Regulation of 11 β -hydroxysteroid dehydrogenase type 2 activity in ovine placenta by fetal cortisol

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Abstract

The effect of fetal cortisol on the activity of the type 2 isoform of the enzyme, 11 β -hydroxysteroid dehydrogenase (11 β -HSD2), was examined in ovine placenta and fetal kidney by measuring tissue 11 β -HSD2 activity during late gestation when endogenous fetal cortisol levels rise and after exogenous cortisol administration to immature fetuses before the prepartum cortisol surge. Placental 11 β -HSD2 activity decreased between 128–132 days and term ($\approx$ 145 days of gestation) in association with the normal prepartum increase in fetal plasma cortisol. Raising fetal cortisol levels to prepartum values in the immature fetus at 128–132 days of gestation reduced placental 11 β -HSD2 activity to term values. In contrast, 11 β -HSD2

activity in the fetal renal cortex was unaffected by gestational age or cortisol infusion. When all the data were combined, there was an inverse correlation between the log fetal plasma cortisol level at delivery and placental 11 β -HSD2 activity, expressed both on a weight-specific basis and per mg placental protein. Fetal cortisol therefore appears to be a physiological regulator of placental, but not renal, 11 β -HSD2 activity in fetal sheep during late gestation. These findings have important implications, not only for glucocorticoid exposure *in utero*, but also for the local actions of cortisol within the placental tissues that are involved in initiating parturition in the sheep.

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Introduction

The enzyme, 11 β -hydroxysteroid dehydrogenase (11 β -HSD), interconverts the active glucocorticoids, cortisol and corticosterone, to their inactive 11-keto metabolites. In mammals, the enzyme has two isoforms (Krozowski *et al.* 1999). The type 1 isoform (11 β -HSD1) is bidirectional and can act either as a dehydrogenase (cortisol to cortisone) or as a reductase (cortisone to cortisol) depending on cofactor availability (Yang 1995a, Krozowski *et al.* 1999). The second isoform, 11 β -HSD2, is unidirectional and converts cortisol to cortisone in the presence of NAD (Yang 1995a). The 11 β -HSD1 isoform is found in a number of adult tissues while 11 β -HSD2 is present primarily in aldosterone-sensitive tissues, such as the kidney, colon and salivary glands (White *et al.* 1997, Burton & Waddell 1999). Studies in several species including the sheep have shown a similar distribution of 11 β -HSD isoforms in fetal tissues with high 11 β -HSD2 expression in the fetal kidneys (Langlois *et al.* 1995, Stewart *et al.* 1995b, Wood & Srun 1995, Brown *et al.* 1996).

Gene expression and bioactivity of 11 β -HSD have also been detected in the placenta of several species, including the sheep, human, baboon, pig, horse, guinea pig, rat and

mouse (Baggia *et al.* 1990, Chavatte *et al.* 1995, Kim *et al.* 1995, Stewart *et al.* 1995a, Brown *et al.* 1996, Klemcke & Christenson 1996, Sampath-Kumar *et al.* 1998, Waddell *et al.* 1998). Both 11 β -HSD isoforms are expressed in the placenta but, based on cofactor preference, 11 β -HSD2 appears to be the predominant placental isoform in most of these species (Yang 1997). By contrast, in ovine placenta, 11 β -HSD1 appears to be the predominant mRNA transcript although both isoforms appear to be equally active in converting cortisol to cortisone in this tissue (Yang 1995b, Yang *et al.* 1997, Murotsuki *et al.* 1998). In primates and rodents, 11 β -HSD2 has been localized specifically to surfaces within the placenta involved in fetal–maternal exchange (Krozowski *et al.* 1995, Brown *et al.* 1996, Pepe *et al.* 1996). Placental 11 β -HSD2, therefore, acts as a barrier to the transplacental passage of bioactive glucocorticoids and, thereby, limits fetal exposure to the higher glucocorticoid concentrations found in the maternal circulation (Sun *et al.* 1998b, Burton & Waddell 1999). This action of placental 11 β -HSD has an important impact on fetal development because glucocorticoids regulate tissue growth and differentiation in the fetus (Fowden *et al.* 1998).

In rodents and baboons, placental 11 β -HSD expression and activity are influenced by gestational age, nutritional

Table 1 The number and gestational ages of the fetuses in the different experimental groups used in the study

		Gestational age (days)		No. of fetuses	
	Subsequent treatment	At catheterization	At tissue collection		
Surgical treatment	Unoperated	Untreated	—	140–142	3
	Catheterized	Saline infused	115–118	128–132	6
		Cortisol infused	115–118	128–132	8
		Untreated	127–132	140–142	4

state and sex steroid concentrations (Baggia *et al.* 1990, Langley-Evans *et al.* 1996, Sun *et al.* 1998b, Waddell *et al.* 1998, Bertram *et al.* 2001). Gestational trends in placental 11β-HSD2 gene expression and/or activity have also been observed in sheep, pigs, guinea pigs and humans (Stewart *et al.* 1995a, Klemcke & Christenson 1996, Sampath-Kumar *et al.* 1988, Whorwood *et al.* 2001). Similarly, there are changes in renal 11β-HSD2 expression and activity between mid and late gestation and in response to hypoxaemia, acidosis and undernutrition in the sheep fetus (Langlois *et al.* 1995, Wood & Srun 1995, Asano *et al.* 1997, Whorwood *et al.* 2001). In sheep and other species, the fetal adrenal output of glucocorticoids increases towards term and in response to adverse intra-uterine conditions, such as undernutrition and hypoxaemia (Fowden & Silver 1995, Challis *et al.* 1999). Glucocorticoids have been shown to regulate 11β-HSD1 activity in fetal ovine liver (Yang *et al.* 1994) but little is known about their effects on 11β-HSD2 activity in the placenta or other fetal tissues, such as the kidney. Hence, in the present study, 11β-HSD2 activity was measured in ovine placenta and fetal kidney during the period of late gestation when endogenous fetal cortisol levels rise and after exogenous cortisol administration to fetuses before the prepartum cortisol surge.

Materials and Methods

Animals

A total of 21 Welsh Mountain sheep fetuses of known gestational age were used in this study. All fetuses were alive at delivery. Food but not water was withdrawn from the ewes (*n*=18) 18–24 h before surgery. All procedures were carried out under the Animals (Scientific Procedures) Act 1986.

Surgical procedures

Under halothane anaesthesia (1.5% in O₂/N₂O), catheters were inserted into the dorsal aorta and caudal vena cava in

17 of the fetuses using the surgical procedures described previously (Fowden *et al.* 1996). The numbers and gestational ages of the fetuses at catheterization are shown in Table 1 (term 145 ± 2 days). Antibiotic was given to the fetus intravenously at the end of surgery (100 mg ampicillin; Penbritin, Smith Kline Beecham, Dun Laoghaire, Co Dublin, Ireland) and to the mother intramuscularly on the day of surgery and for 3 days thereafter (1 g procaine benzyl-penicillin; Depocillin, Intervet, Milton Keynes, Bucks, UK).

Experimental procedures

Arterial blood samples of 2 ml were taken daily during the experimental period to monitor fetal well-being and to determine plasma cortisol concentrations. At least 6 days after catheterization, 14 fetuses were infused intravenously with either cortisol (*n*=8; 1–3 mg/day in 2.5 ml 0.9% (w/v) saline; EF-Cortelan; Glaxo Ltd, Greenford, Middx, UK) or saline (*n*=6; 2.5 ml/day 0.9% (w/v)) for 5 days beginning between 123 and 127 days of gestation. Treatment was continued until tissue collection which occurred at the end of day 5 of infusion. Fetuses treated with cortisol were chosen at random. The dose of cortisol was designed to produce concentrations of plasma cortisol similar to those observed in the immediate prepartum period (Fowden *et al.* 1996). The four remaining fetuses catheterized late in gestation were sampled daily until delivery at 140–142 days of gestation (Table 1).

Tissue collection

All operated fetuses, regardless of previous treatment, and four additional unoperated, untreated fetuses were delivered by Caesarean section under sodium pentobarbitone anaesthesia (20 mg/ml, i.v.); details of the numbers and the ages of the fetuses at delivery are given in Table 1. Blood samples were taken from the fetuses at the time of delivery either through the indwelling catheters immediately before anaesthesia or by venipuncture from the umbilical artery after anaesthesia had been induced.

After administration of a lethal dose of anaesthetic to the fetus and ewe (200 mg/kg sodium pentobarbitone), samples of placenta (two to three placentomes) and fetal kidney were collected and frozen rapidly in liquid nitrogen before storage at -80°C . All blood samples were centrifuged immediately at 4°C and the plasma was stored at -20°C .

Biochemical analyses

Cortisol Plasma cortisol concentrations were measured by radioimmunoassay validated for ovine plasma (Robinson *et al.* 1984). The interassay coefficient of variation was 10% and the minimum detectable quantity of cortisol was 1.5 ng/ml.

Tissue 11 β -HSD2 Tissue 11 β -HSD2 activity was measured using the method described by Wood & Srun (1995). Known weights of placenta and renal cortex were homogenized (0.2 g/ml) in ice-cold Krebs–Henseleit buffer containing sodium bicarbonate (pH 7.4). The homogenate was diluted depending on tissue and treatment before measurement of 11 β -HSD2 activity using NAD as the cofactor. Each assay contained tritiated cortisol (45 000 d.p.m., specific activity 58 Ci/mmol; Amersham Pharmacia Biotech, Amersham, Bucks, UK), unlabelled cortisol (0.1 μM ; Sigma, Poole, Dorset, UK) and NAD (400 μM ; Sigma) in 0.4 ml Krebs–Henseleit buffer (pH 7.4). The mixture of substrate and cofactor was warmed at 37°C for 10 min before 100 μl tissue homogenate containing 0.5–1.2 mg protein was added. After incubation for times chosen to give a linear rate of reaction (2–10 min), the reaction was stopped by addition of ice-cold ethyl acetate (5 ml) containing 20 μg of both unlabelled cortisol and cortisone (Sigma) as internal carriers for the chromatography. After extraction of the steroids, the extracts were dried under an air stream at 37°C , re-dissolved in a small volume (120 μl) of absolute ethanol and spotted onto thin-layer chromatography plates (LK5F Silica gel 150Å; Whatman, Clifton, New Jersey, USA). The plates were developed using a mixture of chloroform and methanol (9:1, v/v). The bands containing cortisol and cortisone were visualized under UV light and excised into scintillation vials. Liquid scintillation cocktail (Optiphase II, Hisafe; Wallac Oy, Turku, Finland) was added and the resulting counts corrected for quenching.

The conversion of cortisol to cortisone was expressed as the percentage conversion of recovered tritium counts in the cortisone band. Using the specific activity of the cortisol and the percentage conversion to cortisone, the conversion rate of cortisol to cortisone was calculated and expressed as the amount of cortisone (pmol) synthesized per min. The assay was performed in duplicate at each time-point and a minimum of three time-points were used per sample assayed. Blanks which contained no tissue homogenate were included in each assay to allow correc-

tion for non-enzymatic oxidation. The protein content of the homogenate was measured using the Lowry assay (Lowry *et al.* 1951). Using a placental sample, the inter-assay coefficients of variation for the protein and 11 β -HSD2 assays were 8.5% and 12.0% respectively. Kinetic parameters were calculated for placental tissue by Lineweaver–Burke plot over substrate concentrations of 0.03–0.3 μM cortisol. The apparent K_m (78–85 nM) and maximum velocity of reaction (11–13 nmol/min per mg protein) were similar to the values published previously for 11 β -HSD-2 in ovine placenta during late gestation (Yang 1995b).

Statistical analyses

Means and S.E.M. have been given throughout and statistical analysis was made using Sigmaplot software (SPSS Inc., Chicago, IL, USA). Statistical significance was assessed by one-way ANOVA using Tukey or Kruskal–Wallis post-hoc tests, as appropriate. Correlation coefficients were calculated by linear regression analyses and assessed for significance using Fisher's *t*-test. Probabilities of less than 5% were considered significant. Since there were no differences between the catheterized and unoperated fetuses at 140–142 days, values from these two groups were combined for all subsequent analyses.

Results

The effects of gestational age

At delivery, the arterial concentration of plasma cortisol was significantly higher at 140–142 days than in the saline-infused animals at 128–132 days of gestation (Table 2). Fetal, but not placental weight, was also significantly greater at 140–142 days than earlier in gestation (Table 2). At 140–142 days, 11 β -HSD2 activity per gram placenta was significantly lower than the value in the saline-infused fetuses at 128–132 days (Table 3). Since there was no significant change in the placental protein content with increasing gestational age (Table 3), placental 11 β -HSD2 activity per mg protein was also significantly less at 140–142 days than in the saline-infused fetuses at 128–132 days of gestation (Table 3). In contrast, there was no apparent gestational trend in 11 β -HSD2 activity in fetal renal cortex either on a weight-specific basis or when expressed per mg protein (Table 3). However, the protein content of the fetal kidney was significantly lower at 140–142 days than in the saline-infused animals at 128–132 days of gestation (Table 3). No significant differences in blood pH or gas tensions were observed with increasing gestational age (Table 2).

The effects of fetal cortisol infusion

Cortisol infusion into the immature fetus raised plasma cortisol concentrations to parturition values. At delivery at

Table 2 Mean (± S.E.M.) values of plasma cortisol concentrations, blood pH and gas tensions and of fetal and placental wet weight at delivery in saline- and cortisol-infused fetuses at 128–132 days and in untreated fetuses at 140–142 days of gestation. Numbers of animals are shown in parentheses

	Treatment	Cortisol (ng/ml)	Weight (g)		Blood pH	Blood gases (mmHg)	
			Fetus	Placenta		pO ₂	pCO ₂
Days of gestation							
128–132	Saline infused	15.1 ± 2.2 ^a (6)	2730 ± 247 ^a (6)	315 ± 30 ^a (5)	7.364 ± 0.014 ^a (5)	18.0 ± 0.8 ^a (5)	51.2 ± 2.2 ^a (5)
	Cortisol infused	59.2 ± 6.9 ^b (8)	2822 ± 152 ^a (8)	318 ± 21 ^a (7)	7.354 ± 0.012 ^a (8)	19.6 ± 1.0 ^a (8)	52.0 ± 1.7 ^a (8)
140–142	Untreated	83.7 ± 13.6 ^b (7)	3632 ± 243 ^b (7)	308 ± 14 ^a (5)	7.340 ± 0.014 ^a (4)	19.0 ± 2.0 ^a (4)	53.7 ± 2.2 ^a (4)

Values in columns with different superscripts are significantly different from each other ($P < 0.01$; ANOVA).

128–132 days, the mean cortisol concentration in the cortisol-infused fetuses was significantly higher than that in the saline-infused fetuses and was similar to the value seen in the older, untreated fetuses at 140–142 days (Table 2). Cortisol infusion had no effect on the weights of the fetus and placenta or on fetal blood pH and gas tensions (Table 2). It also had no apparent effect on the protein content of the placenta (Table 3). In contrast, renal protein content in the cortisol-infused fetuses was significantly lower than that in the saline-infused controls at 128–132 days and was similar to the value found in the untreated fetuses at 140–142 days (Table 3).

Cortisol infusion had no significant effect on 11β-HSD2 activity in either the placenta or kidney when values were expressed on a weight-specific basis (Table 3). However, when the individual differences in protein content were taken into account, placental 11β-HSD2 activity per mg protein was significantly lower in the cortisol- than in the saline-infused animals (Table 3). Cortisol infusion reduced placental 11β-HSD2 activity per mg protein to values similar to those seen in the untreated animals at 140–142 days of gestation (Table 3). Activity of 11β-HSD2 per mg protein in the fetal renal cortex was unaffected by cortisol infusion (Table 3).

The relationship between placental 11β-HSD2 activity and fetal plasma cortisol

Since both groups of fetuses with elevated cortisol levels, whether of exogenous or endogenous origin, had lower

placental 11β-HSD2 activities per mg placental protein than the group with low plasma cortisol values, the relationship between placental 11β-HSD2 activity and fetal plasma cortisol was examined further. When the data from all the fetuses were combined, irrespective of treatment or gestational age, there was a significant inverse correlation between log fetal plasma cortisol at delivery and placental 11β-HSD2 activity expressed either per gram wet weight (Fig. 1A) or per mg placental protein (Fig. 1B). No significant correlation was observed between fetal plasma cortisol and renal 11β-HSD2 activity expressed either on a weight-specific basis ($r = 0.157$, $n = 20$, $P > 0.05$) or per mg renal protein ($r = 0.396$, $n = 20$, $P > 0.05$). There was also no significant correlation between placental 11β-HSD2 activity per mg protein and fetal body weight either at 128–132 days ($r = -0.340$, $n = 13$, $P > 0.05$) or at 140–142 days ($r = 0.076$, $n = 7$, $P > 0.05$).

Discussion

The results of the present study demonstrate that fetal cortisol affects 11β-HSD2 activity in the placenta but not in the kidney of fetal sheep during late gestation. In common with the human, guinea pig and rat (Stewart *et al.* 1995a, Sampath-Kumar *et al.* 1998, Waddell *et al.* 1998), placental 11β-HSD2 activity fell with increasing gestational age in the sheep in association with the endogenous rise in fetal plasma cortisol concentrations towards term. When fetal cortisol levels were raised earlier

Table 3 Mean (± S.E.M.) values of tissue protein content and 11β-HSD2 activity in placenta and kidney from saline- and cortisol-infused fetuses at 128–132 days and from untreated fetuses at 140–142 days of gestation. Numbers of animals are shown in parentheses

	Treatment	Protein (mg/g wet weight)		11β-HSD2 activity (pmol/min per g wet weight)		11β-HSD2 activity (pmol/min per mg protein)	
		Placenta	Kidney	Placenta	Kidney	Placenta	Kidney
Days of gestation							
128–132	Saline infused	122 ± 3 ^a (5)	220 ± 7 ^a (6)	315 ± 35 ^a (5)	4993 ± 542 ^a (6)	2.63 ± 0.36 ^a (5)	22.88 ± 2.30 ^a (6)
	Cortisol infused	132 ± 4 ^a (8)	177 ± 4 ^b (7)	224 ± 19 ^{ab} (8)	4636 ± 534 ^a (7)	1.77 ± 0.17 ^b (8)	26.36 ± 2.70 ^a (7)
140–142	Untreated	117 ± 5 ^a (7)	195 ± 5 ^b (7)	188 ± 17 ^b (7)	5669 ± 165 ^a (7)	1.60 ± 0.13 ^b (7)	26.82 ± 1.05 ^a (7)

Values in columns with different superscripts are significantly different from each other ($P < 0.05$; ANOVA).

Figure 1 The relationship between placental 11 β -HSD2 activity expressed (A) on a weight-specific basis ($y = -146.8 \log_{10} x + 474.8$, $r = -0.708$, $n = 20$, $P < 0.01$) and (B) per mg placental protein in the individual animals ($y = -1.37 \log_{10} x + 4.17$, $r = -0.745$, $n = 20$, $P < 0.01$).

in gestation by exogenous cortisol administration in the present study, there was a premature fall in placental 11 β -HSD2 activity per mg protein to values similar to those found in older fetuses. Cortisol, therefore, appears to suppress 11 β -HSD2 activity in the ovine placenta during late gestation. A similar inhibitory effect of fetal cortisol on 11 β -HSD2 gene expression has been observed in adrenal glands of fetal sheep close to term (Ross *et al.* 2000). The inverse correlation observed in the present study between plasma fetal cortisol and placental 11 β -HSD2 activity also indicates that fetal cortisol is a physiological regulator of the placental conversion of cortisol to cortisone and suggests that it may increase placental exposure to bioactive glucocorticoids during late gestation. These findings are consistent with previous studies that showed reduced placental 11 β -HSD2 gene expression in sheep and rats during conditions such as maternal undernutrition which raise glucocorticoid concentrations *in utero* (Langley-Evans *et al.* 1996, Bertram *et al.* 2001, Whorwood *et al.* 2001). Indeed, cortisol-induced downregulation of placental 11 β -HSD2 activity could explain, at least in part, the lower transplacental passage of cortisol from ewe to fetus observed during late gestation when fetal cortisol levels are reduced by fetal adrenalectomy (Hennessy *et al.* 1982).

In contrast to the placenta, cortisol administration had no effect on 11 β -HSD2 activity in fetal ovine kidneys. There was also no change in renal 11 β -HSD2 activity during the last 15–20 days of gestation when endogenous cortisol levels rise in the sheep fetus. This finding is consistent with previous observations that showed no alteration in renal 11 β -HSD2 mRNA abundance in fetal

sheep over this period of late gestation (McMillen *et al.* 2000). However, earlier studies have shown an increase in both renal 11 β -HSD2 gene expression and activity between 80–100 days and term (Wood & Srun 1995, Langlois *et al.* 1995). Taken together, these observations suggest that renal 11 β -HSD2 activity may rise during nephrogenesis which occurs throughout mid to late gestation but is complete by about 130 days of gestation in the sheep (Wintour 1997). In late gestation, renal 11 β -HSD2 gene expression and activity are enhanced by acidosis and reduced by hypoxaemia in fetal sheep, although neither of these factors alter placental 11 β -HSD mRNA abundance (Asano *et al.* 1997, Murotsuki *et al.* 1998). Reduced renal 11 β -HSD2 expression and activity are also seen in fetal sheep in response to maternal undernutrition during early pregnancy (Whorwood *et al.* 2001). Activity of 11 β -HSD2 in fetal ovine kidneys is, therefore, responsive to certain stimuli, although not to cortisol, during late gestation. Since cortisol reduces renal protein content and is known to regulate the activity of other renal enzymes at this stage of gestation (Fowden *et al.* 1993), its ineffectiveness in altering renal 11 β -HSD2 activity is unlikely to be due to a lack of glucocorticoid receptors (GR) in the fetal kidney. Indeed, GR are present in fetal ovine kidneys from 65 days of gestation and show no change in renal abundance until after birth (Berdusco *et al.* 1993). The effects of cortisol on 11 β -HSD2 activity, therefore, appear to be tissue specific in the sheep fetus close to term.

The activities of placental 11 β -HSD2 per mg protein observed in the present study were within the range of

values reported previously in sheep during late gestation (Kim *et al.* 1995, Murotsuki *et al.* 1998). However, placental 11 β -HSD activity during late gestation is lower in sheep than in other species, such as the human, pig, horse, baboon, guinea pig and rat (Baggia *et al.* 1990, Chavatte *et al.* 1995, Stewart *et al.* 1995a, Klemcke & Christenson 1996, Sampath-Kumar *et al.* 1998, Waddell *et al.* 1998). These animals have higher maternal glucocorticoid concentrations and greater transplacental glucocorticoid gradients than sheep at a similar stage of gestation (Fowden & Silver 1995, Fowden *et al.* 1998). In the present study, the activity of 11 β -HSD2 per mg protein in the fetal kidneys was four- to fivefold higher than observed in previous studies of fetal sheep at a similar gestational age (Wood & Srun 1995, Asano *et al.* 1997, Murotsuki *et al.* 1998). Selective use of the renal cortex in the present study may account, in part, for these higher activities, as 11 β -HSD2 has been localized to the distal convoluted tubules and collecting ducts in the cortex of adult human and rodent kidneys (Cole 1995, Krozowski *et al.* 1995, Roland *et al.* 1995).

In ovine placenta, the types 1 and 2 11 β -HSD isoforms appear to be equally active biologically (Yang 1995b, Murotsuki *et al.* 1998). Since there is no change in either the dehydrogenase or reductase activity of placental 11 β -HSD1 during late gestation (Yang *et al.* 1997), the fall in placental 11 β -HSD2 activity observed during the last 15–20 days of gestation in the present study represents a 15–20% decrease in total dehydrogenase activity of the placenta. This fall in placental cortisol to cortisone conversion will enhance the placental effects of rising fetal cortisol concentrations close to term and lead to increased activation of the glucocorticoid-dependent enzymes involved in placental oestrogen synthesis (Sun *et al.* 1998a, Burton & Waddell 1999, Patel *et al.* 1999). The prepartum fall in placental 11 β -HSD2 activity may, therefore, form part of the endocrine cascade responsible for initiating ovine parturition and contribute to the positive feedback mechanisms that operate during late gestation to ensure delivery of viable lambs at term.

The mechanisms by which cortisol suppresses placental 11 β -HSD2 activity remain unknown. Cortisol may act directly on the 11 β -HSD2 gene as glucocorticoid response elements have been identified in the promotor sequence of the human 11 β -HSD gene (Krozowski *et al.* 1999). Alternatively, cortisol may act indirectly via cortisol-dependent transcription factors or other hormones such as oestrogens. Oestrogens are produced by the ovine placenta in response to cortisol and have been shown to suppress 11 β -HSD2 expression in the human placenta (Sun *et al.* 1998a, Challis *et al.* 1999). However, in baboons, oestrogens increase the dehydrogenase activity of placental 11 β -HSD (Baggia *et al.* 1990). This oestrogen-dependent increase in placental cortisol to cortisone conversion relieves suppression of the fetal hypothalamic–pituitary–adrenal axis and triggers parturition by increasing the

adrenal supply of oestrogen precursors to the placenta in the baboon (Pepe & Albrecht 1995). Although regulation of placental 11 β -HSD activity differs between sheep and baboons in late gestation, the net outcome of the regulatory processes is to facilitate the changes in placental hormone synthesis responsible for initiating labour in both species.

The cortisol dependence of placental 11 β -HSD2 activity in the ovine placenta also has important implications for fetal development before term. Increases in fetal cortisol levels induced by adverse intrauterine conditions before term may reduce placental 11 β -HSD2 activity and, thereby, enhance placental exposure to both fetal and maternal cortisol and increase access of maternal cortisol to fetal tissues. In the placenta, the increased glucocorticoid exposure will alter the production of hormones, such as prostaglandin E₂ and placental lactogen, which favour fetal survival during adverse circumstances (Challis *et al.* 1999, McLaren *et al.* 2000, Ward *et al.* 2000). In the fetus, the increased transplacental passage of maternal cortisol may also aid fetal survival by amplifying the glucocorticoid-induced switch from tissue accretion to tissue differentiation which contributes to the fall in fetal growth rate observed during adverse intrauterine conditions, such as undernutrition and hypoxaemia (Fowden *et al.* 1996, 1998). However, if the reduced placental 11 β -HSD2 activity persists, even after restoration of normal conditions *in utero*, fetal development may be compromised by prolonged exposure to maternal glucocorticoids with long-term consequences for postnatal development and the incidence of adult onset degenerative diseases (Barker 2001). Fetal body weight was unrelated to placental 11 β -HSD activity in the small number of fetuses used in the present study but positive correlations have been observed between placental 11 β -HSD activity and body weight at delivery in larger cohorts of human infants and rat pups (Benediktsson *et al.* 1993, Stewart *et al.* 1995a). The current findings are therefore consistent with the hypothesis that placental 11 β -HSD2 activity has an important role in the intrauterine programming of adult disease (Seckl *et al.* 1995, Sun *et al.* 1998b). However, since cortisol had no effect on renal 11 β -HSD2 activity in the present study, the postnatal hypertension and reduction in renal 11 β -HSD2 gene expression associated with adverse intrauterine conditions, such as undernutrition (Seckl *et al.* 1995, Langley-Evans *et al.* 1996, Whorwood *et al.* 2001), are unlikely to be due to cortisol-dependent changes in renal 11 β -HSD2 activity sustained *in utero*.

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