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Pregnanolone Isomers, Pregnenolone and Their Polar Conjugates Around Parturition

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Summary

The levels of four pregnanolone isomers and their polar conjugates and pregnenolone sulfate were measured in the plasma of 13 and 7 women at delivery with subarachnoidal and epidural analgesia, respectively, and in corresponding samples of umbilical plasma using a simple quadrupole GC/MS system with electron impact ionization (pregnenolone isomers), RIA following HPLC separation (pregnenolone) and specific RIA (pregnanolone sulfate). The concentration of epipregnanolone (3β-hydroxy-5β-pregnan-20-one) in both maternal and umbilical plasma was much lower than that of other pregnanolone isomers. The levels of 3β-hydroxy-pregnanolone isomers were significantly higher in the umbilical plasma than in the maternal, while the differences in 3α-hydroxy-isomers were insignificant. The differences in conjugates were insignificant with the exception of allopregnanolone, the levels of which were lower in umbilical plasma. In all the pregnanolone isomers, a significantly lower conjugated/unconjugated steroid ratio was found in the umbilical plasma than in the maternal plasma. In addition, time profiles of the steroids were measured around parturition and in the postpartum period in the maternal serum. Similarly, the levels of polar conjugates of all pregnanolone isomers were followed during parturition. 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 profiles 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.

Key words

Pregnanolone isomers • Pregnenolone • Maternal plasma • Umbilical plasma • Parturition

Introduction

Neuroactive C21 steroids regulate neuronal activity primarily as modulators of neurotransmitter receptors by influencing the permeability of ion channels (Majewska 1990, Majewska *et al.* 1990a,b, Wu *et al.* 1991, Irwin *et al.* 1992, Hawkinson *et al.* 1996, Poisbeau *et al.* 1997). In addition, they may interact with progesterone receptors (Putnam *et al.* 1991, Rupprecht *et al.* 1996). Pregnenolone sulfate, which is present in body

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fluids in relatively high concentrations, promotes the influx and rapid activation of calcium into the neuron by activating membrane N-methyl-D-aspartate (NMDA) receptors (Wu *et al.* 1991, Irwin *et al.* 1992, Park-Chung *et al.* 1997). 3β-hydroxy-5β-pregnan-20-one-3-sulfate has been reported in *Xenopus laevis* oocytes as a competitor of pregnenolone sulfate known to act in an opposite manner (Park-Chung *et al.* 1997). NMDA receptors are present not only in CNS but also in the periphery (Carlton *et al.* 1998, Coggeshall and Carlton 1998). Pregnenolone sulfate (PregS) and dehydro-epiandrosterone sulfate (DHEAS) act as negative GABA receptor modulators (Majewska 1990, Majewska *et al.* 1990a,b, Wu *et al.* 1991, Irwin *et al.* 1992, Zaman *et al.* 1992, Hawkinson *et al.* 1996, Poisbeau *et al.* 1997) in contrast to their non-conjugated analogues (Majewska 1990, Purdy *et al.* 1990, Lan *et al.* 1991, Putnam *et al.* 1991). The same holds for pregnanolone isomers the action of which can be reversed by their sulfatation at position C-3 (Park-Chung *et al.* 1999).

Progesterone, a precursor of saturated pregnanolone isomers, decreases near parturition. As Leng and Russell (1999) have previously suggested, a decrease in the levels of pregnanolone isomers, which are also produced by the placenta (Dombroski *et al.* 1997, Milewich *et al.* 1979), could trigger the production of oxytocin, thus resulting in an acceleration of delivery.

In this study we have investigated all of the isomers of pregnanolone, i.e. epipregnanolone (3β-hydroxy-5β-pregnan-20-one, P3β,5β), allopregnanolone (3α-hydroxy-5α-pregnan-20-one, P3α,5α), pregnanolone (3α-hydroxy-5β-pregnan-20-one, P3α,5β), and isopregnanolone (3β-hydroxy-5α-pregnan-20-one, P3β,5α). To evaluate the influence of precursors, pregnenolone and its sulfate were included in the study. Considering the differences in steroid metabolism between mother and fetus, attempts were made to evaluate the differences in the levels of individual pregnanolone isomers and their polar conjugates at delivery. Furthermore, the time profiles of steroids in maternal serum were monitored and the changes of steroid levels as well as the proportions between free steroids and their polar conjugates and between 3α- and 3β-pregnanolone isomers were followed.

Methods

Subjects

The patient group consisted of 20 women at delivery, 13 of whom were treated with subarachnoidal and 7 with epidural analgesia. Both types of analgesia have been reported in detail elsewhere (Hill *et al.* 2000). Informed written consent was obtained from all of the subjects both for the collection and the use of the samples for research purposes.

Sample collection

The blood samples for the experiment were collected in 5 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 to 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 an interval of 1 hour, 1 day and 5 days from delivery. Each sample was collected into a cooled plastic tube containing 100 μl of 5 % EDTA and 50 pl of aprotinin (Antilysin from Spofa, Prague, Czech Republic). The serum was obtained using centrifugation for 5 min at 2000 x *g* at 0 °C. Samples of the serum were stored at –20 °C until analyzed.

Steroids and chemicals

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

Instruments

The 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 mass spectrometer with a QP 5050A quadrupole electron-impact detector with a fixed electron voltage of 70eV. The liquid scintillation spectrometer Beckmann, (Fullerton, CA, USA) was used for ^{3}H measurements.

Analytical methods

The pregnanolone isomers P3β,5β, P3α,5α, P3α,5β, and P3β,5α, and their polar conjugated analogues (P3β,5βC, P3α,5αC, P3α,5βC, and P3β,5αC), were measured using the GC/MS analysis described elsewhere (Hill *et al.* 2000).

The pregnenolone (Preg) was measured using the method of Hill *et al.* 1999). The pregnenolone sulfate (PregS) was measured using the RIA following extraction elimination of cross-reactants as described elsewhere (Hill *et al.* 2002)

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 first and the second factor, respectively. Tukey interaction between the factors was not included in the model. For testing the hypothesis it was strictly assumed that the model error is normally and independently distributed with a mean equal to zero and constant variance σ^2, i.e. a homoscedasticity throughout the level treatments. Prior to testing the statistical hypothesis, the data underwent power transformation to stabilize the group variances and to approximate a normal distribution of the data (Tabachnick and Fidell 2001). To avoid the influence of outliers, the normalized residues with absolute values greater than 2 were excluded from the calculations. 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 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 comparisons.

Respecting non-Gaussian distribution of the original data, Spearman correlations were used to evaluate the relations between steroids or between maternal and umbilical serum in individual steroids. Besides simple pair correlations, partial correlations with adjustment to constant levels of the remaining steroids except the pair investigated were applied.

Student's paired t-test and/or Wilcoxon's robust paired test were used for the comparisons between maternal and umbilical serum at delivery. Statistical computations were performed using Statgraphics Plus 3.3 (Manugistics Rockville, MA, USA) statistical software.

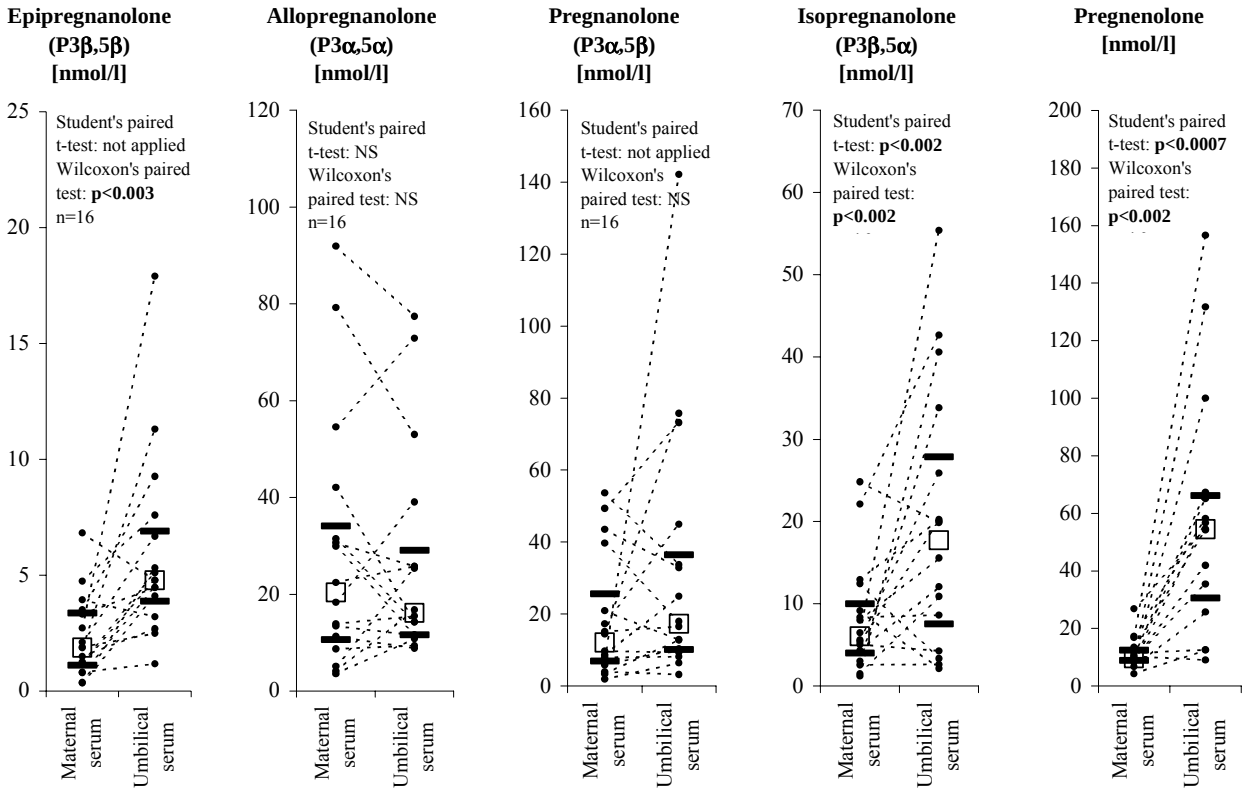

Fig. 1. *Differences between maternal and umbilical serum in pregnanolone isomers and pregnenolone at delivery. The empty squares with error bars represent medians with quartiles, n is the number of subjects. Dashed lines join the corresponding maternal and umbilical steroid levels in individual subjects.*

Results

Levels of steroids in maternal and umbilical serum at delivery

The levels of unconjugated pregnanolone isomers and pregnenolone in maternal and umbilical plasma at delivery are shown in Figure 1. The least abundant pregnanolone isomer in both maternal and umbilical blood was P3β,5β (median concentration at delivery being 1.87 and 4.78 nmol/l in maternal and umbilical plasmas respectively). The proportions of median concentrations of pregnanolone isomers and pregnenolone in maternal plasma at delivery were 0.194 : 2.11 : 1.25 : 0.628 : 1 (P3β,5β, P3α,5α, P3α,5β, P3β,5α, Preg) while those in umbilical plasma were 0.088 : 0.296 : 0.317 : 0.325 : 1 (P3β,5β, P3α,5α, P3α,5β, P3β,5α, Preg).

The levels of 3β-hydroxy-steroids (P3β,5β and P3β,5α) were significantly higher in the umbilical than in the maternal plasma, while the differences in 3α-steroids (P3α,5α and P3α,5β) were insignificant. The proportions of umbilical to maternal plasma medians at delivery were 2.55, 0.79, 1.43, 2.93, and 5.65 for P3β,5β, P3α,5α, P3α,5β, P3β,5α and Preg, respectively.

The levels of conjugated pregnanolone isomers and PregS in maternal and umbilical plasma at delivery are shown in Figure 2. As is the case for the unconjugated steroids, the least abundant pregnanolone isomer in both maternal and in umbilical plasma was P3β,5βC (its median concentration being 15.3 and 11.9 nmol/l in maternal and umbilical plasma, respectively). The proportions of median concentrations of conjugated pregnanolone isomers and pregnenolone in maternal plasma were 0.0099 : 0.1893 : 0.1718 : 0.1255 : 1 for P3β,5βC, P3α,5αC, P3α,5βC, P3β,5αC and PregS, respectively, while those in umbilical plasma were 0.0020 : 0.0234 : 0.0306 : 0.0198 : 1 for P3β,5βC, P3α,5αC, P3α,5βC, P3β,5αC and PregS, respectively.

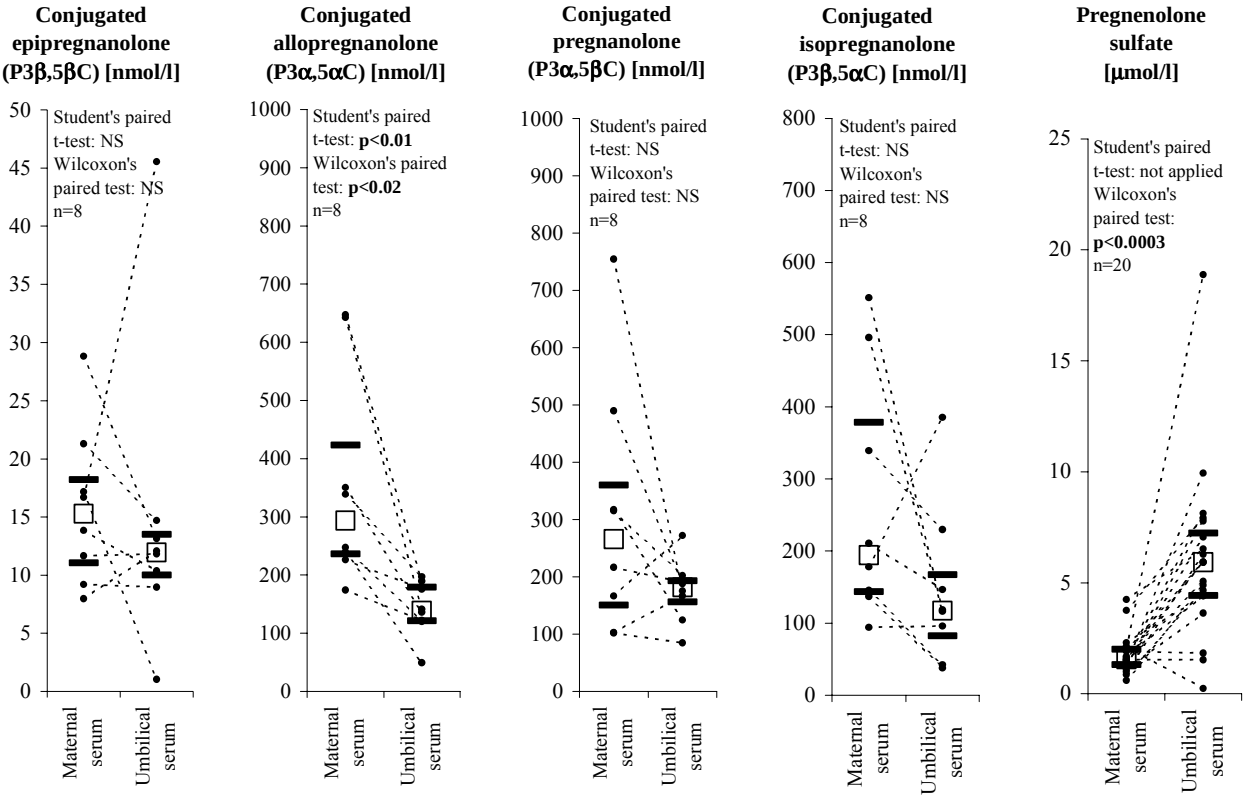

Fig. 2. *Differences between maternal and umbilical serum in polar conjugates of pregnanolone isomers, and pregnenolone sulfate at delivery. The drawings and symbols are the same as in Fig. 1.*

No significant differences were observed between maternal and umbilical plasma in conjugated pregnanolone isomers, except that the levels of P3α,5αC were significantly lower in umbilical plasma. The differences in the levels of P3α,5βC and P3β,5αC did not reach statistical significance due to a broad variance, although the mean values in umbilical plasma were almost half those in maternal plasma. As expected, the levels of PregS were substantially higher in umbilical serum.

The proportions of the median umbilical/maternal plasma at delivery were 0.783, 0.473, 0.683, 0.603, and 3.83 for P3β,5βC, P3α,5αC, P3α,5βC, P3β,5αC, and PregS, respectively.

Differences in proportions between conjugated and unconjugated steroids

In all instances except pregnenolone, significantly lower values of the ratio of conjugated/ unconjugated steroids in pregnanolone isomers in maternal and umbilical sera at delivery were found in the umbilical serum. The significances of the differences were as follows: P3β,5β ($\pi<0.007$, $\nu=8$, Student's paired t-test), P3α,5α β ($\pi<0.03$, $\nu=8$, Wilcoxon's paired t-test), P3α,5β β ($\pi<0.03$, $\nu=8$, Student's paired t-test), P3β,5α β ($\pi<0.03$, $\nu=8$, Wilcoxon's paired t-test). The proportions in maternal serum were 8.2, 14.4, 22, 32.1, and 160.5 for P3β,5β, P3α,5α, P3α,5β, P3β,5α, and Preg, respectively, while those in umbilical serum were 2.5, 8.6, 10.5, 6.6, and 108.8 for P3β,5β, P3α,5α, P3α,5β, P3β,5α, and Preg, respectively.

Mutual correlations of steroid levels

In the maternal serum, P3β,5β, P3α,5α and P3α,5β correlated well, however, partial correlations between the steroids after adjustment to constant value of the remaining steroids indicated correlations only between P3α,5β and P3β,5β ($r=0.633$, $p<0.03$, $n=16$), and between P3α,5β and P3α,5α ($r=0.8941$, $p<0.0001$, $n=16$). A marginal partial correlation was also found between P3α,5β and P3β,5α ($r=0.510$, $p<0.08$, $n=16$). Pregnanolone isomers did not correlate with Preg.

In both umbilical and maternal serum, P3β,5β, P3α,5α and P3α,5β correlated well, but the partial correlations were below the level of statistical significance. In contrast to the maternal serum, the P3α,5α correlated well with Preg both in the simple pair ($r=0.700$, $p<0.004$, $n=15$) and in the partial correlation ($r=0.688$, $p<0.02$, $n=15$).

From pregnanolone isomers, only 3α-isomers P3α,5α ($r=0.618$, $p<0.02$, $n=16$) and P3α,5β ($r=0.547$, $p<0.03$, $n=16$) exhibited significant correlation between maternal and umbilical serum. Pregnenolone did not show significant correlation. As expected, no correlation between maternal and umbilical serum was found in conjugated steroids.

Time profiles of the steroids in maternal serum

All the pregnanolone isomers exhibited significant changes during parturition and postpartum. As apparent from F-statistics, the more apparent changes were found in 5α-isomers (Fig. 3). Nevertheless, the most pronounced changes were observed in pregnenolone sulfate.

The changes in the levels of conjugated pregnanolone isomers are shown in Figure 4. The courses of the time profiles for the steroid conjugates differed from those for their non-conjugated analogues. While in most of the free steroids the changes were already observed during labor or one hour after delivery, the respective changes in the conjugates were most prominent as late as on the first day postpartum. The levels of P3β,5βC, P3α,5αC and P3β,5αC remained nearly constant, while a significant decrease was found in P3α,5βC and PregS during the cervical dilatation 3 cm and 11 cm stages.

The time profiles of the ratios conjugated/free steroid exhibited significant changes around parturition in all pregnanolone isomers except P3α,5α with maximum values one hour after delivery. The significance of the changes were as follows: 3β,5β ($\pi<0.02$, $\nu=8$, ANOVA), P3α,α β ($\pi<0.07$, $\nu=8$, ANOVA), P3α,5β β ($\pi<0.01$, $\nu=8$, ANOVA), P3β,5α β ($\pi<0.0001$, $\nu=8$, ANOVA). In 3α,5α, the ANOVA test did not reach statistical significance, however, LSD multiple comparisons revealed maximum values one hour after delivery similarly as in the other pregnanolone isomers. The ANOVA test was insignificant ($p<0.2$) in pregnenolone.

Changes in the 3α/3β-isomer ratio were evaluated using the index $I_{3\alpha/\beta}$ defined as the square root of the ratio of the product of 3α-isomers to the product of 3β-isomers. 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 conclusion as to the extent of the levels of 3α-isomers exceed those in 3β-isomers regardless of the configuration at the C-5 position.

The 3α/3β- isomer ratio in unconjugated steroids $I_{3\alpha/\beta}$ continually decreased during parturition, primarily in the cervical dilatation 3 cm stage and during the first day after delivery (Fig. 5A). The situation in the conjugated steroids was different (Fig. 5B). The changes, as evaluated by ANOVA, were insignificant.

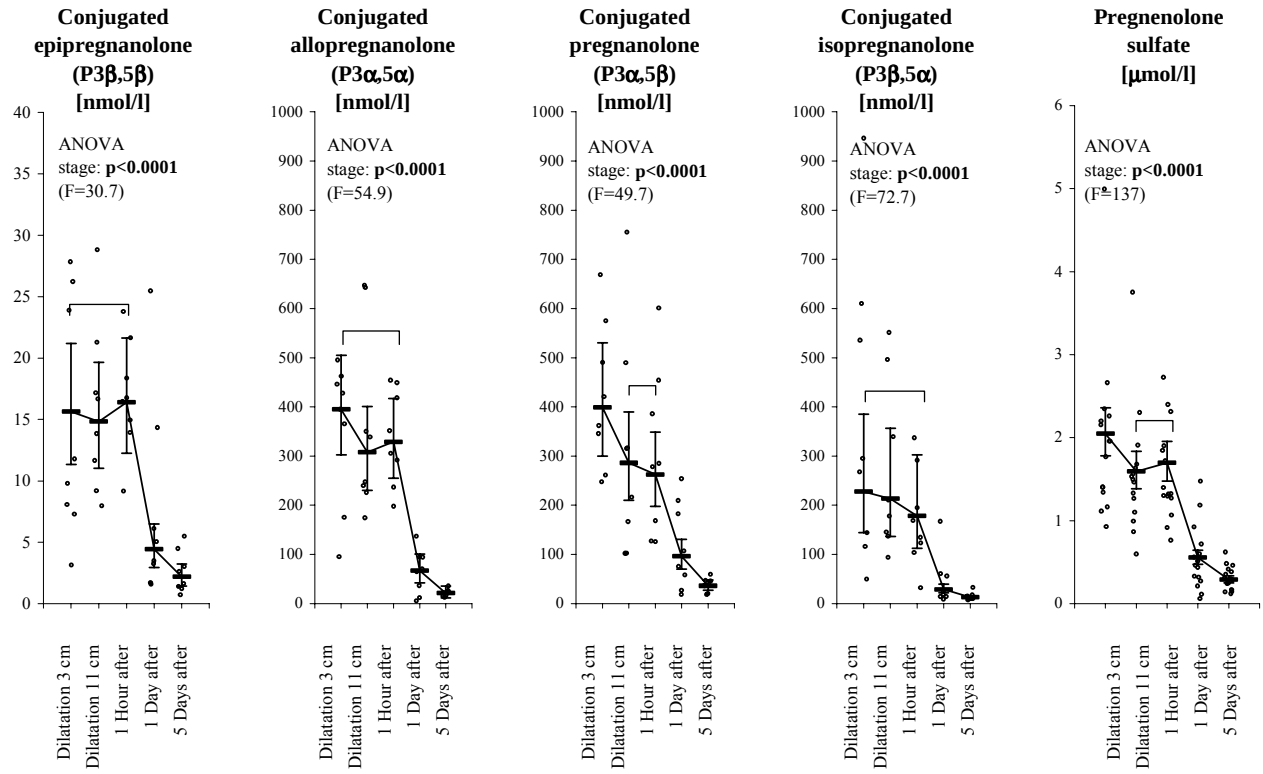

Fig. 3. *Time profiles of pregnanolone isomers and pregnenolone in maternal serum around parturition. The short horizontal lines with error bars represent group mean values with 95 % confidence intervals calculated using least significant difference multiple comparisons. Clamps denote groups with insignificant differences between mean values, F is the explained/random variance ratio (see also the section "Statistical evaluation of the data").*

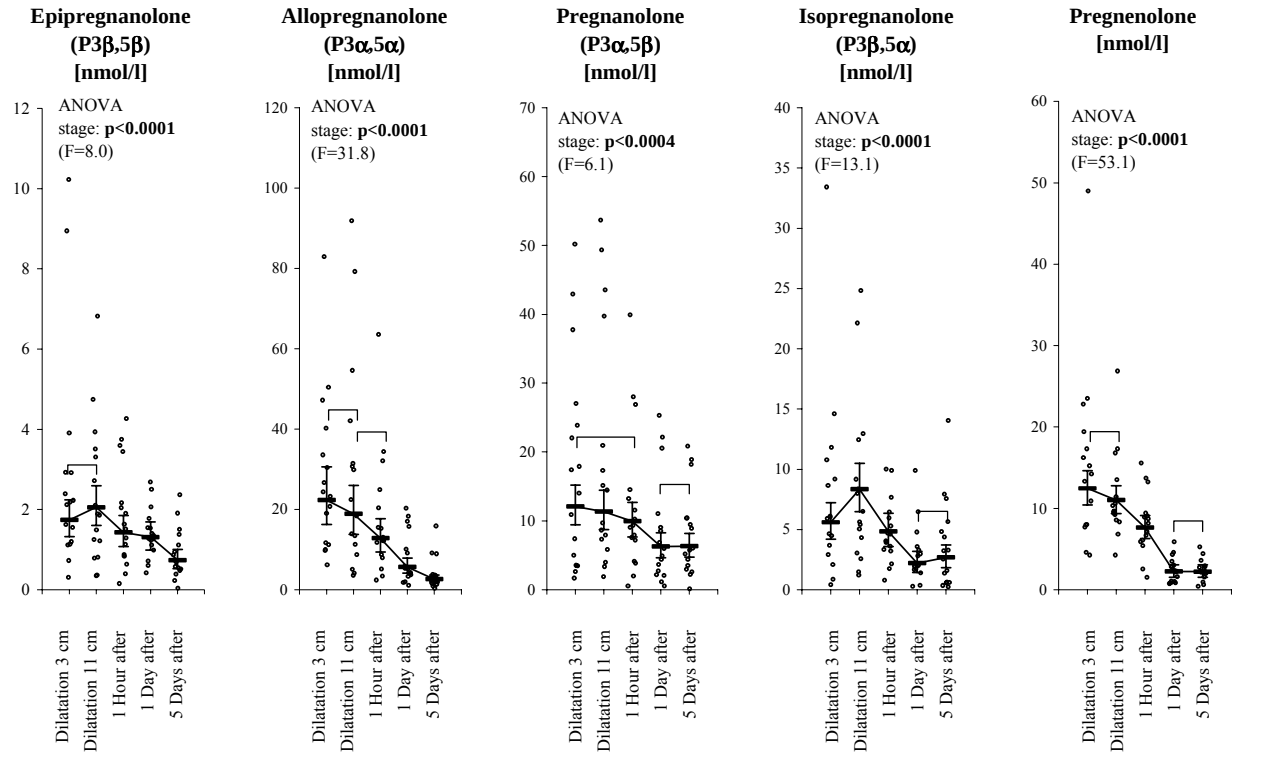

Fig. 4. *Time profiles of conjugated pregnanolone isomers and pregnenolone sulfate in maternal serum around parturition. The drawings and symbols are the same as in Fig. 3.*

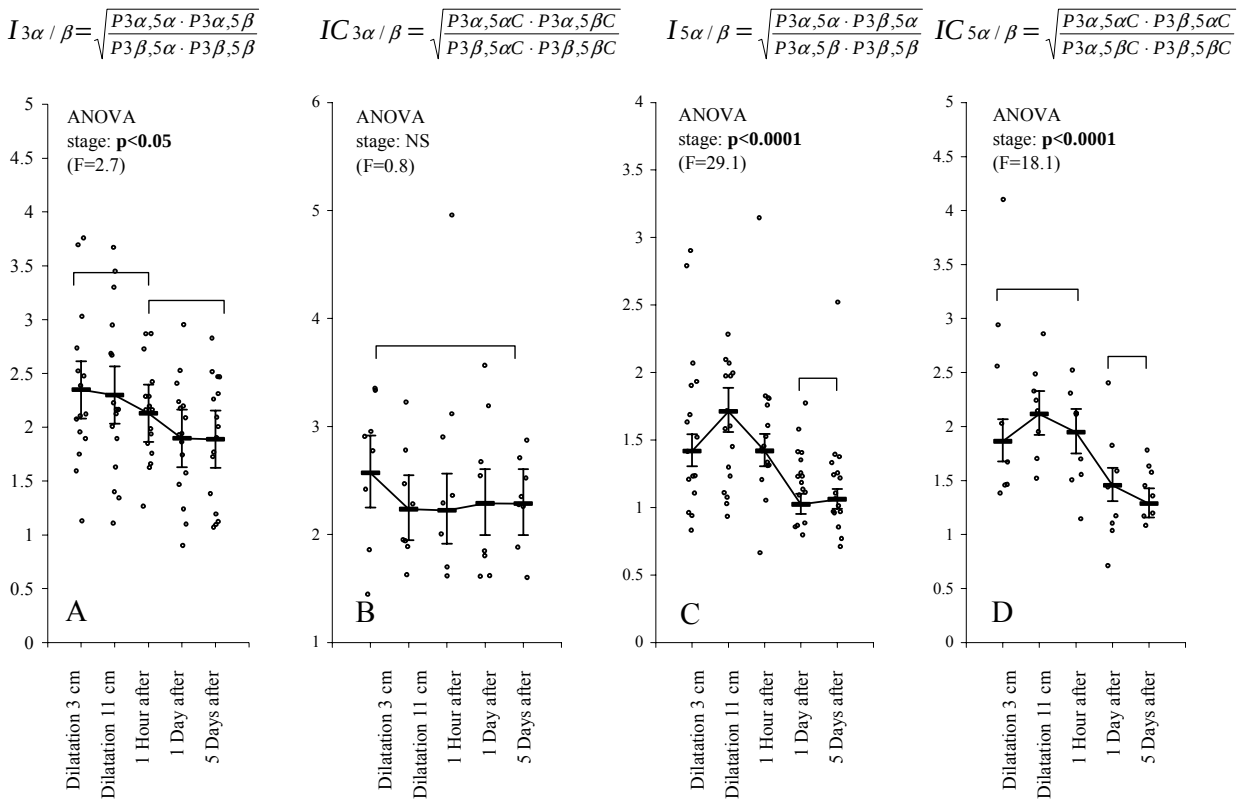

Fig. 5. *Time profiles of the index $I_{3\alpha/\beta}$ characterizing the overall 3α-/3β- isomer ratio of P3α,5β (free steroids – panel A, conjugates – panel B) in maternal serum during parturition and the profiles of the index $I_{3\alpha/\beta}$ characterizing the overall 5α-/5β- isomer ratio. The drawings and symbols are the same as in Fig. 3.*

Changes in the 5α/5β- isomer ratio were evaluated using the index $I_{5\alpha/\beta}$ defined as the square root of the ratio of the product of 5α-isomers to the product of 5β-isomers (Fig. 5C). A significant decrease was found in the free 5α/5β- isomer ratio in the first hour and on the first day after delivery (Fig. 5C); a similar situation was found in the conjugates (Fig. 5D).

Discussion

The discovery of down-regulation of oxytocin production by allopregnanolone during gravidity (Leng *et al.* 1985, 1987, 1988, Leng and Russell 1999), and the identification of changes in GABA receptor affinity to P3α,5α during pregnancy and the lactation period (Brussaard *et al.* 1997, 1999), highlighted the role of neuroactive steroids in human reproduction. The further finding that sulfatation of steroid GABA-receptor activators reversed the original effect of unconjugated steroids (Park-Chung *et al.* 1999) pointed to the importance of such steroid sulfates.

In this connection, the investigation of changes in the levels of both unconjugated and conjugated neuroactive steroids around parturition seems to be reasonable. The current lack of available information is particularly obvious with respect to pregnanolone isomers and their conjugates. The author's aim was to describe the differences in the levels of pregnanolone isomers in maternal and fetal plasma at delivery as the changes of the steroids around parturition, and to suggest the possible physiological consequences of these findings. Although the serum levels of P3α,5α during pregnancy and at delivery were already measured by Luisi *et al* (2000), here we focused on the time changes in the levels of all pregnanolone isomers and their polar conjugates. We tried to elucidate the cooperation between fetus and mother as reflected by the steroid transport between both compartments.

The levels of P3β,5β found in both umbilical and maternal plasma were lower than those for other pregnanolone isomers. However, the physiological importance of P3β,5β and in particular of its sulfate,

another allosteric inhibitor of both GABA (Park-Chung *et al.* 1999) and NMDA (Park-Chung *et al.* 1997) receptors, in human beings needs further investigation.

The levels of both unconjugated 3β-hydroxy-pregnanolone isomers were more than two times higher in umbilical than in maternal serum and the differences were highly significant as documented by paired tests (Fig 1). On the other hand, the differences in 3α-hydroxy-isomers were not significant. The striking difference in unconjugated pregnenolone connected with the fetal adrenals, was in agreement with the previous reports (Buster *et al.* 1979, Mathur *et al.* 1980).

The 3α-/3β-isomer ratios decreased continually, particularly near the delivery (Figs. 5 A). Given the GABA-activating effect of both of the 3α-isomers, the decreasing 3α-/3β-isomer ratios accompanying the decrease in levels of all pregnanolone isomers may be connected to the low physiological requirement for GABA activators after the onset of parturition.

The decrease in the levels of pregnanolone isomers, known to activate GABA receptors during parturition, precedes the decline of their conjugates with an opposite modulating effect (Figs 1 and 2). The question is whether the fall of the levels of steroids attenuating the activity of oxytocin producing cells and, at the same time, maintaining the levels of their sulfated antagonist could influence the initiation and/or the course of parturition *via* hypothalamic oxytocin release or on the peripheral level. The level of P3α,5αC was significantly higher in maternal than in umbilical plasma, while the differences in the other conjugated pregnanolone isomers were not significant, although the tendency to higher levels of the steroids in maternal serum was evident. Moreover, in all of the pregnanolone isomers, significantly higher conjugated/unconjugated steroid ratio was found in maternal than in umbilical plasma in contrast to pregnenolone sulfate in which the ratio did not differ in both sera. This finding could be explained by different biosynthesis of the conjugates. Pregnenolone sulfate originates predominantly from fetal adrenals by the action of sulfotransferase, which is stereospecific to 3β-steroids (Forbes *et al.* 1995, Korte *et al.* 1982, Simonian and Capp 1984). On the other hand, the sulfatation of pregnanolone isomers proceeds by the action of different enzymes selecting between 3α- and 3β-isomers (Driscoll *et al.* 1993).

Besides adrenals, a high sulfotransferase activity is present in the liver (Singer and Sylvester 1976). Dombroski *et al.* (1997) conducted a study addressing the possibility that 5α-dihydro-progesterone (5α-DHP) is synthesized in the placenta from 5α-pregnan-3α/β-ol-20-ones delivered to the trophoblast *via* the fetal umbilical blood. Using the incubation of placental homogenates with radiolabeled 5α-pregnan-3α/β-ol-20-ones, they have found an extensive epimerization and the intermediate, 5α-DHP, was the major product. In other incubations, 5α-pregnan-3β-ol-20-one-sulfate was hydrolyzed and the unconjugated product was further converted to 5α-dihydroprogesterone by homogenates of placental tissue. The oxidation of 5α-pregnan-3α/β-ol-20-ones occurred in microsome-enriched preparations of placental tissue. The authors suggested that progesterone, which enters the umbilical circulation from its site of synthesis in the syncytiotrophoblast, is metabolized in the fetus to 5α-pregnan- 3α/β-ol-ones and to 5α-pregnan-3α/β-yl-20-one sulfates. These metabolites of progesterone, 5α-pregnan-3α/β-ol-20 and 5α-pregnan-3β-yl-20-one sulfate, formed in the fetus, serve as plasma-borne substrates for trophoblast formation of 5α-DHP. Because of the hemochorioendothelial nature of human placentation, 5α-DHP secreted from the trophoblast will preferentially enter the maternal compartment, thus serving as a maternal plasma progesterone-independent source of 5α-DHP. As concerns the correlations between 3α- and 3β-isomers, they were more pronounced in maternal than in the fetal compartment (see Results). The tight correlation between Preg and P3α,5α ($r=0.700$, $p<0.004$, $n=15$), which was only found in umbilical serum, shows that the source of unconjugated pregnanolone isomers is the placenta, from which 5α-DHP penetrates into the maternal compartment. In the mother, it is converted to a 5α, 3α/β isomers and it is further sulfated just before parturition. Though the aforementioned mechanism could explain the deficiency of P3β,5β in both compartments, it is still unable to explain the relative abundance of P3α,5β. In addition, besides the P3α,5α, the correlation between maternal and umbilical serum was found only in P3α,5β and not in P3β,5α and further, the levels P3α,5β are comparable with each other more than the latter metabolite, while those in P3β,5α are about two times lower.

It is known that the sulfatation of pregnanolone isomers reverses the positive modulating effect on GABA receptor activity (Park-Chung *et al.* 1999). The role of GABA receptors in the timing of parturition has been reported in rats (Brussaard *et al.* 1997, 1999, 2000, Leng and Russell 1999). In the view of these findings, the discovery of higher levels of conjugated pregnanolone epimers in maternal serum could be of importance in connection with the timing of parturition. Furthermore,

the ratio conjugates/free steroids exhibited a maximum just one hour after parturition and profiles of conjugated isomers were more prominent than those in the free steroids (Fig. 4).

The decrease in the 5α-/5β- ratio (Figs 5C,D) may be of interest in the context of the different influences of conjugated 5α- and 5β-pregnanolone isomers on NMDA receptor function (Weaver *et al.* 2000). While conjugated 5α-isomers potentiate NMDA receptors, which are present both in the CNS and the periphery (Coggeshall and Carlton 1998, Lin *et al.* 1996), 5β-isomers exert an inhibitory effect (Weaver *et al.* 2000). 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 the peripheral level. A parallel effect might be expected from pregnenolone sulfate, the levels of which are also substantially elevated in the maternal serum near delivery (Fig. 1). The discovery of a marked decrease in the 5α/5β– steroid ratio (Figs 5C,D) probably reflects the absence of placenta after delivery, but the significant decrease in 3α/5β isomers (Figs 5C,D) awaits an unequivocal explanation.

Taking the new results of this study together with the previous findings, it may be concluded that pregnanolone isomers and their conjugates do operate in the onset of labor in humans.

Acknowledgements

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