The effect of prenatal betamethasone administration on postnatal ovine hypothalamic–pituitary–adrenal function

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Abstract

Prenatal exposure to glucocorticoids is associated with alterations in fetal growth and endocrine function. However, few studies have examined the effects of clinically relevant doses of glucocorticoids on postnatal hypothalamic–pituitary–adrenal (HPA) function. To determine the effects of maternal or fetal betamethasone administration (0.5 mg/kg maternal or estimated fetal weight) on postnatal HPA function at 6 months and 1 year postnatal age, pregnant ewes were randomized into the following treatment groups: no treatment ($n=6$); maternal saline ($n=6$); single maternal betamethasone (M1) ($n=6$); repeated maternal betamethasone (M4) ($n=6$); fetal saline ($n=5$); single fetal betamethasone ($n=6$) and repeated fetal betamethasone (F4) ($n=6$). Single injections were given at 104 days of gestation and repeated injections at 104, 111, 118 and 125 days. Lambs were born spontaneously and the ACTH and cortisol responses to i.v. corticotropin-releasing hormone (CRH) (0.5 µg/kg) plus arginine vasopressin (AVP) (0.1 µg/kg) were measured at 6 months and 1 year postnatally. At 6 months postnatal age, neither maternal nor fetal prenatal betamethasone administration altered significantly the ACTH and cortisol responses to CRH+AVP. However, in animals at 1 year postnatal age,

a previous single injection of betamethasone to the mother (M1) resulted in significantly elevated basal and stimulated cortisol levels ($P<0.05$), without significant change in the ACTH response. In contrast, betamethasone administration to the fetus resulted in significantly attenuated ACTH responses to CRH+AVP at 1 year compared with control animals ($P<0.05$), but these were not associated with any significant changes in basal or stimulated cortisol levels. All control animals exhibited a significant increase in peak ACTH responses to CRH+AVP between 6 months and 1 year postnatal age ($P<0.05$). After prenatal betamethasone (F4, M4) the difference in peak ACTH response between animals at 6 months and 1 year postnatal age was abolished. We conclude that in sheep between 6 months and 1 year postnatal age, HPA function undergoes developmental changes that are influenced by prenatal glucocorticoid exposure. Furthermore, the effects of glucocorticoid on postnatal HPA responses may vary according to the time in gestation that the steroid was administered, and whether it was given directly into the fetal or maternal compartment.

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Introduction

The fetus may be exposed to different adverse circumstances during intrauterine development that lead to an increase in concentrations of circulating glucocorticoids. Prenatal stress, resulting in an elevation of maternal corticosteroid levels, has been associated with alterations in postnatal endocrine function of the infant rat and human (Takahashi & Kalin 1991, Weinstock *et al.* 1992, Copper *et al.* 1996). Overexposure to glucocorticoid or stress during fetal life may influence the programming of

different organ systems and increase the predisposition to cardiovascular and metabolic disease in later life (Langley-Evans *et al.* 1996, Seckl 1997, Challis *et al.* 1999). The enzyme 11β-hydroxysteroid dehydrogenase (11βHSD) type 2 is expressed in the human placenta and has been suggested to influence the trans-placental transfer of maternal cortisol to the fetus, by metabolism to inactive cortisone (Stewart *et al.* 1994, Burton & Waddell 1999). However, alterations in the activity or the expression of this enzyme may expose the fetus to increased levels of maternal glucocorticoid that could potentially program

fetal responses leading to cardiovascular and/or metabolic disease in later life (Edwards *et al.* 1993, Seckl 1997).

Synthetic glucocorticoids, such as betamethasone, are poor substrates for the placental 11 β HSD enzyme (Siebe *et al.* 1993), and pass relatively freely across the placenta into the fetal circulation. As a result, synthetic glucocorticoids are used to enhance fetal lung maturation in women at risk of preterm delivery (Liggins & Howie 1972, Lanteri *et al.* 1994, Ballard & Ballard 1995). However, relatively little is known about the longer-term effects of glucocorticoid administration on developing fetal endocrine axes and on postnatal endocrine function. In the rat, synthetic glucocorticoids given late in gestation resulted in significant elevations in basal corticosterone levels with associated hypertension in adult offspring (Levitt *et al.* 1996). Prenatal stress, which presumably elevates maternal and fetal endogenous corticosterone concentrations, also led to altered hypothalamic–pituitary–adrenal (HPA) and metabolic responses in postnatal life (Vallée *et al.* 1996). Low-dose dexamethasone infusion to the ovine fetus prevented hypoxia-induced increases in fetal plasma adrenocorticotropin (ACTH) and cortisol levels, but increased the plasma glycemic response (Fletcher *et al.* 2000). In addition, maternal betamethasone administration at mid-gestation resulted in elevated insulin responses to a glucose load in lambs at 6 months and 1 year after birth (Moss *et al.* 2000, 2001, Sloboda *et al.* 2000b). These observations suggest that fetal and maternal glucocorticoid exposure may have sustained effects on developing endocrine axes that lead to altered responsiveness after birth. The outcomes of administering betamethasone into the maternal or fetal compartment may be different. Maternal betamethasone administration to sheep in late pregnancy resulted in a dose-dependent decrease in lamb birth weights, whereas fetal betamethasone administration, in amounts that produced higher circulating betamethasone concentrations in the fetus, did not alter birth weights (Jobe *et al.* 1996, 1998). Hence there is an obvious need to examine the differential effects of prenatal maternal or fetal glucocorticoid administration on postnatal endocrine function.

Current clinical practice utilizes single or repeated administration of glucocorticoid to promote pulmonary maturity if preterm birth has not occurred, but there is little information on the long-term sequelae of these different treatment regimens. French *et al.* (1999) have shown recently in the human that reduced birth weight and neonatal head circumference are linked to an increased number of maternal antenatal corticosteroid doses. In sheep, maternal betamethasone administration given in amounts that mimic clinical practice, significantly altered fetal basal plasma ACTH, cortisol and cortisol-binding capacity (Sloboda *et al.* 2000a), but it is not known whether these effects persist into postnatal life. Synthetic glucocorticoid administration also provides, in part, a paradigm for reproducing some of the consequences of

stress-induced elevations in maternal and/or fetal cortisol. Given the effects of glucocorticoid in reducing fetal weight (Reinisch *et al.* 1978, Johnson *et al.* 1981, Jobe *et al.* 1998) and the relationship between reduced birth weight with the metabolic syndrome X, postnatal stress responses, and cardiovascular disease in later life (Barker 1998, Phillips *et al.* 1998), we hypothesized that exogenous glucocorticoid administration during pregnancy would alter postnatal HPA responsiveness. Furthermore, we reasoned that there would be different effects of maternal vs fetal administration on the HPA response. The fetal sheep HPA axis undergoes progressive maturation over the last one-third of pregnancy (Challis & Brooks 1989) and we hypothesized that glucocorticoid administration at different times during this development phase would produce different outcomes. To begin to address these issues we have treated maternal and fetal sheep with the synthetic glucocorticoid, betamethasone, as a single injection or with repeated administration at 1 week intervals (to mimic current obstetric practice) during late pregnancy. We allowed the lambs to deliver and have measured the responsiveness of the HPA axis, as reflected in plasma ACTH and cortisol concentrations, to a challenge with corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) in animals at 6 months and 1 year postnatal age.

Materials and Methods

All experimental procedures were approved by the Animal Experimentation Ethics Committee of the University of Western Australia and at the University of Toronto according to guidelines of the Canadian Council of Animal Care.

Experimental procedures

All animal experimentation was performed in Western Australia using a protocol that has been described in detail previously (Jobe *et al.* 1998). Pregnant ewes bearing singleton fetuses were allocated randomly to receive either no treatment (NT) or maternal or fetal injections of saline and/or betamethasone (Table 1). All treated animals (but not NT animals) were injected i.m. with 150 mg medroxyprogesterone acetate (Depo Provera; Upjohn, Rydalmere, NSW, Australia) at ~100 days of gestation to reduce pregnancy losses due to glucocorticoid treatment (Nathanielsz *et al.* 1988). Saline-treated animals were injected with normal saline at 104, 111, 118 and 125 days of gestation (maternal saline (MS), or fetal saline (FS)); animals treated with a single dose of corticosteroid were injected with betamethasone at 104 days of gestation and saline at 111, 118 and 125 days of gestation (maternal (M1) or fetal (F1)); animals treated with repeated doses of corticosteroid were injected with betamethasone at 104,

Table 1 Prenatal treatment protocol using maternal or fetal glucocorticoid

	No treatment control (M n=3; F n=3)	Maternal i.m. injections			Direct fetal i.m. injections		
		MS (M n=3; F n=3)	M1 (M n=3; F n=3)	M4 (M n=4; F n=2)	FS (M n=2; F n=4)	F1 (M n=3; F n=3)	F4 (M n=3; F n=3)
Days of gestation							
~ 100	—	MPA	MPA	MPA	MPA	MPA	MPA
~ 104	—	Saline	Beta	Beta	Saline	Beta	Beta
~ 111	—	Saline	Saline	Beta	Saline	Saline	Beta
~ 118	—	Saline	Saline	Beta	Saline	Saline	Beta
~ 125	—	Saline	Saline	Beta	Saline	Saline	Beta

MPA=medroxyprogesterone acetate, 150 mg; Beta=betamethasone (0.5 mg/kg ewe body weight or estimated fetal body weight); M=male; F=female.

111, 118 and 125 days of gestation (maternal (M4) or fetal (F4)). Maternal betamethasone (Celestone Chronodose; Schering Plough, Baulkham Hills, NSW, Australia) injections were given i.m. in a dose of 0.5 mg/kg body weight; saline injections were of a comparable volume (5–6 ml). Fetal injections were given using an established technique (Newnham *et al.* 1999). Briefly the ewe was held in a supine position, 70% ethanol was applied to the ewe's abdomen as a coupling medium and the fetus was imaged using a 3.5 MHz sector transducer (Echo Camera SSD-500; Aloka, Tokyo, Japan). Betadine solution (Faulding, Welshpool, WA, Australia) was applied to the injection site and a 21-gauge 9 cm spinal needle (Terumo, Nedlands, WA, Australia) was introduced through the maternal abdomen into the muscle of the shoulder or rump of the fetus. Betamethasone (0.5 mg/kg estimated fetal body weight; 1.4 kg at 104 days, 1.9 kg at 111 days, 2.2 kg at 118 days, 2.5 kg at 125 days) or an equal volume of saline (1 ml) was injected by an assistant; the needle tip was imaged throughout the entire procedure. Previous studies have established that the peak fetal betamethasone concentrations, and area under the clearance curve (AUC) were approximately 3-fold higher after fetal injection than after maternal treatment with 0.5 mg/kg betamethasone (Berry *et al.* 1997).

Ewes were permitted to deliver their lambs spontaneously in a field environment and were not disturbed until the time of experimentation. Lambs were raised by their mothers and were observed closely several times each day for the first few postnatal weeks. Two of the M4 lambs were raised exclusively by hand or supplemented with powdered milk (Divetalact; International Animal Health Products, Huntingwood, NSW, Australia) when it became apparent that lactation was affected in some ewes from this group. At approximately 2 months of age, the lambs were immunized, their tails cropped and males were castrated. Weaning occurred at 3 months of age. Prior to experimentation at 1 year postnatal age, estrous cycles of all female animals were synchronized with intravaginal

progesterone sponges (30 mg flugestone acetate, Chrono-Gest30; Intervet Pty Ltd, Castle Hill, NSW, Australia). Six lambs from each of the seven groups were chosen at random as subjects for CRH+AVP challenge tests at 6 months and 1 year postnatal age. At 1 year, supplementation of numbers in the MS, FS, F1 and NT groups (maximum of two animals) was required due to loss such as predation that was unrelated to experimentation. These additional lambs were obtained from identically treated ewes from the original larger flock of animals (Table 1).

CRH+AVP challenges at 6 months and 1 year postnatal age

At both 6 months and 1 year postnatal age, lambs underwent aseptic surgery to implant femoral arterial and venous catheters (halothane anesthesia, 1–2% in O₂, following induction with ketamine/xylazine) and were allowed at least 3 days to recover before performing CRH+AVP challenges. Catheters were removed after the completion of experiments. Food was withdrawn 12 h before challenges, but the animals were allowed free access to water. Basal arterial blood samples (5 ml) were drawn at 30 and 15 min and immediately before the administration of an i.v. bolus of 0.5 µg ovine CRH+0.1 µg AVP (Bachem, Torrance, CA, USA) per kg lamb weight followed by a 10 ml saline flush. Arterial samples (5 ml) were collected at 5, 10, 20, 30, 60, 90, 120, 180 and 240 min after the CRH+AVP administration, centrifuged for 10 min at 4 °C and the plasma was collected and stored at –80 °C. All challenges were administered between 0800 and 0900 h in order to minimize the impact of circadian variability on measurements of plasma ACTH and cortisol.

Measurement of plasma ACTH and cortisol

Plasma immunoreactive ACTH concentrations were measured using a commercial RIA kit (Incstar, Stillwater, MN, USA) previously validated for use in the fetal sheep

Table 2 Basal ACTH (pg/ml) and cortisol (ng/ml) levels (means $\pm$ S.E.M.) at 6 months and 1 year postnatal age following prenatal maternal and fetal betamethasone administration

Treatment groups	Six months		One year	
	ACTH	Cortisol	ACTH	Cortisol
NT	18.0 $\pm$ 5.2	5.8 $\pm$ 1.3	27.9 $\pm$ 1.8	4.3 $\pm$ 0.9
MS	16.4 $\pm$ 3.8	3.9 $\pm$ 0.7	32.1 $\pm$ 2.7	5.3 $\pm$ 0.9
M1	8.2 $\pm$ 3.5	3.7 $\pm$ 0.7	25.9 $\pm$ 2.7	8.5 $\pm$ 0.9*
M4	17.0 $\pm$ 3.5	5.9 $\pm$ 0.6	32.1 $\pm$ 3.0	3.6 $\pm$ 0.9
FS	32.0 $\pm$ 10.7	4.4 $\pm$ 0.5	40.4 $\pm$ 5.4	6.3 $\pm$ 1.1
F1	22.4 $\pm$ 9.5	6.2 $\pm$ 1.3	26.4 $\pm$ 5.4	5.3 $\pm$ 1.1
F4	14.1 $\pm$ 9.5	5.6 $\pm$ 1.2	27.8 $\pm$ 7.0	5.5 $\pm$ 1.4

* $P<0.05$ M1 versus NT, MS and M4. Lack of difference ($P>0.05$) between NT controls, and MS or FS treatment control animals.

(Norman *et al.* 1985). The intra-assay coefficient of variation was 4.5%, and the inter-assay 4%. The mean assay sensitivity was 15 pg/ml. The ACTH antibody cross reacted $<0.01\%$ with α - and β -melanocyte-stimulating hormone, β -endorphin and β -lipotrophin. Plasma cortisol concentrations were quantified by RIA after extraction with diethyl ether. The antibody characteristics have been described previously (Jeffray *et al.* 1995). The intra-assay coefficient of variation was 6%, the inter-assay 15%.

Statistical analysis

Results are expressed as means $\pm$ S.E.M. A comparison of group means was made using ANOVA (SAS, Cary, NC, USA) and S-PLUS (Mathsoft, Seattle, WA, USA). In all cases, basal values represent the mean value of the three samples drawn prior to the administration of the CRH+AVP injection (time 0). The experimental design did not allow us to examine possible sex-specific effects on outcome variables. The numbers of male and female offspring were distributed between different treatment groups (Table 1) such that there was no bias to either sex within any group. Assessment of responses in individual animals indicated overlap between data points for male and female fetuses (data not shown) within treatments, and results are presented as mean values for each group, as a whole.

In order to assess the overall effect of prenatal betamethasone on the response patterns to the challenge, we calculated the AUCs for each group between 5 and 240 min after CRH+AVP. This calculation permitted us to resolve occasional missing values where sample collection may have been impaired. To understand the effects of prenatal betamethasone on both postnatal ACTH and cortisol responses to the challenge and alterations due to age, we compared the observed peak ACTH and cortisol values at 6 months postnatal age with values at 1 year postnatal age, within each treatment group, using

Student's *t*-test (Sigma Stat, Chicago, IL, USA). We also evaluated whether age-related changes in ACTH and cortisol peak responses were different following betamethasone administration, by taking the observations from each animal and analyzing differences using a one-way ANOVA (Sigma Stat). In all cases the level of statistical significance was taken to be $P<0.05$.

Results

Effects of prenatal maternal or fetal betamethasone administration on responses at 6 months postnatal age

At 6 months postnatal age, basal plasma ACTH and cortisol concentrations were similar in all groups following prenatal maternal betamethasone and were similar between NT, FS and MS groups (Table 2). Animals responded to the CRH+AVP challenge with peak ACTH levels (150–250 pg/ml) at approximately 5 min but there was no effect of treatment on peak ACTH values or on the AUC. Peak cortisol values (~ 30 –40 ng/ml) were achieved at ~ 20 min after injection but there were no significant effects of treatment on cortisol responses (data not shown).

Basal ACTH and cortisol concentrations were not different in the F1 and F4 groups from FS controls (Table 2) and there was no effect of prenatal fetal betamethasone treatment on peak or AUC values for ACTH or cortisol after fetal betamethasone treatment (data not shown).

Effect of prenatal maternal or fetal betamethasone administration on responses at 1 year postnatal age

At 1 year postnatal age, basal plasma ACTH concentrations were similar between control groups and amongst all groups following maternal or fetal betamethasone

Figure 1 ACTH (upper panel) and cortisol (lower panel) response patterns to CRH+AVP at 1 year postnatal age following prenatal administration of maternal betamethasone. ○ NT; ▽ MS; ■ M1; ◆ M4. The inset histograms show AUCs for the groups in the same order. Means $\pm$ S.E.M. * $P < 0.05$.

treatment (Table 2). Basal plasma cortisol, however, was significantly higher in the M1 group compared with MS and M4 groups of animals (Table 2; $P < 0.05$).

All animals at 1 year postnatal age from maternal betamethasone treatments responded to the CRH+AVP i.v. bolus with peak ACTH levels at approximately 5 min and peak cortisol at approximately 20 min after administration (Figs 1 and 2). The ACTH response patterns and the AUC for ACTH were not significantly affected by prenatal maternal betamethasone administration (Fig. 1).

However, the peak and AUC for the cortisol values in the M1 group were significantly greater than in the MS and M4 groups of animals (Fig. 1; $P < 0.05$).

All animals treated with fetal betamethasone *in utero* responded to the CRH+AVP challenge with peak ACTH values at 5–10 min and peak cortisol at 20–40 min. The ACTH response in F1 and F4 animals was consistently lower than that in the FS group and the AUCs for ACTH for the F1 and F4 groups were significantly lower than that of the FS group (Fig. 2; $P < 0.05$). Plasma cortisol

Figure 2 ACTH (upper panel) and cortisol (lower panel) response patterns to CRH+AVP at 1 year postnatal age following prenatal administration of fetal betamethasone. ○ NT; ▼ FS; ■ F1; ◆ F4. The inset histograms show AUCs for the groups in the same order. Means $\pm$ S.E.M. * $P < 0.05$.

concentrations at 20 min were significantly higher in the F1 group ($P < 0.05$) but there were no significant differences in the overall AUC for cortisol between groups (Fig. 2).

Comparison of the ACTH and cortisol responses between 6 months and 1 year postnatal age

Peak ACTH and cortisol values in response to CRH+AVP at 6 months and 1 year postnatal age are

illustrated in Figs 3 and 4. NT, MS and FS animals exhibited significantly higher peak ACTH values at 1 year postnatal age compared with 6 months postnatal age (Figs 3 and 4; $P < 0.05$). In contrast, peak cortisol values in untreated (control) animals between 6 months and 1 year postnatal age were unchanged, with the exception of FS animals (Figs 3 and 4). The ACTH and cortisol AUCs following CRH+AVP at 6 months and 1 year postnatal age were consistent with the measurements of peak hormone concentrations in that ACTH values were higher

Figure 3 Peak values for ACTH (upper panel) at 6 months (●) and 1 year (○) postnatal age and for cortisol (lower panel) at 6 months (▼) and 1 year (▽) postnatal age following prenatal maternal betamethasone administration. Means $\pm$ S.E.M. * $P < 0.05$, 6 months vs 1 year; other values not statistically different ($P > 0.05$) from each other.

at 1 year postnatal age but were not associated with any change in cortisol AUC (Figs 5 and 6).

The differences between peak ACTH values and ACTH AUC at 6 months and 1 year were somewhat attenuated after M4 but not M1 betamethasone treatment (Figs 3 and 5), but were completely abolished in both F1 and F4 betamethasone groups of animals (Figs 4 and 6). The lack of difference in peak or AUC cortisol seen generally between control animals at 6 months or 1 year postnatal age was unaffected by maternal betamethasone, although in M1 group animals both peak and cortisol AUC values approached significance. Similarly, there were no significant differences in peak or cortisol AUC values between 6 months and 1 year in the F4 animals but 1 year values were significantly higher than those at 6 months in the F1 group of animals.

Figure 4 Peak values for ACTH (upper panel) at 6 months (●) and 1 year (○) postnatal age and for cortisol (lower panel) at 6 months (▼) and 1 year (▽) postnatal age following prenatal fetal betamethasone administration. Means $\pm$ S.E.M. * $P < 0.05$, 6 months vs 1 year.

Discussion

We have shown that betamethasone administered to sheep during the last two-thirds of pregnancy altered HPA responsiveness in the offspring at up to 1 year postnatal age. The outcomes varied according to the time in gestation when the synthetic glucocorticoid was given, and whether it was administered to mother or fetus. Furthermore, we found that the responsiveness of the HPA axis was altered between 6 months and 1 year postnatal age, and these age-related changes were influenced by prenatal betamethasone administration.

We used bolus CRH and AVP administration to assess changes in plasma ACTH and cortisol as indices of pituitary and adrenal responsiveness following prenatal treatment with glucocorticoids. This method of assessment

Figure 5 AUCs for ACTH (upper panel) and cortisol (lower panel) at 6 months (filled bars) and 1 year (open bars) postnatal age following maternal betamethasone administration. Means $\pm$ S.E.M. * $P < 0.05$, 6 months vs 1 year.

has been utilized previously in fetal (Norman & Challis 1987, Hawkins *et al.* 1999) and adult (Brooks & Challis 1989) sheep. We did not measure changes in metabolic clearance rate of ACTH or cortisol in the lambs at the two postnatal ages, but are not aware of measurements showing changes in clearance that would alter interpretation of our assessment of plasma hormone concentrations.

We were aware that postnatal HPA responses in male and female offspring could be different, but future studies with larger numbers of animals would be required to assess this. Lingas *et al.* (1999) have shown sex-dependent differences in cortisol responses to an ACTH challenge in postnatal guinea pigs subjected to undernutrition during pregnancy. We ensured that there were male and female fetuses in each treatment group to avoid bias in the results based on sex, and found that within each group there was overlap between data points regardless of sex. Hence, while we cannot exclude an influence of sex on HPA responsiveness in postnatal sheep up to 1 year postnatal age, the differences in responses that we report are evident, and presumably greater than any proposed gender influence.

Figure 6 AUCs for ACTH (upper panel) and cortisol (lower panel) at 6 months (filled bars) and 1 year (open bars) postnatal age following fetal betamethasone administration. Means $\pm$ S.E.M. * $P < 0.05$, 6 months vs 1 year.

In the current studies, control animals treated with maternal or fetal saline, as well as the treatment animals, received progesterone during pregnancy. The NT animals did not receive progesterone. The observation that there were no significant differences between saline and NT controls allows one to conclude that progesterone treatment *per se* during pregnancy did not influence the subsequent HPA responses after birth. Postnatal females were synchronized at 1 year postnatal age to eliminate influences of stages of the estrous cycle, and males were castrated at 2 months of age to eliminate influences of postnatal testosterone. Thus, the consistency of responses at 1 year postnatal age demonstrates a remarkable robustness to the influence of glucocorticoid exposure during pregnancy. These effects, for example on the cortisol response in M1 animals at 1 year are clearly quite discrete. It is very likely that exposure to betamethasone on day 104 reflects a particular window in development that hypothetically could be augmented, unaltered or reversed by later glucocorticoid administration. We do know that this is a dynamic period in maturation of the fetal HPA axis (Challis & Brooks 1989). Our results suggest that a specific

influence of glucocorticoid given on day 104 may be attenuated by further glucocorticoid given at 1 week intervals to up to 3 weeks later in pregnancy.

The present observations in animals at 1 year postnatal age are consistent with previous studies that have reported elevated basal corticosterone levels in rats treated prenatally with maternal dexamethasone late in gestation (Levitt *et al.* 1996). Uno *et al.* (1994) have shown that prenatal treatment with maternal dexamethasone resulted in elevated basal and post-stress cortisol levels in juvenile monkeys. These observations were associated with a reduction in fetal hippocampal pyramidal cells (Uno *et al.* 1990, 1994). Many studies have reported significant alterations in glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA levels in the HPA axis following prenatal glucocorticoid exposure (Uno *et al.* 1990, Levitt *et al.* 1996), thus linking glucocorticoid exposure with alterations in negative feedback at the level of the hippocampus. In our study, a single dose of prenatal maternal betamethasone (M1) resulted in significant elevations in basal and stimulated cortisol concentrations at 1 year postnatal age, whereas multiple doses (M4) resulted in a modest attenuation in stimulated cortisol levels. It has been reported previously that administration of as little as one dose of 0.5 mg/kg dexamethasone to the pregnant rhesus monkey significantly depleted fetal hippocampal neurons (Uno *et al.* 1990). It is possible that one dose of prenatal betamethasone given at a critical time point in hippocampal or hypothalamic development could alter the GR pattern of expression and alter the set point of HPA negative feedback function. This effect is altered in the M4 sheep. Previously we found that three injections of maternal betamethasone significantly increased fetal pituitary but not hypothalamic GR mRNA levels (Sloboda *et al.* 2000a), consistent with altered negative feedback function at least at the level of the pituitary. It is not known whether pituitary, hypothalamic or hippocampal GR or MR is altered in the current series of animals. Previous studies have linked increases in fasting cortisol levels in 64-year-old men to a reduction in birth weight, high blood pressure, glucose intolerance, elevated plasma triglycerides and insulin resistance (Phillips *et al.* 1998). These observations further support the hypothesis that the HPA axis is vulnerable to *in utero* 'reprogramming' and that glucocorticoids play a key role in these intrauterine events.

It appears likely that prenatal betamethasone administration results in alterations in adrenal responsiveness irrespective of changes in circulating ACTH. The ovine fetal adrenal undergoes a growth period at 40–90 days of gestation and is capable of secreting cortisol at this time (Boshier & Holloway 1989, Wintour *et al.* 1995). This is followed by a period of relative quiescence (90–120 days of gestation) characterized by a decrease in responsiveness until 120 days gestational age, when adrenal sensitivity increases to a maximum at term (Rose *et al.* 1982, Boshier

& Holloway 1989, Wintour *et al.* 1995). This increase in responsiveness has been associated with an increase in ACTH receptor mRNA levels (Fraser *et al.* 2001). It is possible that an early single exposure to prenatal betamethasone at 104 days of gestation increased ACTH receptors and/or steroidogenic enzymes during this period of relative quiescence in a way that permanently increased the set point of adrenal responsiveness. It has been reported previously that cortisol regulates ACTH activation of fetal adrenal function late in gestation (Challis *et al.* 1985). In addition, dexamethasone treatment of ovine adrenocortical cells increased mRNA levels of all three ACTH receptor transcripts in a dose-dependent manner (Picard-Hagen *et al.* 1997). Multiple maternal betamethasone injections (M4), on the other hand, could have decreased ACTH receptors in the adrenal, consistent with a decrease in adrenal responsiveness. Previous studies have shown that fetal sheep exposed to modest undernutrition early in gestation exhibited a reduction in both pituitary and adrenal responsiveness to i.v. CRH+AVP and to hypoxia late in gestation (Hawkins *et al.* 1998, 1999).

This study is the first to show that fetal betamethasone administration causes alterations in postnatal ovine HPA function. Prenatal fetal betamethasone treatment suppressed pituitary responsiveness at 1 year postnatal age compared with controls. This effect could be exerted at the levels of the hypothalamus or pituitary; for example, an increase in GR in pituitary corticotropes should increase negative feedback and reduce the secretion of ACTH (Matthews & Challis 1997, Sloboda *et al.* 2000a). The fact that animals treated with fetal betamethasone (F1 and F4) responded with values of cortisol similar to controls, in the presence of reduced levels of ACTH, suggests also an increase in adrenal responsiveness. Previous reports have shown that rats exposed prenatally to a low-protein diet had blunted diurnal variation in plasma ACTH levels without any alterations in corticosterone levels. It was suggested that prenatal undernutrition results in a hyper-responsiveness to ACTH in the adrenal of the offspring (Langley-Evans *et al.* 1996). Although the mechanisms are not understood it is apparent that prenatal fetal and prenatal maternal betamethasone administration may work through different pathways.

We have shown that the stimulated HPA axis function in the sheep undergoes a developmental maturation between the postnatal ages of 6 months and 1 year. NT, MS and FS animals exhibited a similar significant increase in peak ACTH responses to CRH+AVP from 6 months to 1 year postnatal age. This observation suggests an increase in pituitary responsiveness with age. Prenatal betamethasone abolished this maturational increase in peak ACTH, an effect that was most apparent in animals that received fetal betamethasone administration. This observation is consistent with alterations in anterior pituitary responsiveness. In contrast to the peak ACTH responses, NT, FS and MS animals did not exhibit any substantial

changes in postnatal peak cortisol values from 6 months to 1 year. It is difficult to discern from these data whether pituitary responsiveness has increased due to a reduction in negative feedback or as a result of increased drive from the hypothalamus. It is clear, however, that although multiple doses of prenatal betamethasone have no effect, one single dose of betamethasone administered to either the mother or the fetus significantly elevated the 1 year peak cortisol levels compared with those at 6 months. Mechanisms by which a single dose of prenatal betamethasone would alter the developmental maturation of the sheep HPA axis are unknown, although it is possible that alterations in adrenal structure and function (as described earlier) could play a role. Further studies are required to understand whether these alterations are at the level of the hippocampus, hypothalamus, pituitary and/or the adrenal, with subsequent alterations in HPA function and negative feedback.

The differences between the effects of fetal and maternal glucocorticoid administration warrant further investigation. Our group has shown previously that maternal betamethasone administration resulted in significant reductions in fetal weight whereas fetal glucocorticoid administration did not (Newnham *et al.* 1999, Moss *et al.* 2000). These observations raise the possibility of an effect of maternal glucocorticoids on placental function, which in turn contributes to influences on growth, and potentially to HPA development.

In conclusion, we have found that prenatal betamethasone administration results in postnatal alterations in ovine HPA axis function that reflect exposure at discrete times in gestation and persist into adulthood. We speculate that these alterations in postnatal HPA axis function may contribute to the mechanisms by which adult health and diseases such as hypertension and diabetes are programmed *in utero*. In light of the number of studies linking alterations in the intrauterine environment with an increased risk of adult-onset disease, further studies seem warranted to understand the mechanisms linking early fetal events to postnatal health.

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