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Changes in the Placental Glucocorticoid Barrier During Rat Pregnancy: Impact on Placental Corticosterone Levels and Regulation by Progesterone[1]

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ABSTRACT

Glucocorticoid excess in utero inhibits fetal growth and programs adverse outcomes in adult offspring. Access of maternal glucocorticoid to the glucocorticoid receptor (NR3C1) in the placenta and fetus is regulated by metabolism via the 11beta-hydroxysteroid dehydrogenase (HSD11B) enzymes, as well as multidrug resistance P-glycoprotein (ABCB1)-mediated efflux of glucocorticoids from the syncytiotrophoblast. This study determined expression of genes encoding the two HSD11B isoforms (*Hsd11b1* and *Hsd11b2*), the two ABCB1 isoforms (*Abcb1a* and *Abcb1b*), and *Nr3c1* in the junctional and labyrinth zones of rat placentas at Days 16 and 22 of normal gestation (Day 23 is term). To assess possible regulation of the *Hsd11b* and *Abcb1* isoforms by glucocorticoids and progesterone, their placental expression was also measured at Day 22 after partial progesterone withdrawal from Day 16 (maternal ovariectomy plus full estrogen and partial progesterone replacement) or after treatment with dexamethasone acetate (1 μg/ml of drinking water from Day 13). Expression of *Hsd11b1* mRNA increased in the labyrinth zone (the site of maternal-fetal exchange) from Day 16 to Day 22, whereas that of *Hsd11b2* fell dramatically. Consistent with these changes, corticosterone levels increased 10-fold in the labyrinth zone over this period. Expression of both *Abcb1a* and *Abcb1b* was markedly higher in the labyrinth zone compared with the junctional zone on both days, consistent with the proposed barrier role of ABCB1 in the placenta. *Nr3c1* mRNA expression was similar in the two placental zones at Day 16 but increased 3-fold in the labyrinth zone by Day 22. Partial progesterone withdrawal increased *Hsd11b1* mRNA and protein expression in the labyrinth zone but decreased *Nr3c1* mRNA expression. These data show that the dynamic expression patterns of the placental HSD11Bs in late gestation are associated with dramatic shifts in placental corticosterone. Moreover, the late gestational rise in labyrinthine *Hsd11b1* seems to be driven by the normal prepartum fall in progesterone level.

11beta-hydroxysteroid dehydrogenase, corticosterone, P-glycoprotein, placental glucocorticoid barrier, progesterone

INTRODUCTION

Intrauterine growth retardation increases the risk of neonatal morbidity and mortality [1] and is implicated in the programming of adult-onset diseases [2, 3]. Glucocorticoids provide key signals for differentiation of fetal and placental tissues (principally via cortisol in humans and corticosterone in rodents), yet excess glucocorticoid exposure limits placental and fetal growth [4–6]. Therefore, regulation of glucocorticoid access to the placenta and fetus is recognized as an important determinant of pregnancy outcome and subsequent development of the postnatal phenotype. Glucocorticoid access to the placenta and fetus is determined principally by the placental glucocorticoid barrier [4–6], the major component of which is the enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2), which catalyzes the conversion of active glucocorticoids to their inert metabolites (11-dehydrocorticosterone in rodents). Previous studies [7, 8] show that late in rat pregnancy HSD11B2 expression falls dramatically in the placental labyrinth zone, the site of maternal-fetal exchange and the region of major growth during the fetal period [8]. In contrast, labyrinthine expression of the bidirectional enzyme HSD11B1, which typically reactivates 11-dehydrocorticosterone via oxoreductase activity [9], increases over the same period [7, 8]. These patterns of placental HSD11B expression are likely to increase levels of corticosterone in the placental labyrinth near term (i.e., reduce the placental glucocorticoid barrier), but such measurements have not been reported, to our knowledge. Moreover, the stimulus for changes in placental HSD11B expression near term is unknown. Results of several in vitro studies of placental [10, 11] and nonplacental [12, 13] cells suggest that progesterone and/or glucocorticoids may regulate expression of the HSD11B isoforms, and maternal levels of both steroids are highly dynamic over the second half of pregnancy. Specifically, maternal corticosterone increases progressively from midpregnancy [14], whereas progesterone declines rapidly near term in preparation for parturition [15].

In the present study, therefore, we measured expression of mRNAs encoding *Hsd11b1* and *Hsd11b2* and levels of endogenous corticosterone in the junctional and labyrinth zones of the placenta at Days 16 and 22 of normal pregnancy. We also tested the hypothesis that placental expression of *Hsd11b1* and *Hsd11b2* is regulated by progesterone and glucocorticoids over the final third of pregnancy. This involved the use of two experimental models: partial progesterone withdrawal from Day 16 (to a level that still maintained pregnancy) [16] and maternal dexamethasone treatment from Day 13 [17]. Most important, both of these treatments are known to reduce placental and fetal growth. We also determined placental expression of multidrug resistance P-glycoprotein (ABCB1) (a membrane-bound efflux protein that may also contribute to the placental glucocorticoid barrier [18–20]) and the glucocorticoid receptor (NR3C1) (the expression of which increases over gestation in other species [21–23]). Rodents express two isoforms of ABCB1 encoded by *Abcb1a* and *Abcb1b* [23, 24–28], both of which increase with advancing gestation in whole rat placenta [27], but their relative expression in the two placental zones of this species has not been quantified. Moreover, results of previous in vitro

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TABLE 1. Primer sequences and conditions for quantitative PCR.

Gene	Sequence[a]	Cycling conditions (45 cycles)	Product size (bp)
Hsd11b1	F: 5′-CTCTCTGTGTCCTCGGCTTC-3′	95°C/1 sec	136
	R: 5′-TTCCATGATCCTCCTTCCTG-3′	59°C/15 sec	
		72°C/5 sec	
Hsd11b2	F: 5′-GATGTTCCCCTCGCCTGAA-3′	95°C/1 sec	349
	R: 5′-ATGAGCAGTGCAATAGCTGCCTTG-3′	59°C/15 sec	
		72°C/5 sec	
Abcb1a	F: 5′-CAAGCTGAAGGACGAAATGA-3′	95°C/1 sec	158
	R: 5′-GATTCATGATGGCATGGAAA-3′	59°C/30 sec	
		72°C/20 sec	
Abcb1b	F: 5′-CTGCCGAGCGTTACTAATCA-3′	95°C/1 sec	238
	R: 5′-TCACGTCAAACCAGCCTATC-3′	59°C/30 sec	
		72°C/20 sec	
Nr3c1	F: 5′-CTTGAGAAACTTACACCTCGATGACC-3′	95°C/1 sec	461
	R: 5′-AGCAGTAGGTAAGGAGATTCTCAACC-3′	62°C/20 sec	
		72°C/30 sec	
Rpl19	F: 5′-CTGAAGGTCAAAGGGAATGTG-3′	95°C/1 sec	195
	R: 5′-GGACAGAGTCTTGATGATCTC-3′	52°C/15 sec	
		72°C/5 sec	

[a] F, Forward; R, reverse.

studies [29–33] suggest that placental expression of the two *Abcb1* isoforms may also be regulated by progesterone and glucocorticoids.

MATERIALS AND METHODS

Animals and Chemicals

Nulliparous albino Wistar rats (n = 4–8 per group) aged between 8 and 12 wk were obtained from Animal Resources Centre (Murdoch, Australia) and were maintained under controlled conditions as described previously [34]. Rats were mated overnight, and the day on which spermatozoa were present in a vaginal smear was designated as Day 1 of pregnancy. All procedures involving animals were conducted after approval by the Animal Ethics Committee of The University of Western Australia. Dexamethasone acetate and progesterone were obtained from Sigma-Aldrich (Sydney, Australia) and [1,2,6,7-^{3}H]corticosterone from Amersham Australia (Sydney, Australia). Primers for PCR were synthesized by Geneworks (Adelaide, Australia).

Steroid Manipulation and Tissue Collection

Maternal progesterone was reduced prematurely by ovariectomy on Day 16, followed by full replacement of estrogen and either full or partial replacement of progesterone as described in a previous study [16]. Briefly, after ovariectomy, the progesterone-reduced animals received estradiol via a subcutaneous miniosmotic pump (Alzet, Sydney, Australia) at a rate of 40 ng/h in propylene glycol. In addition, rats received twice-daily injections of estradiol (s.c. in 0.2 ml of peanut oil) from Day 18 to mimic the normal increase in plasma estradiol levels in late gestation (250 ng per injection on Days 18 and 19 and 500 ng per injection on Days 20 and 21). Progesterone was administered (s.c. in peanut oil) to reflect the normal decline in circulating progesterone over the final week of gestation (for progesterone-restored animals, 10 mg on Day 16 and then twice-daily injections of 10 mg on Day 17, 7.5 mg on Day 18, 5.0 mg on Day 19, and 2.5 mg on Days 20 and 21) or to maintain levels (from Day 16 onward) to approximately one third of those normally observed at Day 22 (for progesterone-reduced animals, a single injection of 0.5 mg on Day 16 and then twice-daily injections of 0.5 mg on Days 17–21). Blood samples from the dorsal aorta at Day 22 collections showed that maternal estradiol levels for progesterone-reduced and progesterone-restored animals were comparable to those for controls. Similarly, Day 22 progesterone levels in progesterone-restored animals were comparable to those of controls, while levels from progesterone-reduced animals were about one third of those of sham-operated animals [16]. Fetal and placental glucocorticoid exposure was increased by administration of dexamethasone acetate (1 μg/ml; Sigma, St. Louis, MO) in the maternal drinking water from Day 13 to Day 22 of pregnancy (Day 23 is term) in intact mothers [17].

Rats were anesthetized with isofluorane/nitrous oxide at Day 16 or Day 22 of gestation, and junctional and labyrinth placental zones were separated by blunt dissection and snap frozen on liquid nitrogen. As previously reported, premature progesterone withdrawal reduced placental and fetal weights in these rats by 10% and 24%, respectively [17], whereas the corresponding reductions after dexamethasone treatment were 32% and 24%, respectively [16]. Neither treatment significantly affected litter size [16, 17].

RNA Sample Preparation

Total RNA was isolated from placental zones using Tri-Reagent (Molecular Resources Center, Cincinnati, OH) according to the manufacturer's instructions. RNA integrity was assessed by agarose gel electrophoresis (data not shown). Total RNA (5 μg) was used to synthesize cDNA using M-MLV Reverse Transcriptase RNase H Point Mutant and random hexamer primers (Promega, Madison, WI) according to the manufacturer's instructions and containing 2.5 mg/ml of Ficoll 400 and 7.5 mg/ml of Ficoll 70 [35]. The resultant cDNAs were purified using the Ultraclean PCR Cleanup kit (MoBio Industries, Solana Beach, CA).

Quantitative RT-PCR

Analyses of expression levels for *Hsd11b* and *Abcb1* isoforms and for *Nr3c1* transcripts were performed by quantitative RT-PCR on the Rotorgene 6000 (Corbett Industries, Sydney, Australia) using Immolase DNA polymerase (Bioline, Alexandria, Australia). Primers for *Hsd11b1*, *Hsd11b2*, *Abcb1a*, *Abcb1b*, and *Nr3c1* (Table 1) were designed using Primer3 software (MIT/Whitehead Institute, http://www-genome.wi.mit.edu) [36]. Each of the selected primer pairs was positioned to span introns to ensure that no product was amplified from genomic DNA. Primers were used at a concentration of 0.2 μM, SYBR Green (Molecular Probes, Eugene, OR) at 1:40 000 of stock, $MgCl_2$ at 3 mM, and 0.5 U of Immolase enzyme per reaction. Ficoll supplementation (2.5 mg/ml of Ficoll 400 and 7.5 mg/ml of Ficoll 70) was used to improve PCR amplification efficiency [35]. Cycling conditions included an initial denaturation at 95°C for 10 min to activate the Immolase enzyme, followed by amplification for 45 cycles of the specific profiles indicated (Table 1). The resulting amplicons were sequenced to confirm specificity (data not shown). All samples were standardized against *Rpl19* as previously described [37]. Standard curves for each product were generated from gel-extracted (QIAEX II; Qiagen, Melbourne, Australia) PCR products using 10-fold serial dilutions and the Rotorgene 6000 software.

Microsomal Preparation and Western Blot Analyses

Microsomal fractions were prepared from labyrinth and junctional zone tissues for determination of HSD11B1 protein levels. Western blot analyses were performed essentially as described by Burton et al. [7]. Briefly, 50 mg of tissue was homogenized in four volumes of 10 mM sodium phosphate buffer (pH 7.0) containing 0.25 M sucrose, 1 mM edetic acid, 1 μM PMSF, and an appropriate amount of Complete-mini protease inhibitor (Roche Biochemicals, Sydney, Australia) with three 10-sec bursts using a Polytron homogenizer

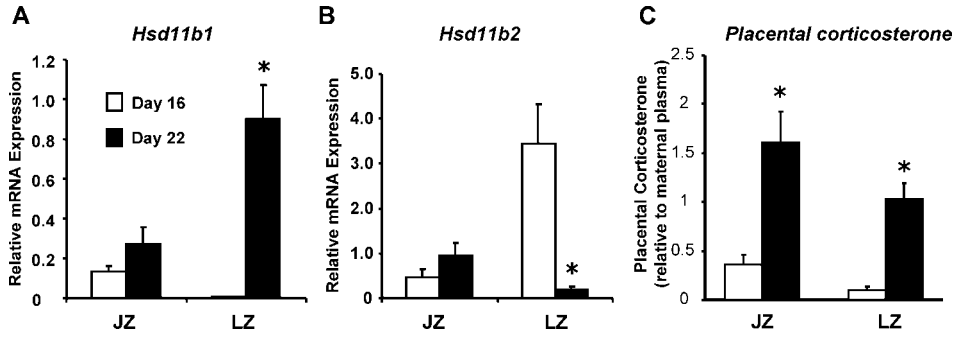

FIG. 1. Relative levels of *Hsd11b1* (**A**) and *Hsd11b2* (**B**) mRNA expression and placental corticosterone levels (**C**) relative to maternal plasma levels in the junctional (JZ) and labyrinth (LZ) zones of the rat placenta at Days 16 and 22 of normal gestation. Values are mean ± SEM (n = 5–8 per group). *$P < 0.01$ compared with corresponding value on Day 16 (two-way ANOVA and least significant difference test).

(Kinematica, Lucerne, Switzerland). The homogenate was centrifuged at 700 × *g* for 30 min at 4°C to remove debris, and nuclear and microsomal fractions were recovered from the supernatant by sequential centrifugations at 10 000 × *g* for 10 min and at 105 000 × *g* for 60 min, each at 4°C. The microsomal pellet was dissolved in 0.2 ml of phosphate buffer containing 0.25 M sucrose, and the protein concentration was determined by Bradford assay. Total microsomal protein (100 μg) was electrophoresed through a 12% polyacrylamide separating gel at 120 V. Following electrophoresis, the separated proteins were transferred to nitrocellulose membranes at 50 mA overnight at 4°C. Membranes were incubated for 1 h in blocking solution containing 5% nonfat milk and then with rabbit anti-HSD11B1 (RAH-113 diluted 1:1000) or rabbit anti-HSD11B2 antibodies (RAH-223 diluted 1:1000); both antibodies were a gift from Dr. Zygmunt Krozowski, Baker Medical Research Institute, Melbourne, Australia). To identify immunoreactive bands, membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (diluted 1:10 000; Santa Cruz Biotechnology, Santa Cruz, CA), and signals were visualized using a chemiluminescence detection kit (SuperSignal West-Pico Substrate; Pierce Chemical, Rockford, IL) and imaged on a Kodak 4000MM Image Station (Eastman-Kodak, Newhaven, CT). Resultant images were quantified by densitometry using Scion Image analysis software (Scion Corporation, Frederick, MD) as previously described [38].

Plasma and Tissue Corticosterone Levels

Placental junctional and labyrinth zones were homogenized in ice-cold PBS. After addition of 1000 cpm of ^{3}H-corticosterone to monitor procedural losses, the homogenate was extracted twice with ethyl acetate (four volumes), and the combined extracts were dried under nitrogen. The residue was reconstituted in 100 μl of charcoal-stripped plasma, and the corticosterone concentration was measured using a corticosterone kit (Active Rat Corticosterone enzyme immunoassay; Diagnostic Systems Laboratories, Webster, TX). Corticosterone concentrations in placental zones were expressed relative to those in maternal plasma from the same animal (measured directly in the enzyme immunoassay).

Statistical Analysis

Changes in *Hsd11b1*, *Hsd11b2*, *Abcb1a*, *Abcb1b*, and *Nr3c1* mRNA or protein levels and placental corticosterone concentrations in normal gestation and following progesterone and glucocorticoid manipulations were assessed by two-way or three-way ANOVAs using GenStat (Hemel Hempstead, Hertfordshire, UK) version 11.0 software, with gestational age, placental zone, and treatment as sources of variation. Where *F* test reached statistical significance ($P < 0.05$), specific differences were assessed by least significant difference test [39].

RESULTS

Placental Expression of the Hsd11b *and* Abcb1 *Isoforms and* Nr3c1 *in Normal Gestation*

Expression of *Hsd11b1* mRNA increased dramatically (>20-fold, $P < 0.01$) in the labyrinth zone from Day 16 to Day 22, whereas that of *Hsd11b2* fell (15-fold, $P < 0.01$) over the same period (Fig. 1, A and B). Consistent with these changes, the mean ± SEM concentration of corticosterone in the labyrinth zone was only 10% ± 4% of that in maternal plasma at Day 16 but had increased 10-fold to 103% ± 16% by Day 22 ($P < 0.01$) (Fig. 1C). The mean ± SEM junctional zone corticosterone level was 36% ± 10% of that in maternal plasma at Day 16 but had increased to 161% ± 32% by Day 22 ($P < 0.01$). The mean ± SEM maternal plasma corticosterone levels were similar at Day 16 (1387 ± 94 ng/ml) and at Day 22 (1322 ± 106 ng/ml). Labyrinthine expression of *Abcb1a* and *Abcb1b* mRNA far exceeded that in the junctional zone (7–10-fold, $P < 0.01$) on Days 16 and 22 of gestation (Fig. 2, A and B), and levels of both transcripts remained unchanged in both zones over this period. Expression of *Nr3c1* mRNA varied with gestational age ($P < 0.02$) and with placental zone ($P < 0.01$), and there was a significant age-×-zone interaction ($P < 0.01$). Thus, expression of *Nr3c1* was similar in the two zones at Day 16 but by Day 22 had increased by almost 3-fold only in the labyrinth zone ($P < 0.01$) (Fig. 2C).

Placental Expression of the Hsd11b *and* Abcb1 *Isoforms and* Nr3c1 *after Progesterone Manipulation*

Partial progesterone withdrawal from Day 16 (progesterone-reduced group) increased *Hsd11b1* mRNA expression in both the junctional (3-fold) and labyrinth (2-fold) zones at Day 22 of gestation ($P < 0.05$) (Fig. 3). There was a corresponding increase (2-fold, $P < 0.05$) in the level of HSD11B1 protein in

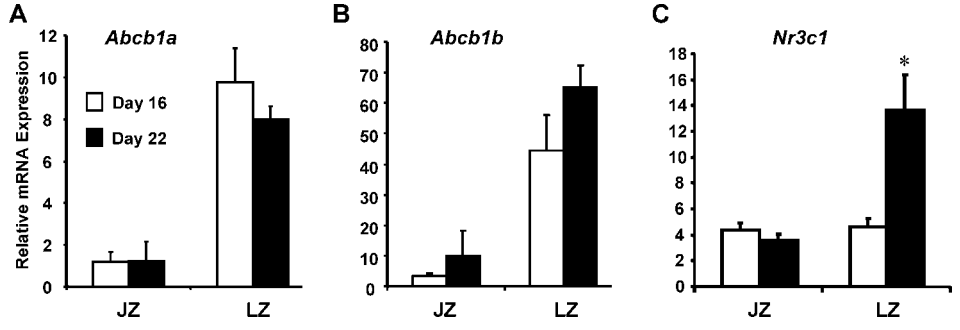

FIG. 2. Relative levels of *Abcb1a* (**A**), *Abcb1b* (**B**), and *Nr3c1* (**C**) mRNA expression in the junctional (JZ) and labyrinth (LZ) zones of the rat placenta at Days 16 and 22 of normal gestation. Values are mean ± SEM (n = 5–8 per group). *$P < 0.01$ compared with corresponding junctional zone (two-way ANOVA and least significant difference test).

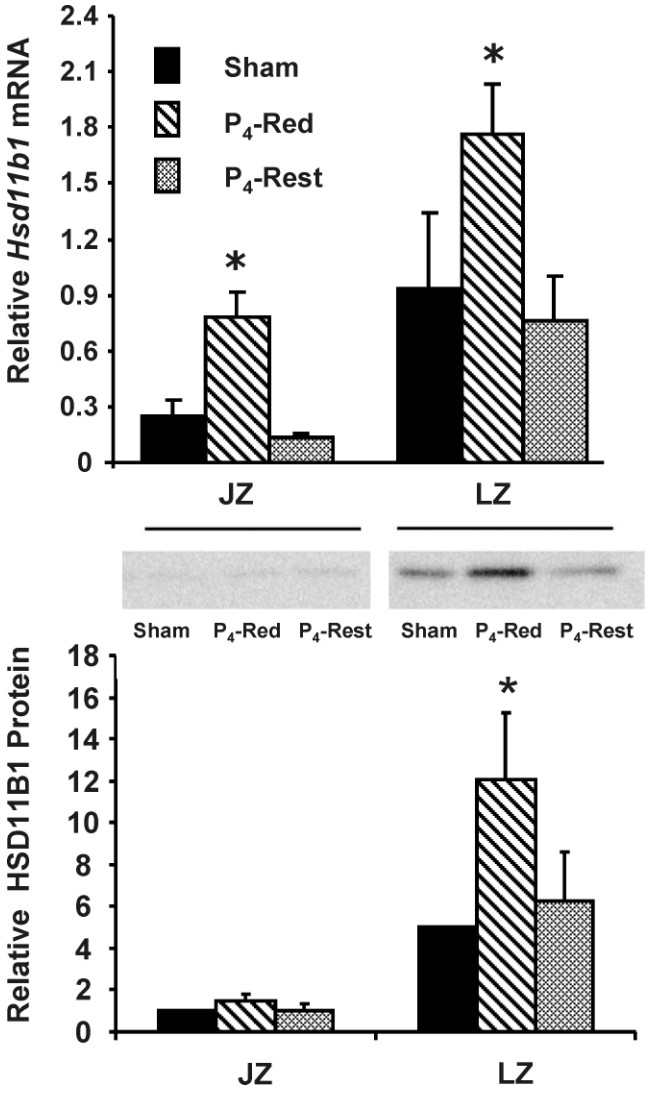

FIG. 3. Relative levels of *Hsd11b1* mRNA and HSD11B1 protein in the junctional (JZ) and labyrinth (LZ) zones of the rat placenta following progesterone manipulation. Animals were ovariectomized on Day 16, and estradiol was replaced to parallel its normal increase over the final week of pregnancy. Progesterone levels were either fully restored (P_4-Rest) or replaced at a reduced level (P_4-Red). Control animals (Sham) were sham operated and injected with vehicle. Values are mean ± SEM (n = 7–13 per group). *$P < 0.05$ compared with corresponding Sham or P_4-Rest value (two-way ANOVA and least significant difference test).

the labyrinth zone following partial progesterone withdrawal (Fig. 3). Most important, these increases in placental *Hsd11b1* mRNA and protein were not evident in ovariectomized mothers that received full replacement of both progesterone and estrogen (progesterone-restored group). Partial progesterone withdrawal had no significant effect on expression of *Hsd11b2* mRNA or protein (data not shown) or *Abcb1a* or *Abcb1b* in either of the placental zones (Fig. 4, A and B). Similarly, progesterone manipulations did not affect the Day 22 mean ± SEM corticosterone levels in maternal plasma (933 ± 113 ng/ml in sham, 697 ± 93 ng/ml in progesterone reduced, and 697 ± 61 ng/ml in progesterone restored) or the mean ± SEM percentages of maternal plasma concentrations in the labyrinth zone (98% ± 12% in sham, 106% ± 21% in progesterone reduced, and 96% ± 18% in progesterone restored) or in the junctional zone (145% ± 63% in sham, 202% ± 54% in progesterone reduced, and 195% ± 32% in progesterone restored). Partial progesterone withdrawal decreased the *Nr3c1* transcript level within the labyrinth zone (2.5-fold, $P < 0.01$), while *Nr3c1* levels in the progesterone-restored animals were comparable to those in controls (Fig. 4C).

Effects of Dexamethasone on Placental Expression of the Hsd11b *and* Abcb1 *Isoforms and* Nr3c1

Maternal dexamethasone treatment from Day 13 decreased labyrinthine expression of *Abcb1a* mRNA by ~45% on Day 22 ($P < 0.05$) but had no effect on junctional zone expression of *Abcb1a* (Fig. 5A) or expression of *Abcb1b* (Fig. 5B), *Hsd11b1*, *Hsd11b2*, or *Nr3c1* in either zone (data not shown). As expected, maternal dexamethasone treatment reduced maternal plasma corticosterone to undetectable levels (<5 ng/ml), and placental concentrations were similarly reduced to very low or undetectable levels.

DISCUSSION

Exposure of the placenta and fetus to glucocorticoids is a key determinant of fetal growth and development and can profoundly influence the subsequent phenotype of adult offspring [40]. The placental glucocorticoid barrier limits exposure of the fetus to maternal glucocorticoids, but this barrier is highly dynamic late in pregnancy, particularly in rodents [7, 8]. In this study, we investigated the expression of placental glucocorticoid barrier components (the HSD11B and ABCB1 isoforms) and their regulation by glucocorticoids and progesterone, as well as placental expression of NR3C1. The major findings were that expression of *Hsd11b1* mRNA in the labyrinth zone (the major site of maternal-fetal exchange) increased in late gestation, whereas that of *Hsd11b2* was almost completely lost. Consistent with these patterns of *Hsd11b1* and *Hsd11b2* expression, the local concentration of corticosterone in the labyrinth zone increased 10-fold during late gestation. The increase in labyrinthine *Hsd11b1* was further enhanced by a premature reduction in progesterone, suggesting that the normal prepartum decline in progesterone stimulates the rise in HSD11B1 that occurs before parturition.

The loss of labyrinthine HSD11B2 and a concomitant rise in HSD11B1 in rat pregnancy has been reported previously on the basis of shifts in bioactivity and mRNA expression measured by S1 nuclease assay and in situ hybridization [7, 8]. The present study confirms these patterns of placental HSD11B expression using the more sensitive quantitative RT-PCR approach and shows that they are associated with a marked increase in local levels of placental corticosterone. Most notably, the concentration of corticosterone in the labyrinth zone increased 10-fold between Days 16 and 22, presumably reflecting the rise in HSD11B1 and the concomitant loss of HSD11B2. This provides further support for loss of the placental glucocorticoid barrier near term, with the associated increase in transplacental passage of maternal corticosterone to the fetus likely ensuring coordinated maturation of vital organs in all fetuses. Most important, this study also shows that labyrinthine expression of *Nr3c1* mRNA increases almost 3-fold between Days 16 and 22, indicative of increased glucocorticoid sensitivity of this tissue. Although previous estimates of rat placental labyrinthine *Nr3c1* mRNA determined by the less quantitative in situ hybridization approach did not show a change with advancing gestation [8], a recent study [23] on the ontogeny of *Nr3c1* expression in the mouse placenta showed an increase toward term. Moreover, higher placental NR3C1 expression has been noted with advancing

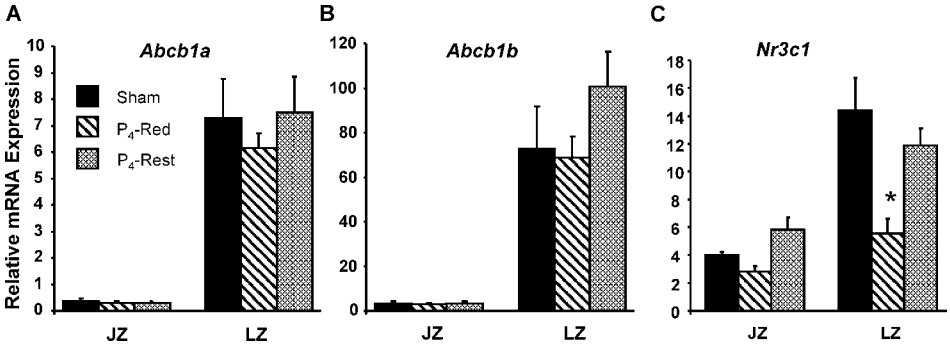

FIG. 4. Relative levels of *Abcb1a* (**A**), *Abcb1b* (**B**), and *Nr3c1* (**C**) mRNA in the junctional (JZ) and labyrinth (LZ) zones of the rat placenta following progesterone manipulation. Animals were ovariectomized on Day 16, and estradiol was replaced to parallel its normal increase over the final week of pregnancy. Progesterone levels were either fully restored (P_4-Rest) or replaced at a reduced level (P_4-Red). Control animals (Sham) were sham operated and injected with vehicle. Values are mean ± SEM (n = 5 per group). $^*P < 0.05$ compared with corresponding Sham or P_4-Rest value (two-way ANOVA and least significant difference test).

gestation in other species [22, 41, 42]. The increase in labyrinthine *Nr3c1* expression, together with the elevation in local levels of corticosterone, suggests that the near-term labyrinth placenta is profoundly affected by glucocorticoids. Indeed, there is now considerable evidence showing that the placenta is an important glucocorticoid target tissue. For example, we have identified several aspects of placental labyrinth function that are influenced by glucocorticoids, including inhibition of leptin receptor (*Lepr*) expression and leptin (*Lep*) transport [38, 43], reduced vascular endothelial growth factor (*Vegfa*) expression and an associated reduction in placental vascularity [17, 44], and reduced expression of peroxisome proliferator-activated receptor-γ (*Pparg*) [17]. Moreover, a recent study [45] of global gene expression in the mouse placenta shows that dexamethasone alters expression of a large number of placental genes. Because many of the glucocorticoid effects observed in these studies relate to growth inhibition, it seems likely that increased glucocorticoid sensitivity of the rat placenta near term contributes to the slowing of placental and thus fetal growth.

Although the dynamic patterns of placental *Hsd11b1* and *Hsd11b2* mRNA expression in rodent pregnancy were previously recognized [7, 8], little was known about the underlying stimulus. Herein, we show that a premature reduction in maternal progesterone, which also inhibits both placental and fetal growth [16], increases placental labyrinth expression of HSD11B1. This suggests that the normal prepartum decline in maternal progesterone (due to reduced ovarian secretion [15]) stimulates HSD11B1 expression in the placental labyrinth zone. Although progesterone is a kinetic inhibitor of *Hsd11b1* in rat hepatocytes [46], we are unaware of any previous studies that show progesterone inhibition of *Hsd11b1* expression. Progesterone receptor isoforms are expressed in the rat junctional zone, but expression in the labyrinth zone is very low [16], which suggests the intriguing possibility that progesterone-mediated suppression of labyrinthine *Hsd11b1* may occur via NR3C1. This is supported by previous studies [47, 48] showing that progesterone regulates gene expression in human trophoblast via interaction with NR3C1. Despite the rise in labyrinthine *Hsd11b1* expression after partial progesterone withdrawal, labyrinthine corticosterone was not different from that in the sham group at Day 22. This may reflect differences in the supply of substrate (i.e., 11-dehydrocorticosterone) to the labyrinthine *Hsd11b*, as local corticosterone levels are the net result of substrate supply and local enzyme activity.

Estrogen is also known to regulate expression of the HSD11B enzymes in the placenta of other species ([49] and in the rat endometrium [50], but whether estrogen regulates rat placental HSD11B is unknown. In the present study, although rats were ovariectomized in the progesterone manipulation experiment, estradiol was fully replaced to mimic the normal increase in its ovarian secretion over late gestation. Thus, the possible role of estradiol in regulating placental HSD11B expression in the rat requires further study.

The presence of ABCB1 within the placenta is known to reduce fetal exposure to xenobiotic compounds that are potentially harmful to the developing fetus [51–53]. Recent evidence in humans and rodents also implicates a role for ABCB1 in restricting penetration of glucocorticoids across blood-tissue barriers [54–56], including that at the maternal-placental interface [18–20]. The present study shows that most placental *Abcb1a* and *Abcb1b* mRNA is expressed in the labyrinth zone, consistent with previous reports of ABCB1 immunolocalization and *Abcb1a* and *Abcb1b* mRNA expression measured by in situ hybridization [18, 27]. This site of expression is consistent with the proposed role of ABCB1 in preventing the transfer of endogenous glucocorticoids and exogenous xenobiotic compounds from the maternal to the fetal circulation. It is unclear whether the amount of ABCB1 present at the maternal-fetal interface is sufficient to make a significant contribution to the placental glucocorticoid barrier

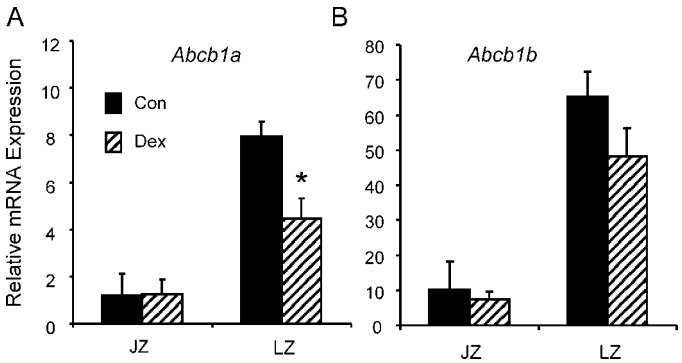

FIG. 5. Relative levels of *Abcb1a* (**A**) and *Abcb1b* (**B**) mRNA in the junctional (JZ) and labyrinth (LZ) zones of the rat placenta at Day 22 in control animals (Con) and following maternal dexamethasone administration from Day 13 (Dex). Values are mean ± SEM (n = 5–8 per group). $^*P < 0.05$ compared with corresponding Con value (two-way ANOVA and least significant difference test).

in vivo (relative to that of HSD11B2). Most important, our data show that, unlike the decline observed in mouse [18, 57] and human [58] placenta, labyrinthine expression of both *Abcb1* isoforms is maintained late in rat pregnancy, coincident with the loss of *Hsd11b2* expression. This suggests that the relative contribution of ABCB1 to the placental glucocorticoid barrier may increase near term. Maternal dexamethasone treatment reduced labyrinthine expression of *Abcb1a*, although the functional significance of this effect is unclear because expression of *Abcb1a* in the rodent placenta is very low compared with expression of *Abcb1b* [18, 27]. In contrast, partial progesterone withdrawal did not affect placental expression of either *Abcb1a* or *Abcb1b*, consistent with the recent observations by Petropoulos et al. [57], who showed that prevention of the prepartum decline in maternal progesterone in the mouse did not influence placental expression of the *Abcb1* isoforms. Progesterone has previously been shown to upregulate *Abcb1* expression in uterine secretory epithelium [29] and in primary human trophoblasts [32], and so the absence of similar effects in the present study is suggestive of tissue-specific regulation of *Abcb1* expression.

In conclusion, the present study shows that changes in the placental labyrinthine expression of *Hsd11b1* and *Hsd11b2* are associated with a marked rise in local corticosterone levels, consistent with a reduced placental glucocorticoid barrier at term. Premature progesterone withdrawal further increased placental expression of *Hsd11b1*, suggesting that the normal rise in this enzyme may be driven by the prepartum decline in circulating progesterone. We also show that *Abcb1a* and *Abcb1b* are expressed predominantly in the labyrinth zone and are thus ideally placed to limit the transfer of endogenous glucocorticoids and exogenous xenobiotic compounds from the maternal to the fetal circulation.

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