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Allopregnanolone has no effect on startle response and prepulse inhibition of startle response in patients with premenstrual dysphoric disorder or healthy controls

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ABSTRACT

Background: Allopregnanolone is an endogenous neuroactive steroid which, through the binding to the $GABA_A$ receptor, enhances inhibitory neurotransmission and exerts anxiolytic, sedative and antiepileptic effects. Following acute administration, allopregnanolone reliably acts as an anxiolytic compound. The primary aim of this study was to investigate if allopregnanolone, administered to healthy women and women with premenstrual dysphoric disorder (PMDD), would have an anxiolytic effect, expressed as a decreased startle response.
Materials and methods: Sixteen PMDD patients and twelve healthy controls completed the study. The participants were scheduled for the startle tests twice in the luteal phase. During the test sessions an intravenous allopregnanolone and placebo bolus injection was administered in double-blinded, randomized order at intervals of 48 h. Following the allopregnanolone/placebo injections startle response and prepulse inhibition of startle response (PPI) were assessed by electromyography.
Results: Following the intravenous allopregnanolone administration the serum concentrations of allopregnanolone increased to 50–70 nmol/l, corresponding to levels that are seen during pregnancy. The obtained serum concentrations of allopregnanolone were significantly lower in PMDD patients than among the healthy controls, $p<0.05$. The allopregnanolone injection resulted in significant increases of self-rated sedation in both groups, $p<0.01$. Allopregnanolone did not induce any changes in startle response or prepulse inhibition of startle response in comparison to placebo. No differences in allopregnanolone-induced changes in startle response or PPI could be detected between PMDD patients and controls subjects.
Conclusion: Startle response and PPI were unaffected by acute intravenous administration of allopregnanolone in PMDD patients and healthy controls.

1. Introduction

Allopregnanolone (3α-hydroxy-5α-pregnan-20-one) is an endogenous neuroactive steroid, which is secreted by the mammalian ovary (Ottander et al., 2005) and adrenal cortex (Genazzani et al., 1998), but also metabolized from progesterone and synthesized in the CNS (Bixo et al., 1997; Compagnone and Mellon, 2000). Through binding to the $GABA_A$ receptor, allopregnanolone enhances inhibitory neurotransmission (Majewska et al., 1986), thus exerting anxiolytic (Bitran et al., 1991), sedative (Timby et al., 2005) and antiepileptic effects (Landgren et al., 1998). In healthy women, the serum concentration of allopregnanolone is approximately 1 nmol/l in the follicular phase, it varies between 0.7–4 nmol/l during the mid-luteal phase and between 0.9–2.0 nmol/l during the late luteal phase (Genazzani et al., 1998; Nyberg et al., 2005; Wang et al., 1996). During pregnancy, serum allopregnanolone levels progressively increase, resulting in serum concentrations of approximately 100 nmol/l at term (Hill et al., 2001; Luisi et al., 2000).

Prior studies have consistently indicated that drugs that alter the GABAergic system are anxiolytic (Laconi et al., 2001) and have a suppressant effect on the startle response. For instance, benzodiazepines such as diazepam (Abduljawad et al., 1997) and alprazolam (Riba et al., 2001) have been shown to inhibit the startle response. Less is known about how neurosteroids affect this behavioral measure, which generally is regarded as an anxiety model. Prior studies have indicated that progesterone and allopregnanolone might induce differential changes in the startle response. Whereas allopregnanolone attenuates CRH-enhanced startle response, medroxyprogesterone acetate, acting via the progesterone receptor, has been shown to amplify CRH-enhanced startle response (Toufexis et al., 2004). However, during withdrawal of progesterone, the acoustic startle response is increased (Gulinello et al., 2003).

We have previously reported that patients with premenstrual dysphoric disorder (PMDD) have an increased startle response in

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comparison to healthy controls (Kask et al., 2008b) and it has also been suggested that startle response is enhanced during the luteal phase as compared to the follicular phase in PMDD patients (Epperson et al., 2007). PMDD is characterized by psychological and physical symptoms appearing in the luteal phase of the menstrual cycle. Symptom severity increases successively during the luteal phase and is most pronounced during the last five premenstrual days and on the first days of bleeding, when progesterone levels are declining (Backstrom et al., 2003). Symptoms occur only during ovulatory menstrual cycles in the presence of a corpus luteum (Hammarback and Backstrom, 1988; Hammarback et al., 1991), or during progesterone administration (Bjorn et al., 2002, 2003; Hammarback et al., 1985). Whether progesterone, or its metabolite allopregnanolone, contributes to the increased startle response previously found in PMDD remains unclear.

Sensorimotor gating can also be assessed by measuring prepulse inhibition (PPI) of the startle response, which is thought to reflect an individual's ability to screen or "gate" sensory stimuli. The PPI paradigm uses a weak, non-startling acoustic stimulus (the prepulse) that typically decreases the reflexive eye blink response (startle) produced by the subsequent startling stimulus (the pulse). Deficits in sensorimotor gating have been associated with impaired cognitive processing and subjects with deficient PPI are characterized by an inability to inhibit irrelevant information in different domains (Braff et al., 2001). Reduced PPI is observed in several psychiatric disorders, specifically schizophrenia but also in a number of anxiety disorders (Geyer et al., 2001; Braff et al., 2001). However, there is also data suggesting that ovarian steroids may influence PPI as women have lower levels of PPI than men do (Swerdlow et al., 1993), and because PPI varies across the menstrual cycle in healthy women (Jovanovic et al., 2004; Kask et al., 2008b; Swerdlow et al., 1997). We have previously shown that PMDD patients have lower levels of prepulse inhibition during the late luteal phase compared to healthy women (Kask et al., 2008b). Furthermore, PMDD patients with high anxiety levels had even more impaired PPI than less symptomatic patients (Kask et al., 2008b). Similar to the startle response, intravenously administered benzodiazepine has been shown to suppress PPI in healthy volunteers (Abduljawad et al., 2001).

Only a limited number of studies have investigated the effect of allopregnanolone in humans. The aim of this randomized, double-blinded, placebo-controlled study was to investigate if allopregnanolone, in analogy with other GABA-active compounds, could affect baseline startle response. A secondary aim was to investigate whether the previously noted luteal phase reduction in prepulse inhibition in PMDD patients is attributable to allopregnanolone, i.e. whether allopregnanolone is able to influence PPI. Finally, the study also aimed to determine whether PMDD patients and healthy controls respond differently in terms of startle response and prepulse inhibition to the allopregnanolone injection.

2. Material and methods

2.1. Subjects

Eighteen PMDD patients and 13 asymptomatic controls, between the ages of 23 and 46 were included in the study. PMDD patients were recruited among women seeking help for premenstrual symptoms at the out-patient ward of the Department of Obstetrics and Gynecology, Uppsala University Hospital, Uppsala, Sweden, and from a newspaper advertisement.

PMDD diagnosis was based on daily, prospective symptom ratings on the Cyclicity Diagnoser (CD) scale during two menstrual cycles (Sundstrom et al., 1999). The CD scale consists of nine negative mood parameters (depression, decreased interest in usual activities, fatigue, irritability, tension, mood swings, lability, difficulties in concentrating, and sleep disturbances), two positive mood parameters (cheerfulness and energy), and four somatic symptoms (food cravings, swelling, breast tenderness and menstrual bleeding). In addition, the CD scale contains a score for measuring the every-day social functioning and work performance. The CD scale is a Lickert scale ranging from 0–8, with zero as complete absence of a particular symptom, and 8 as the maximal severity of the symptom. Patients were considered to have PMDD if they had a 100% increase in at least five symptoms during seven premenstrual days, compared to seven mid-follicular days, associated with a clinically significant social and occupational impairment. The threshold for symptom severity was set at a score of four or more during at least two days during the luteal phase. This scoring indicated that subjects avoided social interaction during these days.

The control group included physically healthy women between the ages of 20 and 46 with regular menstrual cycles and no significant premenstrual dysphoric symptoms in daily prospective ratings on the CD scale.

The exclusion criteria were hearing deficiencies, ongoing pregnancy or breastfeeding, treatment with any hormonal compounds, treatment with benzodiazepines or other psychotropic drugs including serotonin reuptake inhibitors and presence of any ongoing psychiatric disorder. The presence of psychiatric disorders was evaluated using a structured psychiatric interview, the Swedish version of Mini International Neuropsychiatry Interview (MINI.), based on DSM-IV and ICD-10 (Sheehan et al., 1998).

A brief hearing screening with an audiometer (Madsen Midi-mate 622, GN Otometrics, Taastrup, Denmark) confirmed that all subjects were able to detect 40 dB tones at 500, 1000, and 6000 Hz.

The women gave written informed consent prior to inclusion in the study. The study procedures were in accordance with the ethical standards for human experimentation, and the Independent Research Ethics Committee, Uppsala University, Uppsala, Sweden; the Medical Products Agency of Sweden also approved the study.

3. Study protocol

The study had a randomized, placebo-controlled, cross-over design. Participants were tested during the late luteal phase and each subject received an allopregnanolone and placebo bolus injection in randomized order. The allopregnanolone and placebo injections were administered at intervals of 48 h.

Injection of allopregnanolone and assessment of startle response was scheduled in the late luteal phase (postovulatory days 8–13). Ovulation was determined from urine with a urine LH kit (Clearplan, Unipath, Bedford, UK). The luteal phase intervals were chosen to correspond with maximum severity of mood symptoms in the PMDD patients. We chose to examine subjects in the luteal phase, rather than the follicular phase, as increased startle response in PMDD patients appear to be more robust during the luteal phase (Epperson et al., 2007; Kask et al., 2008b). Monitoring of the luteal phase was confirmed by records on the next menstrual bleeding and also by progesterone serum concentrations.

Testing was carried out at the research laboratory of the Department of Women's and Children's Health, Uppsala University between 7:30 AM and 5:00 PM. No subjects consumed alcohol 24 h prior to testing. An intravenous cannula was inserted in each forearm. Thereafter a double-blinded, randomized intravenous bolus injection of either placebo or allopregnanolone (0.05 mg/kg) was given. The injection was given during 1 min. The dose used for the study was based on a previous study by Timby et al. (2005). The startle response test session was started as soon as the allopregnanolone/placebo injection was administered. Finally, a blood sample for allopregnanolone serum concentrations was taken immediately after the startle session was finished. Blood samples for measuring estradiol and progesterone were taken at baseline, but for technical reasons, allopregnanolone baseline levels could not be determined.

The Umeå University Hospital Pharmacy prepared the experimental medications and randomized subjects to the order of injections in blocks of four. Intravenous allopregnanolone solution was formulated with purified allopregnanolone, UC1009 (Umecrine AB, Box 7984, 907 19 Umeå, Sweden) 15 mg dissolved in 100 ml albumin solution (Pharmacia, Stockholm, Sweden, 200 mg/ml) using an ultrasound bath. The solution contained 0.126 ± 0.003 mg/ml (mean ± SEM) allopregnanolone ($n = 9$ random samples). The allopregnanolone concentration of each batch of solution was determined using HPLC and UV absorbance (Turkmen et al., 2004), which is a method with sufficient sensitivity for the high concentrations of allopregnanolone in the solution. The placebo consisted of albumin injection.

3.1. Startle response

The eye blink component of the auditory startle reflex was assessed by EMG measurements of the orbicularis oculi muscles. The delivery of the acoustic startle stimuli and the recording of the eye blink response were controlled by a commercial startle system (SR-HLAB, San Diego Instruments, San Diego, CA, USA). Acoustic startle stimuli were delivered binaurally by Telephonic (TDH-39-P, Maico, Minneapolis, USA) headphones. The sound was calibrated with a Quest Electronics device (model 210 Quest Technologies, Oconomov, WI). After the skin had been scarified with alcohol, two miniature silver/silver chloride electrodes (In Vivo Metric, Healdsburg, CA, USA), with a small amount of electrode gel (Sigma gel, In Vivo Metric, Healdsburg, CA, USA) were positioned below the subject's right eye, over the orbicularis oculi muscle. A ground electrode was placed in the center of the forehead. Electrode impedances were measured and confirmed to be less than 5-kΩ. The EMG was filtered (100–1000 Hz), digitized at 1 kHz and analyzed by the EMG startle response software. The software system recorded 250 1-ms readings starting from the onset of the startle stimulus.

In order to allow subjects to acclimatize to the test situation, the auditory startle reflex test session began with a five-minute acclimatization period, with a background of 70 dB white noise delivered through headphones. After this adaptation period, a series of trials were administered and the startle responses were recorded. Throughout the session, there was background white noise at 70 dB, which continued between the trials. The test session included four trial blocks. Block 1 consisted of five pulse-alone trials (115 dB 40 ms broad-band white noise) and was used to measure the mean baseline startle magnitude. Blocks 2 and 3 each consisted of 15 trials, containing five pulse-alone (115 dB) and 10 prepulse–pulse (PP1 = 78 dB; PP2 = 86 dB) trials presented in pseudorandom order. Prepulse trials of 78 dB and 86 dB were used in the current study as the resulting PPI is affected in PMDD patients during the late luteal phase (Kask et al., 2008b). The last block consisted of five pulse-alone trials, which allowed a measure of within-test habituation. The inter-trial interval was pseudo-randomly variable, averaging 30 s. The entire session lasted approximately 20 min.

3.2. Visual analogue ratings

A visual analogue scale (VAS) was used to rate subjective sedation before and after the allopregnanolone / placebo injections. The scale measured from 0 to 100 mm where 0 equaled complete absence of sedation and 100 represented falling asleep / heavy sedation. Subjective ratings were made at baseline and immediately after the startle session was completed.

3.3. Hormone assays

Serum allopregnanolone levels were analyzed by high performance liquid chromatography followed by radioimmunoassay (HPLC-RIA) (Timby et al., 2005). Plasma samples were analyzed in duplicates with the samples resolved in 1 ml ethanol: water 1:1 (V/V) prior to the analyses. The HPLC system consisted of a Waters 1515 Isocratic Pump, delivering the mobile phase (methanol:water 60:40, V/V) at a flow rate of 1.0 ml/min. A Waters 717 plus Auto-sampler was used for injection of samples (200 µl) into a Symmetry C18 3.5 µm 4.6 × 75 mm separation column (Waters), heated to 45 °C in a Waters 1500 Column Heater. Detection was at 206 nm using a Waters 2487 Dual λ Absorbance Detector. The detector output was recorded on a PC-based Waters Breeze Chromatography Software (version 3.20). Five-milliliter fractions were symmetrically collected with a Waters Fraction Collector around the retention time for allopregnanolone, determined by injection of a standard sample before the start of the analysis. Allopregnanolone antiserum was raised against 3α-hydroxyl-20-oxo-5α-pregnan-11α-yl carboxymethyl ether coupled with BSA. The recovery of allopregnanolone averaged 78% and the results are compensated for recovery. The sensitivity of the assays was 25 pg, with an intraassay coefficient of variation for allopregnanolone of 6.5% and an interassay coefficient of variation of 8.5%.

3.4. Statistical analyses

Patients with negligible startle responses (mean amplitude < 10 µV) were considered as non-responders. According to this definition, two PMDD patients and one control were considered to be non-responders and were removed from the analyses.

Peak startle amplitudes were measured automatically by the software within 20–150 ms following the onset of the startle stimulus. A zero response score was given if no response was detectable, according to the default criteria provided by the software: 1) The peak startle response occurred outside the 20–150 ms time frame 2) a baseline shift exceeded 40 arbitrary units and 3) a startle response was 25 arbitrary amplitude units or less. An arbitrary unit corresponded to 0.076 µV.

Startle magnitude was defined as the total amplitude of all trials with response/total number of trials. Startle magnitude acknowledges zero responses, which is particularly important when assessing the prepulse inhibition. Habituation of startle response was calculated as the reduction in startle amplitude between the first and last blocks of pulse-alone trials by the following formula: [% habituation = 100 × (first block − last block) / first block].

Prepulse inhibition (PPI) in blocks 2 and 3 was computed as the percentage reduction in peak magnitude of startle on pulse-alone trials by the following formula: PPI = 100 × (MPA − MPP) / MPA, where MPA is a mean magnitude of pulse-alone and MPP is a magnitude of prepulse–pulse trials. As there was no difference between blocks 2 and 3 in PPI results ($p > 0.1$) data was collapsed across blocks 2 and 3 for further analyses.

Allopregnanolone effects on startle response and prepulse inhibition were analyzed by three-way analyses of variance (ANOVA) with repeated measures with drug (allopregnanolone vs. placebo), time (pre-injection vs. post-injection) as the within-group variables and group (PMDD patients vs. healthy controls) as the between-subjects variable. The aims of the analysis was to determine whether there was a difference between drugs (main effect of drug) and whether there was any difference between groups (main effect of group), or if PMDD patients and controls responded differently to allopregnanolone (drug by group interaction). The visual analogue ratings of sedation were calculated as Δ-scores (difference from baseline at the end of startle test session) and were analyzed by paired *t*-test.

Demographic variables were analyzed by paired *t*-tests and Chi-square tests. Post-injection allopregnanolone serum concentrations were compared between groups using the Mann–Whitney *U*-test. Correlations between allopregnanolone and startle response and PPI were made with Pearson's Correlation Coefficient.

Table 1
Demographic data and physical characteristics of the study population.

	PMDD patients ($n=16$)	Controls ($n=12$)
Age (years)	39 ± 4.0	37 ± 8.0
Body mass index (kg/m^2)	24.7 ± 3.7	23 ± 3.0
Married or cohabiting (%)	11 (69%)	8 (67%)
Children (n)	0.9 ± 0.9	1.2 ± 1.2
University/college education (%)	11 (69%)	9 (75%)
Employed (%)	16(100%)	11 (100%)
Tobacco users	4 (25%)	3 (25%)

Data are presented as mean ± standard deviation or n (%).

The SPSS statistical package was used for the analyses. p values of less than 0.05 were considered to be statistically significant.

4. Results

Two PMDD patients and one control were considered to be non-responders and were excluded from the analyses. One control subject lacked blood samples from her allopregnanolone test session.

Demographic data of the study group is given in Table 1. There were no differences in physical characteristics or demographic variables between groups. Control subjects and PMDD patients did not differ with respect to the timing of testing during the luteal phase (cycle day −6.6 ± 0.6 vs. −5.0 ± 0.6). All participants were considered to have had an ovulatory cycle when tested, which was also confirmed by the serum progesterone levels >15 nmol/l. PMDD patients and healthy controls did not differ with respect to the plasma estradiol levels or progesterone levels in the late luteal phase, Table 2.

Compared to placebo, the allopregnanolone bolus injection induced a marked increase of self-rated sedation in both groups (PMDD patients $p<0.01$; healthy controls $p<0.01$), Table 3. There was no difference in self-rated sedation scores between groups.

Post-test serum concentrations of allopregnanolone in each group are given in Table 2. The post-test serum concentration of allopregnanolone was lower in PMDD patients than in healthy controls. For this reason, subsequent analyses have included post-test allopregnanolone levels as a co-variate.

4.1. Acoustic startle response

There was no difference in startle response following allopregnanolone injection, either among PMDD patients or controls. The three-way ANOVA indicated no drug ($F(1,26)=0.90$), drug by group interaction ($F(1,26)=0.78$) or group effects, i.e. no difference between PMDD patients and healthy controls in startle response ($F(1,26)=0.78$), Fig. 1. Adjustment for post-test allopregnanolone serum concentrations did not change the results (main effect of drug ($F(1,24)=0.75$; drug by group interaction $F(1,24)=1.35$; main effect of group ($F(1,24)=0.80$).

Percent habituation in startle response throughout the test session did not differ between drugs or groups (PMDD patients following allopregnanolone 37.2% ± 8.9, PMDD patients following placebo 33.0% ± 6.4, healthy controls following allopregnanolone 43.6% ± 6.0, healthy controls following placebo 30.5% ± 8.4). Adjustment for post-test allopregnanolone serum concentrations did not change the results.

Table 2
Mean ± SEM serum concentrations of allopregnanolone following intravenous injection of 0.05 mg/kg allopregnanolone in PMDD patients and healthy controls.

	PMDD patients ($n=16$)	Controls ($n=12$)
Baseline estradiol (pmol/l)	339.3 ± 34.7	266.5 ± 43.8
Baseline progesterone (nmol/l)	26.1 ± 4.0	19.9 ± 3.4
Post-test allopregnanolone, nmol/l	51.4 ± 5.5	70.9 ± 6.4*

* Significantly different from PMDD patients, $p<0.05$, Mann–Whitney U-test.

Table 3
Δ sedatation score after injection of 0.05 mg/kg allopreganolone or placebo in PMDD patients and healthy controls.

Δ sedatation score	PMDD patients ($n=14$)	Controls ($n=11$)
Allopregnanolone injection	26.4 ± 8.2	26.8 ± 6.6
Placebo injection	5.9 ± 9.7**	8.7 ± 4.9**

Data are presented as mean ± SEM.
** $p<0.01$; difference from placebo Δ sedatation scores, paired t-test.

4.2. Prepulse inhibition

PMDD patients had lower levels of PPI compared to controls, but PPI was not affected by the allopregnanolone injection in either group, Fig. 2. The three-way ANOVA indicated a significant effect of prepulse intensity ($F(1,26)=11.56$; $p<0.001$) and group ($F(1,26)=5.00$; $p<0.05$) but no main effect of drug ($F(1,26)=0.13$, or drug by group interaction ($F(1,26)=1.51$). Adjustment for post-test allopregnanolone serum concentrations yielded similar results (main effect of group ($F(1,24)=4.83$; $p<0.05$); main effect of drug ($F(1,24)=0.04$; drug by group interaction ($F(1,24)=1.18$). PPI was reduced in PMDD patients in comparison to controls during the allopregnanolone condition $F(1,26)=4.25$; $p<0.05$, as well as during the placebo condition $F(1,26)=7.16$; $p<0.05$, Fig. 2.

5. Discussion

The main finding of this study was that startle response and PPI were unaffected by acute administration of allopregnanolone. We were also able to replicate prior findings of reduced baseline PPI in PMDD patients, whereas baseline startle response in this study did not differ between PMDD patients and controls (Kask et al., 2008b). The latter finding is presumably due to the smaller sample size used for the current study.

We had hypothesized that allopregnanolone, through its binding to the $GABA_A$ receptor, would induce anxiolytic effects which could be expressed as a decreased startle response. Both animal and human data have consistently indicated that drugs which alter the GABAergic system have a suppressant effect on the startle response. Benzodiazepines such as diazepam (Abduljawad et al., 1997) and alprazolam (Riba et al., 2001) have been shown to inhibit the startle response. There are several reasons why we did not detect any allopregnanolone-induced changes in either the startle response or PPI. First, because of limited knowledge of the safety profile and pharmacodynamic effects

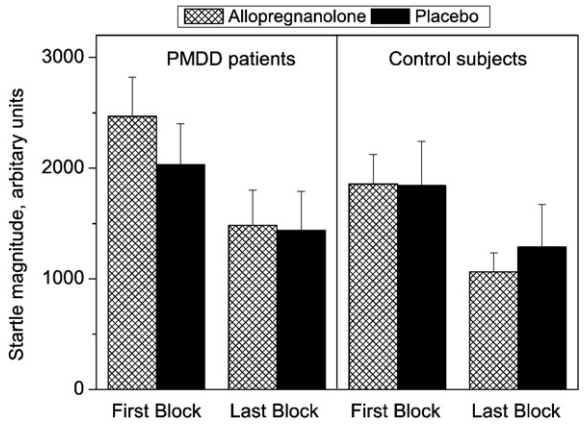

Fig. 1. Mean ± SEM startle response in the luteal phase of the menstrual cycle in 16 women with PMDD and 12 healthy controls following intravenous administration of 0.05 mg/kg allopregnanolone or placebo. Allopregnanolone did not induce any changes in startle response in comparison to placebo. Furthermore, there was no difference in startle response or habituation of the startle response between PMDD patients and controls, or between the allopregnanolone and placebo conditions.

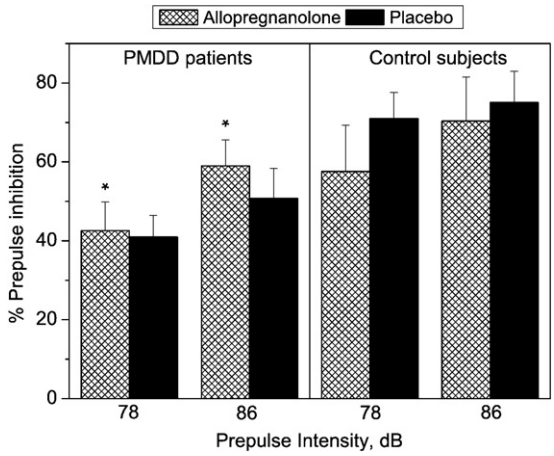

Fig. 2. Mean ± SEM percent prepulse inhibition by prepulse intensity in the luteal phase of the menstrual cycle in 16 women with PMDD and 12 healthy controls following intravenous administration of 0.05 mg/kg allopregnanolone or placebo. PMDD patients had lower levels of PPI compared to controls ($p<0.05$), but there was no difference in PPI between the allopregnanolone and placebo conditions.

of allopregnanolone in humans, a single dose regimen was chosen for the study. It is thus possible that the chosen dose was insufficient to induce any changes in the startle response or PPI. This is, however, unlikely as prior human studies on allopregnanolone, where similar doses have been used (resulting in similar serum concentrations), have indicated that the dose is sufficient to induce objective as well as subjective sedation (Timby et al., 2005) and episodic memory deficits (Kask et al., 2008a). Indeed, the allopregnanolone dose used for the present study induced a significant increase in self-rated sedation in both PMDD patients and healthy controls. Furthermore, oral progesterone resulting in serum concentrations of allopregnanolone of approximately 25 nmol/l has been shown to alter amygdala activity in an fMRI paradigm, although it cannot be established whether the amygdala activation was due to the effects of progesterone or allopregnanolone (van Wingen et al., 2008). Furthermore, the serum concentrations we obtained were similar to what can be expected during the second trimester pregnancy (Luisi et al., 2000), hence within the upper limit of the physiological range in women. If pregnancy levels of allopregnanolone are unable to affect these measures, it is unlikely that allopregnanolone plays a role in ovarian steroid effects on startle response and PPI outcome.

Another plausible reason for our negative finding could rely in the fact that some of the prior pharmacological studies have used an enhanced startle response as their dependent variable. Enhancement of the startle response can be achieved through CRH administration or concomitant presentation of noxious stimuli (fear-potentiated startle response). Possibly, the pharmacological actions of allopregnanolone would have been easier to reveal if the startle response had already been increased at baseline.

Based on the findings of the present study it thus appears unlikely that the previously reported lowering of PPI in the luteal phase among PMDD patients may be attributed to allopregnanolone. Likewise, our previous report on decreased PPI during pregnancy cannot be explained by increased allopregnanolone levels during pregnancy. Most likely, the variability in PPI across reproductive events is due to effects mediated by progesterone or estradiol via their respective receptors.

A secondary aim of the study was to evaluate whether allopregnanolone-induced changes in startle response and PPI differed between PMDD patients and controls. We could not detect any such differences in allopregnanolone response between the two groups. One reason for this may have been that the sample size was too small to demonstrate any differences. From previous studies it is known that women with PMDD have a reduced sensitivity to GABA-active compounds such as benzodiazepines (Sundstrom et al., 1997), alcohol (Nyberg et al., 2004), and certain neurosteroids (pregnanolone, the 5b-stereoisomer of allopregnanolone) (Sundstrom et al., 1998). However, we have previously used saccadic eye velocity (SEV) as dependent measure because it is a sensitive measure of sedation and displays a dose-dependent decrease following administration of sedative drugs. As startle response and PPI were unaffected by allopregnanolone, it is thus not surprising that no group differences were found.

Somewhat surprisingly the post-test serum allopregnanolone concentrations were lower in PMDD patients than in controls. We have previously administered the 5β-stereoisomer of allopregnanolone, pregnanolone, to PMDD patients and controls and obtained similar post-test serum concentrations of pregnanolone (Sundstrom et al., 1998). In the current study, approximately 25 min elapsed between the injection and the blood sampling, whereas blood sampling in our prior study occurred within 5 min of the injection. It is thus possible that allopregnanolone pharmacokinetics differ between PMDD patients and controls. However, to elucidate this possibility, future pharmacokinetic studies of allopregnanolone need to be undertaken.

In conclusion, the present study could not find any evidence that intravenous allopregnanolone, resulting in serum concentrations within the physiological range may influence startle response or prepulse inhibition of startle response in healthy women or in women with premenstrual dysphoric disorder.

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