Gestational age-dependent changes in the levels of mRNAs encoding cortisol biosynthetic enzymes and IGF-II in the adrenal gland of fetal sheep during prolonged hypoxemia

G A Braems^{1,3}, V K M Han^{2,3,4} and J R G Challis^{1,3,4}

¹Department of Physiology, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada, ²Departments of Pediatrics, Obstetrics and Gynecology, Anatomy and Cell Biology, and Biochemistry, University of Western Ontario, London, Ontario, Canada, ³The Lawson Research Institute, St Joseph's Health Center, London, Ontario, Canada and ⁴MRC Group in Fetal and Neonatal Health and Development

(Requests for offprints should be addressed to J R G Challis, Department of Physiology, Faculty of Medicine, 1 King's College Circle, Room 3205/7, University of Toronto, Toronto, Ontario, Canada M5S 1A8)

(G A Braems is now at Department of Obstetrics and Gynecology, University Hospital, Klinikstr. 32, D-35385, Giessen, Germany)

Abstract

Hypoxemia represents a major stress for the fetus, and is associated with alterations and adaptations in cardiovascular, metabolic and endocrine responses, which in turn may affect tissue growth and differentiation. To determine the effects of hypoxemia on fetal adrenal activity and growth, we subjected sheep fetuses at days 126–130 and 134–136 (term 145 days) to reduced PaO₂ by reducing the maternal fraction of oxygen for 48 h (mean reduction of 6.8 mmHg), without change in arterial pH or PaCO₂. This stimulus resulted in similar increases in the plasma immunoreactive ACTH response at both ages. Among adrenal steroids, plasma cortisol (C21Δ₄) rose in both groups of animals, but plasma androstenedione (C19Δ₄) declined marginally, resulting in a pronounced increase in the cortisol:androstenedione ratio in the plasma that was greater and more sustained in the older fetuses. In the younger fetuses, after 48 h of hypoxemia, there were no significant changes in

mRNAs encoding steroidogenic enzymes in the fetal adrenal gland. However, in the older fetuses, hypoxemia resulted in significantly increased levels of mRNAs encoding P450_{SCC}, P450_{C21} and 3β-hydroxysteroid dehydrogenase, but not for P450_{C17}, in the fetal adrenal gland. Levels of IGF-II mRNA in the fetal adrenal gland fell in both groups of fetuses, and this response was greater at the later gestational age. We conclude that sustained hypoxemia is a potent stimulus which activates adrenal steroidogenesis in the late gestation fetal sheep. The resultant increase in cortisol synthesis is associated with decreased expression of adrenal IGF-II mRNA. We speculate that this relationship might influence patterns of fetal organ growth and differentiative function in response to fetal stress such as hypoxemia.

Journal of Endocrinology (1998) **159**, 257–264

Introduction

Hypoxemia is a potent stimulus which increases the activity of the hypothalamic–pituitary–adrenal (HPA) axis in the fetus. In response to short-term (60 min) hypoxemia, there are increases in fetal plasma concentrations of adrenocorticotrophin (ACTH), vasopressin and cortisol (Boddy *et al.* 1974, Rurak 1978, Stark *et al.* 1982, Akagi & Challis 1990). The magnitude of these changes varies at different gestational ages in relation to the progressive maturation of fetal HPA function. Thus, later in gestation, the plasma cortisol response to acute hypoxemia is enhanced, and the ACTH response diminished, presumably as a result of increased fetal adrenal responsiveness, and greater negative feedback by the elevated basal and stimulated glucocorticoid concentrations (Norman *et al.* 1985, Akagi & Challis 1990). However, there is no information available concerning the effects of

hypoxemia on levels of expression of key enzymes in the biosynthetic pathway of fetal adrenal glucocorticoids that might underlie these changes in plasma hormone concentrations.

We have recently developed a model of prolonged (48 h) fetal hypoxemia without concurrent changes in fetal PaCO₂ or pH, to examine effects on fetal HPA function (Braems *et al.* 1996). To identify the gestational age-dependent changes, fetuses were studied on two occasions in late gestation, before and after day 130 of pregnancy (term 145 days), since this is a period of rapid change in HPA responsiveness. Hypoxemia produced similar changes in plasma immunoreactive (ir) ACTH in both groups of animals, but the change in plasma cortisol was greater and more sustained in the older fetuses. The objective of the present study was to determine whether the alteration of fetal adrenal function extended to C19 steroids, and whether age-dependent differences in

fetal adrenal responses were related to differences in the levels of mRNAs encoding key steroidogenic enzymes. We also measured levels of plasma androstenedione, which contributes about 30% of the precursor for fetal-placental estrogen production in sheep during late pregnancy (Mitchell *et al.* 1986). In the sheep fetal adrenal, androstenedione appears to be analogous to dehydroepiandrosterone (DHEA) sulfate from the human fetal adrenal as the precursor for placental estrogen biosynthesis (Albrecht & Pepe 1990).

The increased output of cortisol in the late gestation fetal sheep contributes to fetal organ maturation and to the onset of parturition (Liggins 1994, Matthews & Challis 1996). Fetal hypercortisolemia may also lead to fetal growth restriction. Exogenous glucocorticoids decrease [³H]thymidine incorporation into several fetal tissues, including the brain (Reinisch *et al.* 1978), and reduce insulin-like growth factor-II (IGF-II) expression in many fetal organs (Li *et al.* 1993). We have reported previously that IGF-II mRNA is abundantly expressed in the steroidogenic cells of the ovine fetal adrenal (Han *et al.* 1992) and IGF-II mRNA levels in the adrenal cortex are reduced in fetal sheep infused with either ACTH or cortisol at various times during the last third of pregnancy (Lu *et al.* 1994). Therefore, a further aim of the present study was to determine whether the elevation of cortisol produced in response to hypoxemia was associated with altered expression of the IGF-II gene in the fetal adrenal glands.

Materials and Methods

Animals and surgery

Details of the animals used in these experiments have been described elsewhere (Braems *et al.* 1996). Animal protocols were approved by the Animal Care Committees of the Lawson Research Institute and of the University of Western Ontario, according to the guidelines of the Canadian Council on Animal Care. Briefly, vascular catheters were implanted into two groups of fetal sheep at either days 118–122 ($n=18$) or days 126–128 ($n=10$) of gestation. Experiments were performed in the younger group of animals beginning on days 124–128, and in the older animals beginning on days 132–134 of pregnancy. Fetal hypoxemia was produced by the mother breathing a gas mixture containing a lowered fraction of oxygen, and was continued for 48 h. Control animals continued to breath room air. Fetal hypoxemia was produced in eight fetuses in the younger age group, and in five in the older age group; there were ten and five normoxemic fetuses respectively at the two ages. Blood samples were collected from a fetal artery (2.5 ml) and a maternal artery (5.0 ml) into chilled heparinized syringes at 8 h intervals before and during the period of hypoxemia, for measurement of blood gases with a blood gas analyzer (Radiometer, Copenhagen,

Denmark), plasma ir-ACTH and cortisol (Braems *et al.* 1996) and androstenedione (Mitchell *et al.* 1986) by RIA. The mean intra- and interassay coefficients of variation for the androstenedione assay were 5.3 and 9.0% respectively.

At the end of the experimental period (days 126–130 and days 134–136) the animals were killed by maternal i.v. injection of pentobarbital sodium (Euthanol, Abbott Laboratories, Montreal, Canada). The fetuses were delivered immediately by hysterotomy, and fetal tissues including the adrenal glands were removed, frozen using liquid nitrogen, and stored at -80°C for later analysis. Levels and distribution of proopiomelanocortin (POMC) mRNA in the fetal pituitary were determined by *in situ* hybridization and have been reported elsewhere (Braems *et al.* 1996).

Northern blot analysis

Total RNA was extracted using the lithium chloride–urea method (Auffray & Rougeon 1980). The integrity of the RNA was assessed after electrophoresis on a 1% (w/v) agarose gel, and stained with ethidium bromide. Only the RNA samples without evidence of degradation were used. Electrophoresis of RNA (20 µg) samples was performed as described (Berduco *et al.* 1993) on 1% agarose (w/v), 2.2 M formaldehyde gels, and was followed by capillary transfer to a nylon membrane (Zetaprobe, Bio-Rad, Mississauga, Ontario, Canada). The RNA was covalently cross-linked to the membrane by UV light (GS Gene Linker, Bio-Rad, Hercules, CA, USA). The membrane was prehybridized at 42°C overnight in a buffer containing 50% (v/v) formamide, 7% (w/v) SDS, $4 \times$ SSPE (35 g NaCl, 5.5 g NaH_2PO_4 and 1.5 g EDTA per liter, pH 7.4) and 0.5 mg/ml denatured salmon sperm.

The membranes were then hybridized overnight with one of the following oligonucleotide probes encoding steroidogenic enzymes, or an ovine IGF-II cDNA (Han *et al.* 1992). The difference in loading and transfer among different lanes was assessed by hybridizing the blots with a cDNA encoding mouse 18S ribosomal RNA. The 45-mer oligonucleotide probes were synthesized based on the DNA sequences for ovine P450_{C21} (Crawford *et al.* 1992), P450_{C17} and P450_{sc} (Tangalakris *et al.* 1990) and human 3β -hydroxysteroid dehydrogenase (3β -HSD) type II (Lorrence *et al.* 1990) mRNAs as recorded in the DNA database of the European Molecular Biology Laboratory (EMBL) Gene Bank (Heidelberg, Germany). The probes were prepared by solid phase synthesis using a Beckman Oligo 1000 DNA Synthesizer (Fullerton, CA, USA) and were purified on an 8% polyacrylamide/8 M urea sequencing gel. Probes were complementary to the mRNAs encoding HSD-II (bases 794–838) for 3β -HSD, and SHPCYTOC (bases 1496–1540) for P450_{C21}. The oligonucleotide for P450_{C17} (Tangalakris *et al.* 1990) was extended with GA at the 5' end and GCT GCC CAT CAT C at the 3' end, and the oligonucleotide for P450_{sc}

was elongated by CCAG at the 5' end and AACTT at the 3' end. The probes were 5'-end labeled (Sambrook *et al.* 1989) with [γ ³²P]ATP (specific activity 3000 Ci/mmol; DuPont NEN, Mississauga, Ontario, Canada) using T4 polynucleotide kinase (Promega, Madison, WI, USA). The ovine IGF-II cDNA was labeled with [γ ³²P]dCTP using random priming as described (Han *et al.* 1992). Probes were added ($1.0\text{--}1.5 \times 10^6$ c.p.m./ml buffer) to the membranes, hybridized overnight, and then washed at the highest stringency of $0.1 \times \text{SSC}$ at 50°C . Blots were exposed to X-ray film (XAR, Kodak Laboratories, Rochester, NY, USA) within the linear range of the film and the relative optical densities were determined by computerized image analysis (Imaging Research Inc., St Catharines, Ontario, Canada). Blots were stripped by boiling, and checked by autoradiography before being reprobed. Negative controls for the oligonucleotide probes included Northern blots of fetal sheep liver total RNA, which showed no hybridization when processed identically to the adrenal blots (data not shown).

Data analysis

Results are expressed as means $\pm$ S.E.M. Group comparison was performed by one-way and two-way ANOVA. Corrections were made for repeated measures, and if significance was obtained ($P < 0.05$) these were followed by Duncan's multiple range test or specified contrasts with Student's *t*-test.

Results

Blood gases and plasma ir-ACTH

We have described previously in detail the changes in blood gases, plasma ir-ACTH and levels of pituitary POMC mRNA in these animals (Braems *et al.* 1996). Briefly, fetal PaO₂ values were similar (20 mmHg) at the start of the experiment in all groups of fetuses. During hypoxemia fetal PaO₂ fell by 6.9 ± 1.1 mmHg in the younger fetuses, and by 6.8 ± 1.1 mmHg in the older fetuses. There were no significant effects of hypoxemia on fetal arterial PaCO₂ or pH.

Concentrations of ir-ACTH rose significantly within 2 h of the beginning of hypoxemia at both gestational ages, and then decreased by 18 h to mean values that were higher than those in controls, but they were not different statistically. There was no significant difference in the peak ir-ACTH values, nor in the integrated ACTH response (area under the curve) between the younger and older fetuses (Braems *et al.* 1996).

Plasma cortisol and androstenedione concentrations

The mean plasma cortisol concentrations were 3.1 ± 0.3 ng/ml and 8.9 ± 1.1 ng/ml in the younger and older fetuses respectively during the period before hypoxemia.

During hypoxemia, plasma cortisol concentrations rose to maximum values of 10 ng/ml in the younger fetuses and 30 ng/ml in the older animals. In the fetuses at days 134–136 of gestation, the elevation in plasma cortisol was sustained over the 48 h experimental period. Mean androstenedione concentrations were similar (550–600 pg/ml) at the start of the experiment in all groups of animals (Fig. 1, upper panels). During hypoxemia, there was a gradual decline in androstenedione concentrations such that during the second 24 h period, the mean values of androstenedione had decreased by 15 and 18% for the younger and older fetuses respectively, compared with the 24 h before starting hypoxemia in these animals (both $P < 0.05$; Fig. 1). The different patterns of change in plasma cortisol and androstenedione resulted in a significant increase in the ratio of cortisol:androstenedione concentrations in plasma, which was greater and more sustained in the older animals (Fig. 1, lower panels).

Adrenal steroidogenic enzyme mRNA expression

Northern blotting identified single transcripts for P450_{sc} (size 1.9 kb), P450_{C17} (size 1.7 kb) and 3 β -HSD (size 2.0 kb), and two major transcripts for P450_{C21} (1.7 kb and 2.2 kb) in adrenal tissue from all groups of fetuses. There were no significant differences in the levels of mRNAs encoding P450_{sc}, P450_{C17}, P450_{C21} or 3 β -HSD in adrenal tissue from normoxemic fetuses at the two gestational ages (Fig. 2). However, there was a dramatic difference between the younger and older fetuses in the effect of hypoxemia on levels of mRNA for key steroidogenic enzymes in the fetal adrenal gland (Fig. 2). Hypoxemia had no significant effect on the mRNA levels of any of the four steroidogenic enzymes in the adrenal tissue from the younger fetuses (Fig. 2). In contrast, in the older group of fetuses there were significant increases in the levels of mRNAs encoding P450_{sc} ($P < 0.001$), P450_{C21} 1.7 kb transcript ($P < 0.05$), and for 3 β -HSD ($P < 0.05$), but not for P450_{C17} or P450_{C21} 2.2 kb transcript (Fig. 2).

Adrenal IGF-II mRNA

Seven IGF-II mRNA transcripts (7.2 kb to 0.8 kb) were identified in the adrenal glands of normoxic and hypoxic fetuses from both gestational age groups. The mean levels of total IGF-II mRNA in adrenal tissue from normoxemic fetuses at the two gestational ages were not statistically different. After 48 h hypoxemia there was a decrease in the levels of total IGF-II mRNA in adrenal tissues from both groups of animals, and this was statistically significant in the older fetuses (Fig. 3).

Discussion

We have reported previously that in response to sustained hypoxemia for 48 h, the concentration of cortisol in the plasma of fetal sheep rises, and this response is greater and

Figure 1 Concentrations of androstenedione (upper panels) and the ratio of plasma cortisol:androstenedione (lower panels) in the plasma of fetal sheep at days 126–130 and 134–136 of gestation. Animals were made hypoxemic (solid symbols) or remained normoxemic (open symbols) for 48 h after an initial 24 h (–24 to 0 h) control period. The experimental period (hatched horizontal bars) was between 0 and 48 h. Values are means $\pm$ S.E.M. * $P < 0.05$ between hypoxemic and normoxemic fetuses.

more sustained in fetuses at days 134–136 of gestation compared with those 6–10 days earlier in gestation (Braems *et al.* 1996). This is because adrenal steroidogenesis is directed towards cortisol at the later gestational age. We now report that plasma androstenedione concentrations are relatively unchanged, or even decline gradually over this same period of time. This results in an increase in the plasma cortisol:androstenedione ratio. The sustained nature of these changes in plasma hormone concentrations is consistent with increases in mRNAs encoding P450_{SCC}, 3 β -HSD and P450_{C21} in adrenal tissue from the older fetuses. In both groups of animals there was a concomitant fall in IGF-II mRNA levels in the fetal adrenal gland with a more pronounced change within older fetuses suggesting a relationship between IGF-II gene expression and corticosteroid biosynthesis.

We have shown previously that after hypoxemia, the increases in ir-ACTH in plasma and POMC mRNA in the fetal pars distalis were similar in both groups of animals (Braems *et al.* 1996). Levels of POMC mRNA in the

pars intermedia fell, however, indicating a differential regulation of POMC gene expression in different regions of the pituitary (Braems *et al.* 1996). It remains to be established whether there are changes in the pattern of larger molecular weight ACTH-related peptides in plasma after hypoxemia that could affect the different patterns of adrenal steroidogenic enzymes in the two groups of animals. The ovine fetal adrenal does not have the same relative deficiency of 3 β -HSD (Riley *et al.* 1992, Phillips *et al.* 1996) as the fetal zone of the primate fetal adrenal (Serón-Ferre *et al.* 1978, Pepe & Albrecht 1990, Mason *et al.* 1993) and androstenedione appears to be a reasonable indicator of C19 steroid secretion. Earlier studies showed that the mRNA levels for steroidogenic enzymes in the fetal adrenal reflected changes in enzyme bioactivities (Tangalakakis *et al.* 1989). Thus, increases in P450_{SCC}, 3 β -HSD and P450_{C21} may favor steroidogenesis along the C21 Δ_4 pathway, although we recognize that P450_{C17}, which is required for cortisol biosynthesis, did not change significantly in the present experiments.

Figure 2 Ratio of mRNA encoding P450_{sc}, P450_{C17}, 3β-HSD, and the 1.7 kb P450_{C21} to 18S rRNA in adrenal tissue collected from fetal sheep after 48 h. exposure to normoxemia (open bars) or hypoxemia (hatched bars) at 126–130 and 134–136 days of gestation. Values are mean ± S.E.M. **P* < 0.05.

Figure 3 Levels of mRNA encoding IGF-II (ratio IGF-II mRNA/18S rRNA) in adrenal tissue from fetal sheep exposed for 48 h to normoxemia (open bars) or hypoxemia (hatched bars) at 126–130 or 134–136 days of gestation. There was a significant decrease in IGF-II mRNA/18S rRNA after hypoxemia in fetuses at 134–136 days of gestation. Values are mean ± S.E.M. **P* < 0.05.

Previous studies have shown that there is a triphasic pattern of cortisol output by the adrenal gland of fetal sheep over the course of gestation (Wintour *et al.* 1975). Adrenal responsiveness to ACTH is higher in early (day 50–60) and late pregnancy, but essentially absent at 80–120 days. This pattern of ontogenic change reflects, in part, levels of mRNA, immunoreactive protein and enzyme activity for P450_{sc} and P450_{C17} in the fetal adrenal cortex (Durand *et al.* 1984, Tangalakis *et al.* 1989). P450_{C21} mRNA rises gradually throughout pregnancy (Tangalakis *et al.* 1989), and ir-3β-HSD is present from at least days 50–55 of gestation. In intact fetuses, 3β-HSD activity increases progressively towards term (Manchester *et al.* 1983). A similar pattern of expression for adrenal steroidogenic enzymes during pregnancy has been reported for bovine fetal adrenal tissue (Conley *et al.* 1992).

In the fetal sheep adrenal, pregnenolone can be metabolized by either 3β-HSD or P450_{C17} pathways in competing reactions. Conley & Bird (1997) have reviewed information showing that in this species metabolism through the Δ4 pathway to 17α-hydroxyprogesterone (17αOH-P4) leads to cortisol production, since

17 α OH-P4 is a relatively poor substrate for the C17–20 lyase activity of P450_{C17}. Androstenedione synthesis results from metabolism of pregnenolone through the Δ 5 pathway to DHEA, and then by conversion through 3 β -HSD activity. Hypoxemia may result in greater attenuation of the Δ 5 pathway to DHEA, limiting formation of androstenedione, and favoring formation of cortisol from 17 α OH-P4. In addition, we found a greater increase in 3 β -HSD than in P450_{C17} mRNA, which would favor conversion of pregnenolone through the Δ 4 pathway to cortisol (since the Δ 4 lyase activity is deficient).

It is well documented that ACTH is the major stimulus for acute and chronic phases of adrenal steroidogenesis *in vivo* and *in vitro* (Simpson & Waterman 1988). In fetal sheep, ACTH treatment *in vivo* increases key enzyme activities, including P450_{C17} and 3 β -HSD (Naville *et al.* 1991), and for P450_{C17} and P450_{sc} mRNA levels (Tangalakis *et al.* 1990, 1994). In studies conducted at day 100 of pregnancy, the mRNA levels returned to basal values if the ACTH infusion was stopped for 3 days (Tangalakis *et al.* 1990, 1992), perhaps reflecting loss of labile ACTH-dependent transcription factor(s). In the present experiments some immaturity of the fetal adrenal response still appears to persist, up to 130 days of gestation, compared with the fetuses studied after that time.

It was surprising that the P450_{C17} mRNA levels did not increase significantly, and appeared to be less responsive than the 3 β -HSD mRNA levels to the effect of 48 h hypoxemia. Previous *in vitro* studies have indicated that the P450_{C17} gene appears to be more sensitive to regulation by ACTH and other trophic agents than 3 β -HSD (Naville *et al.* 1991, Cheng *et al.* 1992, Bird *et al.* 1996). It is likely that, during hypoxemia, the adrenal response is influenced by the decreased pulsatility of ACTH secretion or by factors other than ACTH. It is known that continuous infusion of ACTH to the ovine fetus leads to greater output of C21 steroids lacking 17 α hydroxylation than after pulsatile ACTH (Lye *et al.* 1983). The pattern of stimulated ACTH output with hypoxemia may more closely reflect continuous output. It is also possible that the adrenal response to ACTH during hypoxemia is attenuated by factors such as transforming growth factor β or angiotensin II, and that P450_{C17} is affected more than 3 β -HSD (Naville *et al.* 1991). Prostaglandin E₂ (PGE₂) alters levels of P450_{C17} and 3 β -HSD protein and mRNA in bovine adrenal tissue (Railey *et al.* 1991), and PGE₂, likely of placental origin, increases in fetal sheep plasma in some models of prolonged hypoxemia (Hooper *et al.* 1990, Murotsuki *et al.* 1995). The role of PGE₂ in the present experiments is not known, and its possible interactions with other agents acting on the adrenal remain to be explored.

In contrast to the effects on steroidogenic enzymes, IGF-II mRNA levels decreased in adrenal tissue after hypoxemia in both groups of animals, although responses were significant only in the older fetuses. Previously,

we have shown co-localization of IGF-II mRNA in 3 β -HSD immunoreactive cells in the ovine fetal adrenal gland indicating that steroidogenic cells are the source of local IGF-II biosynthesis (Han *et al.* 1992). IGF-II may potentiate mitogenic actions of other growth factors on fetal adrenal growth (Naiman *et al.* 1989, Mesiano *et al.* 1993). In culture, IGFs increase ACTH receptor number, steroidogenic activities and steroid and cAMP responses to ACTH (Naiman *et al.* 1989, Penhoat *et al.* 1989, Mesiano *et al.* 1993). The present results are consistent with *in vivo* studies in fetal sheep showing that infusion of ACTH or cortisol decreases levels of IGF-II mRNA and ir-IGF-II in the adrenal cortex (Lu *et al.* 1994). Furthermore, when fetal sheep are stressed by fasting in late gestation, there is an increase in plasma cortisol concentration, and a decrease in IGF-II mRNA levels in the fetal liver and kidney (Li *et al.* 1993). Exogenous cortisol infusion reproduces the decline in tissue IGF-II mRNA levels, whereas adrenalectomy prevents the prepartum fall of hepatic IGF-II seen in intact animals (Li *et al.* 1993).

We speculate that changes in IGF-II gene expression occurring in response to the endocrine events of hypoxemia may have important consequences for the fetus. It is possible that the stress-induced rises in cortisol during hypoxemia attenuate IGF-II gene expression in various other fetal tissues (Li *et al.* 1993). Such a change, if substantiated, may contribute to long-term decreases in tissue weights. In the adrenal gland, prolonged hypoxemia appears to result in diminished responsiveness to ACTH stimulation (Harvey *et al.* 1993, Murotsuki *et al.* 1996), but there is an increase in the size of the gland (Murotsuki *et al.* 1996), accompanied by increased blood flow (Gagnon *et al.* 1997). We suggest that adrenal cortical hypertrophy may occur as a result of drive from small increments of ir-ACTH and bioactive ACTH in the fetal circulation. ACTH increases cortisol, which decreases IGF-II gene expression, potentially reducing the effects of IGF on the ACTH receptor, cAMP and steroidogenic responses (Naiman *et al.* 1989, Penhoat *et al.* 1989, Mesiano *et al.* 1993). Adrenal cortisol output can be increased by other factors such as PGE₂ (Thorburn & Rice 1990), released in response to hypoxemia (Hooper *et al.* 1990, Murotsuki *et al.* 1995). We speculate that this may result in further downregulation of adrenal responsiveness to acute changes in ACTH. The resulting increase in cortisol biosynthesis of the adrenal may shift the metabolic energy of the steroidogenic cells to this important response to stress, away from the IGF-II biosynthesis which is required for tissue growth.

References

- Akagi K & Challis JRG 1990 Threshold of hormonal and biophysical response to acute hypoxemia in fetal sheep at different gestational ages. *Canadian Journal of Physiology and Pharmacology* **68** 549–555.

- Albrecht ED & Pepe GJ 1990 Placental steroid hormone biosynthesis in primate pregnancy. *Endocrine Reviews* **11** 124–150.
- Auffray C & Rougeon F 1980 Purification of mouse immunoglobulin heavy-chain messenger RNAs from total myeloma tumor RNA. *European Journal of Biochemistry* **107** 303–314.
- Berlusconi ETM, Hammond GL, Jacobs RA, Grolla A, Akaji K, Langlois D & Challis JRG 1993 Glucocorticoid-induced increase in plasma corticosteroid-binding globulin levels in fetal sheep is associated with increased biosynthesis and alterations in glycosylation. *Endocrinology* **132** 2001–2008.
- Bird IM, Pasquarrette MM, Rainey WE & Mason JR 1996 Differential control of 17 α -hydroxylase and 3 β -hydroxysteroid dehydrogenase expression in human adrenocortical H295R cells. *Journal of Clinical Endocrinology and Metabolism* **81** 2171–2178.
- Boddy K, Jones CT, Mantell C, Ratcliffe JG & Robinson JS 1974 Changes in plasma ACTH and corticosteroid of the maternal and fetal sheep during hypoxia. *Endocrinology* **94** 588–591.
- Braems GA, Matthews SG & Challis JRG 1996 Differential regulation of proopiomelanocortin messenger ribonucleic acid in the pars distalis and pars intermedia of the pituitary gland after prolonged hypoxemia in fetal sheep. *Endocrinology* **137** 2731–2738.
- Cheng CY, Fiasch MV & Hornsby PJ 1992 Expression of 17 α -hydroxylase and 3 β -hydroxysteroid dehydrogenase in human fetal adrenocortical cells transfected with SV40 T antigen. *Journal of Molecular Endocrinology* **9** 7–17.
- Conley AJ & Bird IM 1997 The role of cytochrome P450 17 α -hydroxylase and 3 β -hydroxysteroid dehydrogenase in the integration of gonadal and adrenal steroidogenesis via the Δ 5 and Δ 4 pathways of steroidogenesis in mammals. *Biology of Reproduction* **56** 789–799.
- Conley AJ, Head JR, Stirling DT & Mason JI 1992 Expression of steroidogenic enzymes in the bovine placenta and fetal adrenal glands throughout gestation. *Endocrinology* **130** 2641–2650.
- Crawford RJ, Hammond VE, Connell JM & Coghlan JP 1992 The structure and activity of two cytochrome P450 C21 proteins encoded in the ovine adrenal cortex. *Journal of Biological Chemistry* **267** 16212–16218.
- Durand P, Cathiard A-M & Saez JM 1984 *In vitro* maturation of steroidogenic capacity of ovine fetal and neonatal adrenal cells. *Endocrinology* **114** 1128–1134.
- Gagnon R, Murotsuki J, Challis JRG, Fraher L & Richardson BS 1997 Fetal sheep endocrine responses to sustained hypoxic stress after chronic fetal placental embolisation. *American Journal of Physiology* **272** E817–E823.
- Han VKM, Lu F, Bassett N, Yang K, Delhanty PJD & Challis JRG 1992 Insulin-like growth factor-II messenger ribonucleic acid is expressed in steroidogenic cells of the developing ovine adrenal gland is evidence for an autocrine/paracrine role for IGF-II. *Endocrinology* **131** 3100–3109.
- Harvey LM, Gilbert RD, Longo LD & Ducas CA 1993 Changes in ovine fetal adrenocortical responsiveness after long-term hypoxemia. *American Journal of Physiology* **264** E741–E747.
- Hooper SB, Coulter CL, Deayton JM, Harding R & Thorburn GD 1990 Fetal endocrine responses to prolonged hypoxemia in sheep. *American Journal of Physiology* **259** R703–R708.
- Li J, Saunders JC, Gilmour RS, Silva M & Fowden AL 1993 Insulin-like growth factor-II messenger ribonucleic acid expression in fetal tissues of sheep during late gestation: effects of cortisol. *Endocrinology* **132** 2083–2089.
- Liggins GC 1994 The role of cortisol in preparing the fetus for birth. *Reproduction Fertility and Development* **6** 141–150.
- Lorrence MC, Murray BA, Trant JM & Mason JI 1990 Human 3 β -hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase from human placenta: expression in non-steroidogenic cells of the protein that catalyses dehydrogenation/isomerisation of C21 and C19 steroids. *Endocrinology* **126** 2493–2498.
- Lu F, Han VKM, Milne WK, Fraser M, Carter AM, Berlusconi ETM & Challis JRG 1994 Regulation of insulin-like growth factor-II gene expression in the ovine fetal adrenal gland by adreno-corticotrophic hormone and cortisol. *Endocrinology* **134** 2628–2635.
- Lye SJ, Sprague CL, Mitchell BF & Challis JRG 1983 Activation of ovine fetal adrenal functions by pulsatile or continuous administration of adrenocorticotropin-(1–24): effects on fetal plasma corticosteroids. *Endocrinology* **113** 770–782.
- Manchester EL, Lye SJ & Challis JRG 1983 Activation of ovine fetal adrenal function by pulsatile or continuous administration of adrenocorticotropin-(1–24): effects on adrenal cell responses *in vitro*. *Endocrinology* **113** 777–782.
- Mason JI, Ushijima K, Doody KM, Wagai K, Naville D, Head JR, Milewich L, Rainey WW & Ralph MM 1993 Regulation of expression of the 3 β -hydroxysteroid dehydrogenases of human placenta and fetal adrenal. *Journal of Steroid Biochemistry and Molecular Biology* **47** 1–6.
- Matthews SG & Challis JRG 1996 Regulation of the hypothalamo-pituitary-adrenal axis in fetal sheep. *Trends in Endocrinology and Metabolism* **7** 239–246.
- Mesiano S, Mellon SH & Jaffe RB 1993 Mitogenic action, regulation and localization of insulin-like growth factors in the human fetal adrenal gland. *Journal of Clinical Endocrinology and Metabolism* **76** 968–976.
- Mitchell BF, Lye SJ, Lukash L & Challis JRG 1986 Androstenedione metabolism in the late gestation sheep fetus. *Endocrinology* **118** 63–68.
- Murotsuki J, Challis JRG, Johnston L & Gagnon R 1995 Increased fetal plasma prostaglandin E₂ concentrations during fetal placental embolization in pregnant sheep. *American Journal of Obstetrics and Gynecology* **173** 30–35.
- Murotsuki J, Gagnon R, Matthews SG & Challis JRG 1996 Effects of long-term hypoxemia on pituitary-adrenal function in sheep. *American Journal of Physiology* **271** E678–E685.
- Naiman E, Chatelain P, Saez JM & Durand P 1989 *In vitro* effect of insulin and insulin-like growth factor-I on cell multiplication and adrenocorticotropin responsiveness of fetal adrenal cells. *Biology of Reproduction* **40** 570–577.
- Naville D, Rainey WE & Mason JI 1991 Corticotropin regulation of 3 β -hydroxysteroid dehydrogenase/ Δ ⁵- Δ ⁴ isomerase in ovine adrenocortical cells; inhibition by transforming growth factor β . *Molecular and Cellular Endocrinology* **75** 257–263.
- Norman LJ, Lye SJ, Wlodek ME & Challis JRG 1985 Changes in pituitary responses to synthetic ovine corticotropin-releasing factor in fetal sheep. *Canadian Journal of Physiology and Pharmacology* **63** 1398–1403.
- Penhoat A, Jailard C & Saez JM 1989 Synergistic effects of corticotropin and insulin-like growth factor-I on corticotropin receptors and corticotropin responsiveness in cultured bovine adrenocortical cells. *Biochemical and Biophysical Research Communications* **165** 355–359.
- Pepe GJ & Albrecht ED 1990 Regulation of primate fetal adrenal cortex. *Endocrine Reviews* **11** 151–176.
- Phillips ID, Ross JT, Owens JA, Young IR & McMillen IR 1996 The peptide ACTH(1–39), adrenal growth and steroidogenesis in the sheep fetus after disconnection of the hypothalamus and pituitary. *Journal of Physiology* **491** 871–879.
- Rainey WE, Naville D, Cline N & Mason JI 1991 Prostaglandin E₂ is a positive regulator of adrenocorticotropin receptors, 3 β -hydroxysteroid dehydrogenase, and 17 α -hydroxylase expression in bovine adrenocortical cells. *Endocrinology* **129** 1333–1339.
- Reinisch JM, Simon NG, Karow WG & Gandman R 1978 Prenatal exposure to prednisone in humans and animals retards intrauterine growth. *Science* **202** 436–438.
- Riley SC, Boshier DP, Luu-Thé V, Labrie F & Challis JRG 1992 Immunohistochemical localisation of 3 β -hydroxysteroid dehydrogenase/ Δ ⁵- Δ ⁴ isomerase, tyrosine hydroxylase and phenylethanolamine N-methyl transferase in the adrenal glands of sheep fetuses throughout gestation and in neonates. *Journal of Reproduction and Fertility* **96** 127–134.

- Rurak DW 1978 Plasma vasopressin levels during hypoxaemia and the cardiovascular effects of exogenous vasopressin in foetal and adult sheep. *Journal of Physiology* **277** 341–357.
- Sambrook J, Fritsch F & Maniatis T 1989 *Molecular Cloning*, edn 2. Cold Spring Harbor, New York: Laboratory Press.
- Seron-Ferre M, Lawrence C, Siiteri PK & Jaffe RB 1978 Steroid production by definitive and fetal zones of the human fetal adrenal gland. *Journal of Clinical Endocrinology and Metabolism* **47** 603–609.
- Simpson ER & Waterman MR 1988 Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. *Annual Review of Physiology* **50** 427–440.
- Stark RI, Wardlaw SL, Daniel SS, Hussain MK, Sanocka UM, James LS & van de Wiele RL 1982 Vasopressin secretion induced by hypoxia in sheep: developmental changes and the relationship to β -endorphin release. *American Journal of Obstetrics and Gynecology* **143** 204–215.
- Tangalakis K, Coghlan JP, Connell J, Crawford R, Darling P, Hammond VE, Haralambidis J, Penschow J & Wintour EM 1989 Tissue distribution and levels of gene expression of three steroid hydroxylases in ovine adrenal glands. *Acta Endocrinologica* **120** 225–232.
- Tangalakis K, Coghlan JP, Crawford R, Hammond VE & Wintour EM 1990 Steroid hydroxylase gene expression in the ovine adrenal gland following ACTH infusion. *Acta Endocrinologica* **123** 371–377.
- Tangalakis K, Roberts FE & Wintour EM 1992 The time-course of ACTH stimulation of cortisol synthesis by the immature ovine foetal adrenal gland. *Journal of Steroid Biochemistry and Molecular Biology* **42** 527–532.
- Tangalakis K, Crawford R, McFarlane AC & Wintour WM 1994 Regulation of steroid hydroxylase gene expression in the ovine fetal adrenal at 0.4 gestation. *Molecular and Cellular Endocrinology* **103** 21–27.
- Thorburn GD & Rice GE 1990 Placental PGE₂ and the initiation of parturition in the sheep. In *Eicosanoids in Reproduction*, pp 73–86. Ed. MD Mitchell. Boca Raton, FL: CRC Press.
- Wintour EM, Brown EH, Denton DA, Hardy KJ, McDougall JG, Oddie CJ & Whipp GT 1975 The ontogeny and regulation of corticosteroid secretion by the ovine fetal adrenal. *Acta Endocrinologica* **79** 301–316.

Received 18 March 1998

Accepted 30 June 1998