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Tissue-Specific Messenger Ribonucleic Acid Expression of 11β-Hydroxysteroid Dehydrogenase Types 1 and 2 and the Glucocorticoid Receptor within Rat Placenta Suggests Exquisite Local Control of Glucocorticoid Action*

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ABSTRACT

Placental 11β-hydroxysteroid dehydrogenase (11β-HSD) regulates transplacental passage of maternal glucocorticoids to the fetus and is thus a key determinant of fetal glucocorticoid levels. It has also been proposed that placental 11β-HSD expression may influence local glucocorticoid actions by regulating access of corticosterone to the glucocorticoid receptor (GR) or mineralocorticoid receptor (MR). Therefore, the present study used a rat model to assess whether the GR or MR are coexpressed with the two forms of 11β-HSD (types 1 and 2) in the placental labyrinth zone, the major site of maternal-fetal transfer, and in the basal zone, the primary site of placental hormone synthesis. *In situ* hybridization analysis was used to assess messenger RNA (mRNA) expression for the GR, MR, 11β-HSD-1, and 11β-HSD-2 in the two placental zones on days 16, 19 and 22 of pregnancy (term = day 23). Whereas expression of the GR appeared relatively unchanged in both zones at these three stages of pregnancy, that of 11β-HSD-1 clearly increased in the labyrinth zone but fell in basal zone, whereas the opposite pattern of expression was observed for 11β-HSD-2. MR expression was not detected at any stage. The pattern of placental 11β-HSD-2 mRNA expression over days 16, 19, and 22 of pregnancy was paralleled by changes in 11β-HSD-2-specific bioactivity, but despite clear expression of 11β-HSD-1 mRNA, no bioactivity attributable to this enzyme was measurable in either placental zone. To assess the role of fetal adrenal maturation on these changes in 11β-HSD, two experimental models, maternal adrenalectomy and fetectomy, were employed. Maternal adrenalectomy on day 13 advanced maturation of the fetal adrenal cortex but had no effect on 11β-HSD-2 bioactivity in either of the placental zones at day 19. Placental 11β-HSD-2 bioactivity on day 22 was also unaffected by fetectomy 3 or 6 days earlier. In conclusion, the consistent expression of the GR in the two placental zones late in pregnancy suggests that concomitant and marked changes in 11β-HSD-1 and 11β-HSD-2 expression could have a major influence on glucocorticoid action in the placenta at this time. Moreover, the changes in 11β-HSD expression appear to be unrelated to development of the fetal adrenal cortex and are likely to reduce the placental glucocorticoid barrier near the end of pregnancy. (*Endocrinology* **139:** 1517–1523, 1998)

GLUCOCORTICOID action in several target organs is regulated by local, tissue-specific expression of the two recognized forms of 11β-hydroxysteroid dehydrogenase (11β-HSD) (types 1 and 2) (for reviews see Refs. 1–3). These enzymes catalyze the interconversion of active glucocorticoids (corticosterone or cortisol) and their biologically inert 11-keto forms (11-dehydrocorticosterone and cortisone, respectively). In the placenta, 11β-HSD activity regulates passage of active glucocorticoid from the mother to the fetus, a critical role in view of the deleterious effects of excess glucocorticoid on fetal growth and subsequent development of disease in postnatal life (3–6). Both 11β-HSD-1 and -2 are expressed in the rat placenta and each exhibits marked, zone-specific changes in expression over the last third of pregnancy (7) concomitant with development of the fetal hypothalamic-pituitary-adrenal (HPA) axis (8). In the basal zone, the major site of placental steroid and peptide hormone synthesis, 11β-HSD-1 messenger RNA (mRNA) expression falls between day 16 and 22 (term = day 23), whereas that for 11β-HSD-2 increases over the same period. In contrast, the reverse pattern of increasing 11β-HSD-1 and decreasing 11β-HSD-2 mRNA expression is evident in the placental labyrinth zone (7), the major site of maternal-fetal exchange. Whereas such variation is likely to have a major impact on transplacental passage of glucocorticoids, it has also been proposed that placental 11β-HSD may be an important determinant of local glucocorticoid action within the placenta by regulating access of glucocorticoids to their intracellular receptors (7, 9). Therefore, the initial objective of the present work was to assess whether the glucocorticoid receptor (GR) and/or mineralocorticoid receptor (MR) are coexpressed with the two forms of 11β-HSD in the basal and labyrinth zones of the placenta. Secondly, we addressed the possibility that changes in rat placental 11β-HSD expression are linked

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to maturation of the fetal HPA axis as occurs in primates (10). This involved determination of 11β-HSD mRNA expression and bioactivity after manipulation of the fetal HPA axis over the final week of pregnancy using two separate experimental models, fetectomy and maternal adrenalectomy. These were employed on the basis that fetectomy removes all fetal influences whereas maternal adrenalectomy is known to accelerate maturation of the fetal adrenal (11) and so may bring forward changes in placental 11β-HSD-1 and -2 expression.

Materials and Methods

Animals, surgery, and tissue collection

Albino Wistar rats were mated overnight and the day on which a vaginal plug was observed was termed day 1 of gestation; rats in this colony normally deliver on day 23. Placentas were collected from untreated rats on days 16, 19, and 22 of pregnancy (n = 3–4 per group) and frozen rapidly on dry ice (for subsequent *in situ* hybridization analysis) or placed immediately in ice-cold PBS (for enzyme assay). In subsequent experiments, placentas were collected similarly from day 19 pregnant rats that had undergone maternal bilateral adrenalectomy six days earlier (on day 13) by a dorsal approach under halothane anesthesia, or from day 22 pregnant rats that had been unilaterally fetectomized (removal of fetuses but not placentas of one uterine horn) on either day 16 (Fx16) or day 19 (Fx19). Fetectomy was performed under halothane anesthesia by exposure of one uterine horn through a midline abdominal incision. Each fetus was removed through small incisions made in the antimesometrial surface of the uterine wall and in the fetal membranes. The uterine incisions were sutured and the uterine horn returned to the abdomen; placentas in the intact horn served as a within-animal control. All of the above tissue collections were made after rats were killed by cervical dislocation, except for day 19 pregnant rats, which were decapitated to enable blood collection for subsequent analysis of plasma corticosterone by RIA.

In situ hybridization

Rat complementary DNA (cDNA) clones for 11β-HSD-1 (12), 11β-HSD-2 (13), the GR (14), and MR (15) were linearized with appropriate restriction enzymes (Promega Ltd., Southampton, UK), and the resultant templates used with ^{35}S-α-UTP (>1000 Ci/mmol; Amersham, Aylesbury, UK) to synthesize antisense and sense cRNA probes as previously described (16). Cryostat sections (15 μm) of whole placenta were thaw-mounted onto gelatin- and poly-L-lysine-coated microscope slides and stored at −80 C. Tissues sections were postfixed in 4% paraformaldehyde/phosphate (0.1 mol/liter) buffer and prehybridized, hybridized, and washed as previously described (16, 17). Following hybridization and washing, all slides were placed against hyperfilm β_{max} to detect positive signals for mRNAs; selected slides were subsequently dipped in photographic emulsion (NTB2, Eastman Kodak Company, Rochester, NY) to allow cellular localization of these signals which also required counterstaining with hematoxylin and eosin.

11β-HSD bioassays

Placentas from all untreated rats, adrenalectomized rats, and from the untreated horn of fetectomized rats were separated into basal and labyrinth zones as described by Chan and Leatham (18); fetectomized placentas could not be reliably separated into these zones so were left intact. Tissues were then placed in ice-cold PBS (pH 7.4) containing 0.25 M sucrose and homogenized. The homogenate was centrifuged at 750 × *g* for 10 min at 4 C, and the protein concentration of the supernatant determined (Bio-Rad protein assay kit, Bio-Rad, Hertfordshire, UK). 11β-HSD activity was determined by measuring the conversion of [1,2,6,7-^{3}H]corticosterone (SA 90 Ci/mmol; Amersham) (10 nM) to [^{3}H]11-dehydrocorticosterone by 500 μg/ml supernatant protein in the presence of 400 μM NADP (for 11β-HSD-1 activity) or NAD^+ (for 11β-HSD-2 activity) and varying concentrations of added cold corticosterone (0.5, 1, 2, 5, 10, 20 μM for 11β-HSD-1; 0, 12.5, 25, 50, 100, 200 nM for 11β-HSD-2) (16). At each concentration, duplicate incubations were performed at 37 C for 10 min then stopped by the addition of 2.5 ml ethyl acetate, into which steroids were extracted. Extracts were dried and [^{3}H]corticosterone and [^{3}H]11-dehydrocorticosterone purified and quantitated by HPLC as previously described (19). Preliminary experiments established that conversion of [^{3}H]corticosterone to [^{3}H]11-dehydrocorticosterone remained linear beyond the 10-min incubation time under these assay conditions. The reaction velocity (pmol/min per mg protein) for each duplicate incubation set was used to construct Lineweaver-Burk plots for individual placental tissues, from which each K_m and V_{max} were derived (20). For each animal (or treatment within animals), tissues from two placentas were analyzed, each in duplicate, and the derived kinetic data were averaged before calculation of group means.

Statistical analyses

Lineweaver-Burk plots were constructed for individual tissues using least squares regression analysis, and the apparent K_m for corticosterone and the V_{max} derived from the line of best fit (20). Differences among groups for apparent K_m and the V_{max} were assessed by one-way ANOVA, and between placental zones by paired *t* tests (21).

Results

Placental weights

Relative proportions of the two placental zones in untreated rats differed considerably on days 16, 19, and 22 of pregnancy, with the weight of the labyrinth zone increasing more than 3-fold over this period, whereas that of the basal zone remained unchanged (see Table 1). Maternal adrenalectomy on day 13 had no effect on placental weight on day 19 (either basal or labyrinth zones). Following unilateral fetectomy on day 16, placentas were retained *in situ* until collection on day 22 but weighed less than those of untreated rats, whereas after fetectomy on day 19 placental weight on day 22 was similar to, but more variable than, that of untreated rats (Table 1). Histological examination of placentas revealed that fetectomy induced morphological changes in the labyrinth zone, most notably increased edema, indicative of some degeneration, and this effect precluded reliable dissection of the two zones. Importantly, however, the size of the basal zone at day 22 appeared unaffected by fetectomy 3 or 6 days earlier.

TABLE 1. Weights (mg) of basal and labyrinth zones of the rat placenta over the last week of pregnancy and after maternal adrenalectomy (Adx) on day 13 or fetectomy on day 16 (Fx16) or day 19 (Fx19)

	Basal	Labyrinth	Total
Day 16			
Untreated	134 ± 28	86 ± 15	220 ± 17
Day 19			
Untreated	164 ± 55	232 ± 36[a]	396 ± 88[a]
Maternal Adx	148 ± 18	238 ± 10[a]	386 ± 16[a]
Day 22			
Untreated	130 ± 4	284 ± 7[a]	414 ± 6[a]
Fx16	nd[c]	nd	335 ± 23[b]
Fx19	nd	nd	466 ± 51[a]

Values are the mean ± SE (n = 3–9 per group).

[a] $P < 0.05$ compared with day 16 value (one-way ANOVA, LSD test).

[b] $P < 0.05$ compared with day 22 value in untreated rats (unpaired *t* test)

[c] nd, Not determined.

In situ hybridization

Localization of mRNAs for the GR, 11β-HSD-1, and 11β-HSD-2 in sections of whole placenta are shown by representative autoradiographs in Fig. 1; mRNA for the MR was not detectable in placenta at any stage, although signal was clearly evident in positive control kidney sections (Fig. 1j). Messenger RNA for the GR was consistently expressed in the two placental zones and remained effectively unchanged at the three stages of pregnancy examined (Fig. 1, a and b); hybridization of placental sections with the GR sense strand cRNA probe yielded effectively no signal (Fig. 1l). In contrast to the GR, mRNA expression for both 11β-HSD enzymes

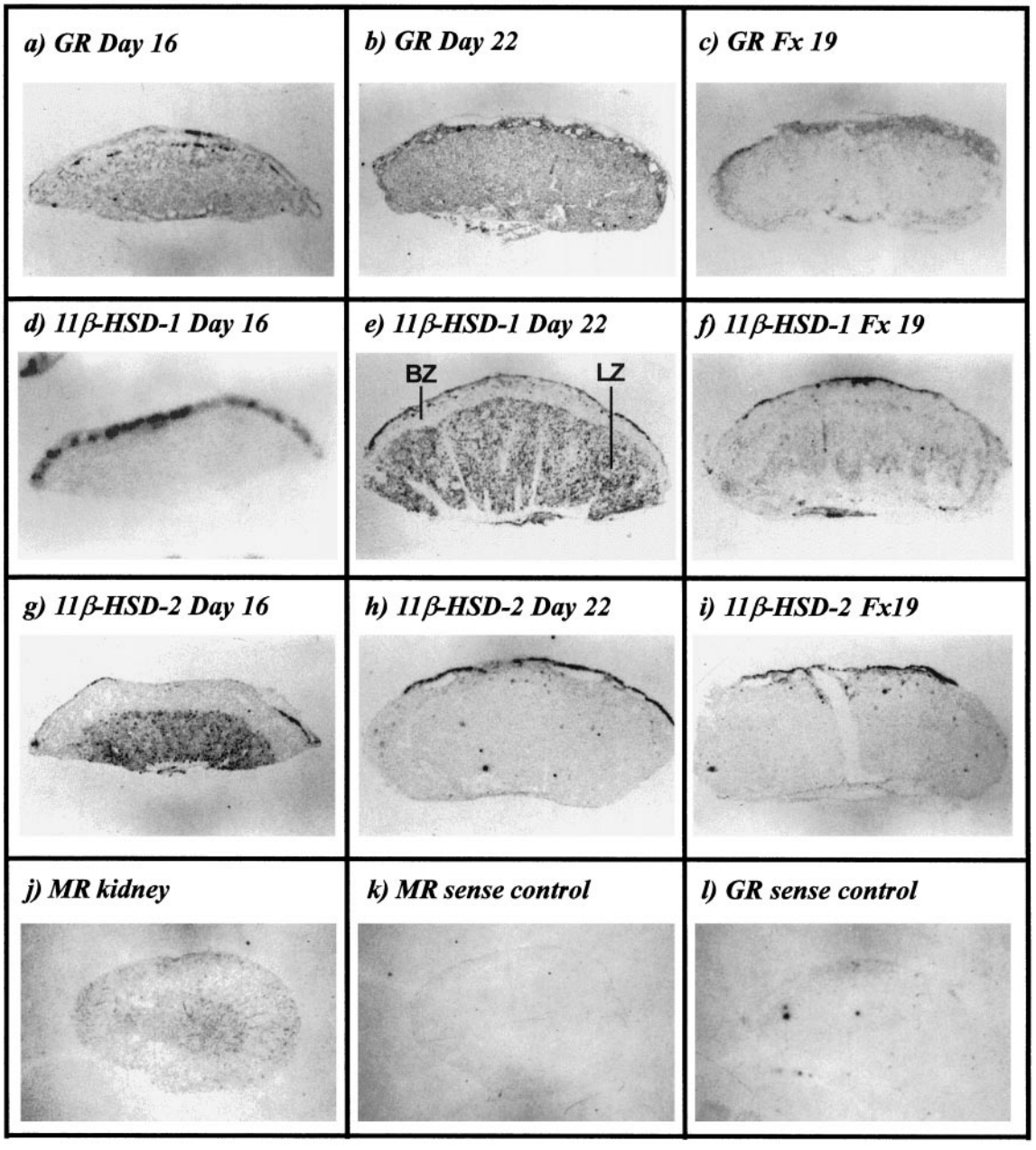

FIG. 1. Autoradiographic localization of mRNAs for the GR, 11β-HSD-1 and 11β-HSD-2 following *in situ* hybridization in sections of whole placenta from control rats (on days 16 and 22) and fetectomized rats (on day 22, 3 days post fetectomy). The outer basal zone (BZ) and inner labyrinth zone (LZ) are labeled only in (e) but are variably apparent in all panels depending on strength of positive signal. Cryostat sections (15 μm) of whole placenta were postfixed in 4% paraformaldehyde, prehybridized for 4 h at 50 C, then hybridized for 12–14 h at 50 C with ^{35}S-labeled riboprobes for 11β-HSD-1, -2, GR, or MR and signal localized by placing slides against hyperfilm β_{max}. Note that expression of the GR mRNA is relatively consistent in the two placental zones on each day (a and b), whereas 11β-HSD-1 mRNA increases in labyrinth zone between day 16 (d) and day 22 (e), and that for 11β-HSD-2 decreases in this zone over the same period (g and h). Following fetectomy, none of the mRNAs appear to be expressed in the labyrinth zone, whereas basal zone expression of each appears unaffected (c, f, and i). No MR mRNA expression was detectable in placenta at any stage, but was evident in positive control kidney sections (j); sense controls are shown for MR in kidney (k) and GR in placenta (l).

varied markedly between days 16 and 22 in the two placental zones; 11β-HSD-1 mRNA was evident in basal zone at day 16 then appeared to fall by day 22, whereas its expression clearly increased over the same period in labyrinth zone (Fig. 1, d and e); sense strand cRNA controls yielded minimal signal, comparable with or less than that observed in negative regions of placenta on the 2 days of pregnancy (*i.e.* labyrinth zone at day 16 for 11β-HSD-1 and day 22 for 11β-HSD-2). Positive signal for 11β-HSD-1 mRNA was observed specifically in trophoblast cells within each placental zone (Fig. 2, a and b). The reverse pattern of change occurred in the two placental zones with respect to mRNA for 11β-HSD-2 (Fig. 1, g and h), consistent with changes in 11β-HSD-2 bioactivity (see below). Messenger RNA for 11β-HSD-2 was also specifically localized to trophoblast cells, and this was especially evident in the labyrinth zone at day 16 (Fig. 2c). Placental expression of mRNAs for 11β-HSD-1, -2, and GR on day 19 were all unaffected by maternal adrenalectomy on day 13 (data not shown). Following fetectomy (both Fx 16 and Fx 19), however, the presumptive labyrinth zone appeared very low or negative for 11β-HSD-1, -2, and GR mRNAs, whereas expression of all three transcripts appeared relatively unaffected in basal zone (Fig. 1, c, f, and i).

Placental 11β-HSD bioactivity

Despite clear expression of the mRNA encoding 11β-HSD-1 in the two placental zones, no 11β-HSD-1 bioactivity (high substrate concentrations in the presence of NADP) was measurable at any stage examined. In contrast, 11β-HSD-2 bioactivity was detectable in both placental zones at each stage of pregnancy and varied in accordance with changes in 11β-HSD-2 mRNA. Characteristics of this bioactivity (V_{max} and apparent K_m) were similar in placentas from untreated day 22 rats and in those from the intact, contralateral horn in fetectomized animals, and so these data were pooled (total n = 9) and used for all subsequent comparisons. The apparent K_m was somewhat higher ($P < 0.05$) in the basal zone (20.7 ± 1.2 nM) compared with the labyrinth zone (15.9 ± 1.3 nM) on day 22 but not on day 16 (15.7 ± 2.4 and 16.2 ± 2.7 nM in basal and labyrinth zone respectively) or day 19 (20.4 ± 2.5 and 19.8 ± 2.0 nM). The V_{max} for 11β-HSD-2 differed between basal and labyrinth zones, and changed in both zones with gestational age. Specifically, V_{max} in basal zone was similar on days 16 and 19 of pregnancy but then increased ($P < 0.01$) by day 22 (2-fold higher than at day 16; see Fig. 3). In contrast, 11β-HSD-2 activity was maximal in labyrinth zone on day 16, remained high on day 19, but then fell ($P < 0.01$) by day 22 (85% lower than day 16 value).

Placental 11β-HSD bioactivity after maternal adrenalectomy

After maternal adrenalectomy on day 13 of pregnancy, maternal plasma corticosterone levels on day 19 (223 ± 31 nmol/liter) were similar to those in untreated rats (256 ± 65 nmol/liter), indicative of advanced maturation of the fetal adrenal cortex. Despite this major shift in the source of maternal corticosterone, there was no effect on the apparent K_m of 11β-HSD-2 for corticosterone, or on the V_{max} in either the basal or labyrinth zones of the placenta (Table 2).

Placental 11β-HSD bioactivity after fetectomy

Although reliable zonal dissection of placentas was not possible following fetectomy, 11β-HSD-2 bioactivity was

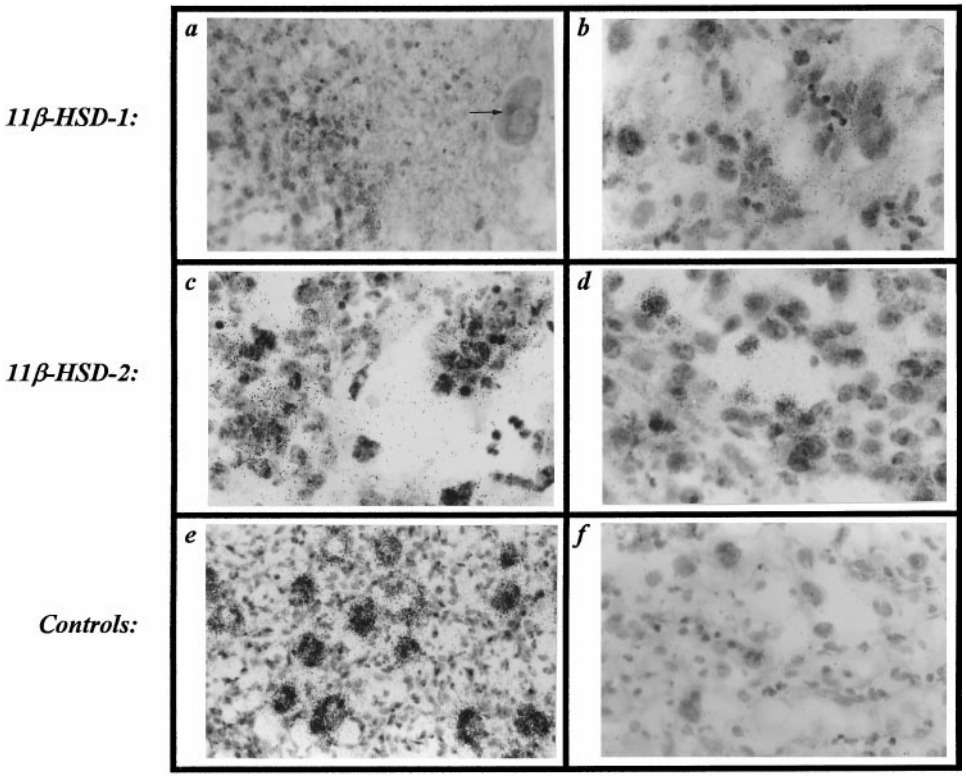

FIG. 2. Cellular localization of mRNAs for 11β-HSD-1 and -2 in basal (BZ) and labyrinth (LZ) zones of the rat placenta on days 16 and 22 of pregnancy. Sections were subjected to *in situ* hybridization as described in legend to Fig. 1, and positive signals identified after slides were dipped in photographic emulsion, subsequently developed and counterstained with hematoxylin and eosin. a, 11β-HSD-1 in day 16 basal zone showing paucity of signal in giant trophoblast cell (*arrow*); b, 11β-HSD-1 in day 22 labyrinth zone showing relatively consistent positive signal in a range of trophoblast cells; c, 11β-HSD-2 in day 16 labyrinth zone with intense signal in effectively all cells; d, 11β-HSD-2 in day 22 basal zone showing clear positive signal in some but not all trophoblast cells; e, 11β-HSD-2 mRNA signal in kidney (positive control); and f, negative control (hybridized with the sense strand of 11β-HSD-1) for day 22 labyrinth zone. Magnification: a, d–f, ×250; b and c, ×400.

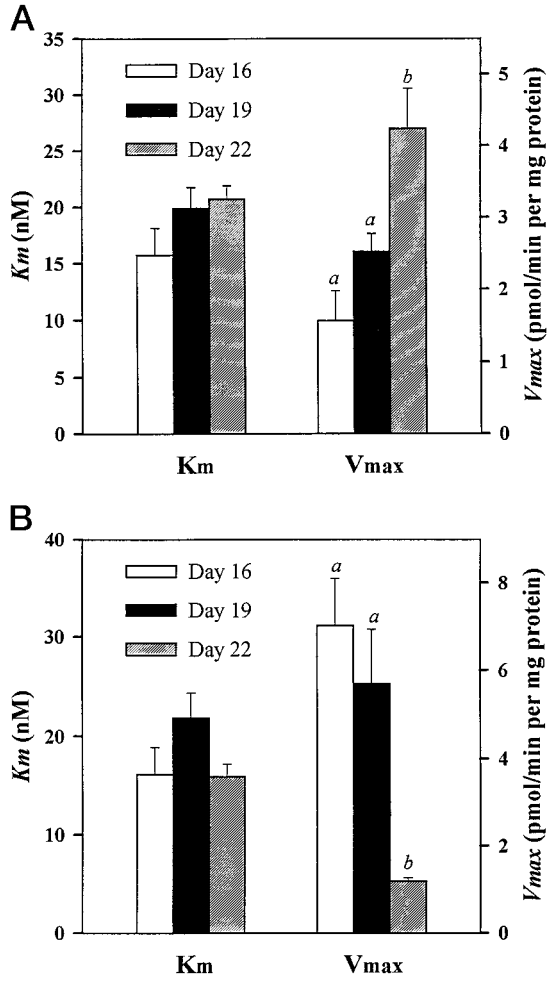

FIG. 3. V_{max} (pmol/min per mg protein) of 11β-HSD-2 in homogenates of (a) basal and (b) labyrinth placental zones at days 16 (n = 4), 19 (n = 3) and 22 (n = 9) of rat pregnancy. Values are the mean ± SE. Homogenates of each placental zone were incubated in duplicate (500 μg protein/ml) with 10 nM [^{3}H]corticosterone, 400 μM NAD^+, and varying concentrations of authentic corticosterone (0–200 nM) for 10 min at 37 C. [^{3}H]corticosterone and [^{3}H]11-dehydrocorticosterone were isolated and quantitated by HPLC. The V_{max} and apparent K_m were derived for each placental tissue by Lineweaver-Burk plots and least squares analysis.

readily detectable in whole placentas on day 22 of pregnancy after removal of fetuses 3 or 6 days earlier (Fig. 4). The apparent K_m of the enzyme in fetectomized placentas was not different from that in either basal or labyrinth zones of control placentas, whereas the V_{max} was intermediate between that in basal and labyrinth zones. The V_{max} in whole placenta at day 22 was similar in the Fx16 group (2.0 ± 0.4 pmol/min per mg protein) and the Fx19 group (1.7 ± 0.2), and neither differed significantly from the value for whole placentas in control rats (2.2 ± 0.2 pmol/min per mg protein; derived from separate estimates made for basal and labyrinth zones and their relative weights).

Discussion

The present study demonstrates colocalization of mRNAs for 11β-HSD-1, -2, and the GR to the basal and labyrinth zones of the rat placenta and thus highlights the potential importance of 11β-HSD-1 and -2 as regulators of glucocorticoid action within these tissues. Moreover, whereas expression of the GR appeared relatively stable during late pregnancy, distinct patterns of change were evident for expression of the two 11β-HSD enzymes and for bioactivity specific to 11β-HSD-2 in the two placental zones. Our data also indicate that these changes in placental 11β-HSD do not appear to be in response to development of the fetal HPA axis because induction of early maturation of the fetal adrenal cortex by maternal adrenalectomy did not alter the patterns of mRNA expression for the two 11β-HSD enzymes, or bioactivity specific to 11β-HSD-2. This lack of regulation by the fetal HPA axis is further supported by the observation that complete removal of the fetus (fetectomy) had no apparent effect on the changes in 11β-HSD-1 and -2 expression in the placental basal zone over the final days of pregnancy.

TABLE 2. Characteristics of 11β-HSD-2 bioactivity in basal and labyrinth zones of the placenta on day 19 of pregnancy in control rats (n = 3) and in rats adrenalectomized six days earlier (Maternal Adx; n = 4)

	K_m (nM)	V_{max} (pmol/min per mg protein)
Basal zone		
Control	20.4 ± 2.5	2.3 ± 0.2
Maternal Adx	18.8 ± 2.7	2.5 ± 0.4
Labyrinth zone		
Control	19.8 ± 2.0	5.0 ± 1.4
Maternal Adx	17.6 ± 2.2	4.4 ± 0.7

Values are the mean ± SE.
There were no significant effects of Maternal Adx in either placental zone (unpaired *t* tests).

The distribution of mRNAs for the 11β-HSD enzymes in the two placental zones identified by *in situ* hybridization is consistent with the pattern recently reported by Burton *et al.* (7) using S1 nuclease analysis. The striking feature of this pattern is that marked yet opposite changes occur in the expression of the two enzymes in the two placental zones over the final week of pregnancy. Thus, whereas mRNA for 11β-HSD-2 virtually disappeared in trophoblast cells of labyrinth zone between days 16 and 22, it was at least maintained in those of the basal zone. Conversely, mRNA for 11β-HSD-1 was effectively absent from the labyrinth zone on day 16 but then increased dramatically to day 22. These contrasting patterns of mRNA expression for 11β-HSD-1 and -2 in the two zones are suggestive of distinct regulatory signals operating at these two locations. This may reflect differences in the supply of maternal blood to the two placental zones, which is directed initially to the labyrinth zone before passing to the basal zone (22). Indeed, because this vascular arrangement effectively represents a portal system, it raises the possibility that products of the labyrinth zone *per se* could influence gene expression in the basal zone, including that for the two 11β-HSD enzymes.

The pattern of 11β-HSD-2 bioactivity in the basal and labyrinth zones closely paralleled the mRNA expression for this enzyme. The observed apparent K_m (in the order of 20 nM) is consistent with 11β-HSD-2 bioactivity (23, 24) and was generally similar between the zones and with advancing pregnancy. One exception was the higher K_m evident in basal compared with labyrinth zone at day 22, possibly reflecting higher levels

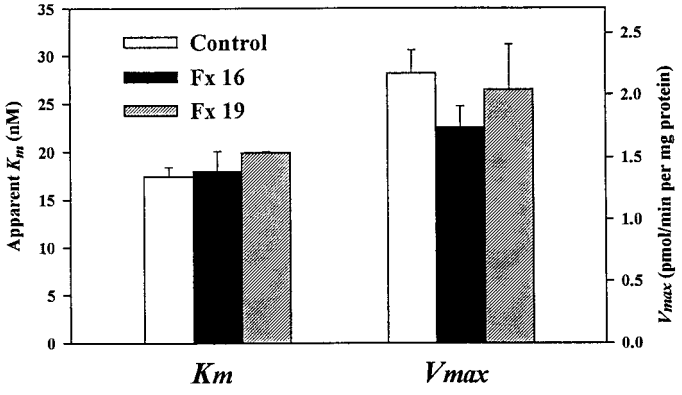

FIG. 4. 11β-HSD-2 bioactivity (apparent K_m and V_{max}) in whole placenta from day 22 pregnant rats fetectomized at either day 16 (Fx16) or day 19 (Fx19), and in control placentas from the same stage of pregnancy. Values are the mean ± SE (n = 3 per group for fetectomized rats and n = 9 for control rats). In control rats separate estimates of 11β-HSD-2 bioactivity were made in the basal and labyrinth zones, and values for whole placenta were derived on the basis of the relative weights of each placental zone (see Table 1). See legend to Fig. 3 for bioassay details.

of progesterone in the basal zone at this time. Progesterone is a potent inhibitor of 11β-HSD (25, 26) and is synthesized locally within the basal zone of the rat placenta (18, 27), albeit at a relatively low level *in vivo* (28). In contrast to 11β-HSD-2, bioactivity characteristic of 11β-HSD-1 was not detectable in either placental zone at any stage of pregnancy, despite the presence of substrate concentrations up to and well beyond the known K_m for this enzyme. This presumably reflects a loss of enzyme activity associated with cellular disruption during homogenization because Burton *et al.* (7) recently showed that 11-oxoreductase activity (generally ascribed to the 11β-HSD-1 enzyme; see Refs. 29–31) in tissue fragments of rat placenta changed in parallel with 11β-HSD-1 mRNA expression and immunoreactivity. An alternative explanation for the absence of 11β-HSD-1-specific bioactivity is that the 11β-HSD-1 mRNA detected by *in situ* hybridization in the present work is an alternative transcript that encodes a protein lacking enzyme activity as occurs in rat kidney (32). This seems unlikely, however, given the considerable 11-oxoreductase activity associated with 11β-HSD-1 in placental fragments (7).

Colocalization of mRNA expression for the GR and the 11β-HSD enzymes in basal and labyrinth zones suggests that placental 11β-HSD could regulate glucocorticoid effects within the placenta. Although previous studies had identified glucocorticoid binding sites indicative of the GR in the placenta of several species including the rat (33–35), to our knowledge the present work provides the first evidence of mRNA expression for the GR in rat placenta. It was recently suggested by Karalis *et al.* (9) that the presence of 11β-HSD in the human placenta may limit local bioactivity of maternal cortisol and thereby influence placental function(s). This is likely to be the effect of 11β-HSD-2 in the rat placenta because this enzyme appears to act exclusively as an 11β-dehydrogenase (23, 24) and as such would reduce levels of active glucocorticoid. On the other hand, the present study shows that 11β-HSD-1 is also coexpressed with the GR in rat placenta, and so glucocorticoid bioactivity could even be enhanced at some stages of pregnancy, depending on the specific placental zone and the direction of the reaction catalyzed by 11β-HSD-1. Although this form of the enzyme has the capacity for bidirectional activity, albeit inherently difficult to measure in homogenates as discussed above, 11-oxoreductase activity appears to be the dominant reaction in intact cells (29–31) and tissues (7, 36) including the basal and labyrinth zones of the rat placenta (7). The very different expression patterns for 11β-HSD-1 and -2 in these adjacent placental zones also raises the possibility of a physiological interaction between the enzymes in relation to substrate supply, similar to that proposed for the uterine endometrium where the two enzymes are differentially expressed in epithelial and stromal cells (37).

With regard to specific glucocorticoid actions within the placenta, several important placental functions are affected by glucocorticoids including synthesis of peptide (9, 38) and steroid (39) hormones. There may also be a number of interactions between glucocorticoids and progesterone such as that recently demonstrated by Karalis *et al.* (9) in which the inhibition of CRH synthesis by progesterone in cultured human trophoblasts was blocked by cortisol. This effect of progesterone is thought to be mediated via progesterone interaction with the GR rather than the progesterone receptor (PR) because the latter is not detectable in human trophoblasts (9). Although the rat placenta does not produce CRH, progesterone does have other important effects on placental function in this species, most notably with respect to growth (40). Moreover, whereas the present study localizes mRNA for the GR to both placental zones, a previous report indicates that the PR is expressed only in basal zone (41). This raises the possibility that any progesterone effects in the labyrinth zone may be mediated via the GR, and as such would be susceptible to inhibition by glucocorticoids as occurs in human placental trophoblasts (9).

Two important species differences are evident with respect to the pattern of placental 11β-HSD expression in the rat and the well characterized baboon model (for review, see Ref. 10). Firstly, the pattern of change in the rat labyrinth zone (*i.e.* reduced 11β-HSD-2 and increased 11β-HSD-1 mRNA expression) is suggestive of a reduction in the placental glucocorticoid barrier between mother and fetus, whereas this barrier is clearly enhanced late in baboon pregnancy (42, 43). Secondly, fetectomy studies in the baboon show that unlike observations of the present work, the shift in placental 11β-HSD activity with advancing pregnancy is dependent on the presence of the fetus (44), specifically through its supply of adrenal androgens for placental estrogen synthesis (26). Indeed, this enhancement of the placental glucocorticoid barrier near term appears to activate maturation of the baboon fetal HPA axis and thus promote fetal autonomy (10, 43). The present study in the rat, however, shows that premature activation of the fetal HPA axis had no effect on 11β-HSD-2 bioactivity in either basal or labyrinth zones, and fetectomy had no apparent effect on mRNA expression for either 11β-HSD-1 or -2 in the basal zone. Moreover, 11β-HSD-2 bioactivity in whole placenta on day 22 of pregnancy was unaffected by fetectomy 3 or 6 days earlier. Fetectomy did prevent the marked increase in 11β-HSD-1 mRNA expression in labyrinth zone, but the biological significance of this effect remains uncertain because there was considerable degenerative change in this zone post fetectomy, consistent with previous analyses of placental morphology after fetectomy (45). It is noteworthy in this regard that GR mRNA expression

was also absent throughout most of the labyrinth zone after fetectomy, presumably reflecting loss of trophoblast, fetal mesenchyme, and fetal vascular tissues. One reason why the influence of the fetus on placental 11β-HSD may be so different in the rat and baboon is the presence in the latter of a feto-placental unit for estrogen synthesis. Thus, the primate placenta is dependent on a continuous and increasing supply of fetal adrenal androgens to ensure rising estrogen levels near term (for review see Ref. 10), and because the trophic drive for this fetal androgen production is derived partly from fetal pituitary ACTH, loss of the placental glucocorticoid barrier could block this trophic support and thereby compromise placental estrogen synthesis. In contrast, the rise in maternal estrogen observed near term in the rat is ovarian in origin (46) and thus not dependent on fetal adrenal status. One could postulate, therefore, that evolution of the feto-placental unit for estrogen synthesis, which is unique to higher primates, required an associated change in the pattern of placental 11β-HSD expression.

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