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Administration of estrogen receptor beta-specific selective estrogen receptor modulators to the hippocampus decrease anxiety and depressive behavior of ovariectomized rats

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Abstract

Estradiol (E_2) may influence some of the sex differences in neuropsychiatric disorders that emerge post-puberty. Studies in our laboratory, and others, have shown that actions at the β isoform of estrogen receptor (ER) are important for E_2's effects for anxiety and/or depressive behavior. Whether ERβ in the hippocampus is a target for these effects was investigated in the present study. We hypothesized that if actions at ERβ in the hippocampus are important for the anti-anxiety and anti-depressive effects, then administration of selective ER modulator (SERMs) with greater affinity for ERβ than ERα to the hippocampus, but not a control region/missed sites (i.e. the ventral tegmental area), should decrease anxiety and depressive behavior, compared to vehicle and that ERα-specific SERMs should not have the same effect. To investigate this, ovariectomized (ovx) rats were surgically-implanted with guide cannulae aimed at the hippocampus (target site) or ventral tegmental area (control site). Rats were administered vehicle, or 17β-E_2 (equal affinity for ERα and ERβ), SERMs with greater affinity for ERα vs. ERβ (17α-E_2 or propyl pyrazole triol), or SERMs with greater affinity for ERβ vs. ERα (coumestrol or diarylpropionitrile) to these sites (2 μg/μl/side) before testing in anxiety (open field, elevated plus maze) or depression (forced swim) tasks. ERβ-selective SERMs to the hippocampus, but not the ventral tegmental area, decreased anxiety and depressive behavior. Rats administered 17β-E_2 or ERβ SERMs entered more central squares in an open field, spent more time on the open arms of the plus maze, and spent less time immobile compared to rats administered vehicle. Administration of ERα-specific SERMs produced similar effects as vehicle administration. Thus, E_2's anti-anxiety and anti-depressive effects may involve ERβ in the hippocampus.

Keywords: SERMs; Estradiol; Affect; Mood; Sex differences; Animal model

1. Introduction

There are sex differences in vulnerability for affective disorders, such that prevalence rates for anxiety disorders and major depression among women are approximately double that seen in men (Breslau et al., 1995; Earls, 1987; Kessler et al., 1993, 1994; Nolen-Hoeksema, 1987; Schneier et al., 1992; reviewed in Seeman, 1997; Young, 1998; Young and Korszun, 2002). One factor that may underlie women's increased vulnerability to affective disorders may be estradiol (E_2). Women's increased vulnerability to mood disorders occurs post-pubertally, with the beginning of cyclical changes in E_2 secretion from the ovaries among women (reviewed by Hayward and Sanborn, 2002; Kessler and Walters, 1998; Lewinsohn et al., 1998; Young and Altemus, 2004). Mood disorders, such as premenstrual dysphoric disorder and post-partum depression, typically occur with fluctuations in endogenous E_2 levels (Bebbington et al., 1981; reviewed in Bloch et al., 2003; Jenkins, 1987; reviewed in Rubinow and

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Schmidt, 1995; Weissman and Klerman, 1977). Moreover, incidence of anxiety and depression disorders increases in post-menopausal women, who typically experience a decline in circulating E_2 levels (Bebbington et al., 1981, 1998; Jenkins, 1987; Weissman and Klerman, 1977; Weissman and Olfson, 1995; Wittchen and Hoyer, 2001). Furthermore, premenopausal women with depression have lower levels of E_2 during the follicular phase of the menstrual cycle than do non-depressed women (Young et al., 2000). E_2 administration to women with affective disorders enhances mood in a regimen-dependent manner (Ahokas et al., 2001; Gregoire et al., 1996; Saletu et al., 1995; Smith et al., 1995). These data suggest that changes in endogenous E_2 may contribute to women's increased vulnerability to affective disorders and that E_2 administration may temper some of these effects.

Animal models have revealed similar effects of E_2 for affective behavior of female rodents. Naturally-receptive rats that have moderately high physiological E_2 levels have decreased anxiety and depression behaviors compared to rats with lower E_2 levels or males (Frye et al., 2000; Frye and Walf, 2002; Mora et al., 1996). Administration of E_2 regimen that mimics endogenous levels observed in naturally-receptive rats to rats that have been ovariectomized (ovx), which surgically removes the main endogenous source of E_2, the ovaries, produces similar anti-anxiety and anti-depressant-like effects (Estrada-Camarena et al., 2003; Frye and Walf, 2004; Frye and Wawrzycki, 2003; Rachman et al., 1998; Slater and Blizard, 1976; Walf and Frye, 2005a,b, 2006; Walf et al., 2004). Together, these data suggest that E_2 can alter affective responses in animal models.

Although there are many possible mechanisms by which E_2 can influence affective behavior, a likely mechanisms involves E_2's ligand-dependent effects through actions at its cognate receptor, intracellular estrogen receptors (ERs), of which there are at least two isoforms (ERα and ERβ). E_2-induced conditioned place preference is attenuated with administration of ER blockers systemically or to the nucleus accumbens, an area of the brain important for hedonic effects (Walf et al., in press). Our laboratory, and others, have previously demonstrated that subcutaneous administration of selective estrogen receptor modulators (SERMs) that have greater affinity for ERβ than ERα produce anti-anxiety and anti-depressant-like effects in ovx rats in several tasks (Lund et al., 2005; Walf and Frye, 2005b; Walf et al., 2004). Co-administration of E_2 or ERβ-selective SERMs with an ER antagonist, tamoxifen, attenuates these effects (Walf and Frye, 2005b). Furthermore, permanent knockout of ERβ increases anxiety and depression behavior of mice and this effect is impervious to E_2 administration (Krezel et al., 2001; Imwalle et al., 2005; Rocha et al., 2005; Walf and Frye, 2006). Thus, ERβ may be important for E_2's modulation of affective behavior, but the brain target for these effects is not known.

The hippocampus may be a target of E_2 for its effects on anxiety and depression behavior. The hippocampus, as part of the limbic region, has long been considered important for modulation of emotional processing and providing inhibitory feedback to the hypothalamic pituitary adrenal axis (HPA) (as reviewed in Herman et al., 2003, 2004). Both ERα and ERβ have been localized to the hippocampus (Shughrue et al., 1997). Moreover, the hippocampus is sensitive to E_2. Administration of an ER blocker, ICI 182,780, to the hippocampus increases anxiety and depressive behavior of naturally-receptive rats (Walf and Frye, 2006). Systemic and intra-hippocampal administration of E_2 produces similar effects to increase central entries in the open field, increase time spent on the open arms of the plus maze, and decrease depressive behavior (immobility) in the forced swim test (Walf and Frye, 2006). Additionally, these effects may be ER-mediated. ER antagonists administered systemically alter ACTH response of E_2-primed rats (Young et al., 2000). Administration of SERMs with higher avidity for ERβ decrease corticosterone levels of ovx rats tested in the elevated plus maze compared to that observed with administration of vehicle or ERα-specific SERMs (Lund et al., 2005). Together, these data suggest that ERs in the hippocampus are a likely target for E_2 to have actions to modify anxiety and depression behavior.

In the present study, we investigated whether E_2's effects for anxiety and depression behaviors may be due to actions at ERβ in the hippocampus. We hypothesized that if actions at ERβ in the hippocampus are involved in E_2's effects on anxiety and depression, then administration of SERMs with greater affinity for ERβ than ERα to the hippocampus, but not a control site (i.e. the ventral tegmental area, which also has high expression of ERβ; Shughrue et al., 1997), should decrease anxiety and depressive behavior, compared to vehicle.

2. Methods

These methods were pre-approved by the Institutional Animal Care and Use Committee at SUNY Albany.

2.1. Animals and housing

Adult (55+ d old), female Long–Evans rats ($N=84$) were obtained from the breeding colony in the Social Sciences and Life Sciences Research Building at SUNY-Albany (original stock from Taconic Farms, Germantown, NY). Rats were group-housed (4–5/cage) in polycarbonate cages (45 × 24 × 21 cm) in a temperature-controlled room (21 ± 1 °C) in the Laboratory Animal Care Facility. Rats were maintained on a 12/12-hour reversed light cycle (lights off at 8:00 am) with continuous access to Purina Rat Chow and tap water.

2.2. Apparatus

2.2.1. Open field

The open field (76 × 57 × 35 cm; with a 48-square grid floor) was situated in a brightly-lit room and was used in accordance with previously published methods (Frye et al., 2000). The number of central and peripheral squares, which were summed for total, that each rat entered during the 5 min test were recorded. An increased number of central entries is an index of anti-anxiety behavior.

2.2.2. Elevated plus maze

The elevated plus-maze was situated in a brightly-lit room and consisted of four arms, 49 cm long and 10 cm wide, elevated 50 cm off the ground. Two of the four arms are enclosed by walls (30 cm high). At the beginning of the test, rats were placed at the junction of the open and closed arms and the number of entries and time spent on the closed and open arms were recorded (as per Frye et al., 2000). An increase in time spent on the open arms indicates anti-anxiety behavior.

2.2.3. Forced swim test

Rats were tested in the forced swim test in a chamber filled with 30 cm of 30 °C water, as per previously published methods (Frye and Walf, 2002). Time spent by the rat in active movement (struggling to escape chamber, or swimming) and immobility was recorded during the ten-minute task. A decrease in time spent immobile indicates anti-depressant-like behavior.

2.3. Surgery

Surgery was performed on rats that were anesthetized with Rompun (12 mg/kg; Bayer Corp., Shawnee Mission, KS) and Ketaset (80 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) at least 1 week before behavioral testing. Rats were ovx and then stereotaxically implanted with 23-gauge cannulae, affixed to the skull with dental cement, aimed at the dorsal (CA1) hippocampus (from bregma AP=−3.8, ML=±2.0, DV=−2.0) or ventral tegmental area (from bregma AP=−5.3, ML=±0.4, DV=−7.0; as per Paxinos and Watson, 1986; Frye and Walf, 2002; Frye and Vongher, 1999).

2.4. Behavioral testing

All rats were administered vehicle or SERMs on two occasions (first, anxiety behavior; second, forced swim test), ten minutes before behavioral testing. Rats were randomly-assigned to drug conditions and were administered the same drug on both testing occasions, which each lasted 10 min. On their first testing occasion, rats were tested in the open field immediately followed by the elevated plus maze. On the second testing occasion, one week later, rats were tested in the forced swim task. Behavioral data was collected by hand by an observer and with a video-tracking system (Any-Maze, Stoelting, Wood Dale, IL) in the Social Sciences and Life Sciences Research Buildings at S.U.N.Y. Albany. There was greater than 95% concordance in data collected from rats in the two buildings with these methods.

2.5. Site analyses

After testing was complete, rats were perfused with 0.9% saline followed by 10% formalin. Brains were removed from the skull, fixed in formalin, followed by 30% sucrose–saline, and then sliced on a cryostat at 40 μm. Slices were then stained with Cresyl violet and infusion location was determined by visual analyses of cannulae tracks with light microscopy, as per previously reported methods (Edinger and Frye, 2005, 2004; Frye and Seliga, 2003). The spread of steroids in 25% β-cyclodextran vehicle is typically 1–2 mm from tip of infusion apparatus (Frye and Rhodes, submitted for publication). Accuracy in cannulae placement was high, such that 43 of 46 had bilateral infusions properly aimed at the dorsal hippocampus and 33 of 35 rats had bilateral infusions properly aimed at the ventral tegmental area. There were differences in behavior of rats that received infusions outside the dorsal CA1 region of the hippocampus and no differences in behavior of rats that received infusions outside the ventral tegmental area; data from these rats are not included in statistical analyses. Data of three rats in which infusion placement to the hippocampus could not be determined due to errors in histological preparation were excluded from statistical analyses.

2.6. SERMs and infusions

Rats were bilaterally infused to the hippocampus 25% β-cyclodextrin vehicle dissolved in 0.9% saline or 17β-E_2 (Steraloids, Newport, RI), which has equal affinity for ERα and ERβ (Kuiper et al., 1997), or one of the following ERα- or ERβ-specific SERMs dissolved in β-cyclodextrin vehicle to a concentration of 2 μg/μl. The infusion volume was 1 μl, which was infused over 60 s, and the infusion needle was left in place for 60 s following infusion. All rats were administered bilateral infusions of these same dosages of SERMs to the hippocampus and ventral tegmental area (i.e. total dosing was 4 μg of each SERM for the two infusions). This concentration was based upon previously reported E_2 regimen to the hippocampus for cognitive performance (Frye and Rhodes, 2002) and to the median raphe nucleus for anxiety behavior (Andrade et al., 2005).

2.6.1. ERα-specific SERMs

Propyl pyrazole triol (PPT; Tocris Cookson, Inc., Ellisville, MO) is highly selective for ERα (Stauffer et al., 2000). 17α-E_2 (Sigma Chemical Co., St. Louis, MO) has moderately higher affinity for ERα than ERβ (Kuiper et al., 1997).

2.6.2. ERβ-specific SERMs

Diarylpropionitrile (DPN; Tocris Cookson, Inc.) is highly selective for ERβ (Meyers et al., 2001). 7,12-dihydrocoumestan (coumestrol; Sigma Chemical Co., St. Louis, MO) has a moderately higher affinity for ERβ than ERα (Kuiper et al., 1998).

2.7. Dependent variables and statistical analyses

The following dependent measures were examined to determine the effects of SERMs and infusion site for anxiety and depression behavior in addition to general motor (control) behavior in each of the tasks. Differences in the primary dependent measures for the open field (central square entries), elevated plus maze (open arm time), and forced swim test (immobility) were considered valid when these were the only substantive differences in behavior in these tasks. In the open field, the number of total and central square entries made by the

Table 1
Open field, elevated plus maze, and forced swim test data (mean±SEM) from rats infused ERα-specific SERMs to the hippocampus or the ventral tegmental area (VTA; n=6–8/group)

Condition	Open field		Elevated plus maze		Forced swim test	
	Central entries	Total entries	Open arm time (s)	Total entries	Time immobile (s)	Time swimming (s)
PPT to the hippocampus	14±4	131±25	7±4	7±1	332±27	128±24
PPT to the VTA	18±5	159±30	17±15	6±3	334±47	69±35
17α-E_2 to the hippocampus	9±5	124±15	10±6	7±1	347±23	137±22
17α-E_2 to the VTA	15±5	101±21	32±16	8±2	258±35	17±13

rat were used to determine effects on motor and anxiety behavior, respectively. For the elevated plus, the dependent measures recorded were the total arm entries (motor behavior) and time spent on the open arms of the maze (anxiety behavior). In the forced swim test, time spent by the rat struggling or swimming (motor behavior) or immobile (depression behavior) were recorded.

Two-way analyses of variance (ANOVAs) were used to examine effects of site (hippocampus vs. ventral tegmental area) and SERMs for the dependent measures described above. The α level for statistical significance was when $P<0.05$ and a trend when $P<0.10$. If a significant main effect of SERM was found, ANOVAs were followed by Fisher's post hoc tests to determine differences among groups. Data for ERα-specific SERMs were not included in these statistical analyses because, despite the timing and dose utilized being the same as with the ERβ-SERMs, reduced expression of ERα compared to ERβ in the brain regions investigated limited interpretation of these effects. These data are included in Table 1.

3. Results

3.1. Open field

In the open field, 17β-E_2 and ERβ-specific SERMs (DPN and coumestrol) [$F(3,40)=6.6$, $P<0.01$] significantly increased central entries made in the open field when administered to the hippocampus, but not ventral tegmental area [$F(1,40)=6.9$, $P<0.01$] (Fig. 1). There was no effect of SERMs for total entries made in the open field, but rats administered SERMs to the hippocampus made fewer total entries than did rats administered SERMs to the ventral tegmental area [$F(1,40)=12.4$, $P<0.01$] (Fig. 1). As Table 1 shows, administration of ERα-specific SERMs to the hippocampus or ventral tegmental area produced similar effects for total or central entries made in the open field.

3.2. Elevated plus maze

There was a tendency for 17β-E_2 and ERβ-specific SERMs (DPN and coumestrol) to increase open arm duration compared to vehicle [$F(3,40)=2.3$, $P<0.08$] when administered to the hippocampus, but not the ventral tegmental area [$F(1,40)=10.8$, $P<0.01$] (Fig. 2). There was no main effect of SERMs condition or infusion site for total entries made in the elevated plus maze (Fig. 2). As Table 1 shows, administration of ERα-specific SERMs to the hippocampus or ventral tegmental area produced similar results for open arm time or total entries made in the elevated plus maze.

3.3. Forced swim test

17β-E_2 and ERβ-specific SERMs (DPN and coumestrol) tended to decrease time spent immobile compared to vehicle [$F(3,40)=2.3$, $P<0.08$] when administered to the hippocampus compared to the ventral tegmental area [$F(1,40)=8.4$, $P<0.01$] (Fig. 3). There was no significant effect of SERMs on struggling behavior, but rats administered SERMs to the hippocampus (145.4±20.1 s) had significantly decreased time spent struggling compared to rats administered SERMs to the ventral tegmental area [$F(1,43)=18.1$, $P<0.01$] (232.3±38.7 s). 17β-E_2 and ERβ-specific SERMs significantly increased swimming

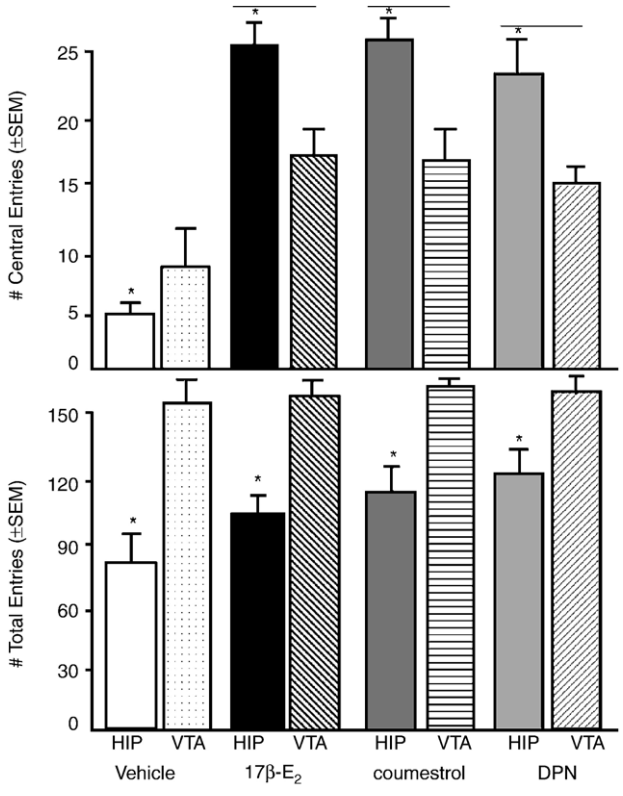

Fig. 1. The mean (±S.E.M.) central (top) and total (bottom) entries made in a brightly-lit open field of rats administered vehicle, 17β-E_2, coumestrol, or DPN to the hippocampus (HIP) or ventral tegmental area (VTA; n=4–7/group). A line above the bars indicates a significant effect of SERMs administration compared to vehicle ($P<0.05$). A * above the bars indicates a significant difference between infusions to the hippocampus versus VTA.

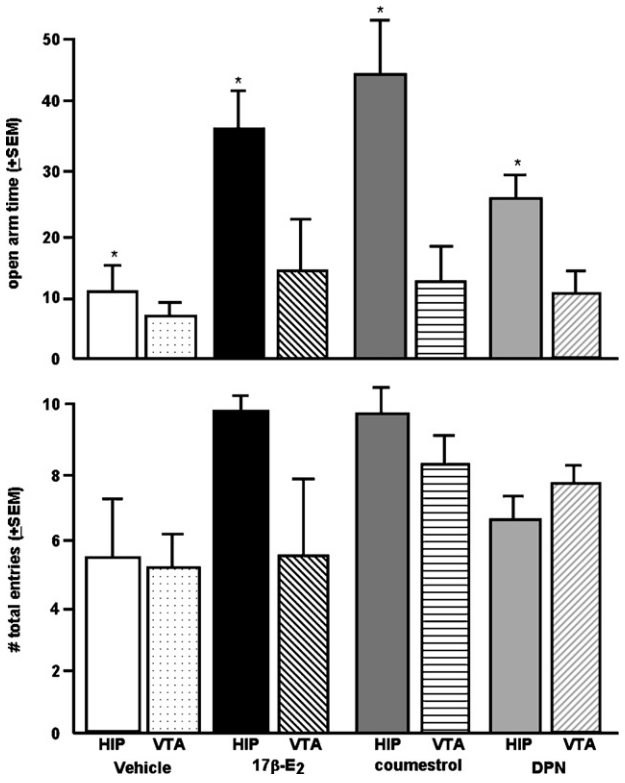

Fig. 2. The mean (±S.E.M.) time spent on the open arms (top) and total arm entries made (bottom) in the elevated plus maze of rats administered vehicle, 17β-E_2, coumestrol, or DPN to the hippocampus (HIP) or ventral tegmental area (VTA; n=4–7/group). A * above the bars indicates a significant difference between infusions to the hippocampus versus VTA.

[$F(3,40)=3.2$, $P<0.03$] when administered to the hippocampus, compared to the ventral tegmental area [$F(1,40)=90.6$, $P<0.01$] and there was a significant interaction [$F(3,40)=4.1$, $P<0.01$] (Fig. 3). As Table 1 shows, administration of ERα-specific SERMs to the hippocampus or ventral tegmental area produced similar behavioral effects in the forced swim test.

4. Discussion

The present results supported our hypothesis that E_2's anti-anxiety and anti-depressive effects may be through actions involving ERβ in the hippocampus. There were similar effects of administration of 17β-E_2, which has equal affinity for ERα and ERβ, and ERβ-specific SERMs, DPN and coumestrol, to decrease anxiety and depressive behavior. Administration of 17β-E_2, DPN, or coumestrol to the hippocampus, but not the ventral tegmental area, increased the number of central entries made in the open field, the time spent on the open arms of the plus maze, and decreased time spent immobile in the forced swim test. Notably, these effects of SERMs occurred independent of gross changes in motor behavior. Furthermore, little differences were observed in these behavioral measures of rats administered vehicle or ERα-specific SERMs. Together, these results suggest that ERβ in the hippocampus may be a target for the anti-anxiety and anti-depressive effects of E_2.

The present results confirm previous reports utilizing animal models that E_2 can alter affective behaviors of female rodents and suggest that the hippocampus is important for these effects. Systemic administration, which would be expected to affect the whole brain, increases anti-anxiety and anti-depressant-like behavior when administered to ovx rodents via injection or subcutaneous implantation of 17β-E_2 pellets or silastic capsules (Estrada-Camarena et al., 2003; Frye and Walf, 2004; Frye and Wawrzycki, 2003; Koss et al., 2004; Rachman et al., 1998; Slater and Blizard, 1976; Walf and Frye, 2005a,b, 2006). We have previously demonstrated that administration of cannulae inserts filled with 1 μg crystalline 17β-E_2 directed towards the same region targeted in the present study, the dorsal hippocampus, but not the ventral tegmental area, of ovx rats increased central entries in the open field, increased open arm time in the plus maze, and decreased time spent immobile in the forced swim test (Walf and Frye, 2006). The present results that infusions of 17β-E_2, to the hippocampus, produced similar anti-anxiety and anti-depressive-like effects in these tasks as did 17β-E_2, administered via other systemic routes or to the hippocampus in other reports, suggest that the hippocampus may be one target of 17β-E_2 for these effects. Whether E_2's actions at ERs may underlie these effects is of interest.

The results of this study confirm previous studies from our laboratory and others on the role of ERβ for affective behavior. Administration of dietary phytoestrogens that have greater affinity for ERβ than ERα increase anti-anxiety behavior in the elevated plus maze of gonadally-intact female

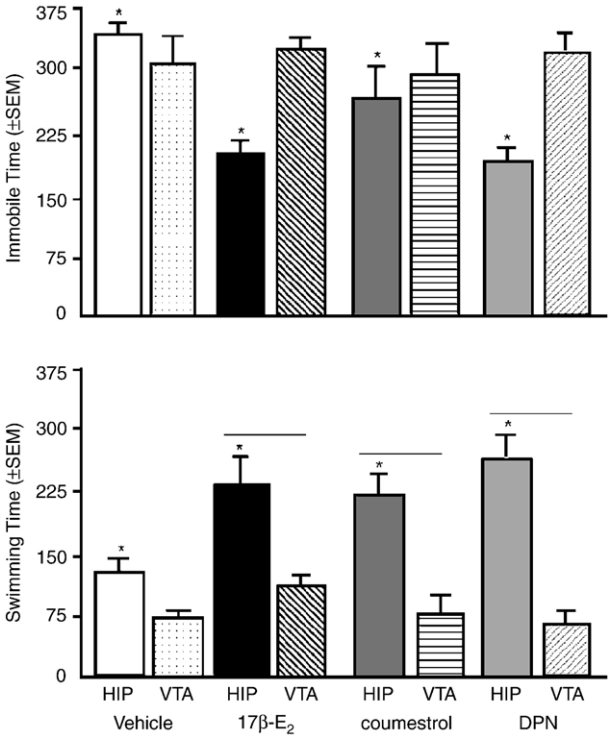

Fig. 3. The mean (±S.E.M.) time spent immobile (top) or swimming (bottom) in the forced swim test of rats administered vehicle, 17β-E_2, coumestrol, or DPN to the hippocampus (HIP) or ventral tegmental area (VTA n=4–7/group). A line above the bars indicates a significant effect of SERMs administration compared to vehicle ($P<0.05$). A * above the bars indicates a significant difference between infusions to the hippocampus versus VTA.

rats (Lund and Lephart, 2001; Patisaul et al., 2005). Subcutaneous administration of ERβ-specific SERMs to ovx rats decrease anxiety behavior in the plus maze (Lund et al., 2005; Walf and Frye, 2005b) as well as several other anxiety models (open field, emergence, light–dark transition, defensive freezing, Vogel punished drinking — Walf and Frye, 2005b) and produce anti-depressant-like behavior in the forced swim test (Walf et al., 2004). Given that these regimen would be expected to have actions throughout the brain, these results are extended by the findings from the present study that administration of ERβ-specific SERMs to the hippocampus, but not another region of the brain that also expresses ERβ, the ventral tegmental area, decrease anxiety and depressive behavior. Interestingly, male rats fed diets rich in ERβ phytoestrogens, which had previously been shown to decrease anxiety behavior, have increased levels of β III tubulin, a neuronal marker of differentiation and survival, in the hippocampus compared to rats fed the phytoestrogen-free diet (Bu and Lephart, 2005). Thus, it may be that the effects of ERβ SERMs to increase anti-anxiety and anti-depressant-like behavior are related to their effects on neuronal integrity, but this remains to be directly addressed.

The present data also suggest that effects of 17β-E_2 and SERMs infusions to the hippocampus can be observed within a short timeframe (10 min). Most studies that have demonstrated E_2's anti-anxiety effects have utilized a longer timeframe for E_2 exposure (i.e. 2–7 d; Estrada-Camarena et al., 2003; Frye and Walf, 2004; Frye and Wawrzycki, 2003; Koss et al., 2004; Rachman et al., 1998; Walf and Frye, 2005a,b, 2006). The timing of E_2's effects is important to consider because it may begin to suggest other mechanisms of E_2 that may be important for these effects. E_2 is known to have rapid effects that involve membrane receptors and initiation of signal transduction pathways (Kelly and Levin, 2001; Moss and Gu, 1999; Nilsen et al., 2002; Toran-Allerand et al., 2002; Wade et al., 2001; Watters et al., 1997). Generally, rapid actions of E_2 are thought to occur in less than 10–15 min (Pfaff and McEwen, 1982). In the present study, functional effects of 17β-E_2 and SERMs were observed between 10–20 min following infusion. As such, these effects of ER ligands occurred in a more rapid timeframe than has been previously reported, leaving open the possibility that these effects involved actions through membrane-bound receptors. Moreover, another possibility to consider in interpretation of these data is the modulatory effects of activation of intracellular ERs on activation of membrane ERs (Kow and Pfaff, 2004; Vasudevan et al., 2001). This could be investigated more directly in the future.

Although the present findings that ERβ-active SERMs have anti-anxiety and anti-depressant-like effects in the hippocampus are intriguing, other factors in interpretation of these data need to be considered. First, other brain areas may be important for these effects. For instance, E_2 administration to the amygdala or median raphe nucleus decreases anxiety behavior in the elevated plus maze and open field and depressive behavior in the forced swim test of ovx rats (Andrade et al., 2005; Frye and Walf, 2003; Walf and Frye, 2006). Second, the effects of E_2 for activity/arousal may have influenced the present results. Naturally-receptive rats or mice, or ovx rats administered E_2, demonstrate more spontaneous motor activity (Becker et al., 1987; Joyce and Van Hartesveldt, 1984; Morgan and Pfaff, 2001; 2002), which may be particularly apparent with a higher dosing of E_2 (Morgan and Pfaff, 2001, 2002). Furthermore, E_2's actions at ERα may be important for E_2's effects on activity/arousal. ERα, but not ERβ, knockout mice differ from their wildtype controls for running wheel activity (Ogawa et al., 2003). However, in the present studies, administration of SERMs to the hippocampus or ventral tegmental area produced modest effects to increase general activity levels in the tasks utilized compared to vehicle-administration, but did not produce discernable differences to suggest effects were ER isoform-specific. Further investigation of SERMs' effects on activity and arousal measures are needed to clarify their role in these and other functional effects. Third, the specificity of the behavioral effects of the compounds used need to addressed. For instance, it is not clear whether the ERβ-SERMs utilized target one of the many variants of ERβ. As well, although both ERα and ERβ have been localized to the hippocampus, there is some indication that the hippocampus expresses ERβ to a greater extent than ERα (Shughrue et al., 1997). It may be that effects of administering a single concentration of ERβ-, but not ERα-specific, SERMs to the hippocampus were effective in altering anxiety and depressive behavior because of higher expression of ERβ in this region. So although these data investigating one regimen of ERα-specific SERMs and findings in the literature of the ineffectiveness of subcutaneously administered ERα SERMs to alter affective behavior suggest that actions at ERα may not be important for regulation of anxiety/depression, caution in interpreting these effects should be taken. Future studies investigating different dosages and timing of administration may be worthwhile.

Together, these data suggest that E_2's effects to reduce anxiety and depressive behavior may involve effects of ERβ in the hippocampus. Administration of 17β-E_2, which has equal affinity for ERα and ERβ, and SERMs with greater affinity for ERβ than ERα (DPN, coumestrol) decreased anxiety behavior in the elevated plus maze and open field and depressive behavior in the forced swim test compared to that in ovx rats administered vehicle. These data are interesting as they begin to dissociate mechanisms of E_2 at ERα and ERβ as well as brain targets for these effects for anxiety and depressive behavior. Given the preponderance of co-morbid affective and substance abuse disorders among women, this is an important topic to pursue (Brooner et al., 1997; Borrelli et al., 1999; Cornelius et al., 1995; Lundy et al., 1995; McCance-Katz et al., 1999). Studies in humans suggest that differences in ER isoform expression in the brain may be related to their functional importance for neuropsychiatric disorders as well as efficacy in treating these disorders (Osterlund and Hurd, 2001; Osterlund et al., 2000, 2005). Indeed, a major criticism of E_2-based therapies to women are their potential proliferative effects on reproductive organs, which are mediated primarily via actions at ERα (Gustafsson, 2003). Thus, it is important to further investigate the functional effects of ERα and ERβ.

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