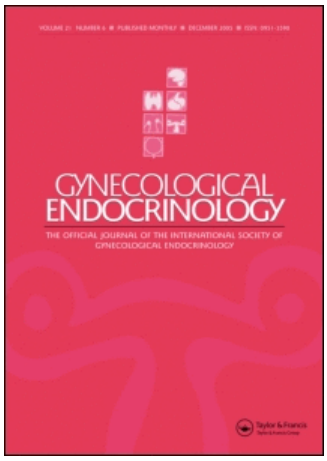

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PREMENSTRUAL SYNDROME

Allopregnanolone decrease with symptom improvement during placebo and gonadotropin-releasing hormone agonist treatment in women with severe premenstrual syndrome

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Abstract

Background. Neurosteroids such as allopregnanolone and pregnanolone are suggested to be of importance for the pathophysiology of premenstrual dysphoric disorder. The aim of this study was to investigate whether the luteal-phase serum concentrations of these neurosteroids are associated with improvement of premenstrual symptoms in 12 women with severe premenstrual syndrome after treatment with low-dose gonadotropin-releasing hormone agonist and placebo.

Methods. Daily ratings for mood and physical symptoms were made prior to treatment and throughout the study. Serum progesterone, allopregnanolone and pregnanolone were assessed in the luteal phase (cycle day -9 to cycle day -1). Based on their symptom ratings, subjects were grouped as either buserelin responders ($n = 6$) or placebo responders ($n = 6$).

Results. Buserelin responders displayed decreased levels of allopregnanolone ($p < 0.05$) and progesterone ($p < 0.05$) in parallel with improvement of symptoms. During the placebo treatment, the placebo responders had lower serum allopregnanolone concentrations than buserelin responders ($p < 0.05$). This was associated with improvement in symptoms compared with pre-treatment ratings.

Conclusion. Treatment response, whether induced by buserelin or placebo, appears to be associated with a decrease in allopregnanolone concentration.

Keywords: *Premenstrual dysphoric disorder, menstrual cycle, gonadotropin-releasing hormone agonist, allopregnanolone, pregnanolone, progesterone*

Introduction

Premenstrual dysphoric disorder (PMDD) or severe premenstrual syndrome (PMS) is characterized by a cluster of physical, affective and behavioral symptoms that occur in the luteal phase of the menstrual cycle. The most prominent affective symptoms are depressed mood, anxiety, irritability and lability, which are also considered as four of the main symptoms according to the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) [1]. The pathophysiology behind the appearance of these symptoms is related to ovarian steroids, as symptoms disappear during anovulatory cycles when no corpus luteum is formed [2]. Likewise, treatment with a gonadotropin-releasing hormone (GnRH) agonist has been proved to relieve PMDD symptoms [3–6].

As serum levels of gonadal hormones are similar between PMDD patients and control subjects [7–9], it has been suggested that women with PMDD have a different sensitivity to the fluctuations during the menstrual cycle of these hormones and/or of their neuroactive metabolites. Differences in sensitivity to the effects of neuroactive steroids and γ-aminobutyric acid A ($GABA_A$)-receptor active substances between PMDD patients and controls have also been reported during the luteal phase [10–13].

Progesterone is metabolized to allopregnanolone (3α-hydroxy-5α-pregnan-20-one) and pregnanolone (3α-hydroxy-5β-pregnan-20-one), which are potent

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$GABA_A$-receptor agonists that exert sedative, anxiolytic and antiepileptic effects in a dose-dependent manner. The findings regarding peripheral concentrations of allopregnanolone in women with PMDD are divergent. Most studies have failed to indicate any difference in peripheral allopregnanolone levels between PMDD patients and control subjects [14–16], although both lower and higher allopregnanolone levels [17–22] have been reported in PMDD patients.

However, within the individual patient, different steroid levels might have an impact on symptom expression. Decreased levels of allopregnanolone have been associated with improvement in PMDD symptoms, irrespective of whether treatment with a selective serotonin reuptake inhibitor (SSRI) or placebo was given [23]. Furthermore, in women receiving postmenopausal hormone therapy (HT), negative mood symptoms are enhanced when allopregnanolone levels increase during progesterone treatment [24].

For this reason, it is of interest to investigate, within an individual patient, the relationship between changes in symptom severity and changes in neuroactive steroids. We have previously shown that a low dose of GnRH agonist is superior to placebo for treatment of severe premenstrual symptoms [25]. Given the varying degree of ovarian and corpus luteum suppression that was induced by the low-dose GnRH agonist [25], this model could be used to investigate changes in the endogenous production of corpus luteum-derived neurosteroids [26] and, at the same time, investigate the symptom profiles of these individuals.

Thus the primary aim of the present study was to investigate whether luteal-phase serum concentrations of progesterone, allopregnanolone and pregnanolone are associated with symptom improvement following low-dose GnRH agonist treatment. A secondary aim was to investigate whether the concentrations of these hormones and neuroactive steroids are associated with symptom improvement during placebo treatment.

Materials and method

Subject study group

The patients included in this study were part of a larger, multi-center, randomized, placebo-controlled, double-blind crossover trial comparing a low dose of the GnRH agonist buserelin with placebo. For the purpose of this study, 18 PMDD patients recruited at the department of Obstetrics and Gynecology, Umeå University Hospital, were asked to give blood samples every second week during the trial.

Hence, 18 otherwise healthy women aged 37.8 ± 1.4 years (mean $\pm$ standard error of the mean), who had suffered from premenstrual mood changes for more than 6 months, were included in the study. All subjects met the criteria for PMDD, as defined in DSM-IV [1]. Diagnosis was based on daily prospective symptom ratings on the Cyclicity Diagnoser (CD) scale [25] during two ovulatory cycles prior to inclusion. The CD scale consists of seven mood parameters (depression, fatigue, irritability, tension, cheerfulness, friendliness and energy), and four somatic symptoms (headache, swelling, breast tenderness and menstrual bleeding). In addition, the CD scale contains one severity item for measuring impairment of everyday family/social functioning and work performance. The CD scale is a Likert scale ranging from 1 to 9, with 1 as complete absence of a particular symptom and 9 as the maximal severity of the symptom [25]. Patients were diagnosed with PMDD if they had a significant worsening in at least five mood symptoms during nine premenstrual days compared with nine midfollicular days, associated with a clinically significant social and occupational impairment [27]. All patients displayed at least one week of sparse symptomatology (scores less than 2) in the follicular phase. Women treated with oral contraceptives, other steroid hormones, benzodiazepines or antidepressants were excluded. In addition, women with irregular menstrual cycles, e.g. variation of more than ± 3 days between cycles, were not included. Those with a current mental disorder or a history of drug abuse during the clinical interview were also excluded from the study. Physical examinations and routine blood chemical tests carried out prior to inclusion were within the normal range. The Umeå University Ethics Committee approved the study, and each participant gave informed consent.

Study design

The PMDD patients were treated with a low dose of the GnRH agonist buserelin 100 μg/day administered intranasally (Aventis Pharma; Hoechst AG, Frankfurt, Germany) or placebo. The placebo spray, prepared in an identical nebulizer, contained the solution for buserelin but without the active drug (Apoteksbolaget AB, Stockholm, Sweden). Prior to the start of the study, all patients were given thorough instructions for the use of the nebulizer. Half of the patients were randomized to start with the GnRH agonist and the remainder started with placebo. The crossover was made after two menstrual cycles. Compliance was assessed by measuring the amount of liquid remaining in the nebulizers at each visit. In addition, patients were questioned about adverse effects of the study drug.

The primary outcome measure for the study was the daily PMDD symptom scores made by the patients on the CD scale throughout the study.

As previously mentioned, a significant relief in premenstrual depression and irritability scores was noted during low-dose GnRH agonist treatment compared with placebo [25].

Blood sampling

Blood samples for analysis of progesterone, allopregnanolone and pregnanolone were obtained every second week throughout the study, but for present purposes only luteal-phase blood samples were used.

Only cycles with a blood sample taken within the stipulated time frame of the luteal phase (day −9 to day −1) have been included in the statistical analyses of this study. The blood sampling was aimed to coincide with the late luteal phase (one week before onset of menses) of each treatment cycle. As buserelin treatment caused irregularities in the bleeding pattern, it was sometimes difficult to schedule the blood sampling in the luteal phase. In these cases, menstrual cycles were either unexpectedly long or onset of menstrual bleeding occurred earlier than expected. Menstrual cycle day was monitored by use of daily ratings of menstrual bleeding.

Second, only those subjects who had a luteal-phase blood sample from a buserelin as well as from a placebo cycle were included in the statistical analyses.

Third, to avoid carry-over effects from buserelin treatment to placebo treatment cycles in subjects starting with buserelin before the crossover to placebo, only blood samples from the second placebo treatment cycle were used.

Hormone assays

Allopregnanolone and pregnanolone were measured by radioimmunoassay (RIA) after diethylether extraction and purification of samples by high-performance liquid chromatography (HPLC).

Extraction. Serum or plasma (0.2–0.4 ml) was pipetted into a cylindrical flat-bottomed glass vial of 20 ml volume, after which water (0.5 ml) and diethylether (3.0 ml) were added. The samples were then allowed to stand on an orbital shaker for 10 min. Following the liquid–liquid extraction, the vials were transferred into an ethanol/dry ice bath. The water phase was frozen, and the ether phase was decanted and evaporated under a stream of nitrogen gas.

Purification. Purification of samples before quantification of allopregnanolone and pregnanolone was achieved by preparative HPLC followed RIA. Plasma samples were analyzed in duplicate. Evaporated samples were re-dissolved in 1 ml of ethanol–water (1:1, v/v) prior to analysis. Our HPLC system consisted of a Waters 1515 Isocratic Pump (Waters Corporation, Millford, MA, USA), 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 separation column (4.6 mm × 75 mm, 3.5 μm; Waters), heated to 45°C in a Waters 1500 Column Heater. Detection of retention times of standards and cross-reacting steroids was at 206 nm using a Waters 2487 Dual λ Absorbance Detector. The detector output was recorded by Waters Breeze Chromatography Software (version 3.20). In the preparative technique, HPLC fractions were collected symmetrically around the retention time for allopregnanolone and pregnanolone. Retention was found from injection of a standard sample before the start of analysis. A Waters Fraction Collector II was used for collection of samples, for further analysis with RIA. It was possible to separate all cross-reacting steroids, even though some had retention times close to that of the collected fraction as analyzed by injection of 20 nmol of standard samples (Table I).

Allopregnanolone. Allopregnanolone was measured by RIA after diethylether extraction and HPLC purification of samples. Recovery was determined for each assay by adding 300–500 cpm of ^{3}H-labeled allopregnanolone, [9,11,12-^{3}H(N)]5α-pregnan-3α-ol-20-one (Perkin Elmer Life Sciences, Boston, MA, USA), to a plasma sample before extraction and measuring the amount recovered after HPLC. The recovery of allopregnanolone averaged 98% and the results are compensated for recovery.

All samples were analyzed using a polyclonal rabbit antiserum raised against 3α-hydroxy-20-oxo-5α-pregnan-11-yl-carboxymethylether coupled to bovine serum albumin (Table I) [28]. The antiserum was used at a dilution of 1:5000 and the antibody solutions were prepared in the same way as described earlier [29]. The sensitivity of the assay was 25 pg; the intra-assay coefficient of variation (CV) for allopregnanolone was 6.5% and the inter-assay CV was 8.5%.

Pregnanolone. After extraction and HPLC, a RIA for pregnanolone was performed as described earlier [12]. Briefly, the antiserum was raised against 3α,21-dihydroxy-5β-pregnan-20-one-21-hemisuccinate coupled to bovine serum albumin in a rabbit by Dr Robert H. Purdy, Department of Psychiatry, College of Medicine, University of California, San Diego, CA, USA. Cross-reactivity is shown in Table I. The antibody was used at a dilution 1:2300 and the solution was prepared using [11,12-^{3}H]pregnanolone custom-synthesized by NEN (New England Nuclear, Boston, MA, USA). The recovery of pregnanolone was 93%. The results are compensated for recovery.

Table I. Retention times of cross-reacting steroids to anti-allopregnanolone and anti-pregnanolone antibodies*. The earlier published cross-reactivity patterns from Purdy's allopregnanolone antiserum [12] and Purdy's pregnanolone antiserum [12 or 16] are also shown. These are the antisera used in the present study.

Steroid	Retention time (min)	Cross-reactivity (%)	
		Purdy's allopregnanolone antiserum	Purdy's pregnanolone antiserum
5α-Pregnan-20β-ol-3-one	26.7	14	–
5α-Pregnan-3α-ol-20-one	24.2	100	4.5
5β-Pregnan-3α-ol-20-one	22.5	5.8	100
5β-Pregnan-3,20-dione	19.4	21	100
5α-Pregnan-3β-ol-20-one	18.9	8.3	<1
4-Pregnen-3α-ol-20-one	18.5	50	–
5β-Pregnan-3β-ol-20-one	17.9	<1	5.8
5α-Pregnan-3,20-dione	17.0	50	1.5
5-Pregnen-3β-ol-20-one (pregnenolone)	14.7	4.0	<1
5α-Pregnan-3α,21-diol-20-one	10.1	–	4.6
Preg-4-ene-3,20-dione (progesterone)	9.4	17	11
4-Pregnen-20α-ol-3-one	9.2	<1	–

*High-performance liquid chromatography performed using a Symmetry C18 separation column (4.6 mm × 75 mm, 3.5 μm; Waters Corporation, Millford, MA, USA), heated to 45°C, and a mobile phase (methanol–water, 60:40, v/v) at a flow rate of 1.0 ml/min.

The sensitivity of the assay was 25 pg; the intra-assay CV was 6.5% and the inter-assay CV, 8.5%.

Progesterone. Measurements of plasma progesterone were taken using Delfia progesterone kits (Wallac Oy, Turku, Finland), a fluoroimmunoassay, according to the manufacturer's instructions.

Reference values for Z-transformation of progesterone and allopregnanolone concentrations. As blood samples were taken from the study patients on different days in the luteal phase, the progesterone and allopregnanolone concentrations were *Z*-transformed. This made it possible to compare serum progesterone and allopregnanolone concentrations taken at different cycle days between cycles and groups. The sample value (*Z*-value) is expressed as the number of standard deviation (SD) units from the mean in the reference group of the particular sampling day. The standard deviation used is the SD in the reference group of that particular cycle day. Z-values were thus calculated using the equation: $Z = X_i - X_{\text{mean reference}}/\text{SD}_{\text{reference}}$, where X_i is the value obtained in the study patient and $X_{\text{mean reference}}/\text{SD}_{\text{reference}}$ is the mean and SD in the reference group for the particular day of the menstrual cycle before the onset of the next menstrual bleeding. No normal curve was available for serum pregnanolone concentrations, which is why this neurosteroid was not transformed.

As reference values for the calculation of *Z*-scores of progesterone and allopregnanolone concentrations in the PMDD patients, daily progesterone and allopregnanolone concentrations during the luteal phase from reference menstrual cycles were used. The mean reference cycle consisted of blood samples of 32 menstrual cycles from a group of 20 women participating in an earlier study without any treatment intervention [15]. The subjects in the reference group were women both with and without PMDD. For the reference cycle, women provided daily blood samples for progesterone and allopregnanolone assays on cycle days 1–4, and from cycle day 10 throughout the remaining cycle until the first four days of menstrual bleeding during the next cycle. Between cycle days 4 and 10, occasional blood samples were taken. The average age of the women was 36.6 years (range 25–44 years). All cycles in the reference group were ovulatory, as defined by plasma progesterone values exceeding 15 nmol/l. These samples were centered on the first day of menstrual bleeding, with reverse counting during the preceding luteal phase and with the day before onset of bleeding as day −1. The mean (SD) concentrations during the menstrual cycle are shown in Figure 1.

Statistical methods

Daily symptom ratings were analyzed separately and in clusters of related symptoms. Related symptoms were grouped together as mean scores of summarized symptoms: 'negative mood symptoms', i.e. tension, irritability and depressed mood.

Analysis of variance with repeated measures was used to evaluate the difference in luteal-phase daily ratings between types of treatments. The within-subjects factors were time (the 7 days prior to onset of menstruation) and treatment (buserelin vs. placebo vs. pre-treatment).

The scores of daily life impairment and summarized negative mood during the pre-treatment cycle were compared with corresponding scores in the placebo cycle. A placebo response was then found in certain individuals. Based on the difference in scores

of daily life impairment and summarized negative mood between pre-treatment cycles and placebo treatment, the women were divided into two groups, buserelin responders and placebo responders, using median split of the rank order of score difference. Two situations in each of the two groups (placebo responders and buserelin responders) were studied, namely: (1) placebo responders on placebo treatment; (2) placebo responders on buserelin treatment; (3) buserelin responders on placebo treatment; and (4) buserelin responders on buserelin treatment.

Comparisons of hormone levels between buserelin responders and placebo responders were made by the Mann–Whitney U test, and between treatments in each group by the Wilcoxon matched-pair signed-rank test. The SPSS statistical package was used for all analyses (SPSS Inc., Chicago, IL, USA). Values $p < 0.05$ were considered statistically significant.

Results

Of the 18 PMDD patients included in the study, 12 (with 24 cycles) had blood samples taken within the stipulated luteal-phase time frame during both a buserelin and a placebo treatment cycle. Of these 12 patients, six were buserelin responders, whereas the remaining six were placebo responders. Table II shows their demographic data.

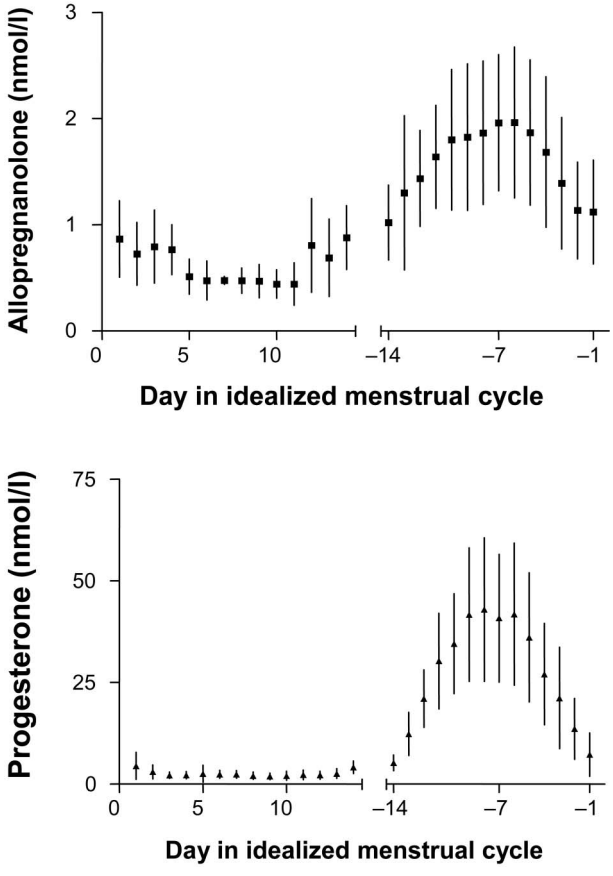

Figure 1. Progesterone (nmol/l) (bottom) and allopregnanolone (nmol/l) (top) concentrations from 32 menstrual cycles in the reference group; data are means with standard deviation shown by vertical bars. The data are centered on the day of onset of menstrual bleeding in 14-day periods.

Buserelin responders

The buserelin responders reported a significant improvement by buserelin treatment in summarized negative mood compared with both pre-treatment and placebo treatment ($F(2,10) = 10.45$, $p < 0.01$). In the *ad hoc* test, negative mood response during buserelin treatment was significantly different from placebo ($p < 0.05$) and pre-treatment ($p < 0.01$). Negative mood scores during placebo were not different from pre-treatment (Figure 2). Likewise, the daily life impairment during the luteal phase was also different with buserelin treatment compared with placebo treatment and pre-treatment ($F(2,10) = 18.85$, $p < 0.001$; Figure 3). The *ad hoc* test indicated a difference between buserelin treatment and placebo ($p < 0.05$), as well as between buserelin treatment and pre-treatment ($p < 0.01$). The placebo treatment did not differ from pre-treatment in this group.

Placebo responders

Placebo responders reported a significant difference in summarized negative mood between treatments and the pre-treatment period ($F(1,10) = 16.86$, $p < 0.001$). They reported improvement in negative mood with both the placebo treatment ($p < 0.01$) and buserelin treatment ($p < 0.01$) compared with pre-treatment (Figure 2). The daily life impairment during the luteal phase was also different between treatments ($F(2,10) = 7.48$, $p < 0.01$). *Ad hoc* tests indicated a significant difference between pre-treatment and placebo treatment ($p < 0.05$), as well as buserelin treatment ($p < 0.05$, Figure 3). However, there was no difference in the mood symptoms

Table II. Demographic data of the study group.

	Buserelin responders ($n = 6$)	Placebo responders ($n = 6$)
Age (years)	38.5 ± 5.5	36.5 ± 5.6
Parity	2.17 ± 0.4	1.3 ± 1.0
Education university/college	3 (50.0)	4 (66.7)
Full-time employment	4 (66.7)	5 (83.3)
Married	4 (66.7)	2 (33.3)
Mothers and/or sisters with PMDD	3 (50.0)	3 (50.0)
Previous psychiatric treatment	2 (33.3)	2 (33.3)
Previous postpartum depression	1 (16.7)	1 (16.7)

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

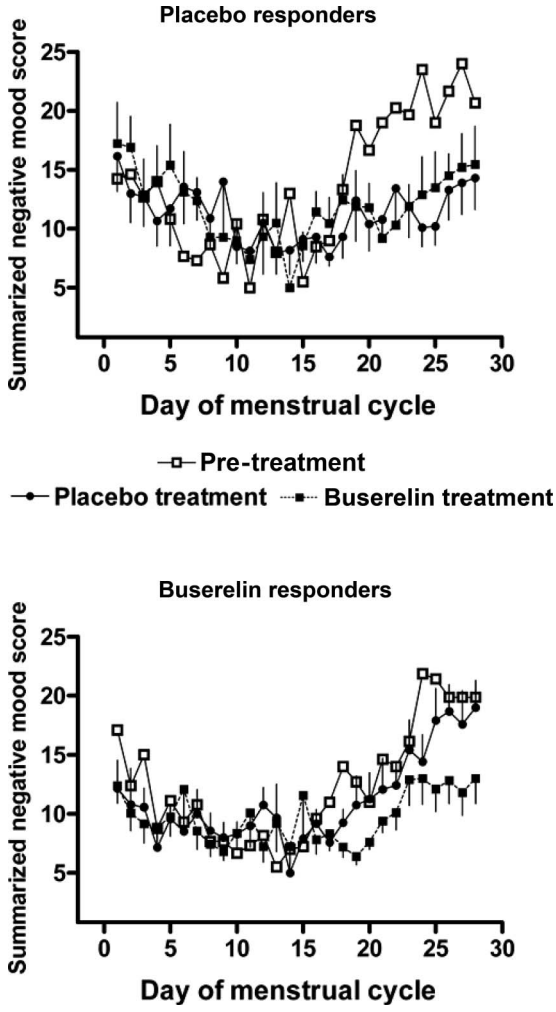

Figure 2. Daily symptom ratings on a 9-point Cyclicity Diagnoser scale of summarized negative mood scores during pre-treatment, placebo and buserelin treatment cycles in placebo responders (top) and buserelin responders (bottom). The cycles represent ideal 28-day cycles showing 14 postmenstrual days and 14 premenstrual days. Each point represents the group mean with vertical bars showing the standard error of the mean; error bars are not displayed during the pre-treatment cycle for clarity. Buserelin responders reported a significant improvement of the buserelin treatment in summarized negative mood symptoms ($F(1,146) = 9.05$, $p < 0.003$) during the luteal phase.

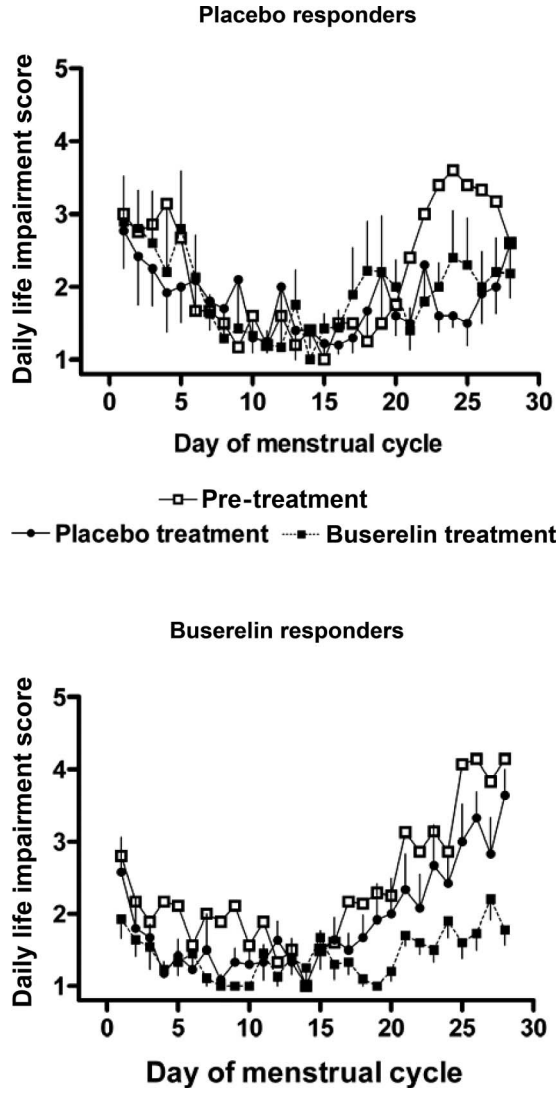

Figure 3. Daily symptom ratings on a 9-point Cyclicity Diagnoser scale of daily life impairment during pre-treatment, placebo and buserelin treatment cycles in placebo responders (top) and buserelin responders (bottom). The cycles represent ideal 28-day cycles showing 14 postmenstrual days and 14 premenstrual days. Each point represents the group mean with vertical bars showing the standard error of the mean; error bars are not displayed during the pre-treatment cycle for clarity. Buserelin responders showed a significant improvement of the buserelin treatment in daily life impairment compared with placebo ($F(1,146) = 26.07$, $p < 0.001$).

between placebo and buserelin treatments in the placebo responder group (Figures 2 and 3).

Neurosteroid and progesterone response to buserelin

Buserelin responders had significantly lower *Z*-scores for progesterone ($p < 0.05$) and allopregnanolone ($p < 0.05$) during buserelin treatment compared with placebo treatment. For the steroid concentrations that were not normalized, there were no significant differences between treatments in either group except for pregnanolone, where placebo responders had significantly lower serum pregnanolone concentrations during buserelin treatment than during placebo treatment ($p < 0.05$; Table III).

There were no differences in neurosteroid or progesterone concentrations or normalized *Z*-score values between buserelin responders and placebo responders during buserelin treatment.

Neurosteroid and progesterone response to placebo

During placebo treatment, placebo responders had lower *Z*-scores of allopregnanolone than buserelin responders ($p < 0.05$). None of the other neurosteroids differed between buserelin responders and placebo responders during placebo treatment.

Table III. Progesterone and neurosteroid levels during the late luteal phase of placebo and buserelin treatment in buserelin responders and placebo responders.

	Buserelin responders ($n = 6$)		Placebo responders ($n = 6$)	
Hormone/neurosteroid	Placebo	Buserelin	Placebo	Buserelin
Progesterone (*Z*-score)	0.39 ± 0.68	-0.94 ± 0.61*	0.11 ± 1.47	0.21 ± 1.42
Progesterone (nmol/l)	28.0 ± 5.0	17.8 ± 2.9	28.0 ± 6.5	19.3 ± 3.0
Allopregnanolone (*Z*-score)	0.34 ± 0.24	-0.37 ± 0.25*	$0.02 \pm 0.20^{\dagger}$	-0.12 ± 0.33
Allopregnanolone (nmol/l)	1.40 ± 0.31	0.99 ± 0.27	1.20 ± 0.16	1.00 ± 0.27
Pregnanolone (nmol/l)	0.83 ± 0.3	0.68 ± 0.1	0.83 ± 0.2	0.69 ± 0.2*

Data are presented as mean $\pm$ standard; *significantly lower compared with placebo cycle (Wilcoxon matched-pair signed-rank test): $p < 0.05$; †significantly lower compared with placebo cycle of buserelin responders (Mann–Whitney *U* test): $p < 0.05$.

Discussion

In the present study, we investigated the effect of a low dose of intranasal buserelin and placebo treatment on symptom improvement and serum steroid levels in women with PMDD. The main finding is that there is an association between the decrease in allopregnanolone concentration and symptom severity when individual patients are investigated.

In buserelin responders luteal-phase allopregnanolone levels decreased together with a decrease in symptom severity between the low-dose GnRH treatment cycles and placebo treatment cycles. Placebo responders, on the other hand, had lower luteal-phase allopregnanolone concentrations during placebo treatment compared with buserelin responders (i.e. women who did not improve on placebo but only on buserelin).

Furthermore, placebo responders who improved on both placebo and buserelin treatment compared with pre-treatment had similar allopregnanolone and progesterone levels during the placebo and low-dose GnRH treatment cycles.

These results thus suggest that there is an association between improved symptoms and decreased serum allopregnanolone concentrations, independent of whether the cause for improvement is a placebo response or an active drug response. An association between a parallel change in severity of negative mood and serum allopregnanolone concentrations has been reported in earlier studies [23,24]. For instance, PMDD patients who reported symptom improvement following treatment with SSRI or placebo had lower levels of allopregnanolone, irrespective of which treatment had been given [23].

However, higher endogenous levels of allopregnanolone in the luteal phase have also been associated with lower symptom severity in PMDD patients [15], with similar results in a study by Girdler and coworkers [22]. The relationship between symptom severity and a decreased sensitivity to different GABAergic substances like pregnanolone, benzodiazepines, and alcohol [10–13], especially in the luteal phase, has previously been reported in women with PMDD. Given the findings of altered functional $GABA_A$-receptor sensitivity in PMDD patients, the absolute level in allopregnanolone concentration might not be the only explanation for the appearance of symptoms. Instead, a combination of an altered $GABA_A$-receptor sensitivity and a possible development of tolerance to these neuroactive agents [30,31] could render these women less sensitive to the effect of allopregnanolone in the luteal phase of the menstrual cycle.

In fertile women, serum allopregnanolone concentration increases from 0.5 nM in the follicular phase to 4 nM in the luteal phase, and is correlated with the level of serum progesterone. The increase in allopregnanolone seems to correlate with the increase in negative mood symptoms during the early luteal phase in women with PMDD [32]. In the present study, the decrease in serum allopregnanolone concentration was, on average, 0.6SD in the buserelin responder group, thus approaching follicular-phase values.

The serum concentration of allopregnanolone seems to be of importance for symptom severity. In postmenopausal women receiving HT with sequential progesterone and estradiol, severity of symptoms increased in parallel with the serum levels of allopregnanolone seen during mid-luteal phase. With further increases in serum allopregnanolone concentrations symptom severity gradually decreases, rendering an inverted U-shaped relationship between symptom severity and allopregnanolone concentration [24,33]. Allopregnanolone is a well-known potent $GABA_A$-receptor agonist, and many $GABA_A$-receptor agonists like benzodiazepines, alcohol, barbiturates and neuroactive steroids have been shown to exert an inverted U-shaped biphasic effect on mood and behavior. With high concentrations, these positive modulators of the $GABA_A$ receptor enhance the effect of GABA and induce an anxiolytic, sedative, hypnotic, antiepileptic and anesthetic

effect in both animals and humans [28,34,35], while in certain individuals low concentrations of allopregnanolone induce loss of impulse control, aggression and irritability [36–43].

This is further substantiated by studies investigating the effect of different doses of progesterone/progestogens in postmenopausal HT. Postmenopausal women taking sequential HT reported more adverse mood effects on 10 mg medroxyprogesterone acetate (MPA) than on 20 mg MPA [44] and, likewise, experienced more negative mood symptoms with vaginal progesterone 400 mg/day compared with 800 mg/day [45]. It is possible that women receiving a low dose of GnRH agonist treatment, resulting in a somewhat downregulated ovarian function, experience symptom improvement secondary to declining allopregnanolone levels. It is also quite possible that the allopregnanolone levels in these women are lower than the peak symptom-inducing allopregnanolone concentration.

The placebo effect in the treatment of PMDD was shown earlier to be substantial [3,25,46,47]. The rate of placebo response for PMDD varies between 6 and 35% [48], but rates up to 94% have been seen in some clinical studies [49]. Placebo response has also been reported in prior GnRH agonist studies, with significant improvement from placebo treatment in between 26 and 70% of patients (depending on the symptom) compared with pre-treatment [3]. The mechanisms behind the placebo response are not known, but explanations of an effect on the opioid system have been forwarded [50,51]. Also, release of dopamine, and expectation of and desire for drug effect, may alter the treatment response. In the present study there was a decrease in allopregnanolone concentration during placebo treatment, indicating that the placebo effect might be related to decreased allopregnanolone concentration or $GABA_A$-receptor stimulation.

There are a number of weaknesses and limitations to the interpretation of this study. First, the number of patients is limited and it is not possible to draw any definite conclusions from this small sample size, although the tendency supports findings from other studies. Another limitation is that with a more frequent blood sampling we could have used actual serum steroid levels instead of transformed *Z*-score levels, although *Z*-scores of serum concentration represent the specific day of the menstrual cycle on which serum is taken. The reason why we failed to take blood samples on a specific day of the menstrual cycle was the variation in cycle length, mainly during GnRH treatment.

In conclusion, this study suggests a relationship between decreased serum allopregnanolone concentrations and decreased symptom severity, independent of active treatment or placebo.

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