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Estrogen increases prepulse inhibition of acoustic startle in rats

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Abstract

Epidemiological studies have shown gender differences in the age of onset and symptoms of schizophrenia. Because sensorimotor gating mechanisms are deficient in schizophrenia, we studied the effect of administration of estrogen on prepulse inhibition of startle in rats, an animal model of sensorimotor gating. Rats were tested in an automated startle apparatus for their responses to random combinations of 115-dB sound pulses and prepulses of various intensity. Startle responses were reduced by increasing intensities of prepulses, indicating prepulse inhibition. Repeated administration of startle pulses caused gradual habituation of startle responses. Ovariectomy did not induce significant changes in either habituation of the startle response or prepulse inhibition of startle. Treatment with 17β-estradiol caused an increase in percentage prepulse inhibition at all prepulse intensities at 18 h, but only at higher prepulse intensities at 30 min after injection. Habituation of startle responses was not affected. The enhancing effect of estradiol on prepulse inhibition was mimicked by testosterone, but not by dihydrotestosterone. Estradiol treatment increased prepulse inhibition similarly in controls or after disruption of prepulse inhibition induced by treatment with apomorphine or dizocilpine (MK-801). Our results may help to explain gender differences in schizophrenia and some of the beneficial clinical effects of estrogen treatment in this disease.

Keywords: Estrogen; Testosterone; Prepulse inhibition; Startle; Dopamine; Glutamate

1. Introduction

The prevalence of schizophrenia in the general population is about 1% (Hirsch and Weinberger, 1995). Symptoms first occur as early as 16–20 years of age, this being approximately 1.5–4.5 years later in women than in men (Angermeyer and Kuhn, 1988; Häfner et al., 1993; Hirsch and Weinberger, 1995; Seeman, 1997). In addition, symptoms of schizophrenia vary with the estrous cycle in women, being most severe during the late luteal and premenstrual phases, when circulating estrogen levels are low (Angermeyer and Kuhn, 1988; Häfner et al., 1993; Seeman, 1997). These epidemiological findings have been taken as evidence for a protective role of sex steroids, in particular estrogen, in the development of schizophrenia (Häfner et al., 1993; Seeman, 1997). While the 'dopamine hypothesis' of schizophrenia suggests increased subcortical dopaminergic activity, estrogen has been suggested to act as an endogenous neuroleptic by reducing this activity at several levels (Di Paolo, 1994; Häfner et al., 1993). On the other hand, several studies have suggested 'pro-dopaminergic' effects of estrogen. For example, administration of estrogen to ovariectomized rats increases amphetamine-induced locomotor hyperactivity, which is widely used as an animal model of psychosis (Menniti and Baum, 1981). In vitro, addition of estrogen enhances stimulated dopamine release from slices of the striatum at low doses and reduces it at high doses (Becker, 1990). One explanation for this paradox could be that these pro-dopaminergic effects are mediated by membrane receptors, leading to rapid non-genomic effects, whereas anti-dopaminergic effects are mediated by classical nuclear receptors, leading to relatively slow genomic modulating (West and Michael, 1986).

The first clinical studies into the effect of estrogen administration in patients with schizophrenia have been encouraging. For example, chronic addition of estrogen

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treatment to standard antipsychotic medication was shown to lead to a more rapid improvement of symptoms in women with schizophrenia than with antipsychotic treatment only (Korhonen et al., 1995; Kulkarni et al., 1996). However, most basic and clinical studies have so far focused mostly on the positive symptoms of schizophrenia, particularly psychosis, or its experimental animal models. Far less is known about a possible role of estrogen in other symptoms of schizophrenia. In the present study, we investigated the effect of sex steroid treatment on prepulse inhibition in rats. Prepulse inhibition is the phenomenon that behavioural responses to a sensory stimulus can be inhibited by another stimulus, delivered 20–500 ms earlier (Braff and Geyer, 1990). The preceding stimulus "sets up an inhibitory network", which dampens the response to the second stimulus. This sensory gating mechanism is suggested to protect the brain from stimulus inundation, which could otherwise lead to cognitive fragmentation and disturbed thought (Braff and Geyer, 1990). Experimental studies on prepulse inhibition usually measure the inhibitory effect of low-intensity prepulses on the startle response to a high-intensity stimulus, such as short sound pulses (Geyer et al., 1990). Prepulse inhibition has been demonstrated similarly in several mammalian species, including rats, mice, guinea pigs, and humans (Geyer, 1999; Geyer et al., 1990). Several studies have shown that prepulse inhibition is impaired in patients with schizophrenia (Braff and Geyer, 1990; Geyer et al., 1990) and that treatment with atypical antipsychotics reverses these deficits (Kumari et al., 1999). Also, in experimental animals, prepulse inhibition is impaired after treatment with dopaminergic drugs, such as the dopamine receptor agonist apomorphine, or glutamatergic drugs, such as the NMDA receptor antagonist dizocilpine (MK-801) (Geyer et al., 1990; Swerdlow and Geyer, 1998). Atypical antipsychotics reverse this deficit (Geyer et al., 1990; Swerdlow and Geyer, 1998).

In women, prepulse inhibition is reduced compared to men, suggesting that female sex steroids, such as estrogen, may not be beneficial or even worsen sensorimotor gating mechanisms (Swerdlow et al., 1993). Indeed, prepulse inhibition is reduced during the midluteal phase of the estrous cycle, when estrogen levels are high (Swerdlow et al., 1997). Similarly, a preliminary study in rats suggested changes in prepulse inhibition across the estrous cycle, with lowest values found during proestrous compared to diestrous and estrous (Koch, 1998). Surprisingly, to the best of our knowledge, no studies have done on the effect of estrogen administration on prepulse inhibition. In the present study, we therefore addressed four questions: (1) what is the effect of ovariectomy, or removing the natural source of sex steroids in female rats, on prepulse inhibition; (2) what is the effect of administration of different doses of estrogen on prepulse inhibition in ovariectomized rats; (3) can this effect be mimicked by other sex steroids, in particular testosterone; (4) does estrogen administration reverse the disruption of prepulse inhibition by treatment with apomorphine or MK-801?

2. Methods

We used female Sprague–Dawley rats that were obtained from the University of Melbourne, Departments of Pathology and Anatomy Animal Services. At the start of the experiment, the rats were 12 weeks of age. All surgical techniques, treatments and experimental protocols were performed 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.

2.1. Prepulse inhibition

Prepulse inhibition experiments were performed using a four-unit automated SRLab startle system (San Diego Instruments, San Diego, CA, USA). Each unit consisted of a plexiglas cylinder on a platform under which a sensitive piezo-electric sensor was mounted. The cylinders were 9 cm in diameter and closed on either end. During the sessions, the animals were kept in the cylinders within a sound-attenuating cabinet where a 70-dB white background noise was delivered through speakers in the ceiling of the box. Stimuli were similarly delivered and responses were measured using the SRLab software (San Diego Instruments) running on a PC in an adjacent room.

For all experiments, an identical protocol was used, adapted from methods developed by the group of Geyer and Swerdlow (1998). A total of 100 trials were delivered with an average (but not constant) interval of 25 s. The first and last 10 trials consisted of single 40-ms, 115-dB pulse-alone startle stimuli. These groups of 10 stimuli and the middle two groups of 10 pulse-alone stimuli (see below) were used to obtain a measure of response habituation in response to repeated delivery of startling stimuli. The middle 80 trials consisted of random delivery of twenty 115-dB pulse-alone trials, 10 trials during which no stimuli were delivered (NOSTIM), and 50 prepulse trials. Prepulse trials consisted of a single 115-dB pulse preceded by 100 ms by a 20-ms non-startling stimulus prepulse of 2, 4, 8, 12, or 16 dB over baseline (i.e., 72, 74, 78, 82 or 86 dB).

2.2. Experiment 1: effect of ovariectomy

Intact female rats were tested for baseline prepulse inhibition at 12 weeks of age, after which they were ovariectomized under general anesthesia with pentobarbital sodium (60 mg/kg, i.p.). Through a midline incision, the ovaries were located and silk-2 sutures were used to ligate the fallopian tubes and blood vessels. The ovaries were then carefully removed using forceps. The abdominal mus-

cle wall and skin were suture closed and the rats were allowed to recover for at least 1 week before the second session of prepulse inhibition was performed. Thus, each rat was tested twice: before and 1 week after ovariectomy.

Rats from this group were used for an estrogen dose response experiment, to test the effect of testosterone, and to test the interaction of estrogen treatment with that of apomorphine and MK-801 (see below).

2.3. Experiment 2: effect of estrogen treatment on prepulse inhibition

Using a randomized, crossover treatment protocol, 10 ovariectomized rats received subcutaneous injections of sesame oil vehicle (1 ml/kg) or 10, 50, or 250 μg/kg of 17β-estradiol benzoate (Sigma, St. Louis, MO, USA) in vehicle. Prepulse inhibition measurements were done 30 min and 18 h after injection. Each rat was thus tested for prepulse inhibition twice after treatment at a total of four occasions, with 4- to 7-day intervals.

2.4. Experiment 3: effect of testosterone treatment on prepulse inhibition

Ten ovariectomized rats were randomly given a subcutaneous injection of sesame oil vehicle (1 ml/kg) or 1 mg/kg of testosterone-proprionate (Sigma) (Eikelis and Van den Buuse, 2001). Prepulse inhibition measurements were done 30 min and 18 h after injection. Thus, each rat was tested on two occasions, with a 1-week interval.

2.5. Experiment 4: effect of dihydrotestosterone treatment on prepulse inhibition

Ten ovariectomized rats were subcutaneously treated with either oil vehicle or 1 mg/kg of dihydrotestosterone (Sigma), 18 h before their prepulse inhibition session. Thus, each rat was tested on two occasions, with a 1-week interval.

2.6. Experiment 5: interaction of estrogen with the effects of apomorphine and MK-801 on prepulse inhibition

Using a randomized, crossover treatment protocol, 10 ovariectomized rats received two subcutaneous injections per prepulse inhibition session. At 18 h before testing, the animals were treated with oil vehicle (1 ml/kg) or 50 μg/kg of 17β-estradiol (Sigma) as above. The next day, 10 min before the prepulse inhibition session, the rats were injected with either saline vehicle (1 ml/kg), 0.1 mg/kg of apomorphine (Research Biochemical, Natick, MA, USA), or 0.1 mg/kg of dizocilpine (MK-801, Research Biochemicals). Thus, all rats were tested a total of six times, with 1-week intervals.

2.7. Data analysis

Responses of individual rats were expressed as the median of the first, second, third and last ten 115-dB pulse-alone trials to determine habituation. We also calculated the median of the middle twenty 115-dB pulse-alone trials and that of each of the groups of 10 prepulse trials. Percentage prepulse inhibition was then calculated as the difference between responses to the 115-dB pulse-alone trial and the respective prepulse trials divided by the response to the pulse-alone trial × 100. Individual responses were then averaged to yield groups means and standard errors of the mean (S.E.M.) (Geyer and Swerdlow, 1998).

Data were analyzed with two-way and three-way analysis of variance (ANOVA) for repeated measures, where appropriate. All experiments were within-animal comparisons, with the change in responses over four blocks of 10 pulse-alone trials in the habituation experiments or prepulse intensity level in the prepulse inhibition experiments as statistical variables. In addition, ovariectomy (experiment 1), estrogen dose (experiment 2), testosterone treatment (experiment 3), or dihydrotestosterone treatment (experiment 4) were additional within-animal treatment factors. In experiment 5, estrogen treatment and apomorphine/MK-801 treatment were within-animal factors. The data for the interaction of estrogen with apomorphine and estrogen with MK-801 were analyzed separately. When $P < 0.05$, differences were considered statistically significant.

3. Results

3.1. Experiment 1: effect of ovariectomy

All rats demonstrated reproducible startle responses to sound pulses. Startle amplitude, as measured by the SRLab startle system, showed significant habituation during the session ($F_{(3,84)} = 28.0$, $P < 0.001$) and this habituation was slightly more rapid in intact rats, as indicated by the block × group interaction ($F_{(3,84)} = 4.5$, $P = 0.005$). Overall, there was no significant effect of ovariectomy on startle amplitude (Table 1).

When preceded by low-intensity prepulses, startle responses to the 115-dB tone were reduced. This reduction was intensity-dependent, with the higher-intensity prepulses producing more inhibition than lower-intensity prepulses. Fig. 1 demonstrates that in both intact and ovariectomized Sprague–Dawley rats, as prepulse intensity increased, the percentage prepulse inhibition also significantly increased ($F_{(4,112)} = 73.5$, $P < 0.001$). Ovariectomy did not cause any significant changes in prepulse inhibition (Fig. 1).

Table 1
Startle amplitude in response to a 40-ms, 115-dB tone and startle habituation in Sprague–Dawley rats before and after ovariectomy and after subcutaneous injection of oil vehicle or estrogen, testosterone or dihydrotestosterone

	Block 1	Block 2	Block 3	Block 4
Effect of ovariectomy				
Intact	463 ± 114	246 ± 26	245 ± 32	209 ± 27
Ovariectomized	311 ± 52	243 ± 27	172 ± 27	150 ± 23
Effect of estrogen 30 min after treatment				
Oil vehicle	865 ± 186	677 ± 165	334 ± 61	455 ± 141
10 μg/kg	596 ± 146	382 ± 58	295 ± 49	280 ± 57
50 μg/kg	522 ± 139	359 ± 75	263 ± 36	234 ± 49
250 μg/kg	482 ± 104	316 ± 54	269 ± 33	221 ± 48
Effect of estrogen 18 h after treatment				
Oil vehicle	594 ± 127	440 ± 108	328 ± 89	329 ± 64
10 μg/kg	480 ± 126	342 ± 53	271 ± 48	220 ± 52
50 μg/kg	523 ± 152	311 ± 60	240 ± 52	217 ± 43
250 μg/kg	441 ± 110	305 ± 52	282 ± 65	196 ± 31
Effect of testosterone 30 min after treatment				
Oil vehicle	273 ± 77	181 ± 30	143 ± 25	191 ± 73
Testosterone	328 ± 123	184 ± 40	140 ± 30	166 ± 41
Effect of testosterone 18 h after treatment				
Oil vehicle	300 ± 113	191 ± 43	128 ± 30	116 ± 28
Testosterone	370 ± 184	195 ± 53	178 ± 44	167 ± 52
Effect of dihydrotestosterone treatment				
Oil vehicle	378 ± 69	334 ± 68	294 ± 77	278 ± 77
Dihydrotestosterone	507 ± 117	335 ± 71	250 ± 50	200 ± 39

Data are mean arbitrary units ± S.E.M. as obtained from the SRLab startle system by calculating the median of blocks of ten 115-dB pulses, before (block 1), during (blocks 2 and 3) and after (block 4) a prepulse inhibition session.

3.2. Experiment 2: effect of estrogen treatment

At 30 min after treatment, startle amplitude was found to habituate significantly during the session ($F_{(3,27)} = 9.4$, $P < 0.001$). There was also a significant effect of treatment on startle amplitude ($F_{(3,27)} = 8.1$, $P = 0.001$), reflecting the slight reduction of absolute startle amplitude after estrogen treatment, when compared to oil-vehicle treatment. However, estrogen treatment had no significant effect on habituation as indicated by the lack of a block × dose interaction.

As expected, at 30 min after injection (Fig. 2) there was a highly significant effect of prepulse intensity ($F_{(4,36)} = 67.1$, $P < 0.001$). In addition, while there was no overall effect of treatment dose, there was a significant interaction of prepulse intensity and treatment dose ($F_{(12,108)} = 2.2$, $P = 0.018$). Analysis of the data of individual prepulse intensities showed that there was no significant effect of different doses of estrogen on the percentage inhibition evoked by prepulse-2 and prepulse-4. However, at higher prepulse intensities, ANOVA showed a significant effect of estrogen treatment (prepulse-8: $F_{(3,27)} = 4.1$, $P = 0.016$; prepulse-12: $F_{(3,27)} = 7.5$, $P = 0.001$; prepulse-16: $F_{(3,27)} = 6.6$, $P = 0.002$). Thus, 30 min after injection, estrogen treatment caused an increase of prepulse inhibition only at higher prepulse intensities (Fig. 2, top panel).

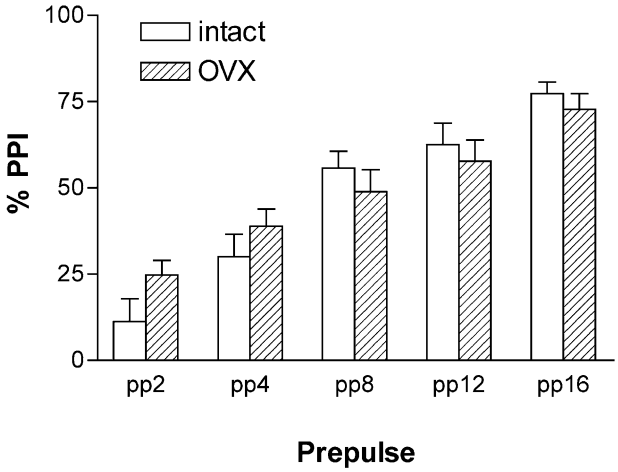

Fig. 1. Prepulse inhibition of acoustic startle in 29 female rats before (white bars) and 1 week after (hatched bars) ovariectomy. Data are expressed as mean % prepulse inhibition ± S.E.M.

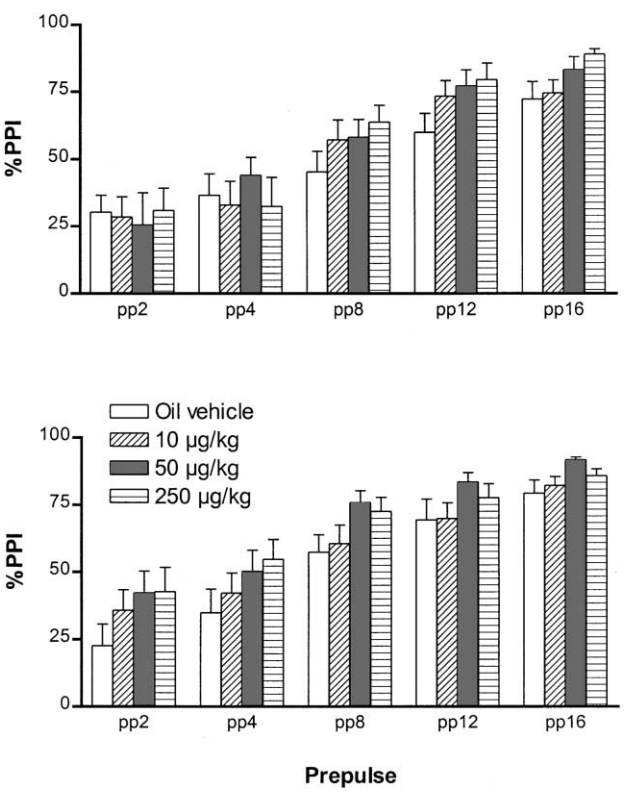

Fig. 2. Prepulse inhibition of acoustic startle in 10 ovariectomized rats after treatment with oil vehicle (white bars), 10 μg/kg of 17β-oestradiol (hatched bars), 50 μg/kg of 17β-oestradiol (dark stippled bars) or 250 μg/kg of 17β-oestradiol (horizontal hatching). Data were obtained 30 min (top panel) and 18 h after treatment (bottom panel) and are expressed as mean % prepulse inhibition ± S.E.M.

At 18 h after injection, startle amplitude again showed significant habituation during the session ($F_{(3,27)} = 10.8$, $P < 0.001$). However, as indicated by the lack of an interaction between block and dose, the extent of habituation was similar whether rats were treated with oil vehicle or different doses of estrogen (Table 1).

At 18 h after injection, the effect of increasing prepulse intensities on percentage prepulse inhibition was again highly significant for all treatments ($F_{(4,36)} = 64.8$, $P < 0.001$). Estrogen treatment induced a significant overall increase in the level of prepulse inhibition ($F_{(3,27)} = 10.7$, $P < 0.001$) (Fig. 2, bottom panel). There was no statistical interaction of treatment and prepulse intensity, suggesting that the effect of estrogen was similar for all prepulse levels. Analysis of the data from individual prepulse intensities confirmed that the effect of estrogen was observed for lower prepulse intensities (prepulse-2: $F_{(3,27)} = 4.3$, $P = 0.014$; prepulse-4: $F_{(3,27)} = 4.2$, $P = 0.015$) as well as for the higher prepulse intensities (prepulse-8: $F_{(3,27)} = 10.1$, $P < 0.001$; prepulse-12: $F_{(3,27)} = 5.9$, $P = 0.003$; prepulse-16: $F_{(3,27)} = 4.9$, $P = 0.008$). Thus, at 18 h after injection, estrogen caused an increase of prepulse inhibition at all prepulse intensities (Fig. 2, bottom panel) while at 30 min after treatment it only increased prepulse inhibition at higher prepulse intensities (Fig. 2, top panel).

3.3. Experiment 3: effect of testosterone treatment

At 30 min after injection of testosterone, startle amplitude showed significant habituation ($F_{(3,24)} = 3.3$, $P = 0.038$), but there was no treatment effect or statistical interaction. At 18 h after injection, startle amplitude tended to habituate during the session, similar to previous experiments (see above), but the extent of habituation failed to reach significance (Table 1).

As shown in Fig. 3, the overall effect of increasing prepulse intensities was again highly significant, both at 30 min ($F_{(4,32)} = 30.2$, $P < 0.001$) and at 18 h after treatment ($F_{(4,32)} = 26.5$, $P < 0.001$). Testosterone treatment significantly increased the level of prepulse inhibition compared to oil vehicle treatment at 18 h after injection ($F_{(1,8)} = 8.2$, $P = 0.021$), but not at 30 min after injection. The effect of testosterone was particularly found at lower prepulse intensities (Fig. 3), although the statistical interaction of prepulse intensity and treatment failed to reach significance ($F_{(4,32)} = 2.3$, $P = 0.077$). Analysis of individual prepulse intensities revealed that the effect of testosterone, 18 h after injection, was significant for prepulse-4.

3.4. Experiment 4: effect of dihydrotestosterone treatment

Startle amplitude significantly habituated during the prepulse inhibition session ($F_{(3,27)} = 6.5$, $P < 0.005$). This effect was similar after both oil-vehicle and dihydrotestosterone administration, as shown by a lack of block × treatment interaction (Table 1).

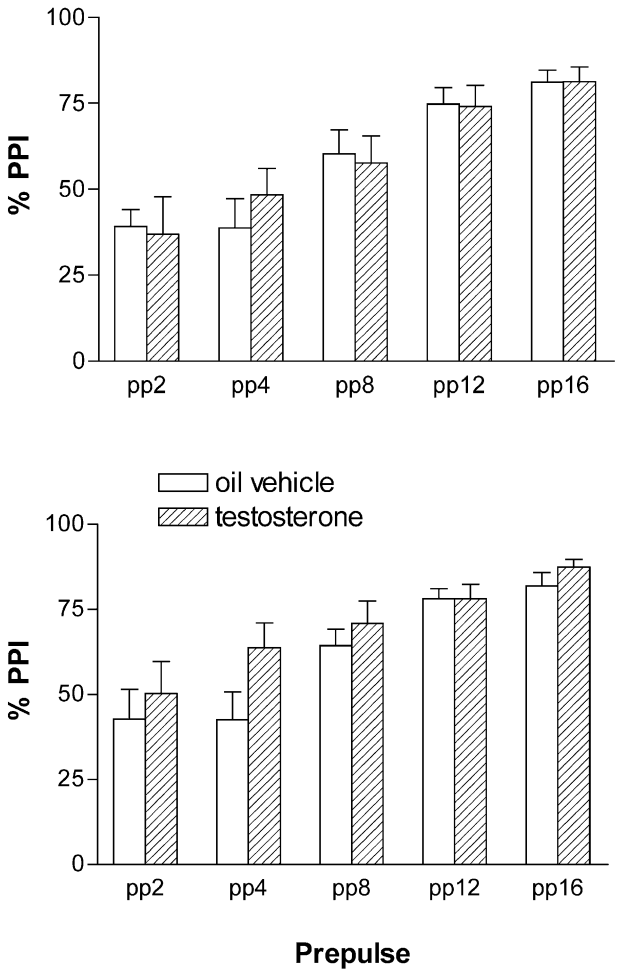

Fig. 3. Prepulse inhibition of acoustic startle in ten ovariectomized rats 18 h after treatment with oil vehicle (white bars) or 1 mg/kg of testosterone (hatched bars). Data were obtained 30 min (top panel) and 18 h after treatment (bottom panel) and are expressed as mean % prepulse inhibition ± S.E.M.

As illustrated in Fig. 4, while there was the expected significant reduction in startle responses by prepulses ($F_{(4,36)} = 41.2$, $P < 0.001$), there was no significant effect of dihydrotestosterone treatment on prepulse inhibition or a statistical interaction.

3.5. Experiment 5: interaction of estrogen with the effects of apomorphine and MK-801 on prepulse inhibition

After all four combinations of pretreatment with either oil vehicle or 50 μg/kg of 17β-estradiol and treatment with either saline or apomorphine, significant habituation of the startle response occurred during the session ($F_{(3,27)} = 32.2$, $P < 0.001$). A significant interaction of block × treatment ($F_{(3,27)} = 5.1$, $P = 0.006$) reflected the finding that apomorphine-treated rats, irrespective whether they were given pretreatment with oil vehicle or estrogen, habituated more rapidly when compared to saline-treated animals (Table 2).

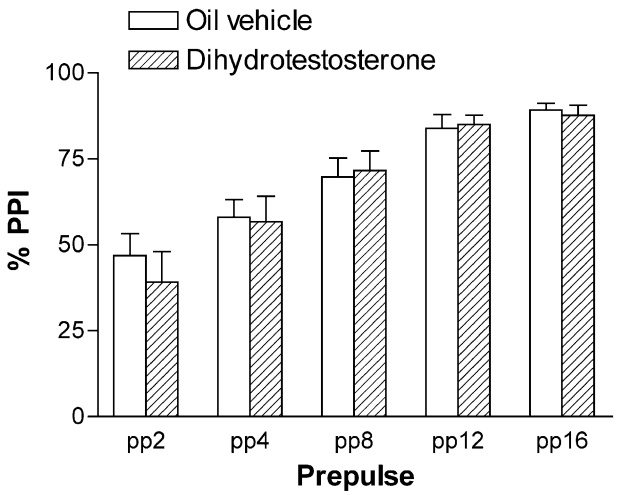

Fig. 4. Prepulse inhibition of acoustic startle in 10 ovariectomized rats 18 h after treatment with oil vehicle (white bars) or 1 mg/kg of dihydrotestosterone (hatched bars). Data are expressed as mean % prepulse inhibition ± S.E.M.

Previous studies have shown that the disruption of prepulse inhibition by apomorphine is most prominent on the lower prepulse intensities. When analyzing data from prepulse-2, prepulse-4 and prepulse-8, there was a significant effect of apomorphine treatment ($F_{(1,9)} = 13.9$, $P = 0.005$). On the other hand, while estrogen pretreatment caused a significant overall increase of prepulse inhibition ($F_{(1,9)} = 6.9$, $P < 0.027$), the lack of an interaction between treatment and pretreatment shows that this effect was similar in saline- or apomorphine-treated animals (Fig. 5). In addition to these drug effects, there was a highly significant overall effect of prepulse intensity ($F_{(2,18)} = 68.2$, $P < 0.001$).

In the MK-801 experiment (Table 2, Fig. 5), again after all four combinations of pretreatments (oil vehicle or estrogen) and treatments (saline or MK-801), a significant overall habituation of startle responses occurred during the session ($F_{(3,27)} = 8.3$, $P < 0.001$). MK-801 treatment caused a marked overall increase in startle amplitude

Table 2
Startle amplitude in response to a 40-ms, 115-dB tone and startle habituation in 10 ovariectomized Sprague–Dawley rats 18 h after subcutaneous injection of oil vehicle or 50 μg/kg of estrogen, and 10 min after subcutaneous injection of saline, 0.1 mg/kg of apomorphine, or 0.1 mg/kg of MK-801

	Block 1	Block 2	Block 3	Block 4
Vehicle/saline	214 ± 22	196 ± 23	155 ± 22	162 ± 23
Estrogen/saline	254 ± 39	182 ± 18	178 ± 24	149 ± 22
Vehicle/Apomorphine	302 ± 63	144 ± 20	138 ± 24	108 ± 26
Estrogen/Apomorphine	265 ± 47	151 ± 21	140 ± 25	123 ± 25
Vehicle/MK-801	576 ± 140	343 ± 48	317 ± 42	309 ± 40
Estrogen/MK-801	611 ± 133	389 ± 66	309 ± 34	303 ± 44

Data are mean arbitrary units ± S.E.M. as obtained from the SRLab startle system by calculating the median of blocks of ten 115-dB pulses, before (block 1), during (blocks 2 and 3) and after (block 4) a prepulse inhibition session.

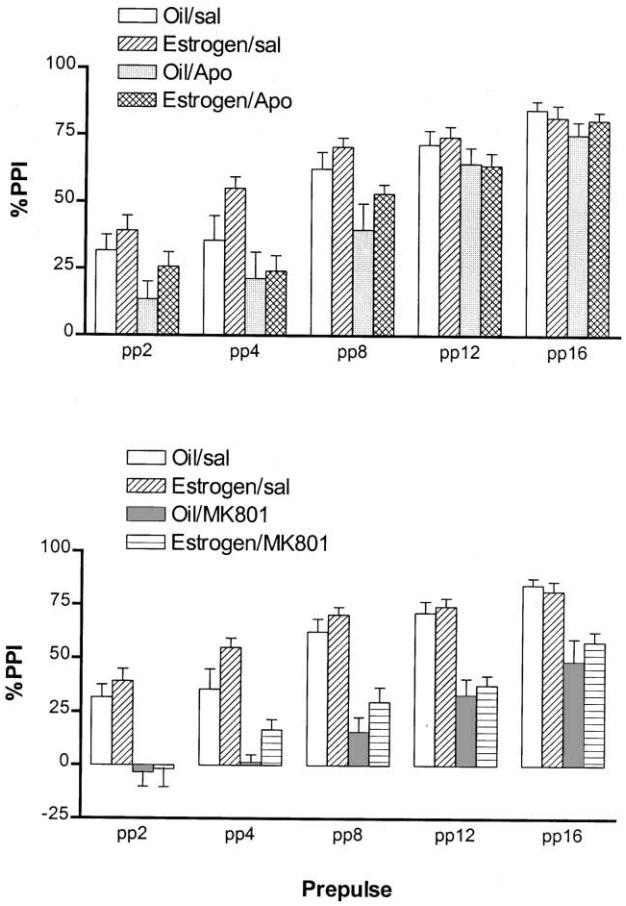

Fig. 5. Prepulse inhibition of acoustic startle in 10 ovariectomized rats 18 h after treatment with oil vehicle (white bars and stippled bars) or 50 μg/kg of 17β-estradiol (hatched bars) and 10 min after injection of either saline, 0.1 mg/kg of apomorphine (top panel), or MK-801 (bottom panel). Data are mean % prepulse inhibition ± S.E.M.

($F_{(1,9)} = 24.1$, $P = 0.001$) and, as with apomorphine, resulted in a more rapid habituation of startle responses compared to saline-treatment (block by treatment interaction ($F_{(3,27)} = 3.5$, $P = 0.030$).

While MK-801 caused a marked disruption of prepulse inhibition ($F_{(1,9)} = 54.5$, $P < 0.001$), the overall effect of prepulse intensity was still significant ($F_{(2,18)} = 28.9$, $P < 0.001$). Estrogen pretreatment significantly increased prepulse inhibition ($F_{(1,9)} = 7.6$, $P = 0.022$), although this effect was similar in saline-treated and MK-801-treated rats, as shown by the lack of statistical interaction between treatment and pretreatment. Thus, estrogen treatment increased prepulse inhibition in rats treated with apomorphine or MK-801, but the extent of this effect was similar as that seen in saline-treated controls (Fig. 5).

4. Discussion

This study produced a number of new findings on the modulatory effects of sex steroids on central nervous system function. We observed that estrogen treatment of

ovariectomized rats induced a dose-dependent increase in prepulse inhibition of the acoustic startle response in ovariectomized rats without changes in startle habituation. The effect of estrogen occurred for all prepulse intensities at 18 h after injection, whereas it was only observed for higher prepulse intensities at 30 min after injection. This prolonged and slow development of the effect of estrogen suggests that it is mediated by a genomic action of the steroid on nuclear receptors, rather than a rapid, non-genomic effect on plasma membrane receptors (Fink et al., 1996; McEwen, 1991). However, further experiments, for example with bovine serum albumin-conjugated estrogen, which does not penetrate the cell membrane, are needed to definitively prove this assumption (Zheng et al., 1996). Whichever the cellular mechanism, the effect of estrogen was mimicked by testosterone, but not dihydro-testosterone. While the effect of testosterone appeared to be more modest than that of estrogen, this could be explained by the relatively low dose of testosterone we used in our study compared to previous studies (Sumner and Fink, 1998). Because dihydrotestosterone cannot be converted by aromatase to estrogen, its lack of effect suggests that testosterone itself is converted to estrogen before it can induce an increase in prepulse inhibition (Sumner and Fink, 1998). One implication of this finding is that testosterone, and presumably estrogen as well, may be acting in the brain in a region rich in aromatase. It is interesting to note that two of the brain regions where moderate to high levels of aromatase gene expression have been detected, the nucleus accumbens and amygdala (Foidart et al., 1995; Jakab et al., 1993), are also implicated in the regulation of prepulse inhibition (Decker et al., 1995; Wan and Swerdlow, 1993). Estrogen receptors, both of the alpha and beta subtype, are found both in the nucleus accumbens and amygdala (Shughrue et al., 1997) and could mediate the effects of estrogen and converted testosterone on prepulse inhibition. However, further studies with local intracerebral injections are needed to verify the site of action of estrogen and testosterone on prepulse inhibition.

While administration of estrogen significantly increased prepulse inhibition, ovariectomy could have been expected to have the reverse effect, i.e. a decrease of prepulse inhibition. However, we observed no significant difference in startle amplitude and prepulse inhibition before and 1 week after ovariectomy. It should be noted that further experiments with sham-operated female rats will need to be done to investigate whether, using this interval, there is normally a time-related change in prepulse inhibition which did not occur in the ovariectomized rats. Such a possibility seems unlikely, however, as we have seen no such time-related effects in intact female rats tested at similar intervals (data not shown). A lack of effect of ovariectomy could indicate that estrogen does not have a tonic modulatory role in the regulation of prepulse inhibition, but is only important in certain physiological states such as during some stages of the estrous cycle (Koch, 1998) or pregnancy. However, this would appear in contrast to the clinical findings that prepulse inhibition is reduced during the period of midluteal elevations of circulating estrogen levels and thus appears to decrease prepulse inhibition (Swerdlow et al., 1997). An explanation could be that changes in prepulse inhibition during the menstrual cycle in women or the estrous cycle in rats (Koch, 1998; Swerdlow et al., 1997) are in fact determined not only by varying levels of estrogen, but also by other ovarian hormonal influences such as progesterone or inhibin. Rupprecht and co-workers recently showed that administration of progesterone caused a dose-dependent, but partial, reversal of the disruption of prepulse inhibition induced by apomorphine treatment (Rupprecht et al., 1999). Baseline prepulse inhibition was not enhanced by progesterone treatment (Rupprecht et al., 1999). It is possible that combined treatment with estrogen and progesterone could lead to a complete restoration of disrupted prepulse inhibition.

The involvement of other hormones is also suggested by neurochemical observations that estrogen appears to enhance stimulated dopamine release from slices of the striatum at low doses and reduces it at high doses (Becker, 1990). Given that dopaminergic stimulation, such as occurs with amphetamine- or apomorphine treatment, disrupts prepulse inhibition, high circulating levels of estrogen during the midluteal phase of the menstrual cycle should reduce this effect and, hence, increase prepulse inhibition. Such an effect is in contrast with the model that it is cycling levels of estrogen only that modulate prepulse inhibition during the menstrual cycle. In the present study, we assessed the effect of estrogen administration without the confounding effect of other hormones that could theoretically oppose or alter its effect. Our conclusion is, that estrogen, at least when administered at the doses we used and at the time intervals we tested, increased prepulse inhibition.

The central neurochemical mechanism for the effect of estrogen on prepulse inhibition is unclear. We administered estrogen in rats that were subsequently treated with apomorphine or MK-801 to assess if estrogen would interact specifically with central dopaminergic or glutamatergic mechanisms. While both apomorphine and MK-801 significantly disrupted prepulse inhibition, the effect of estrogen was essentially similar whether rats received no additional treatment, or either of these drugs. In addition, the effect of estrogen in rats treated with apomorphine or MK-801 was modest at best, possibly indicating that this treatment has little potential to restore sensorimotor gating deficits in patients with schizophrenia. Pretreatment with clinically used antipsychotic drugs, such as haloperidol and clozapine, completely reverses the disruption of prepulse inhibition by apomorphine (Rupprecht et al., 1999; Swerdlow and Geyer, 1993). In contrast, there is discrepancy in the literature as to whether the disruption of prepulse inhibition by MK-801 treatment is blocked by antipsychotic treatment (Bakshi et al., 1994; Hoffman et al., 1993).

While an interaction with dopaminergic or glutamatergic pathways cannot be excluded, these results may indicate that estrogen treatment stimulates a central mechanism completely separate from either of these mechanisms involved in prepulse inhibition. The effects of estrogen are then simply additive to those of apomorphine and MK-801. For example, estrogen has been shown to reduce expression of 5-HT_{1A} receptors in different parts of the brain at doses and time intervals comparable to the ones used in the present study (Osterlund and Hurd, 1998). Administration of 5-HT_{1A} receptor agonists has previously been shown to reduce prepulse inhibition (Rigdon and Weatherspoon, 1992). Thus, endogenous serotonin, acting on 5-HT_{1A} receptors, may reduce prepulse inhibition, an involvement that could be reversed by the down-regulation of these receptors by estrogen. In this way, estrogen acts similar to 5-HT_{1A} receptor antagonists that have been shown to increase prepulse inhibition (Wedzony et al., 2000).

Estrogen has also been shown to increase the expression of 5-HT_{2A} receptors (Sumner and Fink, 1995). However, an involvement of this mechanism in the present results seems unlikely, as 5-HT_{2A} receptors antagonists reverse disruption of prepulse inhibition by drugs such as phencyclidine (Sipes and Geyer, 1994; Varty et al., 1999). Thus, the increased expression of these receptors would lead to the opposite effect, i.e. reduced prepulse inhibition. Furthermore, drugs acting at 5-HT_{2A} receptors have been shown to alter startle amplitude or startle habituation (Geyer and Tapson, 1988), an effect not consistently seen with estrogen treatment.

In addition to serotonergic mechanisms, the activity of several other neurotransmitter systems is modulated by sex steroids (Liaw et al., 1992; Shughrue et al., 2000) and further experiments will be needed to explore these interactions.

In conclusion, the present study demonstrated that estrogen may modulate prepulse inhibition. Startle amplitude was reduced at 30 min, but not 18 h after injection and there was no effect on startle habituation. These results could help to explain the apparent protective effect of estrogen on the psychopathology of schizophrenia (Häfner et al., 1993; Seeman, 1997) and the beneficial effects of estrogen treatment in psychosis (Kulkarni et al., 1996).

References

Angermeyer, M.C., Kuhn, L., 1988. Gender differences in age at onset of schizophrenia: an overview. Eur. Arch. Psychiatry Neurol. Sci. 237, 351–364.

Bakshi, V.P., Swerdlow, N.R., Geyer, M.A., 1994. Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. J. Pharmacol. Exp. Ther. 271, 787–794.

Becker, J., 1990. Direct effect of 17β-estradiol on striatum: sex differences in dopamine release. Synapse 5, 157–164.

Braff, D.L., Geyer, M.A., 1990. Sensorimotor gating and schizophrenia: human and animal model studies. Arch. Gen. Psychiatry 47, 181–188.

Decker, M.W., Curzon, P., Brioni, J.D., 1995. Influence of separate and combined septal and amygdala lesions on memory, acoustic startle, anxiety, and locomotor activity in rats. Neurobiol. Learn. Mem. 64, 156–168.

Di Paolo, T., 1994. Modulation of brain dopamine transmission by sex steroids. Rev. Neurosci. 5, 27–42.

Eikelis, N., Van den Buuse, M., 2001. Cardiovascular responses to 'open-field' stress in rats: sex differences and effects of gonadal hormones. Stress 3, 319–334.

Fink, G., Summer, B.E.H., Rosie, R., Grace, O., Quinn, J.P., 1996. Estrogen control of central neurotransmission: effect of mood, mental state, and memory. Cell. Mol. Neurobiol. 16, 325–344.

Foidart, A., Harada, N., Balthazart, J., 1995. Aromatase-immunoreactive cells are present in mouse brain areas that are known to express high levels of aromatase activity. Cell Tissue Res. 280, 561–574.

Geyer, M.A., 1999. Assessing prepulse inhibition of startle in wild-type and knockout mice. Psychopharmacology 147, 11–13.

Geyer, M.A., Swerdlow, N.R., 1998. Measurement of startle response, prepulse inhibition, and habituation. In: Crawley, J.N., Skolnick, P. (Eds.), Current Protocols in Neuroscience. Wiley, New York, pp. 1–15, Suppl.3: Unit 8.7.

Geyer, M.A., Tapson, G.S., 1988. Habituation of tactile startle is altered by drugs acting on serotonin-2 receptors. Neuropsychopharmacology 1, 135–147.

Geyer, M.A., Swerdlow, N.R., Mansbach, R.S., Braff, D.L., 1990. Startle response models of sensorimotor gating and habituation deficits in schizophrenia. Brain Res. Bull. 25, 485–498.

Häfner, H., Riecher-Rössler, A., An der Heiden, W., Maurer, K., Fätkenheuer, B., Löffler, W., 1993. Generating and testing a causal explanation of the gender difference in age at first onset of schizophrenia. Psychol. Med. 23, 925–940.

Hirsch, S.R., Weinberger, D.R., 1995. Schizophrenia. Blackwell, Oxford.

Hoffman, D.C., Donovan, H., Cassella, J.V., 1993. The effects of haloperidol and clozapine on the disruption of sensorimotor gating induced by the noncompetitive glutamate antagonist MK-801. Psychopharmacology 111, 339–344.

Jakab, R.L., Horvath, T.L., Leranth, C., Harada, N., Naftolin, F., 1993. Aromatase immunoreactivity in the rat brain: gonadectomy-sensitive hypothalamic neurons and an unresponsive "limbic ring" of the lateral septum-bed nucleus–amygdala complex. J. Steroid Biochem. Mol. Biol. 44, 481–498.

Koch, M., 1998. Sensorimotor gating changes across the estrous cycle in female rats. Physiol. Behav. 64, 625–628.

Korhonen, S., Saarijarvi, S., Aito, M., 1995. Successful estradiol treatment of psychotic symptoms in the premenstrual phase: a case report. Acta Psychiatr. Scand. 92, 237–238.

Kulkarni, J., De Castella, A., Smith, D., Taffe, J., Keks, N., Copolov, D., 1996. A clinical trial of the effects of estrogen in acutely psychotic women. Schizophr. Res. 20, 247–252.

Kumari, V., Soni, W., Sharma, T., 1999. Normalization of information processing deficits in schizophrenia with clozapine. Am. J. Psychiatry 156, 1046–1051.

Liaw, J.J., He, J.R., Hartman, R.D., Barraclough, C.A., 1992. Changes in tyrosine hydroxylase mRNA levels in medullary A1 and A2 neurons and locus coeruleus following castration and estrogen replacement in rats. Mol. Brain Res. 13, 231–238.

McEwen, B.S., 1991. Steroid hormones are multifunctional messengers to the brain. Trends Endocrinol. Metab. 2, 62–67.

Menniti, F.S., Baum, M.J., 1981. Differential effects of estrogen and androgen on locomotor activity induced in castrated male rats by amphetamine, a novel environment, or apomorphine. Brain Res. 216, 89–107.

Osterlund, M.K., Hurd, Y.L., 1998. Acute 17beta-estradiol treatment down-regulates serotonin 5HT1A receptor mRNA expression in the limbic system of female rats. Mol. Brain Res. 55, 169–172.

Rigdon, G.C., Weatherspoon, J.K., 1992. 5-Hydroxytryptamine$_{1A}$ receptor agonists block prepulse inhibition of acoustic startle reflex. J. Pharmacol. Exp. Ther. 263, 486–493.

Rupprecht, R., Koch, M., Montkowski, A., Lancel, M., Faulhaber, J., Harting, J., Spanagel, R., 1999. Assessment of neuroleptic-like properties of progesterone. Psychopharmacology 143, 29–38.

Seeman, M.V., 1997. Psychopathology in women and men: focus on female hormones. Am. J. Psychiatry 154, 1641–1647.

Shughrue, P.J., Lane, M.V., Merchenthaler, I., 1997. Comparative distribution of estrogen receptor-α and -β mRNA in the rat central nervous system. J. Comp. Neurol. 388, 507–525.

Shughrue, P.J., Scrimo, P.J., Merchenthaler, I., 2000. Estrogen binding and estrogen receptor characterization (ERα and ERβ) in the cholinergic neurons of the rat basal forebrain. Neuroscience 96, 41–49.

Sipes, T.A., Geyer, M.A., 1994. Multiple serotonin receptor subtypes modulate prepulse inhibition of the startle response in rats. Neuropharmacology 33, 441–448.

Sumner, B., Fink, G., 1995. Estrogen increases the density of 5-HT$_{2A}$ receptors in cerebral cortex and nucleus accumbens in the female rat. J. Steroid Biochem. Mol. Biol. 54, 15–20.

Sumner, B., Fink, G., 1998. Testosterone as well as estrogen increases serotonin$_{2A}$ receptor mRNA and binding site densities in the male rat brain. Mol. Brain Res. 59, 205–214.

Swerdlow, N.R., Geyer, M.A., 1993. Clozapine and haloperidol in an animal model of sensorimotor gating deficits in schizophrenia. Pharmacol. Biochem. Behav. 44, 741–744.

Swerdlow, N.R., Geyer, M.A., 1998. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. Schizophr. Bull. 24, 285–301.

Swerdlow, N.R., Auerbach, P., Monroe, S.M., Hartston, H., Geyer, M.A., Braff, D.L., 1993. Men are more inhibited than women by weak prepulses. Biol. Psychiatry 34, 253–260.

Swerdlow, N.R., Hartman, P.L., Auerbach, P.P., 1997. Changes in sensorimotor inhibition across the menstrual cycle: implications for neuropsychiatric disorders. Biol. Psychiatry 41, 452–460.

Varty, G.B., Bakshi, V.P., Geyer, M.A., 1999. M100907, a serotonin 5-HT$_{2A}$ receptor antagonist and putative antipsychotic, blocks dizocilpine-induced prepulse inhibition deficits in Sprague–Dawley and Wistar rats. Neuropsychopharmacology 20, 311–321.

Wan, F.J., Swerdlow, N.R., 1993. Intra-accumbens infusion of quinpirole impairs sensorimotor gating of acoustic startle in rats. Psychopharmacology 113, 103–109.

Wedzony, K., Mackowiak, M., Zajaczkowski, W., Fijal, K., Chocyk, A., Czyrak, A., 2000. WAY 100135, an antagonist of 5-HT$_{1A}$ serotonin receptors, attenuates psychotomimetic effects of MK-801. Neuropsychopharmacology 23, 547–559.

West, C.H., Michael, R.P., 1986. Time-dependent modulation by estrogen of amphetamine-induced hyperactivity in male rats. Pharmacol. Biochem. Behav. 25, 919–923.

Zheng, J., Ali, A., Ramirez, V.D., 1996. Steroids conjugated to bovine serum albumin as tools to demonstrate specific steroid neuronal membrane binding sites. J. Psychiatry Neurosci. 21, 187–197.