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Type 2 11β-Hydroxysteroid Dehydrogenase in Foetal and Adult Life

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Two isoforms of 11β-hydroxysteroid dehydrogenase (11β-HSD) catalyse the interconversion of active cortisol to inactive cortisone; 11β-HSD1 is a low affinity, NADP(H)-dependent dehydrogenase/oxo-reductase, and 11β-HSD2 a high affinity, NAD-dependent dehydrogenase. Because of the importance of 11β-HSD in regulating corticosteroid hormone action, we have analysed the distribution of the 11β-HSD isoforms in human adult and foetal tissues (including placenta), and, in addition have performed a series of substrate specificity studies on the novel, kidney 11β-HSD2 isoform. Using an RT-PCR approach, we failed to detect 11β-HSD1 mRNA in any human mid-gestational foetal tissues. In contrast 11β-HSD2 mRNA was present in foetal lung, adrenal, colon and kidney. In adult tissues 11β-HSD2 gene expression was confined to the mineralocorticoid target tissues, kidney and colon, whilst 11β-HSD1 was expressed predominantly in glucocorticoid target tissues, liver, lung, pituitary and cerebellum. In human kidney homogenates, 11-hydroxylated progesterone derivatives, glycyrrhetinic acid, corticosterone and the "end products" cortisone and 11-dehydrocorticosterone were potent inhibitors of the NAD-dependent conversion of cortisol to cortisone. Finally high levels of 11β-HSD2 mRNA and activity were observed in term placentae, which correlated positively with foetal weight. The tissue-specific distribution of the 11β-HSD isoforms is in keeping with their differential roles, 11β-HSD1 regulating glucocorticoid hormone action and 11β-HSD2 mineralocorticoid hormone action. The correlation of 11β-HSD2 activity in the placenta with foetal weight suggests, in addition, a crucial role for this enzyme in foetal development, possibly in mediating ontogeny of the foetal hypothalamo–pituitary–adrenal axis.

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INTRODUCTION

11β-Hydroxysteroid dehydrogenase (11β-HSD) is a member of the short-chain alcohol dehydrogenase family responsible for the interconversion of cortisol (F) to hormonally inactive cortisone (E). The biological role of the enzyme in man has come from clinical observations of the enzyme-deficient state (syndrome of Apparent Mineralocorticoid Excess, liquorice ingestion) [1], from which it is clear that 11β-HSD plays a crucial role in conferring specificity upon the mineralocorticoid receptor (MR) [2, 3]. In addition, the enzyme is able to regulate the access of cortisol to the glucocorticoid receptor at several tissue sites [4–6]. 11β-HSD was first characterized and cloned from rat liver [7]; understandably therefore many of the initial functional, distribution and regulation studies were carried out in rodents. Recent studies, however, have increasingly focussed on 11β-HSD activity in human tissues, where two 11β-HSD isoforms have been described and cloned. Type 1 11β-HSD (11β-HSD1) was cloned from a human testis cDNA library using the rat liver 11β-HSD cDNA as a probe [8]. This enzyme encodes for a low affinity, NADP/NADPH-dependent dehydrogenase/oxoreductase (apparent K_m for F, 2 μM; K_m for E, 0.3 μM) [9, 10]. Direct evidence for the existence of a second 11β-HSD isoform (11β-HSD2) has come from the analysis of 11β-HSD activity in rabbit [11, 12] and rat kidney [13], and from further characterization of 11β-HSD expression in human placenta [14], and in the mineralocorticoid target tissues, human kidney [10] and rectal colon [15]. These studies revealed that 11β-HSD2, in contrast to 11β-HSD1, is a high affinity (apparent K_m for F, 50 nM), NAD-dependent

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unidirectional dehydrogenase. Human 11β-HSD2 cDNA was subsequently obtained by expression cloning from human kidney tissue [16].

In this manuscript we present data on the substrate specificity of 11β-HSD2 and summarize our results on the distribution of human 11β-HSD1 and 11β-HSD2 in adult and foetal tissues. In addition, we present data to suggest that placental 11β-HSD2 plays an important role in foetal development, specifically with respect to the regulation of foetal adrenal steroidogenesis.

METHODS

Human foetal tissues and decidua (16–19 weeks gestation) were obtained at elective abortion, in accordance with the Donors Anatomical Gift Act of the State of Texas. Fresh placentae were also obtained from 27 healthy deliveries (gestational age 38.5–41 weeks) and the birth weight noted for each infant. Surface blood clots were removed from the placenta which was then weighed intact. A specimen of each placenta was dissected from foetal membranes and stored at −70°C for a maximum period of 8 weeks. In each case tissue was obtained from a central part of the placenta; preliminary experiments measuring 11β-HSD activity at seven separate sites in a single placenta indicated that activity varied by $< 8\%$ at various sites within the placenta. Placentae were thawed simultaneously and homogenized in ice-cold 0.154 M KCl buffer using an Ultraturrax homogenizer (Janke and Kunchel, Germany). Fresh foetal kidneys ($n = 4$–6) were similarly homogenized. Written consent from the woman about to be delivered or aborted was obtained using a consent form and protocol approved by the Human Research Review Committee of the University of Texas Southwestern Medical Center. Adult human tissues were obtained from operative specimens in accordance with guidelines agreed by the University of Birmingham Pathology Department and local Ethics Committee and were snap frozen at −70°C for mRNA analysis.

11β-HSD activity studies

Placentae and kidney homogenates were centrifuged for 10 min at 1000 *g* to sediment large tissue fragments and a protein assay performed on the supernatant (Coomassie blue kit, Biorad Laboratories, Richmond, CA, U.S.A.). Homogenates (0.25 mg protein/ml) were incubated in 0.5 ml phosphate buffer (0.1 M, pH 7.6), containing 50,000 cpm [^{3}H]cortisol (sp. act. 3.2 TBq/mmol, Amersham International, Arlington Heights, IL, U.S.A.), 0.1 M F and 400 μM NAD for 30 min at 37°C in a shaking water bath. Based on our earlier studies detailing the co-factor preference and apparent K_m for the type 1 and 2 11β-HSD isoforms [10, 15], assays with 0.1 μM F and NAD were designed to detect type 2 activity, which is the predominant, if not exclusive, isoform in placenta and foetal kidney [10, 14]. Aliquots were extracted into 10 volumes of dichloromethane and steroids separated by TLC using ethanol: chloroform (8:92) as a mobile phase. The TLC plates were analyzed on a Bioscan radioimaging detector, the fractional conversion of F to E calculated and enzyme activity expressed as pmol E (or F) formed/mg. protein for each homogenate/h.

To assess the substrate specificity of 11β-HSD2, a series of possible substrates/competitive inhibitors were incubated at varying concentrations (10^{-5}–10^{-10}M) with human foetal kidney homogenates together with 0.1 μM F in triplicate as described above and each experiment repeated three times. Stock solutions of all substrates/inhibitors were prepared in absolute ethanol, diluted in phosphate buffer, and added to the homogenates such that the final ethanol concentration in the incubate was always $< 0.1\%$.

Reverse transcriptase-PCR of human tissue RNA

RNA was isolated from foetal and adult human tissues using a commercially available single step extraction method (RNAzol B, Biotecx Laboratories, Houston, TX, U.S.A.). First strand DNA was synthesized from 10 μg total RNA using avian myeloblastoma viral reverse transcriptase (RT) (Promega, Madison, WI, U.S.A.), driven primer extension from 3′ antisense oligos against the human 11β-HSD1 (5′ ACTTGCTTGCAGAATAGG-3′) and h11β-HSD2 cDNA's (5′-TCACTGACTCTGTCTTGAAGC-3′) [8, 16]. 10% of this reaction served as a template for the PCR amplification of either a 571 bp fragment of h11β-HSD1 using the 3′ oligo with the 5′ sense oligo (5′-CTCGAGTCGGATGGCTTTTTATG-3′) or a 477 bp fragment of h11β-HSD2 using the 3′ oligo with the 5′ oligo (5′-ACCGTATTGGAGTTGAACAGC-3′), and involved 35 cycles of denaturation (94°C for 1 min), annealing (47°C for 1 min) and extension (72°C for 2 min). Negative control experiments included the omission of the RT and template RNA. As a positive control, 8 ng of either the 11β-HSD1/pcDNAI vector or 8 ng of the h11β-HSD2/pGEM4Z vector were used

Table 1. Substrate specificity of human kidney type 2 11β-HSD. The IC_{50} of a variety of competing steroids is shown

	IC_{50}
Potent inhibitor/substrate	
11β-OH-progesterone	1.0×10^{-8} M
Glycyrrhetinic acid	1.6×10^{-8} M
11α-OH-progesterone	1.8×10^{-8} M
Corticosterone (B)	1.8×10^{-8} M
Carbenoxolone	6.3×10^{-8} M
11-dehydrocorticosterone (A)	7.8×10^{-8} M
Moderate inhibitor/substrate	
Deoxycorticosterone	2.1×10^{-7} M
Cortisone (E)	2.4×10^{-7} M
Weak inhibitor/substrate	
Progesterone	5.7×10^{-7} M
Dexamethasone	8.7×10^{-7} M
11β-OH-androstenedione	8.3×10^{-6} M

as templates in separate PCR reactions. To confirm the presence of RNA of adequate integrity for RT, parallel RT–PCR experiments were performed to amplify a 652 bp fragment of the ubiquitously expressed α_1, subunit of human Na–K–ATPase [17] using the 3′ antisense oligo (5′-GGCAATTCTTCCCAT-CACAGT-3′) and the 5′ sense oligo (5′-ATATG-GAACAGACTTGAGCCG-3′).

RESULTS

Inhibition of renal 11β-HSD2 activity

Table 1 shows the IC_{50} for a variety of steroids on renal 11β-HSD2 activity. As shown, 11α- and 11β-hydroxyprogesterone were potent inhibitors of 11β-HSD2 and presumably act as substrates. The liquorice derivatives, glycyrrhetinic acid and carbenoxolone were also potent inhibitors of activity. Corticosterone (B) is also a substrate for 11β-HSD2, but, interestingly, this enzyme appears to be inhibited by its own product, i.e. by E and 11-dehydrocorticosterone (A). Finally DOC, progesterone, dexamethasone and 11β-hydroxyandrostenedione inhibit 11β-HSD2 activity with IC_{50}s of approx. 10^{-6} M.

Analysis of 11β-HSD1 and 11β-HSD2 mRNA expression in foetal and adult tissues

Figure 1(a and b) details the distribution of 11β-HSD2 gene expression in human foetal and adult

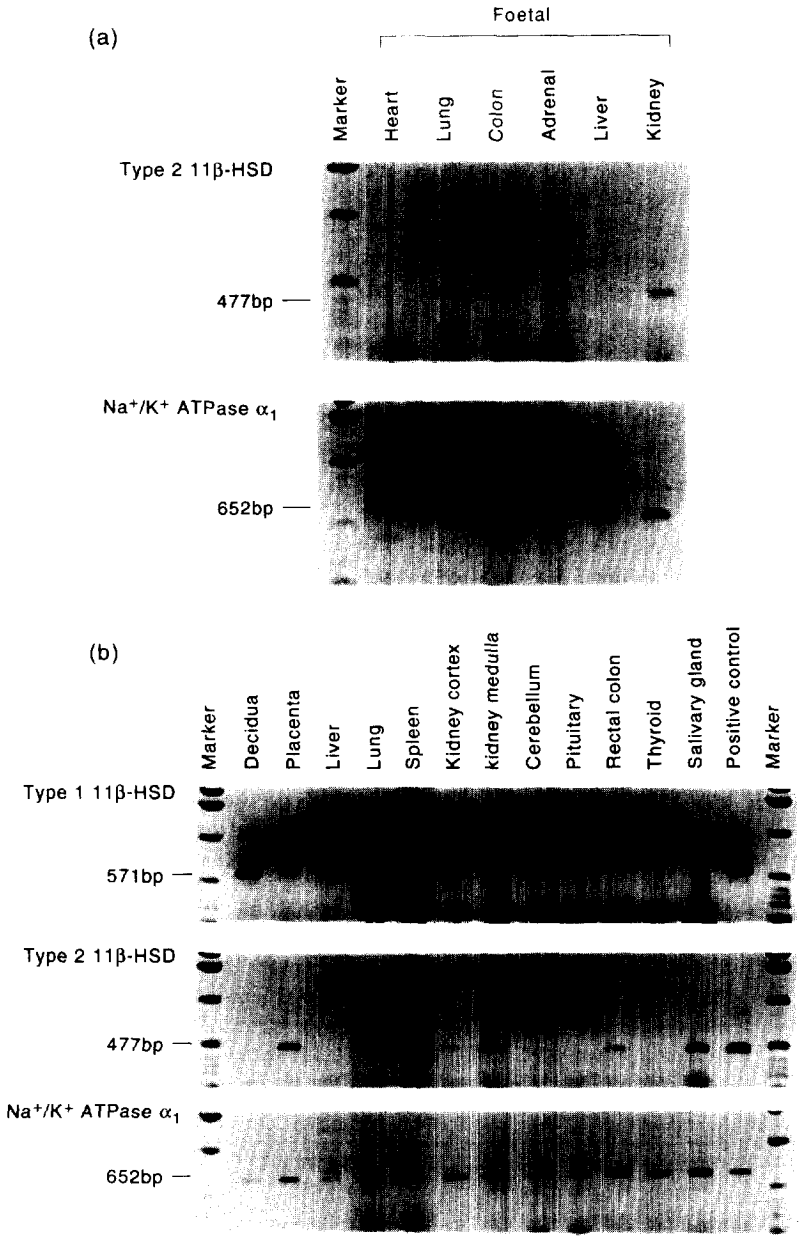

Fig. 1. Parallel RT-PCR analyses of mRNA expression in human foetal tissues (a) and human adult tissues (b). Using specific oligos against 11β-HSD1, 11β-HSD2 and the α1 subunit of Na K-ATPase, PCR amplification of DNA fragments of sizes 571, 477 and 652 bp, respectively, was performed. The detection of the ubiquitously expressed α1 mRNA served as a control for the presence of reverse-transcribable RNA.

tissues. While previous studies have failed to demonstrate 11β-HSD1 mRNA or activity in mid-gestational foetal tissues [18], 11β-HSD2 activity appeared to be present in many foetal tissues and the RT–PCR studies would support this notion. 11β-HSD2 mRNA was detected in foetal adrenal, lung, colon and kidney, but was absent in foetal heart and liver. In adult tissues, 11β-HSD2 gene expression was confined exclusively to the placenta and to the classical mineralocorticoid target tissues, kidney cortex and medulla, colon and salivary gland. In contrast, 11β-HSD1 was localized to the predominantly glucocorticoid target tissues, lung, liver, spleen, pituitary and cerebellum. Some 11β-HSD1 mRNA was present in kidney medulla and placenta, but parallel enzyme activity and Northern blot studies (data not shown) revealed that this isoform was present in low amounts. No 11β-HSD1 mRNA was detected in kidney cortex, colon or salivary gland.

Placental 11β-HSD2 activity and foetal weight

Details of the term birth weights and placental weights from the 27 term placentas are given in Table 2. As shown there was a wide broad distribution of term foetal weight (2580–4990 g) and placental weight (434–1120 g). Indeed 7 foetuses were above the 90th percentile for "normal" gestational-matched controls, and 4 were below the 10th percentile. The capacity of the placenta to metabolize 11-hydroxylated C_{21} steroids varied between 1.73–7.95 μmol F/min. There was a significant positive correlation between foetal weight and 11β-HSD activity ($r = 0.408$, $P = 0.034$), but no significant correlation between placental weight and 11β-HSD activity ($r = 0.043$, $P > 0.05$). (Parallel studies indicated no correlation between placental 3β-hydroxysteroid dehydrogenase (3β-HSD) activity and foetal or placental weight.) When placental 11β-HSD2 activity was expressed in terms of total activity/placenta, the correlation between foetal weight and 11β-HSD activity became statistically stronger ($r = 0.784$, $P < 0.001$), but this could be explained on the basis of a positive correlation between foetal and placental weight ($r = 0.672$, $P < 0.001$).

DISCUSSION

11β-HSD2 has been cloned and characterized from human kidney [10, 16]. The enzyme shares little sequence homology with 11β-HSD1, and is a kinetically distinct isoform. 11β-HSD1 possesses relatively low affinity, NADP-dependent dehydrogenase activity. Furthermore, E has a higher affinity for 11β-HSD1 than F, suggesting that *in vivo* this enzyme may principally act as a reductase. In contrast, 11β-HSD2 is a high affinity, NAD-dependent unidirectional dehydrogenase. As documented here, 11β-HSD2 is inhibited by liquorice derivatives, and appears also to be inhibited by end-product (i.e. E and A). Furthermore, dexamethasone and hydroxyl-derivatives of progesterone are also substrates for 11β-HSD2. This may be of relevance in some tissues not evaluated in this study, such as the ovary, where high affinity 11β-HSD activity has been reported.

The ontogeny of human 11β-HSD1 and 2 is likely to be of interest. In adult tissues, 11β-HSD2 expression is confined to the classical mineralocorticoid target tissues, kidney, colon and salivary gland. In contrast 11β-HSD1 was expressed in predominantly glucocorticoid target tissues, liver, lung, pituitary and cerebellum. 11β-HSD1 mRNA was detected in adult kidney medulla using the sensitive technique of RT–PCR, but parallel activity studies have indicated that the contribution of this isoform to total 11β-HSD activity within the renal medulla is very low. Furthermore, we have consistently failed to demonstrate 11β-HSD1 mRNA in kidney cortex, rectal colon, and salivary gland. Our earlier studies had failed to demonstrate 11β-HSD1 mRNA or activity in mid-gestational foetal tissues [18], and only low levels of 11β-HSD1 activity (if any) in term placenta. The RT–PCR data presented here are consistent with the expression of 11β-HSD2 in human foetal lung, adrenal, kidney, colon and placenta. From the examination of 11β-HSD gene expression in these two distinct developmental periods, the ontogeny of 11β-HSD1 would seem to be a late gestational or post-natal event. In contrast, 11β-HSD2 may "switch off" in some tissues

Table 2. Term birth weight, placental weight and placental 11β-HSD activity in 27 placentas. Results are expressed as mean ± SE and ranges. 11β-HSD rate was expressed as pmol E formed/h/mg.protein, and for "total" 11β-HSD activity, the 11β-HSD rate was multiplied by the placental weight

Parameter	Mean ± SE	Range
Placental weight (g)	585 ± 29	434–1120
Term foetal weight (g)	3441 ± 105	2580–4990
11β-HSD rate (pmol E formed/h/mg.homogenate protein)	312 ± 14	194–448
Total 11β-HSD (μmol/min/placenta)	3.04 ± 0.23	1.73–7.95

postnatally (e.g. lung) to be expressed in the adult only in MR-target tissues.

These tissue localization studies have enabled us to clarify the role of the 11β-HSD isoforms. 11β-HSD2, in adult life is predominantly found in the classical mineralocorticoid-target tissues; its cellular localization within kidney and colon to collecting ducts [19] and surface epithelial cells [15] respectively, indicates that it maintains aldosterone-selectivity upon the MR in an autocrine fashion. In contrast, 11β-HSD1 is found principally in glucocorticoid target tissues, where its role may be to generate active F from E or to inactivate particularly high circulating F levels occurring for example at times of stress. Thus, factors which regulate the tissue-specific expression and regulation of these distinct isoforms will be of major importance in the analysis of corticosteroid hormone action.

It is now clear from this and other studies that 11β-HSD2 plays a key role in foetal development. 11β-HSD activity has been analysed in the placenta and foetal membranes since the 1960's [20–24], but it is only very recently that this activity (with the exception of maternally-derived decidua) has been found to reflect the expression of the 11β-HSD2 isoform [14, 24]. It has been suggested that 11β-HSD may serve to protect the developing foetus from the deleterious effects of maternal cortisol excess. The unusual combination of a low birth weight and large placenta predicting adult hypertension in man [25], and a direct correlation between placental 11β-HSD activity and foetal weight and inverse correlation with placental weight in the rodent [26], suggests that defective placental 11β-HSD activity may play a role in the subsequent development of hypertension in the offspring [27]. Our data suggest that this may not be the case. Firstly, 11β-HSD2 activity and gene expression is present in many foetal tissues in addition to the placenta, suggesting that they can protect themselves from any cortisol excess in an autocrine or paracrine fashion [18]. Secondly, we have failed to identify any relationship between human placental 11β-HSD activity and placental weight in man. Finally, if foetal glucocorticoid excess, mediated through defective placental 11β-HSD activity, was to be a factor in the development of hypertension, then one would expect evidence of suppression of the hypothalamo–pituitary–adrenal (HPA) axis in the low birth weight offspring, yet animal and human studies have, if anything, shown the opposite, ie. activation of the new-born HPA axis [28, 29].

The capacity for the placenta to metabolize cortisol is immense, with an average term placenta capable of converting 3 μmol of cortisol to cortisone per minute. Allowing for a placental whole blood flow of 500–700 ml/min from both foetus and mother, we compute that a maximum of 1 μmol cortisol/min is delivered to the placenta. Thus, rather than considering placental 11β-HSD as protecting the developing foetus from maternally-derived F, we suggest that it co-ordinates a far more crucial role in sustaining foetal adrenal steroidogenesis (Fig. 2). Such a scenario has

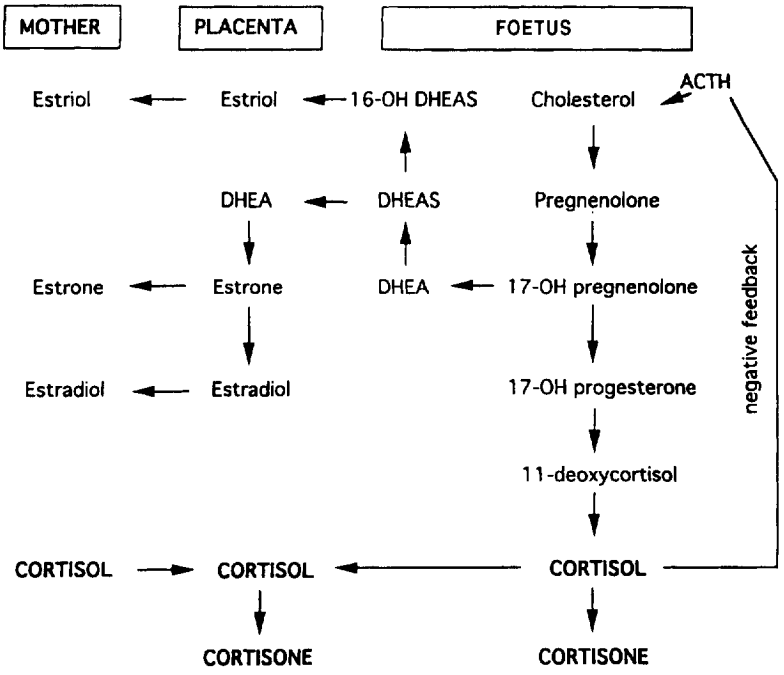

Fig. 2. Steroidogenesis from the foeto–placental unit illustrating the crucial role played by placental 11β-HSD. The capacity of the placenta to metabolise F to E is immense and this increased metabolic clearance of cortisol stimulates foetal ACTH secretion and hence adrenal growth and steroidogenesis through the negative feedback mechanism. Post-partum, following removal of the placenta, the foetal adrenal involutes, and circulating ACTH, DHEA and cortisone levels fall in the new-born.

been presented for the foeto–placental unit of the baboon by Pepe and Albrecht [30]. Here, as gestation advances, oestrogen induction of placental 11 β-HSD activity induces activation of the HPA-axis [31], with increased transcription of the foetal POMC gene, enhanced ACTH secretion [32] and increased foetal adrenal steroidogenesis. Because maternal oestrogen is derived predominantly from foetal adrenal DHEAS, this may serve as a "fast forward" regulatory process.

Data from earlier studies would support such a concept in man [33–36]. The relative size of the foetal adrenal gland is very large compared to the adult gland and foetal ACTH levels are also elevated compared to adult values. Several studies, principally those from anencephalic foetuses, indicate that foetal adrenal growth and steroidogenesis is under the control of ACTH, but the cause of the elevated levels has never been established. Whilst the foetal adrenal exhibits relative attenuation of 3β-HSD activity, accounting for the large secretion of DHEA and DHEAS, cortisol is synthesized by the foetal adrenal, with circulating levels rising from approx. 50 nmol/l at 20 weeks gestation to 150–200 nmol/l at term. The close correlation between the high foetal cortisone levels (150–200 nmol/l) and DHEA levels throughout gestation suggests that the trophic stimulus to the foetal HPA axis is actually enhanced cortisol metabolism rather than impaired secretion [37]. Finally, post partum, after removal of the placenta and therefore the major source of cortisol clearance, the foetal adrenal rapidly involutes, and cortisone and ACTH levels fall immediately to normal adult levels (50 nmol/l and < 5 pmol/l, respectively) [37, 38]. Our observed correlation between placental 11β-HSD activity and birth weight may simply be a reflection that larger foetuses have larger adrenals.

The 11β-HSD story has come a long way since Carl Monder first purified what we now know to be 11β-HSD1 from rat liver microsomes. In man, 11β-HSD1 seems likely to regulate the access of cortisol to the GR and may indeed primarily act as an oxo-reductase. In contrast 11β-HSD2 confers specficity upon the MR, and defects in its activity may explain some forms of hypertensive such as the syndrome of Apparent Mineralocorticoid Excess. Finally the high expression of this isoform in human placenta may be a principal factor driving foetal adrenal steroidogenesis and hence ongoing gestation.

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