Estrogen and Progesterone Prevent Disruption of Prepulse Inhibition by the Serotonin-1A Receptor Agonist 8-Hydroxy-2-dipropylaminotetralin

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ABSTRACT

The aim of the present study was to investigate the effect of estrogen and progesterone treatment on 5-hydroxytryptamine (serotonin)-1A (5-HT₁A) receptor-mediated disruption of prepulse inhibition (PPI) of acoustic startle. The age-at-onset of schizophrenia is later in women than men, and it has been suggested that women may be protected from schizophrenia by the sex steroid hormone estrogen. 5-HT₁A receptors have been implicated in the development of schizophrenia and the action of antipsychotics. PPI is a model of sensorimotor gating that is deficient in schizophrenia and other illnesses. Female Sprague-Dawley rats were ovariectomized (OVX) or sham-operated. Some OVX rats received silastic implants filled with a low dose of estrogen (E20), a high dose of estrogen (E100), progesterone (P), or both the E20- and P-filled (E/P) silastic implants. Two weeks later, the rats were randomly treated with saline, or 0.02 or 0.5 mg/kg of the 5-HT₁A receptor agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT). Treatment with 8-OH-DPAT resulted in a dose-dependent increase in startle amplitude in all rat groups. PPI was significantly reduced after injection of 0.5 mg/kg 8-OH-DPAT in sham-operated rats, untreated OVX rats, E20-treated OVX rats, and P-treated OVX rats. In contrast, in E100- and E/P-treated OVX rats, PPI was not significantly reduced by 0.5 mg/kg 8-OH-DPAT. These data suggest that treatment with a high dose of estrogen, or with a combination of estrogen and progesterone, prevents 8-OH-DPAT-induced disruption of PPI. Thus, these hormones could be protective against sensorimotor gating deficits, at least those induced by 5-HT₁A receptor stimulation, and may therefore be beneficial against some symptoms of schizophrenia.

Schizophrenia is a severe mental illness with symptoms such as hallucinations, delusions, social withdrawal, and cognitive impairment (Harrison, 1999). Several neurotransmitters have been found to be altered in schizophrenia patients, e.g., there are changes in dopamine (D₂ and D₄) and 5-hydroxytryptamine (serotonin) (5-HT₁ and 5-HT₂) receptors (Dean, 2000). Recently, there is increasing literature on the potential importance of the 5-HT₁A receptor in schizophrenia. Post-mortem studies have revealed increased 5-HT₁A receptor density in the prefrontal cortex of patients with schizophrenia (Hashimoto et al., 1991; Simpson et al., 1996). In addition, some atypical antipsychotics, such as clozapine and ziprasidone, have a high affinity for the 5-HT₁A receptor and show a low incidence of extrapyramidal symptoms (Rellena et al., 2000).

Epidemiological evidence indicates that men have an earlier onset of schizophrenia, compared with women, of approximately 3 to 4 years (Hafner et al., 1993). This sex difference in age-at-onset is consistent across cultures and is found by many studies, regardless of the definition of onset and definition of illness used (Hafner et al., 1993; Castle et al., 1995). Furthermore, only in women does the distribution peak for age-at-onset of schizophrenia show a second peak around 50 years of age (Castle et al., 1995). Compared with men, women demonstrate a less severe course of illness in terms of milder symptoms, superior treatment response to antipsychotic medication, and improved social, intellectual, and occupational outcome (Seeman and Lang, 1990; Castle et al., 1995).

The common interpretation of these epidemiological data is that the female hormone, estrogen, plays a neuroprotective role in schizophrenia (Seeman and Lang, 1990; Hafner et al.,

Abbreviations: D, dopamine; 5-HT, 5-hydroxytryptamine (serotonin); PPI, prepulse inhibition; P, progesterone; OVX, ovariectomized; E20, 20% 17β-estradiol; E100, 100% 17β-estradiol; E/P, 20% 17β-estradiol and progesterone; 8-OH-DPAT, 8-hydroxy-2-dipropylaminotetralin; ANOVA, analysis of variance; PP, prepulse; WAY 100,135, N-tert-butyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropamide.
Female schizophrenia patients have lower estrogen levels compared with healthy controls (Oades and Schepker, 1994; Riecher-Rossler et al., 1994). In addition, schizophrenic symptomatology in women increases at times of low circulating estrogen levels (e.g., postpartum) and decreases at times of high estrogen levels (e.g., during pregnancy) (Seeman and Lang, 1990; Riecher-Rossler et al., 1994). Recently, estrogen treatment was used in a placebo-controlled clinical trial in women with acute schizophrenia. It was found that estrogen improved schizophrenic symptoms by accelerating the beneficial effect of the antipsychotic treatment (Kulkarni et al., 2001). Compared with estrogen, however, little is known about the role of other female hormones, such as progesterone, in schizophrenia.

Estrogen has been found to reduce 5-HT1A receptor gene expression in the dorsal raphe nucleus (Pecins-Thompson and Bethea, 1999), regions of the hippocampus (Birzniece et al., 2001) and amygdala (Osterlund and Hurd, 1998). Progesterone further reduces 5-HT1A receptor gene expression in the dorsal raphe nucleus of estrogen-primed animals (Pecins-Thompson and Bethea, 1999); however, it increases 5-HT1A receptor gene expression in the hippocampus (Birzniece et al., 2001). Therefore, in the present study, we focus on the interaction of estrogen and progesterone with 5-HT1A receptors.

To study this interaction, we used prepulse inhibition (PPI) of the acoustic startle response, an operational measure of sensorimotor gating. PPI is shown in humans as well as in experimental animals and has construct, face, and predictive validity as an animal model of sensorimotor gating (Swerdlow et al., 1996). Sensorimotor gating functions as a filter of irrelevant information, which prevents information overload and allows for coherent thought. Deficits in gating incoming sensory information may result in stimulus flooding and misinterpretation of stimuli (Geyer and Markou, 1995), and may cause certain symptoms of schizophrenia, such as disturbed thought (Perry and Braff, 1994), and delusions and hallucinations (Rigdon and Weatherspoon, 1992). It has been shown that patients with schizophrenia have deficient PPI, and treatment with atypical antipsychotic medication may reverse this deficit (Braff et al., 2001). Administration of 5-HT1A receptor agonists to rats causes a marked disruption of PPI (Rigdon and Weatherspoon, 1992).

In intact female rats, PPI has been found to be reduced during proestrus, i.e., the high estrogen phase of the estrous cycle (Koch, 1998). In contrast, ovariectomized rats receiving acute estrogen injections displayed increased PPI (Van den Buse and Eikelis, 2001). In addition, estrogen did not seem to interact with central dopaminergic or glutamatergic mechanisms in PPI (Van den Buse and Eikelis, 2001). In male rats, progesterone was found to partially reverse apomorphine-induced disruption of PPI (Rupprecht et al., 1999). Neither of these studies has yet examined the effects of these hormones on serotonergic mechanisms, nor has progesterone treatment been tested in female rats. Therefore, in the present study, we aimed to assess the interaction of estrogen and progesterone with 5-HT1A receptor-mediated effects on PPI, in female ovariectomized rats.

### Materials and Methods

**Animals.** We used 62 female Sprague-Dawley rats in this study (Department of Pathology and Anatomy Animal Services, University of Melbourne, VIC, Australia). The rats were 12 weeks of age at the time of surgery. They were housed in groups of two to three in standard rat cages, with free access to standard pellet food and tap water. The rats were maintained on a 12-h light/dark cycle (lights on at 6:30 AM), at a constant temperature of 22 ± 2°C. All surgical techniques, treatments and experimental protocols were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990) set out by the National Health and Medical Research Council of Australia.

**Ovariectomy.** Rats were anesthetized with an i.p. injection of sodium pentobarbitone (Nembutal, 60 mg/ml; Merial Australia, Rhone Merieux, QLD, Australia), with an injection volume of 1 ml/kg, and placed upon a heat pad. The abdominal area was shaved and the skin cleaned with an antiseptic solution (0.1% chlorhexidine acetate; Baxter Healthcare, Old Toongabbie, NSW, Australia). A midline incision was made through the skin and the muscle layer, avoiding the intestines. The ovaries were located and a piece of silk suture (Cyanamid Australia, Baulkham Hills, NSW, Australia) was used to ligate the fallopian tubes, after which the ovaries were carefully removed. The muscle layer and skin were suture-closed. Intact rats were sham-operated.

**Hormone Treatment.** During the ovariectomy procedure, rats were subcutaneously implanted with silastic implants at the nape of the neck. These implants were either empty or filled with crystalline steroid hormones and were of a length aimed at maintaining normal circulating levels, as reported previously (Williams and Lipner, 1982; Albert et al., 1991; Suzuki et al., 1995; Lerant and Freeman, 1998). Estrogen implants were 5 mm in length, and filled with either 100% estradiol (17β-estradiol, salt; Sigma-Aldrich, St. Louis, MO), or a 100% estradiol-cholesterol mixture (5-cholesten-3β,20-ol, salt; Steraloids, Newton, RI). Progesterone implants were 35 mm in length and filled with 100% progesterone (4-pregnene-3,20-dione, salt; Sigma-Aldrich). The implants were prepared the day before surgery with siliastic tubing (Dow Corning, i.d. 1.98 mm, o.d. 3.18 mm; Futuremedecis Australia, Balwyn, VIC, Australia) that was cut to the required length plus an additional 6 mm. Each end of the tube was sealed with 3 mm of silicon sealant. Before the implant was inserted, it was immersed in 100% ethanol and then soaked in sterile saline (0.9% sodium chloride; Baxter Healthcare) for 10 min.

**Postoperative Procedures.** After surgery, antiseptic cream (Betadine, povidone-iodine 10%; Faulding Consumer, Salisbury, SA, Australia) was applied over the sutures. Rats were also given a s.c. injection of 5 mg/kg of the nonsteroidal, anti-inflammatory analgesic carprofen (Hoechst Marion Roussel, Rowville, VIC, Australia). Behavioral experiments commenced 2 weeks after surgery. Three days after completion of experiments, the rats were killed by decapitation, and the uteri were removed and weighed. One untreated ovariectomized rat was removed from data analysis due to an abnormally high uterus weight.

**Prepulse Inhibition.** PPI of the acoustic startle response was measured with four automated startle chambers (SR-Lab; San Diego Instruments, San Diego, CA). Rats were placed individually into a transparent Plexiglas cylinder (8.8 cm in diameter) that was closed on each end. Each cylinder was positioned on a platform with a piezoelectric transducer mounted below the platform to detect whole body startle responses. The cylinders were placed in a well lit, sound-attenuating and ventilated cabinet. Background noise and acoustic noise bursts were presented through a speaker, and responses were measured with the SR-Lab software (San Diego Instruments) running on a PC in an adjacent room.

PPI sessions were conducted as described previously (Van den Buse and Eikelis, 2001). In brief, there were 100 trials, including 40 115-dB pulse-alone trials and 50 prepulse-pulse trials. Prepulse-pulse trials consisted of a prepulse (PP) of an intensity of 2, 4, 8, 12, or 16-dB above the 70-dB background (10 each), followed 100 ms later by a startle pulse of 115-dB. Trials were presented with variable intervals (10–37 s) to prevent learning and anticipatory responses. Startle data are measured using the middle 20 115-dB
pulse-alone trials. The percentage of PPI was calculated as the difference in amplitude between the startle response to the pulse-alone trials and the prepulse-pulse trials, divided by the response to the pulse-alone trial × 100%. Each PPI session also included 10 no-stimulus trials to control for nonspecific movement artifacts.

**Experimental Protocol.** For experiment 1, female rats were randomly chosen to become sham-operated controls receiving an empty implant (n = 10); ovariectomized (OVX) rats receiving an empty implant (n = 10); OVX rats implanted with the 20% estradiol mixture (E20, n = 8); or OVX rats treated with the 100% estrogen-filled implant (E100, n = 7). Based on the results of experiment 1, for experiment 2, female rats were randomly chosen to become OVX rats receiving an empty implant (n = 10); OVX rats treated with progesterone (P, n = 7); or OVX rats treated with both E20 and P (E/P, n = 9).

Two weeks after surgery, all rats were randomly injected with saline, or 0.02 or 0.5 mg/kg of the selective 5-HT1A receptor agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT, salt; Sigma/RBI, Natick, MA). These doses were selected on the basis of the literature (Rigdon and Weatherspoon, 1992; Sipes and Geyer, 1995) and preliminary experiments. 8-OH-DPAT was dissolved in sterile saline and administered in the flank (s.c.) 10 min before the rat being placed in the PPI chamber. In a randomized, crossover protocol, all rats in each experiment received all treatments, with 3 to 4 days allowed between each experiment.

**Statistical Analysis.** PPI and startle data are expressed as mean ± S.E.M. Data were analyzed with two-way analysis of variance (ANOVA) with repeated measures, where appropriate, using the statistical software package SYSTAT 9.0 (SPSS Science Inc., Chicago, IL). In both experiments, we assessed main effects of group and of dose, and interactions of dose by group. When significant differences were found in the main analysis, further pairwise ANOVA s were analyzed. Differences between groups or treatments were considered to be significant at p < 0.05.

**Results**

**Experiment 1: Effect of Ovariectomy and Estrogen Treatment**

**Body and Uterus Weight.** There was no significant difference in body weight between either of the groups at the time of surgery (Table 1). However, body weight gain of untreated OVX rats was significantly enhanced [F(1,16) = 61.4; p < 0.001] and E100-treated OVX rats had a significantly reduced body weight gain [F(1,15) = 6.8; p = 0.020], compared with sham-operated rats. The uterus weight of untreated OVX rats was significantly lower compared with sham-operated controls [F(1,18) = 78.1; p < 0.001], whereas the uterus weight of E100-treated OVX rats was slightly higher compared with sham-operated rats [F(1,15) = 4.7; p = 0.048; Table 1]. Importantly, E20-treated OVX rats showed a similar weight gain and uterus weight compared with sham-operated rats.

**Startle Amplitude.** Treatment with 8-OH-DPAT caused a significant dose-dependent increase in startle amplitude [main effect of dose F(2,62) = 10.0; p < 0.001; Fig. 1]. There were no differences between the groups nor of the effect of 8-OH-DPAT on startle amplitude between groups.

With respect to the effect of 8-OH-DPAT, pairwise ANOVA showed that startle amplitude was significantly increased in rats treated with 0.02 mg/kg [F(1,31) = 5.0; p = 0.032] and 0.5 mg/kg 8-OH-DPAT [F(1,31) = 22.6; p < 0.001], compared with saline treatment (Fig. 1). In addition, startle amplitudes were higher after treatment with 0.5 mg/kg 8-OH-DPAT than 0.02 mg/kg [F(1,31) = 4.7; p = 0.038].

**Prepulse Inhibition.** When comparing all the rat groups of experiment 1, there was a highly significant main effect of prepulse intensity [F(4,124) = 227.9; p < 0.001], reflecting the expected progressive reduction of startle responses with increasing prepulse intensity (Fig. 2). There was also a significant main effect of dose [F(2,62) = 15.0; p < 0.001], reflecting the dose-dependent reduction of PPI induced by 8-OH-DPAT. Furthermore, the effect of 8-OH-DPAT seemed to depend on the prepulse intensity, as shown by a significant prepulse by dose interaction [F(8,248) = 4.0; p < 0.001]. No main group effect was found, but the disruption of PPI caused by 8-OH-DPAT was different between the four groups [dose by group interaction F(6,62) = 2.4; p = 0.038]. In addition, there was a significant dose by prepulse by group interaction [F(24,248) = 1.9; p = 0.010].

With respect to the main effect of dose, pairwise ANOVA showed that, compared with saline treatment, PPI was significantly reduced in rats treated with 0.5 mg/kg 8-OH-DPAT [F(1,31) = 26.5; p < 0.001], but not with 0.02 mg/kg 8-OH-DPAT. The significant dose by prepulse interaction reflects that the 0.5 mg/kg dose of 8-OH-DPAT caused a greater disruption at the lower prepulse intensities (data not shown).

The differential effect of 8-OH-DPAT in the different hormone treatment groups was further explored with pairwise ANOVAs. When analyzing data from sham-operated rats and untreated OVX rats only, ANOVA showed no significant dose by group interaction, reflecting the similar effect of 8-OH-DPAT in these two groups (Fig. 2). Similarly, when combin-

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**Table 1**

Body weight (BW) and uterus weights of female rats from experiment 1: sham-operated controls, untreated OVX rats, E20-treated OVX rats, and E100-treated OVX rats; and experiment 2: untreated OVX rats, P-treated OVX rats and E20 and P (E/P)-treated OVX rats.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>BW at Surgery</th>
<th>Final BW</th>
<th>Weight Gain</th>
<th>Uterus Weight</th>
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<td></td>
<td>g</td>
<td>mg</td>
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<td><strong>Experiment 1</strong></td>
<td></td>
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<tr>
<td>Sham-operated</td>
<td>266 ± 5</td>
<td>283 ± 7†</td>
<td>17 ± 4</td>
<td>398 ± 31</td>
<td>1.40 ± 0.10</td>
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<tr>
<td>OVX</td>
<td>290 ± 6</td>
<td>370 ± 10†</td>
<td>81 ± 8**</td>
<td>154 ± 17**</td>
<td>0.43 ± 0.06**</td>
</tr>
<tr>
<td>E20</td>
<td>269 ± 10</td>
<td>294 ± 15†</td>
<td>25 ± 7</td>
<td>423 ± 21</td>
<td>1.45 ± 0.07</td>
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<tr>
<td>E100</td>
<td>277 ± 7</td>
<td>279 ± 6</td>
<td>3 ± 4*</td>
<td>476 ± 22</td>
<td>1.71 ± 0.10*</td>
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<tr>
<td><strong>Experiment 2</strong></td>
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<tr>
<td>OVX</td>
<td>279 ± 4</td>
<td>354 ± 9†</td>
<td>75 ± 8</td>
<td>150 ± 16</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>P</td>
<td>262 ± 6</td>
<td>344 ± 13†</td>
<td>82 ± 9</td>
<td>141 ± 8</td>
<td>0.41 ± 0.03</td>
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<tr>
<td>E/P</td>
<td>276 ± 5</td>
<td>272 ± 4</td>
<td>−4 ± 4*</td>
<td>537 ± 24*</td>
<td>1.97 ± 0.08*</td>
</tr>
</tbody>
</table>

* p < 0.05, † p < 0.001 compared with sham-operated rats for experiment 1, and compared with untreated OVX rats for experiment 2.

† p < 0.05, * p < 0.001 compared with body weight at the time of surgery in the same group.
ing data from untreated OVX rats and E20-treated OVX rats, there was no dose by group interaction. In contrast, there is a significant dose by group interaction \( F_{12,10} = 4.2, p = 0.025 \) when comparing untreated OVX rats and E100-treated rats, reflecting the marked reduction of the effect of 8-OH-DPAT in E100-treated rats compared with untreated OVX rats (Figs. 2 and 5).

Fig. 1. Mean (± S.E.M.) startle responses (in arbitrary units) in sham-operated rats (a, n = 10), untreated OVX rats (b, n = 10), OVX rats implanted with 20% estradiol (OVX + E20; c, n = 8) and OVX rats implanted with 100% estradiol (OVX + E100; d, n = 7). All groups were administered saline (open columns), 0.02 mg/kg 8-OH-DPAT (hatched columns), and 0.5 mg/kg 8-OH-DPAT (solid columns).

Fig. 2. Mean (± S.E.M.) percentage of PPI of sham-operated control rats (a, n = 10), untreated OVX rats (b, n = 10), OVX rats implanted with 20% estradiol (OVX + E20; c, n = 8), and OVX rats implanted with 100% estradiol (OVX + E100; d, n = 7). All groups were administered saline (open columns), 0.02 mg/kg 8-OH-DPAT (hatched columns), and 0.5 mg/kg 8-OH-DPAT (solid columns).
Experiment 2: Effect of Estrogen and/or Progesterone Treatment

**Body and Uterus Weight.** There was no significant difference in body weight between either of the groups at the time of surgery (Table 1). However, although the body weight gain of P-treated OVX rats was not significantly different to the untreated OVX rat group, the E/P-treated OVX rats showed significantly lower body weight gain compared with untreated OVX rats \( F(1,1,7) = 71.9; p < 0.001 \). In addition, the untreated and P-treated OVX rats had similar uterus weights (Table 1), whereas E/P-treated OVX rats had significantly higher uterus weight compared with untreated OVX rats \( F(1,2,0) = 336.6; p < 0.001 \).

**Startle Amplitude.** As in experiment 1, treatment with 8-OH-DPAT caused a significant dose-dependent increase in startle amplitude [main effect of dose \( F_{(2,46)} = 3.6; p = 0.034 \); Fig. 3]. There were, however, overall differences between the groups [main effect of group \( F_{(2,23)} = 4.6; p = 0.029 \)] and the effect of 8-OH-DPAT on startle amplitude tended to be different between groups [dose by group interaction \( F_{(4,46)} = 2.7; p = 0.043 \)].

With respect to the effect of 8-OH-DPAT, pairwise ANOVA showed that startle amplitude was significantly increased in rats treated with 0.02 mg/kg 8-OH-DPAT \( F_{(1,2,3)} = 6.3; p = 0.020 \) but not 0.5 mg/kg 8-OH-DPAT, compared with saline treatment (Fig. 3).

With respect to the main effect of group (and slight dose by group interaction), pairwise ANOVA comparing untreated OVX rats and E/P-treated OVX rats revealed a significant difference in startle amplitude \( F_{(1,1,7)} = 7.8; p = 0.013 \). Inspection of the data (Fig. 3) suggested that whereas E/P-treated OVX rats showed a dose-dependent increase in startle amplitude, untreated OVX rats showed a trend toward greatest startle after treatment with 0.02 mg/kg 8-OH-DPAT. There was no significant difference in startle amplitudes when comparing untreated OVX rats and P-treated OVX rats.

**Prepulse Inhibition.** Similar to experiment 1, in experiment 2 there was a highly significant main effect of prepulse intensity \( F_{(4,49,2)} = 250.5; p < 0.001 \; \text{Fig. } 4 \). There was a significant main effect of dose \( F_{(2,46)} = 13.4; p < 0.001 \), reflecting the dose-dependent reduction of PPI induced by 8-OH-DPAT. In addition, the effect of 8-OH-DPAT depended on the prepulse intensity, as indicated with a significant prepulse by dose interaction \( F_{(8,184)} = 2.5; p = 0.014 \). Furthermore, there was a significant main effect of group \( F_{(3,23)} = 4.3; p = 0.026 \).

With respect to the main effect of dose, pairwise ANOVA showed that, compared with saline treatment, PPI was significantly reduced in rats treated with 0.5 mg/kg 8-OH-DPAT \( F_{(1,2,3)} = 26.8; p < 0.001 \), but not with 0.02 mg/kg 8-OH-DPAT. The significant dose by prepulse interaction reflects that although the 0.5 mg/kg dose of 8-OH-DPAT resulted in a significant disruption at all prepulse intensities (data not shown), inspection of the data reveals that the disruption of PPI tends to be greatest at the lowest prepulse intensities.

In terms of the overall group differences, pairwise ANOVA comparing untreated OVX rats and E/P-treated OVX rats revealed a significant difference in PPI [main effect of group \( F_{(1,1,7)} = 7.0; p = 0.017 \)], reflecting the reduction of the effect of 8-OH-DPAT in E/P-treated (Figs. 4 and 5). In addition, whereas treatment with 0.5 mg/kg 8-OH-DPAT induced a 34% reduction of average PPI in untreated OVX rats, there was only a 16% reduction in E/P-treated OVX rats (Fig. 5). In contrast, there was no significant difference in PPI between untreated OVX rats and P-treated OVX rats (Fig. 4).

**Discussion**

The female sex steroid hormones estrogen and progesterone may be involved in the pathogenesis of schizophrenia (Seeman and Lang, 1990; Rupprecht et al., 1999). The present study examined whether estrogen and progesterone modulate disruption of PPI caused by 5-HT\(_{1A}\) receptor stimulation. We found that either a high dose of estrogen or a low dose of estrogen combined with progesterone, prevented dis-
ruptions of PPI induced by treatment with the prototypical 5-HT1A receptor agonist, 8-OH-DPAT.

As reported previously (Ke et al., 1997), ovariectomy of the rats caused a significant decrease in uterus weight and an increase in body weight gain. Uterus weight and body weight gain in E20-treated OVX rats was similar to that in sham-operated controls, demonstrating that this dose of estrogen was effective in reversing the physiological effects of ovariectomy. In E100-treated and E/P-treated OVX rats, uterus weight was increased and body weight gain decreased compared with sham-operated rats, suggesting that these doses were supraphysiological in terms of these parameters. Progesterone treatment did not influence the effects of ovariectomy on body weight and uterus weight, as has been described previously (Bond et al., 1994).

Previous research has found that estrogen implants similar to the E20 implants used in the present study, restored physiological circulating estrogen levels in OVX rats. Specifically, these implants provided estradiol concentrations equal to the mean level of estradiol present throughout the estrous cycle (16 pg/ml) (Williams and Lipner, 1982; Albert et al., 1991). Implants similar to the E100 implants have been found to produce a serum estradiol concentration of 55 pg/ml in OVX rats (Lerant and Freeman, 1998), which is equivalent to the peak estradiol level reached during noon of proestrus (Williams and Lipner, 1982; Albert et al., 1991). Also, it has been shown that in OVX rats, progesterone implants similar to the ones used in the present study have been found to provide concentrations that are approximately two-thirds of the maximum progesterone level reached during the estrous cycle (Williams and Lipner, 1982; Albert et al., 1991).

In the present study, there was no difference between the hormone-treated groups in terms of the dose-dependent increase in startle amplitude induced by 8-OH-DPAT treatment. This suggests that ovariectomy and estrogen treatment do not influence the level of acoustic startle and its regulation by 5-HT1A receptors. Thus, any differential effects of hormone treatment on PPI cannot be explained by marked changes in startle amplitudes.

Only the high dose of estrogen treatment prevented the disruption of PPI by 8-OH-DPAT treatment. It is unlikely that the low dose of estrogen was simply ineffective, because this dose had physiological effects, i.e., it reversed uterus and body weight and tended to increase baseline PPI, compared with untreated OVX rats, as published previously (Van den
Buuse and Eikelis, 2001). However, with respect to PPI, it seems that an effect of the low dose of estrogen is only observed in the presence of progesterone. In the present study, neither the low dose of estrogen nor progesterone influenced 5-HT<sub>1A</sub> receptor-mediated disruptions of PPI when administered alone, but when combined, the effect was similar to that of the high dose of estrogen. It has been suggested that the site of action of 8-OH-DPAT on PPI is via 5-HT<sub>1A</sub> autoreceptors in the dorsal raphe nucleus (Sipes and Geyer, 1995), increasing dopamine release in the forebrain and subsequently causing a disruption of PPI (Rigdon and Weatherspoon, 1992). We therefore propose that the effect of estrogen and progesterone occurs in the dorsal raphe nucleus. Both estrogen and progesterone receptors are located in this nucleus in the rat (Alves et al., 1998). In addition, estrogen induces progesterone receptor expression in the dorsal raphe nucleus (Bethea, 1994). Previous studies have suggested that estrogen reduces 5-HT<sub>1A</sub> receptor gene expression and the addition of progesterone enhances this effect on 5-HT<sub>1A</sub> autoreceptors (Pecins-Thompson and Bethea, 1999) but reverses the effect of estrogen on the postsynaptic 5-HT<sub>1A</sub> receptors (Birzniece et al., 2001). Therefore, one explanation for the present results could be that estrogen induces progesterone receptor expression in the dorsal raphe nucleus, and together these hormones significantly inhibit 5-HT<sub>1A</sub> autoreceptor expression, reducing the effect of 8-OH-DPAT on PPI. It could be that the high dose of estrogen does not need the presence of progesterone to exert a similar effect. However, further studies are needed to confirm whether the effect of the different hormone treatments is mediated by alterations in 5-HT<sub>1A</sub> autoreceptor density, altered postsynaptic signaling mechanisms, or changes in the activity of other transmitter systems “downstream” from the 5-HT<sub>1A</sub> receptor.

The finding that ovariectomy did not induce any changes in PPI compared with sham-operated rats could indicate that in the intact female rat, estrogen and progesterone do not play a tonic modulatory role on 5-HT<sub>1A</sub> receptor-mediated regulation of PPI. Koch (1998) showed a disruption in PPI during the high estrogen (and low progesterone) phase of the estrous cycle, in the intact female rat (Koch, 1998). Alternatively, the complex nature of hormonal interactions occurring during the estrous cycle, involving hypothalamic, pituitary, and ovarian hormones (Kelly et al., 1999), may mask the role of estrogen and progesterone, a role that becomes clear only when using a chronic treatment regimen, such as used in the present study. Unlike female rats, castrating male rats reduced 8-OH-DPAT-mediated disruptions of PPI (Gogos and Van den Bause, 2003). Treatment of these rats with testosterone restored the effect of 8-OH-DPAT. In addition, the disruption of PPI by 8-OH-DPAT was greater in intact, male rats compared with intact, female rats (Gogos and Van den Bause, 2003). Together, these findings support the concept of a facilitatory role of testosterone on 5-HT<sub>1A</sub> receptor-mediated PPI disruption as opposed to a protective role of estrogen and progesterone.

One methodological limitation of the present study was the chronic treatment regimen of hormone replacement. Chronic treatment was chosen as it has advantages over acute treatment in mimicking the chronic in vivo situation, although a disadvantage is that silastic implants produce stable, continuous hormone release, as opposed to the cyclical levels that occur during the estrous cycle (Albert et al., 1991). Another limitation of this study is that only 8-OH-DPAT was used as a drug stimulus. Although 8-OH-DPAT is the most selective 5-HT<sub>1A</sub> receptor agonist available, it also exhibits some agonist activity at the 5-HT<sub>7</sub> receptor (Barnes and Sharp, 1999). The possibility that 8-OH-DPAT acted on the 5-HT<sub>7</sub> receptor in the present study is unlikely, however, because previous research has found that 8-OH-DPAT induced disruptions of PPI are reversed with the selective 5-HT<sub>1A</sub> receptor antagonist WAY 100,135 (Sipes and Geyer, 1995). Finally, it should be noted that PPI is a model of only one aspect of schizophrenia and the 5-HT<sub>1A</sub> receptor is only one neurotransmitter receptor implicated in schizophrenia. Future studies should therefore address the interaction of estrogen/progesterone with 5-HT<sub>1A</sub> receptors in other behavioral models of aspects of this illness as well as the interaction of estrogen/progesterone with other receptors, e.g., dopamine D<sub>2</sub> or 5-HT<sub>2A</sub> receptors.

PPI is a valid animal model of sensorimotor gating which is disrupted in schizophrenia (Geyer and Markou, 1995) and some other psychiatric illnesses. The findings of the present study on the effects of estrogen and progesterone on PPI may be of importance to sensorimotor gating deficits in schizophrenia. Estrogen and progesterone, administered in the appropriate experimental conditions, seem to modulate 5-HT<sub>1A</sub> receptor-mediated responses in PPI and thus could play a role in schizophrenia through their effects on the 5-HT<sub>1A</sub> receptor. Furthermore, the present results may lead to further insight into the causes of gender differences seen in schizophrenia and may help to explain beneficial effects of hormone treatment on symptoms of the illness.

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References


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