Published in final edited form as:

Vitam Horm. 2017 ; 103: 27–52. doi:10.1016/bs.vh.2016.08.004.

Estrogen Receptors Modulation of Anxiety-Like Behavior

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Abstract

Estrogens exert profound effects on the expression of anxiety in humans and rodents; however, the directionality of these effects varies considerably within both clinical and preclinical literature. It is believed that discrepancies regarding the nature of estrogens' effects on anxiety are attributable to the differential effects of specific estrogen receptor (ER) subtypes. In this chapter we will discuss the relative impact on anxiety and anxiety-like behavior of each of the three main ERs: ER α , which has a generally anxiogenic effect, ER β , which has a generally anxiolytic effect, and the G-protein-coupled ER known as GPR30, which has been found to both increase and decrease anxiety-like behavior. In addition, we will describe the known mechanisms by which these receptor subtypes exert their influence on emotional responses, focusing on the hypothalamic–pituitary–adrenal axis and the oxytocinergic and serotonergic systems. The impact of estrogens on the expression of anxiety is likely the result of their combined effects on all of these neurobiological systems.

1. INTRODUCTION

Changes in circulating estrogen levels across the reproductive lifespan have long been associated with changes in the incidence of anxiety in women. The risk of developing an anxiety disorder is elevated at menarche (Patton et al., 1996), a developmental period characterized by an increase in circulating estradiol from prepubertal to adult levels (Ojeda & Bilger, 2000). By contrast, an increase in anxiety symptoms has also been noted when estradiol levels drop, such as following surgical menopause (Rocca et al., 2008) and in postmenopausal women (Sahingoz, Uguz, & Gezginc, 2011). Moreover, toward the end of the luteal phase of the menstrual cycle, which is characterized by a dramatic decline in circulating estradiol levels, there is an increase in symptoms of anxiety in patients with anxiety disorders (Cameron, Kuttesch, McPhee, & Curtis, 1988), as well as in patients with premenstrual disorders (Yonkers, O'Brien, & Eriksson, 2008).

While the association between anxiety symptoms and low endogenous estradiol levels might suggest a therapeutic effect of estrogens, both clinical and preclinical studies have reported that treatment with estradiol yields contradictory results. For example, in postmenopausal women, anxiety levels have been reported to either decrease (Gleason et al., 2015) or remain unaffected (Demetrio et al., 2011) following estrogen therapy. Using rodent models, studies have shown that the effect of estradiol on anxiety-like behavior may be dependent on both

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the dose administered and the behavioral testing paradigm. A recent study by Kastenberger, Lutsch, and Schwarzer (2012) conducted in ovariectomized mice found that a high dose of estradiol (0.25 mg/kg) but not a low dose (0.025 mg/kg) decreased anxiety-like behavior in the elevated plus maze. In contrast, only the low dose of estradiol increased anxiety-like behavior in the open field test (Kastenberger et al., 2012). A different group also noted that a low dose of estradiol (2.0 µg/day) increased anxiety in ovariectomized mice in the light/dark test, while a dose that was even lower (0.2 µg/day) instead decreased anxiety-like behavior (Tomihara et al., 2009). Other studies have reported either anxiogenic (Mora, Dussaubat, & Diaz-Véliz, 1996), anxiolytic (Tian et al., 2013), or null (Walf & Frye, 2008) effects of estradiol in a variety of rodent models. Consequently, the conflicting effects of estradiol on anxiety and anxiety-like behavior have been proposed to be the result of diverging roles for estrogen receptor subtypes (Kastenberger et al., 2012).

Currently, researchers are aware of at least three types of estrogen receptors. The classic estrogen receptors, ER α and ER β , are highly homologous in structure and belong to one subclass of a large superfamily of nuclear hormone receptors (Burris et al., 2013) which also include the receptors for androgens, glucocorticoids, mineralocorticoids, thyroid hormone, and retinoic acid, to name but a few. A more complete examination of these receptor proteins can be found in several recent reviews (Huang, Chandra, & Rastinejad, 2010; Morrill, Kostellow, & Gupta, 2015), and their distributions into the various subclasses of receptors, specific receptor-related transcriptomics, and reagents are now cataloged online at the nuclear receptor signaling atlas website (www.nursa.org). It is classically thought that the nuclear receptors influence gene expression both directly, as ligand-activated transcription factors, and perhaps indirectly, through the membrane localization of the classical receptors which can rapidly impact intra-cellular signaling cascades (Edwards, 2005). A third, more recently identified receptor, the G-protein-coupled estrogen receptor (GPR30 or GPER1), is not related to the nuclear hormone receptors and exerts its effects through both rapid signaling events and, to a lesser extent, direct transcriptional activation (Prossnitz & Arterburn, 2015). Additionally, receptors such as the STX-sensitive receptor, a membrane-associated receptor which can mediate some of the actions of estradiol (Smith, Bosch, Wagner, Rønnekleiv, & Kelly, 2013), and ER-X, a putative membrane receptor for 17 α estradiol (Toran-Allerand et al., 2002), have recently been described, although our understanding of their effects is currently, at best, very limited. In this chapter, we will discuss the contribution of each of the major estrogen receptors to anxiety and anxiety-like behavior, and their potential roles as therapeutic targets for anxiety disorders.

2. NUCLEAR ESTROGEN RECEPTOR FORM AND FUNCTION

ER α and ER β show a number of similarities, both in their modular structure (Ascenzi, Bocedi, & Marino, 2006) as well as in homology within the central DNA binding domain (Pettersson, Grandien, Kuiper, & Gustafsson, 1997). The two ERs arose from a single duplication event approximately 450 million years ago (Kelley & Thackray, 1999). Since then, they have undergone a parallel evolution resulting in substantial sequence homology, yet they exhibit unique roles in regulated mammalian physiology. The generation of transgenic mouse lines that are null for ER α (Antonson, Omoto, Humire, & Gustafsson, 2012; Dupont et al., 2000), ER β (Dupont et al., 2000; Kregge et al., 1998), or both (Dupont

et al., 2000; Kudwa & Rissman, 2003), has helped to determine the function of the nuclear estrogen receptors. These receptors influence expression of target genes through both genomic and nongenomic mechanisms (Björnström & Sjöberg, 2005) (Fig. 1). Classically, it has been shown that ligand binding to nuclear ERs results in receptor dimerization, followed by binding to promoter regions of target genes at sites known as estrogen response elements, ultimately attracting coregulatory proteins to these areas which can positively or negatively influence gene transcription. In addition, nuclear ERs are able to indirectly influence gene expression via tethering to other transcription factors, as well as through modulating the synthesis of various second messenger proteins (Björnström & Sjöberg, 2005). Despite

3. ESTROGEN RECEPTOR ALPHA

ER α is the original ER to be cloned and characterized and has long been thought of as the ER that is essential for regulating reproductive physiology and behaviors. Knockout models of ER α consistently show impaired reproductive hormone secretion, physiology, and behaviors (Antonson et al., 2012; Dupont et al., 2000; McDevitt et al., 2007). In regard to the regulation of nonreproductive behaviors, the activation of ER α is largely associated with an increase in anxiety-like behavior in the rodent. For example, downregulation of ER α within the medial preoptic area and the posterodorsal amygdala of ovariectomized rats following infusion of an adeno-associated viral vector encoding for a small hairpin RNA (shRNA) targeting the ER α gene produced a decrease in anxiety-like behavior in the light–dark box and open field test (Spiteri et al., 2010; Spiteri, Ogawa, Musatov, Pfaff, & Ågmo, 2012). It is important to note that not all studies have observed effects of ER α on anxiety-like behavior. In ER α knockout mice, there were no observable changes in anxiety-like behavior in the elevated plus maze or open field tests (Kre, el, Dupont, Krust, Chambon, & Chapman, 2001). Additionally, some researchers have observed no effects (Jacome et al., 2010; Walf & Frye, 2005) or mild effects (Lund, Rovis, Chung, & Handa, 2005) of the selective ER α agonist propylpyrazole triol (PPT) on anxiety-like behaviors when tested in the open field and elevated plus maze in rat models. Thus, it is possible that the effects of ER α on anxiety are context or species dependent, and further work is required to tease out the subtleties of this response.

While ER α 's effects appear mostly anxiogenic in the rodent, recent research has suggested an opposing effect of ER α on anxiety-like behavior in female animals following reproductive experience. Expression of ER α in the medial amygdala may change during the postpartum period, demonstrating a downregulation at 3 weeks postpartum, followed by an upregulation at 5 weeks (Furuta et al., 2013). Studies of primiparous rats have shown a decrease in anxiety-like behavior in the elevated plus maze following treatment with PPT at 3 weeks (albeit, not as effectively as estradiol treatment) (Furuta et al., 2013) or at 7 weeks (Byrnes, Casey, & Bridges, 2012; Byrnes, Casey, Carini, & Bridges, 2013) postpartum. It is unknown whether this temporal change in ER α 's effects is the result of hormonal changes during pregnancy and the postnatal period, maternal experience, or some combination of these two factors.

To date, only a small number of studies have considered a link between single nucleotide polymorphisms (SNPs) of the ER α gene (ESR1) and the occurrence of anxiety disorders. A

study of adult men in treatment for substance abuse noted an association between symptoms of anxiety and dinucleotide polymorphisms (Comings, Muhleman, Johnson, & MacMurray, 1999). In an elderly human population, a study investigating SNPs found that, in women only, ESR1 haplotypes consisting of the T allele of rs2234693 and the A allele of rs9340799 were correlated with an increased likelihood of anxiety disorders (Tiemeier et al., 2005). More recently, a study by Ryan et al. noted that these same haplotypes were linked to an increase in panic disorder in elderly women, and that this association was mediated by the use of hormone therapy (Ryan et al., 2011). While further verification in a younger female population is still needed, these studies suggest that specific ESR1 polymorphisms may result in an increased vulnerability to the development of anxiety disorders.

4. ESTROGEN RECEPTOR BETA

ER β was first described in 1996, after being cloned from a rat prostate cDNA library (Kuiper, Enmark, Peltö-Huikko, Nilsson, & Gustafsson, 1996). While studies in the prostate have demonstrated a role for ER β in activating apoptosis and limiting prostate growth, studies utilizing ER β knockout models have suggested that in brain, this receptor is a major component of estradiol's anxiolytic-like effects in the rodent. A number of studies have reported increased anxiety-like behaviors in female ER β knockout mice (Imwalle, Gustafsson, & Rissman, 2005; Kre, el et al., 2001; Walf, Koonce, & Frye, 2008), as well as higher levels of plasma corticosterone (Walf, Koonce, Manley, & Frye, 2009). Further evidence supporting an anxiolytic-like effect of ER β can be found in pharmacological studies utilizing selective ER β agonists. The most commonly used ER β agonist, the selective estrogen receptor modulator (SERM) diarylpropionitrile (DPN), causes decreased anxiety-like behavior in rats and mice in a number of tests, including the open field test (Lund et al., 2005; Oyola et al., 2012; Walf et al., 2008), elevated zero maze test (Walf et al., 2008), elevated plus maze test (Lund et al., 2005; Oyola et al., 2012), and light-dark box test (Lund et al., 2005; Oyola et al., 2012). Administration of a different ER β agonist, WAY-200070, increased anxiolytic-like responding in the open field, the elevated plus maze (Weiser, Wu, & Handa, 2009), the four-plate test, which "punishes" exploration of a novel environment with mild foot shock, and in a test of stress-induced hypothermia (Hughes et al., 2008). Furthermore, DPN administration to the paraventricular nucleus (PVN) decreased restraint stress-induced c-fos mRNA levels within the PVN and decreased the plasma adrenocorticotrophic hormone (ACTH) response to in the male rat (Lund, Hinds, & Handa, 2006), suggesting a central site for ER β action. This mechanism also holds true for mice, since peripheral DPN administration decreased stress-induced plasma corticosterone and ACTH in ovariectomized mice (Oyola et al., 2012). Despite all of the evidence suggesting an anxiolytic-like effect of ER β activation, an influence of this receptor on anxiety-like behavior has not always been observed (Jacome et al., 2010; Patisaul, Burke, Hinkle, Adewale, & Shea, 2009). The absence of effects of ER β agonists on anxiolytic behaviors in some studies may be due to dose or timing phenomena since the selectivity of ER β agonists is rather modest and, therefore, too high or too low a dose may negate its actions by interactions with ER α (at higher doses) or by failing to activate ER β (at lower doses). Nonetheless, there remains strong support for ER β as an inhibitor of rodent anxiety.

Of importance, the activation of ER β is not restricted to estrogens or selective ER β agonists. Naturally occurring metabolites of androgens have been recently described that can also bind and activate ER β and consequently can modulate physiology and behaviors. The two best described androgen metabolites that functionally impact ER β signaling pathways are 5 α -androstene-3 β ,17 β -diol (ADIOL), a metabolite of the adrenal androgen dehydroepiandrosterone (Saijo, Collier, Li, Katzenellenbogen, & Glass, 2011), and 5 α -androstane-3 β ,17 β -diol (3 β -diol) (Weihua, Lathe, Warner, & Gustafsson, 2002), a metabolite of the potent nonaromatizable androgen, dihydrotestosterone. Both of these androgens have been shown to be antiinflammatory (Saijo et al., 2011; Zuloaga, O'Connor, Handa, & Gonzales, 2012), and the effects of 3 β -diol have been described in regulating anxiety-like behaviors. Treatment of rats with 3 β -diol dipropionate reduces anxiety in the elevated plus maze (Handa, Pak, Kudwa, Lund, & Hinds, 2008) and reduces the acute neuroendocrine response to restraint stress (Handa et al., 2008; Lund et al., 2006). Moreover, 3 β -diol has been shown to act through ER β to regulate promoter activity of several neuropeptides, including oxytocin and vasopressin (Hiroi et al., 2013; Pak, Chung, Hinds, & Handa, 2007; Pak et al., 2005).

Unfortunately, despite the large body of literature describing ER β 's effects in the rodent, research regarding the influence of ER β on anxiety in humans is currently very limited. A recent study noted that, for the ER β gene ESR2, the SNP rs1256049 was associated with a greater incidence of generalized anxiety disorder in older women (Ryan et al., 2011). It is unknown if this correlation is observable in younger women or in men. Given the compelling data from preclinical models, further investigation into the role of ER β in human anxiety disorders is critically needed.

5. G-PROTEIN-COUPLED ESTROGEN RECEPTOR

Recent research has identified the existence of a novel estrogen receptor known as GPR30 or GPER1 (Filardo, Quinn, Bland, & Frackelton, 2000; Revankar, Cimino, Sklar, Arterburn, & Prossnitz, 2005; Thomas, Pang, Filardo, & Dong, 2005). This receptor has been described as localized both at the membrane (Filardo et al., 2007; Funakoshi, Yanai, Shinoda, Kawano, & Mizukami, 2006; Thomas et al., 2005) as well as within several intracellular structures, including endoplasmic reticulum (Revankar et al., 2005) and cytokeratin intermediate filaments (Sandén et al., 2011). GPR30 exhibits a widespread distribution across the rodent brain, with high levels of immunoreactivity noted in regions implicated in affective behavior, such as the cortex, hippocampus, hypothalamus, and brainstem (Brailoiu et al., 2007; Hazell et al., 2009). In addition, GPR30 immunoreactivity is increased following acute stress in the basolateral amygdala of ovariectomized mice, which is accompanied by increased inhibitory synaptic transmission in the basolateral amygdala (Tian et al., 2013), suggesting a role in the rodent stress response.

GPR30 is capable of influencing gene expression through several known mechanisms, resulting in both short-term and long-term effects on transcription (see Fig. 1). Agonist binding to GPR30 can cause the activation of adenylyl cyclase, stimulating the production of cAMP, which ultimately activates the transcription factor cAMP response element-binding protein (CREB). Additionally, *in vitro* activation of GPR30 has been found to recruit second

messengers such as Src kinase, producing downstream trans-activation of epidermal growth factor receptors (EGFRs), leading to the activation of mitogen-activated protein kinases (MAPKs) ERK1/2, and the activation of phosphatidylinositol 3-kinases (PI3Ks), ultimately resulting in the activation of proteins that regulate transcription factors (for review, see Maggiolini & Picard, 2010; Prossnitz et al., 2008).

Although several recent studies have implicated GPR30 in modulating the expression of anxiety-like behaviors in the mouse, there are conflicting reports as to the nature of this receptor's effects. Importantly, the impact of this receptor on anxiety-like behavior may be sex dependent and may vary depending on the behavioral testing paradigm employed. For example, following an acute stressor, administration of the GPR30 agonist G-1 to the basolateral amygdala prevented an increase in anxiety-like behavior in ovariectomized mice in the open field test and elevated plus maze (Tian et al., 2013). Peripheral administration of G-1 produced a decrease in anxiety-like behavior in the elevated plus maze in gonadectomized male mice, while ovariectomized subjects were unaffected by treatment (Hart et al., 2014). Conversely, G-1 treatment led to an increase in anxiety-like behavior in ovariectomized mice and intact male mice in the elevated plus maze and open field test (Kastenberger et al., 2012). All told, it is possible that this discrepancy in the effects of G-1 on behavior may result from differences in dose of G-1, as well as differences in the timing of G-1 administration relative to behavioral testing.

While little is known about the nature of GPR30's influence on anxiety-like behavior, even less is known about the potential mechanisms responsible for GPR30's effects on this behavior. Activation of GPR30 increases inhibitory synaptic transmission within the basolateral amygdala of ovariectomized mice (Tian et al., 2013). Based on an observed high level of colocalization of GPR30 with both GABAergic interneurons and glutamatergic pyramidal neurons, coupled with GPR30's prevention of stress-induced downregulation of GABA_A receptor expression and upregulation of glutamate receptors, Tian et al. (2013) have proposed that GPR30 mediates anxiety-like behavior by altering the balance between GABAergic and glutamatergic signaling within the basolateral amygdala (Tian et al., 2013). This receptor's effects may not be the result of activation of the ERK signaling pathway, as two studies investigating anxiety-like behavior following G-1 administration found no changes in phosphorylated ERK in the hippocampus of gonadectomized male (Hart et al., 2014) and female (Anchan, Clark, Pollard, & Vasudevan, 2014) mice. However, potential changes in phosphorylated ERK were not assessed in other regions implicated in regulating anxiety-like behavior that also express GPR30, thereby currently limiting conclusions about the ERK signaling pathway.

6. CROSS TALK BETWEEN ESTROGEN RECEPTORS

The above discussion has focused on the individual contribution of each estrogen receptor in anxiety and anxiety-like behavior. However, evidence suggests that these receptor subtypes are capable of interacting with one another. One such example is the relation between ER α and ER β . Male ER α knockout mice show decreased expression of ER β protein within the medial preoptic area and increased expression within the bed nucleus of the stria terminalis, suggesting that ER β expression can be modulated by ER α . This observation can be coupled

with a relative insensitivity to estradiol's effects on ER β expression, as assessed through gonadectomy and estradiol replacement (Nomura, Korach, Pfaff, & Ogawa, 2003) although this lack of effect may be brain region dependent (Patisaul, Whitten, & Young, 1999). GPR30 and ER α have also shown some interdependence. A recent study by Hart et al. (2014) found that GPR30's agonist G-1 increased protein expression of hippocampal phosphorylated ER α in male mice (Hart et al., 2014). In addition, an in vitro study using ovarian cancer cells reported that both GPR30 and ER α were required for either estradiol or G-1 to induce an upregulation in expression of the oncogene *c-fos* (Albanito et al., 2007). The relation between ER β and GPR30 has not yet been assessed. In sum, these findings demonstrate that estrogen receptors do not function entirely independently of one another, which likely has implications for estrogen receptors' mediation of anxiety.

7. MECHANISMS FOR ESTROGEN RECEPTOR EFFECTS ON ANXIETY

Researchers are continuing to investigate the pathways by which estrogen receptors influence anxiety and anxiety-like behavior. The most well-described mechanisms, discussed later, demonstrate that estrogen receptors are capable of mediating a diverse range of neural systems. Importantly, these systems do not operate in isolation; therefore, estrogen receptor effects on anxiety are likely the result of a number of interactions between pathways.

7.1 Effects on the Hypothalamo-Pituitary-Adrenal Axis

In response to a stressor, neurons within the PVN of the hypothalamus secrete the peptides corticotropin-releasing hormone (CRH) and vasopressin, which act in concert to stimulate the release of ACTH from the anterior pituitary into the peripheral circulation (Fig. 2). Moreover, ACTH acts upon the cortex of the adrenal gland, causing the upregulation of enzymes involved in steroidogenesis and ultimately the enhanced secretion of glucocorticoids. Glucocorticoids then act to prevent further activity of this system through a long-loop negative feedback mechanism that can involve the hippocampus, hypothalamus, and pituitary gland. This neuroendocrine system, known as the hypothalamo-pituitary-adrenal (HPA) axis, is one of the primary ways in which an organism is capable of reacting to a stressor (Herman & Cullinan, 1997) and maintaining homeostasis. Of importance for the current discussion, estrogen receptors have been shown to impact neuroendocrine stress responsivity at several different levels of this axis.

ER α may mediate anxiety-like behavior through its actions on HPA axis function. Glucocorticoids have long been known to activate brain regions that are involved in mediating anxiety and fear responses in rodents. In particular, the central nucleus of the amygdala has been shown to express glucocorticoid receptors, and neurons in this brain area respond to glucocorticoids to enhance anxiety-like behaviors. Thus, increases in glucocorticoid secretion can directly influence behaviors such as anxiety (Shepard, Barron, & Myers, 2003; Weiser, Foradori, & Handa, 2010) and correspondingly, factors that influence HPA activity can have similar actions on anxiety. Administration of estradiol, PPT, or the ER α -selective agonist, moxestrol, to the PVN augmented the ACTH response to restraint stress in the male rat (Lund et al., 2006), which correlated with an elevation in *c-fos*

mRNA levels within this brain region (Lund et al., 2006). Interestingly, ER α is expressed at only low levels within the rat PVN (Shughrue et al., 1997), suggesting an alternative route for the effects of these ER α agonists on behavior. It has been proposed that the activation of the ER α -containing GABAergic neurons surrounding the PVN may inhibit GABAergic inhibition of the PVN, resulting in an increase in HPA activity. Correspondingly, increased stressor-induced *C-fos* immunoreactivity has been shown in CRH neurons following administration of PPT to ovariectomized rats (Thammacharoen, Geary, Lutz, Ogawa, & Asarian, 2009). Furthermore, as shown in vitro by Lalmansingh and Uht (2008) using an amygdaloid cell line, ER α may regulate CRH gene (*crh*) expression through histone acetylation in the cAMP regulatory element region of the *crh* promoter (Lalmansingh & Uht, 2008). This molecular regulation provides another pathway for ER α 's anxiogenic effects.

Substantial evidence for ER β influencing anxiety through HPA axis mediation has also been shown. Peripheral administration of the ER β agonist DPN causes decreased stress-induced plasma corticosterone and ACTH levels in the ovariectomized mouse (Oyola et al., 2012). In contrast to ER α , ER β is robustly expressed within both the rat and mouse (Fig. 3) PVN. A possible target for ER β in the HPA axis is a direct action on a population of neuro-secretory cells in the PVN that produce CRH. Neuroendocrine CRH neurons in the rat PVN express ER β in a subregion-dependent fashion (13–60%) (Laflamme, Nappi, Drolet, Labrie, & Rivest, 1998; Suzuki & Handa, 2005). These CRH-expressing neurons initiate the HPA axis response to stress, ultimately increasing circulating glucocorticoid levels (Herman & Cullinan, 1997). Similar to peripheral DPN injection, implantation of a DPN-containing pellet near the male rat PVN has produced a decrease in stress-induced plasma corticosterone and ACTH (Lund et al., 2006). Thus, ER β may mediate HPA axis activity by actions directly on PVN neurons. It has been proposed that this receptor subtype also regulates *crh* expression through histone acetylation in the cAMP regulatory element region of the *crh* promoter. Unlike ER α , ER β may have a more indirect influence on expression, acting by attracting the cAMP-binding protein-binding protein (CBP) to the CRE element in the *crh* promoter (Lalmansingh & Uht, 2008).

7.2 Effects on the Serotonergic System

The serotonergic system has long been implicated in modulating anxiety in humans and rodent models (Handley, McBlane, Critchley, & Njung'e, 1993; Naughton, Mulrooney, & Leonard, 2000). Estrogens have potent effects on this system, causing changes in levels of serotonin, its metabolite 5-hydroxyindoleacetic acid (5-HIAA), the serotonin transporter, and serotonin receptors (Borrow & Cameron, 2014). These effects may be driven by the actions of ER β and GPR30.

The estrogen receptor that has received the most attention as a facilitator of estradiol's effects on the serotonergic system is ER β . Treatment of male mice with the ER β agonist WAY-200070 has resulted in increased levels of serotonin within the striatum (Hughes et al., 2008), while DPN treatment caused increased levels of 5-HIAA in OVX rats (Jacome et al., 2010). Male ER β knockout mice have been reported to have decreased levels of 5-hydroxytryptophan, the precursor of serotonin, within the frontal cortex (Hughes et al., 2008), while it has been shown that female ER β knockout mice have lower levels of

serotonin within the hippocampus, bed nucleus of the stria terminalis, and the preoptic area (Imwalle et al., 2005). These findings suggest that estrogens may mediate serotonin synthesis through ER β .

One way in which ER β may influence anxiety-like behavior through the serotonergic system is by mediating expression of tryptophan hydroxylase, the rate-limiting enzyme required for the synthesis of serotonin. Knockdown of this enzyme within the caudal dorsal raphe nucleus of OVX rats removes the anxiolytic-like effects of estradiol in the open field test (Hiroi, McDevitt, Morcos, Clark, & Neumaier, 2011). Correspondingly, both male and female ER β -null mice have decreased expression of tryptophan hydroxylase. In addition, OVX decreased the number of tryptophan hydroxylase-positive neurons within the DRN, which was rescued following treatment with estradiol or the ER β agonist LY3201 (Suzuki et al., 2013). Similarly, our laboratory has shown an upregulation in tryptophan hydroxylase 2 in OVX rats following systemic or local treatment with DPN (Donner & Handa, 2009). However, despite evidence implicating ER β as a mediator of anxiety-like behavior through its effects on the serotonergic system, ER β may not act directly on DRN neurons to influence anxiety-like behavior. We have previously reported that direct administration of DPN to this region did not alter anxiety-like behavior in the open field or elevated plus maze in OVX rats (Donner & Handa, 2009). Furthermore, rats may not express ER β within the DRN (Sheng et al., 2004). This suggests an alternative site of action for ER β 's effects on anxiety through serotonergic modulation.

Another potential site for ER β 's effects on the serotonergic system is the amygdala. Kre, el et al. (2001) reported that ER β knockout mice showed an enhanced induction of long-term potentiation within the amygdala relative to wild-type controls. The authors also noted an increase in the receptor 5-HT1A within the medial amygdala of ER β knockout female animals. When coupled with an observed potentiation of synaptic response following GABAergic inhibition and the known effects of serotonin on amygdala GABAergic tone (Koyama, Kubo, Rhee, & Akaike, 1999), this led them to hypothesize that enhanced 5-HT1A receptor activation might mediate long-term potentiation within the amygdala through the inhibition of GABA release (Kre, el et al., 2001). Direct confirmation of changes in anxiety-like behavior as a result of ER β 's effects on the serotonergic system within the amygdala is still needed.

Despite the large body of data implicating ER β in estrogens' mediation of anxiety-like behavior through the serotonergic system, it has recently been proposed that GPR30, not ER β , is the estrogen receptor responsible for some of estrogens' effects. In the female rat PVN, a desensitization of 5-HT1A receptors following administration of estradiol was found to be the result of GPR30 expression, and not of ER β (McAllister, Creech, Kimball, Muma, & Li, 2012). GPR30 is also colocalized with 5-HT1A receptors within the PVN, and administration of the agonist G-1 has attenuated 5-HT1A receptor signaling within this region (Xu et al., 2009). Thus, at least within the PVN, which shows an absence of ER α in the rat (Shughrue et al., 1997), estrogens may influence serotonergic signaling through GPR30 activation.

7.3 Effects on the Oxytocinergic System

Oxytocin (OT) is a nonapeptide produced primarily by magnocellular neurons within the PVN and the supraoptic nucleus of the hypothalamus. Its function has classically been associated with regulation of parturition and lactation, although more recent studies indicate that oxytocin can have a variety of effects on the central nervous system (CNS) to regulate such behaviors as reproductive and parental behavior, anxiety, social bonding, and social recognition (for review, see Borrow & Cameron, 2012; Lee, Macbeth, Pagani, & Young, 2009; Neumann, 2008). OT is secreted by three known methods: release into the peripheral circulation via activation of the neurohypophyseal system, local dendritic release followed by diffusion into local brain structures, and release into regions such as the hippocampus and amygdala by axonal projections from OT neurons within the PVN (Murgatroyd et al., 2004). Following its release, OT acts on its receptors (OTR), which are widely expressed throughout the brain and periphery. OT is an important mediator of anxiety symptoms in both humans and rodents. Plasma OT levels are negatively correlated with anxiety symptoms in depressed patients (Scantamburlo et al., 2007), and intranasal OT administration attenuates emotional fear reactivity in patients with social anxiety disorder (Labuschagne et al., 2010). Furthermore, an SNP within the oxytocin receptor gene, rs2254298, has been correlated with anxiety symptoms in women and adolescent girls (Chen & Johnson, 2012; Thompson, Parker, Hallmayer, Waugh, & Gotlib, 2011). In the rodent, central administration of OT or an OTR agonist decreases anxiety-like behavior and suppresses HPA axis activation in response to stress (Mak, Broussard, Vacy, & Broadbear, 2012; Sabihi, Duroske, Dong, & Leuner, 2014; Windle, Shanks, Lightman, & Ingram, 1997). Estradiol has been found to increase levels of plasma OT (Amico, Seif, & Robinson, 1981; Yamaguchi, Akaishi, & Negoro, 1979), OT mRNA (Hiroi et al., 2013; Nomura, McKenna, Korach, Pfaff, & Ogawa, 2002; Sharma, Handa, & Uht, 2012), and OTR binding (Krege et al., 1998), presenting an additional mechanism by which estrogens are capable of mediating anxiety.

The receptor primarily implicated in the effects of estradiol on OT is ER β . OT and ER β are highly colocalized within the PVN in the female rat (Hrabovszky et al., 2004; Suzuki & Handa, 2005), and colocalization of ER β and OT has also been reported in the human PVN (Hrabovszky et al., 2004). ER β knockout mice do not show the increase in OT mRNA following estradiol administration observed in wild-type controls (Nomura et al., 2002). Administration of the ER β ligand 5 α -androstane-3 β ,17 β -diol (3 β -diol) increased OT mRNA in a mouse hypothalamic cell line (Hiroi et al., 2013; Sharma et al., 2012) and in ovariectomized rats (Hiroi et al., 2013). Finally, the anxiolytic effects of the ER β agonist DPN are blocked in the rat following treatment with an OT antagonist (Kudwa, McGivern, & Handa, 2014). Recent in vitro evidence suggests that ER β regulates OT expression by binding to the composite hormone response element of the *Ot* gene promoter, inducing the recruitment of the coactivators cAMP response element-binding protein (CREB) and steroid receptor coactivator (SRC-1) to the promoter site, resulting in an increase in histone H4 acetylation, ultimately leading to an increase in *Ot* gene transcription (Hiroi et al., 2013; Sharma et al., 2012).

Research investigating the regulation of OT by ER α and GPR30 is more limited, but bears mentioning. ER α knockout mice do not show an increase in OT receptor binding following

estradiol treatment (Krege et al., 1998). Moreover, OT receptors have been shown to be estrogen regulated (de Kloet, Voorhuis, Boschma, & Elands, 1986). Such data indicate that the OT receptor is under estrogenic control, through ER α but not ER β , whereas oxytocin may be differentially regulated by ER β and not ER α . Finally, GPR30 is colocalized with OT neurons within the rat PVN (Sakamoto et al., 2007), but the influence of GPR30 on OT is currently unknown, and warrants further investigation.

8. CONCLUSIONS

There is compelling evidence demonstrating that estrogens influence anxiety and anxiety-like behaviors in both humans and rodents. The nature of this influence differs based on the subtype of estrogen receptor utilized (Table 1). While animal models suggest that activation of ER β may serve to generate anxiolytic-like effects, ER α appears to have largely anxiogenic-like properties. The more recently discovered membrane estrogen receptor, GPR30, has also been implicated in the rodent anxiety response, although, at present, the extent of its participation is not clear. Given the importance of receptor subtype in modulating anxiety, SERMs demonstrate great potential as novel therapeutic agents in the treatment of anxiety disorders. SERMs that target ER β may be particularly effective in patients with specific polymorphisms of ESR1 and ESR2, and in patients with anxiety symptoms temporally associated with declining estrogen levels, such as during the premenstrual period or menopause.

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Fig. 1.

A simplified description of known estrogen receptor signaling pathways. Classically, ligand-activated estrogen receptors (ER) α and β bind directly to promoter regions at estrogen response element (ERE) sites, attracting coregulatory proteins, which influence gene transcription (1). ER α and β can also indirectly affect gene transcription by influencing the activity of other transcription factors (TF) (2) and by mediating the synthesis of second messenger proteins such as Src kinase (3). Ligand binding to GPR30 initiates several second messenger signaling cascades, including the activation of adenylyl cyclase which increases the production of cAMP, ultimately activating the transcription factor cAMP response element-binding protein (CREB) (4), and the recruitment of Src kinase, which activates epidermal growth factor receptors (EGFRs), resulting in the activation of mitogen-activated protein kinases (MAPKs) ERK1/2 and phosphatidylinositol 3-kinases (PI3Ks), ultimately leading to the activation of proteins that regulate transcription factors (5). See Björnström and Sjöbergg (2005), Maggiolini and Picard (2010), and Prossnitz et al. (2008) for review. the marked similarities between ER α and ER β , these receptors differ significantly in tissue distribution (Shughrue, Lane, & Merchenthaler, 1997) and function, particularly in brain.

Fig. 2. Diagrammatic representation of the hypothalamo-pituitary-adrenal (HPA) axis and a summary of the effects of estrogen receptor agonists.

Fig. 3.

Confocal microscopic image showing distribution of immunofluorescent labeling of estrogen receptors α (A) and β (B) in the mouse paraventricular nucleus. ER α was detected by standard immunofluorescent techniques using an AlexaFluor 594-labeled secondary antibody, and ER β -expressing neurons were detected in the same brain section by examining endogenous ER β -EGFP expression in a transgenic mouse model. 3V, 3rd ventricle.

Table 1

The Effect of Estrogen Receptor Subtypes on Anxiety-Like Behavior in the Rodent

Receptor	Method	Species	References	Effect
ERα				
Agonists	PPT s.c., 1 mg/kg	Female ^a and male mice	Kastenberger et al. (2012)	No effect in EPM, LD, OFT
	PPT s.c., 1 mg/kg for 4 days	Female and male rats ^a	Lund et al. (2005)	↑ Anxiety in EPM
	PPT s.c., 10 μ g	Female rats ^a	Walf and Frye (2005)	No effect in DFT, EPM, ET, OFT, VT
	PPT s.c., 3 or 5 mg/kg for 2 days	Female rats ^a	Jacome et al. (2010)	No effect in EPM
	PPT s.c., 1 mg/kg for 7 days	Female rats ^a	Weiser et al. (2009)	No effect in EPM, OFT
	PPT s.c., 1 mg/kg for 3 days	Male rats	Patisaul et al. (2009)	No effect in LD
	PPT s.c., 1 mg/kg for 4 days	Female rats ^a	Bymes et al. (2012)	↓ Anxiety in primiparous females in EPM
	PPT s.c., 1 mg/kg	Female rats ^a	Bymes et al. (2013)	↑ Anxiety in nulliparous females, ↓ anxiety in primiparous females in EPM
	PPT s.c., 1 mg/kg	Primiparous female rats	Furuta et al. (2013)	↓ Anxiety in EPM
	shRNA targeting ER α intra-MPOA, estradiol s.c., 18 μ g/kg	Female rats ^a	Spiteri et al. (2012)	↓ Anxiety in LD, OFT
Genetic models	shRNA targeting ER α intra-meAMYG, estradiol s.c., 18 μ g/kg	Female rats ^a	Spiteri et al. (2010)	↓ Anxiety in LD
	ER α knockout mice	Female and male mice	Kre, el et al. (2001)	No effect in EPM or OFT
ERβ				
Agonists	DPN s.c., 1 mg/kg	Female ^a and male mice	Kastenberger et al. (2012)	No effect in EPM, LD, OFT
	DPN s.c., 1 mg/kg for 4 days	Female and male rats ^a	Lund et al. (2005)	↓ Anxiety in EPM, LD (only females tested), and OFT (only females tested)
	DPN s.c., 3 mg/kg for 2 days	Female rats ^a	Jacome et al. (2010)	No effect in EPM
	DPN s.c., 10 μ g	Female rats ^a	Walf and Frye (2005)	↓ Anxiety in DFT, EPM, ET, OFT, VT
	DPN s.c., 1 mg/kg for 4 days	Female rats ^a	Bymes et al. (2012)	No effect in EPM
	DPN s.c., 0.5 or 2 mg/kg for 4 days, 1 mg/kg for 3 days	Male rats	Patisaul et al. (2009)	No effect in EPM or LD
	DPN s.c., 1 mg/kg	Primiparous female rats	Furuta et al. (2013)	No effect in EPM
	DPN s.c., 0.1 mg/kg	Female mice ^a	Walf et al. (2008)	↓ Anxiety in EPM, EZM, OFT, SIT
	S-DPN s.c., 1 mg/kg	Female mice ^a	Oyola et al. (2012)	↓ Anxiety in EPM, LD, OFT, no effect in MBT

Receptor	Method	Species	References	Effect
	DPN or S-DPN s.c., 2 mg/kg for 7 days	Females rats ^a	Weiser et al. (2009)	↓ Anxiety in EPM, OFT
	Coumestrol s.c., 10 µg	Female rats ^a	Walf and Frye (2005)	↓ Anxiety in DFI, EPM, ET, OFT, VT
	WAY-200070 s.c., 30 mg/kg	Male mice	Hughes et al. (2008)	↓ Anxiety in 4PT, SIH
	WAY-200070 s.c., 2 mg/kg for 7 days	Female rats ^a	Weiser et al. (2009)	↓ Anxiety in EPM, OFT
Genetic models	ERβ knockout mice	Female mice ^a	Oyola et al. (2012)	No effect in EPM, LD, MBT, OFT
	ERβ knockout mice	Female and male mice	Krey et al. (2001)	↑ Anxiety in EPM, OFT (females only)
	ERβ knockout mice, s.c. estradiol (85–100 pg/mL)	Female mice ^a	Imwalle et al. (2005)	↑ Anxiety in EPM
	ERβ knockout mice, s.c. DPN 0.1 mg/kg	Female mice ^a	Walf et al. (2008)	↑ Anxiety in EPM, EZM, OFT, SIT
GPR30				
Agonists	(G-1) s.c., 0.3 µg	Female and male mice ^a	Hart et al. (2014)	↓ Anxiety in EPM (males only), no effect in OFT
	(G-1) s.c., 1 mg/kg	Female ^a and male mice	Kastenberger et al. (2012)	↑ Anxiety in EPM, LD (males only), OFT
	(G-1) intra-BLA, 0.5 µM	Female mice ^a	Tian et al. (2013)	↓ Anxiety in EPM, OFT
Genetic models	GPR30 knockout mice	Female and male mice	Kastenberger and Schwarzer (2014)	↓ Anxiety in EPM (males only) and LD (males only), no effect in OFT

^aGonadectomized subjects.

Note: 4PT, four-plate test; BLA, basolateral amygdala; DFT, defensive freezing test; EPM, elevated plus maze test; ET, emergence test; EZM, elevated zero maze test; LD, light–dark box; MBT, marble burying test; *me-AMYG*, medial amygdala; MPOA, medial preoptic area; OFT, open field test; SIH, stress-induced hypothermia; SIT, social interaction test; VT, Vogel test.