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GLUCOCORTICOID METABOLISM IN HUMAN PLACENTA, DECIDUA, MYOMETRIUM AND FETAL MEMBRANES*

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SUMMARY

The metabolism of cortisone (E) and cortisol (F) by human placenta, decidua, myometrium, chorion and amnion during pregnancy was studied *in vitro*. Early pregnancy, midpregnancy and term placentae metabolized F efficiently yielding E as the major product. The capacity of the placenta to inactivate F to E was observed as early as the 8th week of pregnancy and there was a significantly higher ($P < 0.001$) net production of E in early pregnancy placenta than in term placenta. In contrast to the placenta, midpregnancy and term decidua metabolized mainly E to F with a net production of F. Term chorion demonstrated an equal degree of oxidative and reductive glucocorticoid metabolism while term amnion and myometrium had negligible metabolic activity. Thus the net production of F from E by the decidual membrane unit is due to metabolic activity in the decidua as early as the 13th week of pregnancy and not to activity in the fetal membranes.

INTRODUCTION

In pregnancy there is increasing evidence that corticosteroid production in both mother and fetus rise toward term [1, 2]. In addition, it is now known that corticoids from maternal origin are normally transferred to the fetal circulation [3]. However, the relative importance of this transport mechanism has been a matter of uncertainty because it may depend on the corticoid gradient between mother and fetus, or changes in transcortin levels in the maternal and fetal circulation and on the efficiency of inactivation of F into E by the placenta and other fetal tissues [4–8]. In addition, reverse conversion of E into F has been reported to occur in fetal membranes [9, 10], uterus [11] and lung [12, 13] thus making active F available to tissues *in situ*. Since corticoids have been implicated in the process of lung maturation [14], in the immune mechanism and other physiologic changes associated with pregnancy, understanding their mechanism of production and metabolism is of prime importance. We have therefore undertaken this study with the purpose of elucidating the role of placenta, decidua, membranes and myometrium in handling corticoids.

MATERIALS AND METHODS

[1,2-^{3}H]-F† (SA 40–60 Ci/mmol) and [1,2-^{3}H]-E (SA 40–60 Ci/mmol) were purchased from New England Nuclear Corp. (Boston, MA). The purity of the labeled steroids was checked regularly by thin layer chromatography. Nonlabeled steroids were obtained from Sigma Chemical Co. (St. Louis, MO). Stock solutions of labeled and nonlabeled steroids were prepared in absolute ethanol, and working solutions were made by drying an aliquot of the stock solution under nitrogen and dissolving the steroid in incubation buffer. All solvents were reagent grade from Fisher Chemical Co. (Boston, MA).

Term placentae and fetal membrane samples were obtained immediately after delivery by elective cesarean section. Myometrial samples were obtained from the edges of the uterine incision from women undergoing cesarean section. Early pregnancy (8–12 weeks) and midpregnancy (13–18 weeks) fetal membranes and placentae were obtained immediately following uterine evacuation by suction. Term decidua were isolated by carefully scraping smooth chorion with a razor blade. Term placental cotyledons were dissected and washed free of decidua. Fetal membranes were scraped with a razor blade to remove excess decidua and then the amnion and chorion were separated. Midpregnancy and early pregnancy placental villi were isolated by scraping the villous chorion with a razor blade. Midpregnancy decidua were collected directly by curettage. Samples were reserved for histological confirmation and only data from tissue confirmed by a pathologist has been reported.

Tissue samples were washed extensively in Krebs–

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† The following abbreviations and trivial names are used: E = cortisone-17α,21-dihydroxy-1,4-pregnadiene-3, 11,20-trione; F = cortisol = 11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione; 11β-HSD = 11β-hydroxysteroid dehydrogenase.

Ringer bicarbonate buffer, blotted and weighed. Samples weighing 0.2 g were added to 2 ml Krebs–Ringer bicarbonate buffer, pH 7.4, in glass counting vials containing 10^{-7} M of either [^{3}H]-E or [^{3}H]-F. Incubations were carried out in a shaker bath for 2 h at 37°C in a 95% air–5% CO_2 atmosphere. Under the conditions of the assay, the conversion of E to F and F to E were linear throughout the 2 h period. Controls consisted of incubation buffer without tissue. The incubations were terminated by adding 5 ml ethyl acetate containing 200 μg each of E and F for visualization purposes. After vigorously shaking for 10 min, the organic phase was collected and evaporated to dryness. The average recovery of radioactivity in the controls was 82% for [^{3}H]-E and 78% for [^{3}H]-F. Recovery of radioactivity from tissue incubations ranged between 67 and 90% for [^{3}H]-E and between 68 and 78% for [^{3}H]-F.

The ethyl acetate residues were dissolved in a small volume of methanol–methylene chloride (1:1, v/v), chromatographed on Eastman Chromatogram Sheets (silica gel with fluorescent indicator) and developed in chloroform–methanol (9:1, v/v). Non-radioactive steroids were visualized on the sheets under u.v. light. The E and F spots from each sample were cut out from the sheet and eluted in 2 ml ethanol. The remaining chromatogram was cut up and eluted in 10 ml ethanol. Two ml aliquots of these eluates were mixed with 10 ml of scintillation fluid and counted in a Searle Analytic Delta 300 counter with a tritium efficiency of 55%. In some cases, the identity of E or F eluted from the chromatograms was confirmed by the addition of carrier steroid and crystallization to constant specific activity. Besides F and E, no other metabolites were produced by any tissue under the incubation conditions used. Conversions of F to E and E to F were calculated as follows:

$$A = \text{Percent conversion of E to F}$$

$$= \frac{\text{c.p.m. in F spot of sample} - \text{c.p.m. in F spot of control}}{\text{Total c.p.m. in sample}} \times 100$$

$$B = \text{Percent conversion of F to E}$$

$$= \frac{\text{c.p.m. in E spot of sample} - \text{c.p.m. in E spot of control}}{\text{Total c.p.m. in sample}} \times 100$$

$$A\text{–}B = \text{Net conversion of E to F.}$$

All values are given as means ± SEM and compared for statistical significance by the Student's *t*-test.

RESULTS

Figure 1 shows that in early pregnancy, midpregnancy and term placenta there was a low conversion (4, 10 and 13%, respectively) of E to F and a high conversion (79, 70 and 61%, respectively) of F to E. The difference between the oxidation of F to E and the reduction of E to F was statistically significant ($P < 0.001$) at all stages of pregnancy. There was also a significant increase ($P < 0.01$) in the reduction of E to F and a significant decrease ($P < 0.001$) in the oxidation of F to E in term placenta compared to early pregnancy placenta.

In contrast to the placenta, decidua converted predominantly E to F (45 and 42% for midpregnancy and term decidua, respectively) and to a lesser extent F to E (20 and 15% for midpregnancy and term decidua, respectively). The difference between the reduction of E to F and the oxidation of F to E by the decidua was statistically significant ($P < 0.02$ for midpregnancy decidua and $P < 0.01$ for term decidua). The differences between midpregnancy and term decidua in the reduction of E to F and the oxidation of F to E were not statistically significant. Term chorion reduced E to F (25%) and oxidized F to E (24%) to approximately the same extent.

Term amnion and myometrium showed negligible oxidation of F to E (1 and 5%, respectively) or reduction of E to F (2 and 4%, respectively).

Table 1 lists the percent net conversion of E to F by various tissues *in vitro*. Placental tissue showed a negative E to F net conversion, indicating that there was a net production of E from F. This activity was demonstrated as early as 8 weeks of pregnancy and was highest in the first 8–12 weeks of pregnancy. The net production of E from F was significantly higher in early pregnancy than in term placenta ($P < 0.001$). Decidua showed a net production of F from E as early as the 13th week of pregnancy. No significant difference was observed between the net productions of F from E by midpregnancy and term decidua. Term chorion, amnion and myometrium showed no significant net production of F from E.

DISCUSSION

These studies have shown that the reduction of E to F and the oxidation of F to E vary from one site to another and may be also dependent on the stage of pregnancy.

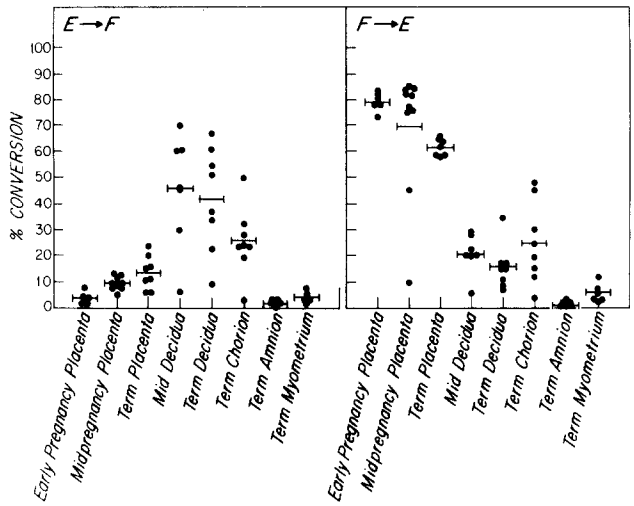

Fig. 1. *In vitro* percent conversion of E to F and F to E by various human tissues during pregnancy. Early pregnancy = 8–12 weeks; midpregnancy = 13–18 weeks; Term = 38–40 weeks.

Placental conversion of F to E predominated and this process of F deactivation was much more efficient at early than in late pregnancy. In particular, the conversion of E to F was almost nonexistent in early pregnancy whereas it was present in term placenta. Our results for term placenta are in agreement with the results of Blandford and Murphy[7] and Levitz *et al.*[8] but they differ from the results of Bernal *et al.*[15] who found undetectable reductive activity in homogenates of placenta at term even in the presence of NADPH. Although transport of F from the maternal to the fetal circulation at the first trimester of pregnancy has not been studied to date, these findings would suggest greater degree of fetal protection from the effect of maternal corticoids in early than in late pregnancy.

In contrast with the placenta, decidual tissue demonstrated an E to F conversion twice as effective as the F to E conversion, resulting in a net E to F conversion of 25 and 27% at mid-term and term, respectively. These results differ from those of Bernal and associates [15, 16] who showed that the E to F conversion in decidual microsomes was lower than the F to E conversion. The net production of F by the amnion was negligible, in agreement with previous results [9]. The chorion, although containing 11β-HSD activity, had little net effect on E to F conversion. Thus it appears that previous reports [9, 10] of net E to F conversion by the fetal membranes can be attributed mainly to decidual contamination. In addition, our results differ from those of Tanswell *et al.*[10] who reported that the capacity of human amniotic membrane (amnion plus chorion plus decidua) to convert E to F appears at 31 weeks of gestation and reaches a maximum at term. In our studies, maximum net conversion of E to F by decidua was observed as early as the 13th week of gestation, whereas no net conversion of E to F was seen in incubations of amnion and chorion. The use of a mixture of amnion, chorion and decidua in the studies of Tanswell *et al.*[10] may explain the discrepancy between their results and the results of the present study.

Another organ of interest is the myometrium. We found that myometrium lacks significant 11β-HSD activity and only 5% E to F interconversion was ob-

Table 1. Net production of F from E by various tissues *in vitro*

Number of determinations	Tissue	% Net conversion* of E to F
6	Early pregnancy† placenta	−75.0 ± 2.07
10	Midpregnancy placenta	−60.1 ± 7.56
7	Term placenta	−48.8 ± 3.31
7	Midpregnancy decidua	+25.2 ± 7.39
8	Term decidua	+26.7 ± 7.35
8	Term chorion	+8.0 ± 6.19
7	Term amnion	+1.7 ± 0.35
6	Term myometrium	−1.3 ± 1.58

* All values represent the mean ± SEM. † Early pregnancy = 8–12 weeks; midpregnancy = 13–18 weeks; term = 38–40 weeks.

served. Thus it appears that the E to F conversion by the human uterus reported by Murphy[11] was due to decidua included in the tissue preparation used.

In summary, these data suggests that the placenta and decidua metabolize E and F in opposite directions and that the F to E interconversion may be stage-of-pregnancy dependent. Other tissues, including the myometrium and fetal membranes, have negligible net effect on active corticoid production.

The proximity of the decidua to the placenta and the discordance of 11β-HSD activity between these two tissues of maternal and fetal origin, respectively, is intriguing. It is conceivable that E reaching the decidua from the placenta is activated into F and that an influx of F from the decidua into adjacent tissues, namely the membranes and the myometrium, may play a role in their function during pregnancy.

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