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Lack of functional estrogen receptor β influences anxiety behavior and serotonin content in female mice

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Abstract

Estrogen has been linked to the modulation of anxiety in females. Here we report results of anxiety tests conducted in female estrogen receptor β (ERβ) knockout (ERβKO) and wild-type (WT) mice. Ovariectomized (OVX) mice treated with chronic estradiol (E2) replacement did not behave differently on the elevated plus-maze when compared with OVX mice that did not experience hormone replacement. However, a genotype difference was noted; WT females were more likely to explore the distal portion of the open arm of the maze than ERβKO littermates. In addition, ERβKO female mice had significantly lower serotonin (5-HT) content than WT littermates in several brain regions including: the bed nucleus of the stria terminalis, preoptic area, and hippocampus. A similar trend was noted in the dorsal raphe nucleus. Dopamine content was reduced within the caudate putamen in ERβKO mice as compared to brains from WT animals. Thus, in the absence of functional ERβ, regardless of the presence or absence of circulating E2 in plasma, female mice exhibited enhanced anxiety and decreased concentrations of 5-HT or dopamine in several brain regions. We hypothesize that ERβ is required during development to modulate the effects of estrogen on anxiety and catecholamine concentrations in female mouse brains.

Keywords: SSRI; Menopause; Estrogen receptor; Depression; Anxiety; Serotonin

1. Introduction

Estrogen is associated with affective disorders such as anxiety or depression. Women are more likely than men to develop affective disorders [3] and this risk increases after menopause [9,24]. The relationship between estrogen and anxiety may be modulated by the serotonergic and dopaminergic systems. Therapeutics directed at enhancing extracellular concentrations of serotonin (5-HT) ease symptoms associated with anxiety [6]. Estrogen treatment concomitant with selective serotonin reuptake inhibitors (SSRI) enhances their effectiveness [35]. Also, estrogen treatment enhances the activity of the dopaminergic system [27,34] and possibly alleviates psychological symptoms in humans [29].

In mice and rat models, the relationship between estrogen and anxiety behavior is controversial and differences in findings may reflect different experimental procedures. Using the elevated plus-maze paradigm, Frick et al. [12] observed that female mice spent less time than males on the open arm (indicating more anxiety) but there were no effects of the estrous cycle. However, Galeeva and Tuohimaa [13] found that exploration of the open arms was reduced in diestrous females, suggesting that when estrogen levels are low, female mice are more anxious. In contrast, ovariectomized (OVX) female mice given estradiol benzoate had enhanced anxiety behavior, in comparison to OVX mice that did not receive hormones in each of three anxiogenic behavior tests [26].

The effects of estrogen are transduced via its two known receptors, estrogen receptor α (ERα) and β (ERβ). Using mice lacking a functional gene for either ERα (ERαKO) or ERβ (ERβKO), Krezel et al. [18] showed that ovary-intact

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random cycling female ERβKO mice explored the open arms of the plus-maze less than wild-type (WT) or ERαKO females. Moreover, ERβKO female mice spent more time next to the edges and in contact with the arena wall in the open field test, a test believed to measure both anxiety and general activity. The results of this experiment [18] were not as complete as they could be because the authors did not control for differences in circulating estrogen concentrations over the estrous cycle. In two other lines of ERαKO [21] and ERβKO [17] mice, OVX and estradiol (E2) replacement increased running wheel activity in mice of all genotypes except ERαKO, suggesting that the effects of E2 on this type of locomotor activity is facilitated by ERα [28]. Moreover, ERβKO females did not differ from other genotypes in time spent in anxiogenic portions of the open field but they were more active in the open field [28].

In the present study, we first examined the effects of a physiological dose of estradiol given after ovariectomy on anxiety-like behavior in ERβKO and WT mice. Because the only study [18] reporting an anxious phenotype in ERβKO female mice was conducted in a strain of ERβKO mice that have only recently been developed [10], we felt it was important to replicate this effect in more commonly used and commercially available (from Taconic labs) ERβKO mice. Here, we employed high-performance liquid chromatography (HPLC) to assess the effects of genotype and hormone treatment on monoamine concentrations in brain. We hypothesized that if ERβ is critical for expression of anxiety-like behavior and monoamine transmission in mice, ERβKO female mice would show reduced exploration of the elevated plus-maze and would have different monoamine profiles that those of WT littermates.

2. Materials and methods

2.1. General methods

Mice used in these experiments were offspring of heterozygous ERβKO breeding pairs [17]. At the time of this experiment, mice had been backcrossed for five generations into C57BL/6J mice. Thus, on average, our animals were 97% genetically similar to C57BL/6J, with the remaining 3% of the genome from SV129 mice. All offspring were genotyped via PCR analysis of tail DNA [17]. After weaning (18–20 days of age), all mice were housed in groups of 2–3 in a temperature-controlled (23±1 °C) and light-controlled (12:12; L/D; lights off at 1900 EST) animal facility. Animals were given Purina mouse chow (#5001) and water ad libitum. All animal maintenance and procedures were in accordance with the University of Virginia Animal Care Committee regulations.

All subjects were sexually naïve, female WT (+/+ for ERβ gene; n=40) or ERβKO (−/− for ERβ gene; n=40) littermates. Behavioral testing began when the animals reached at least 60 days of age (range 60–75 days). All experiments were conducted between 1300 and 1600 h [18].

Mice were bilaterally OVX and one half of the animals were given a single (5 mm) subscapular Silastic implant (1.02 mm ID×2.16 mm OD) containing estradiol-17β (E2 diluted 1:1 with cholesterol) under general anesthesia (100 mg/kg ketamine and 10 mg/kg xylazine, i.p.). Previous work in our laboratory has shown that this implant yields E2 concentrations (85–100 pg/ml) similar to intact mice in proestrus [36]. We opted to use implants instead of daily injections because handling can affect behavior on the elevated plus-maze [19]. Mice were given at least 14 days to recover prior to use in any experiment. In all experiments, the observer was blind to the genotypes of the animals at time of data collection and analysis.

2.2. Elevated plus-maze

The elevated plus-maze is a widely used behavior test for assessing anxiety-like behavior in rodents [7]. Our methods have been described previously [15]. Briefly, testing took place in a dimly illuminated room. Animals were acclimated to the test room for 2 h prior to testing. Mice were gently removed from their cages and were placed in the central portion of the plus-maze facing an open arm and given five minutes to explore the maze while their behavior was videotaped. Between subjects, the maze was cleaned with a paper towel moistened with tap water. The open arms were divided into inner and outer halves based on results of pilot studies. In the pilot work, we noted two types of open arm exploration: WT mice either briefly entered just into the portion of the open arm closest to the closed arm (for less than 1 s) or they would fully explore the entire arm. Time spent on the inner and outer portions of the open arm, total time spent on the open arm, time spent in the closed arms, numbers of entries to the outer and inner half of the open arm, number of entries to the closed arm, and total arm entries were recorded.

Female mice were OVX and given either E2 (n=10 of each genotype) or blank (n=10 of each genotype) implants. These females were exposed to the elevated plus-maze for 5 min. Two to three days after plus-maze exposure, mice were deeply anesthetized with Halothane inhalant, killed by decapitation and brains were frozen on dry ice for HPLC analysis of monoamines. At the time of sacrifice, we confirmed the presence of the hormone implant and examined uteri, which is a bioassay for estradiol activity.

2.3. Monoamine HPLC

Brain levels of dopamine, dihydroxyphenylalanine (DOPA), dihydroxyphenylacetate (DOPAC), norepinephrine (NOR), epinephrine (EPI), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and homovanilic acid (HVA) were measured with a reverse-phase ion-pair high perform-