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Estrogen receptor β deficiency impairs BDNF–5-HT_{2A} signaling in the hippocampus of female brain: A possible mechanism for menopausal depression

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ABSTRACT

Depression currently affects 350 million people worldwide and 19 million Americans each year. Women are 2.5 times more likely to experience major depression than men, with some women appearing to be at a heightened risk during the menopausal transition. Estrogen signaling has been implicated in the pathophysiology of mood disorders including depression; however, the underlying mechanisms are poorly understood. In this study, the role of estrogen receptor (ER) subtypes, ERα and ERβ, in the regulation of brain-derived neurotrophic factor (BDNF) and serotonin (5-HT) signaling was investigated; two pathways that have been hypothesized to be interrelated in the etiology of depression. The analyses in ERα$^{-/-}$ and ERβ$^{-/-}$ mouse models demonstrated that BDNF was significantly downregulated in ERβ$^{-/-}$ but not ERα$^{-/-}$ mice, and the ERβ$^{-/-}$-mediated effect was brain-region specific. A 40% reduction in BDNF protein expression was found in the hippocampus of ERβ$^{-/-}$ mice; in contrast, the changes in BDNF were at a much smaller magnitude and insignificant in the cortex and hypothalamus. Further analyses in primary hippocampal neurons indicated that ERβ agonism significantly enhanced BDNF/TrkB signaling and the downtream cascades involved in synaptic plasticity. Subsequent study in ERβ mutant rat models demonstrated that disruption of ERβ was associated with a significantly elevated level of 5-HT_{2A} but not 5-HT_{1A} in rat hippocampus, indicating ERβ negatively regulates 5-HT_{2A}. Additional analyses in primary neuronal cultures revealed a significant association between BDNF and 5-HT_{2A} pathways, and the data showed that TrkB activation downregulated 5-HT_{2A} whereas activation of 5-HT_{2A} had no effect on BDNF, suggesting that BDNF/TrkB is an upstream regulator of the 5-HT_{2A} pathway. Collectively, these findings implicate that the disruption in estrogen homeostasis during menopause leads to dysregulation of BDNF − 5-HT_{2A} signaling and weakened synaptic plasticity, which together predispose the brain to a vulnerable state for depression. Timely intervention with an ERβ-targeted modulator could potentially attenuate this susceptibility and reduce the risk or ameliorate the clinical manifestation of this brain disorder.

1. Introduction

Depression is a chronic, reoccurring neuropsychiatric disease that currently affects 350 million people worldwide including 6.7% of Americans (World Health Organization, 2012). Although significant progress has been made in the development of antidepressants, current treatments yield a therapeutic efficacy in only 60% of depressed patients (Rush et al., 2009) and are associated with several unfavorable side effects (Feighner, 1999; Holm and Markham, 1999). Moreover, the mechanism of action underlying the therapeutic effect of antidepressants is currently unknown. These facts underscore the importance of understanding the mechanistic etiology of depression and developing novel therapeutic methods based on that understanding.

Depression disproportionately affects men and women. Epidemiological data have revealed that women are 2.5 times more likely to experience major depression than men (Weissman et al., 1984), with some women appearing to be at a heightened risk during the menopausal transition (Campbell et al., 2017; Pratt and Brody, 2014), a period characterized by irregular cycling coupled with fluctuations in gonadal steroid hormone levels. Moreover, several clinical studies have demonstrated significantly reduced depression and mood-related symptomology in menopausal patients undergoing

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estrogen therapy (ET) when compared to placebo controls (Schmidt et al., 2000; Soares et al., 2001). It has been suggested that estrogen receptors, ERα and ERβ, act as the primary regulators of ET-mediated antidepressant effects, however, their specific roles remain controversial. On one hand, post-partum rats treated with an ERα agonist demonstrated reduced depressive behavior in multiple tests suggesting a primary regulatory role for ERα (Furuta et al., 2013). In contrast, ovariectomized (OVX) rats treated with an ERβ agonist (Walf et al., 2009; Weiser et al., 2009) exhibited significantly less depressive behavior than rats treated with vehicle or an ERα agonist (Yang et al., 2014), an effect that was absent in ERβ$^{-/-}$ mice (Walf et al., 2009), indicating that ERβ signaling may play a key role in the mechanism of ET in depression. These disparities require further molecular-level investigations.

Serotonin (5-HT) is central to the monoamine hypothesis of depression (Coppen, 1967). Increased 5-HT bioavailability in the synapses and thus increased 5-HT function has been shown to effectively relieve depressive symptoms (Delgado et al., 1990). 5-HT induces its wide range of actions through a myriad of receptors, the expression and function of which has also been heavily studied in an attempt to unravel the pathophysiology of depression. Studies have reported a decrease in the 5-HT$_{1A}$ receptor signaling (Hirvonen et al., 2008) and an increase in the 5-HT$_{2A}$ receptor signaling (Shelton et al., 2009) in the hippocampus and prefrontal cortex of depressed patients when compared to control subjects, which was reversed in the subjects treated with antidepressants (Celada et al., 2004), implicating the involvement of these receptors in the etiology of depression.

Although 5-HT signaling is highly implicated in depression, contradictory reports (for review see (Hinz et al., 2012)) and the inability of the monoamine hypothesis to explain certain clinical findings such as therapeutic lag (Frazer and Benmansour, 2002) have shifted the research focus towards novel alternative mechanisms of depression (Duman and Monteggia, 2006). Brain-derived neurotrophic factor (BDNF) is essential to the more recent and clinically relevant neurotrophic hypothesis of depression. Several clinical and animal studies (Dwivedi et al., 2003; Lippmann et al., 2007) have reported a decrease in the mRNA and protein expression levels of BDNF and TrkB in the hippocampus and prefrontal cortex of depressed subjects when compared with control subjects, which was reversed by chronic but not acute antidepressant treatment (Xu et al., 2003). In addition, mice heterozygous for a BDNF null allele (BNDF$^{+/-}$) and overexpressing TrkB transgenic gene were found to be resistant to antidepressants (Saarelainen et al., 2003), indicating that BDNF/TrkB signaling is required for the therapeutic effects of antidepressants. Although behavioral and clinical studies strongly support a connection between depression and BDNF signaling, the short half-life of BDNF in systemic circulation (Sakane and Pardridge, 1997) and its blood-brain barrier (BBB) impermeability (Sakane and Pardridge, 1997) hinders its use as a therapeutic agent.

Both the monoaminergic and neurotrophic hypotheses of depression fall short of providing a viable therapeutic window, thus it is highly possible that the key for understanding and successfully treating depression may be found by examining the interaction of these two divergent signaling pathways. 5-HT signaling has been shown to regulate BDNF/TrkB signaling in the hippocampus (Vaidya et al., 1997). Parallel to these studies, BNDF infusion in the midbrain has been reported to increase 5-HT turnover and promote phenotype and function of serotonergic neurons (Siuciak et al., 1996). Additionally, the Val66Met BDNF mouse model exhibits a compromised selective serotonin reuptake inhibitors (SSRIs) response (Yu et al., 2012), while BNDF$^{+/-}$ mice demonstrate reduced 5-HT$_{1A}$ (Hensler et al., 2007) and increased 5-HT$_{2A}$ sensitivity (Trajkovska et al., 2009) in the hippocampus. These observations suggest a possible intertwined interaction of these two different signaling molecules, thus highlighting an important avenue for further investigation.

The goal of this study was to investigate the role of ERs in the regulation of BDNF and 5-HT signaling in the female brain. The analyses in ERα$^{-/-}$ and ERβ$^{-/-}$ animal models demonstrated brain region-specific regulation of BDNF by ERβ but not ERα. Additionally, the data indicate that 5-HT$_{2A}$ and not 5-HT$_{1A}$ is negatively regulated by ERβ. Taken together, these results suggest that ERβ-mediated regulation of BDNF–5-HT$_{2A}$ signaling could play a major role in both the development and intervention of depressive disorders in menopausal women.

2. Materials and methods

2.1. Chemicals

4,4′,4″-(4-Propyl-[1H]-pyrazole-1,3,5-triyl) trisphenol (PPT; ERα agonist), 2,3-bis(4-Hydroxyphenyl)-propionitrile (DPN; ERβ agonist), 4-[2-Phenyl-5,7-bis(trifluoromethyl) pyrazolo[1,5-*a*]pyrimidin-3-yl] phenol (PHTPP; ERβ antagonist), 4-Iodo-2,5-dimethoxy-α-methylbenzeneethanamine hydrochloride (DOI; 5-HT$_{2A/2C}$ agonist), 7,8-Dihydroxy-2-phenyl-4H-1-benzopyran-4-one (7,8 DHF; TrkB agonist), and 4-(4-Fluorobenzoyl)-1-(4-phenylbutyl)piperidine oxalate (4F 4PP oxalate; 5-HT$_{2A}$ antagonist) were purchased from Tocris Bioscience (Ellisville, MO). Chemicals were dissolved at 10–50 mM in analytically pure ethanol and further diluted in culture medium to the final working concentrations (PPT: 100 nM, DPN: 100 nM, PHTPP: 1 μM, DOI: 3 μM, 7,8 DHF: 10 μM, and 4F 4PP oxalate: 10 μM) immediately prior to use. The sources of other materials are indicated in the experimental methods described below.

2.2. Animal models

The use of animals was approved by the Institutional Animal Care and Use Committee at the University of Kansas and followed NIH guidelines for the care and use of laboratory animals. Embryonic day-18 fetuses derived from time-pregnant Sprague-Dawley rats (Harlan, Indianapolis, IN) were used to obtain primary hippocampal neuronal cultures for *in vitro* experiments. *In vivo* experiments were carried out in ERα$^{-/-}$ and ERβ$^{-/-}$ mouse and rat models. ERα$^{-/-}$ and ERβ$^{-/-}$ mouse models (The Jackson Laboratory, Bar Harbor, ME) were created by using a targeting vector containing a neomycin resistance gene driven by the mouse phosphoglycerate kinase promoter to introduce stop codons into exon 2 or exon 3 for ERα$^{-/-}$ and ERβ$^{-/-}$ respectively. The construct was introduced into 129P2/OlaHsd-derived E14TG2a embryonic stem (ES) cells (BK4 subline). Correctly targeted ES cells were injected into C57BL/6J blastocysts to obtain chimeric animals. These mice were then backcrossed to C57BL/6J for eight generations. The line was then bred to C57BL/6NTac from which homozygotes were generated. The ERβ mutant rats were generated using zinc finger nuclease (ZFN) mediated genome editing to target deletion of exon 3 (Ex 3$^{-/-}$) and exon 4 (Ex 4$^{-/-}$) in the ERβ gene (Rumi et al. Defining the role of estrogen receptor beta in the regulation of female fertility. Endocrinology 2017, in press) and are available at the Rat Resource and Research Center (University of Missouri, Columbia, MO). The Ex 3$^{-/-}$ mutation results in a frameshift and a null mutation, whereas the Ex 4$^{-/-}$ mutation results in a DNA binding deficient ERβ variant protein. Animals (mice at 6 months of age and rats at 6 and 10 months of age) were euthanized via thoracotomy and cortical, hippocampal and hypothalamic brain regions were immediately dissected and flash frozen in dry ice (n = 5 for each genotype and age group).

2.3. Primary hippocampal neurons

Primary cultures of hippocampal neurons were prepared from embryonic day-18 fetuses derived from time-pregnant Sprague-Dawley rats. Briefly, after dissected from the brains of the rat fetuses, the hippocampi were treated with 0.02% trypsin in Hank's balanced salt