Placental 11β-hydroxysteroid dehydrogenase: barrier to maternal glucocorticoids

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During mammalian pregnancy, the circulating concentration of cortisol (in rodents, corticosterone) in the mother is much higher than that in the fetus. Since the placenta is the only barrier, apart from the uterus, between the mother and her fetus, this gradient in cortisol concentrations suggests that there is a placental barrier preventing maternal cortisol from crossing into the fetus. The intracellular enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) is an ideal candidate for this barrier because it interconverts cortisol and corticosterone to their inactive metabolites cortisone and 11-dehydrocorticosterone. Indeed, 11β-HSD enzyme is expressed in the placenta of humans and a range of other animal species. Moreover, it is well positioned to serve as the barrier since it is localized to the syncytiotrophoblast, the site of maternal–fetal exchange. Given that fetal exposure to excessive amounts of glucocorticoids leads to intrauterine growth retardation, it has been hypothesized that the physiological significance of this placental 11β-HSD barrier is to protect the fetus from adverse effects of maternal glucocorticoids.

The intracellular enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) is responsible for the interconversion of bioactive glucocorticoids (cortisol and corticosterone) and their inactive metabolites (cortisone and 11-dehydrocorticosterone). Thus, it is considered to be an important tissue-specific modulator of glucocorticoid bioavailability in both glucocorticoid and mineralocorticoid target organs (Monder and Shackleton, 1984). This enzyme was first described in the early 1950s, and was the focus of intense studies in the 1960s and 1970s. The interest in 11β-HSD was revived in 1988 when two groups of investigators, led independently by John Funder (Funder et al., 1988) and Christopher Edwards (Edwards et al., 1988), made the seminal finding that 11β-HSD in mammalian kidney helps to confer the specificity of aldosterone for the renal mineralocorticoid receptor (MR) by rapid local conversion of cortisol to cortisone. The work of the late Carl Monder, who was the first to purify this enzyme from rat liver microsomes (Lakshmi and Monder, 1988), laid the foundation for the subsequent molecular cloning of this elusive enzyme. To date, two distinct isoforms of 11β-HSD (known as 11β-HSD1 and 11β-HSD2) have been identified, characterized and cloned (Table 1); numerous reviews have also been published (for example, Monder, 1993; Monder and White, 1993; Seckl, 1993). This article is intended to provide readers with a brief overview of the current state of knowledge, gaps and future directions with respect to the pattern, regulation and putative role of 11β-HSD expression in the mammalian placenta. Owing to significant differences in placental structure and in the pattern of 11β-HSD expression, humans and other mammals will be discussed separately.

Humans

The human placental 11β-HSD system was the first to be studied and is the best characterized among all the mammals studied to date. Although the presence of 11β-HSD activity in the human placenta was first described by Osinski (1960), it was not until the 1980s that the properties of human placental 11β-HSD were examined in detail (Lopez Bernal et al., 1980, 1982). Both 11β-HSD dehydrogenase (cortisol to cortisone) and reductase (cortisone to cortisol) activities were found to be present in human placental homogenates. The 11β-HSD dehydrogenase activity in tissue homogenates did not change with labour, nor with advancing gestation when expressed per gram of wet tissue but showed a steady decrease when expressed per mg of protein and DNA (Lopez Bernal et al., 1982). The human placental enzyme activity showed comparable preference for NAD and NADP, and the activity in both directions had a K_m value for cortisol in the micromolar range (Lakshmi et al., 1993). The results from these activity studies seemed to indicate the presence of both 11β-HSD1 and -2 in the human placenta. Indeed, recent molecular studies have demonstrated that mRNA encoding both 11β-HSD1 and -2 is expressed in the human placenta, although the amount of mRNA encoding 11β-HSD2 is much higher (Sun et al., 1997). Consistent with its failure to alter 11β-HSD activity, the process of labour had no effect on concentrations of mRNA encoding 11β-HSD1 and -2 (Sun et al., 1997). Seckl and co-workers have purified human placental 11β-HSD2 and have also cloned its cDNA (Brown et al., 1996). The coding sequence of the placental cDNA is identical to that of the kidney except for a single base alteration which probably can be attributed to polymorphism.

The 11β-HSD2 protein is localized exclusively in the syncytiotrophoblast (Krozowski et al., 1995) while the immunostaining for 11β-HSD1 protein is confined to extravillous intermediate trophoblasts (Sun et al., 1997). This distinct pattern of 11β-HSD1 and -2 localization may indicate that they have different physiological functions. It was originally proposed by Murphy (1981), and recently supported by Edwards et al.
The rat placenta expresses mRNA encoding both 11β-HSD1 and -2 as well as displaying 11β-HSD dehydrogenase and reductase activities (Burton et al., 1996). The zonal-specific pattern of 11β-HSD1 and -2 expression is indicative of the distinct functional significance of these two isozymes during rat pregnancy (Burton et al., 1996).

With respect to its function, the placental 11β-HSD system in rats has been the subject of intense study in the past few years (Seckl et al., 1995). The underlying hypothesis was that if placental 11β-HSD protects the fetus from adverse effects of maternal glucocorticoids, inhibition of placental 11β-HSD should have detrimental effects on fetal development. Indeed, when pregnant rats were treated with carbenoxolone (a potent inhibitor of 11β-HSD1 and -2) or dexamethasone (a synthetic glucocorticoid which is not a substrate for 11β-HSD1 and only a poor substrate for 11β-HSD2) during pregnancy, their newborn pups had a 20% reduction in birth weight when compared with those of saline-treated controls. Moreover, the effect of carbenoxolone was dependent upon maternal adrenals since adrenalectomy abolished this effect. In addition, a strong positive correlation between birth weight and placental 11β-HSD2 activity was found. These findings were taken collectively as evidence that the reduced birth weight was the consequence of excessive fetal exposure to maternal corticosterone (owing to the breakdown of the placental barrier as a result of inhibition of placental 11β-HSD by carbenoxolone) or to maternal dexamethasone coming across the placenta. Maternal protein restriction during pregnancy has also been shown to reduce birth weight and attenuate placental 11β-HSD2 activity (Langley-Evans et al., 1996).

There is no doubt that these studies have contributed significantly to our understanding of the role of placental 11β-HSD in mammalian fetal development. However, caution should be exercised in extrapolating these findings to other mammals, especially humans, for the following reasons. First, carbenoxolone is not selective for 11β-HSD2 or placenta but inhibits both 11β-HSD1 and -2 in all tissues expressing these two enzymes. Second, the pattern of relative expression of 11β-HSD1 and -2 in rats is similar in some (for example, sheep, as discussed below) but distinct in other mammals (for example, humans and guinea-pigs). This pattern is considered one of the major determinants of the effectiveness of the placental 11β-HSD barrier because the two isozymes possess distinct catalytic properties with respect to direction and affinity for glucocorticoids (Table 1). Third, the results of these studies would be strengthened greatly by measuring the circulating concentration of corticosterone in the fetus after maternal

### Table 1. Characteristics of human 11β-hydroxysteroid dehydrogenase (HSD)1 and -2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>11β-HSD1</th>
<th>11β-HSD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>34 kDa</td>
<td>40 kDa</td>
</tr>
<tr>
<td>Primary structure</td>
<td>292 aa</td>
<td>405 aa</td>
</tr>
<tr>
<td>Co-factor preference</td>
<td>NADP(H)</td>
<td>NAD</td>
</tr>
<tr>
<td>Direction</td>
<td>Cortisol ↔ Cortisone</td>
<td>Cortisol ↔ Cortisone</td>
</tr>
<tr>
<td>Affinity for cortisol</td>
<td>Low ($K_m = 2–10 \mu M$)</td>
<td>High ($K_m = 10–20 \mu M$)</td>
</tr>
<tr>
<td>Dexamethasone metabolism</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Sites of expression</td>
<td>Widespread</td>
<td>Tissue-specific</td>
</tr>
<tr>
<td>Gene structure</td>
<td>6 exons (&gt; 9 kb)</td>
<td>5 exons (6 kb)</td>
</tr>
<tr>
<td>Gene locus</td>
<td>Chromosome 1</td>
<td>Chromosome 16</td>
</tr>
<tr>
<td>Mutations in AME</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

AME, apparent mineralocorticoid excess syndrome.

(1993), that the role of placental 11β-HSD is to protect the fetus from adverse effects of maternal glucocorticoids. 11β-HSD2 is better suited for this role because of its location (the site of maternal–fetal exchange) and its enzymatic properties (higher affinity for cortisol and possessing only dehydrogenase activity). In a recent study using the intact human placenta ex vivo, it has been demonstrated that most of the maternally administered cortisol is metabolized to cortisone, providing direct evidence of a placental 11β-HSD barrier (Benediktsson et al., 1997). A correlation between the placental 11β-HSD2 activity and birth weight has been reported (Stewart et al., 1995) but not confirmed (Rogerson et al., 1997).

Guinea-pigs

Guinea-pigs are the only non-primate laboratory animal possessing a haemomonochorial placenta, and thus may represent an ideal model for studying the role of placental 11β-HSD in human fetal development. Recently, we have exploited this possibility by determining the characteristics of placental 11β-HSD activity in guinea-pigs. Our results demonstrate that the placental 11β-HSD system of the guinea-pig resembles closely that of the human in that 11β-HSD2 is the predominant, if not the exclusive, isozyme expressed (Sampath-Kumar et al., 1996). Given that guinea-pigs, like humans, display a similar cortisol gradient between the mother and her fetus throughout pregnancy, this animal model may hold the key to unravelling the role of placental 11β-HSD2 in protecting the fetus from being exposed to high concentrations of maternal cortisol.

Rats

The rat placenta expresses mRNA encoding both 11β-HSD1 and -2 as well as displaying 11β-HSD dehydrogenase and reductase activities (Burton et al., 1996). The zonal-specific pattern of 11β-HSD1 and -2 expression is indicative of the distinct functional...
carbenoxolone administration to confirm that the fetal corticosterone is indeed increased as a result of more maternal steroid coming through the placenta.

Baboons
The placental 11β-HSD in baboons has been studied extensively by Pepe and Albrecht (1995). There is an oestrogen-induced change in placental 11β-HSD activity from predominantly reduction (cortisone to cortisol) at mid-gestation to oxidation (cortisol to cortisone) at term. It has been proposed that this change in the placental enzyme activity may play a significant role in regulating the activation of the fetal hypothalamic–pituitary–adrenal (HPA) axis. At mid-gestation, placental 11β-HSD activity favours the formation of cortisol, resulting in cortisol crossing the placenta into the fetus. This placental-derived cortisol would suppress fetal pituitary ACTH release, and thus de novo cortisol synthesis by the fetal adrenal. At term, the increased placental oestrogen stimulates 11β-HSD dehydrogenase activity, resulting in the placenta predominantly converting cortisol to cortisone. This change in placental 11β-HSD activity would result in a decline in fetal cortisol of placental origin, which would then permit increased fetal pituitary ACTH release, culminating in fetal adrenal maturation and de novo cortisol synthesis.

Given that 11β-HSD1 and -2 possess distinct catalytic properties, this changing direction of activity suggests a possible change from 11β-HSD1 at mid-gestation to the induction of 11β-HSD2 at term. However, the results of recent studies have demonstrated that mRNA encoding both 11β-HSD1 and -2 is present from day 50 to term, and concentrations of both types increase progressively during this time (Pepe et al., 1996). Therefore, this switch in the direction of placental 11β-HSD activity cannot be explained by differential expression of 11β-HSD1 and -2. Pepe et al. (1996) have proposed a differential compartmentalization theory but this has yet to be proven. More importantly perhaps, the proposed role of placental 11β-HSD in regulating the fetal HPA axis requires further scrutiny. Furthermore, the precise molecular mechanisms by which oestrogen regulates placental 11β-HSD activity remain to be determined.

Sheep
We have characterized sheep placental 11β-HSD activity, demonstrating that both 11β-HSD1 and -2 activities are expressed (Yang, 1995). Using the ovine cDNAs encoding 11β-HSD1 and -2 as probes, we have shown that 11β-HSD1 gene is expressed predominantly in the ovine placenta (Campbell et al., 1996). Moreover, concentrations of placental mRNA encoding 11β-HSD1 and activity decrease between day 100 and day 125, a period during which the placental transfer of cortisol from mother to the fetus is known to increase (Yang et al., in press). 11β-HSD1 in the ovine placenta may function like 11β-HSD2 in the human placenta because the dehydrogenase activity is always predominant. Thus, the decreasing placental 11β-HSD1 activity may help to explain the increased placental cortisol transfer during that time. Throughout pregnancy, 11β-HSD1 protein is localized to fetal trophoblasts, suggesting that 11β-HSD1 in the ovine placenta is well-positioned to serve as a barrier to maternal cortisol (Yang et al., in press). The localization of, and possible changes in, 11β-HSD2 expression in the ovine placenta during pregnancy remain to be determined. Furthermore, factors responsible for this change in 11β-HSD1 have not been identified, and the physiological significance of the co-expression of two enzymes remains to be explored.

Pigs
In the pig placenta, both 11β-dehydrogenase and 11-oxoreductase activities are present, indicating the co-expression of 11β-HSD1 and -2. Furthermore, changes in these activities during pregnancy, which are consistent with alterations in the rate of placental transfer of cortisol between mother and fetus, have also been found (Kiemcke and Christenson, 1996). However, unlike the situation in humans and rats, there is no correlation between placental 11β-HSD activity and birth weight. The lack of a correlation may reflect differences in placentalification between pigs (epitheliochorial) and humans (haemochorial). Further studies are required to confirm the co-expression of 11β-HSD1 and -2 genes by molecular approaches, and to determine the cellular localization and precise physiological role of these two enzymes during pregnancy in pigs.

Conclusion
It is now established that 11β-HSD1 and -2 genes are co-expressed in mammalian placenta and their expression is specific to the cell and developmental stage. It is also clear that the relative expression of these two enzymes in the placenta differs greatly between species due largely to major differences in placental structure. Correlations and indirect evidence suggest that the 11β-HSD2 in the placenta serves as a barrier to protect the fetus from excessive exposure to maternal glucocorticoids. However, causal relationships and direct experimental evidence in support of this hypothesis are lacking. Moreover, the role of 11β-HSD1 in placenta where 11β-HSD2 is predominantly expressed remains to be defined. In addition, the mechanisms of regulation of 11β-HSD1 and -2 gene expression in mammalian placenta are largely unknown and may prove to be invaluable in understanding the role of these two enzymes in normal and pathological pregnancies.

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