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Platelet Peripheral-Type Benzodiazepine in Pregnancy and Lactation

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The aim of the present study was to investigate the impact of hormonal changes during pregnancy and lactation on the expression of peripheral-type benzodiazepine receptors in platelet membranes. Platelet peripheral benzodiazepine receptor binding characteristics, Hamilton anxiety and depression rating Scores, and progesterone and prolactin (PRL) levels were evaluated during pregnancy and lactation in 17 pregnant women [first (n = 9) and third (n = 8) trimesters], 10 lactating women, and 8 nonpregnant women. A significant decrease (38–41%) in peripheral benzodiazepine receptor density was observed in women during the third trimester of pregnancy when compared to nonpregnant controls and women in their first trimester of pregnancy. The decrease is peripheral benzodiazepine receptors was parallel to the peak in progesterone and PRL secretion. The reduction in peripheral benzodiazepine receptor expression is hormone-dependent and may play a regulatory role geared to prevent pregnancy-related overactivity of the hypothalamic–pituitary-ovarian, hypothalamic–pituitary-adrenal, and hypothalamic-PRL axes. ***[Neuropsychopharmacology 21:513–518, 1999]*** *© 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

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Peripheral-type benzodiazepine receptors have been localized in various peripheral organs as well as in the central nervous system. These receptors are especially abundant in such steroidogenic organs as adrenal gland, testis, and ovary. Peripheral benzodiazepine receptors are involved in the intracellular transport of cholesterol from the outer to the inner mitochondrial membrane, the rate-limiting step in steroid production. Cholesterol is converted into pregnenolone by cytochrome P-450 side-chain-cleavage enzyme, which is located on the inner mitochondrial membrane. Pituitary peptide hormones, such as gonadotropins or ACTH, bind to membranal receptors at their target organs and stimulate, at least partially via peripheral benzodiazepine receptors, mitochondrial cholesterol transport and steroid hormone biosynthesis (Gavish et al. 1992). These receptors are especially sensitive to stress and anxiety (for review, see Weizman and Gavish 1993), but not to depression (Weizman et al. 1995).

One line of evidence suggests that peripheral benzodiazepine receptors are sensitive to changes at the hypothalamic–pituitary-gonadal and hypothalamic–pituitary-adrenal axes. Hypophysectomy has been reported

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to decrease peripheral benzodiazepine receptor density in the adrenal and testis, as well as in ovary, oviduct, and uterus (Gavish et al., 1992). Treatment with gonadotropins and estrogen completely abolishes the effect of pituitary removal in female rat sex organs (Gavish et al. 1992). Adrenalectomy leads to decrease in renal peripheral benzodiazepine receptors; whereas, aldosterone treatment restores the receptor's density to control values (Basile et al. 1987). Surgical castration in rats is associated with depletion of peripheral benzodiazepine receptors in Cowper's gland and adrenal; this decrease is prevented by testosterone replacement therapy (Weizman et al. 1992). Pharmacological suppression of adrogenic activity by cyproterone induces a reduction in peripheral benzodiazepine receptor density in the testis, adrenal, and pituitary (Amiri et al. 1991). Peripheral benzodiazepine receptor density in human granulose–lutein cells of a given follicle increases in association with its growth and with maturational state of the corresponding oocyte; furthermore, peripheral benzodiazepine receptor density in granulosa–lutein cells correlates with serum estradiol measured 24 hours after hCG treatment (Bar-Ami et al. 1991).

Pregnancy is associated with a robust increase in plasma progesterone, estradiol, and prolactin (PRL) (Speroff et al. 1994). The aim of the present study was to investigate whether the hormonal and emotional alterations that normally occur during pregnancy and lactation are reflected in changes in platelet peripheral benzodiazepine receptors. To this end, we evaluated serum progesterone and PRL levels, anxiety and depression levels, and platelet peripheral benzodiazepine receptor characteristics in normal, healthy pregnant women in their first and third trimesters and during lactation, as well as in nonpregnant women.

MATERIALS AND METHODS

Subjects

Thirty-five healthy, drug-free women age 18 to 42 years (mean ± SD, 29.3 ± 5.7 years) were included in the study, which was approved by the Institutional Review Board for Human Investigation. The participants were recruited from a gynecological outpatient clinic, and all consented to participate in the study after its nature was fully explained to them. All the women underwent thorough physical examination and routine blood tests before initiation of the study. Pregnancy was dated by history of last menstrual period, early ultrasound evaluation, and obstetrical examination. All the women were also interviewed by a psychiatrist according to the guidelines of the schedule for Affective Disorders and Schizophrenia—Lifetime Version (Spitzer and Endicott 1978), and only women with no current or past major psychopathology and no history of alcohol and/or substance abuse were included. The women included in the study were assessed using the Hamilton anxiety and depression rating Scales (Hamilton 1959, 1960). The study population was divided into four groups: (1) eight nonpregnant women (3 days after menstruation); (2) nine women at week 8 to 10 of pregnancy (first trimester); (3) eight women at week 34 to 36 of pregnancy (third trimester); and (4) 10 breast-feeding, lactating women (2 to 3 weeks after delivery). Blood samples for hormonal determination and binding assays were collected in all groups between 8:00 and 10:00 AM. In the lactating women, blood samples were collected at least 2 h after breast feeding.

Hormonal Determination

Hormonal assays were performed in the same run to prevent interassay variability. Serum progesterone levels were determined by solid-phase radioimmunoassay (RIA) kit (Diagnostic Products Corporation, Los Angeles, CA). The sensitivity of the progesterone assay is 0.03 ng/ml, and the intra-assay coefficient of variation is 5.1%. Serum PRL levels were determined by double-antibody RIA using materials provided by Diagnostic Products Corporation. The sensitivity of the PRL assay is 2.5 ng/ml, and the mean intra-assay coefficient of variation is 6.5%.

Preparation of Membrane

Blood samples (27 ml) for assessment of [^{3}H]PK 11195 were collected into plastic tubes containing 3 ml of 3.8% sodium citrate and spun at 180 *g* for 15 min at 23°C. Platelet-rich plasma was collected and spun at 1500 *g* for 15 min at 23°C. The platelet-containing pellet was frozen at −70°C until assay. All binding assays were performed in the same run. Before binding assay, the sample were thawed, and each pellet was homogenized in 20 ml of 50 mM Tris-Tris-HCl buffer, pH 7.4, at 4°C with a Brinkmann polytron (setting 10) for 15 s and centrifuged at 49,000 *g* for 15 min at 4°C. The procedures was immediately repeated. The pellet was homogenized in 15 ml of Tris-HCl buffer and used for binding studies.

[^{3}]PK 11195 Binding Assay

[^{3}H] 11195 (92.3 Ci/mol; New England Nuclear, Boston, MA) binding was conducted, as previously described (Diorio et al. 1991). Binding assay in a final volume of 500 μl contained 400 μl platelet membranes (70 to 100 μg protein) and 25 μl [^{3}H] PK 11195 (final concentration 0.2 to 6 nM) in the absence (total binding) or presence (nonspecific binding) of 10 μM unlabeled PK 11195 (similar nonspecific binding was obtained when we used 10 μM unlabeled Ro 5-4864). After incubation for 60 min at 4°C, samples were filtered under vacuum over Whatman GF/B filters and washed three times

Table 1. Clinical and Hormonal Data of the Study Population

	Nonpregnant (n = 8)	First Trimester (n = 9)	Third Trimester (n = 8)	Lactating (n = 10)
Age (years)	28.1 ± 4.7	30.6 ± 7.2	29.6 ± 5.8	28.9 ± 5.5
HAM-A	6.3 ± 3.6	6.9 ± 2.3	8.6 ± 3.7	4.8 ± 2.5
HAM-D	6.0 ± 3.8	5.4 ± 3.0	4.1 ± 3.2	4.5 ± 3.5
Progesterone (ng/ml)	0.45 ± 0.3	26.0 ± 11.5[a,b]	138.8 ± 34.5[c]	0.5 ± 0.3
Prolactin (ng/ml)	10.3 ± 3.8	22.2 ± 4.4	210. 0 ± 32.6[c]	50.0 ± 8.2[d]

Abbreviations: HAM-A = Hamilton anxiety rating scale, HAM-D = Hamilton depression rating scale.
The data represent mean ± SD.
[a] $p < .05$ versus nonpregnant.
[b] $p < .01$ versus lactating.
[c] $p < .001$ versus nonpregnant, first trimester, and lactating.
[d] $p < .001$ versus nonpregnant and first trimester.

with 3 ml of Tris-HCl buffer. Filters were placed in vials containing 5 ml of xylene-Lumax (3:1, vol/vol; Lumac, Schaesberg, The Netherlands) and counted for radioactivity. Equilibrium dissociation constant (K_d) and maximal number of binding sites were determined by Scatchard analyses of saturation curves of [^{3}H] PK 11195 binding. The binding parameters were analyzed for each subject individually.

Statistical Analysis

One-way analysis of variance (ANOVA) and the Student–Newman-Keuls multiple-comparison post hoc test were used for intergroup comparisons. All results are expressed as mean ± SD. Pearson's correlation test was used to assess the relationship between the binding values and the hormonal and psychological measures; $p < .05$ was considered statistically significant.

Table 2. Correlation (r) between Maximal Binding Capacity (B_{max}) and Dissociation Constant (K_d) and the Endocrine and Psychological Measures

	Prolactin	Progesterone	HAM-A	HAM-D
First trimester				
B_{max}	0.56	0.48	−0.77[a]	−0.59
K_d	0.26	0.49	−0.27	−0.46
Third trimester				
B_{max}	−0.15	0.54	−0.61	0.44
K_d	0.11	0.59	−0.41	0.12
Lactation				
B_{max}	−0.26	0.56	−0.67[a]	−0.48
K_d	0.22	0.37	−0.08	−0.27
Nonpregnant				
B_{max}	−0.07	0.39	0.01	−0.19
K_d	0.52	−0.17	0.42	0.39

Abbreviations: HAM-A = Hamilton anxiety rating scale, HAM-D = Hamilton depression rating scale.
[a] $p < .05$.

RESULTS

The women included in the study were of similar ages ($F_{3,31} = 0.26$, $p = .855$) and had no differences in anxiety or depression scores as assessed by Hamilton anxiety and depression rating scores ($F_{3,31} = 2.46$, $p = .08$, and $F_{3,31} = 0.53$, $p = .66$, respectively) (Table 1). The mean Hamilton anxiety and depression rating scores as well as progesterone and PRL levels of the four groups are shown in Table 1. As shown, the Hamilton anxiety scores were 36% higher at the third trimester, as compared to nonpregnant women, but this difference did not reach a significant level, and the scores were within normal range ($p < .10$). As expected, progesterone levels increased gradually during pregnancy, reached a peak during the third trimester, and returned to normal range during lactation ($F_{3,31} = 119.2$; $< .0001$). Similarly to progesterone, PRL levels rose during pregnancy and reached a peak in the third trimester ($F_{3,31} = 256.5$; $p < .0001$). PRL levels remained significantly higher in comparison to nonpregnant levels during the first 2 to 4 weeks of breast feeding (Table 1).

As shown in Table 2, a significant negative correlation was found between Hamilton anxiety scores and maximal binding capacity (B_{max}) values in pregnant women during their first trimester ($r = -.77$; $p < .05$) and in lactating women ($r = -.67$; $p < .05$). No such correlation was obtained during the third trimester ($r = -.61$; $p = .10$) or in nonpregnant women ($r = 42$; $p = .30$). No correlation was found between the endocrine measures (PRL and progesterone) and Hamilton depression scores and either B_{max} or K_d values in any period.

The effect of pregnancy and lactation on the B_{max} and K_d values of platelet peripheral benzodiazepine receptors is depicted in Figure 1. As shown, the third trimester of pregnancy was associated with a significant decrease ($p < .05$) in platelet peripheral benzodiazepine receptors, as compared with nonpregnant (−41%) and first trimester (−38%) women ($F_{3,31} = 3.71$; $p < .05$). The

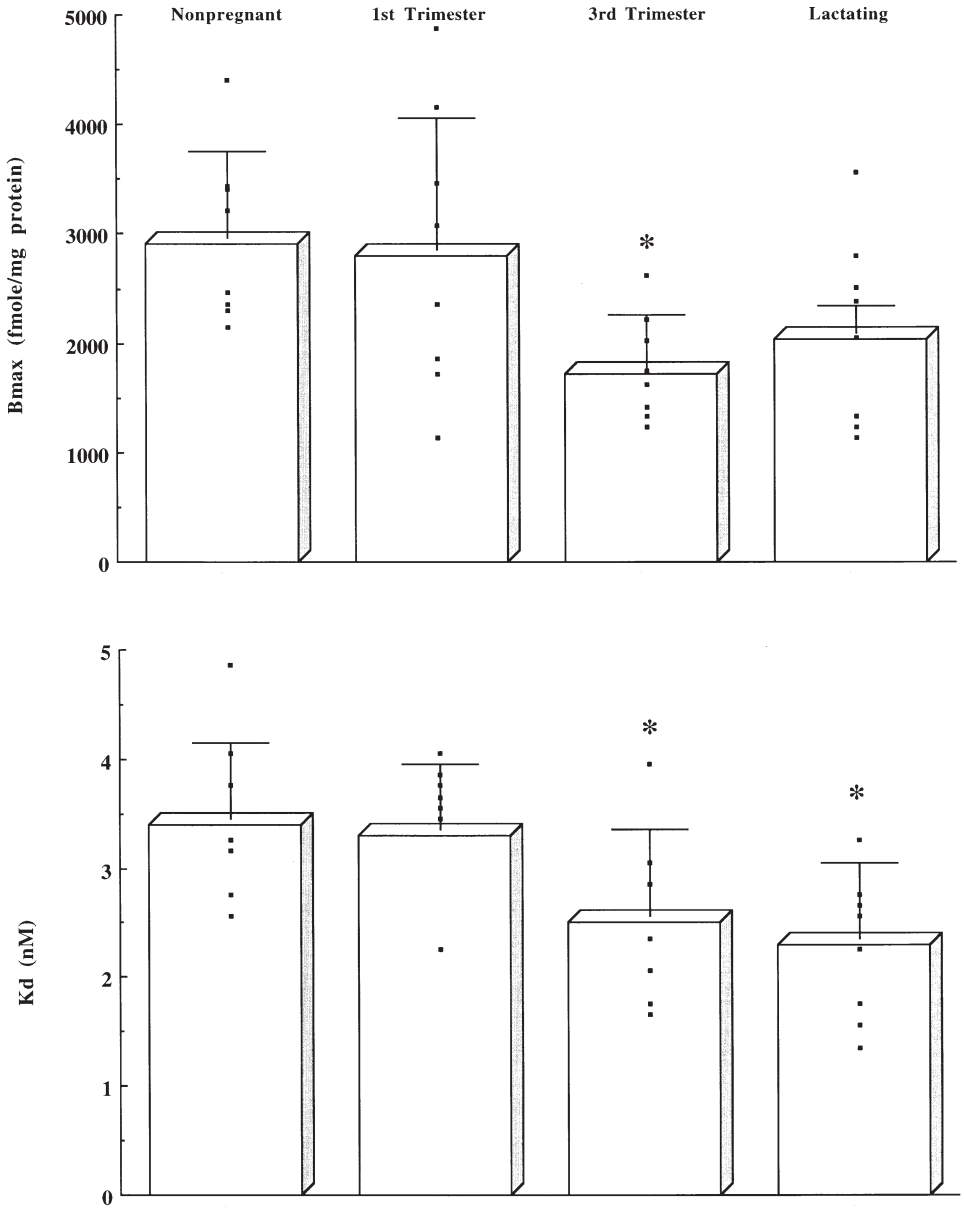

Figure 1. Maximal binding capacity (B_{max}) of [^{3}H]PK 11195 binding sites (fmol/mg protein) and the dissociation constant (K_d) in platelet membranes (nm) of nonpregnant, pregnant (first- and third-trimester), and lactating women. [^{3}H]PK 11195 was assayed at six concentrations (0.2 to 6 nM) in the absence (total binding) or presence (nonspecific binding) of 10 μM unlabeled PK 11195. The binding parameters for each subject were analyzed individually. Results are expressed as means ± SD. *$p < .05$ versus nonpregnant and first trimester pregnancy.

[^{3}H] PK 11195 binding values in the first trimester of pregnancy did not differ from the values obtained in nonpregnant women. The K_d values were significantly reduced in third-trimester and lactating women as compared to first-trimester and nonpregnant women ($F_{3,31} = 5.95$; $p < .01$); however, all values were in the nanomolar range in the four groups (from 1.3 to 4.8 nM).

DISCUSSION

The present study demonstrated that platelet peripheral benzodiazepine receptors are down-regulated during the third trimester of pregnancy, an interval associated with a peak in progesterone and PRL release. Chronic anxiety and stress lead to a decrease in platelet peripheral benzodiazepine receptor density (Weizman et al. 1987; Weizman and Gavish 1993). Pregnancy and the postpartum period are sometimes accompanied by anxiety and depression (Watson et al. 1984). However, psychiatric evaluation of all the women in the present study revealed no signs of anxiety disorder or major depression, and no statistically significant differences in Hamilton anxiety and depression rating scores were detected among the various groups (although an increase of 36% in anxiety scores occurred at the third trimester). The experience of pregnancy and the puerperium is normally predominantly one of fulfillment and joy (Imboden and Adler 1992). However, the tendency toward an increase in anxiety detected in our sample during the third trimester may have played a role in the reduction in platelet peripheral benzodiazepine receptors

during this period, as has been demonstrated in anxious humans (Weizman et al. 1987, 1994; Gavish et al. 1996). Although there was a significant negative correlation between Hamilton anxiety scores and B_{max} values during the first trimester and lactation; that is, increased anxiety associated with diminished peripheral benzodiazepine receptor density, such a correlation was not obtained during the third trimester, when peripheral benzodiazepine receptor densities were the lowest. Thus, it seems unlikely that enhanced anxiety was the major factor responsible for the third-trimester down-regulation of peripheral benzodiazepine receptor expression. However, pregnancy-related anxiety can contribute to hormone-related reduction in peripheral benzodiazepine receptors.

Mitochondrial steroidogenesis can be regulated by the occupy of peripheral benzodiazepine receptors with specific ligands. Thus, it seems that peripheral benzodiazepine receptors in steroidogenic tissues may be the target for endogenous ligands that mediate, at least partially, the stimulatory action of LH on the biosynthesis of ovarian steroids (Gavish et al. 1992). Alterations in ovarian steroid sex hormone (progesterone and estradiol) levels may play a role in platelet peripheral benzodiazepine receptor depletion, because the maximal increase in the levels of these hormones occurs during the third trimester of pregnancy and disappears after delivery (lactation period) (Speroff et al. 1994), a mirror image of the peripheral benzodiazepine receptor profile. If the reduction in platelet membrane peripheral benzodiazepine receptors reflects similar changes in the ovary and pituitary, it could be that the desensitization of peripheral benzodiazepine receptors is a neuroendocrine mechanism aimed at controlling the activity of the hypothalamic–pituitary-ovarian axis. It is noteworthy that short-term ($\sim$9 days) human menopausal gonadotropin treatment in women (Diorio et al. 1991) and gestation less than 30 weeks (Ferrero et al. 1994) are not sufficient to affect peripheral benzodiazepine receptors of platelet and blood mononuclear cells, respectively. Thus, it seems that depletion of platelet peripheral benzodiazepine receptors occurs only following a long-term increase in ovarian steroids, as in the third trimester of pregnancy. Pregnancy is also associated with a marked increase in adrenal cortisol synthesis, probably because of placental production of corticotropin-releasing factor- and proopiomelanocortin-related peptides (Griffing and Melby 1994). The decrease in peripheral benzodiazepine receptors may be geared to slow the rate of glucocorticoid synthesis.

Alternatively, it is possible that PRL plays a role in the suppression of peripheral benzodiazepine receptor expression. PRL secretion increases during pregnancy, beginning at 8 weeks of gestation and reaching a peak at term. The increase in PRL parallels the increase in estrogen. It is assumed that estrogen suppression of the hypothalamic PRL-inhibiting factor (dopamine) and direct stimulation of PRL gene transcription in the pituitary are responsible for the pregnancy-associated hyperprolactinemia. PRL levels decrease about 50% during the first postpartum week. Baseline PRL levels are elevated throughout breast feeding, and suckling provokes a transient twofold increase in the hormone secretion (Speroff et al. 1994). Thus, it seems that there is at least a temporal correlation between the third trimester hyperprolactinemia and the decrease in peripheral benzodiazepine receptor levels. The peripheral benzodiazepine receptor seems to play a role in PRL secretion (Calogero et al. 1990). If the decrease in platelet peripheral benzodiazepine receptors during the third trimester of pregnancy represents a similar reduction in pituitary peripheral benzodiazepine receptors, it is possible that the down-regulation is geared to prevent exaggerated PRL release.

In summary, late pregnancy (third trimester) is associated with diminished peripheral benzodiazepine receptor density. The change occurs regardless of normal psychiatric status and seems to be related to hormonal changes. However, the increase (36%) in anxiety scores during the third trimester may have contributed to suppression of the expression of the receptors (Weizman and Gavish 1993). Peripheral benzodiazepine receptor reduction may be an inhibitory physiological neuroendocrine mechanism aimed to control the activity and sensitivity of the hypothalamic–pituitary-ovarian, hypothalamic–pituitary-adrenal, and hypothalamic-PRL axes.

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