SERUM LEVELS OF NEUROSTEROID ALLOPREGNANOLONE IN PATIENTS WITH PREMENSTRUAL SYNDROME AND PATIENTS AFTER THYROIDECTOMY

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Objective. To evaluate whether altered levels of progesterone and its main neuroactive metabolite allopregnanolone do occur in premenopausal women. The second part of this study deals with allopregnanolone levels in thyreoidectomized women.

Methods. Allopregnanolone, a neurosteroid acting by its allosteric interaction with $GABA_A$ receptors and progesterone, were determined in two groups of patients which have in common certain psychic disorders generally characterized by the depression and anxiety, namely in 23 women with premenstrual syndrome (PMS) and in 52 women after total thyreoidectomy. The control groups consisted of women of the same age without premenstrual complaints and with normal thyroid function, respectively.

Results. Significantly lower values of allopregnanolone in PMS patients than in controls have been found in the follicular phase, indicating the lower peripheral activity of 5α-reductase of C_{21}-steroids detectable at low progesterone levels only. In the thyroidectomized patients significantly higher values of allopregnanolone have been found in the luteal phase.

Conclusion. The increase of allopregnanolone level in thyroidectomized patients may represent one of the counterregulatory mechanisms protecting the organism from some consequences of hormonal disbalance after thyroid ablation.

Key words: Neurosteroids – Allopregnanolone – 5α-reductase – Premenopausal syndrome – Thyroidectomy

During the last decade rapid advances have been recorded in the investigation of neurosteroids. This term has been used for the steroids synthesized and accumulated in the nervous system either from cholesterol *de novo*, or *in situ* from blood-borne precursors (Robel and Baulieu 1994). The new techniques of molecular and cellular endocrinology brought evidence that one of the most important C_{21}neurosteroids, 3α-hydroxy-5α-pregnan-20-one (allopregnanolone, HPO), is predominantly formed in the brain from progesterone by a concert action of steroid 5α-reductase and 3α-hydroxysteroid dehydrogenase (Robel et al. 1995). Allopregnanolone is a positive allosteric modulator of the gamma-aminobutyric acid type A [$GABA_A$] receptors in the brain – similarly as anxiolytics, anticonvulsants and hypnotics and its physiological activity is close to these compounds (Majewska 1990; Lan and Gee 1994; MacDonald 1991).

Female organism in premenopause is being cyclically exposed to considerably differing levels of progesterone. Some women do not tolerate these hormonal changes and during the luteal phase suffer from the so called premenstrual syndrome (PMS),

characterized by behavioral and somatic symptoms, depression and anxiety.

In this respect it would be of interest whether altered levels of progesterone and its main neuroactive metabolite allopregnanolone do occur in these women. This question is addressed in this paper. The second part of this study deals with allopregnanolone levels in thyreoidectomized women, since the psychic disorders often associated with anxiety and depression are typical for the period following the thyroid elimination. The indispensable role of the thyroid hormone for brain development and function is well documented and the relationship between the thyroid function and steroid metabolism including 5α-reductase activity has been known for decades (Tomkins and McGuire 1960). The postoperation stress and lack of these hormones may influence the enzyme system of steroidogenesis in the brain including the formation of allopregnanolone (Barbaccia et al. 1997). Since the depression and anxiety are common for both groups of such patients, the brain 5α-reductase activity may be one of the factors linking both the above mentioned groups of patients.

A close correlation between plasma allopregnanolone and progesterone plasma levels in normal women has been repeatedly reported (Purdy et al. 1990; Bicikova et al. 1995). In this study allopregnanolone and progesterone serum levels were determined in both above mentioned groups of patients and compared with those of controls.

Materials and Methods

Patients with PMS. Twenty three women attending a gynecologist for PMS aged 21-49 years (average 32.5 yr) were examined. The control group consisted of 16 normally menstruating women aged 22-46 years (average 37 yr) without any psychic or somatic problems. All women were asked to fill in the questionnaire in which 30 symptoms of PMS were listed and to mark the degree of each symptom from 0 to 10. The construction of our questionnaire was based on the earlier works of Rubinow and Roy-Byrne (1984) and Bancroft and Bäckström (1985). The patients included in the study suffered from at least two psychic symptoms of a degree above 5 regularly every month. None of the investigated women used any contraception or any other drug treatment. The blood samples were obtained during the follicular phase (4th to 9th day of cycle) and the luteal phase (6th to 1st day before bleeding) of the same menstrual cycle.

Thyroidectomized patients. This group consisted of fifty two thyroidectomized women aged 21-52 years (average 36 years), all complaining for psychic disorders, with average TSH levels of 17.9 ± 28.2 mU/l (range from 0.04 to 100 mU/l), all received thyroid hormones replacement therapy. In addition, the patients had to meet following criteria: 1. the diagnosis of a severe thyreotoxicosis requiring a total thyroidectomy; 2. the first blood sample withdrawal carried out not later than two weeks after surgery; 3. no radioiodine treatment. Sera were divided into two subgroups according to the phase of the menstrual cycle. The control group consisted of 16 euthyroid women (TSH level 0.3 – 3.5 mU/l) without any complains aged 19 – 47 years (average 35). In this group the sera were obtained in both phases of the cycle (with one exception when only the serum from follicular phase was available).

Hormone analyses. Both steroids were determined by radioimmunoassay (RIA). Allopregnanolone was determined by recently described method consisting of n-hexane-diethyl ether extraction followed by permanganate oxidation (in order to eliminate cross-reacting 4-en-3-oxo steroids), second extraction and microcolumn chromatography (Bicikova et al. 1995). [^{3}H]-HPO prepared enzymaticaly from tritiated progesterone was used as a tracer. Antibody to HPO-11-O-carboxymethyloxime bovine serumalbumin conjugate was employed. Progesterone levels were measured in diethylether extract from the sera by the conventional in-house method using rabbit antiserum to progesterone-11α-hemisuccinate bovine serumalbumin conjugate and [^{3}H]-progesterone as a tracer.

Statistical evaluation. The average, S.D., median, quartiles, skewness and kurtosis were calculated for each group. The normality of distribution in the groups was tested by the Jarque-Berra test. Between group differences were evaluated by the modified two tailed Student's t-test for the skewed and heteroskedastic data. Pearson's correlation coefficients has been used for the evaluation of the pair correlations, the statistical significance of the correlations was evaluated by F-test.

Results

Patients with premenstrual syndrome (PMS). The mean serum levels±S.D. and medians (in parentheses) of steroids under study in 23 patients in follicular phase were 0.78 ± 0.48 (0.60) nmol/l for progesterone and 0.09 ± 0.12 (0.04) nmol/l for allopregnanolone. The corresponding values in luteal phase were 12.2 ± 8.46 (10.9) nmol/l and 1.56 ±1.24 (1.7) nmol/l, respectively. In the control group (n= 17) the respective values were 2.30 ± 1.66 (1.97) nmol/l for progesterone and 0.45 ± 0.44 (0.36) nmol/l for allopregnanolone, in the luteal phase 13.3 ± 8.49 (11.4) and 1.20 ± 1.22 (0.7) nmol/l respectively. The interquartile ranges of progesterone and allopregnanolone in both phases of menstrual cycle in the controls and in the PMS patients group are shown in Fig. 1. Significantly lower levels of both progesterone and allopregnanolone have been found only in the follicular phase of PMS patients in comparison to controls, which was in contrast to the luteal one where the differences were not significant.

Patients after thyroidectomy. Allopregnanolone levels and allopregnanolone/progesterone ratios in patients and controls along with additional statistical characteristics are shown in Fig.2. Since the distribution of the data was not normal but strongly skewed, the modified Student's t-test for the skewed data with different group variances was used. Allopregnanolone levels were elevated after the surgery in the luteal phase of the menstrual cycle when compared with controls. The mean serum levels±S.D. and medians (in parentheses) of steroids under study in 36 patients in follicular phase were 1.41 ± 1.13 (0.87) nmol/l for progesterone and 0.38 ± 0.32 (0.22) nmol/l for allopregnanolone. The corresponding values in luteal phase (n=16) were 19.8 ± 13.4 (16.1) nmol/l and 1.91 ± .1.21 (1.63) nmol/l, respectively. In the control group (n= 17) in follicular phase the respective values were 2.30 ± 1.66 (1.97) nmol/l for progesterone and 0.45 ± 0.44 (0.36) nmol/l for allopregnanolone, while these in the luteal phase 13.3 ± 8.49 (11.4) and 1.20 ± 1.22 (0.7) nmol/l, respectively. The allopregnanolone levels in patients and controls were also related to their TSH levels, but no significant correlation was found.

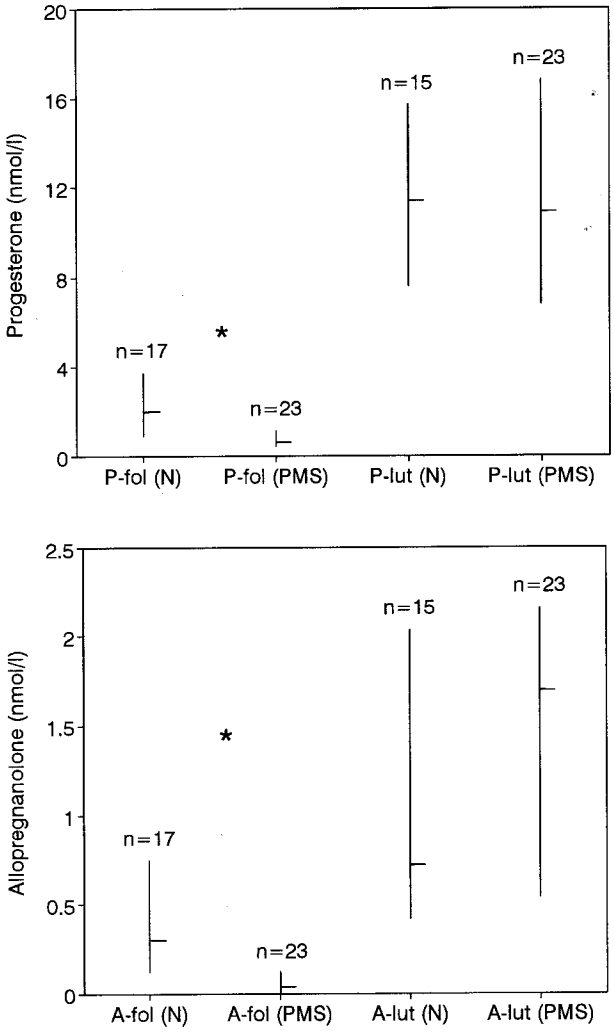

Fig. 1

Progesterone (upper panel) and allopregnanolone (lower panel) levels in the control subjects and in the PMS patients. Vertical lines represent the interquartile ranges of progesterone in follicular (fol) and luteal (lut) phase of menstrual cycle in the control group (N) and patients (PMS). Horizontal segments represent the medians. Significance between patients and controls is marked by an asterisk: * P<0.05.

Discussion

Recent reports brought evidence that known anxiolytic effect of progesterone is mediated by its 5α-reduced metabolites (Bitran et al. 1995) which play an important role in the well-being of women. It was demonstrated in rats that the brain concentrations of steroids follow the variations of circulating conjugated and nonconjugated steroids. Even small dif-

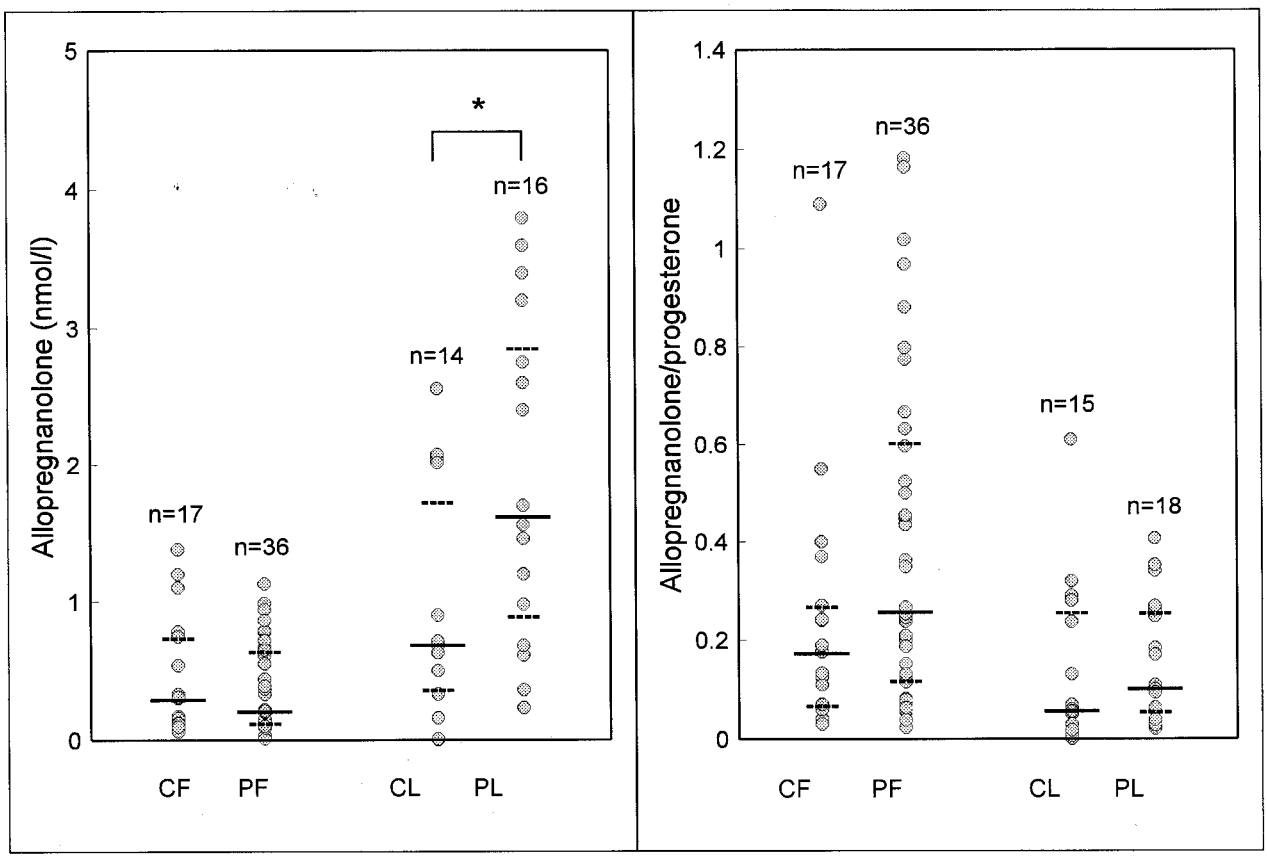

Fig. 2
Allopregnanolone serum levels (left panel) and allopregnanolone/progesterone ratios (right panel) in thyroidectomized female patients and in healthy controls in two phases of menstrual cycle. CF, CL represent sera of the control group in follicular and luteal phase, respectively. PF, PL represent patients sera collected within two weeks after thyroidectomy in follicular and luteal phase respectively. The horizontal bold segments represent medians, the thin ones standard deviations. Significance of the differences between PMS patients and controls: * P<0.05.

ferences between the levels of circulating progestins reflect large differences of steroid concentration in brain (Bixo et al. 1989). Recent study of Bixo (1997) in brains of post-mortem human female showed that there is a variation in brain concentrations of progestins depending on ovarian steroid production, indicating that the secretion pattern during the menstrual cycle is reflected in the brain. Others have shown that a great portion of circulating 5α-saturated progesterone metabolites originates from peripheral conversion taking place especially in the brain (for review see Mac Donald et al. 1991). Therefore plasma levels of allopregnanolone as the most biologically active 5α-reduced progesterone metabolite along with its precursor progesterone may reflect the brain 5α-reductase activity to a considerable extent. In a recent study of daily variations of six steroids including progesterone, its 5-ene precursors and 5α-reduced metabolites during two consecutive cycles in 12 PMS patients and 8 controls, Wang et al. (1996) showed that the levels of 5α-reduced progesterone metabolites in the luteal phase negatively correlated with symptom score reflecting the severity of the syndrome, whereas an opposite trend was observed with progesterone precursors pregnenolone and its sulphate. However, any significant difference between patients and controls has been found in average levels of the 5α-reduced progesterone metabolites and its 5-ene precursors, neither in follicular nor in luteal phase. In contrast to Wang et al. (1996), we have found significantly lower levels of both progesterone and HPO in the follicular phase of PMS patients when compared with controls (cf. Fig 1). It

should be emphasized that randomly selected patients in follicular phase (4th to 9th day of cycle) and luteal phase (6th to 1st day before bleeding), respectively, have been examined. The steroid levels found in this investigation were in average lower than those reported by WANG et al. (1996), probably due to a different technique of separation of the analyte from cross-reacting steroids. However, no significant differences in the concentrations of both steroids between patients and controls were found in the luteal phase. It seems likely that in the luteal phase the small changes in concentrations of 5α-reduced metabolites of progesterone are masked by a huge production of progesterone due to an increased activity of corpus luteum. The finding of lower levels of allopregnanolone at least in the follicular phase, however, allow us to speculate that PMS may be influenced by a lower activity of 5α-reductase in the brain independently on the phase of the cycle, which may be better detectable in the situation when the organism is not flown with progesterone. Anyway, the determination of allopregnanolone may help gynecologists to find an objective criterion for the evaluation as to whether the patient's complaints should be taken into account or not.

To our knowledge no reports exist about allopregnanolone levels in thyroidectomized patients, although the close relationship between thyroid function and steroid metabolism has been repeatedly confirmed (TOMKINS and MCGUIRE 1960; MCEWEN et al. 1991; DELLOVADE et al. 1995. Such relationship may be supported even by the more frequent occurrence of thyroid disorders in women than in men and by the common appearance of goiter during puberty, pregnancy and the menopause (LARSEN and INGBAR 1992). After surgery, postablative hypothyroidism is commonly manifested and replacement therapy with thyroid hormones is required. In adult women hypothyroidism is usually associated with diminished libido and failure of ovulation. The secretion of progesterone falls and endometrial proliferation persists resulting in an excessive and irregular menstrual bleeding. The decrease of thyroid function may predispose patients to the depression and behavioural changes as documented by JOFFE et al. (1984), HALL et al. (1986) and HOWLAND (1993). The broad range of TSH levels in the thyroidectomized patients as measured within two weeks after surgery when the replacement therapy was started may be due to extremely long half-life of T_4 (7-8 days). The fact that allopregnanolone levels are elevated after the surgery at least in the luteal phase indicates that a rapid compensatory mechanism, independent on exogenous substitution, operates in the first days after a severe hit into the hormonal balance. In the later stages, the effect of substitution treatment leading to overall compensation of hormonal balance seems to take place (data not published). This course of enzymatic activity changes corresponds well to clinical observations that psychic disorders are the most severe within the first three months after thyroid ablation. It seems likely that after thyroidectomy the 5α-reductase of C_{21}-steroids is activated which may represent one of counterregulatory mechanisms protecting the organism from undesired consequence of hormonal disbalance.

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