A comparative study of cardiovascular, endocrine and behavioural effects of betamethasone and dexamethasone administration to fetal sheep

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1. Chronically instrumented, late-gestation fetal sheep were prepared to: (1) characterize cardiovascular, endocrine and behavioural effects of fetal treatment with clinical doses of betamethasone and dexamethasone; (2) define specific differences, if any, in the actions of betamethasone and dexamethasone of measured fetal responses; and (3) assess the contribution of changes in peripheral vascular resistance to the glucocorticoid-induced hypertension.

2. Following baseline, either saline ($n = 9$), betamethasone ($n = 9$), or dexamethasone ($n = 6$) was infused for 48 h in fetal sheep commencing at 125 days of gestation. A pronounced increase in fetal blood pressure occurred following both betamethasone and dexamethasone treatment. The nature and magnitude of this increase was similar following treatment with either glucocorticoid.

3. To address possible mechanisms contributing to the glucocorticoid-induced fetal hypertension, fetal plasma catecholamine levels and changes in fetal femoral haemodynamics were assessed following fetal glucocorticoid treatment. A fall in fetal plasma noradrenaline and adrenaline concentrations occurred during betamethasone and dexamethasone treatment. In contrast, a progressive femoral vasoconstriction occurred during betamethasone treatment.

4. A modest fall in the incidence of fetal breathing movements occurred during fetal treatment with either betamethasone or dexamethasone. The magnitude of this reduction was similar with treatment of either glucocorticoid. The fall in fetal breathing during betamethasone and dexamethasone treatment was not associated with a fall in the incidence of fetal low voltage electrocortical activity.

5. Our results indicate that prenatal betamethasone and dexamethasone treatment of late-gestation fetal sheep, in doses similar to those employed clinically, is associated with fetal cardiovascular, endocrine and behavioural effects. Both betamethasone and dexamethasone induce similar increases in fetal blood pressure and similar falls in the incidence of fetal breathing movements. The pronounced betamethasone-induced fetal hypertension is associated with an increase in fetal femoral vascular resistance.

Antenatal glucocorticoid administration to enhance fetal lung maturation has been used in obstetric practice over the last 20 years since first reported by Liggins (1969). In addition to the well-known decrease in Respiratory Distress Syndrome, antenatal administration of either betamethasone or dexamethasone, the only two glucocorticoids used clinically, reduces the incidence of neonatal mortality, periventricular haemorrhage and necrotizing enterocolitis (Crowley, Chalmers & Keirse, 1990). Thus, the National Institutes of Health recently advised routine administration in two to four doses for 48 h of either betamethasome or dexamethasone to all pregnant women at risk of premature delivery before 32 weeks of gestation (NIH Consensus Development Conference, 1994).

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Surprisingly, little information is available on the effects of betamethasone or dexamethasone on the fetus, and that which is available is controversial. While a decrease in human fetal body and breathing movements and a decrease in fetal heart rate variation was reported following maternal betamethasone administration (Mulder, Derks, Zonneveld, Bruinse & Visser, 1994; Derks, Mulder & Visser, 1995), an increase in human fetal heart rate variation (Dawes, Serra-Serra, Moulden & Redman, 1994), with no changes in fetal breathing movements (Mulder, Derks & Visser, 1995), occurred following maternal dexamethasone administration. The reason(s) for these different effects of betamethasone and its stereoisomer dexamethasone on fetal cardiovascular and behavioural responses following maternal administration remains unidentified, but insight to plausible explanations may be obtained from direct fetal treatment with betamethasone or dexamethasone in experimental models. To date, such a study has not been performed.

In contrast, the effects of fetal treatment with cortisol are well established. Short-term intravenous infusion of physiological doses of cortisol to chronically catheterized fetal sheep produced an increase in fetal arterial blood pressure, a fall in heart rate and decreased fetal plasma catecholamine levels (Wood, Cheung & Brace, 1987). Longer-term fetal sheep treatment with cortisol for 24 h (Tangalakis, Lumbers, Moritz, Towstoless & Wintour, 1992) and for 48 h (Dodic & Wintour, 1994) similarly produced an increase in fetal blood pressure but no change in heart rate. The mechanisms mediating cortisol-induced fetal hypertension remain unidentified.

Since cortisol is known to stimulate both glucocorticoid and mineralocorticoid receptors, and dexamethasone and betamethasone do not have any mineralocorticoid activity (Haynes, 1990; see Joels & de Kloet, 1995), one might expect the effects on the fetus of the synthetic steroids used clinically to be different from those produced by natural cortisol.

Thus, the aims of our study were: (1) to characterize fetal cardiovascular, endocrine and behavioural effects of fetal treatment with either betamethasone or dexamethasone; and (2) to define specific differences, if any, in the actions of betamethasone and dexamethasone on fetal cardiovascular, endocrine and behavioural responses. Preliminary evidence from these first series of studies, reported in abstract form (Derks *et al.* 1995*a*), suggested that either betamethasone or dexamethasone treatment of fetal sheep also induced a pronounced increase in fetal arterial blood pressure. To gain insight into the mechanism(s) mediating the synthetic glucocorticoid-induced fetal hypertension, we performed a second series of experiments to (3) address the contribution of any changes in fetal peripheral vascular resistance to the increase in fetal arterial blood pressure.

We aimed to mimic the clinical situation of human antenatal glucocorticoid administration by infusing either betamethasone or dexamethasone directly into the fetal jugular vein at a dose designed to achieve fetal plasma steroid concentrations similar to those measured in human umbilical cord plasma at Caesarean section within 24 h following maternal glucocorticoid treatment (Petersen, Nation, Ashley & McBride, 1980; Kream, Mulay, Fukushima & Solomon, 1983). This approach avoided confounding influences due to differences in transplacental passage of glucocorticoids between the sheep and primate placentae, and possible transplacental passage differences between the two synthetic steroids.

METHODS

Surgical instrumentation

Twenty-four Rambouillet-Colombia ewes (weighing 50–60 kg) bred on a single occasion and carrying fetuses of known gestational ages were used. Following food and water withdrawal for 24 h, the ewes were pretreated with 1 g ketamine I.M. and 1 mg of glycopyrrolate I.M. and induced with 4% halothane for 4 min prior to intubation. Surgery was performed under maintained general anaesthesia with 1·5% halothane at 117 days gestational age (dGA) as previously described in detail (Nathanielsz, Bailey, Poore, Thornburn & Harding, 1980). In brief, in all preparations the ewes and fetuses were instrumented with polyvinyl catheters inserted into the carotid artery and jugular vein, with the tips of the catheters in the ascending aorta and the superior vena cava. Another catheter was placed in the amniotic cavity. Stainless-steel wire electrodes were implanted bilaterally on the fetal parietal dura, 1 cm on either side of the mid-line, for recording of fetal electrocorticogram (ECoG) activity, and on the fetal diaphragm for monitoring of fetal breathing movements (FBM), and on the anterior surface of the pregnant uterine horn for recording of myometrial activity. In addition, to examine whether synthetic glucocorticoid administration had any effect on fetal femoral vascular resistance, a vascular flow probe (2R Transonics Inc., Ithaca, NY, USA) was implanted around the left femoral artery in eight of the fetuses.

All animals were allowed 5 days of post-operative recovery before undergoing any experiment. During this time all ewes received a daily dose of 1 g ampicillin I.M. and 500 mg ampicillin into the amniotic sac. All procedures used were approved by the Cornell University Animal Use and Care Committee and performed in facilities approved by the American Association for the Accreditation of Laboratory Animal Care.

Experimental procedure

The animal preparations were subdivided into three groups. At 125 dGA, at 12 noon, following 2 days of baseline recording, either saline (control group, $n = 5$), betamethasone (Celestone; Schering, $n = 5$), or dexamethasone (Azium; Schering, $n = 6$), was administered into the fetal jugular vein at a rate of 10 $\mu g\ h^{-1}$ over the next 48 h. The ewes underwent elective Caesarean section 3 days after the infusion period at 130 dGA, or earlier, if labour resulted from the glucocorticoid administration. In the additional eight fetuses in which femoral vascular-flow probes had been chronically implanted the experimental protocol was repeated following either saline infusion ($n = 4$) or betamethasone treatment ($n = 4$). These ewes underwent elective Caesarean section at the end of the 48 h infusion period. All fetuses removed by Caesarean were killed by exsanguination while still under halothane anaesthesia and underwent immediate necropsy for tissue retrieval.

Data collection

Data on biophysical variables were recorded continuously throughout the study protocols using a data acquisition system which collected data averaged every second. Fetal arterial blood pressure was measured continuously using a calibrated pressure transducer (Cobe pressure transducers, Lakewood, CO, USA) connected to the fetal carotid catheter. Fetal heart rate was calculated from the blood pressure recording by computed counting of systolic peak-to-peak intervals. The ECoG signal was passed through a 3–30 Hz filter and amplified. The diaphragm EMG signal was filtered through a 80–300 Hz bandpass, preamplified and integrated.

Plasma collection

Daily fetal arterial blood samples (5 ml) were taken at 10.00 h throughout the study for the measurement of blood gases, pH and plasma catecholamines, betamethasone and dexamethasone concentrations. Blood gases and pH were analysed using a blood-gas analyser (ABL500; Radiometer, Copenhagen, Denmark; measurements corrected to 39 °C). All blood samples for hormone analyses were drawn anaerobically and collected in chilled heparinized tubes. The tubes were then spun at 3000 *g* for 5 min. The plasma was collected, flash frozen immediately in liquid nitrogen and stored in a −80 °C freezer until analysis, which was performed within 2 months of plasma collection.

Measurements and calculations

Betamethasone and dexamethasone. Fetal plasma concentrations of betamethasone and dexamethasone were determined by high performance liquid chromatography and UV detection using modifications of the methods described by Tsuei & Ashley (1978). In brief, 0·5–1·0 ml of plasma was extracted with 5 ml ethyl-acetate : hexane (3 : 2). The organic phase was recovered and dried in a Speed-Vac Concentrator. Dried samples were resuspended in methanol and injected into a 3·9 mm × 150 mm 5 μm octadecyl-silane 18 column and eluted with an isocratic mobile phase of 32·5% (v/v) acetonilitrine. The column effluent was monitored by UV absorbance at 235 nm and quantified by peak-height integration. Recovery was monitored by addition of prednisolone as an internal standard to all samples and the reference standards. The sensitivity of the assay was 1–3 ng ml^{-1} for both betamethasone and dexamethasone. The inter-assay and intra-assay coefficients of variation were both < 10%.

Catecholamines. Fetal plasma concentrations of noradrenaline and adrenaline were determined by radioenzymatic assay using minor modifications of the method of Peuler & Johnson (1977). The assay was sensitive to 1–2 pg ml^{-1} of noradrenaline and adrenaline and inter-assay and intra-assay coefficients of variation were both < 5%.

FBM and ECoG. FBM were considered present when fetal diaphragmatic activity occurred for at least 60 s. ECoG activity, which remained below the daily average for at least 3 min, was considered low voltage (LV)-ECoG activity. The incidences of FBM and LV-ECoG activity and LV-ECoG episode duration were quantified, expressed per hour, and averaged over the experimental time periods: baseline, infusion and post-infusion.

Cardiovascular data. Fetal data obtained from daily monitoring of arterial blood gases and pH were similarly averaged over every experimental time period. Fetal femoral vascular resistance was calculated by dividing mean arterial blood pressure by mean fetal femoral blood flow. Data for fetal mean blood pressure, fetal heart rate, mean femoral blood flow and mean femoral vascular resistance were averaged over every hour.

Statistical analysis. All values are expressed as means ± S.E.M. Data were first analysed by the summary of measures method (Matthews, Altman, Campbell & Royston, 1990) to reduce the number of comparisons. During the first series of experiments, mean arterial blood gases and pH, mean incidence of LV-ECoG and FBM, and mean fetal blood pressure and mean fetal heart rate were compared between baseline, the infusion period and the post-infusion period for all treatment groups using ANOVA for repeated measurements. Significant differences were assessed with the Newman–Keuls *post hoc* test. Mean plasma concentrations of noradrenaline and adrenaline were compared between baseline and the infusion period using Student's paired *t* test. During the second series of experiments maximum values in fetal blood pressure and fetal femoral vascular resistance and minimum values in fetal heart rate and femoral blood flow during betamethasone infusion were compared with the corresponding maximum or minimum values during baseline to avoid confounding influences imposed by 24 h rhythmicity using Student's paired *t* test. For all statistical comparisons, differences were considered significant when $P < 0{\cdot}05$.

RESULTS

Outcome of experimental preparations

All ewes were healthy throughout the experimental protocols. The mothers of all nine fetuses treated with betamethasone and two out of six fetuses treated with dexamethasone developed labour-type myometrial activity within 24 h of the end of the infusion period (data not shown). Since all sheep in labour underwent necropsy at 127 dGA no post-infusion data are available for all nine betamethasone-treated fetuses and the two dexamethasone-treated fetuses. All fetuses were healthy at necropsy. There were no significant differences in fetal weights between the control group (3·42 ± 0·13 kg, $n = 9$), the dexamethasone group (3·38 ± 0·27 kg, $n = 6$), or the betamethasone group in labour (3·16 ± 0·13 kg, $n = 9$).

Cardiovascular data

Fetal blood gases and pH. Fetal arterial blood gases and pH remained unchanged throughout the study protocol for the three treatment groups (Table 1).

Fetal blood pressure and heart rate. Fetal blood pressure was similar between the three treatment groups during baseline (Table 2 and Fig. 1). During the infusion period, a pronounced increase in fetal blood pressure occurred in both the betamethasone- and the dexamethasone-infused fetuses. The nature and magnitude of the increase in fetal blood pressure was similar between these two groups. Although an increase in fetal blood pressure with time was detected in control fetuses, this increase was markedly different from that measured in fetuses treated with betamethasone and dexamethasone during the infusion period (Table 2 and Fig. 1). Fetal blood pressure fell towards baseline following the infusion period in the dexamethasone-treated fetuses, however mean fetal blood pressure during the post-infusion period remained elevated from baseline.

Table 1. Arterial blood gases and pH for control, dexamethasone and betamethasone-infused fetuses during the experimental protocol

		pH_a	P_{a,CO_2} (mmHg)	P_{a,O_2} (mmHg)
Controls ($n = 9$)	Baseline	7·38 ± 0·01	46·6 ± 0·7	21·4 ± 0·8
	Infusion	7·37 ± 0·01	47·0 ± 0·9	20·5 ± 0·8
	Post-infusion	7·36 ± 0·01	46·9 ± 1·1	20·4 ± 1·0
Dexamethasone ($n = 6$)	Baseline	7·39 ± 0·01	46·2 ± 1·3	20·1 ± 1·0
	Infusion	7·39 ± 0·01	46·1 ± 1·3	20·5 ± 1·0
	Post-infusion *	7·36 ± 0·01	47·3 ± 0·5	20·3 ± 0·8
Betamethasone ($n = 9$)	Baseline	7·40 ± 0·00	45·7 ± 1·0	21·8 ± 0·4
	Infusion	7·39 ± 0·01	44·7 ± 0·9	22·4 ± 0·4

Values are given as means ± S.E.M. for each of the experimental periods: baseline, infusion and post-infusion. Betamethasone-treated fetuses had no post-infusion values since their mothers went into labour within 24 h following the end of the infusion period. * Two ewes carrying dexamethasone-treated fetuses went into labour during post-infusion. Their data were excluded from the analysis for the post-infusion period.

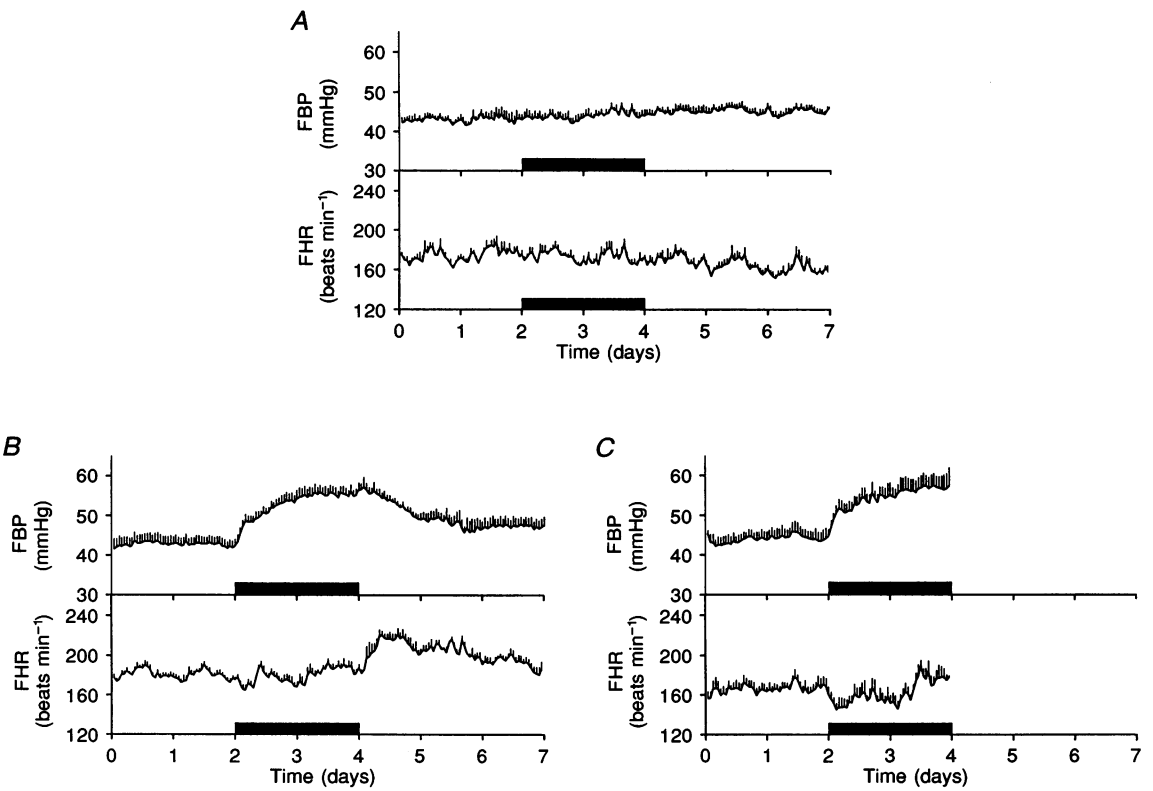

Figure 1. Mean fetal blood pressure (FBP) and fetal heart rate (FHR) values

Data are for control (saline, *A*), dexamethasone-treated (*B*) and betamethasone-treated (*C*) fetuses during the first series of experiments. Values shown are means ± S.E.M. for every hour. The filled bar represents the infusion period. No data were analysed during the post-infusion period in betamethasone-treated fetuses since their mothers went into labour within 24 h of the end of the infusion period. Similarly, two ewes carrying dexamethasone-treated fetuses went into labour during post-infusion. Their data were excluded from the analysis for the post-infusion period.

Table 2. Cardiovascular responses during the first series of experiments

Treatment		FBP (mmHg)	FHR (mmHg)
Controls ($n = 5$)	Baseline	43·6 ± 1·1	178·0 ± 3·3
	Infusion	43·9 ± 1·3	175·2 ± 3·5
	Post-infusion	45·0 ± 1·0[a]	166·3 ± 4·0[a]
Dexamethasone ($n = 6$)	Baseline	42·8 ± 2·0	180·4 ± 2·4
	Infusion	52·7 ± 1·8[a,b]	179·9 ± 2·9
	Post-infusion*	49·8 ± 1·7[a,b]	203·9 ± 3·1[a,b]
Betamethasone ($n = 5$)	Baseline	44·2 ± 2·1	165·9 ± 6·1
	Infusion	53·4 ± 2·7[a,b]	157·6 ± 8·9

Values are shown as means ± S.E.M. Betamethasone-treated fetuses had no post-infusion values since their mothers went into labour within 24 h following the end of the infusion period. * Two ewes carrying the dexamethasone-treated fetuses also went into labour during post-infusion. Their data were excluded from the analysis for the post-infusion period. Significant differences are: [a] different from own baseline (ANOVA for repeated measures, Student–Newman–Keuls, $P < 0·05$) and [b] different from corresponding control period (ANOVA, Student–Newman–Keuls, $P < 0·05$). FHR, fetal heart rate; FBP, fetal blood pressure.

Fetal heart rate was similar between the three treatment groups during baseline (Table 2 and Fig. 1). A decrease in fetal heart rate with time was measured in control fetuses. However, a fall in fetal heart rate was not detected during the infusion of either betamethasone or dexamethasone. Following the end of the infusion period a marked tachycardia occurred in dexamethasone-treated fetuses (Table 2 and Fig. 1*B*).

Femoral haemodynamics. While the fall in fetal femoral blood flow fell outside significance ($P = 0·06$) a progressive increase in femoral vascular resistance was calculated in fetuses treated with betamethasone (Table 3 and Fig. 2). In contrast, in control fetuses femoral blood flow and femoral vascular resistance remained unchanged from baseline.

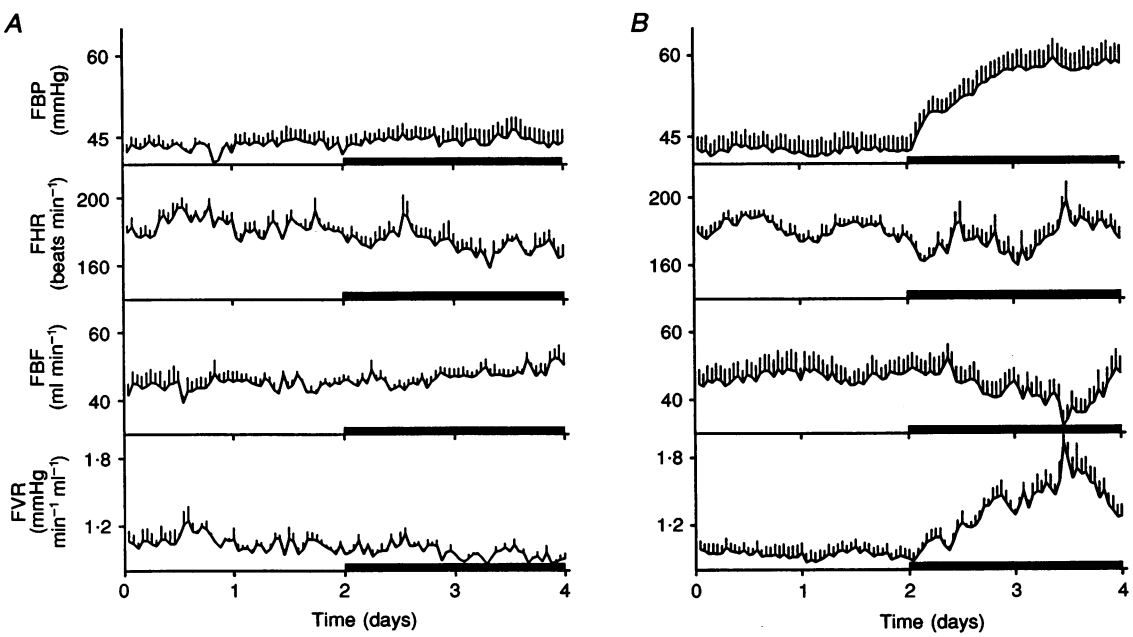

Figure 2. Mean fetal blood pressure (FBP), fetal heart rate (FHR), femoral blood flow (FBF) and femoral vascular resistance (FVR)

Data are for control (*A*) and betamethasone-treated (*B*) fetuses during the second series of experiments. Values shown are means ± S.E.M. for every hour. The filled bar represents the infusion period.

Table 3. Cardiovascular responses during the second series of experiments

Treatment		FBP (mmHg)	FHR (beats min^{-1})	FBF (ml min^{-1})	FVR (mmHg min^{-1} ml^{-1})
Controls ($n = 4$)	Baseline	46·8 ± 1·9	166·2 ± 3·6	37·1 ± 2·7	1·29 ± 0·08
	Infusion	47·0 ± 2·0	162·0 ± 5·9	39·2 ± 0·7	1·22 ± 0·04
Betamethasone ($n = 4$)	Baseline	44·6 ± 2·9	167·9 ± 5·0	41·0 ± 4·0	1·07 ± 0·08
	Infusion	60·8 ± 3·7[a,b]	154·1 ± 6·0[a]	31·9 ± 4·7	1·94 ± 0·23[a,b]

Values are shown as means ± S.E.M. for maximum blood pressure and femoral vascular resistance (FVR) and minimum heart rate (FHR) and femoral blood flow (FBF) during baseline and during the infusion period. Significant differences are: [a] different from own baseline (Student's paired t test, $P < 0·05$) and [b] different from corresponding control period (Student's unpaired t test, $P < 0·05$). FBP, mean fetal blood pressure.

Endocrine data

Fetal plasma beta- and dexamethasone concentrations. Fetal plasma betamethasone and dexamethasone levels achieved 24 h after the start of the infusions were 10 ± 2 and 8 ± 1 ng ml^{-1}, respectively. These plasma levels are in the same range as those measured in human fetal plasma samples obtained during Caesarean section within 24 h following maternal glucocorticoid administration (Petersen *et al.* 1980; Kream *et al.* 1983).

Fetal plasma catecholamine concentrations. Fetal plasma noradrenaline concentrations fell during dexamethasone treatment and fetal plasma adrenaline concentrations fell during the betamethasone treatment (Fig. 3).

Behavioural data

Fetal ECoG. Clear ECoG signals without electrical artifacts were obtained continuously throughout the study from five fetuses in each treatment group. During baseline the incidence of LV-ECoG activity was similar amongst the three groups and a modest, but significant increase in the incidence of LV-ECoG occurred in control and dexamethasone-infused fetuses by the end of the study period (Fig. 4). The mean duration of a period of LV-ECoG during baseline was 20 ± 2 min for controls, 22 ± 2 min for dexamethasone-infused fetuses and 20 ± 2 min for betamethasone-infused fetuses. These durations remained unchanged during infusion of either saline (22 ± 2 min), dexamethasone (25 ± 4 min) or betamethasone (19 ± 2 min).

FBM. During baseline the incidence of FBM was 49 ± 2% for controls, 52 ± 2% for dexamethasone-infused fetuses and 49 ± 3% for betamethasone-infused fetuses. While a modest (but significant) fall in the incidence of FBM occurred during the infusion period in both betamethasone- and dexamethasone-treated fetuses, the incidence of FBM remained unchanged from baseline in control fetuses (Fig. 4). The magnitude of the reduction in FBM was similar between betamethasone- and dexamethasone-treated fetuses. During the post-infusion period, the incidence of FBM returned to baseline in the dexamethasone-infused fetuses.

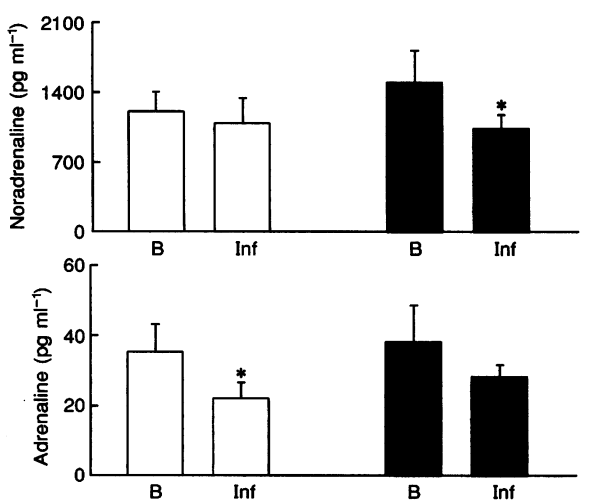

Figure 3. Fetal plasma noradrenaline and adrenaline concentrations

Concentrations during baseline (B) and the infusion period (Inf) for the betamethasone-treated (□) and dexamethasone-treated (■) fetuses. Significant differences are B *vs.* Inf; Student's paired t test, * $P < 0·05$.

DISCUSSION

This is the first report in which the effects of betamethasone and dexamethasone, administered to the fetal sheep to achieve circulating fetal plasma concentrations similar to those measured in human fetal cord plasma obtained at Caesarean section after maternal antenatal steroid administration (Petersen *et al.* 1980; Kream *et al.* 1983), have been studied contemporaneously on fetal cardiovascular, endocrine and behavioural responses.

Both betamethasone and dexamethasone administration to the late-gestation sheep fetus induced a similar, pronounced increase in mean fetal arterial blood pressure and a similar, modest reduction in the incidence of FBM. The steroid-induced increase in fetal blood pressure was associated with an increase in fetal femoral vascular resistance. The fall in the incidence of FBM occurred independently of a fall in fetal LV-ECoG activity.

It is of interest that the increase in fetal arterial blood pressure during either betamethasone or dexamethasone infusion was sustained for the duration of the glucocorticoid treatment. This may have strong clinical implications since a recent clinical trial has reported elevated mean arterial blood pressure for the first 3 days of life in human neonates whose mothers received dexamethasone antenatally (Kari *et al.* 1994). In relation to this, studies performed in rats have demonstrated possible relationships between fetal glucocorticoid exposure and the development of hypertension in adulthood (Benediktsson, Lindsay, Noble, Seckl & Edwards, 1993; Levitt, Holmes, Lindsay & Seckl, 1995).

Differential effects of betamethasone and dexamethasone on fetal cardiovascular and behavioural responses following maternal administration (Mulder *et al.* 1994; Dawes *et al.* 1994; Derks *et al.* 1995*b*) and relatively similar cardiovascular and behavioural effects of both stereoisomers following direct fetal administration reported in the present manuscript may suggest differential transplacental passage. The half-life of betamethasone is longer than that of dexamethasone (Haynes, 1990). As a result, greater effects of betamethasone on the fetus may be expected following administration to the mother in divided bolus doses, as performed clinically.

Cardiovascular effects

An increase in arterial blood pressure in both the adult (see Walker & Williams, 1992) and fetus (Wood *et al.* 1987; Tangalakis *et al.* 1992; Dodic & Wintour, 1994) following cortisol administration is a well-established phenomenon, however the mechanisms mediating this induced hypertension either in the adult or fetus remain to be fully elucidated. Cortisol-induced hypertension may be achieved by the binding of cortisol to either glucocorticoid (GR, type II) and/or mineralocorticoid (MR, type I) classical cystosolic and/or cell-surface receptors (Walker & Williams, 1992). In contrast, synthetic glucocorticoids, such as betamethasone and dexamethasone, do not have any reported mineralocorticoid activity (Haynes, 1990; see Joels and de Kloet, 1995), thus both stereoisomers may be used as powerful tools to identify glucocorticoid- from mineralocorticoid-induced effects on the fetus. In the present study it is reported that fetal treatment with synthetic steroids was associated with an increase in arterial blood pressure and that the nature and magnitude of this increase was similar following either betamethasone or dexamethasone treatment. The occurrence of an increase in fetal blood pressure following betamethasone or dexamethasone treatment provides strong evidence to suggest that reported cortisol MR-mediated effects cannot account for the mechanisms mediating the arterial hypertension. It had been suggested that MR stimulation by cortisol will increase sodium retention, with a subsequent increase in plasma volume and blood pressure and/or promote sodium-mediated endothelial engorgement with a consequent decrease in vessel lumen diameter and increased vascular resistance (Tobian, 1960). Previously reported cardiovascular effects of cortisol on the

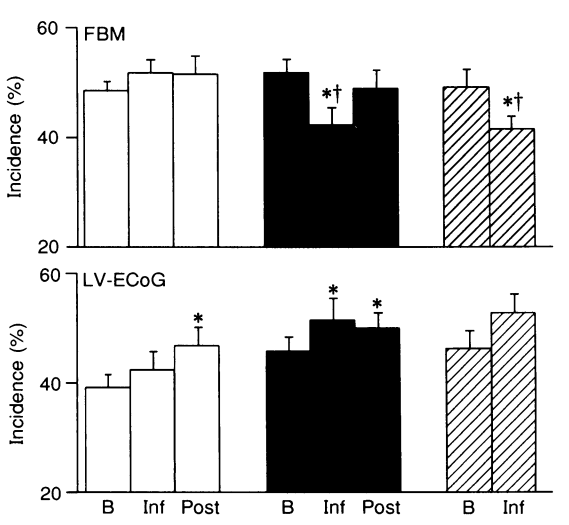

Figure 4. Incidence of fetal breathing movements (FBM) and low voltage electrocorticogram (LV-ECoG)
Data are expressed as a percentage of time in control (□), dexamethasone-treated (■) and betamethasone-treated (▨) fetuses during baseline (B), the infusion (Inf) and the post-infusion (Post) periods. Significant differences are: *, different from own baseline (Student's paired t test, $P < 0{\cdot}05$); †, different from corresponding control period (Student's t test for unpaired data, $P < 0{\cdot}05$).

sheep fetus and those of betamethasone and dexamethasone reported in the present study, are thus likely to be primarily mediated by GR and not MR. This conclusion is in agreement with the study of Wood *et al.* (1987) who reported that blood volume actually decreased, and not increased, following cortisol treatment of fetal sheep.

The mechanisms producing an increase in fetal arterial blood pressure during cortisol, betamethasone or dexamethasone infusion, effected via GR, may be mediated by an increase in fetal cardiac output and/or an increase in fetal total peripheral vascular resistance. The present results are the first to report an increase in femoral vascular resistance following fetal treatment with synthetic steroids. Although an increase in femoral vascular resistance may not represent an increase in total peripheral vascular resistance, changes in resistance in the femoral bed demonstrate a good correlation with changes in peripheral organ vascular resistance (Giussani *et al.* 1996). However, since the increase in femoral vascular resistance following betamethasone treatment was modest and the increase in blood pressure pronounced, relative to these cardiovascular changes during acute fetal hypoxaemia, for example (Giussani, Spencer, Moore, Bennet & Hanson, 1993), we speculate that an increase in cardiac output may have also contributed to the increase in fetal arterial blood pressure.

Several GR-mediated effects have been proposed to contribute to glucocorticoid-induced increases in peripheral vascular resistance. These include an increase in Ca^{2+} entry into vascular smooth muscle (Kornel, 1993) which may explain in part an increase in vascular sensitivity to vasoconstrictor hormones, in particular noradrenaline and angiotensin II, and less consistently to other vasoconstrictors such as vasopressin (Tangalakis *et al.* 1992; Walker & Williams, 1992). Glucocorticoids may also enhance vascular α-adrenoreceptor (Haigh & Jones, 1990) and angiotensin II AT_1 receptor densities (Sato, Suzuki, Nakazato, Shibata, Inagami, Saruta, 1994), and induce inhibition of prostacyclin (Axelrod, 1983) and nitric oxide (Knowles, Salter, Brooks & Moncada, 1990) synthesis. Changes in fetal plasma concentrations of catecholamines cannot contribute to the induced increase in peripheral vascular resistance since fetal plasma catecholamines decreased during cortisol infusion in the study of Wood *et al.* (1987) and during betamethasone or dexamethasone treatment in the present study.

It is interesting that in the current study the pronounced increase in fetal arterial blood pressure during glucocorticoid infusion occurred without any early changes in fetal heart rate. It is of further interest that following the end of the dexamethasone infusion, a pronounced fetal tachycardia was measured. Glucocorticoids are known to play an important role in regulating the coupling of α- and β-adrenergic receptors to cellular post-receptor signal-transduction mechanisms. Low doses of glucocorticoids will promote coupling of α-adrenoreceptors to vasoconstrictor responses (Liu, Haigh & Jones, 1990; Walker & Williams, 1992) and coupling of β-adrenoreceptors to chronotropic responses (Bian, Seidler & Slotkin, 1992; Walker & Williams, 1992). Thus, it is possible that these additional effects of glucocorticoids will promote an increase in peripheral vascular resistance and in cardiac output, both contributing to the observed fetal hypertension. In addition, a baroreflex-induced bradycardia, following glucocorticoid-induced hypertension, may have been masked by the sensitizing effects of glucocorticoids to β-adrenergic-chronotropic stimulation. The effects of glucocorticoids would then be unmasked following the end of the dexamethasone infusion period, at a time when blood pressure started falling towards baseline.

Behavioural effects

A moderate, but significant, increase in the incidence of LV-ECoG was observed in saline-treated fetuses by the end of the experimental period. This increase occurred from 125 to 132 dGA and may represent an increased maturation of organizational states. Increased organization of electrocortical activity may result from the well-reported increase in endogenous fetal cortisol levels which begins at *ca* 120–125 dGA (Magyar *et al.* 1980; Challis & Brooks *et al.* 1989). Accordingly, the greater increase in LV-ECoG incidence measured during the infusion period in beta- and dexamethasone-treated fetuses may be due to augmented fetal glucorticoid concentraton at this time. The fall in the incidence of FBM during betamethasone or dexamethasone infusion reported in the present studies confirms our previous observations in the human fetus following maternal betamethasone administration (Derks *et al.* 1995*b*), but is in contrast with preliminary data in the human fetus in which no effect of maternal dexamethasone administration was observed on FBM (Mulder *et al.* 1995). The difference between human and sheep fetuses may be due to interspecies differences or due to the mode of drug administration. In addition, data collected in the human were restricted to periods of observation of 1 h per day at a specific time of the day.

The fall in the incidence of FBM independent of a fall in LV-ECoG during glucocorticoid infusion reported in the present manuscript is in agreement with our previous observations in human fetuses, in which eye movements, used as an index of behavioural state, were also unaffected despite a decrease in FBM following maternal betamethasone administration (Derks *et al.* 1995*b*).

The effects of betamethasone and dexamethasone on FBM may be mediated by promoting an increase in prostaglandin E_2 (PGE_2) production. Glucocorticoids initiate labour in sheep by increasing the activity of placental 17α-hydroxylase with a resultant increase in the conversion of progesterone to oestradiol (Anderson, Flint & Turnbull, 1975). This altered steroid production results in increased synthesis of PGE_2 (Olson, Skinner & Challis, 1985). PGE_2 has been

demonstrated to inhibit FBM (Kitterman, Liggins, Fewell & Tooley, 1983).

In conclusion, betamethasone and dexamethasone administration to late-gestation fetal sheep, in doses that achieve circulating levels comparable to those measured in the human fetus following maternal administration, induced measurable changes in fetal cardiovascular, endocrine and behavioural responses. Fetal treatment with betamethasone or dexamethasone produced a pronounced increase in arterial blood pressure and a modest fall in the incidence of FBM. The magnitude of these changes were similar following fetal treatment with either glucocorticoid. Betamethasone-induced fetal hypertension was associated with an increase in fetal femoral vascular resistance. The fall in the incidence of FBM after betamethasone or dexamethasone was independent of a fall in the incidence of LV-ECoG.

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