Glucocorticoid-induced apoptosis in human decidua: a novel role for 11β-hydroxysteroid dehydrogenase in late gestation

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Abstract

Glucocorticoids play a fundamental role in the endocrinology of pregnancy but excess glucocorticoids *in utero* may lead to abnormalities of fetal growth. Protection against fetal exposure to cortisol is provided by the enzyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) located in the human placental trophoblast. By contrast, relatively little is known concerning the function of glucocorticoid-activating 11β-HSD1, which is strongly expressed within human maternal decidua. To address this we have assessed: i) changes in decidual 11β-HSD1 expression across gestation and ii) the functional role of glucocorticoids in decidua. Human decidua was collected from women undergoing surgical termination of pregnancy in first ($n=32$) and second ($n=10$) trimesters, and elective caesarean sections in the third trimester ($n=9$). Analysis of mRNA for 11β-HSD1 by real-time RT-PCR showed increased expression in second (9·3-fold, $P<0·01$) and third (210-fold, $P<0·001$) trimesters. Studies using primary cultures of decidual cells also revealed higher levels of cortisol generation in the third trimester. Changes in decidual 11β-HSD1 with gestation were paralleled by increased expression of the apoptosis markers caspase-3 and annexin-V, particularly in cluster designation (CD)10^{-VE} non-stromal cells (20-fold in third trimester relative to first trimester). Apoptosis was also readily induced in primary cultures of third trimester decidual cells when treated with cortisol, cortisone, or dexamethasone (all 100 nM for 24 h). The effect of cortisone but not cortisol or dexamethasone was blocked by an 11β-HSD inhibitor confirming the functional significance of endogenous cortisol generation. These data show that autocrine metabolism of glucocorticoids is an important facet of the feto-placental unit in late gestation and we propose that a possible effect of this is to stimulate programmed cell death in human decidua.

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Introduction

There is strong epidemiological evidence showing an association between smaller size at birth and, in later life, development of hypertension, insulin resistance, type 2 diabetes, and cardiovascular disease-related deaths (Barker *et al.* 1993*a*,*b*, Curhan *et al.* 1996). This association is not evident in all studies (Stanner & Yudkin 2001) but this is likely to be due, at least in part, to modifying effects of early childhood growth (Hanson & Gluckman 2005). Birth weight and other anthropometric markers of size at birth are probably not direct determinants of hypertension in adulthood but are proxy markers of altered fetal development. Current evidence suggests that there are two major environmental influences, which can modulate fetal development and lead to permanent alterations in the cardiovascular system: these are undernutrition and over-exposure to glucocorticoids (Seckl & Meaney 2004).

Evidence that overexposure of the fetus to glucocorticoids can affect its growth and development comes from two sets of observations. Firstly, pregnant women treated with glucocorticoids, because they are at risk of preterm labor, tend to have babies of lower than normal birth weight (Seckl 2004, Seckl & Meaney 2004). Secondly, endogenous fetal plasma cortisol concentrations are increased in pregnancies where there has been intrauterine growth restriction (IUGR; Goland *et al.* 1993). In normal pregnancies maternal plasma cortisol at term is ∼200 ng/ml, while fetal cortisol is about 20 ng/ml (Shams *et al.* 1998). This tenfold difference is probably due to the protective effects of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), an enzyme which is highly expressed in the placenta and converts active cortisol to inactive cortisone (Shams *et al.* 1998). There is reduced placental 11β-HSD2 expression in human pregnancies complicated by IUGR (Shams *et al.* 1998) and babies with

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deleterious mutations in the *11β-HSD2* gene have very low birth weight (Stewart *et al.* 1996). These studies have highlighted the importance of 11β-HSD2 in attenuating fetal exposure to cortisol. Paradoxically, there appears to be a significant capacity for synthesis of this active glucocorticoid during pregnancy via the type 1 isozyme of 11β-HSD1. In contrast to the type 2 isozyme, 11β-HSD1 can catalyze both dehydrogenase (cortisol inactivation) and reductase (cortisol synthesis) activities, although in most tissues the latter predominates (Tomlinson *et al.* 2004). Expression of 11β-HSD1 has been described in placental tissues from humans (Ricketts *et al.* 1998, Pepe *et al.* 1999), rats (Waddell *et al.* 1998), and baboons (Pepe *et al.* 1999), with activity data favoring reductase activity or cortisol generation (Sun *et al.* 1999). The enzyme has been detected in extravillous trophoblasts, chorion, and amnion epithelial cells but is particularly abundant in decidual stromal cells (Sun *et al.* 1997, Ricketts *et al.* 1998).

To investigate further the physiological significance of localized activation of glucocorticoids during pregnancy, we have quantified the expression and activity of 11β-HSD1 in human decidua at different stages of gestation, demonstrating the increased capacity for cortisol synthesis in third trimester tissue. We have also shown that decidual expression of 11β-HSD1 correlates closely with markers of apoptosis, a process intimately associated with glucocorticoid action (Wyllie 1980, Wyllie & Morris 1982). Functional studies using cultured decidual cells suggest that 11β-HSD1 acts in an autocrine or paracrine fashion to increase local cortisol levels and promote apoptosis. We postulate that this mechanism represents a novel but important facet of glucocorticoid action during pregnancy.

Materials and Methods

Subjects

Decidua were obtained from women undergoing elective caesarean section at term (38–41 weeks) at the Birmingham Women's Hospital, University of Birmingham, UK and elective surgical termination of pregnancy in the first trimester (5–12 weeks) and in the second trimester (13–20 weeks) at the Calthorpe Hospital/Birmingham Women's Hospital and at the Royal Victoria Infirmary, Newcastle, UK with informed consent and approval from local ethical committees (LREC 5194/1999).

Primary cultures of human decidual cells and purification of decidual cells into $CD10^{+VE}$ and $CD10^{-VE}$ populations

Decidual tissue was separated from the first and second trimesters samples based on macroscopic appearance and from third trimester samples by blunt curettage of the uterine cavity at the site of placental insertion. Finely minced decidual tissue, under sterile conditions was subjected to two vigorous collagenase (Sigma Chemical Co.) digestions of 30 min at room temperature and a final concentration of 0·03 g/ml. The resulting cell suspension was centrifuged at 300 ***g*** for 1 min, the supernatant removed and then sieved through a 100/40 mm cell strainer followed by further centrifugation at 200 ***g*** for 10 min. The remaining cell pellet was resuspended in serum-free RPMI 1640 medium (Sigma) and cell counting performed. Immunomagnetic bead selection (Miltenyi Biotec, Surrey, UK) was used to isolate cluster designation 10 positive ($CD10^{+VE}$) stromal-enriched cells. Briefly, decidual cells were incubated with primary mouse monoclonal anti-CD10 antibody (1/100 dilution; Novocastra Laboratories, Newcastle upon Tyne, UK) for 30 min at 4 °C. The cells were then washed using ice-cold PBS and recentrifuged for 10 min at 200 ***g***. The resulting cell pellet was resuspended in Midi-Macs buffer (as per manufacturer's instructions) and incubated with 2 μl Macs goat anti-mouse IgG micro beads/1×10^6 cells (Miltenyi Biotec) for 15 min at 4 °C. This cell suspension was then passed through a Midi-Mac separation column (Miltenyi Biotec). A non-bound, $CD10^{-VE}$ stromal cell-depleted fraction was initially isolated. After two washes in Midi-Mac buffer, bound $CD10^{+VE}$ stromal-enriched cells were then eluted. In addition to the isolation of RNA, aliquots of each cell fraction were retained for cytospins to estimate selection efficiency using immunohistochemical analysis (80–90% purity; data not shown). The decidual cells were either used immediately for RNA extraction or incubated for 24 h with cortisone, cortisol, and dexamethasone (at 100 nM) with and without glycerrhetinic acid (at 5 μM) in an atmosphere of 4% CO_2 at 37 °C.

RNA extraction and quantitative PCR

Total RNA was extracted from primary decidual cells (including $CD10^{+VE}$ and $^{-VE}$ cells) immediately after collection, as well as primary decidual cells incubated for 24-h treatment of glucocorticoids and dexamethasone (at 100 nM) with and without glycerrhetinic acid (at 5 μM). RNA was extracted using the StrataPrep total RNA miniprep kit (Stratagene, Amsterdam, The Netherlands). One microgram of RNA from each sample was reversed transcribed using AMV reverse transcriptase (Promega Corp.) and random hexamers in 20 μl reaction volumes according to the manufacturer's instructions. The mRNA levels were analyzed using an ABI7700 sequence detection system (PE Biosystems, Warrington, UK). Briefly, RT-PCR was performed in 25 μl volumes on 96-well plates in reaction buffer containing TaqMan Universal PCR Master Mix, 3 mM $Mn(Oac)_2$, 200 μM dNTPs, 1·25 U AmpliTaq Gold polymerase, 1·25 U AmpErase UNG, 100–200 nmol TaqMan probe, 900 nmol primers and 25–50 ng cDNA. All reactions were multiplexed with 18S control probe (PE Biosystems). Reactions were as follows: 50 °C for 2 min, 95 °C for 10 min, and then 44 cycles of 95 °C for 15 s and 60 °C for 1 min. Data were obtained as C_t values as per the manufacturer's guidelines (the cycle number at, which logarithmic PCR plots cross a calculated threshold line) and used to determine ΔC_t values ($\Delta C_t = C_t$ of the target gene minus C_t of 18S). Measurements

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were carried out on at least three occasions for each sample. Sequences of oligonucleotide primers and probes were as follows: 11β-HSD1 antisense primer 5′-AGGAAAGCT-CAT-GGGAGGACTAG-3′, sense primer 5′-ATGGTGAA-TATCATCATGAAAAAGATTC-3′, probe 5′-CATGCT-CATTCTCAACCACATCACCAACA-3′; hexose-6-phosphate dehydrogenase (H6PDH) antisense primer 5′-CAGG-TGTCCTAGTGCA-CATTGAC-3′, sense primer 5′-GT-AGCCCACTCTCTC-GTCCAA-3′, probe 5′-AAGGC-ACGCCCTCCCAGCG-3′; GRα, sense primer 5′-AAC-TGG CAGCGGTTTTATCAACT-3′, antisense primer 5-AATACTCATGGTCTTATCCAAAAATGTTT-3′, probe ATTCTATGCATGAAGTGGTGGAAAATCTCCTTAAC-TATTG; C/EBPα, forward primer TGGACAAGAACAG-CAACGAG, reverse primer TTGTCACTGGTCAGC-TCCAG, probe CACCTTCTGCTGCGTCTCCACGTT; C/EBPβ forward primer GACAAGCACAGCGACGAGTA, reverse primer GTGCTGCGTCTCCAGGTT, probe ATC-TTGGCCTTGTCGCGGCTCTT; TaqMan gene expression assays for cyclo-oxygenase 2 (COX-2) (Hs00153133_m1), prostaglandin dehydrogenase (PGDH) (Hs00168359_m1), caspase-3 (Hs00234387_m1) and 18S rRNA (QuantumRNA) were purchased from Applied Biosystems.

Measurement of 11β-HSD1 activity

The 11β-HSD enzyme assays were carried out by incubating intact cells with 250 nM cortisone or 50 nM cortisol with appropriate tritiated tracer [^{3}H]-cortisol (specific activity 78·4 Ci/mmol, NEN Life Science Products, Hounslow, UK) or [^{3}H]-cortisone (generated in house as described previously (Bujalska *et al.* 1997)) at 37 °C (Tomlinson *et al.* 2002). After incubation, steroids were extracted using dichlormethane, separated by thin-layer chromatography using silica plates as the solid phase (Fluka, Buchs, Switzerland) with a mobile phase of ethanol/chloroform (8:92). The fractional conversion of cortisol to cortisone and cortisone to cortisol was analyzed using a Bioscan 3000 Imager (LabLogic, Sheffield, UK). Protein levels were assayed using a commercially available kit (Bio-Rad Laboratories Inc). Activity was expressed as picomoles of product formed per mg protein per hour.

Detection of decidual apoptosis

Identification of apoptosis was carried out on decidual cultures treated with cortisone, cortisol, and dexamethasone. Aliquots ($0{\cdot}6\times10^6$) of CD10^{+VE} and CD10^{-VE} decidual cells were washed with cold PBS and then resuspended at concentration of 10^6 cells/ml in 1× flow cytometry binding buffer. Fluorescein isothyocyanate (FITC)-conjugated human annexin-V antibody and propidium iodide were then added to the cell suspensions and incubated at room temperature for 15 min. Analysis of annexin-V and propidium iodide expression in the decidual cells was carried within the next 1 h by flow cytometry.

Immunohistochemistry

Dewaxed and rehydrated decidual sections were boiled in citrate buffer (10 mM citric acid, 0·05% Tween 20, pH 6·0) in a pressure cooker for 1 min and cooled slowly. To reduce nonspecific binding sections were blocked with 10% normal goat serum in PBS for 10 min before incubating with primary antibody (Rabbit anti-active caspase-3 (BD Biosciences 1:100 dilution in PBS) overnight at 4 °C. Negative control sections were incubated in PBS without primary antibody and human tonsil was used as the positive control. Sections were incubated in peroxidase blocking solution for 10 min before detection was performed using Dako REAL Detection System according to the manufacturer's protocol. Washes between each step were performed in PBS (pH 7·4). Sections were counterstained with Mayer's hematoxylin for 45 s.

Statistical analysis

All results were determined as mean ± S.E.M. Statistical analysis was performed on the triplicate average raw on raw ΔC_t values and enzyme activity data using one-way ANOVA with Student–Newman–Keuls Multiple Comparison post-test or Pearson Correlation (Sigma-Stat3 V2.03; Systat Software Inc., Point Richmond, CA, USA).

Results

Change in 11β-HSD1 expression and activity across gestation in human decidua

Results in Fig. 1A show that expression of mRNA for both 11β-HSD1 and glucocorticoid receptor (GR) was increased in whole tissue samples from first trimester decidua (nine- and four-fold respectively) compared with first trimester decidua. However, whereas 11β-HSD1 levels continued to rise in whole tissue from the third trimester (210-fold compared with first trimester), there was a significant reduction in GR expression (50-fold decrease compared with first trimester). Further, studies using primary cultures of decidual cells isolated from either first or third trimester decidua confirmed that the increased expression of 11β-HSD1 in late gestation was associated with enhanced glucocorticoid metabolism (Fig. 1B). Both oxo-reductase (cortisone to cortisol) and dehydrogenase (cortisol to cortisone) activities were higher in cells from third trimester deciduas compared with first trimester ($P<0{\cdot}01$ and 0·05 respectively). However, the ratio of reductase to dehydrogenase activity was significantly higher in third trimester cells (1·44 ± 0·30 S.D. versus 0·73 ± 0·10, $P<0{\cdot}05$).

Analysis of 11β-HSD1 expression in stromal and non-stromal decidual cells

To further characterize the mechanisms associated with enhanced cortisol biosynthesis in third trimester decidua, decidual cells were purified into stromal (CD10^{+VE}) and

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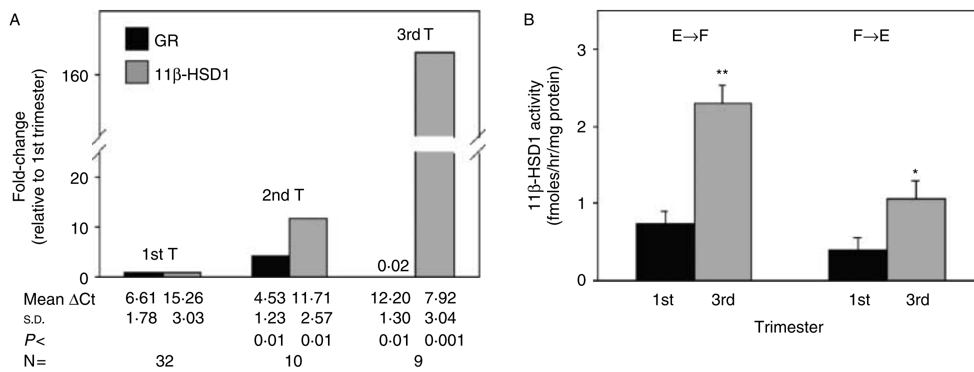

Figure 1 Expression of glucocorticoid receptor (GR) and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in human decidua across gestation. (A) mRNA levels for GR and 11β-HSD1 in first (n=32), second (n=10) and third (n=9) trimesters decidual tissue (first, second, and third trimesters). Values shown are fold-induction of mRNA levels determined by real-time RT-PCR relative to first trimester decidua (value=1). (B) Enzyme activity for 11β-HSD1 in primary cultures of cells isolated from first or third trimester deciduas (n=4). Data shown are for oxido-reductase activity (metabolism of cortisone (E) to cortisol (F)) and dehydrogenase activity (metabolism of F to E). *Statistically different from first trimester cells, $P<0{\cdot}05$. **Statistically different from first trimester cells, $P<0{\cdot}01$.

non-stromal (CD10$^{-\text{VE}}$) populations (Fig. 2). Analysis of gene expression in these sub-populations confirmed that there was increased expression of 11β-HSD1 in third trimester decidual cells compared with first trimester cells, with this increase being greater in CD10$^{-\text{VE}}$ cells (186-fold increase compared with first trimester expression) than in CD10$^{+\text{VE}}$ cells (tenfold increase compared with first trimester). Increased expression of 11β-HSD1 has been reported to be associated with increased expression of CCAAT-enhancer-binding proteins (C/EBP) α and β, known transcriptional regulators of the enzyme (Williams *et al.* 2000). It was, therefore, interesting to note that expression of mRNA for C/EBPα increased 36-fold and 14-fold in CD10$^{-\text{VE}}$ and CD10$^{+\text{VE}}$ cells respectively in the third trimester. Similar effects were also observed for C/EBPβ, which increased 42-fold and 6-fold respectively in CD10$^{-\text{VE}}$ and CD10$^{+\text{VE}}$ third trimester decidual cells. The relatively high levels of 11β-HSD1 in third trimester CD10$^{-\text{VE}}$ cells were also associated with increased expression of H6PDH (threefold higher in third trimester CD10$^{-\text{VE}}$ versus CD10$^{+\text{VE}}$ cells), an enzyme known to be involved in generating co-factor for 11β-HSD1 reductase activity (Draper *et al.* 2003, Hewitt *et al.* 2005, Lavery *et al.* 2005).

Expression of 11β-HSD1 in human decidual cells is associated with the apoptosis marker caspase-3

In tissues similar to decidua where there is close interaction between stromal and non-stromal cells (such as the thymus), glucocorticoids are known to play a key role in modulating programmed cell death (Wyllie 1980, Wyllie & Morris 1982). Therefore, to assess the possible functional impact of enhanced localized generation of cortisol within third trimester decidua, further RT-PCR analyses were carried out to characterize expression of the apoptosis marker caspase-3 (Fig. 3). Caspase-3 expression was increased in decidual biopsies across gestation (Fig. 3A). Furthermore, this occurred primarily in non-stromal CD10$^{-\text{VE}}$ cells (Fig. 3B) and correlated strongly with 11β-HSD1 expression (Fig. 3C). These observations did not appear to be linked to the initiation of parturition, as analysis of laboring and non-laboring choriodecidua showed no significant difference in 11β-HSD1 or H6PDH expression (data not shown).

Immunostaining for active caspase-3 was performed on first trimester (Fig. 4A) and third trimester (Fig. 4B and C) decidua (n=3 each group). Tonsil was used as a positive control (Fig. 4E) and negative controls showed no immunostaining (Fig. 4D). In first trimester decidua, there were

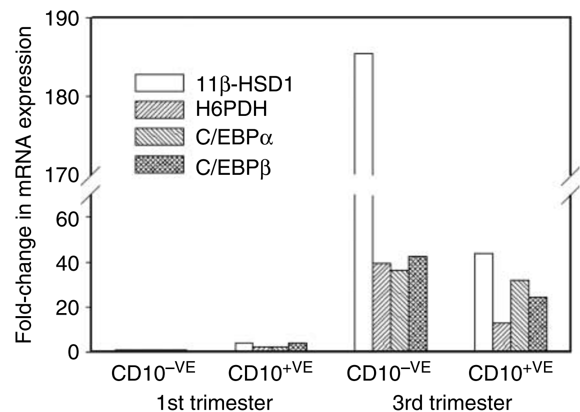

Figure 2 Expression of 11β-HSD1 and associated genes in stromal (CD10$^{+\text{VE}}$) and non-stromal (CD10$^{-\text{VE}}$) decidual cells in first and third trimesters tissue. Cell purified according to CD10 expression were used to isolate RNA for real-time RT-PCR analysis of: 11β-HSD1; hexose-6-phosphate dehydrogenase (H6PDH); ccaat-enhancer binding protein (C/EBP)α and C/EBPβ. Data are shown as fold-change in expression relative to first trimester CD10$^{-\text{VE}}$ cells (value=1).

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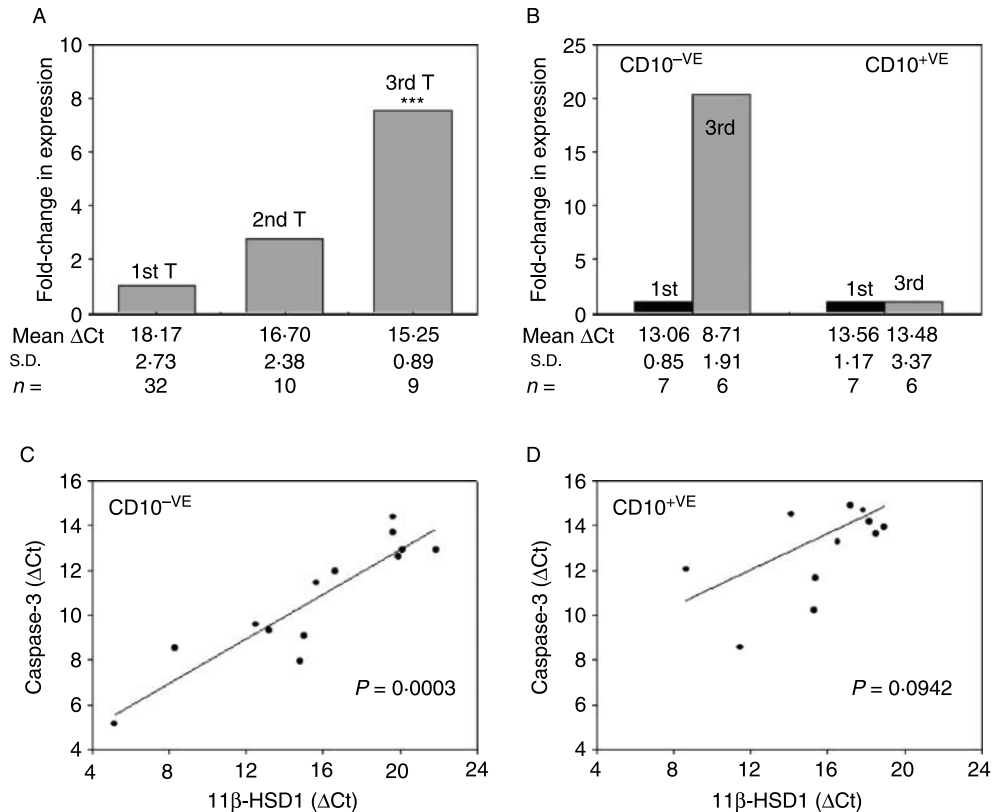

Figure 3 Expression of 11β-HSD1 by CD10^{-VE} third trimester decidual cells is associated with apoptosis. (A) Increased expression of caspase-3 mRNA in second and third trimesters decidual biopsies. Data are shown as fold-change in mRNA expression relative to first trimester decidua (value =1). ***Statistically different from first trimester deciduas, $P<0{\cdot}001$. (B) Increased expression of caspase-3 mRNA in CD10^{-VE} third trimester decidual cells. Data are shown as fold-increase in expression relative to equivalent first trimester cells. (C) Correlation between caspase-3 and 11β-HSD1 in CD10^{-VE} cells. (D) Correlation between caspase-3 and 11β-HSD1 in CD10^{+VE} cells.

scattered immunopositive cells, including decidualized stromal cells, leucocytes (Fig. 4A), and extravillous trophoblast. In third trimester superficial decidua basalis attached to the placenta and there was immunopositivity for active caspase-3 in decidualized stromal cells and extravillous trophoblast cells (Fig. 4B and C). Formal quantification was not performed as sample numbers were limited and caspase 3 immunoreactivity was seen in several different cell types. However, there was no clear quantitative difference in immunostaining of decidual stromal cells between first and third trimester tissues.

Autocrine synthesis of cortisol and the induction of apoptosis in primary cultures of decidual cells

The close association between expression of caspase-3 and 11β-HSD1 in decidual biopsies suggested a possible autocrine mechanism for induction of apoptosis via localized synthesis of cortisol. To investigate this further, primary cultures of decidual cells were used to assess the capacity for induction of apoptosis by 'active' cortisol or 'inactive' cortisone (Fig. 5). RT-PCR analyses showed that both glucocorticoids were able to induce expression of caspase-3 and COX-2 but they had no effect on 11β-HSD1, H6PDH, or PGDH, an enzyme associated with parturition (Fig. 5A). Cortisone also stimulated cell surface expression of annexin-V another marker of apoptosis, which is expressed early in the apoptotic pathway (Fig. 5B). This effect was similar to that seen with cortisol and dexamethasone but in contrast to these two active glucocorticoids, the effect of cortisone was blocked by co-incubation with glycyrrhetinic acid, a known inhibitor of 11β-HSD activity.

Discussion

Glucocorticoids are an essential component of normal pregnancy with pluripotent effects on decidualization and implantation (Arcuri *et al.* 1996, 1997), placental development (Malassine & Cronier 2002), fetal brain development, and lung maturation and parturition (Challis *et al.* 2001, Whittle *et al.* 2001). Conversely, in excess, glucocorticoids are

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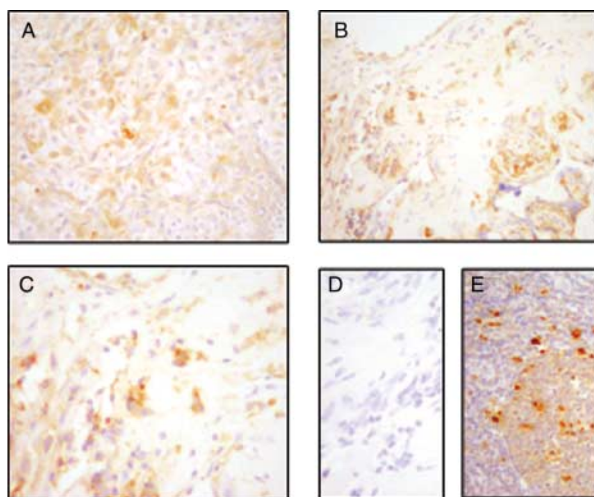

Figure 4 Immunohistochemical localization of activated caspase-3 expression in human decidua across gestation. (A) Activated caspase-3 expression in first trimester decidua showing scattered positive cells (×100 magnification). (B) Third trimester decidua (×100 magnification) showing positive immunoreactivity of villous syncytiotrophoblast and scattered positive cells in superficial decidua basalis. (C) Third trimester decidua (×200 magnification). (D) Third trimester decidua (same specimen as 4C) negative control (×200 magnifaction). (E) Activated caspase-3 expression in human tonsil positive control (×100 magnification).

known to exert detrimental effects on fetal growth (Han & Carter 2001, McMillen *et al.* 2001, Seckl & Meaney 2004), with the consequences of this extending through to adult health (Barker *et al.* 1993*a*,*b*, Goland *et al.* 1993, Curhan *et al.* 1996, Challis *et al.* 2001, Bertram & Hanson 2002, Seckl 2004, Seckl & Meaney 2004).

In the absence of a functionally developed fetal hypothalamic–pituitary–adrenal system, regulation of fetal exposure to glucocorticoids is facilitated by tissue-specific metabolism of glucocorticoids, catalyzed by the enzymes 11β-HSD1 and 11β-HSD2 (Stewart *et al.* 1996, Condon *et al.* 1998, Shams *et al.* 1998, Tomlinson *et al.* 2004). To date, studies have focused primarily on the role of 11β-HSD2 in the placental trophoblast as a mechanism for limiting fetal exposure to glucocorticoids (Stewart *et al.* 1996, Shams *et al.* 1998, McTernan *et al.* 2001). However, it is now clear that tissues within the fetal–placental unit also express 11β-HSD1 and are capable of synthesizing cortisol rather than inactivating it (Baggia *et al.* 1990, Pepe *et al.* 1996, 1999, Sun *et al.* 1997, 1999, Ricketts *et al.* 1998, Waddell *et al.* 1998, Alfaidy *et al.* 2003). The induction of 11β-HSD1 expression, which we have reported for second and third trimesters human decidua is similar to that and which has been previously described for 11β-HSD1 in human fetal membranes and syncytiotrophoblast from baboons (Pepe *et al.* 1996, Alfaidy *et al.* 2003). These studies suggested that, in contrast to 11β-HSD2, which acts to protect the fetus from high levels of circulating maternal steroids, 11β-HSD1 within intrauterine membranes may be more closely linked to the process of parturition through the generation of localized concentrations of cortisol within amniotic fluid (Alfaidy *et al.* 2003).

The most obvious target for glucocorticoid-mediated effects on parturition is prostaglandin synthesis and action (Liggins & Grieves 1971, Potestio *et al.* 1988, Whittle *et al.* 2001). This, in turn, may influence production of a wide range of pro-inflammatory cytokines (Keelan *et al.* 2003) and cellular events such as the induction of

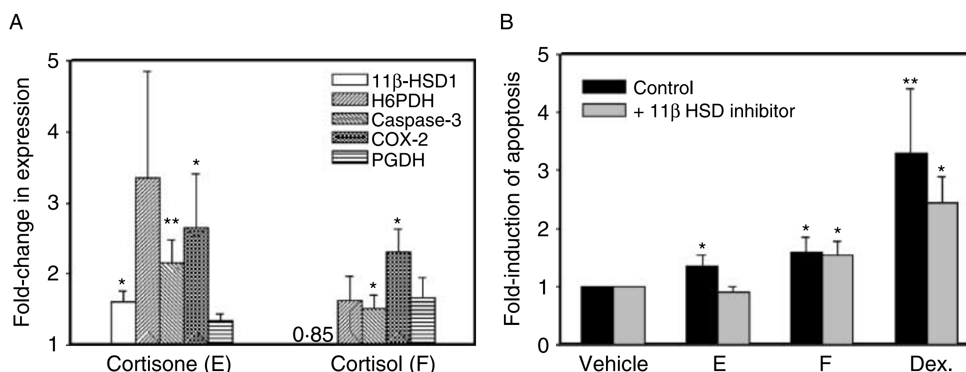

Figure 5 Autocrine induction of apoptosis by glucocorticoids in primary cultures of human decidual cells. (A) Treatment of third trimester decidual cells for 24 h with 'inactive' cortisone (100 nM) and 'active' cortisol (100 nM) induces expression of caspase-3 and COX-2. Data shown are the fold-increase in expression relative to vehicle treated cells (value=1). $n=4$, *Statistically different from vehicle-treated cells $P<0·05$; **Statistically different from vehicle-treated cells $P<0·01$. (B) Autocrine induction of decidual cell apoptosis by cortisone is blocked by the 11β-HSD inhibitor glycyrrhetinic acid (GA). Third trimester decidual cells were cultured for 24 h with cortisone (100 nM), cortisol (100 nM) or dexamethasone (dex.) (100 nM) in the presence (black bars) or absence of GA (grey bars). Cells were then assessed for apoptosis by FACS analysis of cell surface annexin-V expression. Data are shown as the fold-increase in % of apoptotic cells relative to vehicle-treated cells. $n=4$, *Statistically different from vehicle-treated cells $P<0·05$; **Statistically different from vehicle-treated cells $P<0·01$.

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apoptosis (Mu *et al.* 2002, Boos *et al.* 2003, Keelan *et al.* 2003, Correia-da-Silva *et al.* 2005). Indeed, in view of the well established link between the latter and glucocorticoid responses (Wyllie 1980, Wyllie & Morris 1982, O'Brien *et al.* 2004) it is also possible that glucocorticoids will act directly to induce apoptosis in placenta/decidua. Analysis of target genes associated with parturition (COX-2; Kniss 1999, Simmons *et al.* 2004) and PGDH (Challis *et al.* 1999, 2002)), as well as apoptosis (caspase-3) suggests that glucocorticoids can influence both mechanisms (Fig. 5).

Within decidua, apoptosis appears to occur in an autocrine fashion as a consequence of increased generation of cortisol catalyzed by 11β-HSD1. Although mRNA analysis of biopsy material revealed a clear correlation between expression of 11β-HSD1 and caspase-3 across gestation, we were unable to show similar changes in activated caspase expression by immunohistochemistry. This is consistent with previous studies with TUNEL and ApopTag where we have also failed to demonstrate any major differences in apoptosis between first and third trimesters decidual tissues (data not shown). One possible explanation for this is that the apoptosis associated with decidual 11β-HSD1 is restricted to cells with enhanced availability of substrate for the oxo-reductase activity of this enzyme, namely cortisone. The abundance of cortisone within the fetal–placental unit is principally dependent on the activity of trophoblastic 11β-HSD2. As such, decidua that has proximity to trophoblast may act as a 'hotspot' for 11β-HSD1-mediated regeneration of cortisol and, in turn, these cells may be more susceptible to apoptosis. Furthermore, the fact that the enhanced apoptosis observed in third trimester decidua was predominantly associated with $CD10^{-VE}$ non-stromal cells suggests that these cells may be key targets for localized responses to cortisol. It is possible that localized effects of 11β-HSD1 may be difficult to detect in tissue sections which examine a small sample from a limited area, in contrast to the cell culture studies using material from large decidual samples. Although CD10 has relatively limited expression within decidua, being expressed by decidual stromal cells and extravillous trophoblast (D'Ambrosio *et al.* 2003), the $CD10^{-VE}$ population is more diverse, including leukocytes, endothelial and epithelial cells. Possible target cells within third trimester decidua would be the endometrial leucocyte populations. Apoptosis has been reported in leukocytes in the first trimester (Hammer & Dohr 1999) but studies of apoptosis later pregnancy have focused on extravillous trophoblast cells.

The molecular mechanism underlying the induction of 11β-HSD1 mRNA in late gestation remains to be elucidated. However, the coincident upregulation of C/EBPα and C/EBPβ, two transcription factors known to stimulate expression of 11β-HSD1 in the liver (Williams *et al.* 2000), suggests that they play a pivotal role in regulating the enzyme in decidua. Although C/EBPα and β appear to be key determinants of the expression of 11β-HSD1, the actual capacity for cortisol generation is dependent on extra-nuclear regulation of the enzyme. In previous studies, we have shown that cofactor (NADPH) generation within the lumen of the endoplasmic reticulum is a crucial mechanism in maintaining reductase activity of 11β-HSD1 (Draper *et al.* 2003, Hewitt *et al.* 2005, Lavery *et al.* 2005). The enzyme which catalyzes synthesis of NADPH, H6PDH was strongly induced in third trimester decidua, particularly in $CD10^{-VE}$ non-stromal cells. This was coincident with a significant increase in the ratio of reductase/dehydrogenase activity in these samples, suggesting that H6PDH is an important novel consideration in defining the metabolism and action of glucocorticoids across gestation.

A key objective of this study was to assess the possible cell-specific variations in decidual 11β-HSD1 expression. Separation of $CD10^{+VE}$ stromal enriched and $CD10^{-VE}$ non-stromal cells showed that although there was upregulation of 11β-HSD1 mRNA in both populations, this was considerably greater in $CD10^{-VE}$ non-stromal cells. The fact that the $CD10^{-VE}$ population also expressed much higher levels of H6PDH underlines their potential as cortisol-generating cells. It was, therefore, interesting to note that correlation between expression of 11β-HSD1 and the apoptosis marker caspase-3 was only observed for $CD10^{-VE}$ cells. Likewise, the increased expression of caspase-3 across gestation (Fig. 3A) appears to be entirely due to the contribution of $CD10^{-VE}$ cells (Fig. 3B). These data suggest that within decidual cells, the functional significance of 11β-HSD1 expression is not simply due to the magnitude of its transactivation but is also highly dependent on the localized redox potential as defined by the NADPH-generating enzyme H6PDH.

Data presented here provide further evidence that 11β-HSD1 plays a significant role in defining the bioactivity of glucocorticoids in the fetal–placental unit. In particular, we have shown that autocrine conversion of cortisone to cortisol has the potential to act as a local stimulator of decidual cell apoptosis. This effect appears to be restricted to the non-stromal component of decidua, which includes substantial numbers of immune cells. Thus, a possible role of 11β-HSD1 in late gestation may be to regulate the apoptosis of specific decidual leukocyte populations, such as uterine natural killer cells, T cells, or macrophages. Previous studies have shown that expression of Fas ligand was decreased in uNK cells from women with preeclampsia, possibly reflecting a reduced ability of these cells to undergo Fas–Fas ligand-mediated apoptosis (Darmochwal-Kolarz *et al.* 2000). The role of glucocorticoids in this process and the extent to, which decidual apoptotic activity may be linked directly or indirectly to partuition remains unclear. Nevertheless, data presented here suggest that localized expression of 11β-HSD1 is likely to be a key determinant of glucocorticoid responses in decidua, particularly towards the end of gestation.

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