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Influence of acute or chronic administration of ovarian hormones on the effects of desipramine in the forced swim test in female rats

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Abstract

Rationale—Gender may influence antidepressant (AD) treatment outcome. In order to address this pre-clinically, the potential effects of ovarian hormones on AD treatment in ovariectomized female rats were investigated.

Objectives—In the first study, the effect of acute administration of estrogen and progesterone on the antidepressant-like effects of desipramine (DMI), a selective norepinephrine reuptake inhibitor (SNRI), was investigated in the forced swimming test (FST). In the second study, the effect of chronic administration of these hormones on the effects of chronically administered DMI was investigated.

Results—In the acute study, the hormones blocked the effects of DMI in the FST as demonstrated by the absence of either a reduction in immobility or an increase in climbing behavior in animals treated with DMI in combination with the hormones. Concentration-response experiments on hippocampal synaptosomes revealed no changes in the Km or Bmax for uptake of ³H-NE in hormone-treated rats. In the chronic study, the antidepressant-like effects of DMI in the FST were not blocked by chronic administration of hormones. Interestingly, the hormones affected the serum concentrations of DMI. These levels were significantly higher in animals receiving 10 or 15 mg/kg/day in hormone-treated rats as compared to those with placebo.

Conclusions—Acute administration of hormones blocked the effects of DMI (given three times over 24 h) in the FST. However, chronic administration of these hormones failed to block the effects of chronically administered DMI (at a dose that produces clinically relevant serum concentrations).

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Conflict of Interest

Ms. Shah has no financial disclosures to report. Dr. A Frazer has served on advisory boards for Lundbeck and for Takeda Pharmaceuticals International, Inc and Eli Lilly and Co. He has also been a consultant for Dey Pharmaceuticals. No support for this study was received from any pharmaceutical company.

Keywords

depression; estradiol; progesterone; antidepressant; behavior; noradrenergic; desipramine; forced swim test

Introduction

Gender differences in the prevalence of major depressive disorder is well established (Grigoriadis and Robinson 2007) with a ratio of 1.7 for women versus men (Marcus et al. 2005). The 12-month and lifetime prevalence rates for women are about 7% and 17%, respectively, as compared to 3.5% and 9% for men (Hasin et al. 2005). This predominance in women is at least partly due to gonadal hormones. Further, fluctuations in levels rather than absolute levels of these hormones seem more likely to be linked with the onset of symptoms of depression (Soares and Frey 2010).

Preclinical data show antidepressant (AD)-like effects of β -estradiol (E2) and some estrogenic compounds when given either acutely (Estrada-Camarena et al. 2003; Walf et al. 2004) or chronically (Okada et al. 1997; Rachman et al. 1998). Although estrogen may be useful in helping with depression around the perimenopause (see Grigoriadis and Robinson 2007; Soares and Frey 2010), there is no consistent body of data showing its efficacy in postmenopausal depressed patients, either as a stand-alone therapy or as adjunct therapy with selective serotonin reuptake inhibitors (SSRIs) (Huttner and Shepherd 2003; Schneider et al. 2001; Yalamanchili and Gallagher 2012). This is of some interest as estrogen has been shown to modulate neurotransmitter systems, including serotonergic and noradrenergic systems that are involved in mood regulation (McEwen and Alves 1999; Rubinow et al. 1998).

Previously, we have shown that systemic administration of estradiol benzoate (EB) alone in ovariectomized (OVX) rats blocked the function of the serotonin transporter (SERT); however, progesterone (P) did not have this effect. Acute systemic administration of EB and/or P (at doses so as to achieve proestrus serum levels) blocked the ability of SSRIs to slow the clearance of exogenous serotonin (5-HT) from the hippocampus, indicating that they inhibited the ability of SSRIs to block the SERT, a phenomenon also seen specifically during proestrus in naturally cycling female rats (Benmansour et al. 2009). Whereas the AD-like effects of estrogen and P have been demonstrated in the forced swim test (FST) in rats (Estrada-Camarena et al. 2003; Molina-Hernandez and Tellez-Alcantara 2001), the impact of these ovarian hormones on the effects of ADs in the FST has not been investigated in depth. Our group recently found that EB and/or P blocked the decrease in immobility and increase in swimming caused by the SSRI, fluvoxamine (Benmansour et al. 2012).

In light of these findings, in the first part of this study we investigated the effects of acute systemic administration of ovarian steroids, administered using a paradigm to achieve serum concentrations seen during proestrus, on the AD-like effects of desipramine (DMI), a selective norepinephrine reuptake inhibitor (selective NRI), in the FST. In addition, the effect of the hormone treatment on the ability of DMI to block the uptake of ^3H -NE into hippocampal synaptosomes was measured. In the second part, we examined the effects of

chronic systemic administration of these steroids on responses elicited by chronic treatment with DMI. Serum levels of DMI were measured to see if any hormonal effects could be due to changes in the pharmacokinetics of the drug. Further, serum levels of hormones were also measured to verify if we achieved physiological, proestrus serum levels.

Methods

Animals

OVX female rats (Sprague Dawley, 250–350 g) (Harlan, Houston, TX) were housed on a 14:10 h light/dark cycle with lights on at 0700 h. Food and water were provided *ad libitum*. All procedures were carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the local Institutional Care and Use Committee. Behavioral testing was carried out 4–5 weeks after ovariectomy.

Administration of ovarian hormones

Acute study—This treatment paradigm was designed to achieve proestrus levels of hormones at the time of testing, as described by us previously (Benmansour et al. 2009) and originally by Maswood et al. (1999). Estradiol was administered subcutaneously in its esterified form, estradiol benzoate (EB, 25 µg in 100 µl peanut oil; Sigma-Aldrich, St Louis, MO), to OVX rats, followed 48 h later by subcutaneous injection of progesterone (P, 500 µg in 100 µl peanut oil; Sigma-Aldrich). The behavioral experiments were carried out 24 h after administration of P, at which time the hormone levels in serum were measured. This protocol generates peak levels of estrogen and progesterone that are seen during proestrus. Control OVX rats received peanut oil injections.

Chronic study—The hormones were administered in the form of pellets containing 17β-estradiol (E2) and P (0.06 mg and 10 mg/pellet respectively, 21-day release; Innovative Research of America, Sarasota, FL). These were surgically implanted subcutaneously in anesthetized animals under aseptic conditions.

Administration of drugs

Acute study—Desipramine hydrochloride (DMI, 10 mg/kg; Sigma-Aldrich) or saline was administered IP, 23.5, 5 and 1 h before testing the animals. Control OVX animals were injected with physiological saline.

Chronic study—DMI (7.5, 10 or 15 mg/kg/day, IP) or vehicle (distilled water) was delivered via osmotic minipumps as described by Furmaga et al. (Furmaga et al. 2011). Drug solution concentrations were determined based on the mean rat weight over the duration of treatment. One day prior to surgery, osmotic minipumps delivering 2.5 µl/h (Model 2ML4, DURECT Corp., Cupertino, CA) were filled with drug or vehicle, filtered through 0.9-µm nitrocellulose filters (Millipore, Bedford, MA) using a sterile technique. Minipumps were stored in sealed containers filled with sterile saline at room temperature to prime the pumps until the time of surgery. At the time of surgery, rats were anesthetized with an intramuscular injection of a cocktail of 75 mg/kg ketamine and 0.5 mg/kg medetomidine. Drug-filled minipumps were implanted (IP) via a midline incision made on

the lower abdomen. After pump implantation, the muscle and skin were sutured. Rats were injected with penicillin and saline immediately post-op and with atipamezole (1 mg/kg; Antisedan, Pfizer) to reverse the sedative effects of the anesthetic.

The treatment paradigms for acute and chronic studies are shown schematically in Figure 1.

Forced swimming test (FST)

This test was performed essentially as described by Detke et al. (Detke et al. 1995) except the 15-min training session (for acute studies) was carried out 75 h before the 5-min test session, instead of the usual 24 h. The training session for the FST was carried out 75 h prior to testing, as previously described (Benmansour et al. 2012), in order to train all animals in the absence of hormones or drug. There was no training session for chronic studies as the effects of chronic AD treatment in the FST are observed even in the absence of the training session, as shown by others (Cryan et al. 2005) and by us (Furmaga et al. 2011). Briefly, rats were placed into a cylindrical tank (21 × 46 cm) filled with water (23–25°C) at a depth of 37 cm. The behavior of the rats was recorded and scored by a trained rater, who was blind to the treatment conditions, using a time-sampling technique to rate the predominant behavior (climbing, swimming, immobility) over 5 s intervals (bins) over the 5-min testing period. Climbing behavior consists of upward forepaw movement along the sides of the chamber. Swimming behavior is defined as horizontal movement in the tank whereas immobility is the lack of any activity other than that needed to keep the rat's head above the surface of the water.

Locomotor activity test

Rats were individually placed into a novel Plexiglas open field (50 cm × 50 cm × 40 cm). Total distance traveled by the animals was monitored and recorded by automated video tracking software, ANY-Maze (San Diego Instruments, California, U.S.A.). Data for the first 10 min are presented.

Detection of E2 levels in serum

Trunk blood was collected from the animals immediately after behavioral testing and processed to obtain serum. E2 levels in serum were detected by radioimmunoassay (RIA) of duplicate samples using the DSL ultrasensitive estradiol kit (DSL-4800; Beckman Coulter Inc., Brea, CA) as per the manufacturer's protocol.

Detection of DMI levels in serum

Serum concentrations of DMI were determined by HPLC with a Waters spherisorb S5 CN column and UV detection at 214 nm as described by Benmansour et al. (Benmansour et al. 1999).

Synaptosomal preparation and uptake assays

Animals were sacrificed by rapid decapitation and the hippocampi were dissected on ice and homogenized manually in ice-cold homogenization buffer (0.32M sucrose, 4.2mM HEPES, pH 7.4) using a Teflon-glass homogenizer. After centrifugation of the homogenate at 1,500

rpm for 15 min at 4°C, the supernatant fluid was again centrifuged at 10,000 g for 15 min. The final pellet was gently resuspended in 1 ml of Krebs-Ringer/HEPES (KRH) buffer (120 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 10 mM HEPES, 2 mM CaCl₂, 1.2 mM MgSO₄, 10 mM glucose, 100 µM ascorbic acid and 100 µM paragyline, pH 7.4) and the protein content quantified using the Bradford Assay (Sigma-Aldrich, St Louis, MO). Saturation uptake assays were performed by incubating synaptosomal preparations (60 µg/150 µl, in duplicate) with ³H-NE (5.5 nM–1.35 µM, 14.8 Ci/mmol, norepinephrine hydrochloride, DL-[7-³H(N)], PerkinElmer, Boston, MA) for 10 min at 37°C. The reaction was stopped by placing the tubes on ice and samples collected by filtration onto Whatman GF/B filter papers (FP-100, Brandel Inc.), pre-soaked in 0.3% polyetheneimine, using a cell harvester (Brandel, MD). ³H-NE was quantified by a liquid scintillation counter. Non-specific uptake was determined in the presence of 100 µM DMI and subtracted from the total uptake so as to determine specific uptake. For competition uptake assays, samples were incubated in duplicate with 0.15 µM ³H-NE and nonradioactive DMI (7.62 pM – 1350 nM, Sigma-Aldrich).

Statistical Analysis

For all experiments except the uptake assays, two-way analyses of variance were carried out (with hormone treatment as one factor and drug as the other) followed by Duncan's *post hoc* tests. Only a p-value < 0.05 was considered significant. For the uptake assays, an unpaired t-test with Welch's correction was used to compare K_m and B_{max} values between groups. For analyzing the relationship between serum DMI levels and immobility or climbing values in the chronic FST, Spearman's correlation coefficients were calculated.

Results

Acute study

Effect of acute ovarian hormone treatment on the AD-like effects of DMI in the FST

For this study, hormones and drug were administered as shown in Figure 1(a). Previously, we reported that this paradigm leads to mean (±SEM) serum levels of estradiol (52.7 ± 14.21 pg/ml) and progesterone (11.2 ± 1.91 ng/ml) (Benmansour et al. 2009). DMI levels in serum were also measured in samples collected immediately after the FST in this study and were 1638 ± 352 ng/ml.

Results in the FST are shown in Figure 2. The hormone treatment reduced the ability of DMI to cause either a decrease in immobility or an increase in climbing in the FST. For immobility, two-way analysis of variance revealed a significant main effect of DMI (F(1,37) = 6.848, p = 0.01). Duncan's post hoc analysis showed a significant decrease in immobility with DMI (p = 0.01) only in the animals treated with peanut oil and not in those treated with EB plus P. For climbing behavior, two-way analysis of variance revealed a significant main effect of hormone (F(1,37) = 6.96, p = 0.01), DMI (F(1,37) = 12.84, p < 0.001) as well as a hormone-drug interaction (F(1,37) = 4.63, p = 0.04). Duncan's post hoc analysis showed a significant increase in climbing with DMI (p < 0.001) only in the animals treated with placebo (peanut oil) and not those treated with the hormones. Swimming behavior was not altered by either hormone or drug treatment (data not shown).

Effect of acute ovarian hormone treatment alone or in combination with DMI on locomotor activity in an open field—

Locomotor activity in an open field was tested in a different cohort of animals in order to control for potential confounding effects of the ovarian hormones alone or in combination with DMI treatment. The hormone and drug treatment paradigms were identical to those shown in Figure 1(a). The values for distance moved (Mean $\pm$ SEM) during a 10-min period for the 4 groups are as follows: Placebo + Saline (30.7 ± 6.0), Placebo + DMI (15.6 ± 7.7), Hormone + Saline (26.4 ± 2.2), Hormone + DMI (16.0 ± 6.2). DMI reduced locomotor activity in OVX rats whether or not they were given hormone treatment. Two-way analysis revealed a significant main effect of DMI (10 mg/kg, IP, given 3 times over 24 h) on locomotor activity ($F(1, 12)=18.86$, $p < 0.001$). Duncan's post hoc analysis showed that DMI significantly reduced locomotor activity ($p < 0.05$) in both the placebo- and hormone- treated groups. The ovarian hormones themselves had no effect on locomotor activity. As we do not observe an increase in immobility in the FST with DMI, the DMI-induced *reduction* of locomotor activity in the open field does not appear to be a confounding factor for the ability of the hormone treatment to block its effects in the FST.

Effect of hormone treatment on ^3H -NE uptake in hippocampal synaptosomes

—Concentration-response experiments were carried out using synaptosomes from hippocampal homogenates to see if the hormone treatment itself altered the uptake of ^3H -NE. Mean ($\pm$ SEM) K_m values for samples from hormone-treated animals ($0.40 \pm 0.18 \mu\text{M}$) were not significantly different from values for samples from peanut oil-treated animals ($0.15 \pm 0.03 \mu\text{M}$). Similarly, no changes were found in the B_{max} for ^3H -NE between the hormone treated rats ($72.3 \pm 3.95 \text{ pM } ^3\text{H-NE}/\mu\text{g protein}$) and peanut oil treated controls ($61.9 \pm 10.56 \text{ pM } ^3\text{H-NE}/\mu\text{g protein}$). Also, preliminary competition experiments with DMI ($7.62 \text{ pM} - 1350 \text{ nM}$) revealed that there was no change in its affinity to block NE uptake in hormone-treated rats, in comparison with the values in control rats (data not shown).

Chronic study

Hormones were administered *via* subcutaneous pellets containing E2 and P (21-day release). The dose of P (10 mg/pellet) was determined based on a preliminary study where serum levels of P as tested by RIA were $28.0 \pm 4.64 \text{ ng/ml}$ on day 3 and $23.5 \pm 3.96 \text{ ng/ml}$ on day 8 suggesting maintenance of a constant serum level higher than baseline values. For example, in rats, P levels show two peaks of approximately 24 ng/ml during diestrus and 46 ng/ml during proestrus with intervening levels of 2–7 ng/ml (Butcher et al. 1974). A preliminary experiment was carried out to determine the appropriate dose of E2. OVX animals were subcutaneously implanted with pellets containing one of three different doses of E2 (0.06, 0.125 or 0.25 mg/pellet) and 10 mg of P. Serum E2 levels were measured on day 18 by RIA and are shown in Table 1. Based on these data, 0.06 mg E2/pellet was selected for further studies as this dose achieved levels around the peak levels seen during the estrus cycle, 50–90 pg/ml (Butcher et al. 1974; Gibbs 1996). DMI (7.5, 10 or 15 mg/kg/day) was delivered via osmotic pumps that were surgically implanted intraperitoneally on the same day as the hormone pellet implantation. The FST was performed on day 15 and the test for locomotor activity on day 18-post surgery (Figure 1b).

Effect of chronic ovarian hormone treatment on the AD-like effects of chronic administration of DMI

Results for behavior in the FST are shown in Figure 3. Two-way analysis of variance revealed a significant main effect of hormone ($F(1,62) = 5.04$, $p = 0.03$) and of DMI ($F(2,62) = 15.65$, $p < 0.001$) on immobility. However, there was no significant interaction between hormone and drug treatment ($p = 0.1$). Duncan's post hoc analysis revealed a significant reduction in immobility ($p < 0.005$) with DMI (10 mg/kg/day) in the placebo as well as in the hormone treated groups. Interestingly, there was a significant reduction in immobility with DMI at a dose of 7.5 mg/kg/day in the hormone-treated group ($p = 0.003$) but not in the placebo group. Further, the hormone treatment alone increased immobility in comparison with that measured in the vehicle/placebo group ($p < 0.005$). For climbing behavior, two-way analysis of variance showed a significant main effect of hormone ($F(1,62) = 8.12$, $p = 0.01$) and of DMI ($F(2,62) = 25.05$, $p < 0.001$) and no significant interaction between hormone and drug treatment ($p = 0.08$). Duncan's post hoc analysis shows a significant increase ($p < 0.02$) in climbing behavior with DMI (7.5 and 10 mg/kg/day) in both placebo-treated as well as hormone-treated groups. Swimming behavior was not altered by either hormone or drug treatment (data not shown).

DMI levels in serum were determined for all groups (Figure 4). The administration of ovarian hormones influenced the level of DMI in serum. Levels of DMI achieved for doses of 10 mg/kg/day were significantly higher ($p < 0.001$) in animals that received the hormones as compared to those that did not. Consistent with this observation, in a follow-up experiment, 15 mg/kg/day also produced significantly higher levels of DMI in serum ($p < 0.02$) in animals treated with ovarian hormones (1468 ± 207.1 ng/ml) as compared to those treated with placebo pellets (863 ± 93.6 ng/ml).

Effect of chronic ovarian hormone treatment alone or in combination with DMI on locomotor activity in an open field

Locomotor activity in an open field was tested in the same cohort of animals as used for the FST, on day 18, in order to control for potential confounding effects on motoric activity of the ovarian hormones alone or in combination with AD treatment. Results of the locomotor activity during the first ten minutes are shown in Figure 5. Two-way analysis of variance showed a significant main effect of DMI on locomotor activity ($F(2,63) = 29.96$, $p < 0.001$). Duncan's post hoc analysis showed that both doses of DMI significantly reduced locomotor activity ($p < 0.05$) in both placebo and hormone treated groups. The ovarian hormones themselves had no effect on locomotor activity. As we do not observe an increase in immobility, the reduction of locomotor activity in the open field with DMI is not a confounding factor.

Discussion

There are three key findings in these studies. First, acute administration of DMI had a significantly reduced effect in the FST (on both immobility and climbing) in OVX rats treated with EB and P (Figure 2). Second, in contrast to our acute study, chronic administration of DMI still had significant antidepressant-like effects in the FST even in the presence of the ovarian hormones (Figure 3). Third, chronic administration of these hormones affected the steady state serum levels of chronically administered DMI (Figure 4).

There have been several studies looking at the antidepressant-like effects of ovarian hormones and estrogenic compounds. For instance, E2 (5 or 10 µg/rat, for 48 h) decreased immobility and increased swimming behavior in OVX rats in the FST (Estrada-Camarena et al. 2003; Walf et al. 2004). This effect was also seen with chronic administration of E2 (Okada et al. 1997; Rachman et al. 1998) or with chronic administration of P (2.0 mg/kg, daily for 2 weeks) (Molina-Hernandez and Tellez-Alcantara 2001). In our present study, estrogen (as EB or E2) and P were administered in combination. Consistent with our previous report (Benmansour et al. 2012), acute administration of these hormones did not produce an antidepressant-like effect. Our group has previously reported using chronoamperometry to measure the rate of clearance of exogenous serotonin from the CA3 region of the hippocampus, that progesterone blocked the antidepressant-like effect of EB (Benmansour et al. 2009). This may account for the lack of an acute antidepressant-like effect in the FST with the hormone combination.

Chronic administration of E2 and P did not produce antidepressant-like effects either. In fact, this treatment paradigm produced a modest pro-depressive-like effect with a small but significant increase in immobility in the FST. No changes in climbing (or swimming) were seen that would be consistent with a pro-depressive effect.

Few preclinical studies have looked at the ability of ovarian hormones to modify effects produced by ADs. Some groups have used an interesting strategy of combining estrogen with either a noradrenergic or serotonergic AD, with both treatments being used at doses that have no effect in the FST separately. These combination treatments produced antidepressant-like effects in OVX rats (Estrada-Camarena et al. 2004; Recamier-Carballo et al. 2012) as well as in orchietomized male rats (Martinez-Mota et al. 2008). Similarly, the antidepressant-like effect of chronic DMI in OVX animals was seen earlier in the group treated with a single E2 injection than in animals treated with vehicle (Estrada-Camarena et al. 2008). Further, ineffective doses of EB and/or P worked synergistically with sub-optimal doses of DMI to produce anxiolytic-like effects in the shock probe-burying test (Martinez-Mota et al. 2000). These reports highlight an interaction between ovarian hormones and ADs that act through the noradrenergic system.

However, the ability of the female sex steroids to influence the effect of a noradrenergic drug such as DMI might not only be due to pharmacodynamic considerations but pharmacokinetic ones as well. As shown in this report, after chronic administration of DMI, steady state levels of the drug were considerably higher in the OVX rats receiving hormone administration, especially at the higher doses of DMI, than in vehicle-treated rats. In a study carried out to determine sex differences in AD responses, Simpson et al. (2012) measured immobility in the FST in rats given DMI (three times: 24, 5 and 1 h before testing) at 3 different doses (2.5, 5 and 10 mg/kg) and showed a significant main effect of DMI in the male rats but not in the females. Specifically, DMI at 5 mg/kg significantly reduced immobility in the males; however, none of the three doses altered immobility in the females. Whereas the stages of the estrous cycle were not determined in this study, the lack of an effect of DMI in females is consistent with the results of our acute study where DMI has no effect in the OVX females that were treated with hormones. Chronic DMI treatment has been shown to have anxiolytic-like effects in several tests (Bodnoff et al. 1989; Fontana et

al. 1989; Furmaga et al. 2011). In one such test, the elevated plus maze, chronic DMI treatment produced anxiolytic-like effects in OVX females but had no effect in cycling female rats (Fernandez-Guasti et al. 1999).

Although, as just mentioned, there have been a few studies of the impact of ovarian steroids on effects produced by drugs acting through noradrenergic neurons, there have been many studies on the impact of ovarian hormones on the noradrenergic system (McEwen and Alves 1999). Relevant to our study, some have examined the effects of ovarian hormones on the uptake and release of NE. E2 (10^{-7}M - 10^{-5}M) and P (10^{-4}M), when added independently to isolated rat synaptosomes prepared from whole brains of intact female rats, significantly inhibited ^3H -NE uptake. Both steroid hormones also increased the cumulative passive efflux of ^3H -NE from synaptosomes (Janowsky and Davis 1970). Ghraf et al. (1983), showed that E2 (10^{-6}M - 10^{-5}M) inhibited ^3H -NE uptake; however, P (10^{-5}M) failed to do so. We did not see these effects of ovarian hormones in our uptake assays. This apparent discrepancy may be because unlike in our present study, the hormones used in these studies were added independently, *ex vivo* and at high concentrations that may not represent physiological conditions. Further, these experiments were carried out on whole brain tissue from intact animals whereas our study was carried out using hippocampal tissue from OVX animals. The hippocampus was selected for our study as this region has been associated with the pathophysiology of depression (Campbell and Macqueen 2004). The noradrenergic neurons project to the hippocampus and selective NRIs are known to work by increasing norepinephrine in this region (Mongeau et al. 1997). In addition, this region is ideal for studying interactions with estrogen as it contains estrogen receptors (ER) in rats (Weiland et al. 1997).

An effect of ovarian hormones on serum levels of DMI in OVX females by exogenous co-administration of E2 and P was found (Figure 4). This observation is consistent with reports of gender differences in the pharmacokinetics of drugs in humans (reviewed in Bigos et al. 2009). Moody et al. (Moody et al. 1967) have demonstrated higher plasma levels of imipramine and DMI in women than in men. Similar observations were made by Hammer and Sjoqvist (1967) with nortriptyline. Abernethy et al. (1984) have shown that chronic use of oral contraceptives decreased the metabolism of imipramine, thereby increasing its half-life. According to their report, to compensate for this effect of contraceptives on metabolism of imipramine, women on these contraceptives would have to reduce their drug dose to about two-thirds that used for women that are not on contraceptives.

This gender difference in drug metabolism, specifically of DMI, has been demonstrated in rats using ^3H -DMI. Intraperitoneal injection of ^3H -DMI (from $1.7\text{ }\mu\text{g/kg}$ to 20 mg/kg) led to similar patterns of increases in levels in brain and liver in both sexes. However, the absolute amount of DMI in the brain was 2 to 4-fold greater in female as compared to male rats (Biegon and Samuel 1979).

These differences arise due to the influence of gender-specific features on absorption, distribution, metabolism and elimination of drugs. AD drugs are metabolized by cytochrome P450 (CYP) enzymes. DMI is metabolized predominantly by CYP2D6 and to a lesser extent by CYP1A2 (Wishart et al. DrugBank (online)). Estrogen is a substrate for CYP1A2 and can

also inhibit it, thus altering plasma levels of drugs such as DMI that is also a substrate for this enzyme (Bigos et al. 2009). A number of the CYP enzymes including CYP2D6 have been reported to have higher activity in men than in women (Tanaka 1999).

In our study, the serum levels of DMI at the lower dose (7.5 mg/kg/day) were not significantly different between placebo- and hormone-treated animals whereas serum DMI levels at the higher doses (10 and 15 mg/kg/day) were significantly higher in the groups simultaneously receiving hormones. We speculate that this may result from there still being sufficient metabolic capacity for the lower serum concentration of DMI achieved by the low dose even in the presence of the hormones. However, as the serum concentration rises, the effect of the hormones on the metabolic capacity becomes evident.

In our acute study, ovarian hormones were administered using a paradigm that produced proestrus levels at the time of testing whereas DMI (10 mg/kg) was administered as done traditionally, 3 times over a 24 h period (Detke et al. 1995; Porsolt et al. 1977). From a clinical perspective, with this dose of DMI we are already at supra-maximal serum levels and yet blockade of the effect of DMI is still observed. Therefore, results of the acute study indicate a reduced efficacy of DMI in the FST in the presence of ovarian hormones. This phenomenon seems consistent with the diminished SSRI efficacy reported in a previous study from our group (Benmansour et al. 2012) with an identical hormone administration paradigm. In that study, the effect of estrogen on SSRI function was found to be dependent on the ER subtype, with ER α mediating its inhibitory effect on the ability of an SSRI to inhibit the SERT. We can speculate that the same may hold true for DMI; further investigation is required to support this theory. Another possible explanation is the surface redistribution or internalization of the norepinephrine transporter by ovarian hormones. Biotinylation studies would be needed to assess this and such studies with transporters have not been used widely with synaptosomes (Samuvel et al. 2005). However, our results for ^3H -NE-uptake assays do not reveal any effect of the ovarian hormones on the K_m or B_{max} of uptake or on the affinity to DMI to inhibit uptake as potential reasons to explain the observed behavioral phenomenon.

Locomotor activity was monitored to assess the effects of DMI, in the presence or absence of hormone treatment, on ambulation. DMI reduced locomotor activity as expected (Wongwitdech et al. 2006); however, this effect was independent of hormone treatment. It is interesting to note the lack of interaction between hormone and DMI in locomotor activity, as it implies that the significant interaction we see in the FST (acute study), does not necessarily extrapolate to other behaviors involving other systems in the brain.

In our studies, the behavioral tests were carried out approximately 4 – 5 weeks post-OVX surgery. This time period post-OVX is within the appropriate ‘window’ based on the following studies. Barker et al. (Barker and Galea 2009) have shown that there is a decline in E2 levels in the hippocampus 3 weeks post-OVX. In another report, all three doses of DMI tested were effective in reducing immobility in the FST at 3 weeks and 12 weeks but not at 1 week post-OVX (Estrada-Camarena et al. 2011). However, after long-term OVX (12–20 weeks after OVX) animals are not responsive to either acute or chronic E2 treatment in the FST (Estrada-Camarena et al. 2011; Walf et al. 2009).

In our chronic study, hormone pellets containing E2 and P were administered to OVX animals to produce proestrus phase levels of these hormones. The results of this study indicate that ovarian hormones do not interfere with the antidepressant-like effect of chronically administered DMI. In both the acute and chronic study, there is no association between the serum DMI levels and magnitude of its effects in the FST. For instance, in rats treated with 7.5 mg/kg/day DMI without hormones, there was no significant correlation between serum levels of drug and values either for immobility ($r = -0.257$; $p = 0.658$) or climbing ($r = 0.429$; $p = 0.419$) and this was so for 10 mg/kg/day DMI without hormones also, (immobility, $r = 0.293$; $p = 0.34$; climbing $r = -0.06$; $p = 0.834$). No significant correlations were seen between drug levels and behavioral effects in the presence of hormones either. Similarly, this lack of relationship between behavioral effect of DMI in the FST and levels of DMI in brain has been previously reported by Mancinelli et al. (1987). In their study, the animals were given either a single injection of DMI (at 20 or 40 mg/kg), the standard acute 3 injection protocol (20 mg/kg) or 7 days of daily injections of DMI and effects in the FST as well as brain concentrations of DMI were measured. No relationship was found between these two parameters (i.e. positive effects in the FST were seen when brain concentrations of DMI were either equivalent or lower than brain concentrations when an effect was absent).

Limitations when comparing the results of the acute and chronic studies are that the forms of estrogen administered were different (EB for acute vs. E2 for chronic). Also, there was no training session for the chronic study and this factor along with the absence of repeated injections could have caused different levels of stress in the animals during the two studies.

Taken together, these studies also highlight the importance of treatment duration when investigating interaction between the effects of treatments (e.g. hormones, non-AD drugs, etc.) and AD drugs in the FST.

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a) Acute study paradigm b) Chronic study paradigm **Fig. 1.**

Experimental paradigms. Acute treatment paradigm showing time-points at which estradiol benzoate (EB, 25 µg in 100 µl peanut oil; SC), and progesterone (P, 500 µg in 100 µl peanut oil; SC) were administered to OVX rats, 72 and 24 h prior to testing. DMI (10 mg/kg, IP) was injected 3 times. The training session was carried out 75 h prior to testing in the forced swimming test (a). Chronic treatment paradigm showing time-points at which pellets containing E2 and P (0.06 mg and 10 mg/pellet respectively, 21-day release, SC) and osmotic minipumps containing DMI (7.5, 10 or 15 mg/kg/day, IP) were implanted into OVX rats on day 0. Forced swimming test (FST) was carried out on day 15 and locomotor activity on day 18 at the end of which animals were sacrificed for collection of blood samples for further analysis (b)

Fig. 2.

Effect of acute hormone treatment on DMI-induced behaviors in the forced swimming test. OVX rats were treated with hormones (EB plus P) or placebo (peanut oil) 72 and 24 h before testing. DMI (10 mg/kg, IP) or saline was given 3 times (23.5, 5 and 1 h prior to testing). Data are represented as mean of counts $\pm$ SEM of immobility (a) and climbing (b) behavior registered for every 5-s bin during the 5 min test period. * $p < 0.01$, Duncan's post hoc comparison of the effect of DMI as compared to the relevant control value for each behavior, $n = 9-12$ animals/group

Fig. 3.

Effect of chronic hormone treatment on DMI-induced behaviors in the forced swimming test. OVX rats were treated with hormone (E2 plus P) or placebo pellets for 15 days prior to testing. DMI (7.5 or 10 mg/kg/day, IP) or vehicle (water) was administered via osmotic minipumps. Data are represented as mean of counts $\pm$ SEM of immobility (a) and climbing (b) behavior registered for every 5-s bin during the 5 min test period. * $p < 0.02$, Duncan's post hoc comparison of the effect of DMI as compared to the relevant control value for each behavior. $n = 6-20$ animals/group. # $p < 0.005$, Duncan's post hoc comparison of the effect of E2 plus P as compared to placebo in the vehicle treated groups

Fig. 4.

Influence of chronic E2 (0.06 mg/pellet, 21-day release, SC) plus P (10 mg/pellet, 21-day release, SC) on serum DMI levels achieved with osmotic delivery of the drug (7.5 and 10 mg/kg/day, IP). DMI levels reported are those for samples collected on day 18, processed and analyzed by HPLC. Two-way analysis of variance showed a significant main effect of hormone treatment ($F(1, 33) = 8.66, p < 0.01$), of dose of DMI ($F(1, 33) = 16.88, p < 0.001$) and a significant interaction between hormone treatment and dose of DMI ($F(1,33) = 15.7, p < 0.001$). Duncan's post hoc analysis showed a significant increase in serum DMI level between 7.5 and 10 mg/kg/day in the hormone treated group ($\#p < 0.001$). There was a significant difference in serum DMI levels achieved by 10 mg/kg/day in placebo group versus levels achieved by the same doses in the hormone treated group ($*p < 0.001$).

Fig. 5.

Effect of chronic ovarian hormone treatment alone or in combination with DMI on locomotor activity during the first 10 min in an open field. Hormones (E2 plus P) or placebo pellets as well as osmotic pumps containing DMI (7.5 or 10 mg/kg/day) or vehicle were implanted 18 days prior to testing locomotor activity. Distance moved by each animal was recorded by the ANYMaze tracking software. Both doses of DMI significantly reduced locomotor activity as compared to vehicle in placebo and hormone treated groups. The hormones themselves had no effect on locomotor activity. * $p < 0.05$, Duncan's post hoc test. $n = 6-20$ animals/group

Table 1

Serum E2 levels achieved on day 18, post surgical implantation of 21-day release pellets containing one of three doses of E2 (0.06, 0.125 or 0.25 mg/pellet or placebo) and P (10 mg/pellet). Serum levels were measured using RIA and are reported as mean values $\pm$ standard error

Amount of E2/pellet	Serum E2 level on day 15 (pg/ml)
Placebo (0 mg)	4.0 $\pm$ 0.46
0.06 mg	54.3 $\pm$ 6.03
0.125 mg	107.9 $\pm$ 7.88
0.25 mg	322.3 $\pm$ 20.39