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Research paper

Chronic estrogen and progesterone treatment inhibits ketamine-induced disruption of prepulse inhibition in rats

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HIGHLIGHTS

- Ketamine dose-dependently induced disruption of prepulse inhibition (PPI).
- Estrogen (E) implants had no effect on the action of ketamine on PPI.
- E + progesterone (E + P) implants significantly reduced effect of 10 mg/kg ketamine.
- E + P interaction with the effect of ketamine may involve $GABA_A$ receptors.

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ABSTRACT

Ketamine is a dissociative anesthetic and antagonist of *N*-methyl-D-aspartate receptors (NMDAr). Hypofunction of NMDAr may underlie some schizophrenia symptoms and the psychotomimetic effects of ketamine have been used to model this hypofunction. Gender differences exist in the age of onset and symptom profile of schizophrenia and sex steroid hormones have been successfully trialed as adjunctive treatment in this illness; however, the mechanism of action of these hormone treatment strategies remains unclear. The aim of this study was therefore to investigate the effect of sex steroid hormones on ketamine-induced disruption of prepulse inhibition (PPI), an endophenotype of schizophrenia. Female ovariectomized (OVX) rats did not show altered effects of ketamine compared to intact rats. There were also no significant changes in the effect of ketamine on PPI in OVX rats implanted with a high dose of estrogen. In contrast, in OVX rats implanted with a low dose of estrogen plus progesterone, the effect of 10 mg/kg ketamine was significantly reduced. There were no parallel changes in startle amplitude. These results differ from previous studies on the effect of sex steroid hormones on the disruption of PPI by treatment with the NMDAr antagonist, MK-801, or dopaminergic drugs, such as apomorphine. We speculate that this differential effect of sex steroids on the action of ketamine is mediated by mechanisms other than dopaminergic stimulation or NMDA receptor blockade, for example $GABA_A$ receptors. These results extend our understanding of the effects of sex steroid hormones on PPI and their use as potential treatments in schizophrenia.

1. Introduction

Ketamine is a rapidly, but short-acting antagonist at *N*-methyl-D-aspartate (NMDA) receptors with some effects on opioid, muscarinic and a number of voltage-gated receptors [28]. It has been used for many years as an anesthetic in emergency, pediatric and veterinary medicine [28]. More recently, ketamine has been successfully trialed as a rapidly-acting antidepressant [5,23]. Ketamine is also widely abused as an illicit recreational drug, producing psychedelic and dissociative experiences [22,35].

There are prominent sex differences in the incidence and symptomology of psychiatric illnesses. Sex steroid hormones, such as estrogen and progesterone, modulate the antidepressant action of ketamine in rats [1] although less is known about sex differences or the effect of sex hormones in the psychotomimetic action or abuse potential of ketamine [16]. This is despite well-established gender differences in the epidemiology of psychotic illnesses, such as schizophrenia [10,25], and the similarly widely-accepted hypothesis that hypoactivation of NMDA receptors may

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be one major contributor to schizophrenia symptoms [24,31]. Sex steroid hormones, such as oestrogen, have shown promising effects as adjunctive treatments in schizophrenia [15] but the role of NMDA receptors in this clinical effect is unknown.

In the present study, we used prepulse inhibition of acoustic startle (PPI) to investigate the effect of chronic treatment with estrogen or a combination of estrogen and progesterone on the action of ketamine. PPI is a measure of sensory gating which is reduced in psychosis and acutely by treatment with psychotomimetic drugs, including ketamine [6,32]. PPI varies across the estrous cycle [13] and is increased by estrogen treatment [33]. We have shown recently that chronic treatment with estrogen partially reduces the disruption of PPI caused by the NMDA receptor antagonist, MK-801 [8], similar to its effect to prevent the disruption of PPI caused by treatment with the dopamine receptor agonist, apomorphine [7]. Here we extend this work to ketamine and compared its effect in ovariectomized (OVX) rats with or without a chronic estradiol implant [7]. We also included animals with both a combination of a low-dose estrogen and a progesterone implant as we have shown that this combined treatment can produce similar effects to those seen after high-dose estrogen only [9].

Table 1
Body weight (BW) and uterus weight (UW) of animals used in the experiments. Average PPI and average startle following treatment with saline (SAL), 3 mg/kg ketamine (Ket3) or 10 mg/kg ketamine (Ket10).

	Intact	OVX	E100	E20 + P
n	11	13	13	11
Surgery BW	261 ± 9	249 ± 5	252 ± 5	245 ± 6
Weight gain	49.9 ± 5.2*	76.8 ± 6.5	21.3 ± 3.3*	30.5 ± 3.9*
UW	0.52 ± 0.06*	0.13 ± 0.01	0.65 ± 0.04*	0.51 ± 0.04*
%UW/BW	0.166 ± 0.017*	0.041 ± 0.004	0.238 ± 0.013*	0.185 ± 0.014*
Average PPI				
Saline	48.9 ± 4.4	51.5 ± 2.5	55.2 ± 2.9	50.5 ± 3.5
Ket3	45.5 ± 4.7	45.6 ± 2.5	44.0 ± 2.5	48.0 ± 5.6
Ket10	29.6 ± 2.5**	28.2 ± 3.4**	24.5 ± 4.1**	40.0 ± 4.1**,*
Average startle				
Saline	273 ± 22	310 ± 51	209 ± 22	191 ± 25
Ket3	284 ± 25	345 ± 43	248 ± 32	236 ± 26
Ket10	282 ± 28	268 ± 39	257 ± 45	223 ± 29

Data are mean ± S.E.M.

* $P < 0.05$ for difference with OVX group as assessed by one-way ANOVA and Bonferroni-corrected pairwise comparison for weight data and two-way ANOVA for change in PPI after ketamine treatment.

** $P < 0.05$ for difference with PPI after saline treatment.

2. Methods

Forty-eight female Sprague-Dawley rats were obtained from a commercial supplier (Animal Resource Centre, Perth, Australia) and housed in groups of 2–4 in individually-ventilated cages with ad libitum access to food and water. All surgical procedures 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 (OVX) surgery was performed when the rats were 11–12 weeks of age as described previously [9]. The rats were anesthetised with isoflurane and treated with the analgesic/anti-inflammatory agent, Carprofen (Rimadyl, 5 mg/kg), to reduce post-operative pain and discomfort. OVX was done via a 2-cm incision along the dorsal midline and small incisions through the lateral muscle wall. Sham-operated rats (Intact group) underwent the same surgical procedure except their ovaries were not removed. The rats were also implanted with a subcutaneous silastic implant which was either empty (Intact and OVX groups) or one containing either 100% crystalline estrogen (E100 group, 17β-estradiol, Sigma Chemical Company, St. Louis, USA), or one implant containing a 20% crystalline estrogen and 80% cholesterol (5-Cholesten-3β-OL, Steraloids, Newport, RI, USA) mix and one implant containing 100% crystalline progesterone (E20 + P group, 4-pregnene-3,20-dione, Sigma) [9]. The rats were given two weeks to recover before testing. Post-testing, the rats were euthanized with CO_2 inhalation and the uterus was removed and weighed to determine the effect of the hormone replacement (Table 1).

PPI was assessed using SR-Lab startle chambers (San Diego Instruments, San Diego, CA, USA). Each chamber contained a transparent Plexiglas cylinder (8.8 cm in diameter) which had a piezoelectric transducer to measure the whole-body startle responses to acoustic noise bursts which were delivered using the SR-Lab software. There were 100 trials in each PPI session [7,9] with ten no-stimulus trials, forty 115 dB pulse-alone trials and fifty prepulse trials. Prepulse trials consisted of 2, 4, 8, 12 or 16 dB above the 70 dB background noise (10 of each), followed 100 ms later by a startle pulse of 115 dB. Inter-trial interval was varied (10–37 s) to prevent learned and habituated responses. The four blocks of ten 115 dB pulses were used to assess the average startle amplitude. The percentage PPI was calculated as the difference in amplitude between the startle response to the pulse-alone trials and the prepulse trials, divided by the pulse-alone trial X 100% [7,9]. The rats were tested 5–10 min after subcutaneous injection of either saline vehicle, 3 mg/kg of ketamine (Ceva Animal Health, Glenorie, NSW, Australia) or 10 mg/kg of ketamine in a pseudo-randomised order. There was a minimum of three days between testing to allow for wash-out of the drug.

All results are expressed as mean ± Standard Error of the Mean (SEM) and were analysed using Analysis of Variance (ANOVA, SPSS Inc., Chicago, IL, USA). Homogeneity was assessed with Levene's test for equality of variance ($P > 0.05$). When analysing PPI data, Mauchley's test was violated in most cases ($P < 0.05$), therefore the Greenhouse–Geisser correction was used. Where relevant statistical interactions were found, results were further explored by comparing each hormone group to the OVX group. Main effects of Block (startle amplitudes) and prepulse intensity (PPI) were always observed and are not listed here in detail unless relevant interactions with other factors were found. Differences were considered significant at $P < 0.05$.

3. Results

There were no significant differences between the rat groups' pre-surgery body weights (Table 1). Analysis of post-operative weight gain showed a significant difference between hormone groups ($F_{3,44} = 25.9$, $P < 0.001$, Table 1). Post-hoc analysis revealed that weight gain was significantly higher in OVX rats than in all other groups. As expected, uterus weight at the end of the experiment was also significantly different between the hormone groups, both when expressed as absolute weight ($F_{3,44} = 33.7$, $P < 0.001$) or as a percentage of bodyweight ($F_{3,44} = 50.5$, $P < 0.001$). Post-hoc analysis revealed that uterus weight was significantly higher in Intact rats, E100, and E20 + P rats when compared to OVX rats (all, $P < 0.001$; Table 1).

There were no differences between the hormone groups in terms of average PPI or startle after saline injection (Table 1). Ketamine treatment disrupted PPI ($F_{1.8,71.1} = 34.8$, $P < 0.001$) and this effect was significant for both the 3 mg/kg ($F_{1.0,43.0} = 5.6$, $P = 0.023$) and 10 mg/kg dose ($F_{1.0,40.0} = 57.4$, $P < 0.001$). The effect of 3 mg/kg was dependent on prepulse intensity ($F_{2.1,91.4} = 3.3$, $P = 0.038$) but was not different between the groups (Fig. 1). The effect of 10 mg/kg was also dependent on the prepulse intensity ($F_{2.5,100.6} = 8.3$, $P < 0.001$) and analysis revealed a significant ketamine *x* group interaction ($F_{3.0,40.0} = 3.1$, $P = 0.037$) and a trend for a ketamine *x* prepulse intensity *x* group interaction