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ORIGINAL INVESTIGATION

Women with premenstrual dysphoric disorder have altered sensitivity to allopregnanolone over the menstrual cycle compared to controls—a pilot study

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Abstract
Rationale In premenstrual dysphoric disorder (PMDD), a condition that afflicts 3–8 % of women in fertile ages, the cyclic recurrence of debilitating mood symptoms is restricted to the luteal phase of the menstrual cycle. The progesterone metabolite allopregnanolone is produced by the corpus luteum, and circulating levels are reflected in the brain. Allopregnanolone is a modulator of the $GABA_A$ receptor, enhancing the effect of γ-aminobutyric acid (GABA). Previous studies have demonstrated different sensitivity to other $GABA_A$ receptor agonists, i.e., benzodiazepines, alcohol, and pregnanolone, in PMDD patients compared to controls.
Objectives This study aimed to investigate the sensitivity to intravenous allopregnanolone over the menstrual cycle in PMDD patients.
Methods Allopregnanolone, 0.05 mg/kg, was administered intravenously once in the mid-follicular and once in the luteal phase of the menstrual cycle to 10 PMDD patients and 10 control subjects. The saccadic eye velocity (SEV) was recorded by electrooculography as a measurement of functional $GABA_A$ receptor activity, at baseline and repeatedly after the injection. A mixed model was used to analyze data.
Results There was a highly significant group × phase interaction in the SEV response to allopregnanolone ($F(1,327.489) = 12.747$, $p < 0.001$). In the PMDD group, the SEV response was decreased in the follicular phase compared to the luteal phase ($F(1,168) = 7.776$, $p = 0.006$), whereas in the control group, the difference was opposite during the menstrual cycle ($F(1,158.45) = 5.70$, $p = 0.018$).
Conclusions The effect of exogenous allopregnanolone is associated with menstrual cycle phase in PMDD patients and in controls. The results suggest an altered sensitivity to allopregnanolone in PMDD patients.

Keywords Neurosteroid · GABA · Premenstrual dysphoric disorder · Saccadic eye velocity · Menstrual cycle

Introduction

Severe menstrual cycle-related mood changes, with significant impact on quality of life, are experienced by 3–8 % of women in fertile ages (Pearlstein 2007). They show a recurrent cluster of predominantly mental symptoms such as irritability, depressed mood, aggression, and emotional lability during the luteal phase of the menstrual cycle, corresponding with the premenstrual dysphoric disorder (PMDD) defined by DSM-5 (O'Brien et al. 2011; APA 2013). The pathophysiology of PMDD is not yet fully understood, but the temporal association with circulating ovarian steroids, in particular progesterone, produced by the corpus luteum after ovulation, is obvious (Backstrom et al. 2003). Most importantly, symptoms are relieved when ovarian hormones are suppressed (Wyatt et al. 2004) and are only reinstated when progesterone is administered (Segebladh et al. 2009). Since the progesterone receptor antagonist RU-486 has not been shown to diminish PMDD symptom (Schmidt et al. 1991; Chan et al. 1994), an

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interest has emerged for central effects of progesterone metabolites. Progesterone can be metabolized into allopregnanolone (3α-hydroxy-5α-pregnane-20-one), which is an allosteric modulator of the γ-aminobutyric acid $(GABA)_A$ receptor (Majewska et al. 1986). Allopregnanolone is a potent $GABA_A$ receptor agonist which easily crosses the blood–brain barrier and serum levels mirror those of circulating progesterone across the menstrual cycle (Wang et al. 1996; Bixo et al. 1997). Like other GABA agonists, such as benzodiazepines and barbiturates, allopregnanolone has anesthetic, antiepileptic, and anxiolytic properties in animals (Frye 1995; Akwa et al. 1999; Norberg et al. 1999) and is sedative when given in pharmacological doses to humans (Timby et al. 2006; van Broekhoven et al. 2007). In previous studies, no consistent difference between physiological serum levels of allopregnanolone in women with PMDD compared to controls has been shown (Schmidt et al. 1994; Wang et al. 1996; Rapkin et al. 1997; Monteleone et al. 2000). However, treatment with a 5α reductase inhibitor could ameliorate the symptoms in women with PMDD (Martinez et al. 2016) indicating that reduction of allopregnanolone levels could be beneficial for individuals. Could it be that women with PMDD have an adverse response to normal ovarian steroid cyclicity and that this vulnerability is due to a disturbance of GABAergic function? Animal studies have revealed that the plasticity of the $GABA_A$ receptor, which is a chloride channel consisting of five subunits, could change with different reproductive states (Lovick et al. 2005; Maguire et al. 2005). Functional brain imaging studies have further demonstrated normal menstrual cycle-dependent local, neural activation (Fernandez et al. 2003; Protopopescu et al. 2005; Ossewaarde et al. 2011) but abnormal patterns in women with PMDD, e.g., a decreased response in inhibitory cortical areas during negative stimuli compared to controls (Protopopescu et al. 2008; Rapkin et al. 2011; Gingnell et al. 2014).

Furthermore, bimodal responses rather than a linear relationship between mood-derived behavior and GABA agonists are seen in animal studies, e.g., aggressive response to low doses of benzodiazepines or alcohol and calming effects of higher doses, and seem consistent across species (Miczek et al. 2002). Postmenopausal women, with previous PMDD, were shown to react different to estradiol-progesterone combinations depending on how much of the progesterone that was metabolized to allopregnanolone. In these women, concentrations of allopregnanolone corresponding to normal luteal phase levels induced more severe mood changes than both higher and lower levels (Andreen et al. 2006). With the use of functional magnetic resonance imaging (fMRI), several studies report changes in brain reactivity across the menstrual cycle, most notably increased amygdala reactivity in the luteal phase (Toffoletto et al. 2014). In some fMRI studies of human amygdala reaction, a bimodal effect of progesterone/allopregnanolone has been shown. In these experiments, exposure to low concentrations of allopregnanolone increased amygdala activity in response to repulsive pictures compared to a placebo control, whereas high-dose allopregnanolone induced a reduced amygdala response compared to the placebo group (van Wingen et al. 2007, 2011).

Paradoxical reactions to several GABA agonists have been found in animal studies (Miczek et al. 2003) but also in humans (Dougherty et al. 1996; Ben-Porath and Taylor 2002). The frequency of paradoxical reactions to midazolam in studies is, interestingly, roughly the same as the prevalence of PMDD in the population (Weinbroum et al. 2001; Pearlstein 2007), indicating a disturbed $GABA_A$ receptor function in certain individuals (Epperson et al. 2002; Smith et al. 2003; Backstrom et al. 2014). However, no systematic studies showing that this occurs in the same individuals exist. Treatment studies of benzodiazepines for premenstrual anxiety have shown varying results (Pearlstein 2012), and the effect might be dose dependent.

Individual sensitivity to GABAergic substances could be tested in humans by measurement of saccadic eye velocity (SEV) after exposure to the substance in question. SEV represents a stable method with high intra-individual reproducibility and directly related to $GABA_A$ receptor activation (Iacono and Lykken 1981; Hommer et al. 1986). Allopregnanolone has been shown to dose-dependently decrease SEV and increase subjective sedation in humans (Timby et al. 2006).

The aim of the present study was to investigate the sensitivity to intravenous allopregnanolone in terms of SEV and subjectively rated sedation in women with PMDD across the menstrual cycle compared to healthy controls.

Material and methods

Subjects

Ten women with PMDD and 10 healthy controls were recruited by advertisement in local newspapers. All participants were aged 18–40 years, with regular menstrual cycles, physically and mentally healthy, and without any ongoing hormonal or psychotropic drug treatment. PMDD diagnosis in the study group was defined using the Cyclicity Diagnoser (CD) scale, which is a nine-step Likert scale exploring all PMDD symptoms defined in the DSM-5. All women in the PMDD group fulfilled the DSM-5 diagnostic criteria with significant increase in at least five symptoms during the luteal phase of the menstrual cycle, confirmed by daily ratings across two ovulatory cycles. The control group was exempt from cyclical mood changes, confirmed by symptom ratings during two ovulatory, menstrual cycles, on the CD scale. At screening, a structured interview, PRIME-MD, was used to rule out psychiatric disorders in all participants. In addition, routine blood

chemistry scans (total blood cell count, potassium, sodium, creatinine, and glucose) and a pregnancy test were performed.

Subjects gave oral and written informed consent prior to inclusion, and the study was performed in accordance with the Declaration of Helsinki 1964. The Swedish Medical Product Agency and the Regional Ethical Review Board in Umeå approved the study, Dnr 04-125M.

Study protocol

This was an experimental study where the effect of allopregnanolone on SEV and subjectively rated sedation was tested in women with PMDD and healthy controls. Safety parameters were anxiety symptoms, blood pressure, and pulse rate during the experiments. The subjects were tested once in the follicular and once in the luteal phase of the menstrual cycle. All experiments were done in the morning (0800–1100 h) at the university hospital in Umeå, Sweden. IV cannulae were inserted in each forearm, one for allopregnanolone administration and one for blood sampling. The electrodes for SEV recording were placed outside each canthus and one reference electrode in the forehead. Baseline blood samples for allopregnanolone and progesterone concentrations were drawn and baseline SEV recordings made prior to the allopregnanolone injection. The mean of three sets of SEV recordings was defined as the baseline value. Before injection, subjects rated their subjective feeling of sedation and scored anxiety symptoms on the State-Trait Anxiety Inventory (STAI), Anxiety Sensitivity Index (ASI), and Panic Symptoms Scale (PSS) (see "Anxiety and panic scales" section below) for baseline values. A single dose of 0.05 mg/kg allopregnanolone IV was given at 0 min. SEV recordings and subjectively rated sedation scorings were performed at 5, 13, 18, 25, 30, 45, 60, 120, and 180 min after the injection. State Anxiety and Discomfort Scale (SADS) (see "Anxiety and panic scales" section below) anxiety scores were registered at 13, 25, 30, 45, 60, 120, and 180 min, and blood samples for allopregnanolone concentrations were drawn at 5, 18, 30, 45, 60, 120, and 180 min. Subjects were allowed to ambulate after the recording at 60 min. For safety measures, additional scorings on the PSS, STAI, and SADS were done by the subjects 24 h after the experiment to detect late paradoxical reactions to allopregnanolone.

Saccadic eye velocity and subjectively rated sedation

Measurement of SEV by electrooculography is an established method to detect $GABA_A$-mediated sedation and has been used by our group in several studies. The method is fully described earlier (Timby et al. 2006). In short, the induced potential when the vector over the ophthalmic bulb is changed by the quick movement of the eye (the saccade) to focus the fovea on the array of individual lighting diods is registered. The diods are lightened once at a time, and the subject is asked to follow the lights and not to anticipate the target. A software program analyzes and calculates the saccadic eye velocity. We chose saccades of 30° as the saccadic eye velocity reaches a maximum at 30–35° angular movement.

Subjectively rated sedation was scored by the participants on a visual analogue scale where 0 represented complete absence of sedation and 100 represented maximal feeling of sedation, i.e., nearly falling asleep.

Anxiety and panic scales

Allopregnanolone has sparsely been administered to humans. To be able to detect paradoxical reactions over the test sessions, we included several scales measuring subjective anxiety and discomfort in the study protocol. Anxiety Sensitivity Index was used prior to injection to find fear for panic or anxiety. Total score ranges from 0 to 64, and a mean ASI score of 19.01 has been reported for a normal sample (Peterson and Reiss 1992). The DSM-IV-derived PSS has a total score ranging from 0 to 72. The mean score in 12 healthy controls receiving placebo injections has been reported to be 1.02 in the follicular phase and 2.27 in the luteal phase (Bell et al. 2004). STAI has a total range of scores from 20 to 80. A total score below 40 indicates low, between 40 and 59 moderate, and 60 or more severe state anxiety (Spielberger 1970). The SADS is a global measure of subjective discomfort. SADS has six steps from 0 to 5, where 0 represents no discomfort at all and 5 worst imaginable feeling of discomfort. SADS has earlier been used in pharmacological tests on humans in order to detect fluctuations in anxiety level (Radu et al. 2002).

Allopregnanolone solutions and assays

The allopregnanolone solution for IV injection was formulated with Good Manufacturing Practice (GMP) made allopregnanolone (Umecrine AB, Umeå, Sweden), 15 mg dissolved in 100 ml albumin solution (albumin, 200 mg/ml) using an ultrasound bath and sterile filtration. The Umeå University Hospital Pharmacy prepared the allopregnanolone solution which contained 0.126 ± 0.003 (mean ± SEM) mg allopregnanolone/ml ($n = 9$) determined by high-pressure liquid chromatography (HPLC) and UV absorbance (Turkmen et al. 2004). The infusion rate of the allopregnanolone solution was 20 ml/min. Allopregnanolone serum concentrations were analyzed by radioimmunoassay (Schmidt et al.) after celite chromatographic purification. All samples were analyzed using a polyclonal rabbit antiserum raised against 3α-hydroxy-20-oxo-5α-pregnan-11-yl carboxymethyl ether coupled to bovine serum albumin (Purdy et al. 1990). Rabbit antiserum was used in a dilution of 1/5000. The antibody solution was prepared using [11,12]^{3}H-allopregnanolone,

3×10^6 cpm/32 ml (Perkin-Elmer Life Sciences, Boston, USA) solution containing 65 mM boric acid (Merck) buffer, pH = 8.0, bovine serum albumin 100 mg/ml (Sigma, St. Louis, USA), human gamma globulin solution 20 mg/ml (Octapharma, Sweden), and antibody in milliliter ratio of the antibody solution: 30:1:1:0.006. The solution was allowed to equilibrate overnight at 8 °C. Antibody solution (200 μl) was added to all samples (left overnight at 8 °C). After the addition of 200 μl saturated ammonium sulfate, each tube was again mixed and centrifuged at 20,000 RPM for 20 min. Thereafter, the supernatant was aliquoted into a counting vial and diluted with 3.0 ml Optiphase scintillation medium (Wallac, Finland). The samples were counted in a RackBeta (Wallac, Finland) scintillation counter. The sensitivity of the assay was 25 pg, with an intraassay coefficient of variation for allopregnanolone of 6.5 % and an interassay coefficient of variation of 8.5 %. The RIA used does not detect the 3β-epimer (isoallopregnanolone) or the 5β-epimer (pregnanolone).

Statistics

Differences in demographic parameters, baseline values of allopregnanolone, SEV, and anxiety scale scores were analyzed by the non-parametric Mann–Whitney *U* test, between groups, and the Wilcoxon signed-rank test within individuals. The responses on SEV and subjectively rated sedation to the allopregnanolone injection were transformed into delta values (changes from baseline). For comparisons between and within the groups, a mixed model was used, including the interaction variable for phase and group (West et al. 2007). The mixed model handles repeated measurements nested within the individual and non-equivalent time intervals as well as missing values. In the mixed model, subject's intercept was defined as a random coefficient and the other variables (menstrual cycle phase, time point, and group) as fixed coefficients. Area under the curve was calculated for the delta values of SEV during the first hour after the injection in both menstrual cycle phases and groups, to further explore the data. Statistical calculations were performed in the IBM SPSS Statistic version 19 software. We considered *p* values less than 0.05 significant.

Results

Baseline characteristics

Demographic and baseline characteristics for the study group and controls are presented in Table 1. The PMDD group was significantly older than the control group, $p = 0.004$, but there were no differences in BMI, parity, or median menstrual cycle day when the experiment was performed. Ovulatory cycles were confirmed by a serum progesterone value above 10 nmol/l on the luteal phase test day.

Effects of allopregnanolone on saccadic eye velocity and sedation

When comparing PMDD patients with controls, there was a highly significant group × phase interaction regarding the SEV response to allopregnanolone ($F(1,327.489) = 12.747$, $p < 0.001$).

Women with PMDD were significantly less sensitive to allopregnanolone in the follicular phase compared to the luteal phase in terms of SEV response ($F(1,168) = 7.776$, $p = 0.006$) and in terms of subjectively rated sedation ($F(1,168) = 13.735$, $p < 0.001$). In the mixed model, the estimation of the SEV response was 7.8°/s lesser in the follicular phase compared to the luteal phase ($t(168) = 2.789$, $p = 0.006$). For the subjectively rated sedation response, the mixed model estimation in the follicular phase was −11.1 mm lower than in the luteal phase ($t(168) = -3.706$, $p < 0.001$). Also in controls, menstrual cycle phase was significantly associated with the SEV response to allopregnanolone ($F(1,158.45) = 5.70$, $p = 0.018$). However, the response was in the opposite direction across the menstrual cycle compared to PMDD women

Table 1 Demographic and baseline characteristics of women with premenstrual dysphoric disorder (PMDD) and healthy controls

	PMDD, $n = 10$		Controls, $n = 10$	
	Follicular	Luteal	Follicular	Luteal
Age, years median (range)	35.5 (24–38)		26.5* (21–32)	
BMI median (range)	25 (20–37)		23 (19–35)	
Parity, n median (range)	1.5 (0–4)		0 (0–2)	
Menstrual cycle day median (range)	8.5 (6–12)	22 (17–28)	8.5 (6–11)	25 (22–28)
S-allopregnanolone, nmol/l, at baseline median (range)	0.46 (0.20–2.74)	2.15* (0.74–5.18)	0.44 (0.32–0.62)	1.70* (0.82–6.20)

BMI body mass index

*$p < 0.01$, comparison between groups (age) and between menstrual cycle phases (*S*-allopregnanolone)

(Fig. 1). Control women decreased their SEV response in the luteal phase compared to the follicular phase, and the SEV response estimation (β) by the mixed model was 10.2°/s lesser in the luteal phase compared to the follicular phase ($t(158.45) = -2.39$, $p = 0.018$). The finding was further strengthened by a comparison of the area under the curve (AUC) for delta SEV during the first hour of the experiments (Fig. 2). Controls did not rate their subjective sedation on the VAS scale differently during the follicular phase experiment compared to in the luteal phase. Moreover, there were no differences in baseline SEV between menstrual cycle phases or groups.

Allopregnanolone serum concentrations

There were no significant differences in allopregnanolone serum levels at baseline between the groups, neither in the follicular nor in the luteal phase. Already at the first time point (5 min after injection), the maximal allopregnanolone serum concentration was reached in both groups and both menstrual cycle phases, and no significant difference was seen, neither between groups nor between menstrual cycle phases (Fig. 1).

Anxiety scales and other safety parameters

The subjective scorings on the STAI, ASI, and PSS are presented in Table 2. The results displayed neither signs of increased anxiety nor any increased anxiety the day after the experiment in any of the groups. The scores are overall below cutoff levels for anxiety diagnosis and confirm the study groups as psychiatrically healthy. After the allopregnanolone injection, three women in the PMDD group and three control subjects increased their scores on the SADS repetitive scorings across the experimental hours. However, this was not considered clinically significant as the increase among these individuals was restricted to one scale step, from 0 to 1 (in one case from 1 to 2) and all other participants scored 0 at all time points. There was no significant change in vital signs during the experiment, and no other side effects apart from the expected sedation were reported.

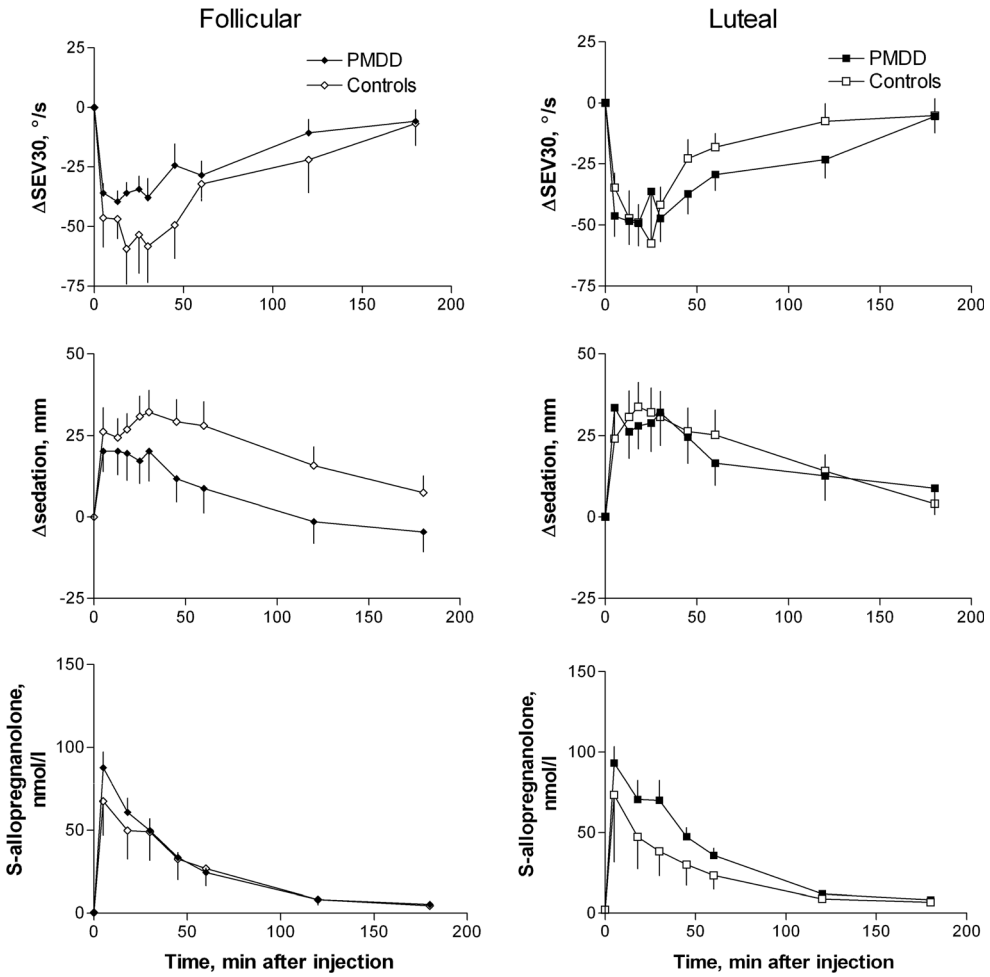

Fig. 1 Changes in saccadic eye velocity (*SEV*) expressed as delta values and subjectively rated sedation (visual analogue scale) following an intravenous injection of allopregnanolone, once in the follicular and once in the luteal phase of the menstrual cycle in women with premenstrual dysphoric disorder (*PMDD*), $n = 10$, and control subjects, $n = 10$. *Bottom panel* shows serum concentrations of allopregnanolone during the experiments. All values expressed as mean ± SEM

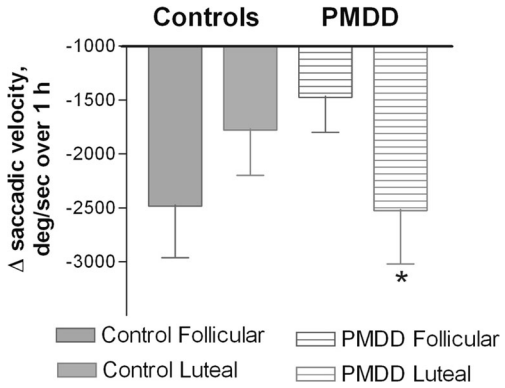

Fig. 2 Area under the curve (AUC) for saccadic eye velocity (SEV) during the first hour after an intravenous injection of allopregnanolone (0.05 mg/kg), once in the follicular and once in the luteal phase in women with PMDD (premenstrual dysphoric disorder) and healthy controls. PMDD women were more sensitive to allopregnanolone in the luteal compared to the follicular phase, $*p < 0.05$

Discussion

The main finding of the present study is that allopregnanolone sensitivity across the menstrual cycle is altered in women with PMDD compared to healthy controls. The difference was manifested as a highly significant group × phase interaction in the SEV response, i.e., that the direction of the change in allopregnanolone sensitivity over the menstrual cycle was the opposite in PMDD patients compared to controls.

Several previous studies of the sensitivity to $GABA_A$ receptor agonists have reported differences in the SEV response between PMDD and control subjects, although there are some discrepancies in the results. In line with the current results, a previous study reported a lesser SEV response to midazolam injections in the follicular phase in women with PMDD compared to controls; however, there were no differences within the groups over the menstrual cycle (Sundstrom et al. 1997). Our finding of a reduced SEV response in the luteal phase within the control group is in contrast to the SEV response following diazepam and pregnanolone exposure since control subjects increased their SEV response in the luteal phase, whereas PMDD patients did not in these studies (Sundstrom et al. 1997, 1998). Moreover, PMDD patients have been found to decrease their SEV response to alcohol in the late luteal phase, whereas control subjects did not (Nyberg et al. 2004). In accordance with the current study, no between-group differences could be detected in the SEV response in either menstrual cycle phase in the alcohol or pregnanolone studies. Pregnanolone is the 5β-epimer to allopregnanolone, with similar actions but with a lower potency to induce anesthesia in rats (Zhu et al. 2001). Pregnanolone and allopregnanolone were inhibitory to each other when given simultaneously in an anesthesia model, suggesting that pregnanolone can act as an antagonist to allopregnanolone following simultaneous exposure to the $GABA_A$ receptor (Norberg et al. 1999). Thus, molecular characteristics of the different ligands with independent interactions at the $GABA_A$ receptor complex could be an explanation to the divergences between previous and present results.

Another phenomenon that could explain the differences between PMDD patients and controls, but also complicate the comparisons with the aforementioned studies, is development of acute tolerance. Acute tolerance to allopregnanolone has been noted in rats during allopregnanolone-induced anesthesia, starting 30 min after allopregnanolone exposure (Zhu et al. 2004). Also, development of acute tolerance to midazolam measured by SEV in healthy humans has been suggested (Ball et al. 1991; Sundstrom et al. 1997). Altered brain sensitivity rather than pharmacokinetic changes has been proposed to explain the mechanism of acute tolerance to GABAergic substances (Wong et al. 1986), and in the present study, no significant pharmacokinetic differences were seen between the groups. The allopregnanolone-induced tolerance in rats, mentioned above, was associated with changes in $GABA_A$ receptor subunit expression (Birzniece et al. 2006). Supposing that acute tolerance might be a regulating factor

Table 2 Subjective scorings of anxiety on the ASI, PSS, and STAI scales, at baseline and 24 h after an intravenous injection of allopregnanolone, 0.05 mg/kg, by women with premenstrual dysphoric disorder (PMDD) and healthy controls

	ASI_0 median (range)	PSS_0 median (range)	$STAI_0$ median (range)	PSS_1 median (range)	$STAI_1$ median (range)
Controls follicular phase	9 (1–14)	1 (0–9)	30 (22–37)	0.5 (0–5)	29 (20–37)
	$n = 9$	$n = 9$	$n = 9$	$n = 8$	$n = 8$
PMDD follicular phase	5 (2–16)	0 (0–7)	26.5 (21–39)	0 (0–5)	26 (22–35)
	$n = 10$	$n = 10$	$n = 10$	$n = 9$	$n = 9$
Controls luteal phase	6 (2–16)	0 (0–7)	30.5 (25–39)	0 (0–5)	29 (21–40)
	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 10$
PMDD luteal phase	5.5 (0–14)	0 (0–3)	26.5 (20–38)	1 (0–8)	26.5 (24–36)
	$n = 10$	$n = 10$	$n = 10$	$n = 6$	$n = 6$

No significant differences between groups or menstrual cycle phases were noted

ASI Anxiety Sensitivity Index, *PSS* Panic Symptoms Scale, *STAI* State-Trait Anxiety Inventory, *0* baseline, *1* 24 h after the experiment

for $GABA_A$ receptor sensitivity, this phenomenon is probably more or less pronounced across the spectrum of $GABA_A$ receptor agonists (Turkmen et al. 2011). Interestingly, indications of acute tolerance to midazolam were only present among controls and only during the luteal phase (Sundstrom et al. 1997). Moreover, the fact that the reduced SEV response to diazepam during the luteal phase in PMDD patients was not replicated for midazolam on the one hand and the divergent SEV response to pregnanolone and allopregnanolone across the menstrual cycle on the other hand is interesting. When it comes to GABAergic facilitation, midazolam is more potent than diazepam (Buhrer et al. 1990), and allopregnanolone is more potent than pregnanolone (Zhu et al. 2001). If tolerance is more easily developed to more potent substances, this could be an explanation for the divergent SEV responses within the benzodiazepin group as well as between the 5α and 5β pregnan epimers. Thus, failure to replicate initial findings within each substance group could in fact be dependent on basal pharmacodynamic properties.

Due to progesterone metabolism, the exposure to endogenous $GABA_A$ active steroids in the brain is increased during the luteal phase (Bixo et al. 1997), likely altering the central responsiveness to allopregnanolone. Allopregnanolone has been shown to activate the $GABA_A$ receptor in a physiologically low range of concentration (Lambert et al. 2001). Regarding PMDD, there are several indications of a deficient adaptation to ovarian cyclicity. Changes of the $GABA_A$ receptor subunit composition could be induced by the oestrus cycle in rats (Lovick et al. 2005), by exogenous steroids (Follesa et al. 2002), or by $GABA_A$ receptor active pharmaceuticals (Uusi-Oukari and Korpi 2010). It is therefore plausible that the altered $GABA_A$ receptor sensitivity over the menstrual cycle in PMDD relates to an abnormal change in subunit composition causing dysfunction of the $GABA_A$ receptor. The dysfunction could depend on a different set of $GABA_A$ receptor subunit composition in a subgroup of the population and/or involve mechanisms of tolerance development. In the present study, no paradoxical, anxiogenic reactions to the supraphysiological levels of allopregnanolone were detected in the PMDD women. It might be that these presumed paradoxical reactions only occur in a certain range of allopregnanolone concentrations similar to the luteal phase of a normal menstrual cycle. This hypothetic speculation is supported by previous studies from our group where women with prior PMDD only developed negative mood symptoms when allopregnanolone levels were medium high, whereas both higher and lower levels did not produce any negative mood (Andreen et al. 2006). This hypothesis is further supported by studies showing that paradoxical reactions to benzodiazepines and PMDD occur in the same range of a population sample (Backstrom et al. 2011).

In another group of patients, namely women with burn-out syndrome, an increased sensitivity to allopregnanolone has been shown compared to controls, but no difference in the effect of flumazenil (a benzodiazepine antagonist) was noted. Surprisingly, flumazenil acted like a positive modulator on the $GABA_A$ receptor in the women with burn-out syndrome, in a manner similar to benzodiazepines (Savic et al. 1991). This is interesting since we know that the $GABA_A$ receptor subtype α4, β, δ is supersensitive to allopregnanolone and that in the presence of α4, flumazenil changes its effect from an inert compound or benzodiazepin antagonist to an agonist (Backstrom et al. 2014). The effect of flumazenil has however not been investigated in PMDD women.

In the present study, we noted a reduced sensitivity to allopregnanolone in the luteal phase among controls and the opposite among PMDD women, and hence these results suggest that PMDD women fail to develop a physiological tolerance to allopregnanolone during the luteal phase.

There are limitations to the present study, mainly the small number of subjects and the absence of placebo control. In addition, the dose of allopregnanolone was chosen to produce a stable reduction in SEV, and subsequently, the serum concentrations of allopregnanolone after injection were supraphysiological compared to the normal fluctuations over the menstrual cycle. However, the post-injection serum concentrations were in line with physiological concentrations in late pregnancy. Future studies will include physiological and supraphysiological concentrations in a dose-finding manner, placebo control, and other GABA agonists as comparison.

Conclusion

In conclusion, the present study shows that women with PMDD have an altered sensitivity to allopregnanolone over the menstrual cycle compared to controls. This finding suggests a $GABA_A$ receptor dysfunction in these patients and that allopregnanolone might be involved in the pathophysiology of PMDD.

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Compliance with ethical standards

Conflict of interest Torbjörn Bäckström has shares in Umecrine AB. None of the other authors declare any conflicts of interest.

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