PREMATURE DELIVERY OF FOETAL LAMBS INFUSED WITH GLUCOCORTICOIDS

G. C. LIGGINS

Postgraduate School of Obstetrics & Gynaecology,
University of Auckland, Auckland, New Zealand

(Received 14 April 1969)

SUMMARY

Dexamethasone caused premature delivery when infused into foetal lambs at rates of 0.06–4.0 mg./24 hr. but it had no effect when administered to pregnant ewes at the rate of 4.0 mg./24 hr. Infusions into the foetus of deoxycorticosterone or corticosterone were ineffective; mixtures of dexamethasone and deoxycorticosterone did not cause parturition more rapidly than dexamethasone alone. Thus, the ability of corticosteroids to cause premature parturition appears to depend on glucocorticoid rather than mineralocorticoid activity.

Parturition induced by dexamethasone was not delayed by administration of 100 mg. progesterone/24 hr. to the ewe or to the foetus. This suggests either that withdrawal of inhibitory effects of progesterone on the myometrium can occur independently of the progesterone concentration in peripheral plasma, or that the mechanism of parturition provoked by corticosteroids in the foetus can override any regulatory influence of progesterone on myometrial contractility.

Partial aeration of the lungs was observed in lambs born vaginally at 117–123 days of gestation after receiving dexamethasone. It is suggested that this may be the result of accelerated appearance of surfactant activity.

INTRODUCTION

The onset of parturition in the ewe has been shown to be profoundly influenced by the foetus (Liggins, 1968). Destruction of the foetal pituitary or hypothalamus leads to marked prolongation of gestation (Liggins, Kennedy & Holm, 1967) and conversely, stimulation of the foetal adrenals by corticotrophin (ACTH) or infusion of cortisol into the foetus leads to premature parturition (Liggins, 1968). Thus it appears likely that the foetal lamb affects myometrial contractility through a pathway which includes the foetal hypothalamus, pituitary and adrenals, and that the activity of the adrenal cortex in this particular function is mediated by a corticosteroid. However, the means by which a corticosteroid in the foetus may influence the myometrium remains obscure.

Cortisol has both mineralocorticoid and glucocorticoid activity. The present experiments were designed to determine which of these components was responsible...
for the ability of cortisol to provoke parturition. In further experiments the possibility was investigated that cortisol might interfere with the regulation of myometrial contractility by progesterone.

MATERIALS AND METHODS

A flock of 43 pure-bred Romney ewes was used. Pregnancies were dated accurately by means of single matings after induction of oestrus with vaginal sponges containing a progestin (Roberts, 1966). A self-retaining catheter was inserted into the foetal peritoneal cavity as described previously (Liggins, 1968) and corticosteroids dissolved in 0-9 % NaCl solution were infused continuously. In two experiments, 100 mg. progesterone/24 hr. dissolved in 70 % propylene glycol in 0-9 % NaCl solution was infused into the foetus together with 1 mg. dexamethasone/24 hr.; in four experiments the same dose of progesterone in the same vehicle was infused into the ewe by means of a catheter inserted into a spinal muscle while dexamethasone was infused into the foetus. Progesterone was kept in solution by maintaining the syringe at approximately 37° with a suitably placed source of radiant heat. The animals were kept in metabolic cages and were fed on lucerne chaff or fresh grass.

Blood samples were taken daily from the jugular veins of one of the ewes receiving a progesterone infusion; the plasma was separated by centrifuging, a tracer amount of [4-14C]progesterone was added, and the progesterone concentration was determined in duplicate by the protein-binding method of Murphy (1967). The results were then corrected for losses during extraction.

Elective Caesarean section was performed on the 7th–14th day of experiments if premature labour did not supervene. All lambs were stored at 4° until autopsy examination which was performed within 24 hr.

RESULTS

The treatment groups and results are shown in Table 1.

Control experiments (group I)

Six single foetuses of 97–120 days gestational age were infused continuously with 0-9 % NaCl solution for 14 days. In each instance pregnancy continued normally. Autopsy examination after delivery by elective Caesarean section revealed no differences in body weight or organ weights from normal lambs of similar maturity. The peritoneal cavities contained a small amount of straw-coloured fluid but were otherwise normal.

Unsuccessful experiments (group X)

There were four postoperative foetal deaths and two maternal deaths. Three of the four dead foetuses showed peritoneal infection with gas-forming organisms; the degenerated state of the 4th foetus precluded a diagnosis. Both maternal deaths occurred within 3 days of operation and were probably due to ovine toxaemia of pregnancy. The six unsuccessful experiments are excluded from the results which follow.
Parturition after glucocorticoid administration to foetus

**Infusions of dexamethasone**

Foetal infusions (group II)

Dexamethasone phosphate was infused continuously into 11 single foetuses of 100–121 days gestational age in daily doses of 0·06–4·0 mg. Two foetuses infused with the lowest dose of dexamethasone were delivered by elective Caesarean section at

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of ewes</th>
<th>Duration of pregnancy* (days)</th>
<th>Treatment during 24 hr.</th>
<th>Interval to delivery† (days)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>97–120</td>
<td>Saline</td>
<td>—</td>
<td>Elective C.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0·06 mg. dex.</td>
<td>—</td>
<td>Single foetus</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>116</td>
<td>Saline</td>
<td>7</td>
<td>Single foetus†</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>109, 117</td>
<td>Saline</td>
<td>3½, 5½</td>
<td>Single foetus</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>116, 116</td>
<td>0·5 mg. dex.</td>
<td>3½, 4</td>
<td>Single foetus</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>115</td>
<td>1·0 mg. dex.</td>
<td>2½</td>
<td>Single foetus</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>110, 114</td>
<td>1·0 mg. dex.</td>
<td>2, 2½</td>
<td>Single foetus</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100</td>
<td>4·0 mg. dex.</td>
<td>2</td>
<td>Single foetus</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>100, 109</td>
<td>Saline</td>
<td>4·0 mg. dex.</td>
<td>Elective C.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saline</td>
<td>—</td>
<td>Single foetus</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>100, 109</td>
<td>1·0 mg. dex.</td>
<td>100 mg. P</td>
<td>4½, 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1·0 mg. dex.</td>
<td>100 mg. P</td>
<td>Single foetus</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>123</td>
<td>12·5 mg. corticosterone</td>
<td>Elective C.S.</td>
<td>Single foetus</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>123</td>
<td>25 mg. corticosterone</td>
<td>Elective C.S.</td>
<td>Single foetus</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>102</td>
<td>10 mg. DOC</td>
<td>Elective C.S.</td>
<td>Single foetus</td>
</tr>
<tr>
<td>VII</td>
<td>2</td>
<td>110, 117</td>
<td>1·0 mg. dex. 100 mg. P</td>
<td>3, 3</td>
<td>Single foetus</td>
</tr>
<tr>
<td>VIII</td>
<td>4</td>
<td>100, 109</td>
<td>1·0 mg. dex.</td>
<td>100 mg. P</td>
<td>4½, 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1·0 mg. dex.</td>
<td>100 mg. P</td>
<td>Single foetus</td>
</tr>
<tr>
<td>IX</td>
<td>1</td>
<td>123</td>
<td>0·125 mg. ACTH (× 2)</td>
<td>6</td>
<td>Both twins infused</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>116</td>
<td>0·125 mg. ACTH</td>
<td>Elective C.S.</td>
<td>One twin infused</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>97, 115</td>
<td>50 mg. cortisol</td>
<td>4, 4</td>
<td>One twin infused</td>
</tr>
<tr>
<td>X</td>
<td>1</td>
<td>115</td>
<td>4 mg. dex.</td>
<td>—</td>
<td>Maternal death</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>125</td>
<td>1 mg. dex.</td>
<td>—</td>
<td>Maternal death</td>
</tr>
</tbody>
</table>

* Duration of pregnancy at start of infusion.
† Latent period to the nearest 12 hr. from start of infusion to delivery.
‡ Cervical dystocia delayed delivery by at least 24 hr.
|| Plasma progesterone concentrations determined.

ACTH = corticotrophin; dex = dexamethasone phosphate; DOC = deoxycorticosterone; elective C.S. = elective Caesarean section performed when treatment had no effect; P = progesterone.

112 and 117 days respectively after 8 days of infusion. The remaining nine lambs were born spontaneously, either living or freshly dead. The duration of infusion preceding parturition at each dose level is shown in Text-fig. 1. Parturition occurred after approximately 48 hr. at all doses from 0·5–4·0 mg./24 hr. With smaller doses the duration of infusion varied inversely with the size of the dose. A dose of 0·06 mg./24 hr. appeared to be marginally effective since parturition occurred in one ewe in which the foetus was infused with dexamethasone at this rate but failed to do so in
two others. It may be significant that the maturity of the foetus receiving the effective infusion (116 days) was greater at the start of the infusion than in the ineffective infusions (104 and 109 days respectively).

Maternal infusions (group III)

Two ewes at the 100th and 109th day of gestation respectively were infused continuously with 4 mg. dexamethasone phosphate/24 hr. while the foetuses were infused simultaneously with 0.9% NaCl solution. Living foetuses were delivered by elective Caesarean section on the 8th day of infusion.

Infusions of corticosterone and deoxycorticosterone (DOC) (group VI)

In two experiments, corticosterone in doses of 12.5 mg./24 hr. and 25 mg./24 hr. respectively was infused into foetuses at the 123rd day of gestation. In a 3rd experiment, 100 mg. DOC/24 hr. was infused on the 102nd day. The experiments were terminated on the 8th day by elective Caesarean section and living, apparently normal, lambs were delivered.

Infusions of mixtures of dexamethasone and DOC (group IV)

Mixtures of dexamethasone phosphate and DOC in a ratio of 1:2.5 (w/w) respectively were infused into four single lambs. The dose of dexamethasone and the time of parturition are shown in Text-fig. 1. In each experiment parturition occurred later than when the same dose of dexamethasone was infused without DOC, but the difference is not significant. In a further experiment, 1 mg. dexamethasone/24 hr. and 2.5 mg. DOC/24 hr. were infused into the amniotic sac on the 91st day of gestation.
Parturition after glucocorticoid administration to foetus

A normal lamb was delivered by elective Caesarean section on the 7th day. The position of the self-retaining catheter was confirmed at the time of insertion by aspiration of amniotic fluid and at the time of delivery by inspection of the amniotic cavity.

**Infusions of dexamethasone and progesterone (groups VII & VIII)**

Six single lambs at gestational ages of 100–118 days were infused with 1 mg. dexamethasone/24 hr. while 100 mg. progesterone/24 hr. were infused simultaneously either into the foetus (2 experiments) or into the ewe (4 experiments). As shown in Text-fig. 1 parturition occurred in five ewes at a time which did not differ significantly from that observed in two experiments in which dexamethasone (1 mg./24 hr.) was infused without progesterone. Parturition was delayed in one ewe receiving progesterone and the membranes ruptured 24 hr. before delivery, which finally took place 4 days after infusion was started.

Blood samples were obtained each day from a jugular vein of one of the ewes receiving progesterone. Immediately before the administration of progesterone the plasma concentration of progesterone on the 117th day of pregnancy was 5-0 ng./ml. The concentrations 24 and 48 hr. after the start of infusion greatly exceeded 100 ng./ml. Delivery of the lamb occurred after 69 hr. The progesterone concentration post partum—the infusion having been discontinued—was 3-5 ng./ml.

**Experiments with twin pregnancies (group IX)**

Corticotrophin (ACTH) (0-125 mg./24 hr.) was infused into both foetuses of a twin pregnancy at 123 days of gestation. Spontaneous delivery occurred after 6 days. At autopsy the adrenals weighed 955 and 416 mg. respectively. In a further twin pregnancy, the same dose of ACTH was infused into one twin at 116 days of gestation. The pregnancy was interrupted by elective Caesarean section after 8 days. The adrenals of the infused lamb weighed 1185 mg. compared with 370 mg. in the control lamb, and the weights of the thymuses were 3-5 and 18-5 mg. respectively.

Spontaneous parturition occurred in two twin pregnancies when cortisol was infused into one foetus in each pregnancy at the same rate (50 mg./24 hr.) as used in previous experiments in which single lambs were delivered prematurely (Liggins, 1968). Although the duration of pregnancy at the start of infusion was 97 days in one ewe and 115 days in the other, the duration of infusion was the same (4 days). The adrenals of the more immature lambs weighed 210 and 205 mg. respectively; those of the other twins weighed 350 mg. (infused) and 380 mg. (not infused).

**Observations on the foetal lungs**

Gross examination of the lungs of ten lambs delivered spontaneously at 117–123 days after dexamethasone infusions showed that in six, none of which had been artificially ventilated, there was partial aeration. In most instances the aeration consisted of patchy expansion in the upper lobes, but in one lamb delivered at 123 days the lower lobes were also partly expanded. This lamb was found alive at least an hour after being born and survived for a further hour until killed. The body weight of 2349 g., the general appearance and the bone age determined by X-ray of the limbs corresponded with the duration of pregnancy calculated from the date of
mating. During the period of observation the lamb bleated and showed some signs of respiratory distress. Histological examination of the lungs removed 4 hr. after death confirmed that alveolar expansion had been maintained (Plate).

DISCUSSION

On the assumption that the ratio of glucocorticoid to mineralocorticoid activity of the various corticosteroids is similar in the foetal lamb to that described in man (Travis & Sayers, 1965), these experiments show that cortisol owes its ability to provoke parturition to its glucocorticoid rather than its mineralocorticoid activity. A dose of a glucocorticoid (dexamethasone) with an activity equivalent to 2-5 mg. cortisol was effective whereas mineralocorticoids (DOC and corticosterone) equivalent to 187-1000 mg. cortisol were ineffective. Furthermore there was no evidence that the mineralocorticoids enhanced the potency of the glucocorticoid, dexamethasone. The major corticosteroids secreted by the adrenals of the foetal lamb are cortisol and corticosterone (Jones, Jarrett, Vinson & Potter, 1964). Since corticosterone is a potent mineralocorticoid but a very weak glucocorticoid and since the reverse is true of cortisol (Travis & Sayers, 1965), it seems likely that cortisol rather than corticosterone is concerned with parturition in the ewe. This conclusion is supported by the experiments in which corticosterone was infused into the foetus without interrupting the pregnancy.

It was found previously (Liggins, 1968) that the dose of cortisol (50 mg./24 hr.) required to provoke parturition when infused into the foetus was considerably in excess of the daily secretion of cortisol of 8 mg. found by Alexander, Britton, James, Nixon, Parker, Wintour & Wright (1968) in a foetal lamb at a gestational age of 143 days. However, in the present experiments, the minimum dose of dexamethasone was 0-06-0-1 mg./24 hr., which is equivalent to 1-25-2-5 mg. cortisol (Travis & Sayers, 1965). It is possible then that the adrenals of the normal lamb at term are capable of secreting cortisol at a rate which can affect uterine activity sufficiently to cause parturition.

The administration of progesterone either to the ewe or to the foetus at a rate several times that of the daily secretion of 16 mg. estimated by Linzell & Heap (1968) failed to delay premature parturition induced by dexamethasone. Bengtsson & Schofield (1963), who observed that daily injections of 80 mg. progesterone did not prevent parturition at term in normal ewes, concluded that the placental contribution of progesterone is not replaceable by progesterone administered systemically. They considered that their findings supported the concept of control of myometrial activity by a local action of placental progesterone. An alternative explanation for the findings in our experiments and those of Bengtsson and Schofield is that progesterone has little to do with the mechanism of parturition whether it is induced prematurely by corticosteroids or occurs spontaneously at term. It is not possible at present to determine which of these views is correct. It is pertinent, nevertheless, to consider the ways in which corticosteroids might influence myometrial activity. If they act by reducing local progesterone influence, the most likely means of accomplishing this is by suppression of placental progesterone secretion. Inhibition of the action of progesterone on the myometrial cell seems less likely since relatively large doses of
dexamethasone fail to cause parturition when given to the ewe despite the likelihood that access to the myometrium is as good from the maternal as from the foetal blood supply. For the same reason, a direct action of corticosteroids on the myometrial cell can probably be excluded; but an indirect action, resulting from the release of an oxytocic material within the foetal compartment, would be consistent with the results of our experiments.

It was reported in a previous paper (Liggins, 1968) that infusions of cortisol into foetal lambs caused premature delivery whereas infusions of larger doses into ewes were ineffective. The present results confirm that corticosteroids probably provoke parturition only when administered to the foetus, since a dose of dexamethasone 40 times greater than that leading to premature delivery when given to the foetus was without effect when given to the ewe. Infusion of dexamethasone into the amniotic fluid also proved ineffective. Pregnancy continued normally to term in 13 ewes injected with 20 mg. betamethasone at 134–137 days of gestation (R. A. S. Welch, unpublished observations). These findings indicate that the ovine placenta is relatively impermeable to corticosteroids, as suggested by Jones et al. (1964). The response of cattle to dexamethasone may be similar to that of sheep since Adams (1969) observed that although parturition occurred within 56 hr. when a single injection of 20 mg. dexamethasone was given to 11 cows at 262–280 days of gestation (0–18 days before term), it failed to occur in three out of four cows receiving the same injection at 235–257 days.

In the experiments in twin pregnancies, administration of ACTH to both foetuses led to premature delivery as it does in single pregnancies (Liggins, 1968). However when ACTH was given to only one twin, parturition did not occur although marked adrenal hyperplasia was induced and cortisol secretion was considerably increased as judged by the degree of involution of the thymus, a degree exceeding that observed in previous experiments in which 50 mg. cortisol/24 hr. was infused (Liggins, 1968). These results are difficult to interpret, particularly when considered together with the previously reported results of foetal hypophysectomy in twin pregnancies (Liggins et al. 1967). In the latter experiments it was shown that hypophysectomy in both twins caused prolonged gestation but when one foetus had an intact pituitary, parturition occurred at term. Thus a normal lamb in a multiple pregnancy on the one hand apparently supplied the stimulus to parturition at term and on the other hand prevented premature delivery when the second foetus was given ACTH. The observations that cortisol, unlike ACTH, caused premature parturition when infused into one of the twins was a further inconsistency. No single mechanism of action of corticosteroids is consistent with all these results; withdrawal of an inhibitory influence from the myometrium and induction of an oxytocic stimulus are equally inadequate explanations.

Cortisol is known to be capable of inducing precocious activity of a variety of enzymes in immature animals. Invertase activity in the intestinal mucosa of both the newborn rat (Doell & Kretchmer, 1964) and the chick embryo (Hijmans & McCarty, 1966) increases after cortisol administration. The same phenomenon occurs with intestinal alkaline phosphatase in chick embryos (Moog & Kirsch, 1955; Moog, 1962; Hayes, 1965), with glutamine synthetase in explants of chick retina (Moscona & Piddington, 1966), with phenylethanolamine-N-methyl transferase in the adrenal
medulla of foetal rats (Parker & Noble, 1967) and with tyrosine aminotransferase in the liver of foetal rats (Holt & Oliver 1968). The dose–response curve shown in Fig. 1 has features that fulfil some of the criteria of enzyme induction described by Wicks (1968). Parturition was observed after a latent period that was dose-dependent below a critical figure (0.5 mg./24 hr.) but that was not reduced by a several-fold increase in dose above this figure.

The observations on the foetal lungs of lambs at 117–123 days gestational age may represent another example of precocious induction of enzyme activity by corticosteroids. According to Howatt, Avery, Humphreys, Normand, Reid & Strang (1965), and Reynolds, Jacobson, Motoyama, Kikkawa, Craig, Orzalesi & Cook (1965), maintenance of alveolar expansion in the lamb is dependent on adequate surfactant activity. These authors and also Brumley, Chernick, Hodson, Normand, Fenner & Avery (1967), found that alveoli collapse and the lungs become airless at maturities of less than 125–127 days. Persistence of partial expansion of the lungs in the corticosteroid-treated lambs in the present series at 117–123 days strongly suggests accelerated appearance of surfactant, possibly as a result of premature activity of enzymes involved in a biosynthetic pathway.

I am grateful to Mr A. Mekkelholt for care of the animals, to Mrs N. Jenkin for technical assistance and to Dr P. C. Rennie for performing the progesterone assays. The work was supported by a grant from the Wellcome Trust.

REFERENCES


Murphy, B. E. P. (1967). Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. J. clin. Endocr. Metab. 27, 973–990.

DESCRIPTION OF PLATE

Section of upper lobe of lung from foetal lamb delivered prematurely at 123 days of gestation after infusion of dexamethasone into foetal peritoneal cavity for 7 days. Most alveoli contain air. Haematoxylin and eosin. (× 80.)