

Placental 11 β -Hydroxysteroid Dehydrogenase in Dahl and Spontaneously Hypertensive Rats

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Studies in normotensive rats showed that excessive fetal exposure to maternal glucocorticoids retards growth and programs hypertension in later life. This excessive exposure is proposed to occur due to a reduction of the placental barrier to maternal glucocorticoids that is provided by 11 β -hydroxysteroid dehydrogenase (11 β HSD). To assess the possible alterations of glucocorticoid placental barrier in two genetic models of hypertension — spontaneously hypertensive (SHR) and Dahl salt-sensitive rats (DS) and their normotensive counterparts Wistar-Kyoto (WKY) and Dahl salt-resistant rats (DR)—we performed real-time reverse transcriptase–polymerase chain reaction analysis and bioactivity measurements of placental 11 β HSD in the last third of gestation. Whereas 11 β HSD2 mRNA expression was not different among the investigated strains, 11 β HSD1 mRNA abundance was 2.4 times higher in WKY than in SHR and 9.6 times higher in DS than in DR placentae. The 11 β HSD2 activity studies performed in placental homogenates revealed activity that did not differ among the strains. Concomitant with 11 β HSD1 mRNA expression 11-oxoreductase activity was clearly

evident in all strains and was higher in WKY and DS rats than in SHR and DR, respectively. Nevertheless, the net 11 β HSD activity of tissue fragments (11 β -dehydrogenase minus 11-oxoreductase) was tended toward dehydrogenase action, ie, toward corticosterone inactivation and was significantly lower in DS than in DR rats. The 11 β -dehydrogenase/11-oxoreductase ratio was less than 2:1 in SHR and WKY rats, whereas this ratio was 9:1 in DR and 4.5:1 in DS rats. These data suggest that the placental glucocorticoid barrier is not decreased in SHR rats in comparison with normotensive WKY but is lower in DS than in DR counterparts. It cannot be excluded, therefore, that the placental glucocorticoid barrier in Dahl rats influences the pathways that might lead to the sensitivity of blood pressure to high salt intake in later life. Am J Hypertens 2003;16:401–406 © 2003 American Journal of Hypertension, Ltd.

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Epidemiologic observations from a number of human populations suggest that low birth weight and large placenta might be a significant risk factor for cardiovascular diseases including hypertension later in life.^{1–3} Some data suggest that increased fetal exposure to maternal glucocorticoids may play a role in determining not only the fetal development but also the association between low birth weight or large placenta and increased blood pressure (BP) in later life. Normally, the fetus has much lower level of glucocorticoids than its mother and this is achieved by placental 11 β -hydroxysteroid dehydrogenase (11 β HSD).⁴ This enzyme catalyzes the interconversion of physiologic glucocorticoids cortisol and corticosterone to their metabolites cortisone and 11-dehydrocorticosterone and is expressed in two different isoforms.⁵ The 11 β HSD1 is a NADP⁺/NADPH-dependent enzyme that has both oxidative and reductive activity. The

11 β HSD2 is a NAD⁺-dependent enzyme that only oxidizes cortisol or corticosterone to inert 11-keto derivatives and thus plays a crucial role in protecting the fetus from maternal glucocorticoids.^{6–9} Although it remains controversial whether human placental 11 β HSD activity correlates with birth weight,^{9,10} such correlation was proved in some mammals. In the rat, placental 11 β HSD activity correlates positively with birth weight and inversely with placental weight,⁷ whereas in the swine a positive correlation has been reported between 11 β HSD and the both fetal and placental weight.¹¹ Furthermore, the inhibition of fetoplacental 11 β HSD by administration of carbenoxolone to pregnant rats retards fetal growth and increases BP in adulthood.¹² Similarly, administration of dexamethasone, a glucocorticoid that is poorly metabolized by 11 β HSD, to pregnant rats reduces the birth weight and stimulates hypertension in the adult offspring.^{7,13}

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These data indicate that excess exposure of rat placenta and fetus to maternal glucocorticoids reduces birth weight and programs hypertension in later life. On the basis of these studies, the hypothesis has been raised that when the fetoplacental barrier to maternal corticosterone is defective, the resulting prenatal exposure to glucocorticoids may support development of hypertension later in life. Therefore, the aim of this study was to determine whether placental 11 β HSD1 and 11 β HSD2 activity and mRNA abundance is different between normotensive and hypertensive rats. To study this question two strains of genetically hypertensive rats and their normotensive counterparts were studied: spontaneously hypertensive rats (SHR), normotensive Wistar-Kyoto rats (WKY), and Dahl rats sensitive (DS) and resistant to salt (DR).

Methods

Animals and Tissue Collection

Female SHR, WKY, as well as Dahl rats of both phenotypes (SS/Jr, SR/Jr) were obtained from the breeding colonies of the Institute of Physiology Czech Academy of Sciences, (Prague). The SHR and WKY rats were maintained on a standard chow, whereas Dahl rats were fed a low-salt diet (0.2 % NaCl). The rats were mated overnight and the day on which a vaginal plug was observed was termed day 1. Placentae and fetuses were collected from the rats on day 20, weighed and placentae frozen rapidly in liquid nitrogen for subsequent reverse transcriptase-polymerase chain reaction (RT-PCR) analysis or placed immediately in ice-cold saline for enzyme assay. The last quarter of gestation was used in this study because rat placental 11 β -dehydrogenation activity increases during this period^{14,15} and 11 β HSD1 mRNA is undetectable before day 15.¹⁶ Blood pressure was measured by a direct puncture of the carotid artery under light ether anesthesia just before sacrifice. The study was approved by the Animal Experimental Ethics Committee.

Isolation of Total RNA

Total RNA was extracted from placental tissue using the guanidium thiocyanate method. Briefly, whole placenta was homogenized in ice-cold denaturation solution (guanidine thiocyanate, 4 mol/L; sodium citrate, 25 mmol/L at pH 7.0; 2-mercaptoethanol, 0.1 mol/L; 0.5 % sarcosyl; 10:1 v/w) using a Polytron homogenizer (Kinematica AG, Littau, Switzerland) at 13,000 rpm for 1 min. Then 0.4 mL of 2 mol/L sodium acetate (pH 4), 4 mL of water-saturated phenol, and 0.8 mL of chloroform-isoamyl alcohol (49:1, v/v) were added to 4 mL of homogenate. After precipitation and reprecipitation with isopropanol and washing with 75% ethanol the RNA was dissolved in water and stored at -70°C .

To remove DNA the isolated RNA was treated with DNase (Promega, Madison, WI) and the contamination of RNA samples by DNA was detected by PCR. The PCR

reaction contained (total volume 25 μL): sample, 0.5 μL ; PCR buffer (1 \times); forward and reverse primers for β -actin, 0.25 $\mu\text{mol/L}$ each; dNTP mix, 100 $\mu\text{mol/L}$ (MBI Fermentas GmbH, St. Leon-Rot, Germany), 3 mmol/L MgCl_2 , 0.5 U Taq-DNA polymerase (MBI Fermentas GmbH). The cycling parameters used were as follows: 95°C for 2 min; 95°C for 40 sec, 60°C for 30 sec, and 72°C for 50 sec, for a total of 35 cycles and 72°C for 5 min. After cooling, sample was treated with RNase for 20 min at room temperature. Amplification products were run on 2% agarose gel and stained with ethidium bromide. RNA was quantified by absorption at 260 nm.

Quantitative RT-PCR

mRNA for three different genes was measured using primers (VBC-GENOMICS, Wien, Austria) with following sequences (5' \rightarrow 3'): for 11 β HSD1, sense GAGTTCAGACCAGAAATGCTCC and antisense TGTGTGATGTGATTGAGAATGAGC; for 11 β HSD2, sense GATGTTCCCCTCGCCTGAA and antisense ATGAGCAGTGCAATAGCTGCCTTG; for β -actin, sense CCGTAAAGACCTCTATGCCA and antisense AAGAAAGGGTGTAAAACGCA.^{17,18}

One-step RT-PCR was performed using a LightCycler instrument and LightCycler-RNA Amplification Kit SYBR Green I (Roche, Mannheim, Germany) in total volume 10 μL containing 50 ng (11 β HSD1, 11 β HSD2) or 1 ng (β -actin) of total RNA, 7 mmol/L MgCl_2 , 0.3 $\mu\text{mol/L}$ (11 β HSD1, 11 β HSD2) or 0.5 $\mu\text{mol/L}$ (β -actin) of each primer, RT-PCR Reaction Mix Sybr Green I (1 \times) and RT-PCR Enzyme Mix. Levels of mRNA generated from placental RNA were calculated from standard curves generated for each pair of primers. For these curves we used as a standard total kidney RNA containing both isoforms of 11 β HSD diluted in the range of 1 to 150 ng (11 β HSD1) or 50 pg to 150 ng (11 β HSD2, β -actin). The LightCycler was programmed as follows: reverse transcription of the RNA template for 20 min at 55°C , initial denaturation of cDNA/RNA hybrid for 30 sec at 95°C , both temperature slope $20^{\circ}\text{C}/\text{sec}$, followed by 45 cycles of amplification of target cDNA: 95°C for 1 sec, 55°C for 10 sec, 72°C for 15 sec. The temperature slope was $20^{\circ}\text{C}/\text{sec}$, except by 72°C when it was $5^{\circ}\text{C}/\text{sec}$. The melting curve analysis was performed at 95°C for 0 sec, 65°C for 60 sec (both temperature slope $20^{\circ}\text{C}/\text{sec}$) and 95°C for 0 sec (the temperature slope $0.1^{\circ}\text{C}/\text{sec}$). The results were calculated as the ratio 11 β HSD1 or 11 β HSD2 to β -actin. Although the amount of 11 β HSD2 mRNA seemed lower than that of 11 β HSD1, more exact quantification at the comparative level of 11 β HSD1 and 11 β HSD2 was not attempted because of possible differences in amplification efficiency with various sets of primers.

Measurement of 11 β HSD2 Activity

The whole placenta was homogenized in ice-cold buffer containing 200 mmol/L of sucrose and 10 mmol/L of

Table 1. Arterial blood pressure and body weight of the dams as well as fetal and placental weight at day 20 of gestation

Strain	Dam			
	SBP (mm Hg)	Body Weight (g)	Fetal Weight (g)	Placental/Fetal Weight Ratio
SHR	195.9 \pm 5.7 (7)†	277 \pm 10 (7)	2.67 \pm 0.09 (44)*	0.17 \pm 0.00 (44)†
WKY	138.7 \pm 6.5 (7)	261 \pm 12 (7)	3.08 \pm 0.17 (31)	0.14 \pm 0.00 (31)
DS	171.6 \pm 3.4 (7)†	363 \pm 11 (7)	2.90 \pm 0.10 (40)†	0.22 \pm 0.01 (40)†
DR	136.9 \pm 5.7 (8)	295 \pm 10 (8)	2.48 \pm 0.10 (35)	0.26 \pm 0.01 (35)

SBP = systolic blood pressure; SHR = spontaneously hypertensive rat; WKY = Wistar-Kyoto rat; DS = Dahl salt-sensitive rat; DR = Dahl salt-resistant rat.

Values are expressed as mean \pm SEM; numbers of dams, placenta, and fetuses are given in parentheses.

* $P < .05$ or † $P < .01$ compared with the normotensive counterparts.

TRIS/HCl at pH 8.5 (1:9 w/v) using Polytron homogenizer (Kinematica AG, Littau, Switzerland). The homogenate was centrifuged at 1000 g for 10 min at 4°C, protein concentration was determined by the Coomassie blue method, and the homogenate used immediately for measurement of 11 β HSD2 activity. Enzyme activity was determined by a radiometric conversion assay as reported previously.¹⁹ Briefly, the assay tube contained 250 μ L of homogenate (1 mg protein), 40 μ L of NAD⁺ (final concentration 0.4 mmol/L), and 750 μ L of incubation buffer (KCl, 100 mmol/L; TRIS/HCl 50 mmol/L at pH 8.5). After 10 min preincubation at 37°C the labeled corticosterone was added (final concentration 14.5 nmol/L) and the reaction followed for 60 min. Preliminary studies indicated that the rate of reaction was linear within this time. The reaction was stopped by cooling and the samples centrifuged for 15 min (3000 g). Steroids were extracted on C₁₈ reversed-phase Sep-Pak columns (Waters, Milford, MA), dried under nitrogen and stored at -20°C. [³H]corticosterone and [³H]11-dehydrocorticosterone were separated and quantified by HPLC as previously described.²⁰ The substrate concentration (14.5 nmol/L) that was used in our experiments corresponded to the apparent K_m for rat placenta.⁶

Measurement of 11 β HSD1 Activity

In contrast to 11 β HSD2, 11 β HSD1 activity was not detected by radiometric assay despite the presence of high substrate concentration (1 μ mol/L) and NADP⁺ instead of NAD⁺. This seemed to reflect the loss of enzyme activity associated with cellular disruption described by other investigators.⁴ Therefore, tissue fragments instead of homogenate were used using a modified method of Pácha and Mikšik.²¹ Briefly, tissue fragments were incubated in sealed vessels (95% O₂/5% CO₂ atmosphere) containing 10 ml of oxygenated buffer (in mmol/L: NaCl, 119.0; CaCl₂, 1.2; MgCl₂, 1.2; NaHCO₃, 21.0; K₂HPO₄, 2.4; KH₂PO₄, 0.6; glucose, 10.0; glutamine, 2.5; β -hydroxybutyrate, 0.5; and mannitol, 10.0, previously gassed for 15 min with 95% O₂/5% CO₂ at pH 7.4) and unlabeled corticosterone (1.45 μ mol/L) or 11-dehydrocorticosterone (1.45 μ mol/L) for 90 min at 37°C. Using this substrate

concentration, the reaction was linearly proportional to the time of incubation. At the end of incubation, an internal standard of deoxycorticosterone (1.5 μ mol/L) was added, the vessel mixed and placed on ice. The incubation medium was centrifuged for 10 min at 3000 g , the supernatant loaded onto a C₁₈ reversed-phase Sep-Pak column (Waters) and the steroids extracted. The analyses of corticosterone and 11-dehydrocorticosterone were performed as described earlier.²⁰

Statistical Analysis

All data are expressed as means \pm SEM and assessed by Student unpaired t test. The relationship between placental and fetal weight was analyzed by a simple linear regression. Correlation coefficients were tested using two-tailed significance levels by the t test. A probability level of $P < .05$ was considered significant.

Results

At the time of the experiment (day 20 of gestation), the BP was significantly higher in SHR and DS dams than in their normotensive counterparts (Table 1). The fetal weights were higher in DS and WKY rats than in DR and SHR animals, respectively. The placental weights of SHR were significantly higher than those of WKY rats. In contrast, the placental weights of DS rats were lower than of DR strain. Statistical analysis revealed a positive correlation between fetal and placental weights in WKY ($r = 0.516$; $P < .01$) and DR rat ($r = 0.440$; $P < .01$). No correlation was demonstrated in both hypertensive models (SHR, DS; $P > .05$). There were significantly fewer fetuses in WKY dams (5.7 ± 0.8 , $P < .01$) than in other strains (SHR: 9.9 ± 0.4 ; DR: 9.0 ± 0.6 ; DS: 10.0 ± 1.1).

Specific mRNA for both isoforms of 11 β HSD was detected in the placenta of all strains (not shown). Because qualitative RT-PCR is severely limited due to differences in the efficiency of priming with different primer pairs, we used the LightCycler system to establish the mRNA abundances of 11 β HSD1 and 11 β HSD2 mRNA. The amount of mRNA was determined with one-step RT-PCR procedure using 11 β HSD1, 11 β HSD2, and β -actin primers.

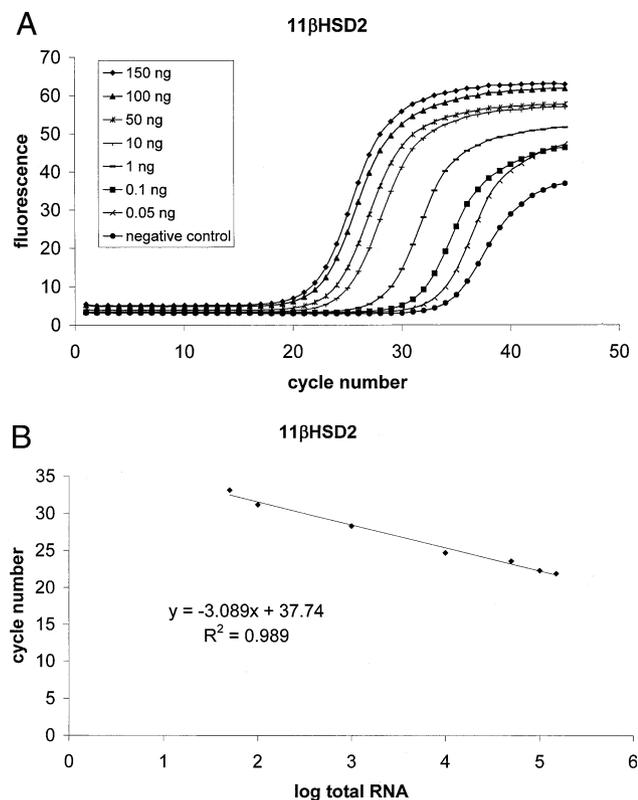


FIG. 1. Amplification plot (A) and standard curve (B) obtained for 11 β HSD2 mRNA. Serial dilutions of RNA used as our standard were used as templates for real-time reverse transcriptase-polymerase chain reaction. The fluorescence is the increase in reporter dye intensity. The amount of RNA in each sample is shown in the key. The level at which the threshold cycle was determined is calculated by the second derivative maximum method. The melting analysis showed that the amplification of negative control represents only primer-dimer. A standard curve generated for the amplification plot is given in **panel B**. 11 β HSD2 = 11 β -hydroxysteroid dehydrogenase 2.

Known amount of RNA was added in serial dilutions from 150 to 0.05 ng to a series of RT-PCR. The reactions were carried out in a LightCycler thermocycler and the fluorescence was monitored throughout the reaction. The results

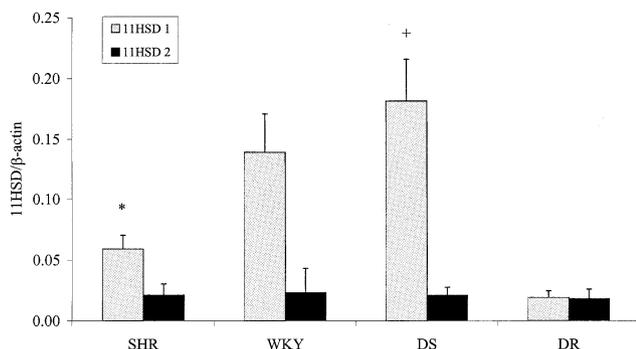


FIG. 2. 11 β HSD1 and 11 β HSD2 mRNA levels in placenta of spontaneously hypertensive (SHR), Wistar-Kyoto (WKY) and Dahl salt-sensitive (DS) and salt-resistant (DR) rats. The values are expressed in arbitrary units relative to those of β -actin (mean \pm SEM). * P < .01 or * P < .05 compared with the normotensive counterparts. Other abbreviation as in Fig. 1.

for 11 β HSD2 mRNA are shown in Fig. 1A and the corresponding calibration curve is given in Fig. 1B. Using this approach we were able to calculate the proportionate quantity of both isoforms of 11 β HSD to β -actin control. As shown in Fig. 2 mRNA levels of 11 β HSD2 did not differ between normotensive and hypertensive counterparts. In contrast, 11 β HSD1 mRNA was significantly less expressed in SHR than in normotensive WKY, whereas in Dahl rats this expression was higher in DS rats than in DR animals (Fig. 2).

The activity of 11 β HSD2 was measured in the placental homogenates using a radiometric assay. To exclude the possible contribution of 11 β HSD1, the substrate concentration in the assay was similar to the apparent K_m for corticosterone of the placental type 2 isoform and NAD⁺ instead of NADP⁺ was used.⁶ Using this assay the activity of 11 β HSD2 was found to be low in placentae of all strains, and in accordance with mRNA abundances no significant differences were observed between normotensive and hypertensive rats (Table 2). In the presence of NADP⁺ or NADPH we have not observed any conversion of corticosterone or 11-dehydrocorticosterone, respectively (data not shown), which supports the conclusion of Burton and Waddell¹⁴ and Waddell et al⁶ that 11 β HSD1 activity is lost during homogenization.

The apparent K_m for corticosterone of the type 1 11 β HSD is in micromolar range,^{5,22} and therefore, 1.45 μ mol/L substrate concentration was used for estimation in 11 β HSD activity in placental fragments. As shown in Table 3 both 11 β -dehydrogenase and 11-oxoreductase activities were clearly evident in placental fragments. The 11-oxoreductase activity followed the expression of 11 β HSD1 mRNA (Fig. 2) and was higher in WKY than in SHR rats and in DS than in DR rats. The data in Table 3 also indicate that 11 β -dehydrogenase activity of placental fragments exceeds 11-oxoreductase activity in all strains, but this fact is more evident in Dahl rats than in SHR and WKY rats. The net 11 β HSD activity calculated as the difference between 11 β -dehydrogenase and 11-oxoreductase activity was significantly different between normotensive and hypertensive Dahl rats.

Discussion

Administration of high doses of carbenoxolone, a 11 β HSD inhibitor, or dexamethasone, a steroid poorly metabolized by 11 β HSD, during rat pregnancy produce lower birth weight and increase BP later in life.^{7,12,13,23} These data suggest that the increased access of maternal glucocorticoids to the fetus represents a great risk for later development of cardiovascular disease even if some studies did not corroborate this conclusion.²⁴ It cannot be excluded that maternal cachexia might produce intrauterine growth retardation and subsequent hypertension of progeny because administration of carbenoxolone or dexamethasone significantly reduced dam weight in some,^{7,12,13} but not all,²³ studies. In addition, epidemio-

Table 2. Placental 11 β HSD2 activity in tissue homogenates

Strain	SHR	WKY	DS	DR
Activity	14.6 \pm 2.1 (10)	12.9 \pm 1.9 (12)	12.0 \pm 1.4 (14)	9.8 \pm 1.4 (12)

11 β HSD2 = 11 β -hydroxysteroid dehydrogenase 2; other abbreviations as in Table 1.

Values are given as mean \pm SEM; numbers of placenta are given in parentheses. The 11 β HSD2 activity is expressed as picomoles of 11-dehydrocorticosterone formed per hour and milligrams of protein.

logic studies revealed an association between low birth weight and adult hypertension later in life.¹⁻³ Some data obtained in rat, swine, and human placenta showed a correlation between placental 11 β HSD activity and weight of the placenta, fetus, or newborn.^{7,9,11} The hypothesis arising from these observations is that pregnancy in hypertensive rat strains might be associated with low placental 11 β HSD activity leading to overexposure of the fetus to maternal glucocorticoids. Such hypothesis is supported by findings of low birth weight and large placenta in SHR rats.²⁵

The key finding of the present study is that placental inactivation of corticosterone to 11-dehydrocorticosterone by 11 β HSD2 and expression of 11 β HSD2 mRNA is similar in SHR and WKY and in DS and DR, respectively. In contrast, 11 β HSD1 is less expressed in SHR than in their normotensive WKY controls and in DR than DS rats. Because 11 β HSD1 operates reversibly,⁵ a critical point is which enzyme direction predominates in the placenta. If 11 β HSD1 operates as 11 β -dehydrogenase instead of 11-oxoreductase, the lower abundance of 11 β HSD1 mRNA might indicate a decreased placental inactivation of corticosterone in vivo both in SHR and DR rats. In contrast, if 11 β HSD1 operates as 11-oxoreductase in vivo, the data indicate that the placenta of WKY and DS rats is able to produce more corticosterone from 11-dehydrocorticosterone than the placenta of SHR and DR rats. Considering that the plasma concentration of 11-dehydrocorticosterone is relatively high and this steroid is much less bound to plasma proteins than corticosterone, the capacity of active glucocorticoid formation in rat placenta might be significant in vivo. This possibility is also indicated by our experiments with tissue fragments. Even if the experiments performed in rat and baboon placenta demonstrated that 11 β HSD1 is associated with the transplacental con-

version of cortisone or 11-dehydrocorticosterone to cortisol and corticosterone, respectively,^{8,26} we cannot exclude the possibility that this enzyme is also involved in the oxidation of glucocorticoids. Nevertheless our experiments with tissue fragments indicate that 11 β -dehydrogenase predominates over 11-oxoreductase and that net 11 β -dehydrogenase activity is similar in both SHR and WKY rats but is lower in DS hypertensive rats than in their normotensive DR controls. These findings are thus consistent with the hypothesis of a net decreased inactivation of corticosterone in hypertensive DS rats. In contrast, no statistical differences of corticosterone inactivation are present in SHR and WKY rats, although the differences of 11 β HSD1 mRNA between normotensive and hypertensive strain are obvious.

In the last third of gestation glucocorticoids largely affect the development of organs related to BP control in rat.²⁷ In agreement with this the prenatal application of dexamethasone or carbenoxolone to pregnant rats during this period programs hypertension in progeny.^{13,23} Thus, the time window for the effect of prenatal glucocorticoid exposure seems to be within the last third of gestation even if it cannot be excluded that the window is longer. Our data show that during this period the placental 11 β HSD2 does not play any role in hormonal programming of hypertension in progeny. In contrast, the possibility that the observed changes in 11 β HSD1 expression in SHR and WKY might have some effects on the development of hypertension cannot be excluded. The 11 β HSD1 is colocalized in placenta with glucocorticoid receptors,⁶ and several important placental functions including steroid, prostaglandin, and peptide hormone synthesis are affected by glucocorticoids.²⁸⁻³⁰ In addition, previous studies showed that the distribution of 11 β HSD1 and 11 β HSD2 is not homogenous in the placenta,^{6,26} and thus the local

Table 3. 11 β HSD activity in tissue fragments

	SHR	WKY	DS	DR
11 β -dehydrogenase	9.3 \pm 1.2	10.7 \pm 2.3	19.2 \pm 2.6	25.6 \pm 4.9
11-oxoreductase	6.1 \pm 0.6*	8.4 \pm 0.9	4.3 \pm 0.4*	2.9 \pm 0.3
Net activity	3.6 \pm 0.9	2.6 \pm 0.6	14.5 \pm 2.0*	22.4 \pm 2.2
Ratio	1.5 \pm 0.4	1.2 \pm 0.3	4.5 \pm 0.9	8.8 \pm 1.6

Abbreviations as in Tables 1 and 2.

Values are given as mean \pm SEM of 9 placentae in each group. The enzyme activity is expressed as picomoles of 11-dehydrocorticosterone (11 β -dehydrogenase or net activity) or corticosterone (11-oxoreductase) formed per hour and milligrams of dry weight.

* $P < .05$ compared with the normotensive counterparts.

concentration of active glucocorticoids might be dependent on the local activity of this enzyme.

In summary, we have demonstrated that differences in placental 11 β HSD2 during the last third of gestation are not involved in programming hypertension in later life of both SHR and DS rats. Although no changes in 11 β HSD2 activity and mRNA expression were observed between hypertensive and normotensive strains, large differences were observed in mRNA level and oxoreductase activity of 11 β HSD1 between hypertensive strains and their normotensive counterparts. It is unlikely that the changes of placental 11 β HSD activity during gestation are the critical determinant of BP in later life but they may be a risk factor in combination with other fetal and maternal factors. The influence of such maternal factors has been shown by reciprocal embryo transfer in Dahl rats³¹ but not in SHR and WKY rats.³²

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