Continuous Intrafetal Infusion of Prostaglandin E$_2$ Prematurely Activates the Hypothalamo-Pituitary-Adrenal Axis and Induces Parturition in Sheep*

I. R. YOUNG, J. M. DEAYTON, S. A. HOLLINGWORTH†, AND G. D. THORBURN

Department of Physiology, Monash University, Clayton, Victoria 3168, Australia

ABSTRACT

This study has investigated the effect of continuous intrafetal infusion of PGE$_2$, or saline on hormone concentrations and the length of gestation in sheep. Fetal and maternal vascular catheters were surgically implanted at 112.3 ± 1.3 days (n = 10), and the infusions were started at 121 ± 1.2 days of gestation (term = 147). Fetuses were infused with either PGE$_2$ (n = 5; 2 μg/min for 48 h and then increased to 4 μg/min for the remainder of the experiment) or the vehicle solution (n = 5, sterile isotonic saline) via the fetal carotid artery. In the PGE$_2$-infused group, fetal and maternal plasma PGE$_2$ concentrations increased (P < 0.001) after the change to the higher dose rate (4 μg/min) and remained elevated, fetal plasma immunoreactive ACTH (ir-ACTH) concentrations dramatically increased after the start of the infusion being maximal at 11 h before decreasing to match concentrations exhibited by the saline-infused group. Fetal plasma cortisol concentrations increased after the start of the PGE$_2$ infusion (P = 0.05) and increased further after the change to the higher dose rate of 4 μg/min (P < 0.001). Concentrations of PGE$_2$, ir-ACTH, and cortisol in the saline-infused group did not change until labor. Plasma concentrations of PGE$_2$ (P < 0.001) and ir-ACTH (P < 0.005) increased on the day of labor in both treatment groups, and fetal cortisol concentrations increased (P < 0.001) in both groups in the last few days before labor. The proportion of low molecular weight ir-ACTH in the plasma of PGE$_2$-infused fetuses was significantly higher than that of saline-infused fetuses (P < 0.001) during the first 15 days of infusion. In the saline-infused group, the proportion of low molecular weight ir-ACTH increased in the last few days before labour (P < 0.001), whereas no change was seen in PGE$_2$-infused fetuses at this time. Maternal plasma progesterone concentrations decreased in both groups in the last few days before labor (P < 0.001). Fetuses infused with PGE$_2$ delivered at 138.4 ± 2.1 days, whereas control fetuses infused with saline delivered at 148.2 ± 0.5 days (P < 0.01). The spontaneous increase in PGE$_2$ preceding normal labor may thus play an important role in activation and maturation of the hypothalamic-pituitary-adrenal axis in fetal sheep (Endocrinology 137: 2424–2431, 1996).

SPONTANEOUS parturition in the sheep depends on a functionally intact fetal hypothalamo-pituitary-adrenal (HPA) axis (1, 2). Some days before birth, the fetal adrenal cortex increases its activity resulting in increased cortisol concentrations in fetal plasma (3), leading to activation of the parturient mechanism (4, 5). The increase in fetal plasma cortisol concentrations is accompanied by increasing ACTH concentrations (6–11), but the magnitude of the ACTH increase in most of these studies is modest and seemingly inadequate to explain the relatively much larger increases in cortisol. Nonetheless, it is unusual that ACTH secretion is not curtailed by negative feedback at this time when cortisol concentrations rise so rapidly. The drive to fetal ACTH secretion in late gestation may not be fully explicable by the actions of the hypothalamic releasing factors, CRH, and arginine vasopressin (AVP) as the response of ACTH to both agents diminishes with advancing gestation (12), and neither appears capable of reliably inducing premature parturition when infused into fetal sheep (13–15).

Intrafetal infusions of PGE$_2$ in sheep have previously been shown to stimulate cortisol (16, 17) and ACTH (18–21) secretion in fetal sheep. The finding that PGE$_2$ infusion releases cortisol from the adrenals of hypophysectomised fetuses (17) suggests a dual site of action, at the adrenal as well as the pituitary. We have also shown that intrafetal infusion of PGE$_2$ for short periods (2 h) stimulates the release of both ACTH and cortisol (11, 21–23). We have also shown that such short-term infusions alter the posttranslational processing of POMC to favor the secretion of low molecular weight (LMW) ACTH-containing species. This may be highly significant for our understanding of the increasing sensitivity of the fetal adrenal gland because the ratio of high molecular weight (HMW) to LMW ACTH-containing peptides may be the critical determinant of adrenal responsiveness to ACTH (9, 10, 24–26).

Unlike CRH and AVP, the ability of short term PGE$_2$ infusions to release ACTH and alter posttranslational processing of POMC do not diminish with advancing gestation (11, 21). Fetal PGE$_2$ concentrations increase in late gestation (27) and appear to parallel the increase in cortisol concentrations in late gestation in fetal sheep. We have argued that PGE$_2$ may contribute to drive in the fetal HPA axis and that an understanding of its actions may help overcome our inability to account for the preparturient fetal cortisol surge based on current knowledge (22, 23, 28–30).

Our knowledge of the effects of PGE$_2$ on the fetal HPA axis...
PGE<sub>2</sub> INDUCES PREMATURE DELIVERY IN SHEEP

2425

is based on short term infusions of doses that yield modest increases in the plasma PGE<sub>2</sub> concentration. The route of administration (carotid artery vs. jugular vein or inferior vena cava) does not appear to influence the ACTH response (21), although the concentration presented to the fetal brain is presumably greatest (by a factor of 10-20) when PGE<sub>2</sub> is infused by the carotid route. The inability to distinguish between the effects of PGE<sub>2</sub> infused by these different routes suggests that the standard dose rate (2 μg/min) exceeds the maximal dose.

The present study investigates the effects of chronically infused PGE<sub>2</sub> on the HPA axis of the fetal sheep and on the timing of parturition. We commenced the infusions at day 121, before the spontaneous activation of the fetal adrenal, which occurs between days 125 and 130. This timing avoids a possible confounding effect of the normally observed increase in fetal cortisol concentrations on the experimental findings at the start of the PGE<sub>2</sub> infusion. In the event that PGE<sub>2</sub> infusion was associated with premature delivery, we were particularly interested to compare the endocrine profiles of the PGE<sub>2</sub>- and saline infused fetuses in relation to the onset of labor. Substantial similarity of the fetal cortisol and maternal progesterone profiles of the experimental and control groups would indicate that chronic PGE<sub>2</sub> infusion induced labor by prematurely activating the mechanisms that lead to spontaneous parturition.

Materials and Methods:

Animals

Ten pregnant cross-bred ewes of known mating date were used in this study. Surgery was performed at 112.3 ± 1.3 days under aseptic conditions and general anesthesia induced with 1 g thiopentone sodium in water (iv) and maintained with 1.5% halothane (oxygen/nitrous oxide, 50:50 vol/vol). Vascular catheters were inserted into the fetal carotid artery, fetal femoral artery, fetal jugular vein, and maternal carotid artery and jugular vein. Electromyographic electrodes were sutured to the myometrium to record uterine activity and determine when the ewes were in labor. The fetal catheters and electrode wires were exteriorized via an incision in the ewe's flank, and all catheters were filled with sterile heparinized saline (0.9% NaCl; 50,000 IU heparin/liter). After surgery, the ewes were housed in metabolic cages and allowed to recover for at least 5 days before the experiment was performed. The ewes were fed once daily and water was provided ad libitum.

Experimental protocol and blood sampling

At 121 ± 1.2 days of gestation, fetuses were infused with either PGE<sub>2</sub> (Upjohn Company, Kalamazoo, MI) (n = 5; 2 μg/min for 48 h, then increased to 4 μg/min until labor) or the vehicle solution (n = 5; sterile isotonic saline) via the fetal carotid artery. Fetal (aortic via femoral artery) and maternal (carotid arterial) blood samples were collected 75 and 15 min before the start of the infusion (1115 h), then 15, 30, and 60 min after the start of the infusion. Further blood samples were collected at 1600 h and 2200 h on the same day. Subsequent blood samples were collected twice daily until labor was apparent. At each sampling time, fetal arterial pH, PO<sub>2</sub>, and PCO<sub>2</sub> were measured to assess fetal wellbeing (ABL30, Radiometer, Copenhagen). Fetal plasma samples (2 ml) for the measurement of ACTH containing peptides (gel filtration chromatography) were taken at 15 min before and 60 min after the start of the infusion and subsequently at 5-day intervals for the remainder of the experiment.

Blood samples were collected into chilled tubes containing 125 IU lithium heparin (Johns Division Mallinkrodt (Australia) Pty. Ltd., South Oakleigh, Victoria, Australia) for the measurement of fetal cortisol. The same tubes were used with the addition of aprotinin (1000 KIU) for immunoreactive ACTH (ir-ACTH) and chromatographic analysis of ACTH-like peptides. Plain tubes containing 15 mg EDTA and indomethacin (final concentration 10 μM) were used for the measurement of fetal and maternal PGE<sub>2</sub>. All blood tubes were centrifuged for 10 mins at 2000 × g at 4°C. The separated plasma for cortisol and ir-ACTH assay was stored immediately at −20°C. Plasma for chromatographic analysis was collected into 1.6% glycine/1 M HCl (150 μl/ml plasma) and stored at −20°C. After centrifugation, plasma for PGE<sub>2</sub> measurement was diluted 1:1 with 0.12 mol/liter methoxyamine hydrochloride (Sigma Chemical Company, St. Louis, MO) in sodium acetate buffer (1 mol/l, pH 5.6) containing 10% absolute ethanol and incubated overnight at room temperature before storage at −20°C.

Hormone assays

Cortisol was measured by RIA in fetal sheep plasma after extraction with dichloromethane using a previously described method (31). Antiserum no. 3368 raised in sheep was supplied by Dr. R. I. Cox (CSIRO, Division of Animal Production, Prospekt, New South Wales). The sensitivity of this assay was 0.59 ± 0.08 ng/ml, and the intra- and interassay coefficients of variation were 9.3% and 16.8%, respectively, as estimated from eight assays required for this study.

ACTH was measured in fetal plasma by RIA as previously described by McMillen et al. (33). Antiserum no. 3 supplied by Dr. C. F. Rice (Department of Physiology, Monash University, Australia) has been raised against synthetic human ACTH<sub>1-24</sub>. As noted below, this antiserum cross-reacts with ACTH<sub>1-39</sub> and with its HMW precursors. It is not, therefore, a specific assay for ACTH<sub>1-39</sub> but measures global concentrations of ACTH-containing peptides in plasma.

The sensitivity of the assay was 46.7 ± 8.3 pg/ml, the intraassay coefficient of variation was 15.9% as estimated from seven assays required for plasma ACTH analysis in this study.

PGE<sub>2</sub> was measured by RIA in fetal and maternal plasma as previously described by us (33) using antiserum no. 9183 raised in sheep against the methyloxime of PGE<sub>2</sub> conjugated to porcine gelatin and supplied by Dr. R. I. Cox (CSIRO, Division of Animal Production, Prospekt, New South Wales). This antisem cross-reacted 100% with PGE<sub>2</sub> methoxime, 4.9% with PGE<sub>1</sub>, 0.5% with 15-keto-PGE<sub>2</sub>, 0.2% with 13,14-dihydro-15-keto-PGE<sub>2</sub>, and less than 0.06% with prostaglandins B<sub>2</sub>, D<sub>2</sub>, F<sub>2α</sub>, F<sub>2β</sub>, 6-keto-PGE<sub>2</sub>, thromboxane B<sub>2</sub>, 15-keto-PGF<sub>2α</sub>, PGFM, 6,15-diketo-13,14-dihydro-PGE<sub>2</sub>, and arachidonic acid. Sensitivity of the assay was 0.43 ± 0.04 nm, and the intra- and interassay coefficients of variation were 14.9% and 18.5%, respectively, as estimated from ten assays required for this study.

Progesterone was measured by RIA as described by us (33). Antiserum S23 supplied by Dr. J. Malecki (Regional Veterinary Institute, Department of Agricultural and Rural Affairs, Bairnsdale, Victoria, Australia) was raised against progesterone-11-α-BSA in sheep. The sensitivity of the assay was 2.88 ± 0.41 nm, and the intra- and interassay coefficients of variation were 12% and 10.8%, respectively, as estimated from eight assays required for this study.

Chromatography and assay of ACTH-containing species

One milliliter of plasma was thawed and loaded onto a 0.9 × 60-cm gel chromatography column (Sephadex G50 fine; Pharmacia LKB, Uppsala, Sweden) and eluted with 1% formic acid containing 1 mg/ml polyeppe (Sigma, St. Louis, MO) at a flow rate of 10 ml/h. Fractions (2 ml) were collected into 10 × 75 mm polypropylene tubes, lyophilised, and stored at −20°C. Before assay, the lyophilised fractions were reconstituted in 0.5 ml assay buffer and allowed to stand for at least 2 h at room temperature. They were then assayed with the ACTH RIA. Data are expressed as the total immunoreactivity in these fractions as a percentage of the total immunoreactivity in the sample. The technique has recently been described in more detail (21). Previous attempts using this approach have not reported the cross-reaction of the antisem used with POMC or pro-ACTH. We have determined the cross-reaction of our antisem to POMC standard derived from AtT-20 cells and donated by Drs. S. Crosby and A. White (University of Manchester, Salford, UK). This standard was prepared from the POMC peak eluting from a Sephadex
G75 column and contains both POMC and pro-ACTH peptides (34, 35). The cross-reaction of this standard in our ACTH assay was 8%, thus the proportions of POMC/pro-ACTH in our chromatographic analyses are probably approximately 12-fold higher than we report here. We have not applied any correction for POMC/pro-ACTH cross reaction because the separate cross-reactions of POMC and pro-ACTH are unknown as are the relative proportions of these two species in the plasma samples. Application of a correction factor of 12 would yield LMW:HMW ratios consistent with those reported by Saphier et al. (9), who used specific immunoradiometric assays to measure closely related forms.

Statistical analysis

All values are expressed as mean ± SEM. Results were analyzed using a program for statistical data analysis (SPSS-X). Because the ewes went into labor at different gestational ages, the data were analyzed in two ways:

1. The animals in the two treatment groups were aligned on the commencement of the infusion and followed for the subsequent 7 days or in the case of the ACTH chromatographic data, the first 15 days.
2. The events in the peripartum period were analyzed by aligning the data on the day of labor and including values obtained up to 20 days before labor.

Hormone concentrations were first tested for homogeneity of variance using Bartlett-Box F and Cochran's C test. A square root, log, or arcsin (ACTH chromatography only; this transformation is applicable to bounded percentage data) transformation was used as appropriate to render homogeneous any hormone data sets that were found to be inhomogeneous. For the analyses of hormone profiles during the initial days of the infusion, the maternal PGE2, data were square root transformed; the fetal PGE2, ACTH, and cortisol data underwent log2 transformation, and arcsin transformation was used for the ACTH chromatographic data. Maternal progesterone data did not require transformation. For the analyses of hormone profiles during the peripartum period, the same transformations were applied with the exceptions of maternal progesterone (square root) and ACTH chromatography (raw data). Data were subsequently analyzed by three-factor repeated measures ANOVA with treatment group, animal within treatment group, and time point (during infusion or as days before labor) as factors. If an interaction was detected between treatment group and time point, those data were reanalyzed separately within the PGE2, or saline infused groups for effect of time using two-factor ANOVA. Student-Newman-Keuls test for multiple comparisons was used to identify differences between mean concentrations. The differences between treatment groups in fetal adrenal or body weight and gestational age at labor were analyzed by Student's t test.

Results

Gestational outcome

Fetal arterial pH, PO2, and PCO2 of all fetuses were within normal limits throughout the study period. All ewes and fetuses were killed by an overdose of barbiturate (Lethabarb, Arnolds of Reading Pty. Ltd., Boronia, Victoria, Australia) while in active labor as determined by electromyographic activity and confirmed at necropsy. Ewes in the PGE2-infused fetal group went into premature labor at 138.4 ± 2.1 days, whereas ewes in the saline-infused fetal group went into labor at 148.2 ± 0.5 days. This difference was significant (P < 0.01) as determined by Student's t test. Fetal body weights at autopsy (PGE2-infused, 3.9 ± 0.4 kg; saline-infused, 4.4 ± 0.4 kg) and combined adrenal weights (PGE2-infused, 564.8 ± 33.3 mg; saline-infused, 616.8 ± 77 mg) of the two groups were not different. The combined adrenal weights corrected for body weight were also not different (PGE2-infused, 150.6 ± 15.5 mg/kg; saline-infused: 141.3 ± 11.3 mg/kg).

Hormone concentrations

Fetal ir-ACTH. Fetal plasma ir-ACTH concentrations in the PGE2-infused group showed a robust increase after the start of the infusion, reaching a maximum value at 11 h (−15 min, 113.4 ± 31.6 pg/ml; +11 h, 1294.3 ± 612.8 pg/ml). These concentrations then decreased within 24 h to match concentrations exhibited by the saline-infused group (Fig. 1). Ir-ACTH concentrations of the two groups of fetuses were subsequently indistinguishable from each other. On the day of labor, ir-ACTH concentrations in both treatment groups increased from an average of 161 ± 10 pg/ml in the week preceding labor to 346.2 ± 50.5 pg/ml during labor (P < 0.005).

Fetal cortisol. There was a nonsignificant (P = 0.051) tendency for fetal plasma cortisol concentrations in the PGE2-infused group to increase from a mean preinfusion value of 2.39 ± 0.41 ng/ml to 4.95 ± 0.98 ng/ml during the first 24 h of PGE2 infusion. A significant P < 0.001) increase occurred after the change to the higher dose rate of 4 μg/min (17.4 ± 1.69 ng/ml) (Fig. 2). Plasma cortisol concentrations in the saline-infused group did not change after the start of the infusion although, in the week preceding labor, these concentrations had risen to match those of the PGE2-infused fetuses. Concentrations in both treatment groups increased significantly in the days immediately preceding labor (3 days, 27.5 ± 6 ng/ml; 0 days, 73.7 ± 8.9 ng/ml; P < 0.001) (Fig. 3). The temporal profiles of plasma cortisol in the two groups were statistically indistinguishable in the lead up to labor, although this event occurred 10 days earlier in the PGE2-infused group.

Fetal and maternal PGE2. Plasma PGE2 concentrations of PGE2- and saline-infused fetuses were not different before commencement of the experiment (PGE2-infused, 1.6 ± 0.4 nM; saline-infused, 2.32 ± 0.93 nM). Plasma PGE2 concentrations of PGE2-infused fetuses increased significantly (P < 0.001) only after the change to the higher dose rate (4 μg/min).

![Fig. 1. Fetal plasma ir-ACTH concentrations during the first 7 days of either continuous PGE2 (○) or saline (□) intrafetal infusion. Arrows indicate the start of infusion and PGE2 doses administered. There was a significant effect of treatment group, with only the PGE2-infused fetuses showing a change with time. Different letters indicate significantly different values (P < 0.001).](image-url)
PGE₂ INDUCES PREMATURE DELIVERY IN SHEEP

30

\( \text{pg/min} \)

25 k

abed

1.00 1.25 1.50 2.00 3.00 4.00 5.00 6.00 7.00 8.00

Days of infusion

FIG. 2. Fetal plasma cortisol concentrations during the first 7 days of either continuous PGE₂ (●) or saline (○) intrafetal infusion. Arrows indicate the start of infusion and PGE₂ doses administered. There was a significant difference between the treatment groups, with only the PGE₂-infused fetuses showing an effect of time. Different letters indicate significantly different values \((P < 0.001)\).

100

1

0 f I I I I I I

125 130 135 140 145 150 155

Gestational age (days)

FIG. 3. Preparturient increase of plasma cortisol in fetuses infused with either PGE₂ (●; \( n = 5 \) except where indicated due to labor) or saline (○; \( n = 5 \) except where indicated due to labor) continuously from 121 days of gestation. Cortisol concentrations of both groups increased significantly over the prepartum period \((P < 0.001)\). PGE₂-infused fetuses delivered 10 days earlier than saline-infused fetuses.

\( \text{pg/min} \)

min) and subsequently remained elevated in the range 8.9 ± 2.8 to 18.1 ± 8.7 nM. PGE₂ concentrations in the saline-infused group did not change from preinfusion concentrations until labor. On the day of labor, fetal PGE₂ concentrations increased significantly in both treatment groups \((\text{PGE}_2\text{-infused}, 43.8 ± 6.3 \, \text{nM}; \text{saline-infused}, 7.5 ± 2.8 \, \text{nM}; P < 0.01 \text{ vs. previous days})\).

PGE₂ concentrations in the arterial plasma of ewes bearing PGE₂- or saline-infused fetuses followed the same temporal patterns as the corresponding fetal values except that no labor-associated increase was seen. During the period of infusion, mean plasma concentrations of PGE₂ in ewes with PGE₂-infused fetuses ranged from 9.1 ± 3.7 to 15.4 ± 5.9 nM, whereas those of ewes with saline-infused fetuses ranged from 3.3 ± 1.0 to 5.6 ± 2.5 nM.

Maternal progesterone. Maternal plasma progesterone concentrations did not change in either group from the start of infusion but decreased in both treatment groups in the last few days before labor \((-3 \text{ days}, 33.4 ± 3.75 \, \text{nM}; 0 \text{ days}, 16.3 ± 3.43 \, \text{nM}; P < 0.001)\) (Fig. 4).

Molecular weight profile of ir-ACTH in fetal plasma

For each plasma sample submitted to chromatography, the immunoreactivity eluting in the position of ACTH(1-39) standard (LMW peak) was expressed as a percentage of total ir-ACTH recovered from the sample (Fig. 5). The percentage of ACTH immunoreactivity eluting in the LMW peak was significantly higher \((P < 0.01)\) in PGE₂-infused fetuses than in the saline-infused group \((\text{PGE}_2, 31.62 ± 3.38\%; \text{saline}, 9.96 ± 1.08\%\). There was an increase \((P = 0.001)\) in the percentage of ACTH(1-39) in the saline-infused group in the last few days before labor from 10.01 ± 0.98% to 24.84 ± 4.53%. In the PGE₂-infused group, no further increase was seen at labor beyond the chronically elevated values associated with PGE₂ infusion.

Discussion

This study shows that chronically administered PGE₂ can induce premature parturition in the sheep and does so by activating the fetal HPA axis. The maternal progesterone profiles and evolution of uterine electrical activity were also consistent with a physiologically normal induction of labor. No other known secretagogue for ACTH has been shown to reliably induce parturition in this way \((13-15)\). In the only report of premature labor associated with CRF infusion, the maternal progesterone concentrations did not exhibit the normal prepartum fall \((13)\), although the neonates were viable.

The concentrations of PGE₂ in the aortic plasma of fetuses receiving PGE₂ at 2 \( \mu g/\text{min} \) did not exceed those of fetuses receiving saline infusion. The PGE₂ was infused into the carotid artery, close to the brachiocephalic trunk, so the concentration of PGE₂ in the contralateral carotid and cerebral arteries may have greatly exceeded those in the periphery. We have previously shown that 2 h intracarotid and jugular venous infusions of PGE₂ result in identical ACTH profiles \((21)\). Because the cerebral concentrations of PGE₂ during jugular venous infusion would have been lower than those resulting from intracarotid administration, this suggests that a maximal response to PGE₂ would have been achieved in this study with a lower rate of PGE₂ infusion. The infusion rate we chose was based on previous studies \((14,16-18)\) and were intended to elicit maximum responses. It is surprising that we were unable to demonstrate increased plasma PGE₂ concentrations during the 2 days of infusion at 2 \( \mu g/\text{min} \), especially so in light of the calculated secretion rate from the placenta into the fetal circulation of 11 ± 2 ng/kg·min \((36)\). At the time we commenced PGE₂ infusion, the fetal body weights would have been approximately 2 kg, so our infusion rate would have exceeded the calculated placental secretion rate by 100-fold. The contribution of tissues other than the placenta may be large, but this remains speculative. An alternative explanation for the lack of a significant in-
increase in plasma PGE2 concentrations in the early part of the infusion would be provided if placental PGE2 secretion were regulated by negative feedback. Although this is a novel and extremely interesting possibility, the present results do not suggest what the other components of such a putative feedback loop may be. The subsequent increase in PGE2 infusion rate from 2-4 μg/min was associated with a statistically significant increase in plasma PGE2 concentration over the remainder of the infusion.

Both PGE2- and saline-infused fetuses exhibited increased plasma PGE2 concentrations on the day of labor. Induction of prostaglandin synthase enzymes is known to occur in the last few days of gestation and is believed to be a late event in the sequence of changes culminating in uterine contraction. We think it likely that the final increase in circulating PGE2 is a consequence of this process. Whether it plays a causative role in the accompanying increases of ir-ACTH and cortisol is unclear at present. The late changes associated with activation of the myometrium and cervical ripening bear many resemblances to the inflammatory response with the involvement of PGE2, PGF2α, and cytokines such as the interleukins. Some of these chemical mediators of the inflammatory response are also known to activate the HPA axis (16, 38, 40), so it is reasonable to propose the existence of multiple positive feedback loops between the fetal HPA axis and the uteroplacental unit in the last day of gestation and the incipient first stage of labor. Whether exogenously infused PGE2 directly activates the inflammatory cascade is unknown, but the long period of stable endocrine profiles between the commencement of infusion and the onset of labor suggests that it did not do so in this instance.

The PGE2 concentration in maternal plasma showed a similar pattern to that observed in the fetus, with no detectable increase until after the infusion rate was increased to 4 μg/min. Because of this resemblance, it appears possible that a proportion of the PGE2 infused into the fetal circulation entered the maternal circulation. Although the mechanisms and kinetics of PGE2 clearance from the maternal circulation are not fully understood, the fact that appreciable concentrations were detectable in arterial blood makes it apparent that not all PGE2 can be cleared in a single passage through the maternal pulmonary circulation.

In the hours after the commencement of the PGE2 infusion, the fetal ir-ACTH concentrations showed the robust increase that has been previously noted in response to short term infusions (19, 21). Within 24 h, this response had dissipated and the ir-ACTH concentrations in the PGE2-infused and control fetuses were subsequently indistinguishable. During the period of PGE2 infusion, the percentage of ACTH-immunoreactive material present as LMW forms was significantly elevated compared to the saline-infused controls. This difference, combined with the marked increase in ir-ACTH during the first 12 h of PGE2 infusion, would have resulted in a considerable increase in the absolute concentration of LMW ACTH, which is believed to be the bioactive form (9, 10, 24, 25). Our finding of a significant difference between the percentages of LMW ACTH in the plasma of PGE2-infused and saline-infused fetuses is consistent with our previous finding that 60 min of PGE2 infusion at the same rate as was used in the present study was associated with a significant increase in the percentage of LMW ACTH in fetal plasma at the same gestational age (21).

The reason for the fall in ir-ACTH concentrations during the second 12 h period of the PGE2 infusion is unclear. The nonsignificant tendency of cortisol to increase during the initial period of PGE2 infusion may have been sufficient to account for the concomitant decline in ir-ACTH secretion through negative feedback. Very low rates of cortisol infusion have been shown to powerfully inhibit ACTH secretion in fetal sheep (37). An alternative explanation for the fall in ir-ACTH is that the initial peak in ir-ACTH concentration represented secretion of the total releasable pool of ACTH from the corticotrophs with subsequent maintenance of circulating ACTH by de novo synthesis. We have no firm
PGE₂ INDUCES PREMATURE DELIVERY IN SHEEP

In a previous study (21), the effect of PGE₂ on the molecular weight profile of ACTH immunoreactive species occurred within 1 h of commencing the infusion. The rapidity of this change argues against it being a transcription-related event. ACTH₁₋₃₉ is cleaved from its parent molecule POMC by specific prohormone convertase enzymes (44, 45). Because the alteration in posttranslational processing of POMC that we observed was probably too rapid to allow for induction of gene expression, another mechanism must be sought. One possible explanation is that the prohormone convertases are constitutively expressed in the corticotroph cell and their activities are modulated by another substance of unknown identity. This putative modulator may be linked to increased trophic drive to the corticotrophs because the alteration in POMC processing also occurred in the saline-infused fetuses during the last few days of fetal life when activity in the HPA axis was increasing. The putative modulator may be PGE₂ itself, although the fact that the alteration in POMC processing occurred in the saline-infused fetuses at a time when plasma PGE₂ concentrations were not increasing argues against this possibility. Alternatively, another factor such as CRF may have been released in response to PGE₂ infusion and acted on the prohormone convertases to effect the rapid alteration in POMC processing.

The plasma cortisol concentrations in the PGE₂-infused fetuses did not parallel those of ACTH. Cortisol concentrations tended to increase during the initial 2 days of the infusion, but statistical significance was not achieved until the infusion rate was increased to 4 µg/min. Previous studies using 2 µg/min infusions of PGE₂ for 2 h have shown a statistically significant increase in plasma cortisol concentration during the period of the infusion (17, 19, 21). The failure to replicate this finding in the present study may reflect immaturity of the adrenals in the fetuses used in this study, although a response to PGE₂ was observed at a similar gestational age in our previous study (21). The discrepancy in cortisol responsiveness between the two studies may be accounted for by a slight difference in gestational age at infusion, with the adrenals of the fetuses involved in this study being less mature (the previous study included animals aged up to 125 days). Exposure of immature fetal adrenals to trophic factors may be expected to cause induction of 17α-hydroxylase and increased ability to secrete cortisol within 1–2 days (46). At least two trophic factors may have been acting on the adrenal cortices of the PGE₂-infused fetuses: ACTH and PGE₂ itself (17).

As noted above, the net bioactivity of ACTH in fetal plasma would have been markedly increased during the first 12 h of PGE₂ infusion when both the ir-ACTH concentration and the proportion of ACTH in LMW forms were increased. Although the plasma ir-ACTH concentration subsequently returned to basal values, the proportion of LMW ACTH remained elevated throughout the remainder of the PGE₂ infusion. Because the net bioactivity of ACTH in plasma...
appears to be determined by the balance of LMW-HMW immunoreactive species (9, 24-26), the increased proportion of LMW ACTH-containing species throughout the PGE₂ infusion would favor cortisol production relative to the saline-infused controls. The proportion of LMW ACTH containing peptides in the plasma of the saline-infused fetuses increased in the 5 days before labor, reaching similar values to those of the PGE₂-infused fetuses. Such an increase in LMW ACTH in the immediate prepartum period is consistent with previous reports (9, 24, 47).

The increase in plasma cortisol concentration that occurred on the third day of PGE₂ infusion may thus have been a reflection of increased adrenal drive brought about by initial maturation of the steroidogenic pathway, subsequently maintained by increased bioactivity of ACTH. Alternatively, since the increase in cortisol output coincided with the increases in both the PGE₂ infusion rate and the plasma concentration of PGE₂, a direct effect of PGE₂ on the fetal adrenal cortex may have occurred. PGE₂ has been reported to stimulate cortisol secretion in hypophysectomized fetal sheep (17) and in cultured bovine adrenal cells (48). The similarity of the plasma PGE₂ and cortisol profiles in the PGE₂-infused fetuses during the first week of infusion suggests a direct effect of PGE₂ on adrenal cortisol secretion.

The profiles of ACTH and cortisol in the plasma of the PGE₂-infused fetuses and the progesterone profiles of their dams in the days preceding labor were indistinguishable from those of the saline-infused group. These similarities of concentration and temporal profile strongly suggest that, whatever the site of PGE₂ action, the parturient mechanism was the same in both groups. This, together with the long latency from the commencement of the PGE₂ infusion to labor, suggests that PGE₂ acts as a modulator or amplifier of the established processes that control parturition in the fetal sheep. As noted above, likely points of PGE₂ action are on the trophic drive to the corticotrophs via CRF, the posttranslational processing of POMC, and the maturation of the steroidogenic pathway in the fetal adrenal.

The infusion rate of PGE₂ in the present study was supraphysiological, and further studies in this area should utilize doses calculated to bring about alterations within the physiological range. This may allow a more sophisticated assessment of the significance of the naturally occurring increase in PGE₂ that has been reported in the fetus and ewe in late gestation (27, 33, 49).

In summary, this study demonstrates that exogenous infusion of PGE₂, which is naturally present in high concentrations in late gestation fetal plasma, prematurely activates several mechanisms in the hormonal cascade leading to parturition.

Acknowledgments

We wish to thank The Upjohn Company (Kalamazoo, MI) for the supply of PGE₂ used in these studies and Mr. A. N. Satragno and Mrs. C. McKechnie for surgical assistance. Dr. P. McCurdy, Director, Statistical Consulting Service, Monash University, advised on the statistical methods, and Mr. D. J. Caddy supervised the statistical analyses.

References

PGE₂ INDUCES PREMATURE DELIVERY IN SHEEP

32. McMillen IC, Antolovich GC, Mercer JE, Perry RA, Silver M 1990 Proopiomelanocortin messenger RNA levels are increased in the anterior pituitary of the sheep fetus after adrenalectomy in late gestation. Neuroendocrinology 52:297–302
40. Watanabe H, Takebe K 1994 Effects of intravenous administration of interleukin-1β on the release of prostaglandin E₂, corticotropin-releasing factor, and arginine vasopressin in several hypothalamic areas of freely moving rats; estimation by push-pull perfusion. Neuroendocrinology 60:8–15
44. Benjannet S, Rondeau N, Day R, Chretien M, Seidah NG 1991 PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. Proc Natl Acad Sci USA 88:3564–3568
48. Rainey WE, Naville D, Cline N, Mason JI 1991 Prostaglandin E₂ is a positive regulator of adrenocorticotropic receptors, 3β-hydroxysteroid dehydrogenase and 17α-hydroxylase expression in bovine adrenocortical cells. Endocrinology 129:1333–1339