Estrogen Treatment Blocks 8-Hydroxy-2-dipropylaminotetralin- and Apomorphine-Induced Disruptions of Prepulse Inhibition: Involvement of Dopamine D1 or D2 or Serotonin 5-HT1A, 5-HT2A, or 5-HT7 Receptors

Andrea Gogos, Perrin Kwek, Carolina Chavez, and Maarten van den Buuse

ABSTRACT

Prepulse inhibition (PPI) is a measure of sensorimotor gating and an endophenotype of schizophrenia. We have shown previously in rats that estrogen treatment prevents disruption of PPI by the 5-HT1A/5-HT2A receptor agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT). The aim of the present study was to examine the role of dopamine D1 and D2 and serotonin 5-HT1A, 5-HT2A, and 5-HT7 receptors in these effects. Part 1 of this study investigated the ability of estrogen treatment to reverse PPI disruption induced by 8-OH-DPAT or the dopamine D1/D2 receptor agonist apomorphine. Part 2 of this study compared these effects to the ability of various antagonists in reversing the action of 8-OH-DPAT and apomorphine on PPI. Female Sprague-Dawley rats were ovariectomized (OVX), and, where appropriate, they received silastic implants containing either a low (E20) or high dose (E100) of estrogen, and functional outcome (Häfner et al., 1993). In women, the hypothesis that the “female” sex steroid hormone estrogen plays a protective role in schizophrenia (Seeman and Lang, 1990; Häfner et al., 1993). Men and women with schizophrenia differ in terms of age at onset, symptom severity, and functional outcome (Häfner et al., 1993).

Heritable, clinical, and animal studies have led to the hypothesis that the “female” sex steroid hormone estrogen may play a protective role in schizophrenia (Seeman and Lang, 1990; Häfner and co-workers, 1993). Men and women with schizophrenia differ in terms of age at onset, symptom severity, and functional outcome (Häfner et al., 1993). Women, in general, are less severely affected than men. Estrogen prevents information overload and allows for coherent thought. Patients with schizophrenia have deficient PPI, and treatment with atypical antipsychotic medication may reverse this deficit (Braff et al., 2001). The PPI disruption in schizophrenia has been identified as one of the most consistently observed endophenotypes of the illness (Braff et al., 2001). It is interesting to note that, as with schizophrenia, symptoms worsen during the low estrogen stage of the menstrual cycle (Riecher-Rössler et al., 1994). However, the mechanism of action of a protective effect of estrogen is unclear.

Prepulse inhibition (PPI) is a measure of sensorimotor gating that functions as a filter of irrelevant information, preventing information overload and allowing for coherent thought. Patients with schizophrenia have deficient PPI, and treatment with atypical antipsychotic medication may reverse this deficit (Braff et al., 2001). The PPI disruption in schizophrenia has been identified as one of the most consistently observed endophenotypes of the illness (Braff et al., 2007). It is interesting to note that, as with schizophrenia,
gender differences in PPI have been described where men show greater PPI than women (Swerdlow et al., 1993). Furthermore, women have greater PPI during the early follicular (low-estrogen) phase of the menstrual cycle (Swerdlow et al., 1997; Jovanovic et al., 2004).

PPI can be reliably assessed in humans and experimental animals in a technically similar way (Geyer and Markou, 1995; Gogos and Van den Buuse, 2004; Gogos et al., 2006). Several studies using a variety of experimental conditions in animals have implicated dopamine and serotonin in the regulation of PPI (Geyer et al., 2001). For example, treatment with the dopamine D₁/D₂ receptor antagonist apomorphine or the 5-HT₁₆/5-HT₇ receptor agonist 8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) caused a disruption of PPI in male rats (Gogos and Geyer, 1995; Gogos and van den Buuse, 2003; Van den Buuse, 2003; van den Buuse and Gogos, 2007). The 8-OH-DPAT-induced disruption of PPI has been found to be prevented by the 5-HT₁₆ receptor antagonist WAY 100,135 (Sipes and Geyer, 1995). We previously showed that the effect of 8-OH-DPAT on PPI in female rats could be prevented by estrogen treatment (Gogos and Van den Buuse, 2004). Likewise, we showed that, in humans, disruption of PPI by treatment with the 5-HT₁₆ receptor partial agonist buspirone could be prevented by treatment with estrogen (Gogos et al., 2006). Thus, our results support the notion that estrogen exerts a functional protection against mechanisms that disrupt PPI, in this case 5-HT₁₆ receptor activation. However, the mechanisms underlying this estrogen effect remain unclear.

It is well established that alterations in dopamine release in the brain play a major role in the symptoms of schizophrenia. Most antipsychotic drugs are dopamine D₂ receptor antagonists and the therapeutic effects of antipsychotic drugs are correlated with the level of dopamine receptor blockade in the brain (Harrison, 1999). In addition to dopamine, several studies have suggested a role for serotonin receptors in schizophrenia, particularly the 5-HT₁₆ and 5-HT₂₅ receptor subtypes (Dean, 2003). For example, post-mortem research has shown a significant increase in 5-HT₁₆ receptor binding density and a decrease in 5-HT₂₅ receptor binding density in the frontal cortex from patients with schizophrenia (Bantick et al., 2001; Dean, 2003). There is considerable evidence for an interaction of dopaminergic and serotonergic receptor mechanisms in schizophrenia, which may be particularly important in the efficacy of atypical antipsychotic treatment (Alex and Pehek, 2007). 5-HT₁₆ receptor agonists selectively influence dopamine release in particular brain regions (Bantick et al., 2001). In vivo animal studies report that activation of 5-HT₁₆ receptors preferentially increases cortical dopamine release but does not affect release in the striatum (Bantick et al., 2001; Alex and Pehek, 2007). In terms of schizophrenia, this may improve symptoms and not cause extrapyramidal side effects, respectively (Bantick et al., 2001).

Previous studies have shown that estrogen dose-dependently modulates dopamine-related behaviors (Van Hartesveldt and Joyce, 1986) and can alter serotonin levels, firing rate, receptor levels, metabolism, and transporter binding and function (Bethea et al., 1998). Thus, the proposed functional protection exerted by estrogen in schizophrenia may be mediated by an action on either dopaminergic or serotonergic activity or both. Therefore, the aim of the present study was to further investigate the effect of estrogen on PPI. In part 1 of this study, the action of estrogen on the disruption of PPI induced by 8-OH-DPAT was compared with its effect on the disruption of PPI induced by apomorphine. In part 2 of the study, we compared the action of estrogen with that of dopamine D₁ and D₂ and 5-HT₁₆, 5-HT₂₅, and 5-HT₇ receptor antagonists.

Materials and Methods

Animals. We used 160 female Sprague-Dawley rats in this study (Monash Animal Services, Monash University, Victoria, 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 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.

Surgery. For ovariectomy surgery, rats were anesthetized using an isoflurane/oxygen gas mixture and placed upon a heat pad in the prone position. The medial region of the lower back was shaved, and a 2- to 3-cm midline incision was made through the skin that was then separated from the underlying muscle in a lateral direction on either side of the incision. A 5-mm incision was made through the abdominal wall, and the ovaries were bilaterally located and removed. The abdominal incision was sutured with silk thread, and the skin layer was closed using surgical staples. Intact rats were sham-operated, i.e., these rats underwent the same procedure as for ovariectomy, with the exception of removal of the ovaries.

During the ovariectomy procedure, rats were subcutaneously implanted with Silastic implants at the nape of the neck as described previously (Gogos and Van den Buuse, 2004). In brief, these implants (Dow Corning, 1.98 mm i.d., 3.18 mm o.d.; Futuremedics Australia, Victoria, Australia) were either empty or filled with crystalline steroid hormones. Estrogen implants were 5 mm in length and filled with either 100% estradiol (17β-estradiol; Sigma-Aldrich, St. Louis, MO) or a 20% estradiol/cholesterol mixture (cholesterol: 5-cholest-3β-ol; Steraloids, Newport, RI).

After surgery, rats received a subcutaneous injection of the nonsteroidal, anti-inflammatory analgesic carprofen (Zenecarp, 5 mg/kg; Heriot AgVet, Victoria, Australia). Behavioral experiments commenced 2 weeks after surgery. Three days after completion of experiments, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbitone (Nembutal, 60 mg/ml; Merial Australia, Parramatta, Australia) and killed by decapitation. The uterus was removed, weighed, and inspected for any abnormalities. Trunk blood was collected for determining 17β-estradiol levels using a radioimmunoassay kit (Diagnosics Systems Laboratories, Beckman Coulter, Webster, TX).

Prepulse Inhibition. PPI of the acoustic startle response was measured with eight automated startle chambers (SR-Lab; San Diego Instruments, San Diego, CA) as described previously (Gogos and van den Buuse, 2003). In brief, rats were placed individually into a transparent Plexiglas cylinder in a sound-attenuating cabinet. The PPI session comprised of 80 trials presented with variable intervals (8–27 s), including 32 pulse-alone trials (four blocks of eight 115-dB trials) and 40 prepulse-pulse trials. Prepulse-pulse trials consisted of a prepulse of an intensity of 2, 4, 8, 12, or 16 dB above the 70-dB background (eight of each), followed 100 ms later by the startle pulse. Startle data were measured using all four blocks of pulse-alone trials. The %PPI value was calculated as (pulse-alone trials startle amplitude − prepulse-pulse trials startle amplitude)/ (pulse-alone trials startle amplitude) × 100%. The middle 16 pulse-alone trials were used to calculate %PPI.
Experimental Protocol. All drugs were dissolved or diluted in saline to doses that were selected on the basis of the literature and preliminary experiments. All drugs were administered in a volume of 1 ml/kg. In a randomized, crossover protocol, all rats in each experiment received all treatments, with 3 to 4 days allowed between each experiment.

Pretreatments were administered subcutaneously in the flank 30 min before the next injection. These included the 5-HT\textsubscript{3R} receptor antagonist WAY 100,635 (Sigma-Aldrich), the 5-HT\textsubscript{4A/C} receptor antagonist ketanserin [3-(4-(4-fluorobenzoyl)-1-piperidinyl)ethyl]-2,4,11H,2H-quinazolinolinedione (+)-tartrate salt; Tocris Bioscience, Bristol, UK), the 5-HT\textsubscript{7} receptor antagonist SB-269970 (Sigma-Aldrich), the typical antipsychotic and dopamine D\textsubscript{2} receptor antagonist haloperidol [Serenace, 5-mg/ml ampule; 4-{4-[4-(4-fluorobutyrophenone; Sigma-Aldrich), or the dopamine D\textsubscript{3} receptor antagonist SCH 23390 (Sigma/RBI, Natick, MA). The treatments 8-OH-DPAT (Tocris Bioscience) and apomorphine [R-(-)-apomorphine hydrochloride hemihydrate; Sigma-Aldrich] were administered subcutaneously in the flank 10 min before the rat being placed in the PPI chamber.

In experiment 1, rats were randomly chosen to become sham-operated controls receiving an empty implant (n = 13), ovariotomized (OVX) rats receiving an empty implant (n = 14), OVX rats implanted with the 20% estradiol mixture (E20; n = 13), or OVX rats receiving the 100% estradiol implant (E100; n = 14). Rats in this experiment were tested for PPI after administration of saline and 0.5 mg/kg 8-OH-DPAT. In experiment 2, rats were randomly chosen to become sham-operated controls receiving an empty implant (n = 13), OVX rats receiving an empty implant (n = 15), E20-treated OVX rats (n = 13), or E100-treated OVX rats (n = 12). Rats in this experiment were tested for PPI after administration of saline and 0.3 mg/kg apomorphine.

In experiments 3 to 7, all rats were OVX rats receiving an empty implant to eliminate the confounding effects of sex hormones on PPI. In experiment 3, rats (n = 12) were tested for PPI after pretreatment with saline or 1 mg/kg WAY 100,635 and treatment with either saline, 0.3 mg/kg apomorphine, or 0.5 mg/kg 8-OH-DPAT. In experiment 4, rats (n = 12) were tested for PPI after pretreatment with saline or 2 mg/kg ketanserin and treatment with either saline, 0.3 mg/kg apomorphine, or 0.5 mg/kg 8-OH-DPAT. In experiment 5, rats (n = 8) were tested for PPI after pretreatment with saline or 10 mg/kg SB-269970 and treatment with saline or 0.5 mg/kg 8-OH-DPAT. In experiment 6, rats (n = 9) were tested for PPI after pretreatment with saline or 0.25 mg/kg haloperidol and treatment with either saline, 0.3 mg/kg apomorphine, or 0.5 mg/kg 8-OH-DPAT. In experiment 7, rats (n = 12) were tested for PPI after pretreatment with saline or 0.1 mg/kg SCH 23390 and treatment with either saline, 0.3 mg/kg apomorphine, or 0.5 mg/kg 8-OH-DPAT.

Statistical Analysis. All data are expressed as mean ± S.E.M. and were analyzed using the statistical software package SYSTAT 9.0 (SPSS Inc., Chicago, IL). Average %PPI was used for graphical presentation only and reflects the average of the five prepulse intensities. Body weight, uterus weight, and radioimmunoassays results (i.e., circulating estradiol levels) were analyzed with one-way analysis of variance (ANOVA) for group. Body and uterus weights were statistically analyzed for the rats of experiments 1 and 2; estradiol levels were measured in a sample of rats from experiments 1 and 2. PPI and startle data were analyzed with a two-way ANOVA, with repeated measures where appropriate. For experiments 1 and 2, a four group (intact, OVX, E20, and E100) × two treatment (saline or 8-OH-DPAT/apomorphine) × five prepulse intensity (PP2–PP16) ANOVA was used. For startle amplitude, a four group × two treatment × four block ANOVA was used. For experiments 3 to 7, a two pretreatment (saline or antagonist) × two treatment (saline or 8-OH-DPAT/apomorphine) × five prepulse intensity (PP2–PP16) ANOVA was used. For startle amplitude, a two pretreatment × two treatment × four block ANOVA was used. Main effects of prepulse intensity or startle block were always observed and are not be reported here in detail unless there were relevant interactions with other factors. Significant main effects and interactions were further explored with pairwise ANOVAs. Differences are considered to be significant at p < 0.05.

Results

Body Weight, Uterus Weight, and Circulating Estradiol Levels. Table 1 shows the body and uterus weights of all the rats in the seven experiments. Overall, there was no significant difference in body weight at the time of surgery. In experiments 1 and 2, there were significant group differences in body weight gain [experiment 1, F(3,50) = 64.7, p < 0.001; experiment 2, F(4,49) = 75.5, p < 0.001]. Compared with sham-operated controls, weight gain was significantly enhanced in untreated OVX rats and significantly reduced in E20- and E100-treated OVX rats (Table 1). Uterus weight was also significantly different between the groups [uterus weight as a percentage of BW: experiment 1, F(3,50) = 20.0, p < 0.001; experiment 2, F(3,49) = 22.1, p < 0.001].

TABLE 1

<table>
<thead>
<tr>
<th>Experiment 1: 8-OH-DPAT</th>
<th>Surgery BW</th>
<th>Wt Gain</th>
<th>Uterus Wt</th>
<th>% UW/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>252 ± 8</td>
<td>32 ± 6</td>
<td>0.47 ± 0.03</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>OVX</td>
<td>244 ± 5</td>
<td>96 ± 5*</td>
<td>0.14 ± 0.01*</td>
<td>0.04 ± 0.00*</td>
</tr>
<tr>
<td>E20</td>
<td>270 ± 12</td>
<td>10 ± 5*</td>
<td>1.03 ± 0.12*</td>
<td>0.39 ± 0.05*</td>
</tr>
<tr>
<td>E100</td>
<td>257 ± 7</td>
<td>10 ± 4*</td>
<td>0.82 ± 0.08*</td>
<td>0.32 ± 0.05*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 2: Apomorphine</th>
<th>Surgery BW</th>
<th>Wt Gain</th>
<th>Uterus Wt</th>
<th>% UW/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>258 ± 10</td>
<td>26 ± 4</td>
<td>0.46 ± 0.02</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>OVX</td>
<td>250 ± 7</td>
<td>84 ± 4*</td>
<td>0.15 ± 0.01*</td>
<td>0.04 ± 0.00*</td>
</tr>
<tr>
<td>E20</td>
<td>250 ± 7</td>
<td>8 ± 4*</td>
<td>1.14 ± 0.15*</td>
<td>0.45 ± 0.06*</td>
</tr>
<tr>
<td>E100</td>
<td>263 ± 14</td>
<td>7 ± 4*</td>
<td>0.85 ± 0.10*</td>
<td>0.34 ± 0.05*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 3: WAY 100,635</th>
<th>Surgery BW</th>
<th>Wt Gain</th>
<th>Uterus Wt</th>
<th>% UW/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>204 ± 6</td>
<td>83 ± 5</td>
<td>0.18 ± 0.01</td>
<td>0.06 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>241 ± 5</td>
<td>64 ± 6</td>
<td>0.22 ± 0.05</td>
<td>0.08 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 4: Ketanserin</th>
<th>Surgery BW</th>
<th>Wt Gain</th>
<th>Uterus Wt</th>
<th>% UW/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>224 ± 6</td>
<td>82 ± 5</td>
<td>0.13 ± 0.01</td>
<td>0.04 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 5: SB-269970</th>
<th>Surgery BW</th>
<th>Wt Gain</th>
<th>Uterus Wt</th>
<th>% UW/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>243 ± 6</td>
<td>50 ± 4</td>
<td>0.12 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 6: Haloperidol</th>
<th>Surgery BW</th>
<th>Wt Gain</th>
<th>Uterus Wt</th>
<th>% UW/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>226 ± 4</td>
<td>82 ± 5</td>
<td>0.13 ± 0.01</td>
<td>0.04 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 7: SCH 23390</th>
<th>Surgery BW</th>
<th>Wt Gain</th>
<th>Uterus Wt</th>
<th>% UW/BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>241 ± 6</td>
<td>76 ± 3</td>
<td>0.12 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>

UW, uterus weight.

* P < 0.05 compared with sham-operated rats (in experiments 1 and 2).

† P < 0.05 compared with untreated OVX rats (in experiments 1 and 2).
with sham-operated rats, untreated OVX rats showed reduced uterus weight, whereas E20- and E100-treated OVX rats had larger uteri. Furthermore, the untreated OVX rats of experiments 3 to 7 had comparable body weight gain and uterus weight compared with those in experiments 1 and 2 (Table 1).

Based on the radioimmunoassay results, the levels of circulating 17β-estradiol were as follows: sham-operated rats, 7.2 ± 1.1 pg/ml (n = 13); untreated OVX rats, 3.4 ± 0.4 pg/ml (n = 12); E20-treated OVX rats, 16.2 ± 1.6 pg/ml (n = 15); and E100-treated OVX rats, 19.3 ± 2.6 pg/ml (n = 14). ANOVA indicated that there was a significant difference between the groups [F(3,50) = 18.3, p < 0.01]; Bonferroni post hoc tests showed that both E20- and E100-treated OVX rats had significantly higher levels of circulating estradiol compared with sham-operated and untreated OVX rats (all p < 0.01).

**Experiment 1: Estrogen Treatment and 8-OH-DPAT.** By comparing PPI in the four hormone-treated groups, we showed that 8-OH-DPAT caused a significant disruption of PPI [F(1,50) = 61.3, p < 0.001; Fig. 1a]. However, the effect of 8-OH-DPAT was different among the four groups [8-OH-DPAT × group interaction, F(3,50) = 4.9, p < 0.01]. Pairwise ANOVA showed no differences in the effect of 8-OH-DPAT between sham-operated and untreated OVX rats or between untreated OVX and E20-treated OVX rats. In contrast, by comparing untreated OVX with E100-treated OVX rats, we showed that there was a significant reduction in the effect of 8-OH-DPAT when rats received E100 treatment [8-OH-DPAT × group interaction, F(1,26) = 10.9, p < 0.01]. Specifically, treatment with 0.5 mg/kg 8-OH-DPAT induced a significant 22% reduction of average PPI in untreated OVX rats [F(1,13) = 25.3, p < 0.001] but only a nonsignificant 4% reduction in E100-treated OVX rats (Fig. 1a). Treatment with 8-OH-DPAT caused a slight increase in startle amplitude [F(1,50) = 4.1, p < 0.05], which was not different between the groups (Fig. 1c).

**Experiment 2: Estrogen Treatment and Apomorphine.** By comparing PPI in the four hormone-treated groups, we showed that there was a significant main effect of apomorphine [F(1,49) = 87.3, p < 0.001] and an apomorphine × prepulse intensity interaction [F(4,196) = 5.3, p < 0.001], indicating that apomorphine treatment disrupted PPI and that this effect was dependent on the prepulse intensity (Fig. 1b). Inspection of the data suggested that apomorphine had its greatest effect at the middle prepulse intensities (data not shown). The disruption of PPI caused by treatment with apomorphine was different among the four groups [apomorphine × group interaction, F(3,49) = 4.8, p < 0.01]. Similar to 8-OH-DPAT, there was no difference in the effect of apomorphine on PPI between sham-operated and untreated OVX rats. However, by comparing untreated OVX rats with E20- or E100-treated OVX rats, we showed that there was a significant apomorphine × group interaction [F(1,26) = 4.3, p < 0.05 and F(1,25) = 15.4, p < 0.01, respectively], reflecting the reduction of the effect of apomorphine with estrogen treatment.

---

**Fig. 1.** Average %PPI (a and b) and startle amplitude (arbitrary units; c and d) of female sham-operated controls (Sham), untreated ovariectomized rats (OVX), 20% estradiol-treated OVX rats (E20), or 100% estradiol (E100)-treated OVX rats (n = 12–15/group). All rats were administered saline and 0.5 mg/kg 8-OH-DPAT (left) or saline and 0.3 mg/kg apomorphine (right). *, p < 0.05 compared with saline treatment.
treatment, in particular with E100 treatment. Treatment with 0.3 mg/kg apomorphine induced a 32% reduction of average PPI in untreated OVX rats \(F_{1,14} = 66.9, p < 0.001\), 21% reduction in E20-treated OVX rats \(F_{1,12} = 40.3, p < 0.001\), but only a nonsignificant 9% reduction in E100-treated OVX rats (Fig. 1b). Apomorphine treatment caused a significant increase in startle amplitude \(F_{1,49} = 7.7, p < 0.01\), which was not different between the groups (Fig. 1d).

**Experiment 3: WAY 100,635 versus 8-OH-DPAT and Apomorphine.** Although the main effect of 8-OH-DPAT was not reached significance, there was a significant pretreatment \(\times\) 8-OH-DPAT interaction \(F_{1,11} = 12.6, p < 0.01\), reflecting the lack of an 8-OH-DPAT-induced PPI disruption after WAY 100,635 pretreatment (Fig. 2a). Indeed, post hoc analysis showed that 8-OH-DPAT significantly disrupted PPI after saline pretreatment \(F_{1,11} = 17.5, p < 0.01\) but not after WAY 100,635 pretreatment (Fig. 2a). There was also a significant main effect of WAY 100,635 pretreatment \(F_{1,11} = 6.7, p < 0.05\); however, this was not significant by comparing saline/saline and WAY 100,635/saline.

As expected, apomorphine treatment significantly disrupted PPI \(F_{1,11} = 22.3, p < 0.01\). There was no main effect of WAY 100,635 pretreatment or a significant pretreatment \(\times\) apomorphine interaction, indicating that the apomorphine-induced PPI disruption was similar regardless of WAY 100,635 pretreatment (Fig. 2b). Indeed, PPI was significantly disrupted by apomorphine treatment both after saline pretreatment \(F_{1,11} = 10.8, p < 0.01\) and after WAY 100,635 pretreatment \(F_{1,11} = 16.8, p < 0.01\). In addition, there was no difference in PPI after apomorphine either in the saline or WAY 100,635 pretreatment condition.

Analysis of the effect of 8-OH-DPAT on startle amplitude revealed a trend for a pretreatment \(\times\) treatment interaction \(F_{1,11} = 4.3, p = 0.06\) but no main effects. This reflected the tendency for startle amplitude to be reduced by 8-OH-DPAT treatment alone but not after pretreatment with WAY 100,635 (Fig. 2c). Apomorphine treatment tended to cause an increase in startle \(F_{1,11} = 4.4, p = 0.06\) (Fig. 2d). There was also a significant main effect of WAY 100,635 pretreatment \(F_{1,11} = 8.0, p < 0.05\) and a pretreatment \(\times\) treatment interaction \(F_{1,11} = 6.1, p < 0.05\), indicating that the apomorphine-induced increase in startle amplitude was not seen after WAY 100,635 pretreatment. Startle was significantly enhanced by apomorphine treatment after saline pretreatment \(F_{1,11} = 5.4, p < 0.05\) but not after WAY 100,635 pretreatment (Fig. 2d).

**Experiment 4: Ketanserin versus 8-OH-DPAT and Apomorphine.** Also in this cohort, 8-OH-DPAT treatment significantly disrupted PPI \(F_{1,11} = 16.3, p < 0.01\). There was no significant main effect or interactions of ketanserin pretreatment (Fig. 3a), suggesting that the 8-OH-DPAT-induced PPI disruption occurred regardless of the presence of ketanserin. Pairwise comparison confirmed significant PPI disruption by 8-OH-DPAT after saline pretreatment \(F_{1,11} = 7.4, p < 0.05\) and ketanserin pretreatment \(F_{1,11} = 8.4, p < 0.05\). In addition, there was no difference in PPI after 8-OH-DPAT either in the saline or ketanserin pretreatment condition (Fig. 3a).

Likewise, apomorphine treatment significantly disrupted PPI \(F_{1,11} = 9.1, p < 0.05\), and this was not affected by ketanserin pretreatment (Fig. 3b). PPI was significantly disrupted by apomorphine treatment after saline pretreatment \(F_{1,11} = 13.8, p < 0.01\), although it failed to reach significance after ketanserin pretreatment \(F_{1,11} = 2.5, p = 0.14\).
However, there was no difference in PPI after apomorphine between the saline or ketanserin pretreatment condition.

Analysis of the effect of 8-OH-DPAT on startle amplitude revealed no main effects of ketanserin pretreatment or 8-OH-DPAT treatment and no interactions (Fig. 3c). Apomorphine treatment caused an increase in startle amplitudes \( F(1,10) = 17.7, p < 0.01 \); but there was no effect of ketanserin pretreatment or interaction, indicating that the apomorphine-induced increase in startle amplitude was not affected by ketanserin pretreatment. Startle was significantly enhanced by apomorphine treatment after saline pretreatment \( F(1,10) = 5.4, p < 0.05 \) as well as after ketanserin pretreatment \( F(1,11) = 13.5, p < 0.01 \); Fig. 3d).

**Experiment 5: SB-269970 versus 8-OH-DPAT.** 8-OH-DPAT treatment significantly disrupted PPI \( F(1,7) = 36.7, p < 0.01 \). There was no significant main effect or interactions of SB-269970 pretreatment (Fig. 4a), suggesting that the 8-OH-DPAT-induced PPI disruption occurs regardless of the presence of SB-269970. Pairwise comparison confirmed significant PPI disruption by 8-OH-DPAT after saline pretreatment \( F(1,7) = 20.1, p < 0.01 \) and SB-269970 pretreatment \( F(1,7) = 30.3, p < 0.01 \). In addition, there was no difference in PPI after 8-OH-DPAT either in the saline or SB-269970 pretreatment condition (Fig. 4a). Pretreatment with SB-269970 slightly reduced startle \( F(1,7) = 5.9, p < 0.05 \), but there were no significant effects of 8-OH-DPAT or any interactions (Fig. 4b).

**Experiment 6: Haloperidol versus 8-OH-DPAT and Apomorphine.** As expected, 8-OH-DPAT treatment significantly disrupted PPI \( F(1,8) = 16.9, p < 0.01 \); Fig. 5a). There was a significant main effect of haloperidol pretreatment \( F(1,8) = 8.6, p < 0.05 \), although comparison of the saline/saline and haloperidol/saline conditions was not significantly different. It is important to note that the significant pretreatment \( \times \) 8-OH-DPAT interaction \( F(1,8) = 5.6, p < 0.05 \) reflected that haloperidol pretreatment blocked the 8-OH-DPAT-induced PPI disruption (Fig. 5a). Post-hoc testing confirmed that 8-OH-DPAT disrupted PPI after saline pretreatment \( F(1,8) = 26.4, p < 0.001 \) but not after haloperidol pretreatment.

Apomorphine treatment significantly disrupted PPI \( F(1,8) = 19.5, p < 0.01 \); Fig. 5b). In addition, there was a significant effect of haloperidol pretreatment \( F(1,8) = 12.6, p < 0.01 \) and pretreatment \( \times \) apomorphine interaction \( F(1,8) = 10.9, p < 0.05 \), indicating that haloperidol pretreatment blocked the apomorphine-induced PPI disruption (Fig. 5b). Pairwise comparison showed that apomorphine disrupted PPI after saline pretreatment \( F(1,8) = 26.9, p < 0.01 \) but not haloperidol pretreatment.

In the 8-OH-DPAT experiment, haloperidol pretreatment slightly reduced startle amplitude \( F(1,8) = 26.0, p < 0.01 \); Fig. 5c), but there was no significant effect of 8-OH-DPAT or interactions. Also in the apomorphine experiment, haloperidol pretreatment slightly reduced startle \( F(1,8) = 5.9, p < 0.05 \); Fig. 5d). Apomorphine treatment increased startle \( F(1,8) = 8.2, p < 0.05 \), but there was no pretreatment \( \times \) treatment interaction (Fig. 5d).

**Experiment 7: SCH 23390 versus 8-OH-DPAT and Apomorphine.** 8-OH-DPAT treatment significantly disrupted PPI \( F(1,11) = 13.5, p < 0.01 \); Fig. 6a) and pretreat-

---

Fig. 3. Average %PPI (a and b) and startle amplitude (arbitrary units; c and d) of untreated ovariectomized rats \((n = 12)\) that were pretreated with saline or 2 mg/kg ketanserin, followed 30 min later by saline, 0.5 mg/kg 8-OH-DPAT (left), or 0.3 mg/kg apomorphine (right). *, \( p < 0.05 \) compared with saline treatment.
ment with SCH 23390 slightly but significantly increased PPI [$F_{(1,11)} = 20.6, p < 0.01$]. It is important to note that there was no significant pretreatment × 8-OH-DPAT interaction, suggesting that SCH 23390 pretreatment did not affect 8-OH-DPAT-induced PPI disruption (Fig. 6a). Post hoc testing confirmed that 8-OH-DPAT disrupted PPI after saline pretreatment [$F_{(1,11)} = 5.5, p < 0.05$] and after SCH 23390 pretreatment [$F_{(1,11)} = 17.7, p < 0.01$].

Apomorphine treatment significantly disrupted PPI [$F_{(1,11)} = 13.8, p < 0.01; $ Fig. 6b]. In addition, there was a significant effect of SCH 23390 pretreatment [$F_{(1,11)} = 40.4, p < 0.001$] and pretreatment × apomorphine interaction [$F_{(1,11)} = 8.6, p < 0.05$], indicating that SCH 23390 pretreatment blocked the apomorphine-induced PPI disruption (Fig. 6b). Pairwise comparison showed that apomorphine disrupted PPI after saline pretreatment [$F_{(1,11)} = 14.6, p < 0.01$] but not SCH 23390 pretreatment.

In the 8-OH-DPAT experiment, there were no main effects of SCH 23390 pretreatment or 8-OH-DPAT treatment. However, a significant pretreatment × 8-OH-DPAT interaction [$F_{(1,11)} = 8.8, p < 0.05$] reflected a trend for an 8-OH-DPAT-induced decrease in startle after saline pretreatment as opposed to a slight but significant [$F_{(1,10)} = 6.3, p < 0.05$] 8-OH-DPAT-induced increase in startle after SCH 23390 pretreatment (Fig. 6c). In the apomorphine experiment, SCH 23390 pretreatment slightly reduced startle [$F_{(1,10)} = 13.8, p < 0.01; $ Fig. 6d]. Although there was no main effect of apomorphine treatment, a significant pretreatment × apomorphine interaction [$F_{(1,10)} = 7.2, p < 0.05$] reflected a trend for an apomorphine-induced increase in startle after saline pretreatment as opposed to a slight but significant [$F_{(1,10)} =

---

Fig. 4. Average %PPI (a) and startle amplitude (arbitrary units; b) of untreated ovariectomized rats ($n = 8$) that were pretreated with saline or 10 mg/kg SB-269970, followed 30 min later by saline or 0.5 mg/kg 8-OH-DPAT. *, $p < 0.05$ compared with saline treatment.

Fig. 5. Average %PPI (a and b) and startle amplitude (arbitrary units; c and d) of untreated ovariectomized rats ($n = 9$) that were pretreated with saline or 0.25 mg/kg haloperidol, followed 30 min later by saline, 0.5 mg/kg 8-OH-DPAT (left), or 0.3 mg/kg apomorphine (right). *, $p < 0.05$ compared with saline treatment.
6.3, \( p < 0.05 \) apomorphine-induced decrease in startle after SCH 23390 pretreatment (Fig. 6c).

Discussion

In the present study, we aimed to examine the effects and underlying mechanisms of estrogen action on PPI. We showed that estrogen treatment not only reversed disruption of PPI by 8-OH-DPAT treatment but also by apomorphine treatment. Subsequent experiments confirmed that the action of apomorphine on PPI was mediated by dopamine D₁ and D₂ receptors, whereas the effects of 8-OH-DPAT were mediated by 5-HT₁A receptors. It is interesting to note that 8-OH-DPAT-induced PPI disruption was also reversed by the dopamine D₂ receptor antagonist haloperidol but not by the dopamine D₁ receptor antagonist SCH 23390, the 5-HT₂ₐ/₂₉C receptor antagonist ketanserin, or the 5-HT₇ receptor antagonist SB-269970. Thus, estrogen may prevent disruptions of PPI induced by both 8-OH-DPAT and apomorphine by an action on dopamine D₂ receptors “downstream” of 5-HT₁A receptors.

The present results confirm our previous study on the protective effect of estrogen treatment against 8-OH-DPAT-induced disruptions of PPI in rats (Gogos and Van den Buuse, 2004). We showed previously a similar effect of estrogen treatment in healthy women, where estrogen treatment prevented a disruption of PPI induced by the 5-HT₁A receptor partial agonist buspirone (Gogos et al., 2006). Although 8-OH-DPAT is commonly used as the prototypical 5-HT₁A receptor agonist, it also has some agonist activity at 5-HT₇ receptors (Barnes and Sharp, 1999). This is the first study to show that the specific 5-HT₁A receptor agonist WAY 100,635 blocks 8-OH-DPAT-induced PPI deficits in rats but that the selective 5-HT₇ receptor antagonist SB-269970 had no effect. Our results suggest that the action of 8-OH-DPAT on PPI is mediated by 5-HT₁A rather than 5-HT₇ receptors. On its own, this result could then suggest that the action of estrogen on 8-OH-DPAT-induced disruption of PPI is mediated by an effect on 5-HT₁A receptor function. Alternatively, the present results showed estrogen also inhibited the effect of apomorphine treatment to disrupt PPI in female rats. This
supports the notion that estrogen exerts a functional protection against PPI disruption by two neurotransmitter systems, specifically 5-HT₁A and dopamine D₂ receptor activation. Others have also shown that estrogen can modulate both dopaminergic and serotonergic activity in the brain. For example, estrogen treatment reduces behaviors induced by 8-OH-DPAT, such as the lordosis reflex (Jackson and Etgen, 2001) and hypothermia (Matsuda et al., 1991). In addition, estrogen treatment reduces apomorphine-induced stereotypes in the rat (Van Hartesveldt and Joyce, 1986). Estrogen receptors are located in regions of high 5-HT₁A receptor density, such as the presynaptic autoreceptors of the dorsal raphe nucleus as well as postsynaptic regions such as the hippocampus (Azmitia and Whitaker-Azmitia, 1995; Shughrue et al., 1997). Estrogen receptors are also located in regions of high dopamine D₂ receptor density, such as the ventral tegmental area (Shughrue et al., 1997).

Apomorphine is a mixed D₁/D₂ receptor agonist but has greater affinity for the D₂ receptor (Keibanian and Calne, 1979). Dopamine D₂ receptor antagonists, such as ziperone and raclopride (Swerdlov et al., 1991), and D₁ receptor antagonists, such as SCH 23390 (Wan et al., 1996), attenuated apomorphine-induced disruptions of PPI. In previous studies, apomorphine effects on PPI involved both D₁ and D₂ receptors; however, it has also been suggested that dopaminergic regulation of PPI is predominantly mediated by dopamine D₂ receptors (Wan et al., 1996). In our experiments, 8-OH-DPAT-induced disruption of PPI was blocked by pretreatment with haloperidol but not SCH 23390, suggesting that D₂ receptors play an important downstream role in the effects of 5-HT₁A receptors on PPI. 5-HT₁A and dopamine D₂ receptors closely interact in terms of PPI (van den Buuse and Gogos, 2007). Serotonin can normally inhibit dopamine cell firing, and removal of this inhibition by activation of 5-HT₁A autoreceptors may therefore increase dopamine cell firing (Alex and Pehek, 2007). Furthermore, studies show that there are direct synaptic contacts between serotonin terminals and dopamine cells in the midbrain (Alex and Pehek, 2007). The 5-HT₁A receptor has been found to be located on dopaminergic neurons within the ventral tegmental area (Doherty and Pickel, 2001). This model of primary 5-HT₁A receptor activation followed by secondary dopamine D₂ receptor activation also helps to explain the similar effect of estrogen on both 8-OH-DPAT- and apomorphine-induced disruption of PPI. Thus, these results suggest that the “protective” effect of estrogen occurs via an action on dopamine D₂ receptor mechanisms, blocking both the effect of apomorphine and the “upstream” effect of 8-OH-DPAT. An interaction of estrogen with D₁ receptor antagonists is unlikely, as pretreatment with SCH 23390 did not inhibit the action of 8-OH-DPAT on PPI. Previously, acute or chronic estrogen treatment in OVX rats increased 5-HT₂A receptor binding in the frontal cortex, nucleus accumbens, and dorsal raphe nucleus (Sumner and Fink, 1995; Cyr et al., 2000). However, in our experiments, pretreatment with ketanserin did not affect the disruption of PPI either by apomorphine or 8-OH-DPAT, making 5-HT₂A receptors less likely as a target for the action of estrogen treatment, at least under these experimental conditions. It is also unlikely that 8-OH-DPAT directly acts on dopamine D₂ receptors, as it is not known to have significant affinity for any of the dopamine receptors (Protais et al., 1998). Furthermore, haloperidol has very little affinity for 5-HT₁A receptors and a high affinity for dopamine D₂ receptors (Newman-Tancredi et al., 2005).

Treatment with 8-OH-DPAT caused a slight increase in startle amplitude, but only in some of the experiments. Previous studies have similarly found either an increase in startle amplitude after 8-OH-DPAT treatment (Gogos and Van den Buuse, 2004), no effect, or a partial effect (Sipes and Geyer, 1995; van den Buuse and Gogos, 2007), suggesting that the role of 5-HT₁A receptors on startle amplitude is modest and unlikely to have influenced the present PPI results. Apomorphine treatment significantly increased startle amplitude in several of the experiments of the present study. This is in contrast with previous work where apomorphine treatment had no effect on startle amplitude (Koch, 1998; Van den Buuse, 2003). In the present study, the majority of animals were OVX rats, and it may be that ovariectomy and thus the loss of endogenous sex steroid hormones results in an apomorphine-induced increase in startle amplitude. In conclusion, these data suggest that 8-OH-DPAT activates 5-HT₁A receptors, and not 5-HT₂ receptors, which subsequently activate downstream dopamine D₂ receptors, resulting in a disruption of PPI. Haloperidol, which blocks the effects of apomorphine treatment on PPI, may act directly on these downstream dopamine receptors to block the action of 5-HT₁A receptor activation. WAY 100,635 appears to act directly on 5-HT₁A receptors to block the effects of 8-OH-DPAT treatment on PPI. The protective effect of estrogen against 8-OH-DPAT- and apomorphine-induced PPI disruptions may be mediated by modulating dopamine D₂ receptor function. This may support an antipsychotic-like action of estrogen, at least on PPI. PPI is a valid animal model of sensorimotor gating that is disrupted in schizophrenia (Geyer and Markou, 1995) and some other psychiatric illnesses (Gogos et al., 2009). The findings of the present study suggest an interaction between 5-HT₁A and dopamine D₂ receptors in mediating PPI and may be of importance in schizophrenia. For example, the newer atypical antipsychotic aripiprazole is clinically effective and has partial agonist activity at 5-HT₁A and D₂ receptors and acts as a dopamine-serotonin system stabilizer (Burris et al., 2002). The present findings suggest a mechanism of action for a protective role of estrogen in PPI and therefore schizophrenia, that is, estrogen acts on the dopamine D₂ receptor. Furthermore, the present results may help to explain the beneficial effects of estrogen treatment on symptoms of the illness (Kulkarni et al., 2001).

References


Burris KD, Molski TF, Xu C, Ryan E, Tottori K, Kikuchi T, Vercio FD, and Molinoff


Address correspondence to: Prof. Maarten van den Buuse, Behavioral Neuroscience Laboratory, Mental Health Research Institute, 155 Oak St., Parkville (Melbourne), Victoria 3052, Australia. E-mail: m.vandenbuuse@mhri.edu.au