Psychoneuroendocrinology 87 (2018) 93–107

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Sex differences in behavioral and neurochemical effects of gonadectomy and aromatase inhibition in rats

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ARTICLE INFO

Keywords:
Letrozole
Rat
Monoamines
Amino acids
Ovariectomy
Castration
Estrogens
Testosterone
Hippocampus
Prefrontal cortex
Forced swim test
Open field
Stress

ABSTRACT

Aromatase inhibitors, which are widely used for the treatment of estrogen-dependent cancers, have been associated with psychiatric side effects ranging from mania to depression. In the present study, we investigated sex differences in the behavioral and neurochemical effects of aromatase inhibition on male and female, sham-operated or gonadectomized adult rats. Three weeks after surgery, rats received chronic treatment with the aromatase inhibitor letrozole or vehicle and were then subjected to the open field test, which assesses general activity. Half of the subjects were subsequently exposed to the stressful procedure of the forced swim test (FST), which is also a test of antidepressant activity. Aromatase activity was analyzed in the hypothalamus and testosterone and corticosterone were assayed in the blood serum of all rats. The hippocampus and prefrontal cortex (PFC) were analyzed for monoamine (noradrenaline, dopamine and serotonin), as well as amino acid (GABA, glutamate, glycine, taurine, alanine and histidine) levels. The observed decrease in hypothalamic aromatase activity confirmed the efficacy of letrozole treatment in both sexes. Moreover, letrozole enhanced testosterone levels in sham-operated females. In the open field test, females were overall more active and explorative than males and gonadectomy eliminated this sex difference. In the FST, females exhibited overall higher immobility than males and gonadectomy further enhanced this passive behavior in both sexes. However, sustained aromatase inhibition had no effect on open field and FST behaviors. Head shakes during FST, which were fewer in females than in males, were reduced by castration in males and by letrozole treatment in ovariectomized females, suggesting a role of testosterone and extra-gonadal estrogens in the expression of this behavior. Sustained aromatase inhibition also decreased noradrenaline and the dopaminergic turnover rates [DOPAC/DA, HVA/DA] in the hippocampus and PFC of male and female rats, irrespectively of gonadectomy. Moreover, letrozole treatment enhanced the serotonergic turnover [5HIAA/5HT] rate in the hippocampus of males and females, irrespectively of gonadectomy. Amino acid levels were not influenced by letrozole, but sex differences were demonstrated with higher levels in the PFC of females vs. males. Present findings suggest that the neuropsychiatric effects of aromatase inhibition can be attributed to the inhibition of extragonadal estrogen synthesis, presumably in the brain, and could be further associated with serotonergic and catecholaminergic changes in brain regions involved in mood and cognition. Importantly, present data could be linked with the neurobiology of affective side-effects in post-menopausal women receiving aromatase inhibitors.

1. Introduction

Aromatase inhibitors, which are widely used for the treatment of estrogen-dependent cancers, have been associated with psychiatric side effects ranging from mania to depression (Goodwin, 2006; Rocha-Cadman et al., 2012). Aromatase is the rate-limiting enzyme that catalyzes the conversion of androgens into estrogens. It is mainly located in the ovaries and in the testes (Lephart, 1996). However, it has been shown that other organs, such as the liver, bones, placenta and importantly the brain can produce estrogens locally (Santen et al., 2009). Emerging evidence suggests that brain-derived estrogens are responsible for the fine-tuning of neuronal circuits in males and females (Rudolph et al., 2016; Srivastava et al., 2011).

Our previous studies have shown that estrogen-deprived female, but

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http://dx.doi.org/10.1016/j.psyneuen.2017.10.007
Received 11 April 2017; Received in revised form 5 October 2017; Accepted 9 October 2017

not male, aromatase knockout (ArKO) mice display altered coping behavior in the forced swim test (FST) and serotonergic activity in the hippocampus (Dalla et al., 2004, 2005b). However, these mice are not an appropriate model to differentiate the role of peripheral versus brain-derived estrogens in adulthood. Although there is a wealth of studies investigating the effects of extra-gonadal estrogens on sexual/social behaviors, cognition, ischemia/stroke and epilepsy (Arevalo et al., 2015; Cornil et al., 2006; de Bournonville et al., 2016; Sato and Woolley, 2016; Srivastava et al., 2011; Srivastava et al., 2013), there are only limited studies on the role of extra-gonadal estrogens in stress response and antidepressant action (Wei et al., 2014). Moreover, several studies have implicated extra-gonadal estrogens in the regulation of long-term potentiation, glutamate NMDA receptors and spine density in the hippocampus of female mice (Tuscher et al., 2016; Vierk et al., 2012; Zhou et al., 2010), suggesting an effect on substrates of stress, anxiety and mood.

We recently showed that acute but not sustained treatment with letrozole, an aromatase inhibitor, exerts an antidepressant effect on intact female rats in the FST (Kokras et al., 2014). In the present study, we sought to assess sex differences and further investigate the behavioral and neurochemical effects of sustained aromatase inhibition in both male and female rats. We chose the open field test, which assess general activity and anxiety levels, as well as the FST, which is a test of antidepressant activity and stress coping strategies (Kokras and Dalla, 2014). To differentiate the effects of gonadal- and extra-gonadal (mainly brain-derived) estrogens, we investigated the effect of gonadectomy on these parameters, predicting that gonadectomy would only decrease periphery-derived estrogens, whereas gonadectomy combined with aromatase inhibition would eliminate both peripheral and central sources of estrogens. We focused on the prefrontal cortex (PFC) and the hippocampus that form a circuit, which is important for stress and depression (Kafetzopoulos et al., 2017). Specifically, we investigated effects on monoamine (serotonin, noradrenaline and dopamine), as well as amino acid (such as glutamate, glutamine, GABA, taurine, histidine and alanine) levels, as some of these neurotransmitter systems have been found to be sexually differentiated and affected by estrogens and stress (Arevalo et al., 2015; Barth et al., 2015; Srivastava et al., 2013). Finally, we measured aromatase activity in the hypothalamus to a) confirm estrogen depletion in the brain and b) investigate sex differences and stress effects on aromatase activity in the brain.

2. Material and methods

2.1. Animals

Male (n = 51) and female (n = 48) adult Wistar rats, aged 3 months old and weighing 350 ± 13 and 250 ± 8 g (mean ± S.E.M.) respectively, were group-housed under standard laboratory conditions at the Dept. of Pharmacology, Medical School of Athens. All experiments were performed in accordance with the EU directive 2010/63. Half (n = 25 males and 23 females) of the animals received a sham-operation while the others were gonadectomized under anesthesia with 100 mg/kg ketamine, 10 mg/kg xylazine and 0.5 mg/kg atropine intraperitoneally (i.p.). Females (n = 25) received a bilateral ovariectomy and males (n = 26) a castration (orchidectomy) as previously described (Dalla et al., 2008b). After surgery, rats were kept warm and under observation until recovery from anesthesia and were provided with 100 mg/kg of acetaminophen (24 mg/ml syrup), administered orally. All animals were left undisturbed to recover in their cages for 3 weeks. Estrous cycle was assessed with vaginal smears as previously described (Kokras et al., 2015). Sham-operated females were cycling normally, whereas ovariectomized females were not. Subsequently, all rats received a daily i.p. injection for 8 days, of the aromatase inhibitor letrozole (1 mg/kg; Novartis Pharma AG, Switzerland) (n = 50) or vehicle (saline with 5% Tween 80, 5% ethanol) (n = 49), as previously described (Kokras et al., 2014). Letrozole-treated females stopped cycling and only sham-operated, vehicle-treated females were cycling randomly and all phases of the estrous cycle were equally represented in this group.

2.2. Behavioral testing

On the 7th day of letrozole treatment, all rats were subjected for 10 min to the open field test (OF), as previously described (Kafetzopoulos et al., 2017). All rats were acclimated to the test room for 1 h and thereafter placed in a clear Plexiglas chamber (Med Associates Inc., St Albans, VT) measuring 43 × 43 × 30 cm with arrays of 16 × 16 photo detectors, positioned 2.5 cm and 10 cm above the floor of the chamber. Interruption of adjacent photo beams provided an index of ambulatory activity (horizontal activity), while interruption of the upper line of photo beams provided an index of rearing behavior (vertical activity). The latency to escape from the center of the open field and the time spent in the center served as indices of anxiety (Kokras et al., 2012). One day later (Day 8) and approximately 24 h after the 7th injection, half of the rats (n = 50) remained undisturbed in their home cages and served as controls while the other half (n = 49) were subjected to the forced swim test (FST), which is a test of antidepressant activity, but is also a stressful procedure that elicits coping behaviors (de Kloet and Molendijk, 2016; Slattery and Cryan, 2012). For the FST, each rat was individually placed in a cylindrical tank measuring 50 cm height * 20 cm width, filled with water (24 ± 1 °C) at a height of 40 cm. On the first day, rats were forced to swim for 15 min. All rats received the final 8th letrozole or vehicle injection after the first FST session. Twenty-four hours later (day 9), they were subjected to a 5 min FST session and the total duration of immobility (passive behavior), swimming and climbing (active behaviors), as well as frequency of head shakes were manually measured with the in-house developed software *Kinoscope* (Kokras et al., 2017a). Rats were considered to be immobile when they were making only movements necessary to keep their heads above the water. Swimming was recorded when they were actively swimming around in circles (horizontal movement). Climbing was scored when rats were climbing at the walls of the cylinder (vertical movement). The head shakes were recorded when rats exhibited headshake responses, as described in detail elsewhere (Kokras et al., 2017b). Immediately after the second FST session, all rats (controls and FST) were killed by rapid decapitation and the hypothalamus, hippocampus and PFC were dissected, weighed and stored at −80 °C. Trunk blood was collected for serum extraction.

2.3. Aromatase activity

Aromatase activity was determined in the hypothalamus by the production of tritiated water associated with the conversion of [1β-^{3}H]-androstenedione into estrone (Roselli et al., 1984). Briefly, hypothalamus samples were homogenized and triplicate aliquots (50 µl) of homogenate containing approximately 5 mg wet weight were added to 50 µl of 1.2 µM [1β-^{3}H]-androstenedione and 50 µl of buffer with or without Vorozole, a potent aromatase inhibitor, to determine the specific enzymatic activity. To initiate the assay, 50 µl of NADPH (4.8 mM) were added. The reaction was stopped by cooling the samples and adding 0.4 ml ice-cold 10% trichloroacetic acid containing 2% activated charcoal. After centrifugation, supernatants were applied to columns with a Dowex cation exchange resin AG 50W-X4, 100–200 mesh. The columns were then eluted and effluents were collected in scintillation vials and 10 ml Ecoscint-A were finally added. Vials were counted for 3 min on a Wallac Winspectral 1414 Liquid Scintillation Counter. Enzyme activity is expressed in fmol/h/mg fresh weight.

2.4. Hormonal assays

Blood samples were processed to recover serum (centrifugation at 4000*g*, 30 min, 4 °C) and serum samples were stored at −20 °C before