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Estrogen receptor β and oxytocin interact to modulate anxiety-like behavior and neuroendocrine stress reactivity in adult male and female rats

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HIGHLIGHTS

- An ER beta agonist reduces anxiety more effectively in female versus male rats.
- An ER beta agonist reduces depressive like behaviors in female but not male rats.
- An ER beta agonist reduces CORT responses to restraint-stress.
- An oxytocin antagonist prevents some of the actions of ER beta agonist.

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ABSTRACT

The hypothalamic–pituitary–adrenal (HPA) axis is activated in response to stressors and is controlled by neurons residing in the paraventricular nucleus of the hypothalamus (PVN). Although gonadal steroid hormones can influence HPA reactivity to stressors, the exact mechanism of action is not fully understood. It is known, however, that estrogen receptor β (ERβ) inhibits HPA reactivity and decreases anxiety-like behavior in rodents. Since ERβ is co-expressed with oxytocin (OT) in neurons of the PVN, an ERβ-selective agonist was utilized to test the whether ERβ decreases stress-induced HPA reactivity and anxiety-like behaviors via an OTergic pathway. Adult gonadectomized male and female rats were administered diarylpropionitrile, or vehicle, peripherally for 5 days. When tested for anxiety-like behavior on the elevated plus maze (EPM), diarylpropionitrile-treated males and females significantly increased time on the open arm of the EPM compared to vehicle controls indicating that ERβ reduces anxiety-like behaviors. One week after behavioral evaluation, rats were subjected to a 20 minute restraint stress. Treatment with diarylpropionitrile reduced CORT and ACTH responses in both males and females. Subsequently, another group of animals was implanted with cannulae directed at the lateral ventricle. One week later, rats underwent the same protocol as above but with the additional treatment of intracerebroventricular infusion with an OT antagonist (des Gly-NH_2 $d(CH_2)_5$ [$Tyr(Me)^2$, Thr^4] OVT) or VEH, 20 min prior to behavioral evaluation. OT antagonist treatment blocked the effects of diarylpropionitrile on the display of anxiety-like behaviors and plasma CORT levels. These data indicate that ERβ and OT interact to modulate the HPA reactivity and the display of anxiety-like behaviors.

1. Introduction

In female rodents, the response of the hypothalamo-pituitary-adrenal (HPA) axis to stress is greater than that of males, as evidenced by a larger and more prolonged secretion of adrenocorticotropic hormone (ACTH) and adrenal corticosterone (CORT) [1–3]. Much of this sex difference is attributed to activational effects stemming from sex differences in circulating testosterone (T) and estradiol (E2), since adult gonadectomy reduces, and hormone replacement reinstates, the sex difference [4–7]. In particular, studies show that E2 enhances, whereas T inhibits, HPA axis reactivity [8–11], although some studies

Abbreviations: 3β-diol, 5 alpha androstane 3β,17β diol; ACTH, adrenocorticotrophic hormone; CORT, corticosterone; CRH, corticotropin releasing hormone; DRN, dorsal raphe nucleus; E2, estradiol; HPA, hypothalamo-pituitary-adrenal; PVN, paraventricular nucleus; T, testosterone; TPH2, tryptophan hydroxylase 2; ERβ, estrogen receptor beta; ERα, estrogen receptor alpha; OT, oxytocin; EPM, elevated plus maze; VEH, vehicle; AR, androgen receptor; GABA, gamma amino butyric acid; ERβKO, estrogen receptor beta knockout; WT, wild type; OTKO, oxytocin knockout; FST, forced swim test; R-DPN, R-diarylpropionitrile; ICV, intracerebroventricular; EDTA, ethylenediaminetetraacetic acid; CSF, cerebrospinal fluid.

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also have shown E2-mediated inhibition of the HPA response to stress [12,13]. It is known that E2 and T act by binding the classic estrogen receptors α or β (ERα, ERβ) or the androgen receptor (AR) in neuropeptide-containing cells located within, or projecting to, the paraventricular nucleus (PVN) [14–16], the principal site for regulation of the HPA axis.

Estrogen receptors are localized within the PVN and surrounding hypothalamic regions, albeit with differing patterns specific for ERα and ERβ. Whereas few ERα-expressing neurons are found in the PVN proper [17,18], ERα is expressed by GABA containing neurons in the periPVN region [14]. By contrast, ERβ is highly expressed by OT-containing neurons in the parvocellular PVN of both rats and mice [17–20]. Within the rat PVN, approximately 85% of OT-containing neurons co-express ERβ [18]. Furthermore, in wild-type mice, exogenous E2 increases OT expression in the brain, but this increase is not observed in ERβ knockout mice (ERβKO) [21,22]. Thus, substantial overlap in the anatomical distribution of OT and ERβ indicates the potential for interactions in the control of neuroendocrine function and behavior.

Estrogen receptor β knockout mice [23,24] and OT knockout mice [25,26] display increased anxiety-like behavior and enhanced stress-induced plasma CORT levels, suggesting that both ERβ and oxytocin are normally involved in the control of the adult stress response [27–30]. Moreover, activation of ERβ by a variety of ERβ agonists attenuates stress-induced hypothalamic–pituitary–adrenal (HPA) activity and decreases the display of anxiety-like behaviors in rodents [31,32]. Correspondingly, an endogenous ERβ ligand, 5α androstane 3β,17β diol, a metabolite of the non-aromatizable androgen, dihydrotestosterone, has similarly been shown to increase PVN OT mRNA expression, likely through direct actions of ERβ on the OT promoter [33]. Nonetheless, the degree to which ERβ and OT regulatory mechanisms intersect in the control of HPA activity and anxiety-like behaviors has not yet been explored.

Oxytocin is a hypothalamic neuropeptide that was originally shown to regulate parturition. Release of OT from parvocellular PVN neurons that project to the median eminence and release of OT into the hypophyseal portal vessels enhances HPA function and increases adrenal glucocorticoid release by modulating the actions of CRF at the level of the anterior pituitary [34]. However, OT neurons in the PVN also provide the predominant OTergic projections to the forebrain where OT is released in response to psychological and physiological stressors [35,36] to exert anxiolytic actions and enable social interactions that may otherwise be avoided [37]. When applied to the PVN, OT acts to inhibit HPA axis activity [38] apparently through modulation of CRH neuron activity. Although baseline diurnal rhythms of CORT do not differ between OTKO and wild-type (WT) mice [25,26], OTKO mice do display more anxiety-related behavior and have a greater plasma CORT response to a stressor as compared to their WT counterparts [25,30], further supporting a specific role for OT in the HPA reactivity to stress.

Oxytocin receptors are expressed at high levels in limbic brain regions [39]. Following testing of female OTKO and WT mice on the elevated plus maze, c-Fos expression in the medial amygdala of female OTKO mice was greater than that observed in WT counterparts [25]. The medial amygdala is a limbic region important for the processing of psychogenic stress and anxiety and also contains OT receptor expressing neurons [39]. Moreover, following restraint stress, upregulation of CRH mRNA in the PVN was observed with OTKO mice exhibiting a greater increase in CRH mRNA than did WT mice [40]. Correspondingly, central administration of an oxytocin antagonist, increases anxiety-like behaviors and HPA function as indicated by increased levels of plasma ACTH and CORT [38] supporting the hypothesis that central release of OT in response to stress may act to attenuate the stress-induced increase in HPA reactivity, thereby enabling social interactions beneficial to the animal such as reproduction and feeding [37].

In the current experiments, we tested the hypothesis that activation of ERβ neurons with a selective ERβ agonist inhibits the reactivity of the HPA-axis and reduces anxiety-like behaviors through activation of OT-containing neurons in both males and females. To our knowledge, this is the first study directly comparing effects of the ERβ agonist, R-diarylpropionitrile (R-DPN) on neuroendocrine stress responses and stress related behaviors across both sexes. Our studies demonstrate a sex difference in response to ERβ activation and further activation of ERβ utilizes an oxytocinergic pathway to temper anxiety-like behaviors and HPA activation.

2. Methods

2.1. Animals

Male and female adult Sprague–Dawley rats were purchased from Charles River Laboratories (San Diego, CA) and housed at the laboratory animal research facility at Colorado State University. A total of 118 animals were used in these studies. Animals were group housed in temperature and humidity controlled rooms on a 12:12 light:dark cycle (lights on at 0600 h) and placed onto a phytoestrogen free diet (AIN-93G modified, DYETS Inc, Allentown PA, with corn oil substituted for soy oil) with water and food available ad libitum. Young adult rats were bilaterally gonadectomized under isoflurane anesthesia one week after arrival as described [41]. Following gonadectomy, animals were returned to their home cage and allowed to awaken prior to the administration of post-operative buprenorphine hydrochloride (0.05 mg/kg). 7 days later, animals began an experimental treatment regimen consisting of daily subcutaneous (S.C) injections of the biologically active R-isomer of the ERβ agonist diarylpropionitrile (R-DPN, 2 mg/kg S.C.) [42,43] or vehicle (VEH; 27% hydroxypropyl β-cyclodextran in 0.9% saline S.C.). Beginning two days preceding the initiation of peripheral R-DPN treatment, animals were handled for 3–5 min daily and then daily throughout the course of the study. All behavioral tests and restraint testing were performed in the morning between 0800 and 1200 h in order to avoid the diurnal rise in corticosterone that occurs in the afternoon in rodents. All animal protocols followed NIH guidelines and were approved by the Animal Care and Use Committee at Colorado State University.

2.1.1. Experiment 1. Effect of R-DPN on anxiety and depressive-like behaviors and hormonal response to restraint stress in gonadectomized male and female rats

Male and female Sprague–Dawley rats (30 males, 32 females) were gonadectomized and one week later, were started on a regimen of once daily injections of R-DPN (2 mg/kg BW, S.C.) or vehicle (250 μl/animal). 2–4 h after the 5th injection, animals were tested for behaviors in the elevated plus maze (EPM, see below). Following testing, animals were returned to their home cage. Animals were allowed to rest for two days and following the 7th injection, animals were introduced to the forced swim test (FST, see below). Behaviors in the FST were scored 2–4 h after the 8th daily injection the following day. Following the FST, animals were returned to their home cage. Restraint testing (see below) was initiated 4 days later, 2–4 h after the 12th injection and trunk blood was collected by decapitation immediately following the 20 min restraint stress. Control animals were killed immediately after being removed from their home cage and trunk blood collected.

2.1.2. Experiment 2. Effect of central treatment with an OT antagonist on ERβ agonist effects

One week following gonadectomy, a separate group of 56 male and female rats received an intracerebroventricular (ICV) cannula directed at the lateral ventricle. Five days later, animals were started on a treatment regimen of 5 daily injections of either R-DPN (2 mg/kg) or VEH. On the fifth day, animals received a central infusion of either an oxytocin receptor antagonist (des Gly-NH_2 d$(CH_2)_5$ [Tyr(Me)^2, Thr^4] OVT; Sigma) or VEH (aCSF) 3–4 h after R-DPN treatment and 20 min prior to being tested on the elevated plus maze. Animals were returned to