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Neuroactive steroids, their precursors and polar conjugates during parturition and postpartum in maternal blood: 2. Time profiles of pregnanolone isomers

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Abstract

Time profiles of the pregnanolone isomers epipregnanolone (3β-hydroxy-5β-pregnan-20-one), allopregnanolone (3α-hydroxy-5α-pregnan-20-one), pregnanolone (3α-hydroxy-5β-pregnan-20-one), and isopregnanolone (3β-hydroxy-5α-pregnan-20-one) were measured around parturition and in the postpartum period in the serum of 13 and three women with subarachnoidal and epidural analgesia, respectively. In addition, the levels of polar conjugates of all pregnanolone isomers were followed during parturition. GC/MS analysis was used for the measurement of steroid levels. Changes in concentrations of free steroids exhibited a similar pattern, with a fall primarily within the first hour after delivery. The decrease in conjugated steroids was shifted to the interval within the first hour and first day after delivery, and the changes were more pronounced. The time profile of the conjugated/free steroid ratio exhibited a significant decrease within the first hour and the first day after delivery in all of the isomers investigated. A decrease was also observed in the ratio of 3α/3β-isomers and 5α/5β-isomers around parturition. The possible physiological consequences of the findings are indicated.

Keywords: Neuroactive steroids; Parturition; Postpartum; Maternal blood; Pregnanolone isomers

1. Introduction

The role of some neuroactive steroids has recently been evaluated in connection with the onset of parturition. These are effective primarily as modulators of the neurotransmitter receptors influencing the permeability of ion channels [1–7], and some also act at progesterone receptors [8,9]. Epipregnanolone sulfate has been reported in *Xenopus laevis* oocytes as a competitor of pregnenolone sulfate [10] known to act as activator of the membrane *N*-methyl-D-aspartate (NMDA) receptors regulating the permeability of calcium channels [4,5,10].

The well-known activating effect of pregnanolone isomers on γ-aminobutyric acid (GABA) receptors can be reversed by their sulfatation at position C-3 [11]. A precursor of pregnanolone isomers, progesterone, decreases near parturition. As Leng and Russell [12] have previously suggested, a decrease in the levels of pregnanolone isomers (which are also produced by placenta [13,14]) could trigger the production of oxytocin [15–17], thus resulting in a rapid delivery.

Abbreviations: AlloPal, allopregnanolone (3α-hydroxy-5α-pregnan-20-one); AlloPalC, conjugated allopregnanolone; EpiPal, epipregnanolone (3β-hydroxy-5β-pregnan-20-one); EpiPalC, conjugated epipregnanolone; GABA, γ-aminobutyric acid; GC/MS, gas chromatography/mass spectrometry; HPLC, high performance liquid chromatography; IsoPal, isopregnanolone (3β-hydroxy-5α-pregnan-20-one); IsoPalC, conjugated isopregnanolone; NMDA, *N*-methyl-D-aspartate; Pal, pregnanolone (3α-hydroxy-5β-pregnan-20-one); PalC, conjugated pregnanolone.

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In this study the authors investigated the time profiles of all of the isomers of pregnanolone, i.e. epipregnanolone (3β-hydroxy-5β-pregnan-20-one, EpiPal), allopregnanolone (3α-hydroxy-5α-pregnan-20-one, AlloPal), pregnanolone (3α-hydroxy-5β-pregnan-20-one, Pal), and isopregnanolone (3β-hydroxy-5α-pregnan-20-one, IsoPal), around parturition. The primary aim was to evaluate patterns in the time profiles of pregnanolone isomers—including their polar conjugates (the mixture of sulfates and glucuronides)—in maternal and umbilical serum during parturition and postpartum. To evaluate the differences in the production of individual pregnanolone isomers, differences in their time profiles and in those of their polar conjugates around delivery were investigated. The following questions were addressed:

1. What are the changes in serum levels of pregnanolone isomers and their polar conjugates during parturition?
2. Are there any changes in the proportions of the 3α- and 3β-isomers or their polar conjugates?
3. Are there any changes in the proportions of the 5α- and 5β-isomers or their polar conjugates?
4. Are there any changes in the proportions of conjugated and unconjugated pregnanolone isomers around parturition?

2. Experimental

2.1. Subjects

The patient group consisted of 16 women at delivery, of whom 13 were treated with subarachnoidal and three with epidural analgesia. Both types of analgesia have been reported in detail elsewhere [18].

Informed written consent was obtained from all of the subjects both for the collection and utilization of the samples.

2.2. Sample collection

The blood samples for the experiment were collected in five stages during parturition and in the postpartum period. The first stage named 'cervical dilatation 3 cm' was characterized by a diameter of the Os uteri of 3–4 cm. The border that remained of the Os uteri after 30 min from the 'cervical dilatation 3 cm' stage, when the cervical dilatation reached a diameter of 10–11 cm, identified the second stage, named 'cervical dilatation 11 cm'. The third, fourth and fifth stages, named '1 hour after', '1 day after' and '5 days after' respectively, corresponded to samples collected after lapses of 1 h, 1 day and 5 days from delivery. Each sample was collected in a cooled plastic tube containing 100 μl 5% EDTA and 50 pl aprotinin (Antilysin from Spofa, Prague, Czech Republic). The serum was obtained using centrifugation for 5 min at $2000 \times g$ at 0 °C. The samples of serum were stored at −20 °C until analyzed.

2.3. Steroids and chemicals

The non-radioactive steroids and their conjugates were from Steraloids (Wilton, NH, USA). The solvents for extraction and high performance liquid chromatography (HPLC), and pyridine, were of analytical grade, from Merck (Darmstadt, Germany). The derivatization agent Sylon BFT was purchased from Supelco (Bellefonte, PA, USA).

2.4. Instruments

The gas chromatography/mass spectrometry (GC/MS) system was supplied by Shimadzu (Kyoto, Japan). The system consisted of a GC 17A gas chromatograph equipped with automatic flow control, AOC-20 autosampler and for the MS a QP 5050A quadrupole electron-impact detector with a fixed electron voltage of 70 eV. The liquid scintillation spectrometer was supplied by Beckmann Instruments, (Fullerton, CA, USA).

2.5. Analytical methods

The pregnanolone isomers EpiPal, AlloPal, Pal, and IsoPal, and their polar conjugated analogues (EpiPalC, AlloPalC, PalC, and IsoPalC), were measured using the GC/MS analysis described elsewhere [18].

2.6. Statistical evaluation of the data

For the evaluation of changes in the time profiles, two-way ANOVA was used with stage and subject as the factor A and B, respectively. Tukey interaction between the factors was not included in the model. For hypothesis testing it is strictly assumed that the model error is normally and independently distributed with a mean of zero and variance σ^2, i.e. a homoscedasticity or a constant variance throughout the level treatments. Prior to statistical hypothesis testing, the data underwent power transformation to stabilize the group variances and to approximate a normal distribution of the data [19] The group means calculated from the transformed data with their lower and upper limits of confidence intervals were re-transformed to the original scale, and the values thus obtained were used for a graphical demonstration of the time profiles. The re-transformed mean values were close to the medians and their confidence intervals were more or less asymmetrical, reflecting the skewness of the original data. Individual differences between the stages were evaluated by the use of the least significant difference multiple compari-

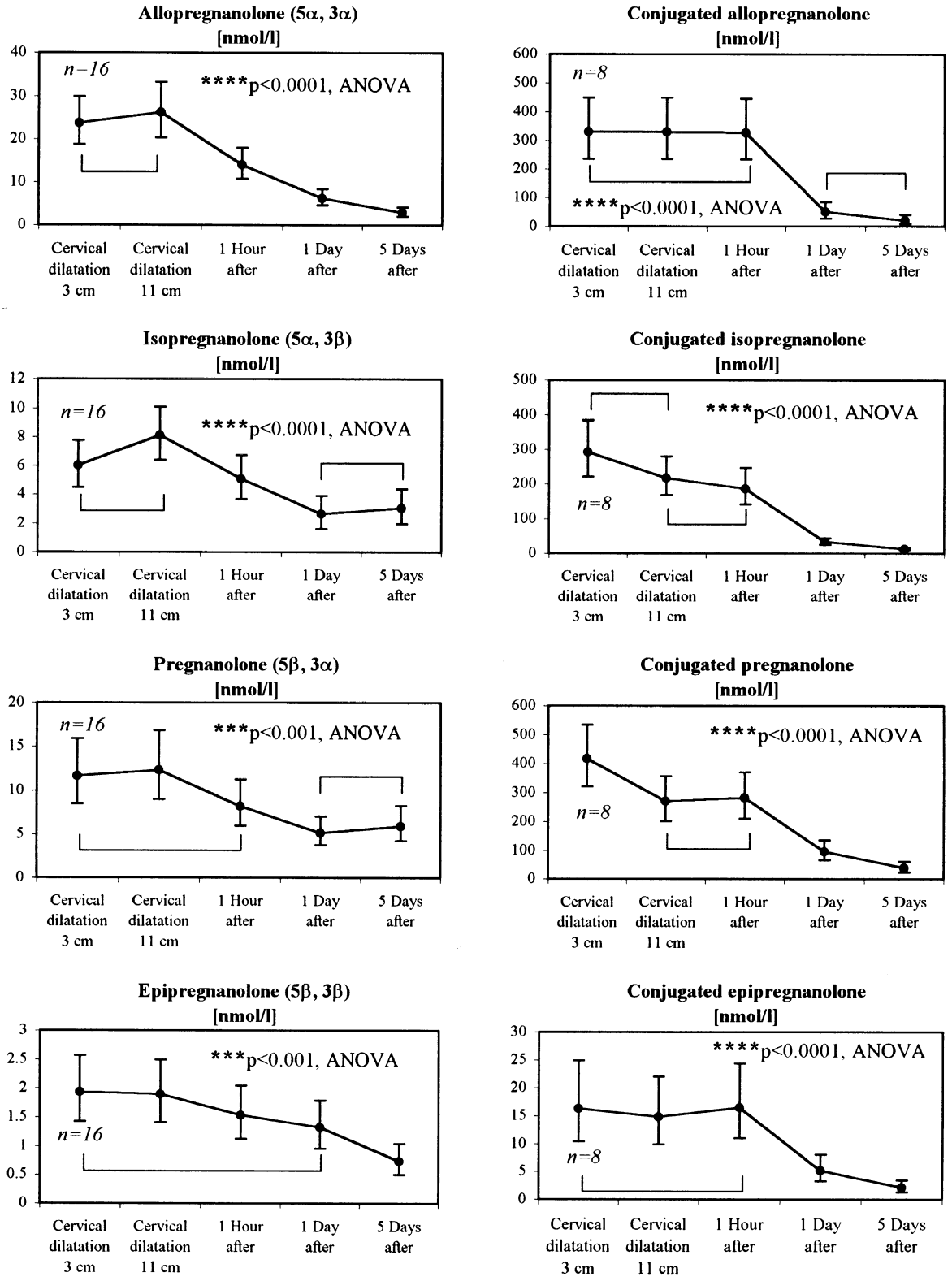

Fig. 1. Time profiles of pregnanolone isomers and their polar conjugates (mixture of sulfates and glucuronides) in maternal serum during parturition. The squares with whiskers represent group mean values with 95% confidence intervals calculated using the least significant difference multiple comparison. Asterisks denote the statistical significance (***$P < 0.001$, ****$P < 0.0001$) of the differences in all stages around parturition. Clamps denote groups with insignificant differences between mean values. (For statistical treatment see Section 2.6).

son. Statistical computations were performed using Statgraphics Plus 3.3 (Manugistics Rockville, MA, USA) statistical software.

3. Results

3.1. Time profiles of free steroids

All of the pregnanolone isomers exhibited significant changes during parturition and postpartum. More pronounced changes were found in 5α-isomers (Fig. 1).

3.2. Profiles of conjugated steroids

The changes in the levels of conjugated pregnanolone isomers are demonstrated in Fig. 1. The shapes of the profiles for the conjugates differed from those for their non-conjugated analogues. While in the free steroids the major change was observed during labor and 1 hour after delivery, the change in the conjugates was most prominent over the first day postpartum. The levels of AlloPalC and EpiPalC remained constant, while a tendency to decrease was found in the levels of IsoPalC and PalC during the cervical dilatation 3 and 11 cm stages. For PalC, this decline even reached statistical significance.

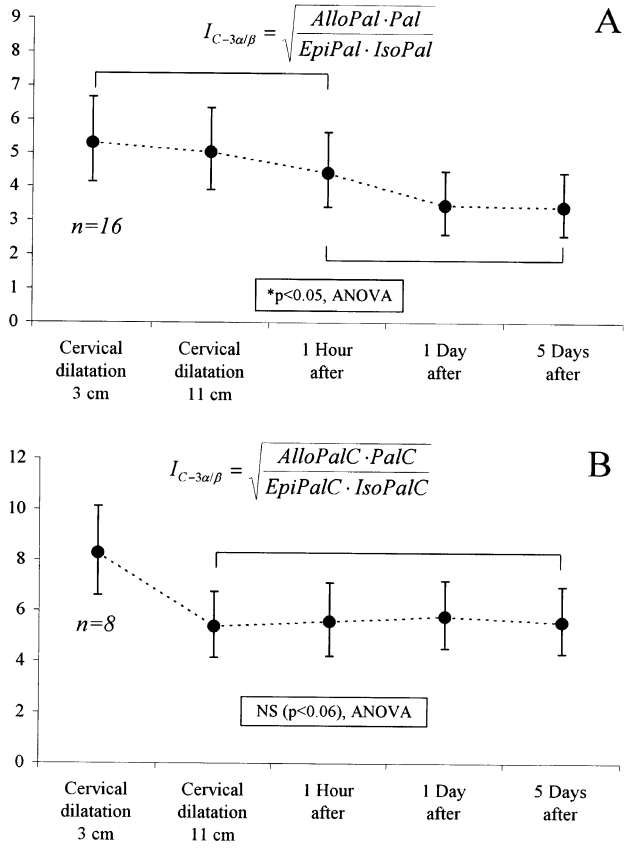

Fig. 2. Time profiles of the index $I_{C\text{-}3\alpha/\beta}$ characterizing the overall 3α-/3β-isomer ratio of pregnanolone (free steroids: panel A, conjugates: panel B) in maternal serum during parturition. The squares with whiskers represent group mean values with 95% confidence intervals. Asterisks denote the statistical significance ($*P < 0.05$) of the differences in all stages around parturition. Clamps denote groups with insignificant differences between mean values. (For statistical treatment see Section 2.6).

3.3. Time profiles of the 3α-/3β-isomer ratio

Changes in the 3α-/3β-isomer ratio were evaluated using the index $I_{C\text{-}3\alpha/\beta}$ defined as the square root of the ratio of the product of 3α-isomers to the product of 3β-isomers (Eq. (1), Fig. 2):

$$I_{C\text{-}3\alpha/\beta} = \sqrt{\frac{\text{AlloPal}\cdot\text{Pal}}{\text{EpiPal}\cdot\text{IsoPal}}} \quad (1)$$

The square root was used to approach the same scale as in the comparison of the two steroids. The formulation mentioned above enables a simple notion of how many times the levels of 3α-isomers exceed those in 3β-isomers regardless of the configuration in the C-5 position.

The free 3α-/3β-isomer ratio continually decreases, primarily in the cervical dilatation 3 cm stage and during the first day after delivery (Fig. 2A). The situation in the conjugated steroids is different (Fig. 2B). The decline, as evaluated by ANOVA, is below the level of significance. However, a least significant difference multiple comparison revealed a significant decrease at the cervical dilatation 3 and 11 cm stages.

3.4. Time profiles of the 5α-/5β-isomer ratio

Changes in the 5α-/5β-isomer ratio were evaluated using the index $I_{C\text{-}5\alpha/\beta}$ defined as the square root of the ratio of the product of 5α-isomers to the product of 5β-isomers (Eq. (2)):

$$I_{C\text{-}5\alpha/\beta} = \sqrt{\frac{\text{AlloPal}\cdot\text{IsoPal}}{\text{Epipal}\cdot\text{Pal}}} \quad (2)$$

A significant decrease was found in the free 5α-/5β-isomer ratio in the first hour and the first day after delivery (Fig. 3A); a similar situation was found in the conjugates (Fig. 3B).

3.5. Time profile of the conjugates/free pregnanolone isomer ratio

Overall changes in the conjugated/free steroid ratio were evaluated using the index $I_{\text{conjug./free}}$ defined as the fourth root of the ratio of the product of the conjugates to the product of the free steroids (Eq. (3)):

$$I_{\text{conjug./free}} = \sqrt[4]{\frac{\text{AlloPalC}\cdot\text{IsoPalC}\cdot\text{PalC}\cdot\text{EpiPalC}}{\text{Allopal}\cdot\text{IsoPal}\cdot\text{Pal}\cdot\text{EpiPal}}} \quad (3)$$

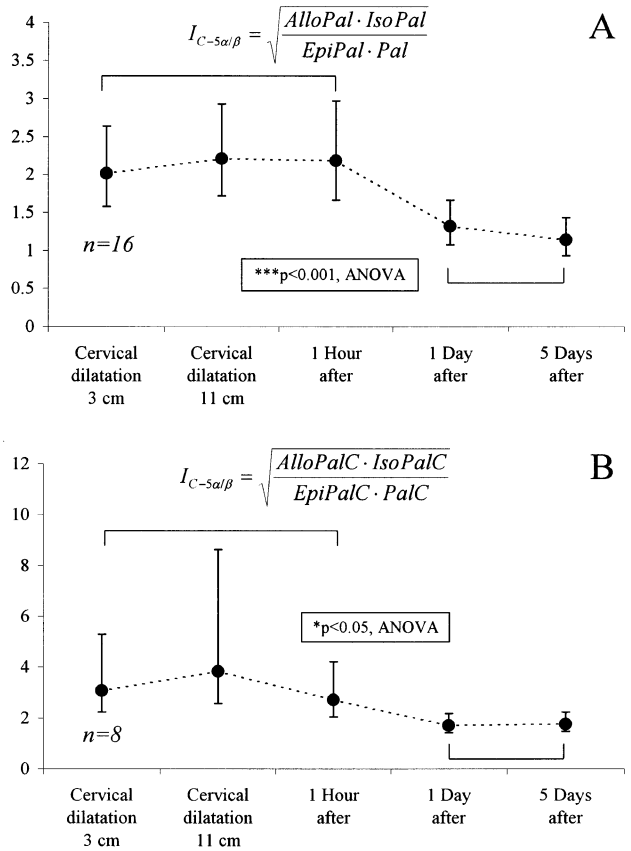

Fig. 3. Time profiles of the index $I_{C\text{-}5\alpha/\beta}$ characterizing the overall 5α-/5β-isomer ratio of pregnanolone (free steroids: panel A, conjugates: panel B) in maternal serum during parturition. The squares with whiskers represent group mean values with 95% confidence intervals. Asterisks denote the statistical significance (***$P < 0.001$, *$P < 0.05$) of the differences in all stages around parturition. Clamps denote groups with insignificant differences between mean values. (For statistical treatment see Section 2.6).

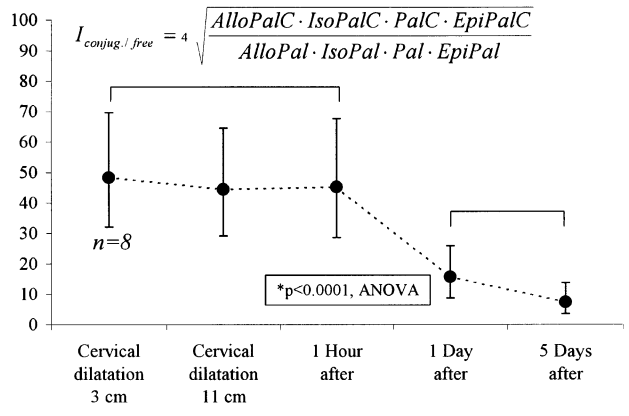

Fig. 4. Time profiles of the index $I_{conjug./free}$ characterizing the overall conjugated/free isomer ratio of pregnanolone in maternal serum during parturition. The squares with whiskers represent group mean values with 95% confidence intervals. Asterisks denote the statistical significance (***$P < 0.001$, *$P < 0.05$) of the differences in all stages around parturition. Clamps denote groups with insignificant differences between mean values. (For statistical treatment see Section 2.6).

As shown in Fig. 4, no changes were observed within the cervical dilatation 3 cm stage or during the first hour after delivery. A significant change was found in the first hour and the first day after labor. As is evident from Fig. 4, the conjugate/free steroid ratio decreases to about 1/5 of the levels observed close to labor by the fifth day after delivery.

4. Discussion

The discovery of the effect of allopregnanolone down-regulating production of oxytocin during gravidity [12,20–22] and the finding of changes in GABA receptor affinity to AlloPal during pregnancy and the lactation period [15,16], resulted in an increased interest on the part of investigators in the role of neuroactive steroids in human reproduction. Recently, the importance of the polar conjugates of pregnanolone isomers has grown as a consequence of the discovery of retrogression in the effect of GABA-receptor steroid activators after their sulfatation on C-3 hydroxyl [11]. In this connection, the investigation of changes in the levels of neuroactive steroids around parturition suggests itself; the current lack of available information is particularly obvious for pregnanolone isomers and their conjugates. Recently, results have been reported on the relationship between levels of pregnanolone isomers and their polar conjugates in maternal and umbilical serum at delivery [18]. The serum levels of AlloPal during pregnancy and at delivery were measured by Luisi et al. [23]. The authors' aim in the present study was to describe the time changes in the levels of all pregnanolone isomers and their polar conjugates in maternal serum during parturition and postpartum. Particular attention was paid to changes in the relative proportions of 3α- and 3β-isomers and of 5α- and 5β-isomers, and to the conjugated/free isomer ratio.

The decrease in the levels of pregnanolone isomers that activates GABA receptors during parturition is in advance of the decline in their conjugates that has a negative modulating effect (Fig. 1). The question is whether the fall of the levels of steroids attenuating the activity of oxytocin producing cells while concurrently maintaining the levels of their sulfated antagonist could influence the initiation and/or the course of parturition via hypothalamic oxytocin production cells or on the peripheral level. It is known that the sulfatation of 3α-pregnanolone isomers reverses the positive modulating effect on GABA receptor activity [11]. Moreover, the role of GABA receptors in the timing of parturition in rats has been reported [12,15–17].

The proportions of 3α- and 3β-isomers decreased continually, particularly in the stages near delivery. Given the GABA-activating effect of both of the 3α-isomers, the decreasing 3α-/3β-isomer ratio accompany-

ing the decrease in levels of all pregnanolone isomers may be connected to the low physiological requirement for GABA activators after the onset of parturition.

A time change in the proportions of both free and conjugated 5α- and 5β-isomers that was even more pronounced than that in the 3α-/3β-isomer ratio was found, especially marked during the first day after delivery. The decrease in the 5α-/5β- ratio may be of interest in the context of the different influences of conjugated 5α- and 5β-pregnanolone isomers on NMDA receptor function [24]. While conjugated 5α-isomers potentiate NMDA receptors (which are present both in CNS and peripherally [25,26]), 5β-isomers exert an inhibitory effect [24]. The question is whether the higher levels of conjugated 5α-isomers in the earlier stages of parturition (relative to those in the postpartum period) could positively influence the onset and course of parturition at least at a peripheral level. A parallel effect might be expected from pregnenolone sulfate, levels of which are also substantially elevated in maternal serum near delivery [27].

The fall in the conjugated/free steroid ratio during the first day after delivery may be of importance. This pattern of change was found in all of the individual pregnanolone isomers. The question arises as to whether the elevated activity of sulfotransferase inverting the effect of GABA-activators [11] could be connected to the initiation and course of parturition. Further evaluation of the conjugated/free pregnanolone isomer ratio (particularly as applied to 3α-isomers) in the interval of several days before the onset of parturition could usefully contribute to answering this question.

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