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Co-expression of Two Distinct Isoforms of 11β-Hydroxysteroid Dehydrogenase in the Ovine Placenta

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We have previously described two distinct isoforms of 11β-hydroxysteroid dehydrogenase (11β-HSD) with respect to enzymatic activity in the ovine liver and kidney. To determine which isoform(s) is expressed in the ovine placenta, we studied the characteristics of 11β-HSD activity in placental tissues collected at days 140–143 of pregnancy. 11β-HSD activity was determined by a radiometric conversion assay using cortisol and cortisone as physiological substrates. At 100 nM cortisol, the placental 11β-HSD utilized NAD as cofactor, but displayed preference for NADP at 10 μM cortisol. Kinetic characteristics were examined in the presence of alternate cofactors, in order to determine whether this difference in the cofactor requirement represents distinct enzymes. With NAD as cofactor, the placental 11β-dehydrogenase had a K_m (110 ± 18 nM) compatible with the kidney enzyme, but displayed a K_m (12 ± 2 μM) similar/identical to the liver 11β-HSD when NADP was used. By contrast, the placental 11-oxoreductase showed preference for NADPH regardless of cortisone concentration. Kinetic analysis, using NADPH as cofactor, revealed a single species of 11-oxoreductase activity with a K_m of 4 ± 0.9 μM and a V_{max} of 3.1 ± 0.5 pmol/mg/min. Finally, since the NAD-dependent 11β-HSD in the ovine placenta displayed similar/identical kinetic characteristics to the enzyme described previously in the ovine kidney where a truncated 11β-HSD transcript was identified, we have also determined whether this transcript is expressed in the placenta by Northern blotting. It was found that the truncated 11β-HSD transcript was undetectable in the total RNA samples. These results demonstrate that both liver- and kidney-types of 11β-HSD activities are expressed in the ovine placenta, thus providing further evidence for the existence of a NAD-dependent 11β-HSD distinct from the well-characterized hepatic NADP-dependent enzyme. Furthermore, the lack of the truncated 11β-HSD transcript in the placenta suggests that the NAD-dependent enzyme identified in placenta and kidney is the product of a gene distinct from 11β-HSD.

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INTRODUCTION

Recently, there has been renewed interest in elucidating the structure and function of 11β-hydroxysteroid dehydrogenase (11β-HSD), an enzyme responsible for the interconversion of active glucocorticoids (cortisol and corticosterone) and their inactive metabolites (cortisone and 11-dehydrocorticosterone) [1]. It is now widely accepted that at least two isoforms of 11β-HSD are present in mammals [2–6]. The first is a NADP-dependent enzyme which is ubiquitously expressed with liver being the major site of its expression [7–15]. It contains both dehydrogenase and reductase activities and has a K_m in the μM range. It has been purified from the rat [7] and most recently from the mouse liver microsomes [15]. It is a glycoprotein with an apparent molecular weight of 34 kDa [7, 15]. The cDNAs encoding this isoform have been cloned from a number of species, including rat [8], human [12], sheep [13] and squirrel monkey [16]. Although its biological function in individual organs remains largely speculative, it is thought to regulate the access of glucocorticoids to their receptors [17–24]. The other isoform is NAD-dependent, contains only dehydrogenase activity and has a K_m in the nM range [3, 4, 6, 25, 26]. Very recently, the purification of this

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isoform from human placenta has been reported in an abstract form [27]. It is a 40 kDa protein with little sequence homology to the liver-type 11β-HSD. It is predominately expressed in placenta and in aldosterone target organs, such as kidney. In kidney, this enzyme confers aldosterone specificity on the mineralocorticoid receptor [28–30]. It achieves this by rapid conversion of active glucocorticoids into their inactive metabolites, and thus preventing glucocorticoids which circulate at 100–1000-fold higher levels than aldosterone from competing with aldosterone for the non-selective mineralocorticoid receptor [31, 32]. The physiological role of this isoform in placenta is relatively poorly understood although it has been proposed to be a mechanism by which the fetus is protected against high circulating levels of maternal glucocorticoids [33, 34].

Despite its potential physiological significance, placental 11β-HSD has been relatively poorly characterized until recently when a NAD-dependent 11β-HSD was purified from human placenta [27]. Furthermore, studies on placental 11β-HSD have been largely using human tissues [33, 35–43], and there is a lack of comparative studies in other mammals. In addition, those human studies have generated controversy as to its reversibility and cofactor dependence. We have previously cloned the ovine liver 11β-HSD cDNA [13], and have demonstrated the presence of 11β-HSD mRNA in ovine placenta. However, there are no reports on the characterization of ovine placental 11β-HSD. In the present study, we determined the characteristics of 11β-HSD activity, and also examined the expression of 11β-HSD mRNA, in ovine placental tissues at late pregnancy.

EXPERIMENTAL

Reagents and supplies

[1,2,6,7-^{3}H(N)]cortisol (70.1 Ci/mmol) was purchased from Du Pont Canada Inc. (Markham, Ontario). Purity was improved regularly by thin layer chromatography. Non-radioactive steroids were obtained from Steraloids Inc. (Wilton, NH). Co-factors (NAD, NADH, NADP, and NADPH) were purchased from Sigma Chemicals (St Louis, MO). Polyester-backed thin-layer chromatography (TLC) plates were obtained from Fisher Scientific Ltd. (Unionville, Ontario). All solvents used were OmniSolv grade from BDH Inc. (Toronto, Ontario).

Preparation of [1,2,6,7-^{3}H(N)]cortisone

[1,2,6,7-^{3}H(N)]cortisone was prepared from [1,2,6,7-^{3}H(N)]cortisol by the method of Shaw and Quincey [44]. Briefly, the labelled cortisol (10 μCi) was incubated in 1 ml of 50% aqueous acetic acid containing 1% (w/v) chromium trioxide at room temperature for 20 min. The residue from the dichloromethane extract of the reaction products was chromatographed by TLC using chloroform/methanol (9:1, v/v) as solvent, and non-radioactive cortisol and cortisone as reference markers. The cortisone-containing region was scraped off and eluted with ethyl acetate.

Protein estimation

Protein concentration was determined by the Bradford method using a Bio-Rad (Mississauga, Ontario) protein assay kit with bovine serum albumin as standard.

Assay of 11β-HSD activity

Preparation of tissue homogenates. Placental tissues (4–5 g), obtained from pregnant sheep at days 140–143, were homogenized in 5 vol. of ice-cold 10 mM sodium phosphate buffer, pH 7.0, containing 0.25 M sucrose (Buffer A). The homogenate was used immediately in assays for the respective 11β-dehydrogenase and 11-oxoreductase activity, as described below.

Assay of 11β-dehydrogenase activity. 11β-Dehydrogenase activity was determined by measuring the rate of conversion of cortisol to cortisone, as described previously [26, 45]. Briefly, the assay tubes contained approx. 100,000 cpm of the labelled cortisol, non-radioactive cortisol at final concentrations of 0.1, 5 or 10 μM, and 250 μM cofactor (NAD or NADP). Buffer B (0.1 M sodium phosphate buffer, pH 7.5) was added to bring the volume up to 0.4 ml. After 10 min incubation at 37°C, 100 μl of tissue homogenate containing 200–300 μg protein was added. After incubation for 30 min (preliminary studies indicated that the rate of reaction was linear with time from 15 to 120 min, and with the amount of tissue homogenates containing between 0.1–1.2 mg protein), the reaction was stopped and the steroids were extracted with 4 ml ethyl acetate containing 40 μg mixture of non-radioactive cortisol and cortisone as carrier steroids. The extracts were dried, and the residues were resuspended in 100 μl methanol. A fraction of the resuspension was spotted on a TLC plate which was developed in chloroform/methanol (9:1, v/v). The bands containing the labelled cortisol and cortisone were identified under UV light by the non-radioactive carriers, cut out into scintillation vials and counted in Scientisafe™ Econol 1 (Fisher Scientific, Toronto, Canada). From the specific activity of the labelled cortisol and the radioactivity of cortisone, the rate of cortisol to cortisone conversion was calculated, and expressed as the amount of cortisone (pmol) formed per min per mg protein.

Assay of 11-oxoreductase activity. 11-Oxoreductase activity was determined similarly except that cortisone was used as substrate, NADH or NADPH as cofactor, and Buffer C (0.1 M phosphate buffer, pH 6.0) replaced Buffer B.

Kinetic analysis. Kinetic analyses were performed as described previously [26], for the respective dehydrogenase and reductase, and also separately for the dehydrogenase using NAD or NADP as cofactor.

Conversion assays were conducted using a fixed amount of cofactor (250 μM), enzyme preparation (200–300 μg protein), and reaction time (30 min), but with varying amounts of substrate. The conditions were chosen so that the initial velocity was linear with the reaction time and the amount of enzyme preparation. In addition, the amount of the labelled substrate was increased proportionally with increasing amounts of non-labelled substrate in the kinetic studies such that the rate of conversion in the experimental tubes was always more than twice that in the blank tubes (no enzyme). In all cases, the rate of conversion in the blank tubes (ranging from 1 to 3% of the total substrate radioactivity) was subtracted from that in the experimental tubes (ranging from 48 to 8% of the total substrate radioactivity) before analysis. Each experiment was carried out in duplicate, and a total of four experiments were conducted. The data were plotted as a straight line of s/v against s according to the Michaelis–Menten Equation [46], and the K_m and V_{max} values were calculated from the intercepts of these plots as described [46].

RNA extraction and Northern blot analysis

Total cellular RNA was extracted from the same placental tissues as were used in enzyme activity assays, and also from one maternal liver and kidney, with lithium chloride/urea [47]. The size as well as the relative abundance of 11β-HSD mRNA was assessed by Northern blot analysis as described previously [13]. Briefly, denatured RNA samples (30 μg) were subjected to agarose gel (1%) electrophoresis in the presence of formaldehyde, and transferred overnight by capillary blotting to a Zeta-Probe membrane (Bio-Rad Canada Ltd., Mississauga, Ontario). The RNA was fixed by UV cross-linking (Gene Cross-Linker, Bio-Rad) to the membrane which was then baked under vacuum at 80°C for 60 min. The blot was hybridized at 42°C for 16 h in the presence of formamide (50%) and [^{32}P]sheep 11β-HSD cDNA. The ovine liver 11β-HSD cDNA [13] was labelled with [^{32}P]dCTP (ICN Biomedicals Canada Ltd., St Laurent, Quebec; 3000 Ci/mmol) by random priming [48]. We used a cDNA for mouse 18S rRNA as an internal control for gel loading and efficiency of RNA transfer. The same blot was stripped and reprobed with the radiolabelled 18S rRNA cDNA, as described previously [13, 49].

RESULTS

Cofactor preference

At 10 μM cortisol, the placental 11β-dehydrogenase displayed a tendency towards NADP preference, but showed clear preference for NAD ($P < 0.05$ when compared with NADP activity) at 100 nM cortisol (the activity in the presence of NAD and NADP was 270 ± 40 and 150 ± 30 fmol/min/mg protein, respectively) (Fig. 1). By contrast, the placental 11-oxoreductase displayed marked preference for NADPH regardless of cortisone concentration ($P < 0.05$ in both cases; Fig. 2).

Kinetic characteristics

With cortisol as physiological substrate, the placental 11β-HSD exhibited two distinct levels of 11β-dehydrogenase activity, one with higher affinity (K_m = 110 ± 20 nM) and lower capacity (V_{max} = 630 ± 80 fmol/min/mg protein) and NAD-dependent, the other with lower affinity (K_m = 12 ± 2 μM) and higher capacity (V_{max} = 6.3 ± 1.5 pmol/min/mg protein), and NADP-dependent (Fig. 3 and Table 1). However, the placental 11β-HSD showed a single NADPH-dependent 11-oxoreductase activity (K_m = 4.0 ± 0.9 μM and V_{max} = 3.1 ± 0.5 pmol/min/mg protein) (Fig. 3 and Table 1).

11β-HSD mRNA

As shown in Fig. 4, there was a single 1.8 kb 11β-HSD mRNA in the placenta, although its abundance was much lower than that in the liver. However, the 1.5 kb truncated 11β-HSD mRNA,

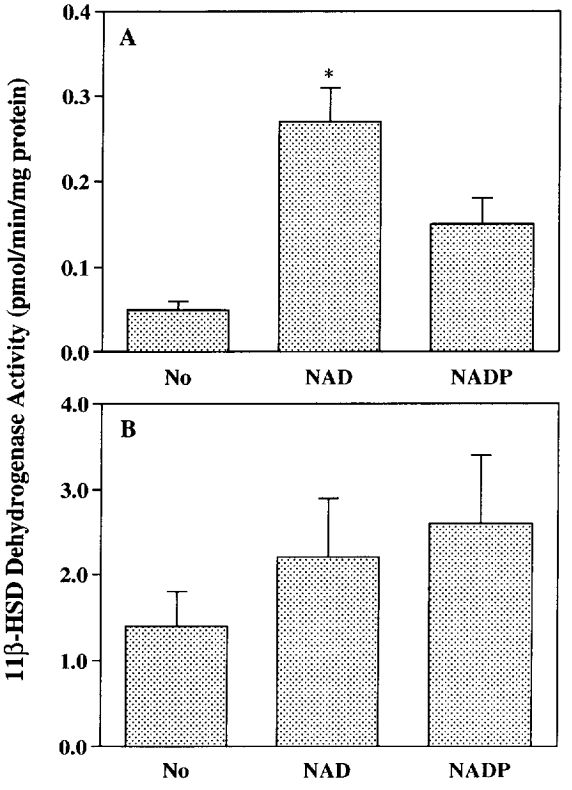

Fig. 1. Cofactor dependence of 11β-dehydrogenase activity in the presence of different substrate concentrations. Placental homogenates were incubated with 0.1 (A) and 10 μM (B) of cortisol in the absence and presence of NAD or NADP for determining 11β-dehydrogenase activity, as described in the Experimental. Each bar represents mean ± SEM (n = 4). Differences in NAD- and NADP-activities were analyzed by Student's t-test (*$P < 0.05$).

Table 1. Kinetic parameters of placental 11β-HSD in the presence of different cofactors

11β-HSD activity	K_m (μM)	V_{max} (pmol/min/mg protein)
Dehydrogenase (NAD)	0.11 ± 0.02	0.63 ± 0.08
Dehydrogenase (NADP)	12 ± 2	6.3 ± 1.5
Reductase (NADPH)	4.9 ± 0.9	3.1 ± 0.5

Kinetic studies were carried out using the placental homogenate as described in the Experimental. 50, 100, 200 and 500 nM of cortisol were used for the NAD-dependent 11β-dehydrogenase, and 5, 10, 20 and 50 μM of cortisol for the NADP-dependent 11β-dehydrogenase, respectively. For placental 11-oxoreductase, NADPH was used as cofactor, and 1, 2, 5 and 20 μM of cortisone were used as substrate. Values are mean ± SEM of four separate experiments.

which was present in the kidney, was not detectable in the placental total RNA samples by Northern blotting.

DISCUSSION

Placental 11β-HSD regulates the transfer of maternal glucocorticoids to the fetus, and therefore protects the fetus from exposure to the growth-inhibiting effects of maternal glucocorticoids [33, 34, 50]. In the present study, we have characterized 11β-HSD activity in the ovine placenta. Our results demonstrate that two distinct species of 11β-HSD are expressed in the ovine placenta. One is a reversible NADP(H)-dependent enzyme, and the other is NAD-dependent with little reductase activity. Furthermore, since the truncated 11β-HSD mRNA is absent from the placental tissues, our results reinforce the notion that the NAD-dependent enzyme identified in placenta and kidney is the product of a gene distinct from 11β-HSD [3, 25].

In our previous studies, we cloned the ovine liver 11β-HSD cDNA which encodes a protein of 292 amino

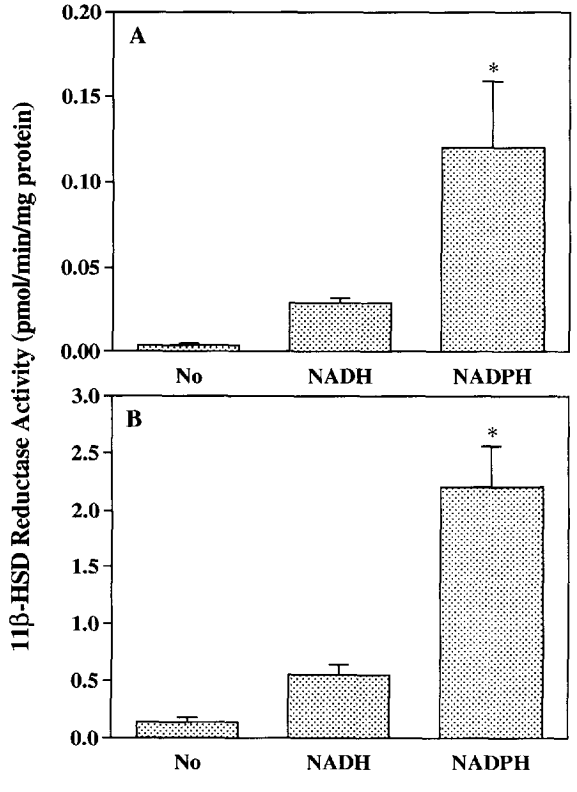

Fig. 2. Cofactor dependence of 11-oxoreductase activity in the presence of different substrate concentrations. Placental homogenates were incubated with 0.1 (A) and 5 μM (B) of cortisone in the absence and presence of NADH or NADPH for determining 11-oxoreductase activity, as described in the Experimental. Each bar represents mean ± SEM ($n = 4$). Differences in NADH- and NADPH-activities were analyzed by Student's t-test ($^*P < 0.05$).

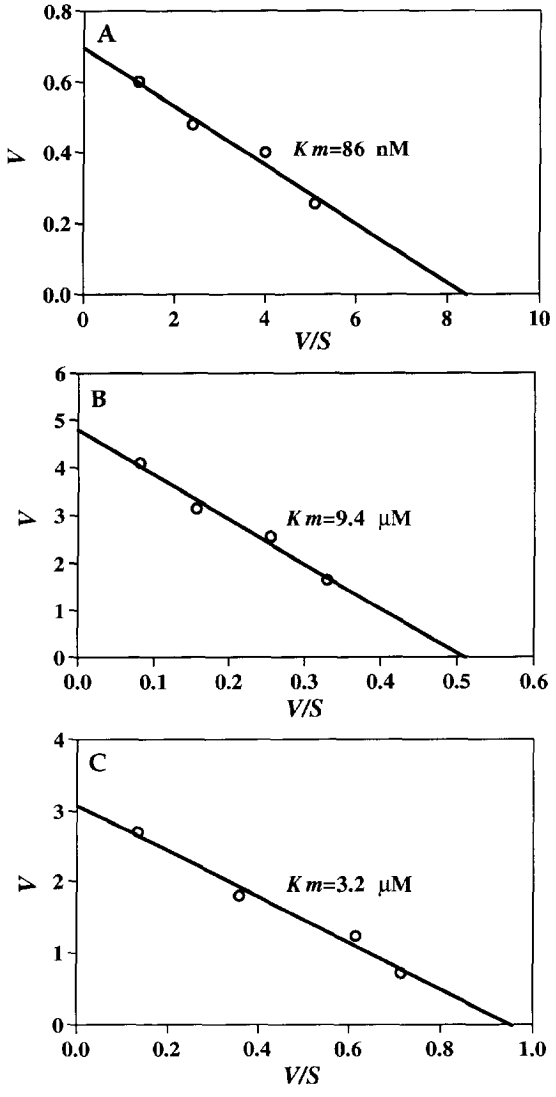

Fig. 3. Plots according to the Michaelis–Menten equation for determining kinetic parameters. Data from a representative experiment for the NAD-dependent dehydrogenase (A), NADP-dependent dehydrogenase (B) and NADPH-dependent reductase (C) are shown. Assays were conducted with varying amounts of cortisol (for dehydrogenase) or cortisone (for reductase), as described in the Experimental. s, substrate concentration (μM); v, velocity (pmol/min/mg protein).

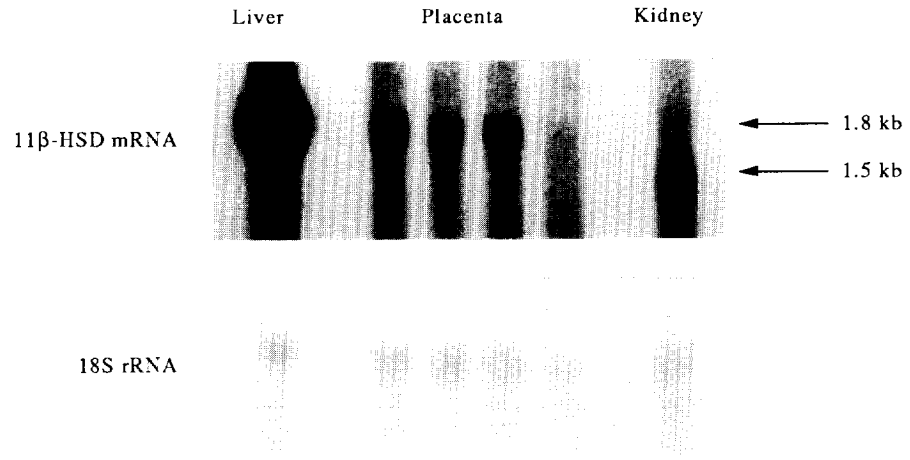

Fig. 4. Northern blot of 11β-HSD mRNA. Total RNA samples (30 μg) were analyzed, and the top panel is an autoradiograph of the blot probed with [^{32}P]sheep 11β-HSD cDNA (3 days exposure time). As a control, the same blot was rehybridized with [^{32}P]mouse 18S rRNA cDNA (bottom panel; exposure time 1 h).

acids with two potential sites for N-glycosylation [13]. We also characterized 11β-HSD activity in the ovine liver and kidney [26]. We showed that the hepatic enzyme was reversible, NADP(H)-dependent, and had a K_m of 8 μM for cortisol. By contrast, the renal 11β-HSD contained only dehydrogenase activity that was NAD-dependent and had a K_m of 69 nM for cortisol [26]. In the present study, we have shown that the ovine placenta contains NADP-dependent dehydrogenase and NADPH-dependent reductase activities. Furthermore, the kinetic characteristics of these activities are similar/identical to those described previously for the liver 11β-HSD [26]. In addition, the 1.8 kb 11β-HSD mRNA, which is predominantly expressed in the liver [13], is present in the placental tissues. Thus, the NADP(H)-dependent 11β-HSD in the ovine placenta represents the well-defined liver-type enzyme and is encoded by the 11β-HSD gene. On the other hand, the placental NAD-dependent activity represents the kidney-type enzyme since it has a similar K_m for cortisol and lacks the corresponding NADH-dependent reductase activity. Taken together, these results provide strong evidence for the co-existence of two distinct species of 11β-HSD in the ovine placenta.

Unlike the rat kidney where both the 1.8 kb and the 1.5 kb truncated 11β-HSD transcripts are expressed [51], the sheep kidney contains only the truncated transcript [13]. It is known that the truncated 11β-HSD transcript gives rise to a truncated protein lacking 26 (rat) [52] or 30 (human and sheep) [12, 13] amino acids at the N-terminus of the liver protein. If the distinct 11β-HSD activity we characterized previously in the ovine kidney is associated with this truncated protein, it is reasonable to expect that the placenta, which contains the kidney-type activity, expresses the truncated transcript. Our results indicate that this is unlikely to be the case since under the conditions of the present study we were unable to detect the truncated 11β-HSD transcript in the placenta. This conclusion is consistent with the findings that the expressed truncated protein in CHO cells is enzymatically inactive [53]. Thus, the NAD-dependent 11β-HSD activity in the placenta resides in an enzyme distinct from the well-defined liver protein. Direct evidence in support of this came from a recent study in which a 40 kDa NAD-dependent 11β-HSD was purified from human placental tissues [27]. Although the characteristics of NAD-dependent 11β-HSD activity are similar in kidney and placenta, it remains to be determined whether the same protein is produced in the two organs.

11β-HSD activity in human placenta was first demonstrated by Osinski in 1960 [35]. This was later confirmed and extended by several independent studies in which the question of cofactor-dependence [36, 38, 42, 54] and reversibility [33, 37–39, 55] was addressed. Although conflicting results were reported, the general consensus, based largely upon more recent studies [43], is that placental 11β-HSD in the human, as we showed here in the sheep, is reversible and displays preference for both NAD and NADP. Although the K_m of the placental NAD-dependent 11β-HSD is similar between human [3] and sheep, the K_m of the placental NADP-dependent 11β-HSD in the sheep is about five times higher than in the human [42, 43]. The precise reasons for this difference are not clear at present, although it remains possible that this may reflect species differences in the placental 11β-HSD-mediated control of cortisol transfer between mother and fetus. If these *in vitro* data can be extrapolated to the physiological reactions occurring in the intact placenta, they suggest that the NAD-dependent 11β-HSD may function much more effectively than the NADP-dependent enzyme since the former has an affinity for cortisol similar to the physiological level of this hormone in circulation [56]. The potential physiological significance of placental

11β-HSD will be fully appreciated when the relative cellular distribution of these two isozymes, and changes associated with advancing pregnancy have been determined. It has been shown that 11β-dehydrogenase activity in human placenta decreases towards term [39, 42]. However, there were no changes in human placental 11β-HSD activity in relation to parturition [40]. In the baboon placenta, 11β-HSD activity changed from reduction at mid-gestation to oxidation at term [57]. Further studies in other mammalian species are required to establish whether species differences exist with respect to characteristics of placental 11β-HSD activity and changes associated with pregnancy and labor.

The physiological significance of placental 11β-HSD has been, for many years, a matter of speculation. It has been proposed that placental 11β-HSD protects the fetus from the growth-retarding effects of maternal glucocorticoids [33, 34, 50]. Thus, abnormally low placental 11β-HSD activity, by increasing fetal exposure to maternal glucocorticoids, leads to intrauterine growth retardation and high blood pressure in adulthood. This exciting hypothesis is supported by the experimental evidence that rat placental 11β-HSD activity correlated positively with term fetal weight. Furthermore, offspring of rats treated during pregnancy with dexamethasone (which is not metabolized by 11β-HSD) had lower birthweights and higher blood pressure when adult than did offspring of control rats [50]. If this proves to be true in the human, it will have significant clinical implications.

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REFERENCES

1. Monder C. and Shackleton C. H. L.: 11beta-Hydroxysteroid dehydrogenase: fact or, fancy? *Steroids* **44** (1984) 383–417.
2. Kenouch S., Coutry N., Farman N. and Bonvalet J. P.: Multiple patterns of 11beta-hydroxysteroid dehydrogenase catalytic activity along the mammalian nephron. *Kidney Int.* **42** (1992) 56–60.
3. Brown R. W., Chapman K. E., Edwards C. R. and Seckl J. R.: Human placental 11beta-hydroxysteroid dehydrogenase: evidence for and partial purification of a distinct NAD-dependent isoform. *Endocrinology* **132** (1993) 2614–2621.
4. Náray-Fejes-Tóth A., Rusvai E., Denault D. L., St. Germain D. L. and Fejes-Tóth G.: Expression and characterization of a new species of 11β-hydroxysteroid dehydrogenase in *Xenopus* oocytes. *Am. J. Physiol. Renal, Fluid Electrolyte Physiol.* **265** (1993) F896–F900.
5. Low S. C., Assaad S. N., Rajan V., Chapman K. E., Edwards C. R. W. and Seckl J. R.: Regulation of 11β-hydroxysteroid dehydrogenase by sex steroids *in vivo*: further evidence for the existence of a second dehydrogenase in rat kidney. *J. Endocrinol.* **139** (1993) 27–35.
6. Náray-Fejes-Tóth A., Rusvai E. and Fejes-Tóth G.: Is the renal type III corticosteroid-binding site the collecting duct-specific isoform of 11β-hydroxysteroid dehydrogenase? *Endocrinology* **134** (1994) 1671–1675.
7. Lakshmi V. and Monder C.: Purification and characterization of the corticosteroid 11beta-dehydrogenase component of the rat liver 11beta-hydroxysteroid dehydrogenase complex. *Endocrinology* **123** (1988) 2390–2398.
8. Agarwal A. K., Monder C., Eckstein B. and White P. C.: Cloning and expression of rat cDNA encoding corticosteroid 11β-dehydrogenase. *J. Biol. Chem.* **264** (1989) 18,939–18,943.
9. Monder C. and Lakshmi V.: Corticosteroid 11beta-dehydrogenase of rat tissues: immunological studies. *Endocrinology* **126** (1990) 2435–2443.
10. Moisan M. P., Seckl J. R. and Edwards C. R.: 11beta-hydroxysteroid dehydrogenase bioactivity and messenger RNA expression in rat forebrain: localization in hypothalamus, hippocampus, and cortex. *Endocrinology* **127** (1990) 1450–1455.
11. Lakshmi V., Sakai R. R., McEwen B. S. and Monder C.: Regional distribution of 11beta-hydroxysteroid dehydrogenase in rat brain. *Endocrinology* **128** (1991) 1741–1748.
12. Tannin G. M., Agarwal A. K., Monder C., New M. I. and White P. C.: The human gene for 11β-hydroxysteroid dehydrogenase. *J. Biol. Chem.* **266** (1991) 16,653–16658.
13. Yang K., Smith C. L., Dales D., Hammond G. L. and Challis J. R.: Cloning of an ovine 11beta-hydroxysteroid dehydrogenase complementary deoxyribonucleic acid: tissue and temporal distribution of its messenger ribonucleic acid during fetal and neonatal development. *Endocrinology* **131** (1992) 2120–2126.
14. Monder C.: The forms and functions of 11beta-hydroxysteroid dehydrogenase. *J. Steroid Biochem. Molec. Biol.* **45** (1993) 161–165.
15. Maser E. and Bannenberg G.: The purification of 11β-hydroxysteroid dehydrogenase from mouse liver microsomes. *J. Steroid Biochem. Molec. Biol.* **48** (1994) 257–263.
16. Moore C. C. D., Mellon S. H., Murai J., Siiteri P. K. and Miller W. L.: Structure and function of the hepatic form of 11beta-hydroxysteroid dehydrogenase in the squirrel monkey, an animal model of glucocorticoid resistance. *Endocrinology* **133** (1993) 368–375.
17. Phillips D. M., Lakshmi V. and Monder C.: Corticosteroid 11beta-dehydrogenase in rat testis. *Endocrinology* **125** (1989) 209–216.
18. Edwards C. R. and Stewart P. M.: The cortisol-cortisone shuttle and the apparent specificity of glucocorticoid and mineralocorticoid receptors. *J. Steroid Biochem. Molec. Biol.* **39** (1991) 859–865.
19. Seckl J. R., Kelly P. A. and Sharkey J.: Glycyrrhetinic acid, an inhibitor of 11beta-hydroxysteroid dehydrogenase, alters local cerebral glucose utilization *in vivo*. *J. Steroid Biochem. Molec. Biol.* **39** (1991) 777–779.
20. Walker B. R., Yau J. L., Brett L. P., Seckl J. R., Monder C., Williams B. C. and Edwards C. R.: 11beta-hydroxysteroid dehydrogenase in vascular smooth muscle and heart: implications for cardiovascular responses to glucocorticoids. *Endocrinology* **129** (1991) 3305–3312.
21. Stewart P. M. and Sheppard M. C.: Novel aspects of hormonal action: intracellular ligand supply and its control by a series of tissue specific enzymes. *Molec. Cell. Endocrinol.* **83** (1992) C13–C18.
22. Whorwood C. B., Franklyn J. A., Sheppard M. C. and Stewart P. M.: Tissue localization of 11β-hydroxysteroid dehydrogenase and its relationship to the glucocorticoid receptor. *J. Steroid Biochem. Molec. Biol.* **41** (1992) 21–28.
23. Seckl J. R., Dow R. C., Low S. C., Edwards C. R. and Fink G.: The 11beta-hydroxysteroid dehydrogenase inhibitor glycyrrhetinic acid affects corticosteroid feedback regulation of hypothalamic corticotrophin-releasing peptides in rats. *J. Endocrinol.* **136** (1993) 471–477.
24. Monder C., Miroff Y., Marandici A. and Hardy M. P.: 11β-Hydroxysteroid dehydrogenase alleviates glucocorticoid-mediated inhibition of steroidogenesis in rat Leydig cells. *Endocrinology* **134** (1994) 1199–1204.
25. Rusvai E. and Naray Fejes Toth A.: A new isoform of 11beta-hydroxysteroid dehydrogenase in aldosterone target cells. *J. Biol. Chem.* **268** (1993) 10,717–10,720.
26. Yang K. and Yu M.: Evidence for distinct isoforms of 11beta-hydroxysteroid dehydrogenase in the ovine liver and kidney. *J. Steroid Biochem. Molec. Biol.* **49** (1994) 245–250.
27. Brown R. W., Chapman K. E., Edwards C. R. W. and Seckl J. R.: Isolation of 11beta-hydroxysteroid dehydrogenase type 2 from human placenta reveals the product of a novel gene.

76th Annual Meeting of The Endocrine Society, Anaheim, CA (1994) Abstr. 1428.
28. Stewart P. M., Corrie J. E., Shackleton C. H. and Edwards C. R.: Syndrome of apparent mineralocorticoid excess. A defect in the cortisol-cortisone shuttle. *J. Clin. Invest.* **82** (1988) 340–349.
29. Edwards C. R., Stewart P. M., Burt D., Brett L., McIntyre M. A., Sutanto W. S., de Kloet E. R. and Monder C.: Localisation of 11beta-hydroxysteroid dehydrogenase—tissue specific protector of the mineralocorticoid receptor. *Lancet* 2 (1988) 986–989.
30. Funder J. W., Pearce P. T., Smith R. and Smith A. I.: Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* **242** (1988) 583–585.
31. Krozowski Z. and Funder J. W.: Renal mineralocorticoid receptors and hippocampal corticosterone-binding species have identical intrinsic steroid specificity. *Proc. Natn. Acad. Sci. U.S.A.* **80** (1983) 6056–6060.
32. Arriza J. L., Weinberger C., Cerelli G., Glaser T. M., Handelin B. L., Housman D. E. and Evans R. M.: Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* **237** (1987) 268–275.
33. Murphy B. E.: Ontogeny of cortisol-cortisone interconversion in human tissues: a role for cortisone in human fetal development. *J. Steroid Biochem.* **14** (1981) 811–817.
34. Edwards C. R., Benediktsson R., Lindsay R. S. and Seckl J. R.: Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? *Lancet* **341** (1993) 355–357.
35. Osinski P. A.: Steroid 11beta-ol dehydrogenase in human placenta. *Nature* **187** (1960) 777.
36. Meigs R. A. and Engel L. L.: The metabolism of adrenocortical steroids by human tissues. *Endocrinology* **69** (1961) 152–162.
37. Pasqualini J. R., Marfil J., Garnier F., Wiqvist N. and Diczfalusy E.: Studies on the metabolism of corticosteroids in the human foeto-placental unit. 4. Metabolism of deoxycorticosterone and corticosterone administered simultaneously into the intact umbilical circulation. *Acta Endocrinol. Copenh.* **64** (1970) 385–397.
38. Lopez Bernal A., Flint A. P. F., Anderson A. B. M. and Turnbull A. C.: 11beta-hydroxysteroid dehydrogenase activity in human placenta and decidua. *J. Steroid Biochem.* **13** (1980) 1081–1087.
39. Giannopoulos G., Jackson K. and Tulchinsky D.: Glucocorticoid metabolism in human placenta, decidua, myometrium and fetal membranes. *J. Steroid Biochem.* 17 (1982) 371–374.
40. Lopez Bernal A., Anderson A. B. M. and Turnbull A. C.: The lack of influence of labor on human placental 11beta-hydroxysteroid dehydrogenase activity. *J. Clin. Endocrinol. Metab.* 54 (1982) 1251–1254.
41. Lopez Bernal A. and Turnbull A. C.: Cortisol metabolism in human placenta and decidua is resistant to anoxia. *Horm. Metab. Res.* **17** (1985) 167.
42. Blasco M. J., Lopez Bernal A. and Turnbull A. C.: 11beta-Hydroxysteroid dehydrogenase activity of the human placenta during pregnancy. *Horm. Metab. Res.* **18** (1986) 638–641.
43. Lakshmi V., Nath N. and Muneyyirci Delale O.: Characterization of 11beta-hydroxysteroid dehydrogenase of human placenta: evidence for the existence of two species of 11beta-hydroxysteroid dehydrogenase. *J. Steroid Biochem. Molec. Biol.* **45** (1993) 391–397.
44. Shaw D. A. and Quincey R. V.: The preparation of tritium-labelled cortisol metabolites of high specific activity. *J. Endocrinol.* **26** (1963) 577–578.
45. Yang K., Berdusco E. T. M. and Challis J. R. G.: Opposite effects of glucocorticoid on hepatic 11beta-hydroxysteroid dehydrogenase mRNA and activity in fetal and adult sheep. *J. Endocrinol.* **143** (1994) 121–126.
46. Henderson P. J. F.: Statistical analysis of enzyme kinetic data. In *Enzyme Assays: A Practical Approach* (Edited by R. Eisenthal and M. J. Danson). Oxford University Press, NY (1992) pp. 277–316.
47. Auffray C. and Rougeon F.: Purification of mouse immunoglobulin heavy-chain mRNAs from total myeloma tumor RNA. *Eur. J. Biochem.* **107** (1980) 303–314.
48. Feinberg A. P. and Vogelstein B.: A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132** (1983) 6–13.
49. Yang K., Hammond G. L. and Challis J. R. G.: Characterization of an ovine glucocorticoid receptor cDNA and developmental changes in its mRNA levels in the fetal sheep hypothalamus, pituitary gland and adrenal. *J. Molec. Endocrinol.* 8 (1992) 173–180.
50. Benediktsson R., Lindsay R. S., Noble J., Seckl J. R. and Edwards C. R.: Glucocorticoid exposure *in utero*: new model for adult hypertension. *Lancet* **341** (1993) 339–341.
51. Krozowski Z., Stuchbery S., White P., Monder C. and Funder J. W.: Characterization of 11beta-hydroxysteroid dehydrogenase gene expression: identification of multiple unique forms of messenger ribonucleic acid in the rat kidney. *Endocrinology* **127** (1990) 3009–3013.
52. Krozowski Z., Obeyesekere V., Smith R. and Mercer W.: Tissue-specific expression of an 11beta-hydroxysteroid dehydrogenase with a truncated N-terminal domain. A potential mechanism for differential intracellular localization within mineralocorticoid target cells. *J. Biol. Chem.* **267** (1992) 2569–2574.
53. Obeid J., Curnow K. M., Aisenberg J. and White P. C.: Transcripts originating in intron 1 of the HSD11 (11beta-hydroxysteroid dehydrogenase) gene encode a truncated polypeptide that is enzymatically inactive. *Molec. Endocrinol.* 7 (1993) 154–160.
54. Pepe G. J. and Albrecht E. D.: Comparison of cortisol-cortisone interconversion *in vitro* by the human and baboon placenta. *Steroids* 44 (1984) 229–240.
55. Roy S. and Pasqualini J. R.: Transformation of corticosterone in the foetal and placental compartments of the rat. *Acta Endocrinol. Copenh.* **69** (1972) 689–700.
56. Bettins I. Z., Kowarski A., Shermeta D. W., De Lemos R. A. and Migeon C. J.: Fetal and maternal secretion rate of cortisol in sheep: diffusion resistance of the placenta. *Pediat. Res.* 4 (1970) 129–134.
57. Pepe G. J. and Albrecht E. D.: Transuteroplacental metabolism of cortisol and cortisone during mid- and late gestation in the baboon. *Endocrinology* **115** (1984) 1946–1951.