied, is larger in men than in women, and change with aging [23,32,33], but no differences have been found between homosexual and heterosexual men [34]. In this nucleus, named INAH1 by Allen and coworkers, they failed to find sex differences [35]. Other small cell groups, ventral to the SDN, InM or INAH1, have revealed sex differences and sexual orientation divergences between heterosexual and homosexual men. Thus, Allen and Gorski described the second and third interstitial nuclei of the anterior hypothalamus (INAH2 and INAH3) with a clear sexual dimorphism [35] and by sexual orientation [36]. LeVay found that the INAH3 is larger in men than in women and that its volume is smaller in homosexual men compared with that of heterosexuals and with a similar size to the one in women [37]. Interestingly, in relation to gender identity, male to female transsexuals also have a reduction in both the volume and number of cells in this nucleus (also termed hypothalamic uncinate nucleus) [33]. Based on its position and cytoarchitecture, Byne and coworkers proposed that the human INAH3 resembles the rat SDN [38].

Vasopressin is a neuropeptide produced in different brain areas with clear sex differences [39]. In rats, the vasopressinergic system is sensitive to changes in gonadal hormones; for example, castration eliminates vasopressin fibers in the lateral septum, whereas testosterone restores it [40]. In relation to sexual orientation, the population of vasopressin neurons in the suprachiasmatic nucleus (SCN) is higher in homosexual men in comparison with heterosexuals [34]. Interestingly, perinatal treatment with the aromatase inhibitor ATD (1,4,6-androstatriene-3,17-dione), of male rats, changed their partner preference: males showed bisexual behavior, accompanied by an increase in the number of vasopressinergic neurons (AVP+) in the SCN [41] and a reduced volume of the SDN [31].

Although ATD has been useful for analyzing sexual differentiation of sexual behavior, this steroidal compound, at higher concentrations, affects other androgen metabolizing pathways [42], and possesses affinity for androgen, estrogen and progesterone receptors [43,44], which may veil the conclusion that its actions are solely due to aromatase inhibition. Letrozole is a non-steroidal member of the third generation of aromatase inhibitors, acting by non-competitive or mixed action mechanisms [45]. Another difference between letrozole and ATD is that letrozole has an IC_{50} of 9.9 nM (the concentration needed to achieve 50% inhibition of aromatase activity), whereas ATD has a value of 71 nM [46,47]. Recently we reported that prenatal administration of letrozole resulted in a subpopulation of males with same-sex preference when examined in a partner preference test where the male had free choice to interact with a receptive female or a sexually active male [14,48]. After subcutaneous prenatal treatment with letrozole (0.56 μg/kg from gestation day 10 to day 22) usually one or two males per litter (mean 1 ± 0.3) spent more time with the sexually experienced male, displaying sexual interest (mounting the stimulus male, sniffing its genital area and hop/darting), and showed no or little interest in the receptive female. Interestingly, these males with same-sex preference were sexually aroused by a sexually-experienced male, measured as non-contact penile erections, and most of them displayed lordosis when mounted by the stimulus male. However, these males retained the full capacity to display male sexual behavior (mounts, intromissions and ejaculation) when caged together with a sexually receptive female. In contrast, the remainder of the males of the same litter was behaviorally unaffected by letrozole, spent more time with the receptive female, and displayed typical male sexual behavior and penile erections when exposed to a female. Remarkably, the hormonal profile of serum steroid hormones and gonadotropins in adulthood was similar between the males of the vehicle control group (with female preference) and those that received the prenatal treatment with letrozole, independent of their partner preference [14].

The aim of present study was to determine if there was an association between sex preference and morphometric parameters (number of cells and volume) in the SDN and SCN. For this last nucleus, these parameters were established in AVP+ cells. We hypothesize that males with same-sex preference induced by prenatal treatment with letrozole would have a reduction in the volume of the SDN (female-like) together with an increased volume and number of AVP+ cells in the SCN (observed in homosexual men and in male rats perinatally treated with ATD and with same-sex preference).

2. Material and methods

2.1. Animals

All rats were housed in a room with controlled temperature conditions (22 ± 2 °C) under a 12:12 light-dark inverted cycle (lights off at 10:00 h); with ad libitum access to water and food throughout the experiment. Animal management was done according to the general principles of laboratory animal care (NIH publication 85-23, 1985) and all experimental procedures were performed in accordance with the Mexican Official Norm for the use and care of laboratory animals "NOM-062-ZOO-1999" and approved by the local Ethics Committee (CICUAL-Cinvestav). Five control males (coming from two litters) and ten letrozole-treated males (coming from four litters) were tested in the early phase of the dark-light cycle (between 12 and 16 h) for partner preference at the age of three months and with a weight between 280 and 340 g.

2.2. Treatment and experimental design

The procedure of letrozole administration has been described in detail in Olvera-Hernández et al. [14]. Briefly: female rats in proestrus were time mated (day of mating = day 0 of pregnancy). Prenatal treatment consisted of daily subcutaneous injections to the mothers from day 10 of pregnancy until one day before delivery of vehicle (corn oil) or letrozole at a dose of 0.56 μg/kg/ml (Sigma-Aldrich, St. Louis, USA). At the day of birth, gestational days 22 or 23, the pups were culled to five males and five females. The pups were weaned at 21 days of age and housed in groups of 6–8 rats with the same treatment per cage. The rats were kept undisturbed until we performed the sex preference test at 90 days. Three groups of males were included and selected according to their sex preference: controls, treated prenatally with vehicle