



Year: 2009

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DOI: <https://doi.org/10.1159/000235879>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-29014>

Journal Article

Published Version

Originally published at:

Wächter, R; Masarik, L; Bürzle, M; Mallik, A; von Mandach, U (2009). Differential expression and activity of 11beta-hydroxysteroid dehydrogenase in human placenta and fetal membranes from pregnancies with intrauterine growth restriction. *Fetal Diagnosis and Therapy*, 25(3):328-335.

DOI: <https://doi.org/10.1159/000235879>

Differential Expression and Activity of 11 β -Hydroxysteroid Dehydrogenase in Human Placenta and Fetal Membranes from Pregnancies with Intrauterine Growth Restriction

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Key Words

11 β -Hydroxysteroid dehydrogenase · Placenta · Fetal membranes · Intrauterine growth restriction

Abstract

Objectives: To study the expression and the function of the 11 β -hydroxysteroid dehydrogenase enzyme 1 (11 β -HSD1) and 2 (11 β -HSD2) in placenta and the fetal membranes from pregnancies with intrauterine growth restriction (IUGR) and from controls. **Methods:** Amnion, chorion, decidua and cotyledon were separated from placenta; mRNA was analyzed by TaqMan real-time technology and proteins by Western blot; enzyme activities were measured by the conversion of ³H-cortisol to ³H-cortisone and vice versa. **Results:** Predominant mRNA expression ($p < 0.001$) was found for 11 β -HSD1 in chorion and for 11 β -HSD2 in decidua and cotyledon. In pregnancies with IUGR, 11 β -HSD1 was upregulated in chorion (mean Δ Ct 11 β -HSD:18S mRNA 193.5 vs. 103.0 in controls respectively, $p < 0.05$) and 11 β -HSD2 was downregulated in decidua (mean Δ Ct 11 β -HSD2:18S mRNA 0.18 vs. 15.88 in controls respectively, $p < 0.05$). 11 β -HSD1 protein levels were reduced in amnion and 11 β -HSD1 and 11 β -HSD2 oxidase activity in decidua and cotyledon were reduced from pregnancies with IUGR. **Conclusion:** Reduced synthesis or activity of 11 β -HSD1 or 2 in cases of IUGR is shown in some but not in all tissues. The local mRNA expression of 11 β -HSD1 in cho-

riion may reflect a mechanism on the post-transcriptional gene regulation to stimulate the formation of cortisone in IUGR. To provoke increasing activity with oxidase stimulators could be a future therapy in cases of IUGR.

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Introduction

Intrauterine growth restriction (IUGR) is a major cause of fetal morbidity and mortality. It has already been shown that fetal exposure to high glucocorticoid levels during pregnancy results in a low birth weight [1, 2], associated with higher risk for cardiovascular and metabolic disorders such as hypertension and diabetes type 2 in adult life (fetal programming) [3, 4]. Moreover, newborns after IUGR show a higher blood glucocorticoid level than normally developed infants with corresponding gestational age [5].

In human pregnancy, maternal circulating cortisol concentrations are 5–10 times higher than fetal concentrations [6, 7]. This difference is maintained by the 11 β -hydroxysteroid dehydrogenase enzyme (11 β -HSD). Two isoforms of 11 β -HSD exist: the 11 β -HSD1 is NADP(H)-dependent and catalyzes the conversion of cortisol to cortisone and vice versa, whereas the 11 β -HSD2 requires NAD and has (in vivo) only oxidase (dehydrogenase) ac-

tivity (conversion of the hormonally active cortisol to the inactive form cortisone) [8, 9]. Both isoenzymes have been identified in human placenta. However, conflicting results exist on its expression (mRNA, protein) and the activity in human placental tissue depending on gestational age, delivery mode, cellular localization of the sample and preparation of the tissue: it seems that the 11 β -HSD1 is more abundant in chorion and amnion [10, 11]. Its reductase activity is stimulated by prostaglandins and may be therefore an additional regulator for the onset of labor and birth [12]. Its role in fetal growth is still unclear, although for pregnancies with preeclampsia a reduced activity for 11 β -HSD1 oxidase (but not for the reductase form) has been found [13]. A recent study about the gene expression found reduced mRNA levels for both the 11 β -HSD1 and the 11 β -HSD2 in placentae of small-for-gestational-age neonates [14]. The 11 β -HSD2 is abundant in the syncytiotrophoblast [15] or in placental tissue without fetal membranes [10]. It protects the fetus from high maternal cortisol level and its primary role is therefore that of a barrier [16]. Additionally it protects the placental mineralocorticoid receptor in the placenta itself and regulates the fetal-maternal electrolyte and water transport [17]. Both functions may be associated with the regulation of fetal growth. Indeed, a reduced expression as well as a reduced activity for the 11 β -HSD2 has been found in pregnancies with IUGR or preeclampsia [13, 14, 18–22]. Conflicting findings about the expression and activity of both 11 β -HSD isoenzymes in pregnancies with IUGR led us to initiate the present study. The objective was therefore to provide detailed information on the expression as well as on the function (activity) of the 11 β -HSD1 and 11 β -HSD2 in placenta (decidua and cotyledon) and in the fetal membranes (amnion, chorion) from pregnancies with IUGR and from gestational age-matched normal pregnancies (controls).

Material and Methods

Selection of the Patients

Tissue samples were taken from women with singleton pregnancies delivered by elective caesarean section at our department. All women provided written informed consent for the study which was approved by the local institutional review board. The group with IUGR consisted of 10 placentae from pregnancies where growth restriction was defined as gestational-related birth weight below the 10th centile (SGA) [23] and/or where ultrasound growth rates fall without maternal pathology such as preeclampsia or abnormal Doppler in umbilical artery. The control group (n = 12) consisted of placentae from pregnancies without growth restriction but with matched gestational age. Controls parallel to

cases were recruited consecutively during 10 months. Exclusion criteria were: multiple pregnancy, neonatal infection, amnion infection syndrome, CRP ≥ 5 mg/l, BMI ≥ 30 kg/m², gestational diabetes, diabetes mellitus, cardiovascular diseases, neoplasia, hepatitis, HIV infection, nicotine, alcohol and drug abuse, and treatment with corticosteroids for fetal lung maturation.

Tissue Preparation

Immediately after delivery a representative piece of placenta (20–50 g in ≥ 2 cm distance from the cord and the margin) was taken. From the fetal side, fetal membranes, i.e. amnion and chorion, were separated from each other. From the maternal side the first grayish layer consisted of decidua tissue and the cotyledon consisted of villous tissue without decidua and without fetal membranes. Contamination with maternal blood was avoided in all samples. Histological and immunohistochemical characterizations were performed to assess the purity of the isolated amnion, chorion and decidua. The samples of each layer (2 g) were snap-frozen in liquid nitrogen and ground in a metal mortar stored before at -80°C and filled up with liquid nitrogen. The powdered sample was stored at -80°C until RNA extraction and microsomal preparation for Western analysis of proteins and for activity assay.

mRNA Expression

RNA Isolation

350 mg of the frozen powdered tissue was lysed in 350 μl lysis buffer (guanidine salt) and total RNA extraction was performed with the RNeasy Mini Kit from Qiagen AG (Hombrechtikon, Switzerland) according to the manufacturer's protocol. Potential DNA was digested with DNase in the same kit. RNA concentration was determined with a spectrophotometer (Eppendorf Bio-Photometer).

Reverse Transcription to cDNA

Reverse Transcription System ImProm-IITM and the PCR kit were from Promega AG (Wallisellen, Switzerland). Agarose was from AppliChem GmbH (Darmstadt, Germany) and all other chemicals were from Sigma-Aldrich GmbH (Buchs, Switzerland). 600 ng RNA and 1 μl of primer were heated at 70°C for 15 min and cooled at 4°C for 5 min. 14 μl of the RT master mix were added to the primer/RNA mix and cycled according to the manufacturer's protocol. To test if RNA extraction and RT were successful, a PCR with β -actin was performed according to the standard manufacturer's protocol.

Quantitative (Real-Time) PCR

We used the TaqMan method [24] already described for 11 β -HSD mRNA in placental tissue [19, 20, 25]. The PCR reaction was performed with the TaqMan Universal PCR Master Mix and the TaqMan Gene Expression Assay Mix designed by Applied Biosystems, Inc. (Foster City, Calif., USA) containing the following primers: *11 β -HSD1*: Gene ID 3290, 77 bp, assay location 626; Hs00194153_m1 (NM 181755.1); *11 β -HSD2*: Gene ID 3291, 50 bp, assay location 387; Hs00388669_m1 (NM 000196.2); *18S rRNA*: Gene ID 36162, 187 bp, assay location 60; Hs99999901_s1 (X 03205.1). According to the manufacturer's protocol the reaction was performed on a reaction plate in 20 μl volume/well containing 40 ng cDNA. Measurements were done with ABI Prism 7000 Sequence Detection System (Applied Biosystems, Inc.). All reac-

tions were duplicated with the housekeeping gene 18S. Data were obtained as Ct values (the cycle number at which logarithmic PCR plots cross a calculated threshold line) to determine Δ Ct (Ct of the target gene - Ct of the housekeeping gene = 11β -HSD:18S). Measurements were carried out in triplicates. On each plate a triplicate of a control cotyledon sample from 1 volunteer (always the same) was measured as internal standard. Final results of 11β -HSD mRNA were expressed as relative fold increase in Δ Ct mRNA of the target related to the Δ Ct of the internal standard (arbitrary value of 1).

Protein

Microsomal Preparation

100 mg of the frozen powdered tissue from the four different sources were homogenized in four independent charges each in 1 ml cooled TS2 buffer (NaCl 100 mM, EDTA 1 mM, EGTA 1 mM, MgCl₂ 1 mM, sucrose 250 mM, Tris-HCl pH 7.4 20 mM) using the Fast Prep technique (Bio 101 Savant Fast Prep FP120; twice 20 s, speed 4.0, 4°C). Tissue homogenates were centrifuged at 4°C at 805 g for 12 min and the supernatants were used for Western blot analysis (and the assay of activity, see below). The protein content was measured by the Bradford method using a protein assay kit (Bio-Rad) with BSA as standard [26].

Western Blot Analysis

We followed the principle already described by Alfaidy et al. [21] with some modifications. Thawed samples from microsomal preparation were diluted 1:5 (final concentration of protein 30 µg) with Laemmli sample buffer (62.5 mM Tris-HCl pH 6.8, 25% glycerol, 2% sodium dodecyl sulfate and 0.01% bromophenol blue (Bio-Rad Laboratories, Inc., Hercules, Calif., USA, ordered by Bio-Rad Laboratories, Reinach, Switzerland) and heated at 100°C for 5 min. Polyacrylamide gels were prepared (stacking gel 15%, separating gel 15%). Proteins (7.5 µg per lane) were separated by SDS-PAGE and then transferred electrophoretically onto a nitrocellulose membrane (Bio-Rad, Inc., USA). The Western blot was run at 400 V for 80–90 min. Transfer was confirmed by protein visualization with Ponceau S (Sigma-Aldrich). Membranes were carefully rinsed with distilled water. Blocking was made in 4% BSA (4 g bovine serum albumin in 100 ml Tris-buffered saline, pH 7.5). Subsequently, blots on membranes were cut into halves and one-half was incubated with primary antibodies for rabbit anti-mouse 11β -HSD1 and rabbit anti-human 11β -HSD2 respectively (all from Alpha Diagnostic International, ordered by VWR Int., Dietikon, Switzerland) for 1 h at room temperature. Membranes with the same samples but without primary antibody were used for negative controls. Membranes were then rinsed 4 times for 5 min in Tris-buffered saline and incubated with secondary polyclonal rabbit antiserum conjugated with horseradish peroxidase (DakoCytomation, Zug, Switzerland; Ref. P 0448) for 1 h. Membranes were washed 4 times, 5 min each and the antibody-antigen complex was detected using the Hypercassette from Amersham Pharmacia Biotech containing an X-ray film. A major band of 34 kDa corresponding to the known molecular mass of 11β -HSD1 and a second major band of 41 kDa corresponding to the molecular mass of 11β -HSD2 were clearly visible. All Blue Precision Plus Protein Standard (Bio-Rad, Inc., USA) was thereby used as a marker and β -actin was used to control loading errors. Protein bands were quantified by Image J Software. Relative optical density (OD) units were obtained from each band by analyzing mean OD.

Activity

Reagents and Supplies

[1,2,6,7-³H]cortisol (69 Ci/mmol) and [1,2,6,7-³H]cortisone (42 Ci/mmol) were purchased from Amersham Biosciences (High Wycombe, Bucks., UK). Nonradioactive steroids, coenzymes and other chemicals were obtained from Sigma-Aldrich. Thin-layer chromatography plates (SIL G-25 UV254) were obtained from Machery-Nagel GmbH (Oensingen, Switzerland).

Frozen aliquots of the microsomal preparations were used. No significant change in 11β -HSD activity was detected after storage of frozen aliquots at -80°C for 4 months. Oxidase and reductase activities were assessed by measuring the conversion rate of [³H]cortisol to [³H]cortisone and vice versa. The method was modified from assays already published [27, 28]. Reactions were carried out in 96-well optical PCR reaction plates (Nunc-Immuno™ 96 MicroWell™ Plates, Nunc, Wiesbaden, Germany). Parallel assays in the same wells containing no placental homogenate were performed to control the spontaneous interconversion between 11-dehydro and 11-hydroxy forms of the steroids. For the oxidase activity (11β -HSD1 and 11β -HSD2) 10 µl of the homogenate corresponding to 20 µg of protein were incubated with 10 µl TS2 buffer in the presence of saturating substrate ([³H]cortisol and 5 nM cortisol) and coenzyme (400 µM NADP for 11β -HSD1 or 400 µM NAD for 11β -HSD2 respectively) for 15 min at 37°C on a ThermoShaker. For the reductase activity (11β -HSD1), 10 µl of the homogenate corresponding to 20 µg of protein were incubated with 10 µl TS2 buffer in the presence of substrate ([³H]cortisone and 5 nM cortisone) and coenzyme (400 µM NADPH) for 15 min at 37°C on a ThermoShaker. The reactions were stopped by immediate transfer of the tubes to ice, addition of methanol and an excess of unlabeled steroids. [³H]cortisol and [³H]cortisone were separated by thin-layer chromatography and the radioactivity was counted using a beta counter. Activities were expressed as nanomoles of cortisone or cortisol respectively formed per milligram of microsomal protein per minute. The percentage conversion of cortisol to cortisone and vice versa should be >2%. Data of each layer were obtained from two independent charges of homogenates.

Statistics

For statistical analysis the program StatView 5.0.1 for Windows XP was used. From data mean and standard deviation (SD), standard error of the mean (SE) and the median were calculated. Data in graphs are given mean \pm SE. Differences between the two groups (IUGR vs. control) were tested by the Mann-Whitney test (for nonparametric data) and the unpaired t test respectively (for log-normal distributed data). Differences between placental layers were tested by the Wilcoxon signed test and the Kruskal-Wallis test (all four placental layers in one comparison). $p < 0.05$ was defined as significant in all tests.

Results

Demography of the Volunteers

Most of the women with IUGR were primiparae reflecting the higher incidence of IUGR in primi- than in multiparae (table 1). There were 3 pregnancies in the con-

Table 1. Demographic data of the volunteers

	Control (n = 12)	IUGR (n = 10)	p
Age, years	31.4 ± 4.4 (30.0)	34.0 ± 6.7 (33.0)	0.3
Caucasian, n	10	10	0.48
Parity >1, n	9	2	0.01
GA at delivery, weeks	36.9 ± 1.6 (37.4)	35.1 ± 2.7 (35.3)	0.07
Elective caesarean section, n	12	10	1.0
Placental weight wet, g	566.7 ± 91.6 (560.0)	419.2 ± 124.2 (387.5)	0.01
Fetal weight, g	3,002.5 ± 540.9 (3,225.0)	2,005.0 ± 688.7 (2,240.0)	0.001
Fetal weight, centile	52.3 ± 27.2 (55.4)	3.6 ± 3.8 (2.6)	<0.0001

GA = Gestational age. Continuous values = mean ± SD (median); numeric values = n.

Differences between the groups were tested by unpaired two-tailed t test or Fisher's two-tailed exact test as indicated.

Table 2. 11β-HSD1 and 11β-HSD2/18S mRNA (relative units) in placental tissues from all subjects (n = 22)

	Amnion	Chorion	Decidua	Cotyledon
11β-HSD1	19.51 ± 10.10 (0.11; 161.15)	144.14 ± 28.40 ^a (110.84; 527.45)	7.62 ± 2.21 (3.81; 35.68)	5.34 ± 2.17 (1.38; 41.54)
11β-HSD2	0.03 ± 0.01 (0.005; 0.224)	0.01 ± 0.003 (0.004; 0.04)	8.75 ± 5.86 ^b (0.03; 106.02)	4.16 ± 3.30 ^b (0.39; 72.79)
p ^c	<0.0001	<0.0001	<0.0001	<0.005

Data = mean ± SE (median; range).

^a p < 0.001 compared with amnion, decidua or cotyledon respectively tested by Wilcoxon's signed rank test.

^b p < 0.001 compared with amnion or chorion tested by Wilcoxon's signed rank test.

^c Differences between log values of 11β-HSD1 and 11β-HSD2 were tested by unpaired two-tailed t test.

control group with delivery at <37.0 weeks who had idiopathic preterm labor without tocolysis (gestational age 34 weeks).

mRNA Expression

Both isoenzyme mRNA and 18S amplified with the same efficiency. No significant differences existed in the rRNA of the 18S housekeeping gene between the IUGR (Ct 16.56 ± 0.15) and the control group (Ct 16.84 ± 0.15; mean ± SE), p = 0.19, nor within the groups between the layers rendering the 18S as a valid housekeeping gene for fetal membranes and for placental tissue. The highest expression of the 11β-HSD1 mRNA was measured in chorion (p < 0.001 vs. amnion, decidua or cotyledon respectively). The 11β-HSD2 mRNA was highly expressed in the decidua and in the cotyledon (p < 0.001 vs. amnion

or chorion respectively); only small amounts were found in the fetal membranes (table 2). After log transformation the values were log normally distributed and the 11β-HSD1 mRNA was significantly higher than that of 11β-HSD2 in all layers (p < 0.0001 and p < 0.005, unpaired t test).

In chorion the 11β-HSD1 mRNA expression was approximately 2 times higher in IUGR (193.52 ± 50.17) than in the control group (103.0 ± 27.71 relative fold increase; p < 0.05; Mann-Whitney test) (fig. 1a). No differences between pathological and normal pregnancies existed in the other layers. The 11β-HSD2 mRNA expression in decidua was significantly lower in pregnancies with IUGR than in normal pregnancies (0.18 ± 0.10 vs. 15.88 ± 10.49 relative fold increase; p < 0.05) (fig. 1b).

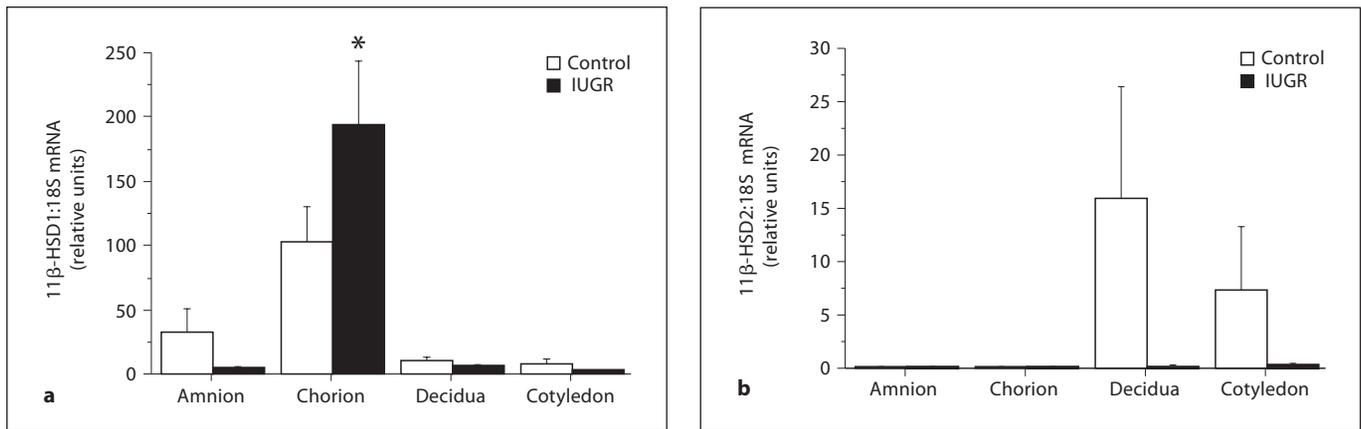


Fig. 1. Mean \pm SE mRNA expression (relative fold increase in Δ Ct related to the Δ Ct of the internal standard 18S) in fetal membranes and placenta from pregnancies with IUGR (n = 10) vs. normal pregnancies (controls, n = 12). **a** 11 β -HSD1, **b** 11 β -HSD2. * p < 0.05 IUGR vs. controls in chorion.

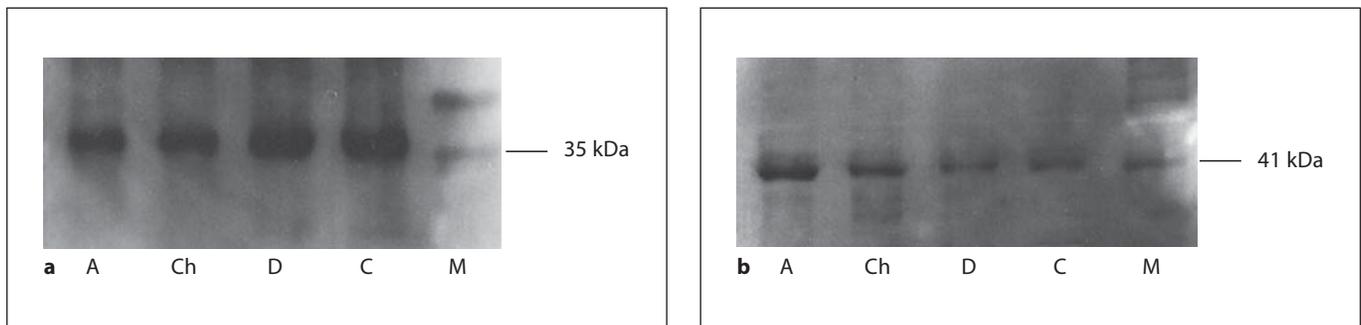


Fig. 2. Protein expression of **a** 11 β -HSD1 (35 kDa) and of **b** 11 β -HSD2 (41 kDa) (Western blot) in amnion (A), chorion (Ch), decidua (D) and cotyledon (C). M was the used marker (Precision Plus Protein Standard from Bio-Rad). β -Actin was used to control loading errors and did not show any expression.

Protein Expression

The 11 β -HSD1 and 11 β -HSD2 proteins were both present in all tissues (fig. 2, 3). 11 β -HSD1 levels (relative ODs) were reduced in pregnancies with IUGR in amnion, chorion and decidua but not in cotyledon; however, only in amnion the difference was significant (4.42 ± 1.46 vs. 8.24 ± 1.16 , mean \pm SE, p < 0.05). Levels were higher in chorion, decidua and cotyledon than in amnion [p < 0.05 chorion vs. amnion (IUGR) and decidua vs. amnion (IUGR and controls); p < 0.001 cotyledon vs. amnion (IUGR and controls)]. 11 β -HSD2 protein levels in IUGR and controls were similar. Differences in levels between the tissue were only found in normal pregnancies (14.56 ± 1.75 in decidua vs. 9.54 ± 1.63 in amnion, mean \pm SE, p < 0.05).

Activity

Oxidase Activity

No difference between 11 β -HSD1 (NADP-dependent) and 11 β -HSD2 (NAD-dependent) activities (nmol/mg protein/h) was found; however, activities were significantly higher in decidua (2.53 ± 0.83) and in cotyledon (2.05 ± 0.57) than in amnion (0.18 ± 0.12 , p < 0.0001) or in chorion (0.81 ± 0.29 , mean \pm SE, p < 0.001) (fig. 4a). Oxidase activities were decreased in tissues from pregnancies with IUGR; it was not measurable in IUGR amnion samples. The difference in 11 β -HSD2 (or 11 β -HSD1) between IUGR and controls was significant in cotyledon (1.23 ± 0.75 vs. 2.72 ± 0.82 nmol/mg protein/h in controls; p < 0.05) (fig. 4b).

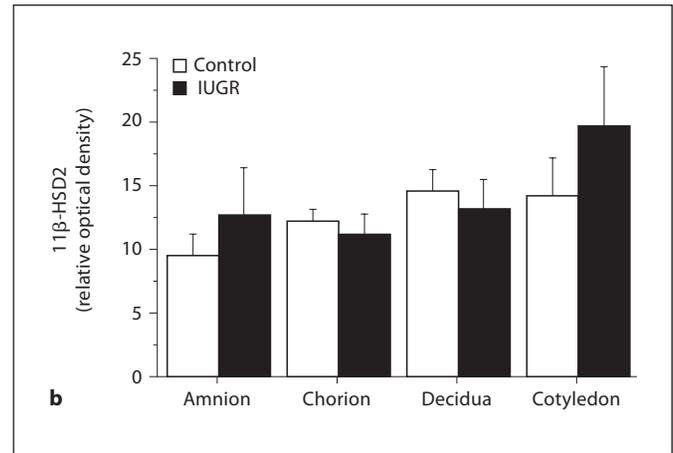
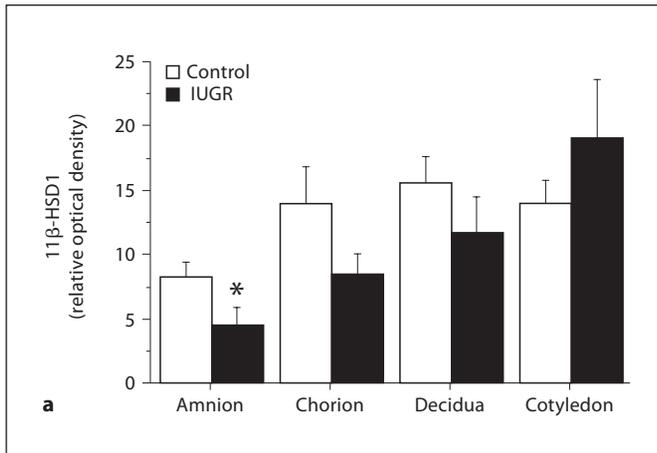


Fig. 3. Mean \pm SE protein expression from Western blot (relative OD) in fetal membranes and placenta from pregnancies with IUGR (n = 10) vs. normal pregnancies (controls, n = 12). **a** 11 β -HSD1, **b** 11 β -HSD2. * p < 0.05 IUGR vs. controls in amnion.

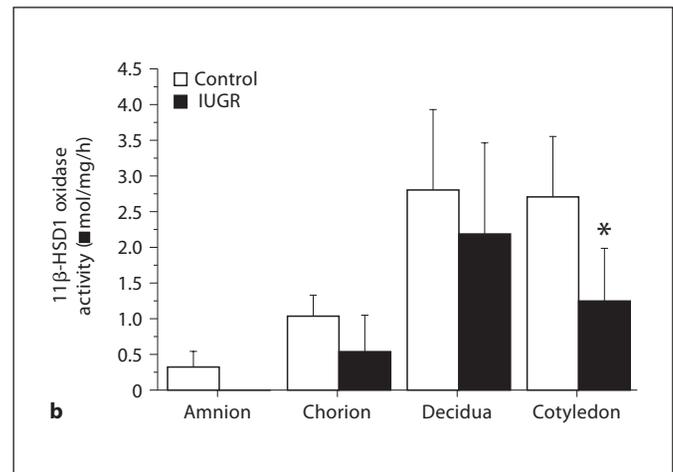
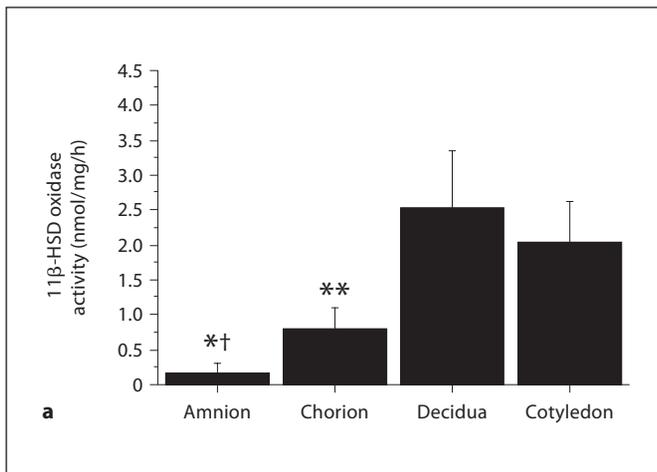
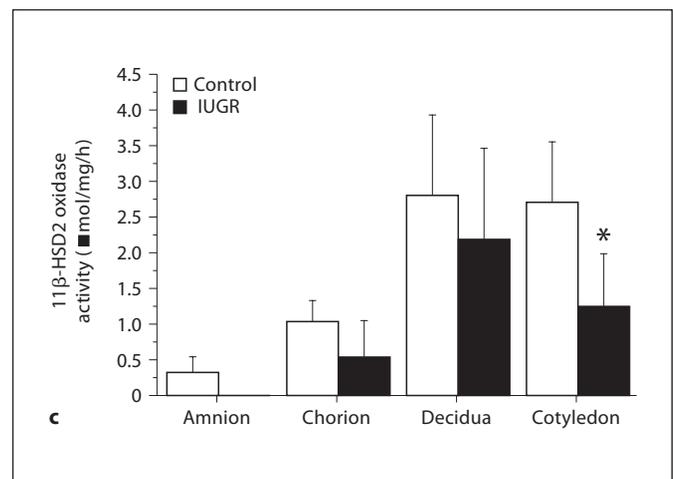


Fig. 4. a Mean \pm SE. 11 β -HSD oxidase activity (nmol/mg protein/h) in microsomal preparation of fetal membranes and placenta from all pregnancies (n = 22). * p < 0.01 amnion vs. chorion, † p < 0.0001 amnion vs. decidua or cotyledon, and ** p < 0.001 chorion vs. decidua or cotyledon, respectively. There was no difference between oxidase activity of 11 β -HSD1 and that of 11 β -HSD2. **b** Mean \pm SE 11 β -HSD1 oxidase activity (nmol/mg protein/h) in microsomal preparation of fetal membranes and placenta from pregnancies with IUGR (n = 10) vs. normal pregnancies (controls, n = 12). **c** Mean \pm SE. 11 β -HSD2 oxidase activity (nmol/mg protein/h) in microsomal preparation of fetal membranes and placenta from pregnancies with IUGR (n = 10) vs. normal pregnancies (controls, n = 12). Oxidase activity was not measurable in IUGR amnion samples. * p < 0.05 IUGR vs. controls in cotyledon.



Reductase Activity

The 11 β -HSD1 (NADPH)-dependent reductase activity was not measurable in fetal membranes (amnion and chorion) or was too low (conversion of cortisone to cortisol <2% in decidua, cotyledon); no comparison between pregnancies with IUGR and controls was therefore possible (data not shown).

Comment

To our knowledge the present study may be the first focusing on all elements (mRNA, protein, activity) of both isoenzymes, the 11 β -HSD1 and the 11 β -HSD2 in placenta and in fetal membranes from normal and IUGR pregnancies. We demonstrated a significantly higher mRNA expression of 11 β -HSD1 than of 11 β -HSD2 in all four tissues. The 11 β -HSD1 mRNA was abundant in chorion whereas the protein levels as well as the oxidase activity were more abundant in placenta than in fetal membranes. Therefore, we found little concordance between mRNA and protein expression although all analyses were performed from the same samples and by the same analyzers. Our data confirm the apparent discrepancy of the results from previous studies: The first reports about 11 β -HSD1 have shown that mRNA (Northern blot) and reductase activity (from homogenates) are expressed in maternal derived decidua but not in fetal membranes [29]. Surprisingly, subsequent studies demonstrated abundant 11 β -HSD1 mRNA in chorion and small amounts in amnion as well as in placenta (no separation of decidua) [10]. It was also shown that 11 β -HSD1 protein is upregulated during pregnancy in the fetal membranes at term [11] and that local PGF 2α may contribute to the initiation of labor by increasing 11 β -HSD1 reductase activity to promote cortisol production [12].

For 11 β -HSD2 mRNA we found downregulated levels in fetal membranes and agree therefore with the results of Sun et al. [10] who also missed 11 β -HSD2 mRNA in fetal membranes. However, we found again little consistency between mRNA and protein: the 11 β -HSD2 protein was expressed in all layers. In our study, oxidase activity was significantly higher in decidua and cotyledon than in fetal membranes. We could not find any difference in the oxidase activity between the two isoenzymes. Neither were we able to detect relevant 11 β -HSD1 reductase activity in any studied tissue. Already McCalla et al. [13] have shown absent reductase activity in homogenates of the whole placenta. However, in subsequent studies, reductase activity could be demonstrated in homogenates

of separated placental tissue. It was therefore hypothesized that in placenta (syncytiotrophoblast) and in decidua both isoenzymes act as oxidase whereas in fetal membranes the 11 β -HSD1 acts only as reductase [11].

In cases of IUGR, we found upregulated 11 β -HSD1 mRNA in chorion but no superior protein level. Although an overall match in gestational age for the groups was carefully attained, we were unable to prevent that the IUGR group delivered up to 10 days earlier. It is unlikely that the higher level in chorion for the IUGR than for the control group is associated with the lower gestational age in that group. For 11 β -HSD2 mRNA, we found downregulated levels in pregnancies with IUGR only in decidua and in cotyledon and agree therefore with results from studies performed with placental tissue (without fetal membranes) of IUGR that showed significantly depressed mRNA [19, 20]. However, we could not find reduced 11 β -HSD2 protein levels whereas oxidase activity was also depressed in decidua and cotyledon. Other studies have shown a significantly lower 11 β -HSD2 activity in placental tissue from IUGR than from normal pregnancies [10, 13, 21, 30].

To conclude, our results demonstrate the different contribution of the two 11 β -HSD isoenzymes on the level of mRNA, protein and activity in fetal/placental metabolism. However, in cases of IUGR the oxidase activity of both isoenzymes is reduced in placenta. The underlying mechanism may be a post-transcriptional gene regulation relying on specific or different RNA-protein interactions in case of IUGR and in normal pregnancies respectively. The importance of these regulatory mechanisms is illustrated by the clinical relevance: epidemiological studies have linked low birth weight with the later occurrence of cardiovascular and metabolic disorders, particularly hypertension [1–4]. To provoke increasing activity with oxidase stimulators could therefore be a future therapy in cases of IUGR preventing also complications such as cardiovascular disorders in adult life. Otherwise, attention should be given in pregnancies with IUGR and the requirement of glucocorticosteroids which additionally decrease the activity of the 11 β -HSD. The most relevant therapy with glucocorticosteroids in obstetrics is that for fetal lung maturation in preterm labor, which could consequently be a problem in pregnancies with IUGR.

Acknowledgements

We thank Brigitte Frey from the Department of Clinical Research, University of Berne, and Alex Odermatt from the Department of Pharmaceutical Sciences, University of Basel, for help and support with the analysis of 11 β -HSD activity.

References

- Reinisch JM, Simon NG, Karow WG, Gandelman R: Prenatal exposure to prednisone in humans and animals retards intrauterine growth. *Science* 1978;202:436–438.
- Benediktsson R, Lindsay RS, Noble J, Seckl JR, Edwards CR: Glucocorticoid exposure in utero: new model for adult hypertension. *Lancet* 1993;341:339–341.
- Rich-Edwards JW, Stampfer MJ, Manson JE, Rosner B, Hankinson SE, Colditz GA, Willett WC, Hennekens CH: Birth weight and risk of cardiovascular disease in a cohort of women follow-up since 1976. *BMJ* 1997;315:396–400.
- Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ: Birth weight and adult hypertension, diabetes mellitus and obesity in US men. *Circulation* 1996;94:3246–3250.
- Goland RS, Jozak S, Warren WB, Conwell IM, Stark RI, Tropper PJ: Elevated levels of umbilical cord plasma corticotropin-releasing hormone in growth-retarded fetuses. *J Clin Endocrinol Metab* 1993;77:1174–1179.
- Predine J, Merceron L, Barrier G, Sureau C, Milgrom E: Unbound cortisol in umbilical cord plasma and maternal plasma: a reinvestigation. *Am J Obstet Gynecol* 1979;135:1104–1108.
- Gitau R, Cameron A, Fisk NM, Glover V: Fetal exposure to maternal cortisol. *Lancet* 1998;352:707–708.
- Tannin GM, Agarwal AK, Monder C, New MI, White PC: The human gene for 11 β -hydroxysteroid dehydrogenase. Structure tissue distribution, and chromosomal localization. *J Biol Chem* 1991;266:16653–16658.
- Albiston AL, Obeyesekere VR, Smith RE, Krozowski ZS: Cloning and tissue distribution of the human 11 β -hydroxysteroid dehydrogenase type 2 enzyme. *Mol Cell Endocrinol* 1994;105:R11–R17.
- Sun K, Yang K, Challis JR: Differential expression of 11 β -hydroxysteroid dehydrogenase types 1 and 2 in human placenta and fetal membranes. *J Clin Endocrinol Metab* 1997;82:300–305.
- Alfaidy N, Li W, MacIntosh T, Yang K, Challis J: Late gestation increase in 11 β -hydroxysteroid dehydrogenase 1 expression in human fetal membranes: a novel intrauterine source of cortisol. *J Clin Endocrinol Metab* 2003;88:5033–5038.
- Alfaidy N, Xiong ZG, Myatt L, Lye SJ, MacDonald JF, Challis JR: Prostaglandin F_{2 α} potentiates cortisol production by stimulating 11 β -hydroxysteroid dehydrogenase 1: a novel feedback loop that may contribute to human labor. *J Clin Endocrinol Metab* 2001;86:5585–5592.
- McCalla CO, Nacharaju VL, Muneyyirci-Delale O, Glasgow S, Feldman JG: Placental 11 β -hydroxysteroid dehydrogenase activity in normotensive and pre-eclamptic pregnancies. *Steroids* 1998;63:511–515.
- Struwe E, Berzl GM, Schild RL, Beckmann MW, Dörr HG, Rascher W, Dötsch J: Simultaneously reduced gene expression of cortisol-inactivating enzymes in placentas of small-for-gestational-age neonates. *Am J Obstet Gynecol* 2007;197:43.e1–e6.
- Krozowski Z, Maguire JA, Stein-Oakley AN, Dowling J, Smith RE, Andrews RK: Immunohistochemical localization of the 11 β -hydroxysteroid dehydrogenase type II enzyme in human kidney and placenta. *J Clin Endocrinol Metab* 1995;80:2203–2209.
- Murphy VE, Smith R, Giles W, Clifton VL: Endocrine regulation of human fetal growth. The role of the mother, placenta and fetus. *Endocr Rev* 2006;27:141–169.
- Driver PM, Rauz S, Walker EA, Hwison M, Kilby MD, Stewart PM: Characterization of human trophoblast as a mineralocorticoid target tissue. *Mol Hum Reprod* 2003;9:793–798.
- Shams M, Kilby MD, Sommerset DA, Howie AJ, Gipta A, Wood PJ, Afnan M, Stewart PM: 11 β -Hydroxysteroid dehydrogenase type 2 in human pregnancy and reduced expression in intrauterine growth restriction. *Hum Reprod* 1998;13:799–804.
- McTernan CL, Draper N, Nicholson H, Chalder SM, Driver P, Hewison M, Kilby MD, Stewart PM: Reduced placental 11 β -hydroxysteroid dehydrogenase type 2 mRNA levels in human pregnancies complicated by intrauterine growth restriction: an analysis of possible mechanisms. *J Clin Endocrinol Metab* 2001;86:4979–4983.
- Schoof E, Girstl M, Frobenius W, Kirschbaum M, Dorr HG, Rascher W, Dötsch J: Decreased gene expression of 11 β -hydroxysteroid dehydrogenase type 2 and 15-hydroxyprostaglandin dehydrogenase in human placenta of patients with preeclampsia. *J Clin Endocrinol Metab* 2001;86:1313–1317.
- Alfaidy N, Gupta S, DeMarco C, Caniggia I, Challis JR: Oxygen regulation of placental 11 β -hydroxysteroid dehydrogenase 2: physiological and pathological implications. *J Clin Endocrinol Metab* 2002;7:4797–4805.
- Kajantie E, Dunkel L, Turpeinen U, Stenman UH, Wood PJ, Nuutila M, Andersson S: Placental 11 β -hydroxysteroid dehydrogenase-2 and fetal cortisol/cortisone shuttle in small preterm infants. *J Clin Endocrinol Metab* 2003;88:493–500.
- Voigt M, Schneider KT, Jahrig K: Analysis of a 1992 birth sample in Germany. 1. New percent values of the body weight of newborn infants. *Geburtshilfe Frauenheilkd* 1996;56:550–558.
- Ginzinger DG: Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. *Exp Hematol* 2002;30:503–512.
- Van Beek JP, Guan H, Julian L, Yang K: Glucocorticoids stimulate the expression of 11 β -hydroxysteroid dehydrogenase type 2 in cultured human placental trophoblast cells. *J Clin Endocrinol Metab* 2004;89:5614–5621.
- Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–254.
- Atanasov AG, Tam S, Rocken JM, Baker ME, Odermatt A: Inhibition of 11 β -hydroxysteroid dehydrogenase type 2 by dithiocarbamates. *Biochem Biophys Res Commun* 2003;308:257–262.
- Schweizer RAS, Atanasov AG, Frey BM, Odermatt A: A rapid screening assay for inhibitor of 11 β -hydroxysteroid dehydrogenases: flavanone selectively inhibits 11 β -HSD1 reductase activity. *Mol Cell Endocrinol* 2003;212:41–49.
- Stewart PM, Rogerson FM, Mason JI: Type 2 11 β -hydroxysteroid dehydrogenase messenger ribonucleic acid and activity in human placenta and fetal membranes: its relationship to birth weight and putative role in fetal adrenal steroidogenesis. *J Clin Endocrinol Metab* 1995;80:885–890.
- White PC, Mune T, Agarwal AK: 11 β -Hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr Rev* 1997;18:135–156.