



Dexamethasone and betamethasone administration during pregnancy affects expression and function of 11 β -hydroxysteroid dehydrogenase type 2 in the rat placenta

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ABSTRACT

Placental 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) is the key enzyme which protects the fetus from overexposure to glucocorticoids (GCs) by their oxidation into inactive derivatives. Several recent studies suggest that 11 β -HSD2 expression is subjected to regulation by antenatal steroid therapy. In our study we investigated the effect of two commonly used synthetic steroids, dexamethasone (DXM) and betamethasone (BTM), on the expression and function of 11 β -HSD2 in the rat placenta. Pregnant rats were pretreated with low (0.2 mg/kg) or high (5 mg/kg and 11.5 mg/kg for DXM and BTM, respectively) i.m. doses of GCs. 11 β -HSD2 expression was investigated using real-time RT-PCR and Western blotting; conversion capacity of 11 β -HSD2 was assessed by dual perfusion of the rat placenta. Significant increase in placental 11 β -HSD2 mRNA expression was found in rats treated with DXM, however, this alteration was not observed on protein level. BTM had no effect on either mRNA or protein levels of 11 β -HSD2. Functional studies revealed that both GCs significantly reduced the metabolism of corticosterone by the placenta. Our data indicate that placental barrier function mediated by 11 β -HSD2 might be considerably impaired by the antenatal therapy with DXM and BTM. In addition, the discrepancy between expressional and functional studies suggests that sole analysis of expressional changes of 11 β -HSD2 at mRNA and/or protein levels cannot convincingly predict the role of GC treatment on 11 β -HSD2 function in the placental barrier.

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1. Introduction

It is well documented that the intrauterine environment and specific factors acting during critical developing periods determine both the neonatal and adult well-being. In particular, prenatal exposure to endogenous glucocorticoids (GCs) is in the centre of interest since GCs are essential for the maturation of fetal organs, growth and preparing the fetus for birth [1–3]. Although GCs are highly lipophilic and can readily cross the placenta, during the whole course of pregnancy the levels of circulating corticosteroids in the fetus are considerably lower than those in the mother. This gradient is maintained by the activity of placental 11 β -hydroxysteroid dehydrogenase (11 β -HSD), the enzyme responsible for the inter-conversion of cortisol and corticosterone and their inactive 11-keto products cortisone and 11-dehydrocorticosterone, respectively [4].

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Two types of 11 β -HSD are now distinguished. Type 1 (11 β -HSD1) is a low affinity NAD(P)H preferring enzyme which appears to act predominantly as a reductase with high K_m in GC target tissues [5]. Conversely, type 2 (11 β -HSD2) is a NAD⁺-dependent oxidase with K_m in the nanomolar range for cortisol and corticosterone which catalyses their unidirectional conversion to inactive 11-oxo metabolites [6,7]. Although the presence of both types has been demonstrated in the placenta [8–10], it is generally accepted that the predominant form limiting the materno-fetal passage of GCs is 11 β -HSD2 with its oxidative activity. 11 β -HSD2 has been localized to the syncytiotrophoblast layer of the placenta, the main site of materno-fetal exchange [8,11]. It forms an active component of the placental barrier with the potency to protect the fetus against GC overexposure. Attenuated expression and activity of 11 β -HSD2 allowing enhanced transplacental passage of GCs has been linked to the intrauterine growth restriction and increased occurrence of cardiovascular and metabolic diseases in adult life (for detailed review see [12]).

The expression and activity of placental 11 β -HSD2 is not uniform across gestation. The variation most likely reflects tissue-specific regulation of 11 β -HSD2 by diverse endogenous agents

Table 1
Sequences of primers used in real-time RT-PCR.

Gene	NCBI sequence	Sequence 5' → 3'	Product length (bp)	Localization
<i>rβ-2-microglobulin</i>	NM.012512	TGC CAT TCA GAA AAC TCC CCA (f) TAC ATG TCT CGG TCC CAG GTG A (r)	303	64–366
<i>r11β-hsd1</i>	NM.01780	GGCTTCATAGACACAGAAACAG (f) TTCCCAAGCAGAAGTGGAG (r)	161	721–881
<i>r11β-hsd2</i>	NM.017081	CAG CAG GAG ACA TGC CAT AC (f) CAC ATT AGT CAC TGC CTC TGT C (r)	161	785–945

[13–15]. Moreover, the changes in 11β-HSD2 activity and expression throughout pregnancy express marked variability among species. Increasing activity of placental 11β-HSD2 with advancing pregnancy was demonstrated in the human, baboon and rat placenta [16–18]. However progressive increase in 11β-HSD2 expression with advanced pregnancy was demonstrated only in human [19,20] and baboon [17] placenta while an opposite trend was demonstrated in the rat [21,22]. Furthermore, it has been recently suggested that 11β-HSD2 is subjected to regulation by various exogenous agents; particularly, by synthetic GCs. However, the results of recent studies remain controversial. Clarke et al. found that administration of cortisol to pregnant ewes reduced placental 11β-HSD2 activity [23]. Likewise, prenatal exposure to dexamethasone (DXM) was followed by decreased 11β-HSD2 expression in the ovine placenta during mid-gestation [24]. Contrary to these observations, betamethasone (BTM) and DXM treatment was demonstrated to stimulate the expression of 11β-HSD2 in baboon placenta [25] and expression and activity of 11β-HSD2 in primary culture of human trophoblast [26], respectively.

In the present study we have investigated the effect of synthetic GCs on 11β-HSD2 expression and function in the rat placenta. In particular, we have focused on DXM and BTM since these steroids are commonly administered to pregnant women in managing premature delivery [27]. Changes in the expression of 11β-HSD2 on mRNA and protein levels were investigated using quantitative real-time RT-PCR and Western blotting techniques. In addition, we have recently validated the *in-situ* method of perfused rat placenta as a viable model to study placental steroid metabolism by 11β-HSD2 [21]. Using this method we were able to examine the effect of antenatal administration of GC on functional capacity of placental 11β-HSD2 to metabolize corticosterone at term.

2. Materials and methods

2.1. Animals

All experiments were approved by the Ethical Committee of the Faculty of Pharmacy (Hradec Kralove, Charles University in Prague) and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, 1996 and the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes, Strasbourg, 1986. Pregnant Wistar rats were purchased from Biotest Ltd. (Konarovice, Czech Republic) and were maintained in 12/12-h day/night standard conditions with water and pellets *ad libitum*. A group of 20 dams was pretreated with five doses of DXM ($n=9$) (Medochemie Ltd., Limassol, Cyprus) or BTM ($n=11$) (Schering-Plough, Herouville, France) administered intramuscularly into the hindlimb muscles in 24-h intervals (regularly at 9:00 a.m.) at 16–20 gestation days. This treatment regime was chosen to investigate the effect of GC treatment in the third term of pregnancy and with respect to previously published studies [28,29]. Two different doses of each GC were applied; low doses (0.2 mg/kg of DXM; $n=5$ and BTM; $n=6$) or high doses (5 mg/kg and 11.5 mg/kg of DXM; $n=4$ and BTM; $n=5$, respectively). The administered volume was 0.5 ml. A control group of five dams received vehicle treatment (0.9% NaCl). Experiments were performed on day 21 of gestation. Fasted rats were anesthetized with pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL) in a dose of 40 mg/kg administered into the tail vein and dual perfusion of the rat placenta was performed. At the end of the experiment the perfused placenta along with four randomly selected placentas, were dissected free of maternal tissues and fetal membranes and stored at -70°C until analysis.

2.2. Real-time RT-PCR

Total RNA from frozen tissue samples was isolated using TriReagent (Molecular research centre, Inc., Cincinnati, USA). The RNA concentrations were determined from the A_{260} measurement. Purity and integrity of RNA was confirmed by calculation of the UV absorbance ratio A_{260}/A_{280} and electrophoresis on 2.5% agarose gel.

Before the initiation of the real-time RT-PCR analysis, stability of the following genes was compared: *β-2-microglobulin*, *Surf1*, *Nup54* and *Polr2a*. For subsequent PCR analyses, *β-2-microglobulin* gene was chosen as the least regulated one. In addition to *β-2-microglobulin* gene normalization, we have related the expression of 11β-HSD to the total amount of RNA. The results did not change significantly and there was no influence of the denominator.

Single strand cDNA was prepared from 1 μg of total RNA by reverse transcription with M-MLV transcriptase (Finnzymes Oy, Espoo, Finland) using oligo(dT)₁₅ VN primer (Generi Biotech Ltd., Hradec Kralove, Czech Republic) and porcine RNase inhibitor (TaKaRa Bio Inc., Otsu, Shiga, Japan). Primers for amplification of segments of rat target (*11β-hsd1* and *11β-hsd2*) and housekeeping (*β-2-microglobulin*) genes were designed using the Vector NTI Suite software (Infomax, Bethesda, MD, USA). Specifications are given in Table 1. Primers for housekeeping genes *Surf1*, *Nup54* and *Polr2a* were purchased from Generi-Biotech Ltd. (Hradec Kralove, Czech Republic). Real-time PCR analysis was performed on iCycler iQ (Bio-Rad Laboratories, Inc., Hercules, CA). HotStart Taq DNA polymerase (AB gene, Epsom, UK) was employed for cDNA amplification under the following conditions: 1.5 mM MgCl₂, 0.2 mM dNTP, 0.025 U/μl polymerase, 0.3 μM of each primer, SYBR[®] Green I in 1:100,000 dilution. The temperature profile was 95 °C for 14 min and 40 repeats of cycle consisting of 95 °C for 15 s, 60 °C for 20 s and 72 °C for 20 s. Calibration curves used for the calculation of reaction effectivity were generated from amplification of dilutions of randomly selected cDNA sample, the dilution factor was 5. The amplification efficiencies varied between 95% and 105%. Amplification of the desired sequence was confirmed by melting curve analysis (T_m were 86.5 °C, 84.5 °C and 87.5 °C for *β-2-microglobulin*, *11β-hsd1* and *11β-hsd2*, respectively). PCR products were separated on 2% agarose gel in the presence of ethidium bromide, visualized under ultraviolet light and compared with low-molecular-weight ladder (25–766 bp) (New England Biolabs, Hertsfordshire, UK). The analysis of real-time amplification curves and subtraction of Ct values was performed using iCycler iQ 3.0 software (Bio-Rad Laboratories, Inc., Hercules, CA). Relative expression of 11β-HSD mRNA was calculated from the real-time RT-PCR efficiencies and the Ct deviation of a sample versus control using the method of Pfaffl [30]. Results are expressed as a percentage of control ± SEM.

2.3. Western blotting analysis

Western blots were performed as described previously [21]. Briefly, placenta and kidney as a positive control were homogenized, centrifuged at 400 × g for 10 min and the supernatant centrifuged again at 100,000 × g for 60 min. The pellet was resuspended and sonicated in Laemmli buffer containing β-mercaptoethanol (2%), boiled for 3 min and stored at -20°C until analysis.

Proteins were separated by 10% SDS-PAGE gel electrophoresis and electroblotted in a semi-dry blotting system. The blots were incubated with a sheep anti-rat 11β-HSD2 polyclonal antibody (Chemicon International, Inc., Temecula, CA, USA) and subsequently incubated with peroxidase-conjugated rabbit anti-sheep immunoglobulin G (Pierce Biotechnology, Inc., Rockford, IL, USA). The protein bands were visualized using Super Signal West Femto substrate (Pierce Biotechnology, Inc., Rockford, IL, USA) and detected by luminescence analyzing system LAS-1000 (Fuji, Tokyo, Japan).

For quantification, equal amounts of protein from each sample (13 μg of total protein) were used and the chemiluminescent signal of the measured immunoreactivity was in the linear range in terms of protein amounts used in this analysis. The results were calculated as a ratio of chemiluminescent signal to mg of protein ± SEM. Four samples from each group were analyzed.

2.4. Dual perfusion of the rat placenta

The method of dually perfused rat placenta was used as described previously [21]. Briefly, one uterine horn was excised and allowed to dive in the heated Ringer saline (37 °C). A catheter was inserted into the uterine artery proximal to the blood

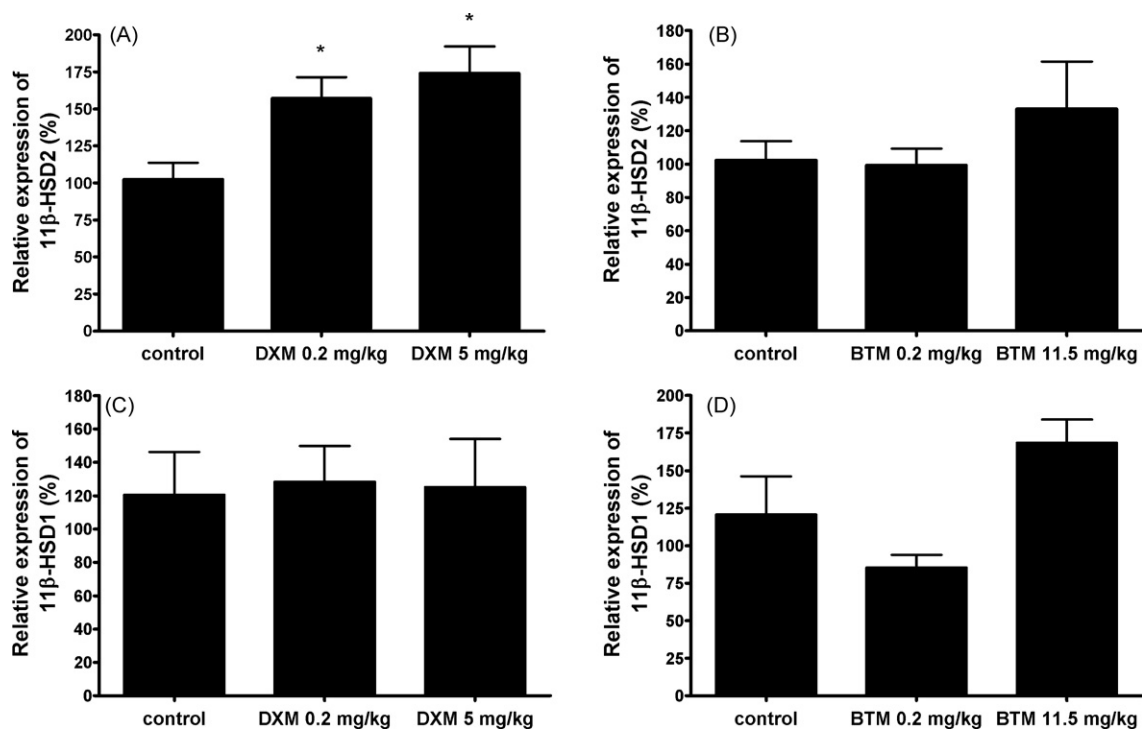


Fig. 1. Real-time RT-PCR analysis of mRNA expression of 11β-HSD2 (A and B) and 11β-HSD1 (C and D) in the rat placenta. Pregnant rats were pretreated with DXM (0.2 mg/kg or 5 mg/kg), $n=9$ or BTM (0.2 mg/kg or 11.5 mg/kg), $n=11$. Relative expression was calculated by using the method of Pfaffl [30] and is expressed as mean percentage of control \pm SEM; * $p < 0.05$.

vessel supplying a selected placenta and connected with the peristaltic pump. Krebs' perfusion liquid (exposed to the mixture of 95% oxygen and 5% carbon dioxide) containing 1% dextran was brought from the maternal reservoir at a rate of 1 ml/min. The uterine vein, including the anastomoses to other fetuses, was ligated behind the perfused placenta and cut so that maternal solution could leave the perfused placenta. The selected fetus was separated from the neighboring ones by ligatures. The umbilical artery was catheterized using 24-gauge catheter and connected with the tubing by which the fetal perfusion liquid from the fetal reservoir was supplied at a rate of 0.5 ml/min. The umbilical vein was catheterized in a similar manner and the selected fetus was removed. Before the start of each experiment, the fetal vein effluent was collected into pre-weighted glass vials to check a possible leakage of perfusion solutions from the placenta. In the case of leakage, the experiment was terminated. Maternal and fetal perfusion pressures were monitored continuously throughout the perfusion experiments.

Immediately after successful surgery maternal side of the placenta was perfused with either 50 nM or 200 nM concentration of corticosterone with [3 H]corticosterone as a tracer. After 10 min stabilization period sample collection started. Fetal effluent samples were collected for 30 min of perfusion and the steroids extracted using Sep-Pak cartridges. The analysis of steroids was performed by HPLC using an Agilent 1100 system (Agilent, Palo Alto, CA, USA) and radioactivity detector (Radiomatic 150TR, Canberra Packard, Meriden, CT, USA) with a flow cell. Corticosterone and its reaction products were detected according to a previously described procedure [31]. Conversion capacity of 11β-HSD2 was calculated as a ratio of 11-dehydrocorticosterone (metabolite) concentration to the concentration of total corticosteroids (corticosterone and 11-dehydrocorticosterone) and presented as means \pm SEM.

2.5. Statistical analysis

Data from real-time RT-PCR were analyzed using the REST@software [32] to assess statistical significance. Results of Western blot analyses and dual perfusion experiments were analyzed by ANOVA followed by Bonferroni's comparison test. Differences of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of GC pretreatment on birth weights

Decline in fetal weights was observed in rats treated with GCs when compared to control group, however, statistical significance was found only in high dose DXM group of rats ($p < 0.001$).

Means of fetal weights were 3.52 ± 0.54 g, 3.23 ± 0.36 g, 1.75 ± 0.3 g, 3.48 ± 0.18 g and 3.05 ± 0.31 g for control, 0.2 mg/kg DXM, 5 mg/kg DXM, 0.2 mg/kg BTM and 11.5 mg/kg BTM, respectively. No significant changes in placental weights were observed.

3.2. Effect of GC pretreatment on mRNA and protein levels of 11β-HSD in the rat placenta

Real-time RT-PCR analysis demonstrated the presence of both 11β-HSD1 and 11β-HSD2 mRNA in the rat placenta. Rat liver and kidney were used as positive controls (data not shown). Quantitative data evaluation revealed a distinct enhancement of placental 11β-HSD2 mRNA in dams pretreated with DXM (Fig. 1A). Both dosage regimens lead to statistically significant enhancement in 11β-HSD2 mRNA expression. The mRNA expression was increased up to 57.1% and 74% at 0.2 mg/kg DXM and 5 mg/kg DXM, respectively. In contrast, antenatal treatment of rats with either 0.2 mg/kg or 11.5 mg/kg of BTM had no effect on placental 11β-HSD2 mRNA expression (Fig. 1B). In the case of 11β-HSD1, neither DXM nor BTM administration affected the expression at mRNA level (Fig. 1C and D).

The immunoreactive signal of 11β-HSD2 was localized to microsomal fractions and was detected as a major band of 40 kD both in the kidney and placenta. Contrary to the real-time RT-PCR data, Western blotting analysis of placentas obtained from pregnant rats treated with high doses of DXM did not reveal any changes in placental protein content. Similarly, no alterations of placental 11β-HSD2 protein expression were observed in animals treated with high doses of BTM (Fig. 2).

3.3. Effect of GC pretreatment on corticosterone metabolism in perfused rat placenta

In perfusion studies corticosterone was used as a substrate of 11β-HSD2. Conversion capacity of placental 11β-HSD2 was investigated at two corticosterone concentrations: 50 nM

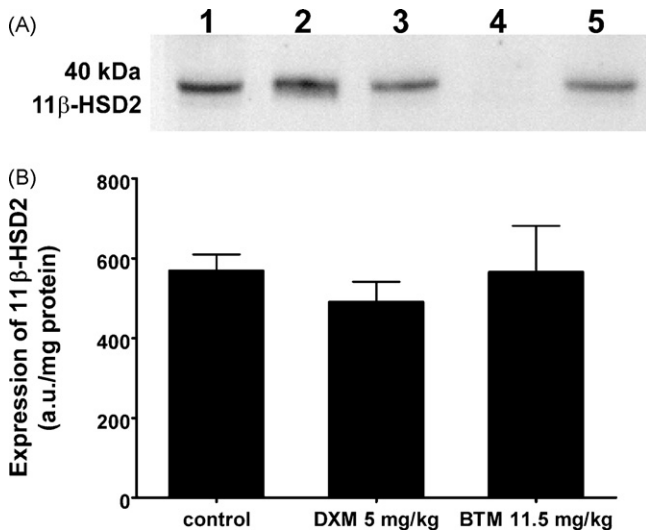


Fig. 2. Western blotting analysis of 11 β -HSD2 expression in the rat placenta. (A) Representative Western blot of four similar analyses: (1) rat placenta – control (2) rat kidney, (3) rat placenta – pretreatment with BTM 11.5 mg/ml, (4) cytosolar fraction – negative control, (5) rat placenta – pretreatment with DXM 5 mg/kg. Protein load: 13 μ g of total protein. (B) Expression of 11 β -HSD2 calculated as a ratio of chemiluminescent signal to mg of protein. Results are expressed as mean values \pm SEM, $n=4$.

(reflecting physiological concentrations [33]) and 200 nM (supra-physiological concentrations). 11 β -HSD2 conversion capacity was influenced by both substrate concentration and GC pretreatment. As in our previously published study [21], significant reduction in 11 β -HSD2 conversion capacity due to enzyme saturation was observed at supra-physiological corticosterone concentration inflow (conversion capacity = $40.2 \pm 8.3\%$ and $16.3 \pm 4.3\%$ at 50 nM and 200 nM corticosterone, respectively) (Fig. 3).

Pretreatment with DXM reduced 11 β -HSD2 conversion capacity in a dose dependent manner (conversion capacity = $30.2 \pm 4\%$ and $17.2 \pm 3.2\%$ at 0.2 mg/kg DXM and 5 mg/kg DXM, respectively) (Fig. 3A). In BTM treated group of rats low doses did not affect the conversion capacity of 11 β -HSD2, however, high doses of BTM lead to significant reduction in corticosterone metabolism (conversion capacity = $40.9 \pm 2.8\%$ and $15.3 \pm 4.9\%$ at 0.2 mg/kg and 11.5 mg/kg BTM, respectively).

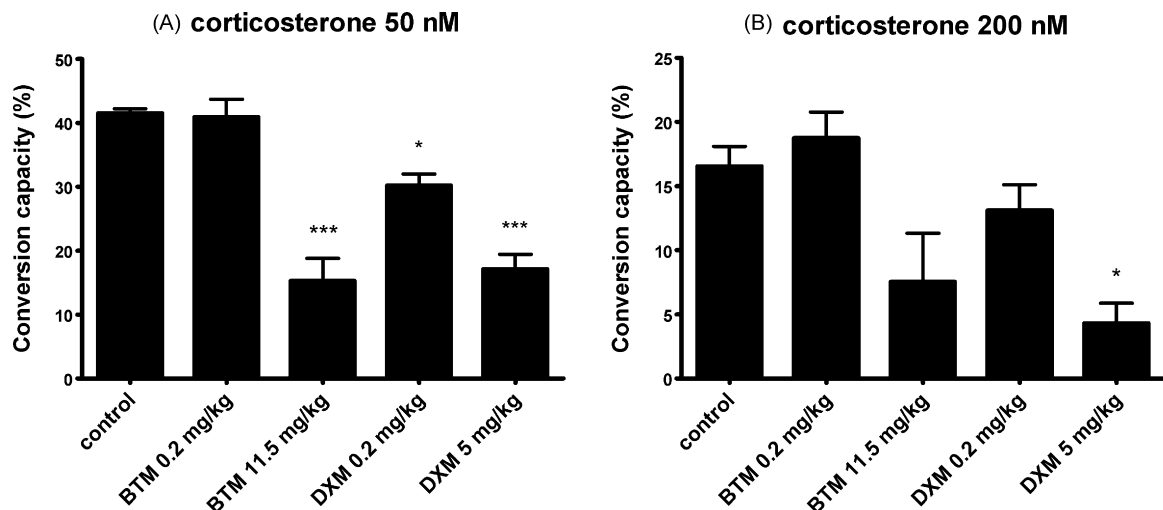


Fig. 3. The effect of GC pretreatment on 11 β -HSD2 conversion capacity in the rat placenta. Corticosterone was infused into the placenta via uterine artery at a concentration of 50 nM (A) or 200 nM (B) and fetal effluent was sampled and assayed for corticosterone and 11-dehydrocorticosterone. Conversion capacity of 11 β -HSD2 was calculated as a ratio of 11-dehydrocorticosterone concentration to the concentration of total corticosteroids and expressed as a percentage of mean \pm SEM; * $p < 0.05$, *** $p < 0.001$.

Similar pattern of changes in placental steroid metabolism caused by GC administration was found at 200 nM corticosterone inflow concentration (Fig. 3B). However, the fall in conversion capacity of 11 β -HSD2 was statistically significant only in rats pretreated with high doses of DXM (conversion capacity = $4.3 \pm 1.6\%$).

4. Discussion

Preservation of normal activity and expression of 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) in the placenta is very important in the view of healthy development of a new individual [34]. 11 β -HSD2 forms an active component of the placental barrier which controls the transplacental passage of glucocorticoids protecting the fetus against excessive GC exposure [35]. It has been demonstrated that 11 β -HSD2 expression and activity is regulated by a diversity of endogenous as well as exogenous substances [36–40]. Synthetic GCs, for example, have been recently suggested to alter the expression and activity of placental 11 β -HSD2 [26]. Taking into account that synthetic GCs, particularly, DXM and BTM, are often administered to pregnant women to prevent respiratory distress syndrome in premature babies [41], the elucidation of the effect of GCs on 11 β -HSD2 expression and activity is strongly demanded. Several studies using variety of experimental approaches have been employed to clarify the effect of synthetic GCs on placental 11 β -HSD2 [23–25], however, no definite conclusions have been drawn up to date.

We have recently investigated the effect of DXM and BTM on 11 β -HSD2 mRNA expression in the human choriocarcinoma Jeg3 cells [42]. 11 β -HSD2 expression tended to decline with increasing steroid concentration and incubation period, however, this trend was not statistically significant. In the present study we followed the effect of DXM and BTM pretreatment on expression and function of 11 β -HSD2 in the rat placenta. Our data show that antenatal administration of DXM significantly enhanced the mRNA expression of rat placental 11 β -HSD2. This increase was apparent at both low and high doses of DXM. The mechanism by which DXM induces expression of 11 β -HSD2 was not examined in our study, however, van Beek et al. proposed that this effect is caused by increased 11 β -HSD2 transcription, enhanced mRNA stability and prolonged mRNA half life mediated by activated glucocorticoid receptor [26].

Unlike DXM, antenatal BTM treatment did not cause any significant changes in 11 β -HSD2 mRNA level. These results suggest that DXM and BTM differ in their tissue specific effects. The species-

specific variability in transactivation response to DXM and BTM has been recently studied by Tanigawa et al. [43]. They documented that esterified BTM had only partial transactivation agonistic activity mediated by rat glucocorticoid receptor when compared with esterified DXM and non-esterified BTM and DXM. Since the injections applied to pregnant rats contain esterified DXM or BTM we speculate that the lower transactivation effect of esterified BTM may underlie the variation in gene transcription induction between DXM and BTM.

Furthermore, the effect of GCs on placental 11 β -HSD2 protein content has been investigated. BTM treatment has been recently documented to stimulate 11 β -HSD2 expression in the baboon placenta [25], however, in our study neither DXM nor BTM affected the expression of 11 β -HSD2 in the rat placenta at the protein level. Inconsistencies between changes at mRNA and protein levels have been previously described for other placental proteins as well [44,45]. We assume that the processes involved in post-transcriptional/translational regulation may mask the stimulatory effect of DXM; nevertheless, detailed examination is needed to clarify these observations.

Although, functional studies have demonstrated that the net dehydrogenase activity in total rat placenta increases with advanced pregnancy [16], the expression of 11 β -HSD2 declines as term approaches [21,22]. An opposite pattern of changes in expression was found for 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) [8] indicating that 11 β -HSD1 participates in placental GC metabolism at term in the rat. Furthermore, it has been recently reported that mRNA expression and reductase activity of placental 11 β -HSD1 is stimulated by cortisol and DXM [46,47]. In our experiments, however, no changes in the expression of 11 β -HSD1 at mRNA level were observed in placentas of rats pretreated with DXM or BTM. Nevertheless, since neither the protein content nor functional activity of 11 β -HSD1 were investigated in our study, the overall effect of GCs treatment on 11 β -HSD1 expression and activity remains to be elucidated.

The impact of synthetic GCs on placental 11 β -HSD2 has been recently studied in rats [16], ovines [24], primates [25] and human trophoblast cells *in vitro* [26]. However, direct evidence of the effect of antenatal GC therapy on placental barrier function of 11 β -HSD2 has not been investigated to date. This is the first report showing the influence of GCs on functional activity using perfused rat placenta. We have previously established the *in-situ* technique of dually perfused rat placenta to study the placental corticosteroid metabolism mediated by 11 β -HSD2 [21]. This technique enables us to investigate the effects of maternal drug administration on the placental 11 β -HSD2 steroid metabolism which is not feasible in humans. Surprisingly, our findings from *in situ* perfusion experiments did not correspond with RT-PCR and Western blotting data; on the contrary, functional studies revealed that both GCs are capable to significantly impair the steroid metabolism within the rat placenta. The administration of both doses of DXM and high doses of BTM caused decreased corticosterone inactivation, allowing higher amounts of corticosterone to reach fetal circulation. At physiological concentrations of corticosterone (50 nM) the calculated conversion capacity of 11 β -HSD2 was reduced by 24.9%, 57.5% and 62% at 0.2 mg/kg DXM, 5 mg/kg DXM and 11.5 mg/kg BTM, respectively. Similar pattern of changes, although, less pronounced, was observed at 200 nM corticosterone placental inflow.

The differences between expressional and functional experiments observed in our study could be attributed to other interacting factors such as the interference of 11 β -HSD1, up-regulated by GCs treatment. However, results from RT-PCR do not support this hypothesis. Furthermore, in our previous study [21], no other metabolites than 11-dehydrocorticosterone were detected in the fetal effluent. Another possible factor affecting perfusion experiments could result from the competition between corticosterone

and synthetic GCs on binding sites of 11 β -HSD2 which could result in decreased conversion of corticosterone. However, in our experimental setup, the selected placenta was initially washed out by perfusion solution for 10 min before the collection of samples started; subsequently no synthetic glucocorticoid was present in the perfusion liquid during the perfusion experiment. Only trace amounts of DXM or BTM could have remained in the placenta after maternal pretreatment. Nevertheless, considering their short biological half lives ($T_{1/2} = 2-3$ h) [48,49] it is evident that 24 h after the last administered dose the level of steroids in the organism is negligible. Therefore, interference of corticosterone with DXM or BTM during perfusion studies can be ruled out. The discrepancies between expressional and functional studies thus remain to be elucidated; nevertheless, they strongly suggests that sole analysis of expressional changes of 11 β -HSD2 at mRNA and/or protein levels cannot reliably predict the role of GC treatment on 11 β -HSD2 function in the placental barrier. Without functional studies, results from real-time RT-PCR and Western blotting analyses could have lead to a false conclusion that DXM and BTM administration has only little impact on 11 β -HSD2 function in the placenta.

In conclusion, we show the effect of synthetic GCs, DXM and BTM, on expression and function of placental 11 β -HSD2 in rats. Our results reveal that only DXM but not BTM enhances 11 β -HSD2 mRNA expression; however, no alterations in 11 β -HSD2 protein content were detected. Despite the elevated expression observed at mRNA level, antenatal GC administration considerably decreased the metabolism of GCs in the perfused rat placenta enabling higher amounts of corticosterone to reach the fetal circulation. The impairment of corticosterone metabolism was apparent in both DXM and BTM treated groups of rats. Finally, our observations highlight the importance of functional analysis in the investigation of the effect of GCs on placental barrier.

Conflict of interest

None.

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