



Available at www.sciencedirect.com



journal homepage: www.elsevier.com/locate/psyneuen



Estrogen recruits the endocannabinoid system to modulate emotionality

Matthew N. Hill, Eda S. Karacabeyli, Boris B. Gorzalka*

Department of Psychology, University of British Columbia, 2136 West Mall, Vancouver, BC, Canada V6T1Z4

Received 18 August 2006; received in revised form 22 January 2007; accepted 1 February 2007

KEYWORDS

Fatty acid amide hydrolase;
Antidepressant;
Anandamide;
Depression;
Gender

Summary

Estrogen administration elicits anxiolytic and antidepressant-like effects in female rats; however, the mechanism of this effect is unknown. Fatty acid amide hydrolase (FAAH), the enzyme which degrades the endocannabinoid anandamide, has been shown to be regulated by estrogen. Thus, we examined if the anxiolytic and antidepressant effects of estrogen implicated the endocannabinoid system. In the first experiment, ovariectomized female rats were administered a single injection of 17β -estradiol (10 μ g) or oil, and 48 h later were given an injection of the cannabinoid CB₁ receptor antagonist AM251 (1 mg/kg) or vehicle. One hour after AM251 or vehicle administration, subjects were tested in either the open field test (OFT), elevated plus maze (EPM) or the forced swim test (FST). Estradiol treatment resulted in a significant increase in open arm entries in the EPM and time spent in the center quadrant of the OFT, which were reversed by co-treatment with AM251, suggesting that endocannabinoids are integral to the anxiolytic effects of estrogen. No significant effects of estradiol or AM251 were seen in the FST. In the second experiment, administration of the FAAH inhibitor URB597 (0.1 and 0.3 mg/kg) increased open arm entries in the EPM and time spent in the center quadrant in the OFT as well as significantly reduced immobility in the FST. Collectively, these data demonstrate that estrogen may elicit changes in emotional behavior through an endocannabinoid mechanism, and suggest that inhibition of FAAH represents a therapeutic target for anxiety and depression in women.

© 2007 Elsevier Ltd. All rights reserved.

1. Introduction

The effects of estrogen on mood and emotion have been well documented. For example, menopausal declines in

circulating estrogen levels have been associated with an increase in mood disturbances, including symptoms of anxiety and depression in women (Weissman and Klerman, 1977; Wittchen and Hoyer, 2001). Ovariectomy in female rats mirrors the depletion of estrogen in menopause, and concurrently elicits a reliable increase in anxiety and depression-like behaviors in tests of emotionality, such as the elevated plus maze (EPM) and forced swim test, which

*Corresponding author. Tel.: +1 604 822 3095;
fax: +1 604 822 6923.

E-mail address: bgorzalka@psych.ubc.ca (B.B. Gorzalka).

are reversible by treatment with physiological doses of estradiol (Estrada-Camarena et al., 2003, 2006; Walf et al., 2004; Andrade et al., 2005a; Walf and Frye, 2005a; Picazo et al., 2006). These behavioral changes are likely mediated, at least in part, by classical nuclear actions of estrogen on gene transcription given the temporal lag required for consistent behavioral effects to be seen. There is evidence that the serotonergic 5-HT_{1A} receptor is involved in these behavioral changes (Andrade et al., 2005; Estrada-Camarena et al., 2006); however, this role appears to be more related to rapid effects of estrogen rather than longer term effects. However, other neurotransmitter systems and neuromodulators warrant further investigation.

The endocannabinoid system is a neuromodulatory system and potential target to mediate the behavioral effects of estrogen. Activation of the cannabinoid CB₁ receptor by both exogenous ligands, such as tetrahydrocannabinol, the psychoactive constituent of cannabis, and endogenous ligands, such as the arachidonate derived lipids arachidonyl ethanolamine (anandamide; AEA) and 2-arachidonylglycerol (2-AG), elicits profound effects on emotional behavior (Onaivi et al., 1990; Kathuria et al., 2003; Hill and Gorzalka, 2004, 2005a; Viveros et al., 2005; Bortolato et al., 2006; Patel and Hillard, 2006). More specifically, inhibition of the primary enzyme involved in AEA metabolism, fatty acid amide hydrolase (FAAH), reduces anxiety on several rodent indices such as ultrasonic vocalizations and open arm avoidance in the EPM (Kathuria et al., 2003; Patel and Hillard, 2006). Similar effects on anxiety have been documented with the combined AEA transport/FAAH inhibitor AM404 (Bortolato et al., 2006). Inhibition of FAAH and/or AEA transport has also been found to induce an antidepressant-like effect in the forced swim test (Gobbi et al., 2005; Hill and Gorzalka, 2005a). However, it should be noted that opposing effects have also been demonstrated such that administration of antagonists to the CB₁ receptor have been found to elicit similar anxiolytic and antidepressant-like effects (Griebel et al., 2005; Shearman et al., 2003; Tzavara et al., 2003), indicating that this system exhibits a complex regulation of emotional behavior. Regardless of this complexity, these behavioral effects elicited by enhancement of AEA/CB₁ receptor signaling are quite similar to those seen following estrogen administration (Estrada-Camarena et al., 2003, 2006; Walf et al., 2004; Andrade et al., 2005; Walf and Frye, 2005a).

At the molecular level, the FAAH enzyme is a potential locus for an interaction between estrogen and endocannabinoid signaling. The FAAH enzyme possesses an estrogen response element in its genetic sequence, and translocation of the estrogen receptor to the nucleus results in inhibition of FAAH transcription (Waleh et al., 2002). In the periphery, estrogen has been found to downregulate FAAH expression and activity in uterine tissue (Maccarrone et al., 2000). Estrogen-induced downregulation of FAAH would result in a consequent increase in AEA signaling, which, if it occurred in the central nervous system could influence emotional processes. We performed a series of experiments to explore this hypothesis. First, we examined if the ability of estrogen to reduce anxiety in the EPM and open field test, and elicit an antidepressant response in the forced swim test, were sensitive to CB₁ receptor antagonism. Second, we examined the effect of pharmacological inhibition of FAAH in these

behavioral tests in ovariectomized female rats. Our data demonstrate that estrogen engages the endocannabinoid system, potentially through a FAAH-related mechanism, to modulate emotional behavior in the female rat.

2. Methods

2.1. Subjects

Female Long-Evans rats that were 10 weeks of age, at the time of testing, and weighed between 225 and 275 g were used in this study. All subjects were housed in groups of three in triple mesh wire cages in a colony room that had a maintained temperature of 21±1 °C and a 12h:12h light dark cycle (lights on at 0900h). All females were ovariectomized under either Halothane anesthesia (2–4% flow rate as required to maintain stable respiration) or a combination of 75 mg/kg ketamine hydrochloride (intraperitoneal) and 7 mg/kg xylazine (intraperitoneal) anaesthesia. Subjects were given a two week recovery period following surgery before behavioral testing occurred, and each subject was only used once per behavioral test. All rats had ad libitum access to tap water and Purina Rat Chow, and were handled 4 times a week prior to testing. All experimental protocols were in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University of British Columbia.

2.2. Elevated plus maze (EPM)

The EPM consists of two open arms (50 cm × 12.5 cm) and two enclosed arms (50 cm × 12.5 cm × 50 cm) which extend from a common middle platform (12.5 cm × 12.5 cm). The apparatus was constructed from wood, with all walls and platforms painted black. The extending arms were elevated above the ground at a height of 60 cm by four pedestals on which each of the extended arms was resting. All animals were placed in the center square of the EPM (facing an open arm) and were tested for 5 min in the apparatus. Behavior was monitored and scored blind by observers employing an overhead camera (Hitachi 2500A) in a dimly lit room. The EPM examines a natural conflict between a desire to explore and a vulnerability in open spaces, thus it is believed that time spent in and entries into the open arms represent a reduction in anxiety (File, 2001). Both the total time spent in the open arms and the frequency of entries into open and closed arms were scored. From this, the ratio of time spent in the open arms relative to time spent in the closed arms and the ratio of entries into the open arms relative to entries into the closed arms was calculated. Arm entries were defined by at least three of the rats' paws entering an area and as animals left a closed to an open arm, or vice versa, they were considered to be in the prior arm until all three paws had entered the new arm. Thus, any time in the central area was counted as time in the previous arm. After individual rats finished their trial in the apparatus, the walls and platform floors were wiped with a 1% hibitane solution (an antiseptic solution) and dried to remove any scent trail that the subject might have left.

2.3. Forced swim test (FST)

Plexiglas cylindrical containers (diameter 35 cm and height 45 cm) were used during forced swim testing. Given the documented influence of water depth on forced swim behaviors (Abel, 1994), each container was filled to 30 cm so that an animal could only touch the bottom with the tip of its tail, and water temperature was maintained at a constant $23 \pm 1^\circ\text{C}$. To remove the influence of potential alarm substances on behaviours in the FST (Abel, 1991), fresh water was introduced prior to each test. All test sessions were recorded with a video camera (Hitachi 2500A) under dim light conditions positioned such that the entire container was in full sight, and videotapes of test sessions were subsequently scored by blind, trained observers. The time spent struggling (thrashing behavior against the side of the container in a vertical alignment), swimming (active movement around the chamber with the use of all four paws) and immobility (no movement other than the minimum required to stay afloat) was scored for each subject. The expression of immobility is believed to represent the engagement of maladaptive coping behaviors, thus antidepressant agents are identified by their ability to reduce time spent immobile (Porsolt et al., 1977).

2.4. Open field test (OFT)

The open field arena was 120 cm \times 120 cm with walls 60 cm high. The arena was painted white and was divided into 16 equal quadrants (30 cm \times 30 cm) by black lines. For testing, animals were placed in the central quadrant and left for 5 min. No experimenters were in the room and behavior was recorded by an overhead camera (Hitachi 2500A) and scored later blindly. The total number of line crosses (defined as all four paws crossing a line) and fecal boli were tallied and time spent in the central quadrant (central four squares) was also measured. After individual rats finished their trial in the apparatus, the walls and platform floors were wiped with a 1% hibitane solution and dried to remove any scent trail that the subject might have left.

2.5. Treatment procedure

In Experiment 1, we aimed to investigate if the effects of estradiol on behaviors in the EPM, OFT and FST were sensitive to blockade of the cannabinoid CB₁ receptor. The estradiol treatment protocol was based on an established protocol employed in other laboratories that elicits both anxiolytic and antidepressant-like effects (Walf et al., 2004; Walf and Frye, 2005a). Forty eight hour prior to testing, all subjects received a 0.1 ml subcutaneous injection of peanut oil or 10 μg 17 β -estradiol (Sigma-Aldrich, Canada). One hour prior to testing, all subjects received an injection of 1 mg/kg AM251 (a cannabinoid CB₁ receptor antagonist; Tocris-Cookson, USA) or vehicle (1:1:8 Dimethyl Sulfoxide: Tween 80: 0.9% Saline). No subjects were used more than once.

In Experiment 2, we aimed to investigate if pharmacological inhibition of FAAH elicited similar behavioral effects to estradiol treatment in ovariectomized rats. For this purpose, we administered the FAAH inhibitor URB597 (0.1 and 0.3 mg/kg; Cayman Chemical, USA) or vehicle (1:1:8

Dimethyl Sulfoxide: Tween 80: 0.9% Saline) 1 h prior to testing in the EPM, OFT and FST.

2.6. Statistics

For Experiment 1, data were analyzed using a univariate analysis of variance (ANOVA) with estrogen treatment and AM251 administration as fixed factors. Post hoc analysis was performed using a Tukey's test. For Experiment 2, data were analyzed using a one way ANOVA and post hoc analysis was performed using a two-tail Dunnett's test. All analyses used $p < 0.05$ as an indication of significance.

3. Results

3.1. Elevated plus maze

In the EPM, there was a significant interaction between estradiol and AM251 administration on the ratio of open arm entries relative to closed arm entries [$F(1, 22) = 6.51$, $p < 0.02$; Fig. 1(a)]. Post hoc analysis revealed that estradiol administration increased the ratio of open arm entries relative to oil/vehicle treated animals ($p < 0.05$); additionally, animals that had been co-treated with estradiol and AM251 were not different from those receiving oil/vehicle treatment ($p > 0.05$) but were significantly different from those receiving estradiol/vehicle treatment ($p < 0.04$).

A similar effect was seen with the ratio of time spent in the open arms relative to time spent in the closed arms, such that there was a significant interaction between estradiol treatment and AM251 administration [$F(1, 22) = 6.83$, $p < 0.02$; Fig. 1(b)]. Consistent with data for ratio of open arm entries, estradiol treatment significantly increased the ratio of time spent in the open arms relative to time spent in the closed arms ($p < 0.01$) and this effect was completely blocked by AM251 administration ($p < 0.01$; estradiol/vehicle vs. estradiol/AM251).

These effects were not an artifact of alterations in locomotor function as there was no significant interaction [$F(1, 22) = 0.05$, $p > 0.05$] nor main effects of either estradiol treatment [$F(1, 22) = 2.80$, $p > 0.05$] or AM251 administration [$F(1, 22) = 1.71$, $p > 0.05$] on closed arm entries (Fig. 1(c)).

There was a significant effect of URB597 administration on ratio of open arm entries relative to closed arm entries [$F(2, 19) = 4.10$, $p < 0.04$; Fig. 2(a)]; analysis with a Dunnett's test revealed that both the 0.1 ($p < 0.05$) and 0.3 mg/kg ($p < 0.02$) dose of URB597 significantly increased the ratio of entries into open arms relative to closed arms.

A similar finding was seen with ratio of time spent in the open arms relative to closed arms; however, there was only a near significant effect of URB597 treatment [$F(2, 19) = 2.59$, $p = 0.08$; Fig. 2(b)]; however, Dunnett's test revealed that administration of the 0.3 mg/kg dose of URB597 increased the ratio of time spent in the open arms relative to the closed arms ($p < 0.05$), whereas the 0.1 mg/kg dose elicited a similar, but non-significant, effect ($p = 0.11$).

Similar to what was seen with estradiol treatment, these effects are not an artifact due to alterations in motor

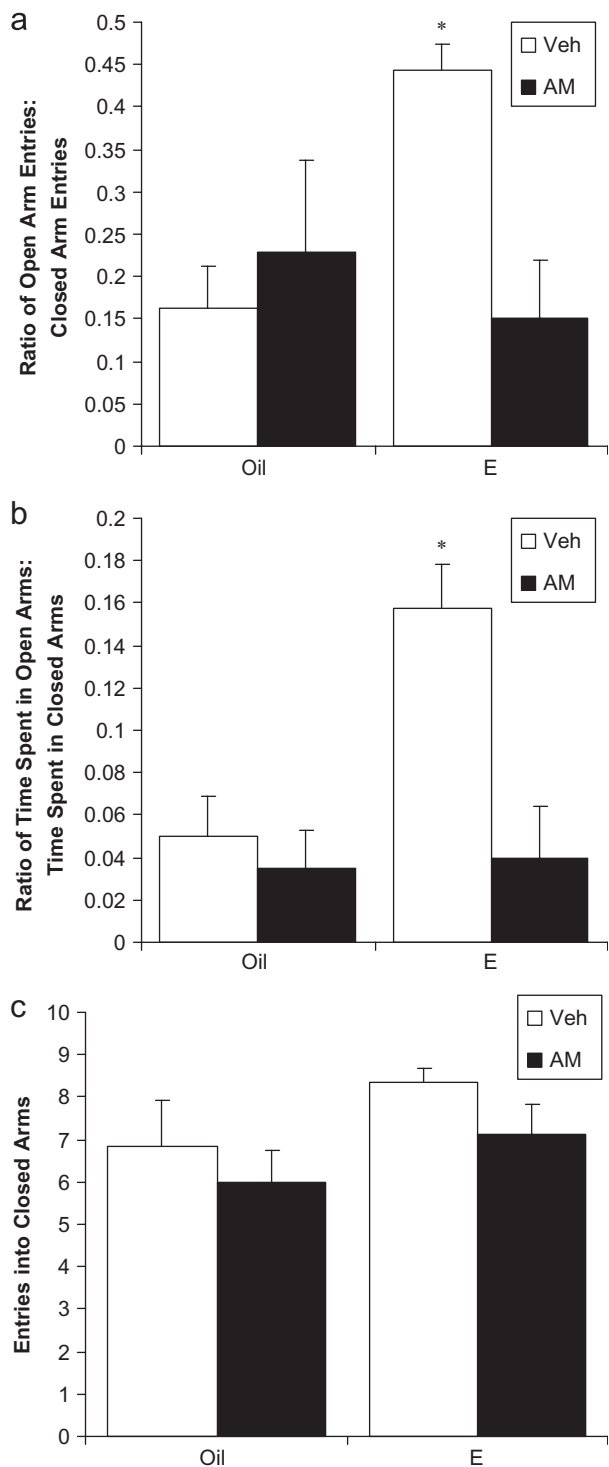


Fig. 1 The effect of treatment with 17β-estradiol (E; 10 μg; 48 h prior to testing) or oil and administration of the cannabinoid CB₁ receptor antagonist AM251 (AM; 1 mg/kg; 1 h prior to testing) or vehicle (Veh) on (a) the ratio of entries into open arms relative to entries into closed arms, (b) the ratio of time spent in the open arms relative to time spent in the closed arms and (c) total entries into closed arms in the elevated plus maze. Data are presented as mean ± SEM. * denotes significant difference with all other treatment conditions ($p < 0.05$).

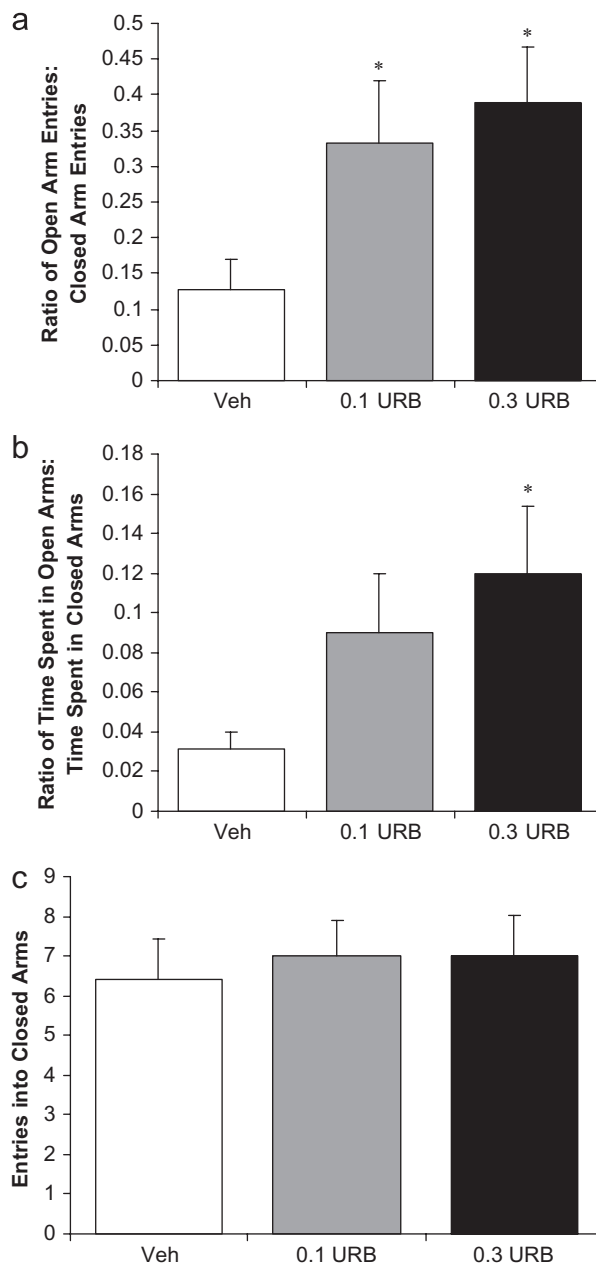


Fig. 2 The effect of treatment with the fatty acid amide hydrolase (FAAH) inhibitor URB597 (0.1 and 0.3 mg/kg; 1 h prior to testing) or vehicle (Veh) on (a) ratio of entries into open arms relative to entries into closed arms, (b) ratio of time spent in the open arms relative to time spent in the closed arms and (c) total entries into closed arms in the elevated plus maze. Data are presented as mean ± SEM. Significant differences ($p < 0.05$) relative to vehicle treated animals are denoted by *.

activity as there was no effect of URB597 treatment on closed arm entries [$F(2, 19) = 0.12, p > 0.05$; Fig. 2(c)].

3.2. Open field test

There was no interaction between estradiol and AM251 [$F(1, 23) = 0.63, p > 0.05$] nor main effects of either

estradiol [$F(1, 23) = 0.04, p > 0.05$] or AM251 [$F(1, 23) = 0.17, p > 0.05$] on total line crosses in the OFT (Table 1). With respect to time spent in the central quadrant, there was no significant interaction between AM251 and estradiol [$F(1, 23) = 0.89, p > 0.05$; Table 1]; however, there was a significant main effect of estradiol treatment [$F(1, 23) = 4.43, p < 0.05$] and a near significant main effect for AM251 [$F(1, 23) = 3.98, p < 0.06$]. There was no significant interaction between estradiol and AM251 [$F(1, 23) = 0.16, p > 0.05$], nor significant main effects of either estradiol [$F(1, 23) = 2.04, p > 0.05$] or AM251 [$F(1, 23) = 1.32, p > 0.05$] on total boli during the 5 min OFT (Table 1).

There was no significant effect of URB597 treatment on line crosses in the OFT [$F(2, 19) = 0.70, p > 0.05$; Table 1] or total boli during the OFT [$F(2, 19) = 1.10, p > 0.05$; Table 1]. There was a significant effect of URB597 treatment on time spent in the central quadrant of the OFT [$F(2, 19) = 4.32, p < 0.03$; Table 1], with Dunnett's test demonstrating that treatment with 0.1 mg/kg URB597 increased time spent in the central quadrant ($p < 0.02$), whereas the 0.3 mg/kg dose of URB597 had no significant effect ($p > 0.05$).

3.3. Forced swim test

There was no significant interaction between estradiol and AM251 [$F(1, 23) = 0.49, p > 0.05$], nor main effects of

either estradiol [$F(1, 23) = 1.11, p > 0.05$] or AM251 [$F(1, 23) = 0.25, p > 0.05$] on immobility in the FST (Table 2). Similarly, there was no interaction [$F(1, 23) = 0.43, p > 0.05$] nor main effects of either estradiol [$F(1, 23) = 0.16, p > 0.05$] or AM251 [$F(1, 23) = 0.03, p > 0.05$] on swimming in the FST (Table 2), and no interaction [$F(1, 23) = 2.50, p > 0.05$] and no main effects of either estradiol [$F(1, 23) = 0.64, p > 0.05$] or AM251 [$F(1, 23) = 0.27, p > 0.05$] on struggling behavior in the FST (Table 2).

There was a near significant effect of URB597 administration on immobility in the FST [$F(2, 20) = 3.48, p = 0.053$; Fig. 3(a)]; however, Dunnett's test demonstrated that 0.1 mg/kg URB597 significantly reduced immobility in the FST ($p > 0.02$), and 0.3 mg/kg URB597 produced a near significant reduction in immobility ($p = 0.06$). There was, however, no effect of URB597 treatment on either swimming [$F(2, 20) = 0.13, p > 0.05$; Fig. 3(b)] or struggling [$F(2, 20) = 1.19, p > 0.05$; Fig. 3(c)] in the FST.

4. Discussion

The results of Experiment 1 confirmed previous research by demonstrating that administration of physiological doses of estradiol to ovariectomized female rats produced an anxiolytic effect in the open field test and EPM. Our results also provided the first evidence to date that

Table 1 The effect of treatment with the fatty acid amide hydrolase (FAAH) inhibitor URB597 and combined treatment of 17 β -estradiol and the cannabinoid CB₁ receptor antagonist AM251 on behavior in the open field test.

	Time in center (s)	Total line crosses	Fecal boli
Vehicle	13.5 \pm 4.0	77.8 \pm 7.0	1.5 \pm 0.9
0.1 mg/kg URB597	24.9 \pm 2.6*	68.9 \pm 3.8	4.3 \pm 1.5
0.3 mg/kg URB597	16.6 \pm 1.8	69.3 \pm 6.7	1.9 \pm 1.1
Oil-vehicle	22.1 \pm 5.5	71.9 \pm 4.7	3.7 \pm 0.6
Estradiol-vehicle	34.2 \pm 3.6*	75.7 \pm 4.2	2.7 \pm 0.8
Oil-AM251	18.0 \pm 1.6	78.1 \pm 7.0	5.3 \pm 1.1
Estradiol-AM251	22.6 \pm 3.9	73.7 \pm 4.0	3.4 \pm 1.3

Treatment with URB597 (0.1 but not 0.3 mg/kg) 1 h prior to testing resulted in an increase in time spent in the central quadrant in the open field test without affecting total line crosses or fecal boli. Similarly, treatment with 17 β -estradiol (10 μ g) 48 h prior to testing resulted in an increase in time spent in the central quadrant that was not seen when AM251 (1 mg/kg) was co-administered 1 h prior to testing. Neither 17 β -estradiol nor AM251 altered total line crosses or fecal boli in the open field test. Data are presented as mean values \pm SEM. Significantly different values ($p < .05$) denoted by *.

Table 2 The effect of the combined treatment of 17 β -estradiol and the cannabinoid CB₁ receptor antagonist AM251 on behavior in the forced swim test.

	Immobility time (s)	Swimming time (s)	Struggling time (s)
Oil-vehicle	108.0 \pm 26.6	389.5 \pm 22.9	101.8 \pm 7.4
Estradiol-vehicle	65.7 \pm 20.1	383.6 \pm 22.3	150.7 \pm 20.4
Oil-AM251	103.3 \pm 16.6	373.0 \pm 17.7	123.7 \pm 21.8
Estradiol-AM251	94.7 \pm 30.8	397.4 \pm 28.0	107.6 \pm 24.9

Treatment with 17 β -estradiol (10 μ g) 48 h prior to testing resulted in a non-significant reduction in immobility in the forced swim test, that was absent when 17 β -estradiol was co-administered with AM251 (1 mg/kg). AM251 treatment alone had no effect on immobility in the forced swim test. Administration of 17 β -estradiol and AM251, alone or in combination, did not alter either swimming or struggling behavior. Data are presented as mean values \pm SEM.

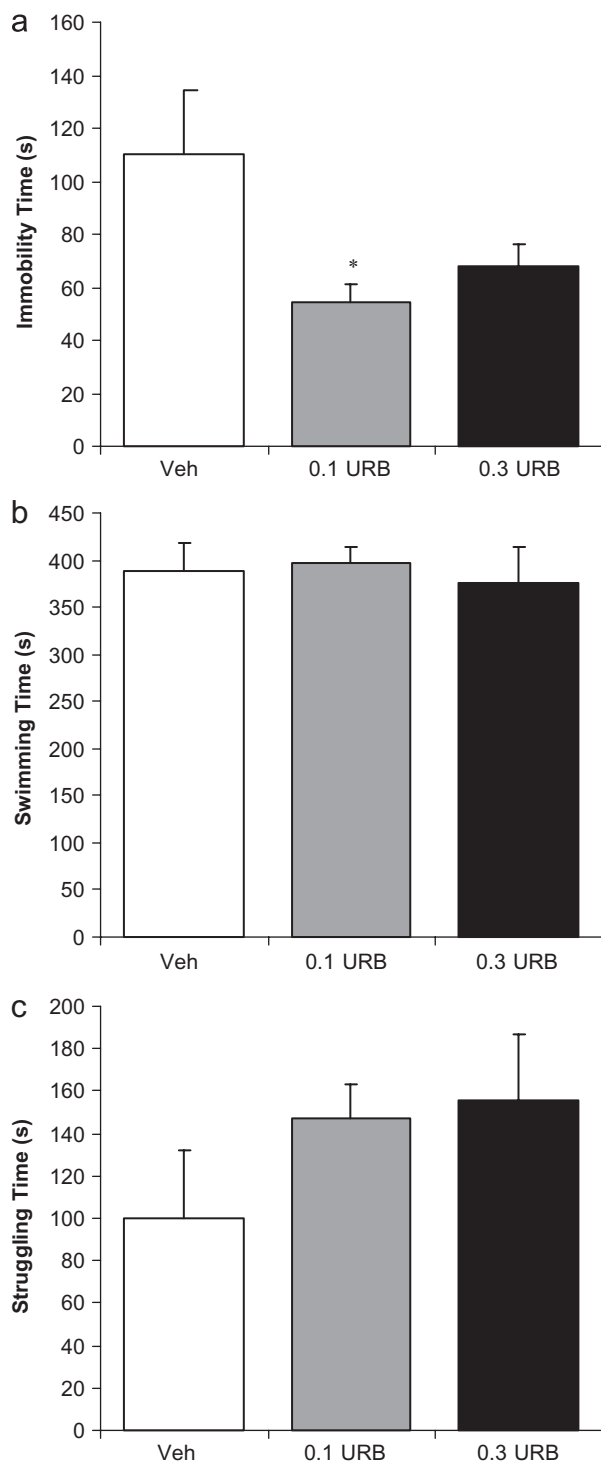


Fig. 3 The effect of treatment with the fatty acid amide hydrolase (FAAH) inhibitor URB597 (0.1 and 0.3 mg/kg; 1 h prior to testing) or vehicle (Veh) on time spent in (a) immobility, (b) swimming and (c) struggling in the forced swim test. Data are presented as mean \pm SEM. Significant differences ($p < 0.05$) relative to vehicle treated animals are denoted by *.

estradiol-induced alterations in emotionality are sensitive to antagonism of the cannabinoid CB₁ receptor. Surprisingly, estradiol treatment did not elicit a significant

antidepressant-like effect in the forced swim test, which contrasts with previous reports (Estrada-Camarena et al., 2003, 2006; Walf and Frye, 2005a). However, inspection of the data reveals that estradiol treatment alone did induce a notable, but highly variable, reduction in immobility in the forced swim test, which was absent in animals that were co-treated with the CB₁ receptor antagonist AM251. In addition, Experiment 2 revealed that pharmacological inhibition of FAAH, the primary enzyme that regulates AEA metabolism, resulted in a significant reduction in anxiety-like behaviors in the open field test and EPM in ovariectomized female rats, and significantly reduced immobility in the forced swim test.

The finding that estradiol-induced changes in emotionality are sensitive to blockade of the CB₁ receptor suggests that estradiol treatment increases endocannabinoid signaling. This increase in endocannabinoid signaling is likely due to either an increase in endocannabinoid biosynthesis or release, or a reduction in endocannabinoid metabolism. Estradiol has been shown to rapidly stimulate anandamide release from endothelial cells (Maccarrone et al., 2002); however, if a comparable phenomenon is occurring in nervous tissue, this effect would likely not account for the behavioral results as testing occurred 48 h following estradiol treatment. These delayed effects are likely reflective of estradiol-mediated alterations in gene expression. Of interest, the gene sequence for FAAH possesses an estrogen response element, which when bound by the estrogen receptor, inhibits transcription of FAAH (Waleh et al., 2002). Thus, declines in estrogenic steroids following ovariectomy, may result in a disinhibition of FAAH transcription and consequent increases in FAAH expression and AEA metabolism. Administration of estradiol may in turn, inhibit FAAH transcription and downregulate FAAH expression resulting in elevated endocannabinoid content. Consequently, these changes in endocannabinoid activity may contribute to estradiol's ability to modulate mood and affect. While this hypothesis would require biochemical validation for confirmation, estrogen has been shown to decrease FAAH expression and activity and increases AEA levels in the uterus (Maccarrone et al., 2000), and the vasorelaxant effects of AEA in female rats have been found to be decreased by ovariectomy and restored by concurrent estradiol administration (Peroni et al., 2004). These data are consistent with the hypothesis that ovariectomy results in a facilitation of FAAH activity, and that estradiol treatment attenuates AEA hydrolysis. Thus, it is plausible that a comparable phenomenon may be occurring in the central nervous system. In support of this, pharmacological inhibition of FAAH in ovariectomized female rats elicited comparable, if not more robust, behavioral responses in the EPM, open field test and forced swim test. Thus, the current data suggest that estradiol may modify emotional behavior by downregulating FAAH expression and increasing AEA/CB₁ receptor signaling. Given that several reports have demonstrated that the ability of estradiol to modulate emotional behavior is mediated by activation of β -estrogen receptors, and not α -estrogen receptors (Walf et al., 2004; Walf and Frye, 2005b; Lund et al., 2005), examination of whether the FAAH gene is a molecular target of β -estrogen receptors is a necessary step in this line of research.

The mechanism by which endocannabinoids regulate emotional behavior is largely unknown; however, CB₁ receptors are distributed in key limbic regions such as the amygdala, prefrontal cortex and hypothalamus, where they regulate excitatory and inhibitory neurotransmission (Freund et al., 2003). Alternatively, endocannabinoids could exert their effects on behavior by modulating monoaminergic neurotransmission. Specifically, a recent study demonstrated that the antidepressant effects of the FAAH inhibitor URB597 were associated with increased firing of serotonergic neurons in the dorsal raphe (Gobbi et al., 2005). Previous research has suggested that the effects of estradiol on emotional behavior may in part be mediated by serotonergic activity. Specifically, if 5-HT_{1A} receptor antagonists are co-administered with estradiol, when testing occurs at a later time point, the behavioral effects of estradiol treatment are attenuated (Andrade et al., 2005; Estrada-Camarena et al., 2006). Thus, it appears that both the serotonergic and endocannabinoid systems may be involved in the effects of estradiol on emotionality, and it is possible that endocannabinoids may act as a mediator between estrogen and increased serotonergic activity, or vice versa.

The fact that pharmacological inhibition of FAAH with URB597 elicited such robust alterations in tests of emotional behavior supports and replicates previous research examining this effect in male rodents (Kathuria et al., 2003; Gobbi et al., 2005; Patel and Hillard, 2006). However, it should also be noted that several studies have found that antagonism of the CB₁ receptor also elicits both anxiolytic and antidepressant-like effects in several of the same paradigms employed in this research (Griebel et al., 2005; Tzavara et al., 2003; Shearman et al., 2003). The factors that determine when facilitation or antagonism of endocannabinoid signaling will modulate emotionality are not well understood. Some studies suggest that environmental conditions such as lighting conditions or previous stress history of the individual could alter the responses of these drugs (Hill and Gorzalka, 2004; Haller et al., 2004; Patel et al., 2005). The current study adds gender to this list of factors, as potentially the endocannabinoid system plays a more prominent role in emotionality in females than it does in males. Additionally, these data do not demonstrate any behavioral effects of antagonism of the cannabinoid CB₁ receptor in tests of emotionality in female rats, at least at the dose employed here. Future research should directly examine the conditions under which emotionality is altered by inhibition of FAAH or antagonism of the CB₁ receptor. Regardless, the current data suggest that FAAH inhibition represents a potential target for the development of novel therapeutics for depression and anxiety in women. Given that these mood disorders are more prevalent in women than men (Nolen-Hoeksema, 1987; Kessler et al., 2003), identification of pharmacological targets that exhibit efficacy in preclinical tests in females as well as males is essential.

Several (Schmidt et al., 2000; Soares et al., 2001; Cohen et al., 2003), but not all (Brunner et al., 2005), clinical studies have demonstrated that estrogen administration to post-menopausal women results in an improvement in mood and reductions in measures of anxiety and depression. Despite these mood elevating properties of estrogen, administration of estrogenic compounds to post-menopausal women is controversial given the potential risk of increasing the development of breast cancer (Rossouw et al., 2002;

Chlebowski et al., 2003). Determination of the mechanism by which estrogen alters mood may help to bypass the health risks of direct estrogen administration. The current data suggest that estradiol may facilitate endocannabinoid signaling, which in turn could be a mechanism by which estrogen modulates affect. Together, these data highlight the potential importance of endocannabinoid signaling in emotional behavior in females, and support previous suggestions that pharmacological inhibition of FAAH is a plausible candidate for the development of novel pharmacotherapeutics for mood disorders (Gobbi et al., 2005; Hill and Gorzalka, 2005b).

Role of the funding source

There was no role of the funding source in the collection, analysis or interpretation of this data nor the writing or submission of this paper.

Conflict of interest statement

No authors on this paper have any conflict of interest to report with the data presented in this manuscript.

Matthew N. Hill
Eda S. Karacabeyli
Boris B. Gorzalka

Acknowledgments

This research was supported by operating grants provided to BBG by the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada (NSERC) and graduate fellowships to MNH provided by NSERC and the Michael Smith Foundation for Health Research. The authors would like to thank Stephanie Lieblich, Larissa Froese, Anna Morrish, Cedric Gabilondo and Sarah Thompson for their technical assistance.

References

- Abel, E., 1991. Gradient of alarm substance in the forced swimming test. *Physiol. Behav.* 49, 321–323.
- Abel, E., 1994. Behavioral and physiological effects of different water depths in the forced swim test. *Physiol. Behav.* 56, 411–414.
- Andrade, T.G., Nakamuta, J.S., Avanzi, V., Graeff, F.G., 2005. Anxiolytic effect of estradiol in the median raphe nucleus mediated by 5-HT_{1A} receptors. *Behav. Brain Res.* 163, 18–25.
- Bortolato, M., Campolongo, P., Mangieri, R.A., Scattoni, M.L., Frau, R., Trezza, V., La Rana, G., Russo, R., Calignano, A., Gessa, G.L., Cuomo, V., Piomelli, D., 2006. Anxiolytic-like properties of the anandamide transport inhibitor AM404. *Neuropsychopharmacology* 31, 2652–2659.
- Brunner, R.L., Gass, M., Aragaki, A., Hays, J., Granek, I., Woods, N., Mason, E., Brzyski, R., Ockene, J., Assaf, A., LaCroix, A., Matthews, K., Wallace, R., 2005. Women's Health Initiative Investigators. Effects of conjugated equine estrogen on health-related quality of life in postmenopausal women with hysterectomy: results from the Women's Health Initiative Randomized Clinical Trial. *Arch. Intern. Med.* 165, 1976–1986.
- Chlebowski, R.T., Hendrix, S.L., Langer, R.D., Stefanick, M.L., Gass, M., Lane, D., Rodabough, R.J., Gilligan, M.A., Cyr, M.G.,

- Thomson, C.A., Kandekar, J., Petrovich, H., McTiernan, A., 2003. Womens Health Initiative Investigators. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. *J. Am. Med. Assoc.* 289, 3243–3253.
- Cohen, L.S., Soares, C.N., Poitras, J.R., Prouty, J., Alexander, A.B., Shifren, J.L., 2003. Short-term use of estradiol for depression in perimenopausal and postmenopausal women: a preliminary report. *Am. J. Psychiatry* 160, 1519–1522.
- Estrada-Camarena, E., Fernandez-Guasti, A., Lopez-Rubiclava, C., 2003. Antidepressant-like effect of different estrogenic compounds in the forced swimming test. *Neuropsychopharmacology* 28, 830–838.
- Estrada-Camarena, E., Fernandez-Guasti, A., Lopez-Rubiclava, C., 2006. Participation of the 5-HT_{1A} receptor in the antidepressant-like effect of estrogens in the forced swimming test. *Neuropsychopharmacology* 31, 247–255.
- File, S.E., 2001. Factors controlling measures of anxiety and responses to novelty in the mouse. *Behav. Brain Res.* 125, 151–157.
- Freund, T.F., Katona, I., Piomelli, D., 2003. Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* 83, 1017–1066.
- Gobbi, G., Bambico, F.R., Mangieri, R., Bortolato, M., Campolongo, P., Solinas, M., Cassano, T., Morgese, M.G., Debonnel, G., Duranti, A., Tontini, A., Tarzia, G., Mor, M., Trezza, V., Goldberg, S.R., Cuomo, V., Piomelli, D., 2005. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proc. Natl. Acad. Sci. USA* 102, 18620–18625.
- Griebel, G., Stemmelin, J., Scatton, B., 2005. Effects of the cannabinoid CB₁ receptor antagonist rimonabant in models of emotional reactivity in rodents. *Biol. Psychiatry* 57, 261–267.
- Haller, J., Varga, B., Ledent, C., Barna, I., Freund, T.F., 2004. Context dependent effects of CB₁ cannabinoid gene disruption on anxiety-like and social behavior in mice. *Eur. J. Neurosci.* 19, 1906–1912.
- Hill, M.N., Gorzalka, B.B., 2004. Enhancement of anxiety-like responsiveness to the cannabinoid CB₁ receptor agonist HU-210 following chronic stress. *Eur. J. Pharmacol.* 499, 291–295.
- Hill, M.N., Gorzalka, B.B., 2005a. Pharmacological enhancement of cannabinoid CB₁ receptor activity elicits an antidepressant-like response in the rat forced swim test. *Eur. Neuropsychopharmacol.* 15, 593–599.
- Hill, M.N., Gorzalka, B.B., 2005b. Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? *Behav. Pharmacol.* 16, 333–352.
- Kathuria, S., Gaetani, S., Fegley, D., Valino, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., La Rana, G., Calignano, A., Giustino, A., Tattoli, M., Palmery, M., Cuomo, V., Piomelli, D., 2003. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.* 9, 76–81.
- Kessler, R.C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K.R., Rush, A.J., Walters, E.E., Wang, P.S., 2003. National Comorbidity Survey Replication. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *J. Am. Med. Assoc.* 289, 3095–3105.
- Lund, T.D., Rovis, T., Chung, W.C., Handa, R.J., 2005. Novel actions of estrogen receptor-beta on anxiety-related behaviors. *Endocrinology* 146, 797–807.
- Maccarrone, M., De Felici, M., Bari, M., Klinger, F., Siracusa, G., Finazzi-Agro, A., 2000. Down-regulation of anandamide hydrolase in mouse uterus by sex hormones. *Eur. J. Biochem.* 267, 2991–2997.
- Maccarrone, M., Bari, M., Battista, N., Finazzi-Agro, A., 2002. Estrogen stimulates arachidonylethanolamide release from human endothelial cells and platelet activation. *Blood* 100, 4040–4048.
- Nolen-Hoeksema, S., 1987. Sex differences in unipolar depression: evidence and theory. *Psychol. Bull.* 101, 259–282.
- Onaivi, E.S., Green, M.R., Martin, B.R., 1990. Pharmacological characterization of cannabinoids in the elevated plus maze. *J. Pharmacol. Exp. Ther.* 253, 1002–1009.
- Patel, S., Hillard, C.J., 2006. Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. *J. Pharmacol. Exp. Ther.* 318, 304–311.
- Patel, S., Cravatt, B.F., Hillard, C.J., 2005. Synergistic interactions between cannabinoids and environmental stress in the activation of the central amygdala. *Neuropsychopharmacology* 30, 497–507.
- Peroni, R.N., Orliac, M.L., Becu-Villalobos, D., Huidoboro-Toro, J.P., Adler-Graschinsky, E., Celuch, S.M., 2004. Sex-linked differences in the vasorelaxant effects of anandamide in vascular mesenteric beds: role of oestrogens. *Eur. J. Pharmacol.* 493, 151–160.
- Picazo, O., Estrada-Camarena, E., Hernandez-Aragon, A., 2006. Influence of the post-ovariectomy time frame on the experimental anxiety and the behavioural actions of some anxiolytic agents. *Eur. J. Pharmacol.* 530, 88–94.
- Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.* 229, 327–336.
- Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A., Howard, B.V., Johnson, K.C., Kotchen, J.M., Ockene, J., 2002. Writing Group for the Womens Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative Randomized Controlled Trial. *J. Am. Med. Assoc.* 288, 321–333.
- Schmidt, P.J., Nieman, L., Danaceau, M.A., Tobin, M.B., Roca, C.A., Murphy, J.H., Rubinow, D.R., 2000. Estrogen replacement in perimenopause-related depression: a preliminary report. *Am. J. Obstet. Gynecol.* 183, 414–420.
- Shearman, L.P., Rosko, K.M., Fleischer, R., Wang, J., Xu, S., Tong, X.S., Rocha, B.A., 2003. Antidepressant-like and anorectic effects of the cannabinoid CB₁ receptor inverse agonist AM251 in mice. *Behav. Pharmacol.* 14, 573–582.
- Soares, C.N., Almeida, O.P., Joffe, H., Cohen, L.S., 2001. Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: a double-blind, randomized, placebo-controlled trial. *Arch. Gen. Psychiatry* 58, 529–534.
- Tzavara, E.T., Davis, R.J., Perry, K.W., Li, X., Salhoff, C., Bymaster, F.P., Witkin, J.M., Nomikos, G.G., 2003. The CB₁ receptor antagonist SR141716A selectively increases monoaminergic neurotransmission in the medial prefrontal cortex: implications for therapeutic actions. *Br. J. Pharmacol.* 138, 544–553.
- Viveros, M.P., Marco, E.M., File, S.E., 2005. Endocannabinoid system and stress and anxiety responses. *Pharmacol. Biochem. Behav.* 81, 331–342.
- Waleh, N.S., Cravatt, B.F., Apte-Deshpande, A., Terao, A., Kilduff, T.S., 2002. Transcriptional regulation of the mouse fatty acid amide hydrolase gene. *Gene* 291, 203–210.
- Walf, A.A., Frye, C.A., 2005a. Antianxiety and antidepressive behavior produced by physiological estradiol regimen may be modulated by hypothalamic-pituitary-adrenal axis activity. *Neuropsychopharmacology* 30, 1288–1301.
- Walf, A.A., Frye, C.A., 2005b. ERbeta-selective estrogen receptor modulators produce antianxiety behavior when administered systemically to ovariectomized rats. *Neuropsychopharmacology* 30, 1598–1609.
- Walf, A.A., Rhodes, M.E., Frye, C.A., 2004. Antidepressant effects of ERbeta-selective estrogen receptor modulators in the forced swim test. *Pharmacol. Biochem. Behav.* 78, 523–529.
- Weissman, M.M., Klerman, G.L., 1977. Sex differences and the epidemiology of depression. *Arch. Gen. Psychiatry* 34, 98–111.
- Wittchen, H.U., Hoyer, J., 2001. Generalized anxiety disorder: nature and course. *J. Clin. Psychiatry* 62 (Suppl. 11), 15–19.