The Mechanism by which Foetal Cortisol Controls the Onset of Parturition in the Sheep

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The sheep is one of the few species in which there is good evidence that the foetus is involved in determining the length of pregnancy. Parturition in the sheep is prevented by foetal hypophysectomy (Liggins et al., 1967; Comline et al., 1970; Bosc, 1972) or adrenalectomy (Drost & Holm, 1968), and can be induced prematurely by intrafoetal administration of ACTH* or glucocorticoid (Liggins et al., 1967). The foetal adrenal hypertrophies towards the end of pregnancy, doubling in weight during the last 2 weeks (Comline & Silver, 1961), and its ability to respond to ACTH with increased cortisol output also increases during this time (Bassett & Thorburn, 1973). High concentrations of ACTH have been reported in foetal plasma near term (Rees et al., 1973), although it is not clear that the increase precedes the onset of labour. There is a dramatic and sustained increase in the concentration of cortisol in the foetal plasma during the last week of pregnancy (Bassett & Thorburn, 1969; Comline et al., 1970).

In addition to these events in the foetus, important endocrine changes occur in the mother before parturition. Placental progesterone production declines, leading to a decrease in the maternal peripheral plasma progesterone concentration (Bassett et al., 1969; Fylling, 1970), and maternal plasma concentrations of oestrogens, both unconjugated and sulpho-conjugated, rise (Challis, 1971; Currie et al., 1973). Uterine prostaglandin production increases, and high concentrations of prostaglandin F₂α appear in the uterine venous effluent (Liggins & Grieves, 1971; Thorburn et al., 1972). These changes can be monitored in the foetus and mother by the use of indwelling vascular catheters implanted at surgery some weeks before delivery.

Investigation of the temporal interrelationships between these endocrine changes has shown that the progesterone concentration in maternal plasma falls at about the same time as that of cortisol in foetal plasma rises, 3–7 days before delivery. Oestrogen concentrations in plasma increase later, generally about 24–48 h before delivery, and are accompanied by rising uterine venous concentrations of prostaglandin F (Thorburn et al., 1972). These changes precede the onset of uterine contractions (Rawlings & Ward, 1973; Flint et al., 1974). Since oestrogen administration stimulates uterine prostaglandin production in a number of species (including the sheep) both during pregnancy and otherwise, it has been suggested (Liggins et al., 1973) that the elevated oestrogen concentration in maternal plasma is responsible for the increased concentration of prostaglandin F. Prostaglandins cause marked increases in uterine contractility in sheep at term (M. D. Mitchell, A. P. F. Flint & A. C. Turnbull, unpublished work), as in other species, and it is probable that the oestrogens, which themselves cause labour when administered to pregnant sheep (Hindson et al., 1967), act through prostaglandin synthesis.

These endocrine events play an important part in controlling the onset of labour in sheep. The foetal cortisol surge is a necessary prelude to parturition at term: foetal hypophysectomy prevents delivery. Involvement of various maternal organs has been ruled out by extirpation: parturition occurs normally after maternal hypophysectomy (Denamur & Martinet, 1961; Bosc, 1972), ovariectomy or adrenalectomy (Thompson & Wagner, 1974). The fall in the progesterone concentration in maternal plasma is also a pre-requisite for normal labour. Labour induced with foetal glucocorticoid can be blocked by the simultaneous administration of large doses of progesterone to the ewe (Liggins et al., 1972); and induction of labour with oestrogens (when there is no fall in progesterone concentration) is associated with failure of the cervix to dilate. There

* Abbreviation: ACTH, adrenocorticotrophin.
Scheme I. Synthesis of oestrogens from progesterone in sheep placenta

The star (*) indicates enzymes controlled by foetal cortisol.

appear to be no reports of parturition, either spontaneous or induced with glucocorticoid, occurring in the absence of elevated concentrations of either unconjugated or conjugated oestrogen.

The endocrine changes preceding the onset of labour induced by intrafoetal adminis-
tration of ACTH or glucocorticoid are similar to those preceding the spontaneous onset of labour, except that with intrafoetal glucocorticoid there is no rise in the cortisol concentration in foetal plasma (Currie et al., 1973; Liggins et al., 1973). Therefore it appears likely that the decrease in placental progesterone secretion and the increase in the uterine production of oestrogens are caused by the foetal cortisol surge. The mechanism by which cortisol has these effects is the subject of the present investigation.

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**Fig. 1.** Radiochromatogram scans of t.l.c. plates after separation of products obtained from the incubation of (a) minced placenta with [3H]pregnenolone and (b) minced placenta with [3H]pregnenolone, tissue obtained 18 h after intrafoetal administration of dexamethasone.

The silica gel G t.l.c. plates were developed in chloroform-acetone (17:3, v/v). Key: 1, progesterone; 2, pregnenolone; 3, 17α,20α-dihydroxypregnen-4-en-3-one. Metabolites were identified by recrystallization with authentic carrier. O, Origin; Sf, solvent front.
Effect of foetal glucocorticoid on placental progesterone metabolism

Assay of cholesterol side-chain cleavage enzyme and 3β-hydroxy steroid dehydrogenase in unfractionated homogenates of placentae obtained before and after intrafoetal administration of glucocorticoid indicates that the decrease in progesterone secretion resulting from this treatment cannot be accounted for by any change in the total activities of these enzymes (Anderson et al., 1975). However, by incubating placentalt minces or homogenates with labelled pregnenolone or progesterone the appearance, in response to glucocorticoid, of a steroid 17α-hydroxylase can be demonstrated (Anderson et al., 1975).

In conjunction with the 20α-hydroxy steroid dehydrogenase present in ovine placenta, this enzyme results in the formation of 17α,20α-dihydroxyprogren-4-en-3-one in vitro (Scheme 1 and Fig. 1). To confirm this effect in vivo a radioimmunoassay for 17α,20α-dihydroxyprogren-4-en-3-one has been developed that uses an antibody raised in sheep against 17α,20α-dihydroxyprogren-4-en-3-carboxymethylxime-bovine serum albumin. By means of this assay it can be shown that the peripheral plasma 17α,20α-dihydroxyprogren-4-en-3-one concentration increases near term, as the progesterone concentration falls (Flint et al., 1975). This finding suggests that, as is the case with other steroids of the placenta, the intermediates involved in placental biosynthetic pathways are secreted.

The observation that placental steroid 17α-hydroxylase is controlled by glucocorticoid in vivo raises the possibility that after the foetal cortisol surge the tissue may be capable of oestrogen synthesis (Scheme 1). This has been confirmed by incubating microsomal fractions from placental homogenates with labelled 17α-hydroxyprogesterone and isolating labelled oestrone by t.l.c. (Table 1). Oestrogen production with treated tissue is considerably higher than with control tissue, although the pathway is present before exposure to glucocorticoid. By using microsomal fractions the steroid C(17)-C(20)-lyase and steroid aromatase responsible for oestrogen synthesis are separated from the bulk of the 20α-hydroxy steroid dehydrogenase in the tissue; this is important since in vitro (as apparently in vivo) 20α-hydroxy steroid dehydrogenase competes with steroid C(17)-C(20)-lyase for 17α-hydroxyprogesterone, forming 17α,20α-dihydroxypropregn-4-en-3-one, which is not a substrate for the lyase.

Steroid aromatase is present in placenta throughout the last third of pregnancy, and the increase in oestrogen synthesis after exposure to glucocorticoid therefore suggests induction or activation of the steroid C(17)-C(20)-lyase. This is also suggested by the finding that androstenedione, the intermediate in the conversion of 17α-hydroxyprogesterone into oestrone, does not accumulate during incubations in vitro; thus the steroid aromatase is not rate-limiting, at least in vitro. On the other hand, trapping experiments, in which unlabelled androstenedione is added to placental microsomal fraction incubated with labelled 17α-hydroxyprogesterone, indicate that androstenedione is the interme-

Table 1. Conversion of 17α-hydroxy[7α-3H]progesterone into [3H]oestrone by microsomal fractions from sheep placenta

The microsomal fractions were incubated for 1 h in a sucrose-Tris-EGTA [ethanedioxybis(ethylamine)tetra-acetic acid]–mercaptoethanol buffer containing 0.75 mg of NADPH/ml, 10 μCi of substrate and 1–2 mg of microsomal protein/ml; the total volume was 2 ml. The percentage conversions are given as means ± S.D. with the numbers of animals in parentheses.

<table>
<thead>
<tr>
<th>Tissue obtained</th>
<th>Conversion into oestrone (%)</th>
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<tr>
<td>From untreated animals (121–138 days gestational age)</td>
<td>3.2 ± 0.44 (5)</td>
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<tr>
<td>After induction of labour with intrafoetal dexamethasone</td>
<td>24.4 ± 2.13 (4) (P &lt; 0.001)</td>
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<tr>
<td>After the spontaneous onset of labour at term</td>
<td>20.6 ± 10.2 (5) (P &lt; 0.01)</td>
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mediate in this pathway. Measurement of androstenedione by radioimmunoassay shows that the concentration in the circulation increases at term, at about the same time as the concentration of 17α,20α-dihydroxypregn-4-en-3-one.

These findings suggest that the foetal cortisol surge at term stimulates the endocrine changes leading to parturition by raising the activities of steroid 17α-hydroxylase and steroid C_{17,20}-lyase in the placenta, thus converting that organ from being primarily a progesterone-secreting gland into an oestrogen-secreting gland. However, although 17α,20α-dihydroxypregn-4-en-3-one and androstenedione concentrations increase in the peripheral plasma at term, this in itself is no indication that these intermediates are being secreted by the placenta. Other possible sources are the maternal adrenal and ovary and the foetal adrenal and gonad. The foetal adrenal particularly has been considered a possible source of oestrogen precursors, since it fulfils this role in man. Experiments to prove that the secretion of 17α,20α-dihydroxypregn-4-en-3-one, androstenedione and oestrone reflects the formation of these compounds by the placenta have involved the removal of alternative sources and the measurement of arteriovenous differences across the uterus. By extirpating the maternal ovary, foetal adrenal and foetal gonad and measuring uterine production of 17α,20α-dihydroxypregn-4-en-3-one, androstenedione and oestrogen sulphates it has been possible to rule out other possible sources of these compounds and to implicate the placenta directly in the total synthesis of oestrogens from progesterone.

Conclusions

The mechanism of action of foetal cortisol involves an increase in the activities of placental enzymes responsible for the synthesis of oestrogens from C_{21} steroids. The resulting rise in maternal oestrogen synthesis stimulates uterine prostaglandin production, thus leading indirectly to the onset of labour. The degree to which the rise in these placental enzyme activities contributes to the decrease in the peripheral concentration of progesterone is not fully elucidated. However, by comparing maternal progesterone and oestrogen sulphate production rates (calculated from blood concentrations and metabolic clearance rates) it appears likely that, without any change in the rate of synthesis of C_{21} steroids by the placenta, the fall can be accounted for quantitatively. Whether the temporal relationship between falling progesterone and rising oestrogen concentrations is consistent with this mechanism is yet to be seen.

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