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ORIGINAL INVESTIGATION

Allopregnanolone concentration and mood—a bimodal association in postmenopausal women treated with oral progesterone

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Abstract
Rationale Allopregnanolone effects on mood in postmenopausal women are unclear thus far.
Objectives Allopregnanolone is a neuroactive steroid with contradictory effects. Anaesthetic, sedative, and anxiolytic as well as aggressive and anxiogenic properties have been reported. The aim of this study is to compare severity of negative mood between women receiving different serum allopregnanolone concentrations during progesterone treatment.
Materials and methods A randomized, placebo-controlled, double-blind, crossover study of postmenopausal women (n=43) treated with 2 mg estradiol daily during four treatment cycles. Oral micronized progesterone at 30, 60, and 200 mg/day, and placebo were added sequentially to each cycle. Participants kept daily symptom ratings using a validated rating scale. Blood samples for progesterone and allopregnanolone analyses were collected during each treatment cycle.
Results During progesterone treatment, women had significantly higher negative mood scores when allopregnanolone serum concentration was in the range of 1.5–2 nmol/l compared to lower and higher concentrations. In addition, women displayed a significant increase in negative mood during the progesterone treatment period, compared to the estradiol-only period when 30 mg progesterone daily was used. On the other hand, treatment with higher doses of progesterone had no influence on negative mood.
Conclusions Mood effects during progesterone treatment seem to be related to allopregnanolone concentration, and a bimodal association between allopregnanolone and adverse mood is evident.

Keywords Progesterone · Allopregnanolone · GABA · Mood · Biphasic

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Introduction

The addition of progestagens and vaginal progesterone in sequential hormone therapy (HT) provokes negative mood (Andreen et al. 2003; Bjorn et al. 2000; Hammarback et al. 1985; Magos et al. 1986) and the progesterone/progestagen-induced symptoms are similar to the mood deterioration encountered during the luteal phase of ovulatory cycles in women with premenstrual dysphoric disorder (PMDD) (Backstrom et al. 1983; Bixo et al. 2001). The negative mood symptoms appear shortly after the progestagen is added, rise in severity until the end of the progestagen treatment, and thereafter decline (Hammarback et al. 1985; Magos et al. 1986). To understand progesterone-induced adverse mood effects, it is important to note that progesterone is metabolized to allopregnanolone (3α-OH-5α-pregnan-20-one) and pregnanolone (3α-OH-5β-pregnan-20-one), both acting as agonists on the γ-aminobutyric acid A ($GABA_A$) receptor complex in the brain (Majewska et al. 1986).

Thus far, studies have indicated a certain degree of disparity with regard to the effects of these neuroactive progesterone metabolites. Some studies have reported

beneficial effects, such as sedation (Sundstrom et al. 1998; Timby et al. 2005), anaesthesia (Carl et al. 1990), and anti-epileptic effects (Backstrom et al. 1984; Landgren et al. 1998) of high doses of allopregnanolone and pregnanolone in animals and humans. Furthermore, allopregnanolone has been reported to have anxiolytic effects in animals (Bitran et al. 1995; Wieland et al. 1991). In studies by Pinna et al., it was shown that aggression was related to decrease in allopregnanolone brain content. Aggression and brain allopregnanolone levels were normalized by fluoxetin treatment in their studies (Pinna et al. 2003; Pinna et al. 2005). These beneficial properties are similar to the effect of other $GABA_A$ receptor modulators, e.g., benzodiazepins.

On the other hand, studies have also reported negative effects by allopregnanolone and other $GABA_A$ receptor modulators. Allopregnanolone has been shown to increase aggression (Fish et al. 2005; Miczek et al. 1997) and inhibit learning and memory (Johansson et al. 2002). Short-term, as opposed to acute, treatment with progesterone resulted in increased anxiety in rats, an effect related to an up-regulation of the alpha4 subunit of the $GABA_A$ receptor, which in turn is attributed to an allopregnanolone effect (Gulinello et al. 2001). Similar results were obtained by Beauchamp et al., who showed that place aversion as a measure of anxiety in rats was produced by a low dosage of allopregnanolone (Beauchamp et al. 2000).

Besides the neuroactive progesterone metabolites, benzodiazepines, barbiturates, and alcohol also act as positive modulators of the $GABA_A$ receptor (Majewska et al. 1986). Recent reports from human and animal studies support the hypothesis of a bimodal effect of these $GABA_A$ receptor modulators, which in turn could provide a possible explanation for the reported discrepancies of neurosteroid effects. In animal studies, allopregnanolone and other $GABA_A$ receptor modulators induce irritability, aggression (Fish et al. 2001; Miczek et al. 1997; Yoshimura and Ogawa 1989), and anxiety (Beauchamp et al. 2000) in a bimodal pattern. Whereas low doses or concentrations increase an adverse, anxiogenic effect, high doses or concentrations decrease this effect.

Paradoxical findings of $GABA_A$ receptor modulators are also reported from human studies. Adverse effects, such as aggressive and depressive behavior, occur in a proportion of patients with cardiac conditions undergoing transesophageal echocardiography after intravenous administration of midazolam (Wenzel et al. 2002). Moreover, low doses of diazepam given to humans elicit more aggression than placebos during experimental conditions (Ben-Porath and Taylor 2002), while higher doses have sedative and anxiolytic properties. High-dose oral progesterone treatment in women caused significant increases in fatigue, delayed verbal recall, and symbol coping in subjects receiving high levels of allopregnanolone and pregnanolone, while those with lower metabolite levels reported no negative effects (Freeman et al. 1993).

Progesterone can be added to estradiol therapy in the form of vaginal suppositories or oral micronized progesterone. The metabolism of progesterone differs depending on the route of administration. After oral administration of micronized progesterone, plasma concentration of allopregnanolone is higher than the concentration of progesterone, while pregnanolone concentrations are similar to progesterone levels (de Lignieres et al. 1995). On the other hand, after vaginal administration of progesterone, a smaller increase in allopregnanolone levels occurs as well as a slight or insignificant increase in pregnanolone, compared with progesterone concentration (Andreen et al. 2006; de Lignieres et al. 1995).

Given the reported findings of both beneficial and adverse mood effects, in addition to a bimodal action of allopregnanolone in certain animal studies, the aims of this randomized, double-blind, placebo-controlled, crossover study were to investigate (1) the severity of negative mood effects, and (2) whether allopregnanolone has bimodal effects after administration of oral micronized progesterone. Furthermore, the study was aimed to evaluate the differences in plasma concentrations of progesterone and allopregnanolone between three different doses of oral micronized progesterone.

Materials and methods

Subjects

Forty-three women with climacteric symptoms including hot flushes or sweating were recruited and randomly assigned to treatment in a double-blind, crossover study. All subjects were more than 6 months postmenopausal, had intact uterus and ovaries, and had not used any HT for 3 months before inclusion in the study. They had no contraindications to HT and were considered physically healthy. The women were not receiving any steroid treatment, had no history of psychiatric illness, and had not been treated with psychopharmacological drugs within the past 6 months. Subjects with ongoing psychiatric illness were excluded through the use of the Primary Care Evaluation of Mental Disorders (PRIME-MD) questionnaire. PRIME-MD has been developed to help primary care physicians screen, evaluate, and diagnose mental disorders. This diagnostic tool is constructed to conform to Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria and has been validated for use in a primary care setting (Spitzer et al. 1994). Before inclusion, subjects gave written informed consent and agreed to keep a daily record of symptoms. Before taking part, the patients underwent a

Table 1 Demographic data and physical characteristics of the study group (n=37)

Age y, median (IQR)	53 (51–57)
Weight kg, median (IQR)	71 (66–79)
BMI, median (IQR)	25 (24–27)
Having a partner (%)	89
Education, college or university (%)	84
Parity (%)	87
Climacteric symptoms y, median (IQR)	3 (1–7)
Years after menopause y, median (IQR)	1 (1–5)
Previous HT (%), y, median (IQR)	70, 1 (1–2)

IQR Inter-quartile range, *BMI* body mass index, *HT* hormone therapy

physical and gynecological examination, including routine vaginal ultrasonography.

Thirty-seven women completed the study and were included in the analysis. Demographic data and physical characteristics of the study group are shown in Table 1. Of the 43 women who were originally included for the study, four dropped out during the study course (one due to depression, one due to abdominal bloating and fatigue, and two due to fear of side effects). Two women were excluded, one due to a major life event during the study (hospital treatment of severe disease) and one due to protocol violation (failed to fill in the daily symptom rating scale). The study included women with and without a history of PMS. Premenstrual symptoms during fertile life were defined by a retrospective report of mood deterioration before menstruation, which decreased and disappeared within 4 days after the onset of menstrual bleeding. The influence of PMS symptoms on daily life was graded, and women whose family relations, social activities, or work were negatively affected by the symptoms were considered to have a history of PMS. Eleven subjects had a history of PMS and 26 had no prior PMS, according to these criteria. Only four out of 11 women with a reported history of PMS showed symptom cyclicity. Retrospective history of PMS is an insecure method to define PMS or PMDD, and according to DSM-IV criteria daily prospective symptom ratings are needed for two or more menstrual cycles to diagnose PMDD, and this is not possible after the menopause. Therefore, we abandoned the retrospective reports of PMS.

Study design

The effect on mood, physical symptoms, and progesterone and allopregnanolone plasma concentrations of 30, 60, and 200 mg/day of micronized progesterone given orally in two doses (morning and evening) was evaluated in a randomized, placebo-controlled, double-blind, crossover design. The study began with a 33-day run-in cycle during which the patients were treated with 2 mg/day of estradiol valerate (Schering AG, Germany) orally and 10 mg of medoxyprogesterone acetate (MPA) (Leo Pharma, Sweden) orally on days 20–33 of the cycle. Given the positive effects of estrogen on well-being due to the reduction of vasomotor symptoms during the first month of treatment, this run-in cycle was included to avoid interference with mainly estrogen-dependant effects on climacteric symptoms in the subsequent analyses (Holst et al. 1989). The drawback to this procedure is that all cycles after a progestagen treatment will have a period of 3–4 days in the beginning of the next cycle where the symptoms from the previous cycle decline (Bjorn et al. 2000).

In the following four 33-day cycles, the women were treated with estradiol valerate orally in a dose of 2 mg daily throughout the study period. Subjects were randomly assigned to start with either placebo or one of the three progesterone doses according to a Latin square design. Progesterone or placebo was added twice a day on days 20–33 of each cycle. A crossover to a new treatment was carried out after each cycle. The oral formulation of progesterone consisted of soft gelatin capsules containing placebo or 15, 30, and 100 mg of micronized progesterone in monohydrous lactose. The capsules were made to appear identical, and were prepared, packed, and randomized by Apoteket AB, the Swedish national pharmacy company (Production and Laboratories, Stockholm, Sweden). The three progesterone doses were chosen because earlier studies have indicated that they provide serum concentrations of progesterone and allopregnanolone in the physiological (Andreen et al. 2006) and supraphysiological range (de Lignieres et al. 1995). The study ended with a 33-day run-out cycle during which patients were treated with 2 mg of estradiol valerate daily and 10 mg of MPA on days 20–33. This run-out cycle was included to secure endometrial shedding and continued daily symptom scoring after the last treatment cycle (when symptoms from the previous cycle decline).

The primary outcome measure was the daily symptom ratings made by the patients throughout the study. We used a modified form of the Cyclicity Diagnoser (CD), an instrument for diagnosing cyclic symptoms that has been validated for the diagnosis of PMDD (Sanders et al. 1983; Sundstrom et al. 1999), but is also used to evaluate HT-related symptom changes in postmenopausal women (Andreen et al. 2003; Andreen et al. 2005; Bjorn et al. 2000). The CD included four physical symptoms (breast tenderness, hot flushes, abdominal bloating, and withdrawal bleeding) and seven psychological symptoms (cheerfulness, friendliness, libido, anxiety/tension, irritability, fatigue, and depression). The effects on daily life caused by symptoms were graded. The CD is a Likert scale, graded from 0 to 8, where 0 indicates complete absence of a particular symptom and 8 represents the maximum severity of the symptom. The patients can detect one scale step as a

difference in mood experience, as shown in a study of symptom severity in women with PMDD (Seippel and Backstrom 1998).

A gynecologist saw the patients twice, at inclusion and at termination of the study (at 28 weeks). During the study, patients made scheduled visits to a research nurse at the end of each treatment cycle (six times). Weight, blood pressure, and gynecological examination, including a routine vaginal ultrasound, were followed up after 28 weeks. The Umeå University Ethical Committee and the National Medical Products Agency approved the design of the study.

Steroid assays

Two blood samples for estradiol, progesterone, and allopregnanolone analyses were collected each cycle, on 2 different days, during the last week of progesterone treatment. The blood samples were drawn immediately before administration of progesterone in the morning. Measurements of plasma progesterone were made by Delfia progesterone kits (Wallac Oy, Turku, Finland), a fluoroimmunoassay, according to the manufacturer's instructions. Estradiol was assayed with Delfia estradiol kits (Wallac Oy, Turku, Finland), a fluoroimmunoassay, according to the manufacturer's instructions.

Assay of allopregnanolone

Extraction

Allopregnanolone was measured by radioimmunoassay (RIA) after diethylether extraction and celite chromatographic purification of samples. Serum or plasma (0.2–1.0 ml) was pipetted into a cylindrical flat bottom glass vial of 20 ml volume, where after water (0–0.5 ml) and diethyl ether (3.0 ml) were added. The samples were then allowed to stand on an orbital shaker for 10 min. After 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.

Celite chromatography

The evaporated sample was dissolved in 1.0 ml isooctane (Merck) saturated with ethylene glycol (J. T. Baker), before application to the column. Celite column chromatography was performed as follows. Glass columns (50×5 mm i.d.) were tightly packed with a mixture of Celite (Mansville, Denver, CO, USA, heated to 600°C overnight) and propylene glycol (Merck), w/v, 1/1. Isooctane (10 ml) was percolated through the columns before sample applications. The sample was applied, followed successively by a 1.0-ml isooctane wash, 1.5 ml isooctane to elute 5α- and 5β-dihydroprogesterone, 4 ml isooctane to elute progesterone, an additional 4 ml isooctane to elute allopregnanolone, and in the next 4.0 ml isooctane 3α-hydroxy-pregn-4-en-20-one and pregnanolone were eluted. 20β-Hydroxy-5α-pregnan-3-one, which cross-reacts with the antiserum, is not eluted with isooctane. The allopregnanolone-containing fraction was evaporated under nitrogen. Recovery was determined for each assay using 300–500 cpm of tritium-labeled allopregnanolone, [9,11,12-^{3}H(N)]-5α-pregnan-3α-ol-20-one (Perkin-Elmer Life Sciences, Boston, USA) added to a serum sample before extraction and by measuring the amount recovered after chromatography. The recovery of allopregnanolone averaged 78%, and the results are compensated for recovery.

Radioimmunoassay

All samples were analyzed using a polyclonal rabbit antiserum (Purdy et al. 1990). The antiserum was raised against 3α-hydroxy-20-oxo-5α-pregnan-11-yl carboxymethyl ether coupled to bovine serum albumin. The cross-reactivity of the antibody is shown earlier (Purdy et al. 1990; Timby et al. 2006). The standard curve was established by preparing duplicate tubes containing eight concentrations of unlabeled allopregnanolone to give a range from 0 to 5,000 pg. The antiserum was used in a dilution of 1/5,000. The antibody solutions were prepared using [11,12]^{3}H allopregnanolone, 3×106 cpm/32 ml solution containing 65 mM boric acid (Merck) buffer, pH=8.0, bovine serum albumin 100 mg/ml (Sigma, St Louis, MO, USA), human gamma globulin solution 20 mg/ml (Octapharma, Sweden): 30:1:1:0.006. The solution was allowed to equilibrate overnight at 8°C. Antibody solution (200 μl) was added to all standard and sample tubes, and incubated 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) scintillation counter. The sensitivity of the assays was 25 pg, with an intra-assay coefficient of variation for allopregnanolone of 6.5% and an inter-assay coefficient of variation of 8.5%.

Statistics

Symptoms were analyzed separately and in clusters of related symptoms based on an earlier principal component analysis (Sanders et al. 1983). Related symptoms were

Table 2 Serum progesterone and allopregnanolone concentrations given as median and inter-quartile range in postmenopausal women (n=37) during treatment with progesterone at 30, 60, and 200 mg/day, and placebo

Progesterone treatments					
Median serum concentration (IQR)	30 mg/day	60 mg/day	200 mg/day	Placebo	$F(3, 96)$; P value
Progesterone (nmol/l)	2.4 (1.8–3.3)	3.9 (3.0–5.1)	8.4 (6.3–12.)	1.6 (1.1–2.1)	F=78.9; P<0.001
Allopregnanolone (nmol/l)	0.9 (0.6–1.1)	1.5 (1.0–2.1)	4.2 (2.9–5.5)	0.2 (0.1–0.2)	F=73.4; P<0.001

In the post hoc test, all steroid concentrations were significantly different from each other (P<0.01–0.001)
IQR Inter-quartile range

grouped together as summarized symptom scores: 'negative mood symptoms', including tension, irritability, depression, and fatigue; 'positive mood symptoms', including cheerfulness and friendliness; and 'physical symptoms,' such as breast tenderness and bloating.

Differences in symptom scores during the treatment cycles within individuals were analyzed by two-way analysis of variance (ANOVA) with repeated measures. Independent variables were cycle phases (estrogen vs progesterone phase) and cycle days in the repeated measures analyses. One-way ANOVA was used for comparing summarized negative, positive, and physical symptom scores between different steroid concentrations. The least significant difference test was used as a post hoc method when applicable. In cases of missing values, the last value brought forward was used in an intention to treat in the manner of the repeated measure analysis. The SPSS statistical package was used for the analyses. P<0.05 was considered statistically significant.

Results

Steroid concentrations

During treatment with placebo and oral micronized progesterone at 30, 60, and 200 mg/day, median serum concentrations of progesterone and allopregnanolone differed significantly [$F(3, 96)$=78.9; P<0.001 and $F(3, 96)$=73.4; P<0.001, respectively] (Table 2). During continuous treatment with 2 mg of estradiol, median (inter-quartile range) plasma concentration of estradiol was 0.21 (0.14–0.25) nmol/l.

Symptom cyclicity in all subjects

Cyclicity in symptoms was first evaluated in the entire study group. The period of the estrogen phase, when positive mood reached its highest and negative mood and physical symptoms their lowest, occurred during the last 7 days of the estrogen phase (days 13–19). In the progesterone phase, the worst period peaked during the last 4 days of the treatment cycle and continued the first 3 days of the next cycle (days 30–3).

When treated with progesterone at 30 mg/day, the women scored significantly more negative mood symptoms during the progesterone treatment period (days 30–3), compared to the estradiol-only period (days 13–19) [$F(1, 36)$=4.19; P<0.05] (Table 3).

While receiving 60 mg of progesterone per day, the women scored significantly less negative mood symptoms during the progesterone treatment period, compared with the estradiol-only period [$F(1, 36)$=7.69; P<0.01] (Table 3).

Finally, while treated with progesterone at 200 mg/day, the women scored significantly more physical symptoms during the progesterone treatment period, compared with the estradiol-only period [$F(1, 36)$=5.53; P<0.05]. Furthermore, women scored a significant increase in daily life impairment during the progesterone treatment period compared with the estradiol-only period [$F(1, 36)$=9.83; P<0.01] (Table 3). During placebo treatment no cyclicity was seen.

Symptom changes in women who experience worsening of symptoms during progesterone treatment (cyclicity)

Certain women experience cyclicity with worsening of negative mood and physical symptoms during progestagen treatment in sequential HT (Bjorn and Backstrom 1999; Hammarback et al. 1985; Magos et al. 1986). In our group, mood symptom cyclicity was seen during the treatment cycle with estradiol and progesterone at 30 mg/day. For this reason, we identified women with an increase in negative mood symptoms with two scale steps or more during progesterone treatment (30 mg/day), compared with estrogen-only treatment. Furthermore, it was required that their negative mood symptoms were not merely an exacerbation of previously existing symptoms (i.e., high negative mood scores throughout estrogen only treatment), and finally that they did not deteriorate during placebo treatment. Sixteen women (43%) fulfilled the criteria for symptom cyclicity and 21 women did not (Fig. 1, Table 4). The group of women with symptom cyclicity scored, as expected,

Table 3 Summarized symptom scores in all subjects (n=37) during treatment with unopposed estrogen (E-phase, treatment days 13–19) and estrogen + progesterone at 30, 60, and 200 mg/day, or placebo (P-phase, treatment days 30–3)

Progesterone treatments												
	30 mg/day			60 mg/day			200 mg/day			Placebo		
Symptom scores Mean±SEM	E-phase	P-phase	E vs P $F(1, 36)$; P value	E-phase	P-phase	E vs P $F(1, 36)$; P value	E-phase	P-phase	E vs P $F(1, 36)$; P value	E-phase	P-phase	E vs P $F(1, 36)$; P value
Summarized negative mood	3.8±0.8	5.5±1.0	4.19; $P<0.05$	4.8±0.9	3.5±0.7	7.69; $P<0.01$	4.4±0.8	4.7±0.9	N.S	4.1±0.8	4.6±0.8	N.S
Irritability	0.9±0.2	1.3±0.2	5.49; $P<0.05$	1.2±0.2	0.8±0.2	5.12; $P<0.05$	1.0±0.2	1.3±0.2	N.S	1.0±0.2	1.1±0.2	N.S
Anxiety/tension	0.7±0.2	1.1±0.3	N.S	1.0±0.3	0.7±0.2	6.98; $P<0.05$	1.0±0.3	0.9±0.2	N.S	0.9±0.2	1.0±0.2	N.S
Depression	0.8±0.2	1.4±0.3	5.56; $P<0.05$	0.9±0.2	0.6±0.1	N.S	0.9±0.2	1.0±0.2	N.S	0.9±0.2	1.0±0.2	N.S
Fatigue	1.4±0.3	1.7±0.3	N.S	1.7±0.3	1.4±0.3	7.40; $P<0.05$	1.6±0.3	1.5±0.3	N.S	1.4±0.3	1.6±0.3	N.S
Summarized positive mood	11.±0.7	10.±0.7	N.S.	10.±0.7	9.5±0.5	N.S	10.±0.7	10.±0.7	N.S	11.±0.6	1.1±0.6	N.S
Cheerfulness	5.3±0.3	5.1±0.4	N.S	5.0±0.4	5.5±0.3	12.2; $P<0.01$	4.9±0.4	4.8±0.4	N.S	5.2±0.3	5.3±0.3	N.S
Friendliness	5.4±0.3	5.2±0.4	N.S	5.0±0.4	5.5±0.3	9.80; $P<0.01$	5.3±0.3	5.2±0.3	N.S	5.4±0.3	5.6±0.3	N.S
Summarized physical symptoms	1.3±0.3	1.2±0.3	N.S	1.3±0.3	1.4±0.3	N.S	1.1±0.3	1.6±0.3	5.53; $P<0.05$	1.4±0.4	1.6±0.4	N.S
Abdominal bloating	0.7±0.2	0.7±0.2	N.S	0.8±0.2	0.7±0.2	N.S	0.5±0.1	0.8±0.2	4.33; $P<0.05$	0.7±0.2	0.8±0.2	N.S
Breast tenderness	0.6±0.2	0.4±0.1	N.S	0.5±0.2	0.7±0.2	N.S	0.5±0.2	0.8±0.2	N.S	0.7±0.2	0.8±0.2	N.S
Hot flushes	0.5±0.1	0.4±0.1	N.S	0.5±0.1	0.5±0.1	N.S	0.5±0.1	0.8±0.2	N.S	0.6±0.2	0.6±0.2	N.S
Impairment	0.7±0.1	1.2±0.3	N.S	0.8±0.2	0.7±0.1	N.S	0.6±0.1	1.0±0.2	9.83; $P<0.01$	0.7±0.1	0.8±0.1	N.S

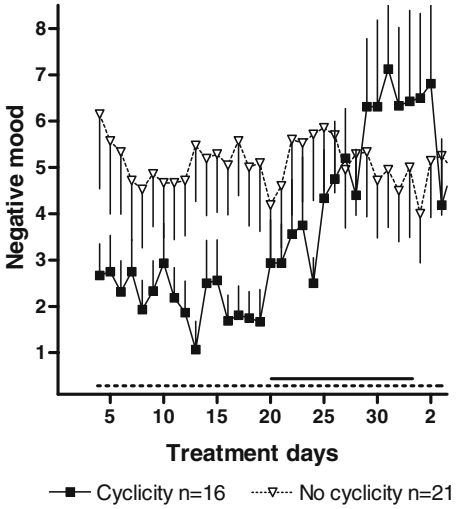

Fig. 1 Mean±SEM of summarized negative mood in postmenopausal women treated with 2 mg of estradiol continuously (*dotted line*) and with progesterone at 30 mg/day on treatment days 20–33 (*filled line*). Women were divided into two groups: one group with cyclicity in the mood (n=16) and the other group without cyclicity (n=21). Significant cyclicity with worsening of negative mood during progesterone phase was seen in the group of women with cyclicity [F(1, 15)=8.37, P<0.01]. Women without cyclicity had higher negative mood scores during estrogen phase, compared with women with cyclicity [F(1, 35)= 5.13; P<0.05]

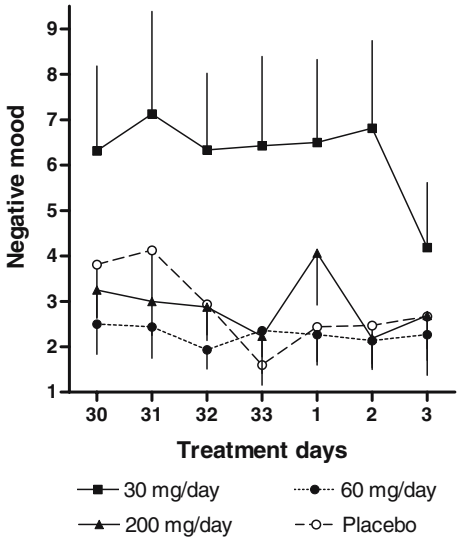

Fig. 3 Mean±SEM of summarized negative mood in postmenopausal women (n=16) who experienced worsening of mood symptoms during progesterone treatment. Women were treated with 2 mg of estradiol continuously and with progesterone at 30, 60, and 200 mg/day, or placebo on days 20–33. Negative mood symptoms were analysed when they reached their lowest during progesterone phase (days 30–3). During that period, women had significantly higher negative mood scores when treated with progesterone at 30 mg/day, compared with the other treatments [F(3, 45)=4.77; P<0.01]

significantly more negative mood symptoms and less positive mood symptoms during treatment with 30 mg/day of progesterone, compared with the estradiol-only period [F (1, 15)=8.73; P<0.01 and F(1, 15)=9.63; P<0.01, respectively] (Fig. 2, Table 4). Furthermore, these women scored a significant increase in daily life impairment during the progesterone treatment period, compared with estradiol only period [F(1, 15)=5.31; P<0.05] (Table 4).

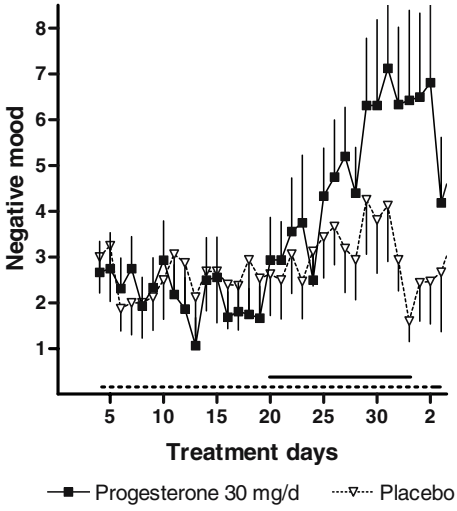

Fig. 2 Mean ± SEM of summarized negative mood in postmenopausal women (n=16) who experienced worsening of mood symptoms during progesterone treatment. Women were treated with 2 mg of estradiol continuously (*dotted line*) and with progesterone at 30 mg/day or placebo treatment days 20–33 (*filled line*). During progesterone treatment, a significant cyclicity with worsening of negative mood during progesterone phase was observed [F(1, 15)=8.37; P<0.01]

During treatment with progesterone at 200 mg/day, the group of women with symptom cyclicity reported more physical symptoms during the progesterone treatment period, compared with the estradiol only period, although the difference was not statistically significant (P=0.055). Women scored a significant daily life impairment during the progesterone treatment period, compared with the estradiol only period [F(1, 15)=6.37; P<0.05] (Table 4).

Differences in symptom severity between different progesterone doses in women with symptom cyclicity

The women with symptom cyclicity reported significantly more negative mood symptoms during the progesterone phase when treated with 30 mg/day progesterone, compared with 60 and 200 mg/day, or placebo [F(3, 45)=4.38; P<0.01]. In the post hoc test, the score of negative mood during treatment with 30 mg/day of progesterone was higher, compared with negative mood scores during 60 mg/day (P<0.05), 200 mg/day (P<0.05) and placebo (P<0.05) (Fig. 3, Table 4).

During estrogen phases, a significant difference occurred in negative mood scores between the treatment cycles [F(3, 45)=3.36; P<0.05]. Women with cyclicity reported adverse mood during estrogen phase in the treatment cycle with

Table 4 Summarized symptom scores in women with cyclicity (n=16) and women without cyclicity (n=21) during treatment with unopposed estrogen (E-phase, treatment days 13–19) and estrogen + progesterone at 30, 60, and 200 mg/day, or placebo (P-phase, treatment days 30–3)

Progesterone treatments												
Symptom scores Mean± SEM	30 mg/day			60 mg/day			200 mg/day			Placebo		
Women with cyclicity (n=16)	E-phase	P-phase	E vs P $F(1,15)$; P value	E-phase	P-phase	E vs P $F(1,15)$; P value	E-phase	P-phase	E vs P $F(1,15)$; P value	E-phase	P-phase	E vs P $F(1,15)$; P value
Summarized negative mood	1.9±0.5[a]	6.6±1.7[b]	8.73; P<0.01	4.0±0.8	2.3±0.5[b]	N.S	2.4±0.7[c]	2.9±0.6[b]	N.S	2.5±0.8	2.9±0.8[b]	N.S
Summarized positive mood	11.±0.9	9.4±1.1	9.63; P<0.01	9.7±1.1	9.2±0.8	N.S	11.± 0.9	10.±0.9	N.S	11.±0.9	11.±0.8	N.S
Summarized physical symptoms	0.8±0.4	1.0±0.3	N.S	1.3±0.5	0.8±0.3	N.S	0.6±0.3	1.3±0.4	N.S (P=0.055)	0.8±0.5	0.9±0.4	N.S
Impairment	0.3±0.1	1.7±0.6	5.31; P<0.05	0.7±0.2	0.6±0.1	N.S	0.3±0.1	0.7±0.2	6.37; P<0.05	0.4±0.2	0.6±0.2	N.S
Women without cyclicity (n=21)	E-phase	P-phase	E vs P $F(1,20)$; P value	E-phase	P-phase	E vs P $F(1,20)$; P value	E-phase	P-phase	E vs P $F(1,20)$; P value	E-phase	P-phase	E vs P $F(1,20)$; P value
Summarized negative mood	5.2±1.2[a]	4.7±1.1	N.S	5.5±1.4	4.9±1.1	N.S	5.9±1.3[c]	6.1±1.4	N.S	5.3±1.1	5.9±1.3	N.S
Summarized positive mood	10.±1.0	11.±1.0	10.1; P<0.01	10.±1.0	11.±0.9	10.3; P<0.01	9.9±1.0	9.9±1.0	N.S	10.±0.9	11.±0.8	4.69; P<0.05
Summarized physical symptoms	1.7±0.5	1.3±0.4	N.S	1.3±0.4	1.9±0.5	N.S	1.5±0.4	1.9±0.5	N.S	1.9±0.6	2.2±0.6	N.S
Impairment	0.9±0.2	0.8±0.2	N.S	0.9±0.3	0.8±0.2	N.S	0.7±0.2	1.3±0.3	N.S	0.9±0.2	0.9±0.2	N.S

[a] $F(1,35)$=5.13; P<0.05
[b] $F(3,45)$=4.38; p<0.01

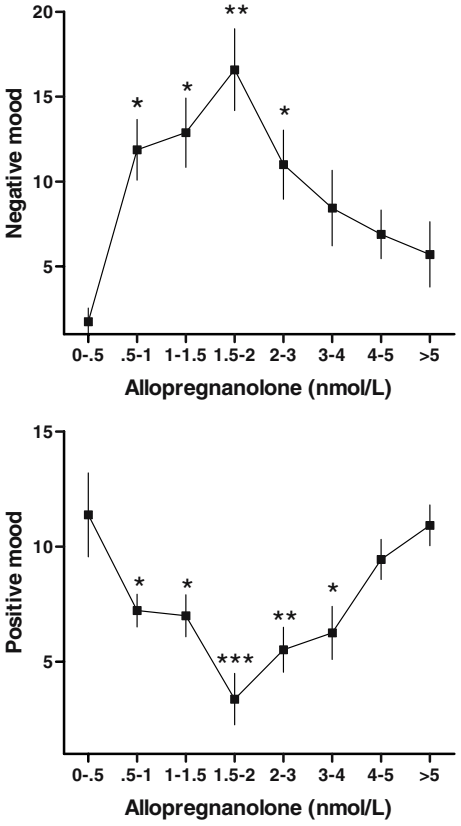

Fig. 4 Mean±SEM of summarized negative mood (*top panel*) and positive mood (*bottom panel*) in postmenopausal women (n=37) treated with estrogen and progesterone. The mood scores were from the same day as the serum allopregnanolone analysis. Blood samples from the women were grouped by their allopregnanolone concentrations: 0–0.5 nmol/l (n=8), 0.5–1 nmol/l (n=55), 1–1.5 nmol/l (n=41), 1.5–2 nmol/l (n=22), 2–3 nmol/l (n=31), 3–4 nmol/l (n=16), 4–5 nmol/l (n=18), and >5 nmol/l (n=24). *Significant difference in mood from 0–0.5 nmol/1: *P<0.05, **P<0.01, ***P<0.001. All statistics were calculated by one-way ANOVA. Detailed description for the *top panel*. Differences in summarized negative mood: 0–0.5 nmol/l differ from 0.5–1 nmol/l, 1–1.5 nmol/l, 1.5–2 nmol/l, and 2–3 nmol/l (P<0.05–0.01); 3–4 nmol/l and 4–5 nmol/l differ from 1.5–2 nmol/l (P<0.05 and P<0.01, respectively), >5 nmol/l differ from 0.5–1 nmol/l, 1–1.5 nmol/l, 1.5–2 nmol/l (P<0.05–0.001). Detailed description of the *bottom panel*. Differences in summarized positive mood: 0–0.5 nmol/l differ from 0.5–1 nmol/l, 1–1.5 nmol/l, 1.5–2 nmol/l, 2–3 nmol/l, 3–4 nmol/l (P<0.05–0.001); 1.5–2 nmol/l differ from 0.5–1 nmol/l, 1–1.5 nmol/l, 4–5 nmol/l, >5 nmol/L (P<0.01–0.001). 4–5 nmol/l differ from 2–3 nmol/l (P<0.05); >5 nmol differ from 0.5–1 nmol/l, 1–1.5 nmol/l, 2–3 nmol/l (P<0.01–0.001)

60 mg/day of progesterone, compared with the estrogen phase in the 30 mg/day cycle (P<0.05) and 200 mg/day cycle (P<0.05).

Differences in symptom severity between different progesterone doses in women without symptom cyclicity

Women without symptom cyclicity scored more positive mood symptoms during the progesterone/placebo treatment period, compared with the estradiol only period while treated with placebo [F(1, 20)=4.69; P<0.05], progesterone at 30 mg/day [F(1, 20)=10.1; P<0.01], and 60 mg/day [F(1, 20)=10.3; P<0.01] (Table 4).

Differences between women with and without symptom cyclicity

Women without symptom cyclicity generally scored more negative mood, compared with women with symptom cyclicity. During estrogen phases (cycle days 13–19) of treatment cycles with progesterone at 30 and 200 mg/day, women without cyclicity scored significantly higher negative mood, compared with women who experienced cyclicity [F(1, 35)=5.13; P<0.05 and F(1, 35)=4.85; P<0.05, respectively) (Table 4). However, no significant difference was revealed between these groups in negative mood scores during the 60 mg progesterone treatment cycle.

Allopregnanolone and mood

The relationship between allopregnanolone serum concentration and mood and physical symptoms was evaluated using one-way ANOVA. Serum concentration of allopregnanolone from each blood sample was used to divide the women with progesterone treatment into eight groups without accounting for the dose of progesterone administered. The eight groups, based on range of allopregnanolone concentration, were 0–0.5 nmol/l (n=8), 0.5–1.0 nmol/l (n=55), 1.0–1.5 nmol/l (n=41), 1.5–2 nmol/l (n=22), 2–3 nmol/l (n=31), 3–4 nmol/l (n=16), 4–5 nmol/l (n=18), and >5 nmol/l (n=24). The total range of allopregnanolone concentrations during progesterone treatment was 0.19–19.6 nmol/l, which corresponds to values from below follicular to far above luteal phase concentration in the menstrual cycle (Genazzani et al. 1998; Schmidt et al. 1994; Wang et al. 1996).

A significant difference in summarized negative mood scores between the eight different allopregnanolone concentration groups was noted [F(7, 207)=2.90; P<0.01] (Fig. 4). The post hoc test indicated a significant increase in negative mood scores between allopregnanolone concentrations in the range of 0–0.5 nmol/l compared with allopregnanolone concentrations in the ranges of 0.5–1.0 nmol/l (P<0.05), 1.0–1.5 nmol/l (P<0.05), 1.5–2 nmol/l (P<0.01), and 2–3 nmol/l (P<0.05). Detailed description of the differences between the groups is given in the legend of Fig. 4.

The summarized positive mood scores were negatively related to serum allopregnanolone concentration [F(7, 207)= 5.23; P<0.001] (Fig. 4). The post hoc test indicated a significant decrease of positive mood scores of the women with allopregnanolone concentrations in the range 0–0.5 nmol/l, compared with 0.5–1.0 nmol/l (P<0.05),

1.0–1.5 nmol/l ($P<0.05$), 1.5–2 nmol/l ($P<0.001$), 2–3 nmol/l ($P<0.01$), and 3–4 nmol/l ($P<0.05$). Detailed description of the differences between the groups is given in the legend of Fig. 4. No correlation between summarized physical symptoms and allopregnanolone concentration was seen.

Discussion

The main finding of this study is the bimodal association between allopregnanolone serum concentration and negative mood in postmenopausal women treated with different doses of oral micronized progesterone. The women scored significantly highest on the negative mood scale when allopregnanolone concentration was in the range of 1.5–2 nmol/l, compared with lower and higher concentrations where symptoms gradually decreased and eventually disappeared, as in an inverted U-shaped curve. In addition, women displayed a significant deterioration of negative mood during the progesterone phase in sequential HT when a daily dose of 30 mg of progesterone was used. On the other hand, treatment cycles with higher doses of progesterone had no influence on negative mood.

Our study shows that the mood-deteriorating effect of oral progesterone treatment is related to allopregnanolone concentration in a bimodal pattern. Women receiving allopregnanolone concentrations in the range of 1.5–2 nmol/l responded with the highest negative mood scores, and the mood-deterioration is less evident at lower and higher concentrations. In agreement with the present observation are reports from human studies where $GABA_A$ receptor modulators in certain situations induce anxiety, irritability, and aggression in a bimodal fashion. This has been shown with benzodiazepines (Ben-Porath and Taylor 2002; Wenzel et al. 2002), barbiturates (Kurthen et al. 1991; Lee et al. 1988; Masia et al. 2000), and ethanol (Cherek et al. 1992; Dougherty et al. 1996). In postmenopausal women receiving sequential HT, more intense adverse mood symptoms are reported during treatment with vaginal progesterone at 400 mg/day, compared to 800 mg/day (Andreen et al. 2003), and MPA 10 mg/day, compared to 20 mg/day (Bjorn et al. 2002).

In the study by Andréen et al. (2003), a significant increase in serum progesterone concentrations (mean± SEM) from 17±2.2 to 27±3.5 nmol/l was evident between the doses. An earlier study by our group has also indicated that allopregnanolone, but not progesterone, has a bimodal association with negative mood in postmenopausal women treated with vaginal progesterone. In that study negative mood symptoms occurred at serum concentrations of allopregnanolone similar to endogenous luteal phase levels, whereas lower and higher concentrations had no significant effect on mood (Andreen et al. 2005).

In the present study, cyclical mood deterioration was only reported during treatment with the lowest progesterone dose (30 mg/day), but not with the placebo or higher doses of progesterone. These findings further support the association between mood deterioration and allopregnanolone concentration. However, there is a complex interaction between neurosteroids and neurotransmitter systems in CNS. Estradiol is known to influence the effect of progestagens and allopregnanolone on mood symptoms and excitability (Bjorn et al. 2003; Landgren and Selstam 1995). To minimize the variation in symptoms due to changes in estradiol levels, the subjects in this study were postmenopausal and the treatment with estradiol was continuous and dosages kept constant. To control for estradiol variations, measurements of estradiol were made every treatment cycle and there was no difference in concentrations between the treatment cycles.

Similar findings with a biphasic action of $GABA_A$ receptor active substances, including allopregnanolone, have been shown in animal studies (Beauchamp et al. 2000; Fish et al. 2001; Miczek et al. 2003). In a study by Gourley et al. (2005), a biphasic benzodiazepine-heightened aggressive behavior was evident in rats treated with different doses of benzodiazepines. The effect was antagonized by flumazenil (a benzodiazepine antagonist) and with β-CCt and 3-PBC (both subunit-specific $GABA_A$ receptor antagonists). The exact mechanism for the biphasic phenomena is not known, but it has been suggested that this contradictory effect is linked to the $GABA_A$ receptor by a mechanism called disinhibition (Miczek et al. 2003). It has been hypothetically argued that failed inhibition leads to increased, instead of inhibited, excitation. The failed inhibition might be caused by a change in the sensitivity of the $GABA_A$ receptor, which may be influenced by several factors, including the subunit composition of the receptor. The decrease in sensitivity could also be induced by environmental factors, such as stress or HT, as shown in a study by Gulinello and co-workers (2001). In that study, an increase in anxiety and a modulation of the $GABA_A$ receptor subunits were seen after a short time exposure to neuroactive steroids.

Treatment with progesterone in postmenopausal women can be expected to be related to mood effects only in certain subjects. In an earlier retrospective study, about 30% of women stated occurrence of negative mood during HT with progestagens and 35% stated negative side effects as reason for discontinuing HT (Bjorn and Backstrom 1999). In our study, 43% of the subjects fulfilled our criteria for symptom cyclicity (i.e., mood deterioration during progesterone phase). Women in this group showed a dose-dependent cyclicity, with mood deterioration only during treatment with low-dose progesterone, compared to higher doses and placebo. Apparently, this group of women is more sensitive

to the effects of allopregnanolone and its 5β-stereoisomer pregnanolone. Using saccadic eye movement parameters as objective measures of sedation, our group has previously shown that postmenopausal women who display significant symptom cyclicity are more sensitive to the sedative effects of an intravenous pregnanolone injection, compared to women without cyclical symptoms (Wihlback et al. 2005).

An unexpected finding was the improvement in negative mood reported during the progesterone phase, compared to the estrogen phase during treatment with 60 mg/day of progesterone. It appears that this apparent mood improvement during progesterone phase is explained by increased adverse mood during estrogen phase, rather than a change in mood during progesterone phase in women with symptom cyclicity. One can assume that this is not caused by the treatment, though the same dose of estradiol was given to the same subjects during estrogen phases in the other treatment cycles without negative effects. However, the reason for the increased negative mood during the estrogen-only phase is not known. No life events were reported among the subjects, and the increased negative mood was present in several individuals. In addition, women without cyclicity reported symptom cyclicity with improvement in positive mood during progesterone phase while on progesterone 60 mg/day. However, this group of women reported similar improvement during treatment with placebo and 30 mg/day, and the impact of these mood scores are not clear. A similar effect of estradiol has, however, been reported in women with PMDD receiving estradiol while on gonadotropin-releasing hormone (GnRH) agonist to inhibit ovarian endogenous hormone production (Schmidt et al. 1998).

Just more than half of the subjects showed no cyclical mood changes during the treatment cycle. They reported high negative mood scores during the entire rating period. It has been shown in several HT studies that certain women will develop negative mood while on progestagens, but others will not (Bjorn and Backstrom 1999; Greendale et al. 1998; Kirkham et al. 1991). The reason for the discrepancy between individuals is not known. Whether the women with constant high negative mood scores in our study have a different personality or if they, in fact, were suffering from a latent depression not diagnosed by PRIME-MD was not investigated in the present study.

A hypothetical explanation why certain women in our study scored constant high negative mood scores without deterioration during progesterone phase may be that due to constant increase in negative mood, the women might have developed tolerance to $GABA_A$-active substances. Tolerance development has been described earlier as a result of prolonged exposure to GABA-agonists, as in cases of endogenous rise in allopregnanolone concentration during stress, pregnancy, and exogenous treatment with progestagens (Czlonkowska et al. 2001; Palmer et al. 2002; Smith et al. 1998; Turkmen et al. 2006; Zhu et al. 2004).

In this study, treatment with oral micronized progesterone at 30, 60, and 200 mg/day resulted in significant differences in progesterone and allopregnanolone concentrations. Earlier studies have indicated a correlation between progesterone, allopregnanolone, and pregnanolone serum concentrations during oral progesterone treatments (de Lignieres et al. 1995; Freeman et al. 1993). In the present study, the nadir concentration was analyzed, and the concentration of progesterone was 2–2.6 times higher than the concentration of allopregnanolone. One must assume that the progesterone treatment in our study also produced pregnanolone, which has affected the $GABA_A$ receptor complex, in addition to allopregnanolone. Therefore, the concentration of allopregnanolone related to adverse mood effects in the present study is somewhat lower, compared with our earlier study where we investigated the effect of vaginally administered progesterone and found an association between a slightly increased allopregnanolone concentration and adverse mood effects (Andreen et al. 2005). This difference is likely explained by the difference in the metabolism between oral and vaginal administration of progesterone (de Lignieres et al. 1995).

In conclusion, the present study demonstrated a bimodal association between serum allopregnanolone concentration and adverse mood effects in postmenopausal women treated with oral micronized progesterone.

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