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Relationship between Symptom Severity and Steroid Variation in Women with Premenstrual Syndrome: Study on Serum Pregnenolone, Pregnenolone Sulfate, 5α-Pregnane-3,20-Dione and 3α-Hydroxy-5α-Pregnan-20-One*

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ABSTRACT

The relationship between symptoms of premenstrual syndrome (PMS) and serum levels of pregnenolone (Pe), pregnenolone sulfate (PS), 5α-pregnane-3,20-dione (5α-DHP), 3α-hydroxy-5α-pregnan-20-one (5α-THP), LH, 17β-estradiol (E_2), and progesterone (P) was investigated during 2 consecutive menstrual cycles in 12 patients using daily measurements. Corresponding hormones were also measured during 1 cycle in 8 control women. Pe, PS, 5α-DHP, and 5α-THP showed a significant cyclicity within menstrual cycles and a high rate of correlation with P variation in both PMS patients and controls. No significant difference was found between PMS patients and controls in average serum concentrations of Pe, PS, 5α-DHP, 5α-THP, and LH during the luteal phase, whereas a significantly higher level of E_2 and a lower level of P were observed in PMS patients. The variation in symptom scores was compared with that in hormone levels within each woman. The symptom peak showed a delay of 3–4 days after the serum P, Pe, 5α-DHP, and 5α-THP peaks. However, the plasma PS peak appeared on the same day or only 1 day before the symptom peak in PMS patients. When comparing the 2 cycles studied, more negative symptoms occurred in cycles with higher luteal phase E_2, Pe, and PS concentrations, whereas higher luteal phase 5α-DHP and 5α-THP concentrations were associated with improved symptom ratings in PMS patients. These results suggest that the mentioned steroids are related to the severity of distressing symptoms in PMS patients. (*J Clin Endocrinol Metab* **81:** 1076–1082, 1996)

MENSTRUAL cycle-linked mood change, termed premenstrual syndrome (PMS), is characterized by the cyclic appearance of distressing somatic and behavioral symptoms during the premenstrual phase. This syndrome appears to be caused by a response of the central nervous system (CNS) to factors produced by the human corpus luteum, because the menstrual cycle-linked symptom variation is not manifested in spontaneous or GnRH analog-induced anovulatory cycles (1, 2). Our earlier study on the relationship between symptoms and serum steroid levels during two consecutive cycles in PMS patients indicated that a higher number of adverse premenstrual complaints occurred in cycles with high luteal phase serum 17β-estradiol (E_2) and progesterone (P) concentrations. In particular, a high luteal phase serum E_2 concentration was positively correlated with PMS symptom severity (3).

In general, E_2 exerts excitatory action and P exerts inhibitory effects on the CNS. Elevated levels of E_2 are associated with an activating effect on mood and activity (4), whereas P and its naturally occurring 5α-reduced metabolite, 3α-hydroxy-5α-pregnan-20-one (5α-THP), are potent general anesthetics (5). Recent biochemical and electrophysiological studies have demonstrated that 5α-THP is a benzodiazepine/barbiturate-like agonist of central γ-aminobutyric $acid_A$ ($GABA_A$) receptors and is responsible for P-induced anesthesia (6, 7). On the other hand, there exist steroids with opposite effects on $GABA_A$ receptors, acting as inverse agonists of $GABA_A$ receptors, which are known to be anxiogenic (8). Recent studies support the view that the metabolic precursor of P, pregnenolone sulfate (PS), interacts with the $GABA_A$ receptor as an inverse agonist as well as potentiates *N*-methyl-D-aspartate (NMDA) receptor-mediated intracellular calcium responses in the CNS (8). PS can also be hydrolyzed to pregnenolone (Pe) via steroid sulfatases. It is, therefore, possible that neuroactive anxiogenic steroids induce abnormal responses in CNS and thus cause PMS symptoms in the luteal phase. 5α-Pregnane-3,20-dione (5α-DHP) is the intermediate in the conversion of P to 5α-THP and also of interest to study.

The aim of the present study was to evaluate the menstrual cycle-linked variation in serum levels of Pe, PS, 5α-DHP, and 5α-THP in women diagnosed with PMS compared with those in non-PMS controls and to determine the correlation

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between the serum levels of the corresponding steroids and symptom severity in PMS patients.

Subjects and Methods

Patients

Patients were recruited among women seeking help for PMS at the gynecological out-patient department. All had a history of recurrent cyclical mood changes, with at least 1 psychological symptom for more than 1 yr. They underwent 2 consecutive months of clinical evaluation for diagnosis by daily rating their symptoms, using a previously validated visual analog scale (9). These patients provided blood samples for hormone assays on cycle days 1–4 and from cycle day 10 through the cycle to the first 4 days of menstrual bleeding during both cycles. The diagnosis was based on established criteria (9). All patients showed significant cyclical mood changes and fulfilled the criteria for late luteal phase dysphoric disorder, according to the Diagnostic and Statistical Manual of Mental Disorders, 3rd ed., revised (10). A dozen patients were finally selected of 21 patients who had given daily blood samples and symptom rating as described above. The only criteria for selection was that the patients showed a difference in symptom severity between 2 studied cycles, which could be differentiated into a high symptom cycle and a low symptom cycle. None of these patients received any medication. Their average age was 37.8 yr (range, 27–44 yr).

Controls

Eight volunteers were recruited as controls from hospital personnel and from women requesting intrauterine devices at the Family Planning Unit. They were not taking any medications and had no history of recurrent cyclical mood changes. The controls completed daily prospective symptom rating for one menstrual cycle, and blood samples were taken as described above. None of the controls showed significant cyclicity of mood change by the above criteria on the daily ratings. Their average age was 35.5 yr (range, 25–43 yr). All cycles studied in both patients and controls were ovulatory as defined by plasma P values above 15 nmol/L. The day of ovulation was taken as the day after the LH surge. The study was approved by the Umea University ethics committee, and informed consent was given by each participant.

Daily rating scale

Every evening during the studied cycles, the women filled out a daily rating scale. In total, four negative mood parameters, four positive mood parameters, and three somatic signs were rated (see Table 2) as was the severity of menstrual bleeding. For each item, the volunteer marked on a 10-cm line to indicate how she had experienced a particular symptom during that day, with 0 as a complete absence of the symptom, and 10 as the maximal severity of the symptom. The women were also asked to describe events during the day that might have influenced their mood and to record any medication taken.

Hormone analyses

Analyses of LH, E_2, P, Pe, PS, 5α-DHP, and 5α-THP were performed on the serum samples by RIA. The standard for serum LH RIA (Farmos Diagnostica, Oulu, Finland) was human pituitary LH, WHO 68/40. The intra- and interassay coefficients of variations were 8.7% and 8.7%, respectively. E_2 was measured as previously described (11). The intra- and interassay coefficients of variation were 10% and 12%, respectively. Measurements of P, 5α-DHP, and 5α-THP were made by RIA after separation using Celite chromatography (12–14). For P and 5α-DHP assays, an antiserum against P 11α-succinyl BSA (Endocrine Science, Tarzana, CA) was used. The cross-reactivity of this antiserum is 100% to P and 31% to 5α-DHP (14). The intra- and interassay coefficients of variation of the P assay were 8% and 11%, whereas those for 5α-DHP assay were 10% and 11%, respectively. The RIA for 5α-THP was performed using an antiserum raised against 3α-hydroxy-20-oxo-5α-pregnan-11α-yl carboxymethyl ether coupled with BSA (developed by Dr. R. H. Purdy), with intra- and interassay coefficients of variation of 6.5% and 8.5%, respectively (13). Measurements of Pe and PS were performed by RIA after separation of the steroids using a C_{18} column (Amersham International, Amersham, UK), where the chromatographic method of Belanger *et al.* (15) completely separated Pe from PS. An antiserum raised against Pe 3-hemisuccinate BSA (ICN Biomedicals, Costa Mesa, CA) that has 100% cross-reaction to both Pe and PS was used for Pe and PS assays. The intra- and interassay coefficients of variation for the Pe assay were 8% and 9%, respectively; those for PS assay were 11% and 13%, respectively. All samples were analyzed in duplicate.

Analysis of data

The difference in steroid concentrations between patients and controls was tested using a two-way ANOVA, followed *ad hoc* by the least significant difference test. Differences between follicular and luteal phase steroid concentrations were tested using one-way ANOVA. Relationships between serum steroid levels and the symptoms were calculated using an autocorrelation test to find the day of maximal correlation and to determine whether there was a delay of maximal symptoms in relation to the peak levels of steroids. Before any group calculations were performed, separate study groups for each steroid were defined. This was conducted by comparing the luteal phase (14 premenstrual days) concentration of each steroid in each patient between the two studied cycles using the area under the curve (AUC) method. The AUC of a 5-day period centered at the day of maximal steroid concentration was calculated for individual steroid. A difference in AUC larger than or equal to 10% between two cycles was demanded for each patient used in further comparison. For example, if the AUC for the E_2 concentration in the first luteal phase was 10% higher than that in the second, the woman was selected for further group calculations of E_2 levels.

Otherwise, the woman was excluded from the study group for that steroid. Therefore, the number of women included in the study groups differed depending on how many had a 10% difference in the AUC in their luteal phase concentrations of each particular steroid (n = 9–11; see *Results*). In women with different luteal phase steroid concentrations, the cycle with higher steroid concentration is described as the high cycle. The cycle with the lower luteal phase steroid concentration is termed the low cycle. In calculating the group results, the high cycle was grouped against the low cycle, and the symptom severity in the luteal phase of the high cycle was compared with that in the low cycle luteal phase using two-way ANOVA. The daily symptom scores were also added to three summarized scores per day in each patient, namely summarized negative, summarized positive, and summarized somatic symptom scores (see Table 2). To divide the two studied cycles into high and low symptom cycles, a method of symptom-free counting was used to test the symptom severity between two studied cycles (16). The total number of symptom-free days per symptom in the 14-day premenstrual period was counted for the negative mood and somatic symptoms. A day with a negative symptom score or a somatic symptom score of 0–1 was considered symptom free. The sum of the symptom free counting of each symptom yields a symptom-free counting for each luteal phase. The higher the symptom-free counting, the less the symptom severity in that cycle. Thus, the cycle with lower symptom-free counting was called the high symptom cycle, and the cycle with higher symptom-free counting was called the low symptom cycle. The serum steroid concentrations were compared between high and low symptom cycles using two-way ANOVA. The average summarized scores for negative, positive, and somatic symptoms within the 14 premenstrual days were also compared between two cycles in each patient to confirm the results from symptom-free counting. The rating scores were used only for comparisons in the same woman and not between different women, because the scale limits were subjectively defined and not directly comparable between individuals. For example, irritability rated at 5 by one woman was not necessarily higher than irritability rated at 4 by another woman. Within individuals, however, the scale is of the ordinal type. Calculation of symptom-free days is comparable between individuals, as symptom free is definable between individuals.

Results

Hormone variation during the menstrual cycle

In both patients and controls, the serum Pe, PS, 5α-DHP, and 5α-THP showed a cyclical pattern during the menstrual cycle, with significantly higher concentrations during the

luteal phase (14 premenstrual days) compared to the follicular phase (14 postmenstrual days; Table 1). There were no significant differences in the plasma LH, Pe, PS, 5α-DHP, or 5α-THP concentrations between patients and controls during the luteal phase. Significantly lower luteal phase P concentrations were observed in PMS patients [$F(1,13) = 3.68$; $P < 0.02$, by two-way ANOVA; Table 1]. A significantly higher concentration of E_2 was observed in PMS patients compared to controls in the luteal phase [$F(1,13) = 7.26$; $P < 0.01$, by two-way ANOVA; Table 1].

Figure 1 shows the average serum concentrations of Pe, PS, P, 5α-DHP, 5α-THP, and LH during the menstrual cycle synchronized with the onset of menstrual bleeding at the beginning of the cycle and day of ovulation. Data from both patients and controls were used. The menstrual cycle-linked variation in Pe, PS, 5α-DHP, and 5α-THP showed a high degree of correlation with the serum P concentration, with the correlation rate to P ranked as 5α-THP ($r = 0.85$; $P < 0.001$), Pe ($r = 0.70$; $P < 0.001$), 5α-DHP ($r = 0.57$; $P < 0.005$), PS ($r = 0.44$; $P < 0.005$). In the follicular phase, the mean Pe, PS, 5α-DHP, and 5α-THP concentrations reached the follicular baseline 1 day after P.

Relationship between PMS symptoms and plasma steroid concentrations

The mean symptom rating started to increase shortly after ovulation and reached a peak during the last 5 premenstrual days. After the onset of menstrual bleeding, the symptoms declined and reached a minimum during the preovulatory period. The maximum decline occurred during the first 4 days of the cycle. The initial rise in serum P, Pe, PS, 5α-DHP, and 5α-THP concentrations during the luteal phase preceded the development of symptoms. This pattern was observed in all 12 patients when they were analyzed individually. By use of the autocorrelation test, the maximum correlation between individual symptoms and serum P, Pe, 5α-DHP, and 5α-THP levels revealed that the symptom peak during the luteal phase appeared 3–4 days after the steroid peaks. The delay of the symptom peak was 6 days after the E_2 peak, whereas the symptom peak occurred on the same luteal phase day or with a 1-day delay from the serum PS peak (Table 2).

Difference in luteal phase symptom severity between cycles with high and low steroid concentrations

The serum steroid concentrations during the 2 luteal phases were compared to determine the number of women who showed more than a 10% difference in AUC of the steroid levels between the 2 studied cycles. Of the 12 PMS patients, 11 (92%) showed a difference in serum E_2 concentration during the luteal phase of the 2 cycles, 11 (92%) showed a difference in serum P concentration, 10 (83%) showed a difference in serum PS concentration, and 9 (75%) showed a difference in serum Pe concentration. The numbers of women with a difference in serum 5α-DHP and 5α-THP concentrations in the luteal phase between the 2 cycles were 12 (100%) and 11 (92%), respectively.

The women with a significant difference in individual serum steroid levels between the two cycles were then grouped together for further analysis. Figure 2 indicates the comparison of

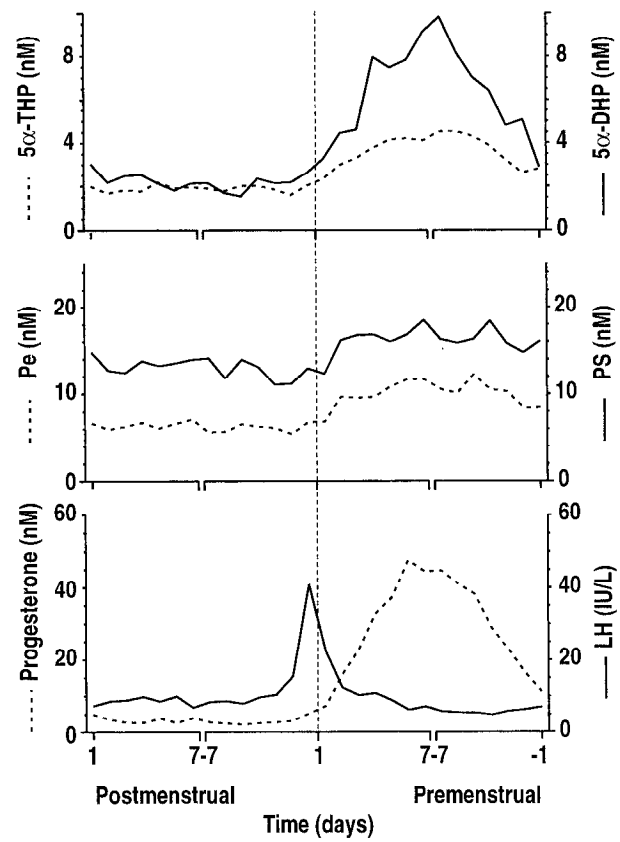

FIG. 1. Mean plasma steroid hormone concentrations in the menstrual cycle of PMS women and controls (n = 20). The data are centered around the day of ovulation ± 7 days and the day of onset of menstrual bleeding ± 7 days. The *central broken line* indicates the day of ovulation.

TABLE 1. Comparison of plasma steroid concentrations between patients and controls

Steroid	Patients		Controls		Statistics	
	Folli. Phase	Lut. phase	Folli. Phase	Lut. phase	P^a	P^b
Pe (nmol/L)	6.3 ± 0.2	10.4 ± 0.3	6.4 ± 0.3	9.8 ± 0.4	NS	NS
PS (nmol/L)	13.7 ± 0.4	17.0 ± 0.5	11.2 ± 0.6	15.2 ± 0.8	NS	NS
P (nmol/L)	3.2 ± 0.2	30.4 ± 1.1	5.0 ± 0.5	34.7 ± 2.4	NS	<0.02
5α-DHP (nmol/L)	2.5 ± 0.2	7.00 ± 0.5	2.2 ± 0.2	5.8 ± 0.8	NS	NS
5α-THP (nmol/L)	1.9 ± 0.1	3.6 ± 0.1	1.8 ± 0.1	3.6 ± 0.2	NS	NS
E_2 (pmol/L)	362.1 ± 21.4	362.3 ± 9.9	317.6 ± 39.9	308.4 ± 25.7	NS	<0.01
LH (IU/L)	13.1 ± 0.8	7.3 ± 0.3	13.8 ± 1.0	7.6 ± 0.3	NS	NS

Folli. phase, Follicular phase; Lut. phase, luteal phase. Values are the mean ± SEM.
[a] Comparison of follicular phase steroid concentrations between patients and controls.
[b] Comparison of luteal phase steroid concentrations between patients and controls.

TABLE 2. Maximum correlation between symptom scores and steroid concentrations in luteal phase and delay of symptom peak from the steroid peak

Symptom	P		E_2		5α-THP		5α-DHP		Pe		PS	
	r[a]	Delay[b]	r[a]	Delay[b]	r[a]	Delay[b]	r[a]	Delay[b]	r[a]	Delay[b]	r[a]	Delay[b]
Sum of negative	0.51	3	0.27	6	0.52	3	0.52	3	0.55	3	0.62	0
Anxiety	0.47	3	0.24	6	0.51	3	0.47	3	0.56	3	0.54	1
Irritability	0.40	4	0.23	6	0.41	3	0.41	3	0.48	3	0.57	1
Fatigue	0.60	4	0.27	6	0.46	4	0.42	4	0.36	4	0.44	0
Depression	0.33	4	0.23	6	0.39	4	0.42	4	0.33	4	0.36	0
Sum of positive	−0.45	3	−0.30	6	−0.44	3	NS	NS	−0.46	3	−0.43	0
Cheerfulness	−0.46	4	−0.30	6	−0.43	3	NS	NS	−0.55	3	−0.53	1
Well-being	−0.44	3	NS	NS	−0.41	3	−0.46	3	−0.56	4	−0.41	1
Friendliness	−0.38	3	−0.30	6	−0.39	3	NS	NS	−0.44	3	−0.36	1
Feel energetic	−0.54	3	−0.28	6	−0.53	3	−0.34	3	−0.54	4	−0.45	1
Sum of somatic	0.60	4	0.30	6	0.57	4	0.44	4	0.38	4	0.36	0
Headache	0.34	3	NS	NS	0.38	3	NS	NS	NS	NS	−0.34	0
Feel swelling	0.65	4	0.24	6	0.52	4	0.47	4	0.40	4	NS	NS
Breast tenderness	0.50	4	0.29	6	0.45	3	0.38	4	0.45	4	0.38	0

[a] Maximal correlation coefficient (autocorrelation test) between symptom scores and plasma steroid concentrations, $P < 0.05$.
[b] The delay of individual symptom peak in days after the plasma steroid peaks at the maximal correlation coefficient.

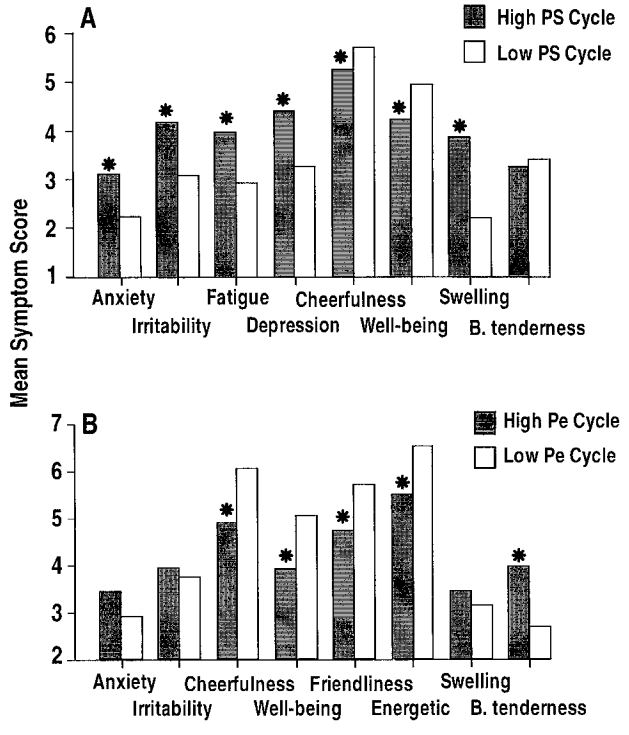

FIG. 2. Comparison of mean symptom scores between cycles with high and low PS (A) and Pe (B) concentrations in the luteal phase. *, Significantly different from the low steroid cycles ($P < 0.05$ to $P < 0.001$). B. tenderness, Breast tenderness.

symptom ratings between the high and low Pe and PS cycles. The mean serum PS concentration in the luteal phase was 16.8 ± 0.6 nmol/L in the high PS cycles *vs.* 14.5 ± 0.5 nmol/L in the low PS cycles. Severe symptom ratings of anxiety [F(1,13) = 4.00; $P < 0.05$], irritability [F(1,13) = 4.22; $P < 0.05$], fatigue [F(1,13) = 4.04; $P < 0.05$], depression [F(1,13) = 5.90; $P < 0.05$], and feeling of swelling [F(1,13) = 9.73; $P < 0.01$] were observed in the high PS cycles compared to the low PS cycles. The patients also rated themselves worse in cheerfulness [F(1,13) = 3.96; $P < 0.05$] and well-being [F(1,13) = 3.92; $P < 0.05$] in high PS cycles (Fig. 2A). Of the summarized symptoms, only summarized negative symptom score was significantly different between two cycles with the high score in the high PS cycles. In serum steroid concentrations, Pe was significantly high in higher PS cycles [11.2 ± 0.5 *vs.* 8.7 ± 0.5 nmol/L; F(1,13) = 5.33; $P < 0.05$].

The mean serum Pe concentration in the luteal phase was 11.8 ± 0.4 nmol/L in the high Pe cycles *vs.* 8.9 ± 0.5 nmol/L in the low Pe cycles. In the low Pe cycles, significantly improved positive symptom ratings in cheerfulness [F(1,13) = 13.03; $P < 0.01$], well-being [F(1,13) = 4.69; $P < 0.05$], friendliness [F(1,13) = 7.83; $P < 0.05$], and feeling energetic [F(1,13) = 7.84; $P < 0.05$] were found. Patients rated themselves worse in feeling more breast tenderness in high Pe cycles compared with low Pe cycles [F(1,13) = 10.45; $P < 0.01$; Fig. 2B]. The summarized positive symptom score was significantly higher in low Pe cycles as well. The only plasma steroid that showed a significant difference during the luteal phase in high Pe cycles compared to low Pe cycles was PS [16.4 ± 0.5 vs. 14.5 ± 0.5 nmol/L; F(1,13) = 8.67; $P < 0.01$].

Figure 3 demonstrates the symptom ratings between the high and low 5α-DHP and 5α-THP cycles. The mean serum 5α-DHP concentration in the luteal phase was 7.2 ± 0.9 nmol/L in the high 5α-DHP cycles *vs.* 5.7 ± 0.6 nmol/L in the low 5α-DHP cycles. Significant severe symptom ratings were observed in low plasma 5α-DHP cycles for fatigue [F(1,13) = 15.53; $P < 0.01$], depression [F(1,13) = 14.48; $P < 0.01$], feeling of swelling [F(1,13) = 9.11; $P < 0.01$], and breast tenderness [F(1,13) = 3.89; $P < 0.05$; Fig. 3A]. Both summarized negative and somatic symptom scores were significantly lower in high 5α-DHP cycles. In the low 5α-DHP cycles, a higher PS concentration was found [15.2 ± 0.6 *vs.* 17.1 ± 0.5 nmol/L; F(1,13) = 11.2; $P < 0.01$]. A higher 5α-THP concentration was found in high 5α-DHP cycles [3.1 ± 0.1 *vs.* 2.8 ± 0.1 nmol/L; F(1,13) = 6.19; $P < 0.05$].

The mean serum 5α-THP concentration in the luteal phase was 3.6 ± 0.1 nmol/L in the high cycles *vs.* 2.9 ± 0.1 nmol/L in the low cycles. In the high 5α-THP cycles, improved positive symptom ratings in cheerfulness [F(1,13) = 5.32; $P < 0.05$], well-being [F(1,13) = 10.28; $P < 0.01$] and feeling energetic [F(1,13) = 4.55; $P < 0.05$] were found. Patients rated

lower in irritability [F(1,13) = 6.03; $P < 0.05$] and feeling of swelling [F(1,13) = 6.66; $P < 0.05$] in high 5α-THP cycles compared with low plasma 5α-THP cycles (Fig. 3B). The summarized positive symptom score was also significantly higher in high 5α-THP cycles. The serum steroid concentration of 5α-DHP was higher in high 5α-THP cycles [6.9 ± 0.8 *vs.* 5.4 ± 0.6 nmol/L; F(1,13) = 10.3; $P < 0.005$].

In the E_2 group, the mean serum E_2 level was 433.7 ± 24.0 pmol/L in the high E_2 cycles *vs.* 315.5 ± 13.4 pmol/L in the low E_2 cycles. A significantly higher summarized negative symptom score and lower summarized positive symptom score were observed in the high E_2 group compared to the low E_2 group [F(1,13) = 4.72; $P < 0.03$ and F (1,13) = 10.91; $P < 0.01$ respectively, by two-way ANOVA]. No significant difference was found in the summarized somatic symptom score, nor did the scores for feeling of swelling and breast tenderness show any difference between the high and low E_2 cycles. Among the other steroids, Pe and PS concentrations were significantly higher in high E_2 cycles [F(1,13) = 16.12; $P < 0.001$ and F(1,13) = 14.24; $P < 0.001$, respectively].

In the P testing, the mean serum P concentration in the luteal phase was 36.9 ± 2.1 nmol/L in the high P cycles *vs.* 27.9 ± 1.7 nmol/L in the low P cycles. No significant differences were found in the summarized negative, summarized positive, and summarized somatic symptom scores. However, significantly higher scores were found in the high P cycles for four symptoms: fatigue [F(1,13) = 7.14; $P < 0.01$], depression [F(1,13) = 6.67; $P < 0.01$], feeling of swelling [F(1,13) = 4.53; $P < 0.05$], and breast tenderness [F(1,13) = 4.03; $P < 0.05$]. None of the other steroids showed a significant difference between high and low P cycles.

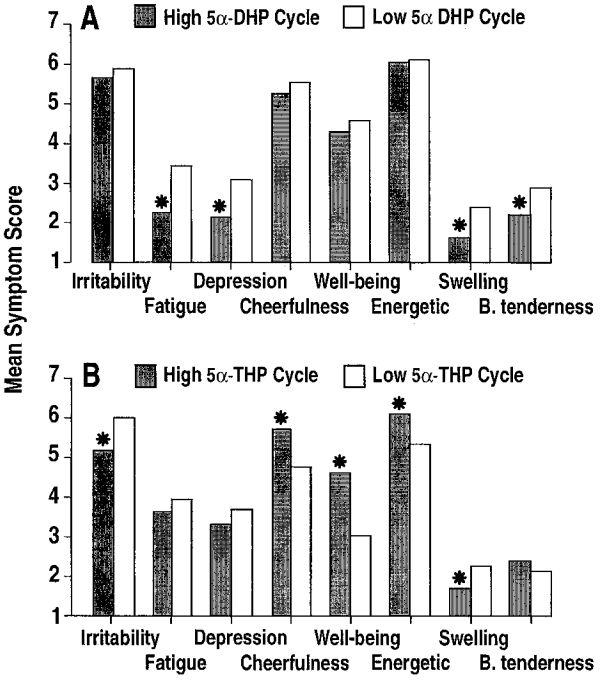

FIG. 3. Comparison of mean symptom scores between cycles with high and low 5α-DHP (A) and 5α-THP (B) concentrations in the luteal phase. *, Significantly different from the low steroid cycles ($P < 0.05$ to $P < 0.001$).

Mean luteal phase steroid concentrations in cycles with high and low PMS symptom severity

All 12 PMS patients had significant differences in symptom severity in the luteal phase between the 2 studied cycles. These 2 studied menstrual cycles were differentiated into a high symptom severity cycle and a low symptom severity cycle, according to symptom-free counting. The symptoms in the high cycle were generally more severe than those in the low cycle, with significantly higher means of the summarized negative and somatic symptom scores as well as a significantly lower summarized positive symptom score (Table 3). The serum concentrations of E_2 and PS differed between the 2 cycles, with higher luteal phase concentrations in the high symptom cycle (Table 3).

Discussion

The serum concentrations of Pe, PS, 5α-DHP, and 5α-THP showed a high degree of correlation to the serum P level during the menstrual cycle. This could probably be explained by the short synthetic pathway to and from P. It is well known that Pe is a direct metabolic precursor of P, whereas 5α-DHP and 5α-THP are the initial and second 5α-reduced metabolites of P, respectively. The menstrual cycle-linked variation in steroids and symptoms revealed a high degree of correlation. PS variation showed maximal correlation to symptoms with the shortest delay, whereas all other steroids showed a longer delay between steroid peak and symptom peak. In addition, there was a positive relation among PS, Pe,

TABLE 3. Summarized symptom scores and plasma steroid concentrations in luteal phase between high symptom and low symptom cycles

	High symptom cycle	Low symptom cycle	P^a
Sum of negative symptoms	15.2 ± 0.7	11.6 ± 0.7	<0.01
Sum of positive symptoms	17.0 ± 0.5	21.7 ± 0.6	<0.05
Sum of somatic symptoms	9.8 ± 0.6	7.2 ± 0.6	<0.01
Steroid concentrations			
E_2 (pmol/L)	422.8 ± 23.2	349.2 ± 17.5	<0.01
P (nmol/L)	32.7 ± 2.7	31.2 ± 1.9	NS
Pe (nmol/L)	10.7 ± 0.4	10.0 ± 0.5	NS
PS (nmol/L)	18.4 ± 0.7	15.2 ± 0.7	<0.01
5α-DHP (nmol/L)	8.6 ± 1.0	6.2 ± 0.6	NS
5α-THP (nmol/L)	3.8 ± 0.2	3.4 ± 0.1	NS
LH (IU/L)	8.1 ± 0.6	7.1 ± 0.4	NS

Values are the mean ± SEM.
[a] By two-way ANOVA.

and symptoms with higher symptom severity in cycles with higher concentrations of Pe and PS. On the other hand, serum 5α-DHP and 5α-THP concentrations were reversibly correlated with the severity of certain PMS symptoms. The symptoms showing a significant difference between the high and low cycles in the steroid comparison were the most typical PMS symptoms. They have been reported to vary cyclically during the menstrual cycle, with a clear relationship to E_2 and P variations (4). These results indicated that the serum steroid concentrations are related to the development of PMS symptoms within the menstrual cycle. The delay of symptom peak after serum P peak was also observed in our earlier study (17). The underlying reason is still unclear. One can speculate that the PMS symptom-provoking factors are different from those steroids we measured. They can have a different cyclical pattern compared to P. PS, for example, shows a closer relationship to symptom development during the luteal phase. Another hypothesis is that the delay is due to the synthesis of proteins (receptors, *etc.*) that can continue after the P peak. Therefore, a following protein peak could appear later in the luteal phase. The synthesis of PGs in the endometrium demonstrates such a pattern, where the maximum PG concentrations appear in the late luteal phase whereas synthesis starts earlier (18).

The group calculation showed higher negative and somatic symptom scores in the cycles with a high luteal phase level of PS than in the cycles with a low PS levels. In addition, improved positive symptom ratings were found in the cycles with low luteal phase levels of Pe. The plasma PS and Pe concentrations showed a high degree of correlation based on the fact that the Pe level was also significantly higher in the high PS level cycles and *vice versa*. These results suggest that PS and Pe may be involved in the provocation of PMS symptoms. Recent electrophysiological and biochemical studies support the view that PS is active in the CNS by interacting with $GABA_A$-gated chloride ion channels in an antagonistic fashion (8, 19, 20). In micromolar concentrations, PS inhibits TBPS binding, decreases muscimol binding, blocks GABA agonist-stimulated chloride uptake in synaptoneurosomes (19), and diminishes chloride ion conductance by reducing the channel opening frequency (21, 22). In behavioral studies, PS, and more distinctly Pe, showed memory-enhancing effects in mice (23). A single oral dose of Pe increased the time spent in slow wave sleep and depressed electroencephalograph σ power, whereas sleep-associated nocturnal cortisol and GH secretion remained unchanged (24). Given the biochemical and electrophysiological data demonstrating that PS acts as an antagonist of $GABA_A$ receptors, and that Pe given peripherally has behavioral effects, it is possible that there is a relationship among PS, Pe, and behavioral measurements. There is now unpublished evidence that PS injected iv in pharmacological doses can effectively cross the blood-brain barrier in rats (Wang, M., Wahlström G, Bäckström T, unpublished data). However, it is uncertain whether peripheral PS in women body can pass the blood-brain barrier without being hydrolyzed to lipophilic Pe and, as such, being taken up by the CNS. It is, therefore, unclear what a correlation between PMS symptom severity and peripheral PS variation represents. Within the CNS, a conversion of Pe back to PS can take place. Another possibility is that the peripheral PS concentration reflects an overall PS status in humans within both the brain and the peripheral compartment.

When we compared the symptom rating between the high and low 5α-DHP and 5α-THP cycles, we observed a reverse correlation between symptom severity and circulating levels of 5α-DHP and 5α-THP opposite to that found for Pe and PS. Less severe symptom ratings of negative and somatic symptoms were observed in high 5α-DHP cycles. On the other hand, improved positive symptom ratings were found in the high 5α-THP cycles. However, Schmidt *et al.* (25) studied the circulating level of 5α-THP in the luteal phase in women with PMS and observed no significant correlation between the severity of mood and behavioral symptoms and serum levels of P and 5α-THP. The disagreement could be explained by the fact that Schmidt *et al.* only studied one cycle per individual and compared the symptom severity and steroid levels between individuals, whereas we studied the correlation between cycles with different steroid levels in the same individual. In addition, blood samples were taken only once from the late luteal phase by them, whereas we compared the symptom rating and steroid concentration over 14 premenstrual days. This gave a better manifestation of both symptoms and steroids. 5α-THP was believed to be a P metabolite with anxiolytic properties, acting as an $GABA_A$ receptor agonist and, therefore, might be of importance for the well-being of the patients (4, 26). Consideration should also be given to an interaction of P with other steroid-binding sites, *e.g.* glucocorticoid or androgen receptors, in mediating behavioral effects (27). An abnormal neurotransmitter response to the multifactorial variation of steroids secreted by the ovary is, however, more likely to be the etiological basis of PMS symptoms. The difference in serum progestin concentrations between high and low symptom cycles was modest. It is known from our earlier animal work that steroid concentrations in the brain are higher than those in plasma. The postovulatory P concentrations in certain brain areas were 300 times higher than those during the preovulatory period, whereas the post- to preovulatory ratio of plasma P concentration was only 2 (28). On the other hand, we know that PMS symptoms develop during a low corpus luteum activity, as indicated by a rise in P from 2 to 7 nmol/L (17). Therefore, one can speculate that a modest difference in plasma progestin concentration might give a large difference in brain concentration and, as such, trigger behavioral change.

We confirmed our previous observation that increased plasma E_2 and decreased plasma P levels were detected during the luteal phase in PMS women compared to controls (29). It is anticipated that the correlation between plasma steroids and PMS symptoms could reflect the actions of these steroids within the brain. We know that the brain concentration of steroids varies with the plasma conjugated and unconjugated steroids (28). However, it is also known that P, Pe, PS, 5α-DHP, and 5α-THP are neurosteroids derived from cholesterol in brain tissue independent from their peripheral resources (30, 31). A growing body of evidence indicates that many natural and synthetic steroids have effects in the brain and can, therefore, induce both positive and negative behavioral changes in humans. The effects of steroids on the brain, probably mediated by influencing neurotransmitter

functions, are complicated and involve both gnomic and nongenomic actions. Further work is required for better understanding of the behavioral and endocrine consequences of neuroactive steroid secretion in both physiological and pathological states.

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