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Sequential serotonin and noradrenalin associated processes involved in postpartum blues

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ABSTRACT

Objective: We investigated whether postpartum blues was related to changes in parameters of noradrenergic and serotonergic functioning.
Methods: From 26 healthy pregnant women blood was collected at the end of pregnancy and 5 days and 6 weeks postpartum. Serotonergic parameters were: platelet serotonin content; paroxetine binding to platelet membranes as an index of serotonin transporter activity; the serotonin precursor tryptophan in proportion to the large neutral amino acids, as an estimate of its cerebral influx. Noradrenergic indices were the noradrenaline precursor tyrosine and its metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG). The Kennerly and Gath blues questionnaire was applied at day five postpartum.
Results: The incidence of postpartum blues was 30%. The tryptophan ratio and serotonin content of platelets were decreased ($p<0.01$) at day five postpartum in all women. B_{max} paroxetine at day five was correlated with blues score ($\beta=0.460$; $p=0.031$). MHPG levels at 6 weeks were increased in women with blues ($p<0.001$). In a regression model MHPG at 6 weeks was related to blues score ($\beta=0.477$; $p=0.002$) and MHPG at day five ($\beta=0.550$; $p=0.001$), explaining >50% of the variation ($R^2=0.588$; $p<0.001$).
Conclusions: A decreased serotonergic activity was found at the fifth day postpartum in all subjects. Increased SERT activity, reflected by higher paroxetine binding to platelets might be involved in the onset of blues. The elevated MHPG levels in women with blues are compatible with a higher stress sensitivity, or a decreased stress coping in those and is suggested to be involved with the onset of depression.

1. Introduction

Postpartum blues is a transient affective syndrome occurring in about half of the women (15.3%–84%) in the first week after delivery (Gitlin and Pasnau, 1989; Henshaw, 2003). Symptoms of postpartum blues include crying, grief, anxiety, sadness, confusion, headache and also exuberance (Yalom et al., 1968; Kennerley and Gath, 1989a; Henshaw, 2003). Postpartum blues is considered as a risk factor for postpartum depression (Henshaw et al., 2004; Bloch et al., 2005). In the etiology of postpartum blues much attention has been given to the effects of the postpartum drop of estrogen and progesterone on neurophysiology and behavior (Abou-Saleh et al., 1998; Bloch et al., 2003). But, only about half of the women suffer from postpartum blues, and no consistent differences in hormone levels have been found between women with and without blues (Hendrick et al., 1998; Bloch et al., 2003), indicating that other factors are involved in the etiology of postpartum blues.

We hypothesized that differences in serotonin (5-HT) metabolism and neurotransmission might be a factor underlying the individual risk for postpartum blues. Alterations in serotonin metabolism and transmission have been associated with affective disorders (Owens and Nemeroff, 1994; Kendler et al., 2005). In the peripartum the synthesis of both peripheral and cerebral 5-HT is limited due to the decreased availability of tryptophan, caused by the increased catabolism of tryptophan in the placenta during pregnancy (Schrocksnadel et al., 1996; Munn et al., 1998), and the immune activation and increased liver metabolism following delivery (Fuchs et al., 1996; Maes et al., 2002; Schrocksnadel et al., 2003a). Changes in tryptophan

Abbreviations: 5-HT, serotonin; MHPG, 3-methoxy-4-hydroxyphenylglycol; LNAAs, large neutral amino acids; tyr, Tyrosine; phe, phenylalanine; SD, standard deviation; SERT, serotonin transporter.

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concentration (Handley et al., 1977; Abou-Saleh et al., 1999; Kohl et al., 2005; Bailara et al., 2006), tryptophan catabolism (Maes et al., 2000, 2002) and platelet 5-HT content (Maurer-Spurej et al., 2007) have all been associated with postpartum blues. Moreover, postpartum depression has been associated with differences in functioning of the serotonin transporter (SERT) as reflected by a decreased Kd of both imipramine and paroxetine (Hannah et al., 1992b; Newport et al., 2004). During pregnancy serotonergic activity is further modulated by estrogen, progesterone and some of their metabolites (Bethea et al., 2002) resulting in a sudden change in the serotonergic neurotransmission postpartum. All together, the early postpartum period is characterized by a sudden change, mostly a reduction, of serotonergic activity and this condition may contribute to the development of mood disorders.

Another factor contributing to postpartum blues might be stress experienced during the delivery and subsequent days. Stress and life events are well documented risk factors for depression (Kendler et al., 1999). Virtually all kinds of stress induce activation of noradrenergic neurons of both peripheral (sympathetic) system and the cerebral system, in particular that located in the locus coeruleus, leading to increased formation of the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) (Bremner et al., 1996a,b). Therefore the impact of stress can, to some extend, be assessed by measuring MHPG in the circulation.

Here we investigate if peripartum noradrenergic and serotonergic plasma parameters are associated with postpartum blues. In contrast to most previous studies, we use a longitudinal design and combine several peripheral measures of serotonergic activity. Assessment of those parameters are limited to measurements in plasma. More direct methods like CSF-analyses or PET scanning, are difficult to perform frequently, and could be harmful for the fetus. These peripheral measures are frequently used in psychiatric research, but their translation to central functioning is not straightforward, and criticized in literature (Muller-Oerlinghausen et al., 2004).

Serotonergic activity was measured by 1) The density and activity of 5-HT binding sites of blood platelets, as assessed by platelet paroxetine binding, which is a measure for activity of the SERT. The SERT has a key role in regulating cerebral extracellular serotonin levels; 2) The serotonin content of the platelets, reflecting the recent serotonin production 3) Plasma levels of the serotonin precursor tryptophan in relation to the large neutral amino acids (LNAA), as factor linked to central serotonin production. Noradrenaline activity was measured by plasma levels of its precursor tyrosine in relation to LNAAs and its metabolite MHPG.

The following hypotheses were tested: 1) Postpartum blues is associated with a decreased plasma tryptophan ratio and/or decreased platelet 5-HT levels; 2) Serotonin transporter capacity and functioning, as reflected by platelet paroxetine binding, correlate with blues score; 3) Women with postpartum blues experience more stress, represented by increased MHPG levels.

2. Subjects, materials and methods

2.1. Subjects and experimental protocol

Twenty-six healthy pregnant women who visited the maternity clinic of the Erasmus University Medical Center in Rotterdam participated in this study, after they gave written informed consent. The protocol was approved by the ethical committee of Erasmus Medical Center. Women, all Caucasian, and aged between 21 and 35 years, were in good physical health and did not take any medication. None of the subjects suffered from affective disorders or other mental illnesses at baseline. Blood was collected at the end of the third trimester (after 36 weeks), and postpartum at day 5 and 6 weeks. The following biochemical parameters were measured: the platelet serotonin content; the paroxetine binding to platelet membranes; the plasma concentrations of the LNAA, i.e. tryptophan, tyrosine, phenylalanine, valine, leucine and isoleucine, and the plasma levels of MHPG. A Dutch version of the Kennerley and Gath blues questionnaire was used at the fifth postpartum day (Iles et al., 1989; Kennerley and Gath, 1989a). This questionnaire is the most used instrument for measuring blues. According to this scale women have blues when the score was higher than the mean of the whole group (Kennerley and Gath, 1989b). Because such a measure is strongly influenced by outliers, and complicates the comparisons with other samples, we use the widely used cut-off score of 12.

2.2. Biochemical analyses

Blood (20 ml) was collected between 9:00 and 10:30 AM in siliconated vacutainer tubes containing 0.15% K_3-EDTA as anticoagulant. Platelet-rich plasma was obtained by centrifugation of the blood at 90 ×g for 20 min at 20 °C. A sample of 200 µl was frozen at −80 °C for the determination of 5-HT and platelets were counted in a sample of 50 µl. The rest of the platelet-rich plasma was centrifuged at 2650 ×g for 20 min. The supernatant (plasma) was frozen at −80 °C for the determination of amino acids and MHPG. The platelets were isolated from the pellet after two centrifugation runs, pooled and washed in 10 ml buffer (Tris–HCl 50 mM, NaCl 150 mM, Na2-EDTA 20 mM, pH 7.35) and finally frozen at −80 °C for determination of paroxetine binding.

The LNAA tryptophan, tyrosine, phenylalanine, valine, leucine and isoleucine were analysed in the plasma samples by reversed phase high performance liquid chromatography (HPLC) after pre-column derivatisation with *o*-phthaldialdehyde and detected fluorometrically (Fekkes et al., 1995). The platelet 5-HT concentrations were measured by a reversed phase HPLC method described previously (Fekkes et al., 1997). Plasma free MHPG was measured by a reversed phase HPLC method after extraction using a slightly modified published procedure (Moleman and Borstrok, 1982). In short, 0.5 ml plasma, 20 µl internal standard (2 µg *iso*-MHPG/ml) and 3 ml ethylacetate were added to a seraclear tube containing 0.75 g NaCl and 0.1 g florisil. The tube was shaken for 20 min and centrifuged for 10 min at 2750 g. Two ml of the organic layer was evaporated to dryness at 40 °C under vacuum in a Buchler Vortex Evaporator (Lenexa, KS, U.S.A.) and the residue was dissolved in 0.5 ml of the mobile phase. Ten or 20 µl samples were injected onto a reversed phase column (ODS-Hypersil, 5 µm particle size, 200×2.1 mm, Hewlett Packard) which was protected by a guard column (20×2.1 mm) of the same material. The mobile phase consisted of 50 mM sodium phosphate and 0.67 mM disodium EDTA containing 1% isopropanol, pH 2.7. The flow rate was set at 0.25 ml/min and the column temperature was 28 °C. The detection system consisted of a Model 5100A Coulochem detector equipped with a 5021 conditioning cell and a 5011 high sensitivity cell (ESA, Bedford, MA, U.S.A.). The potentials for the conditioning cell and detectors 1 and 2 were +0.45, −0.05 and −0.43 V, respectively. The gain was 15×100 and quantification was done by measuring peak heights. The limit of detection at a signal to noise ratio of 2 was 10 fmol (approx. 2 pg) MHPG per injection. The retention times of MHPG and *iso*-MHPG were 7.6 and 12.8 min, respectively. The intra- and inter-day coefficients of variation of duplicate analysis of plasma samples were 2.6% (n=8) and 4.9% (n=18), respectively. The recovery of *iso*-MHPG added to the plasma samples was 79±4% (n=14). Paroxetine binding to blood platelets was determined using ^{3}H-paroxetine (New England Nuclear) as radioligand and clomipramine as displacing agent to correct for nonspecific binding (Klompenhouwer et al., 1990).

2.3. Calculations

The tryptophan ratio indicated the tryptophan availability in the brain for subsequent 5-HT synthesis. This ratio was computed with the formula: plasma tryptophan×100/sum of the plasma LNAA minus tryptophan. The tyrosine ratio indicated the tyrosine availability in