No evidence for HPA reset in adult sheep with high blood pressure due to short prenatal exposure to dexamethasone

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No evidence for HPA reset in adult sheep with high blood pressure due to short prenatal exposure to dexamethasone. Am J Physiol Regulatory Integrative Comp Physiol 282: R343–R350, 2002; 10.1152/ajpregu.00222.2001. — Exposure of pregnant ewes to dexamethasone, for only 2 days (term ~150 days) at 27 days of gestation (group D), results in adult offspring with high blood pressure. In this study, hemorrhage stress has been used to see whether in these animals the responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis is altered. In addition, we studied mineralocorticoid (MR) and glucocorticoid (GR) receptor gene expression in the hippocampus and GR gene expression in the hypothalamus using real-time PCR. Calculated areas under the adrenocorticotropin, arginine vasopressin, and cortisol plasma concentration curves in response to hemorrhage were similar between the control and group D. In addition, there was no significant difference in the expression of MR and GR in the hippocampus or GR in the hypothalamus between the control and group D. Taken together, it is unlikely that reset in the HPA axis plays a major role in this particular model of “programmed” hypertension.

There is solid epidemiologic evidence to suggest that small size at birth (for gestational age) is associated with an increased incidence of adult-onset diseases or dysfunction, including syndrome X (hypertension, non-insulin-dependent diabetes mellitus, and hyperlipidemia) (2, 16, 19). It is thought that an adverse intrauterine environment during a critical stage of development permanently alters or “programs” the development of fetal tissues, which enables the fetus to survive, but with adverse consequences in postnatal life.

Low birth weight adults showed greater rates of urinary glucocorticoid (GR) excretion (6), elevated basal plasma cortisol (F) concentrations (28), and greater adrenocortical responsiveness to ACTH (20, 30). These studies proposed that the link between the size at birth and altered hypothalamic-pituitary-adrenal (HPA) axis function in later life might be a mechanism via which programming of adult cardiovascular and metabolic diseases occurs.

Animal studies to test the “programming” hypothesis have imposed perturbations such as moderate-to-severe maternal undernutrition, restriction in specific dietary components (iron, protein), or restricting normal placental growth either throughout pregnancy or during parts of gestation and have confirmed that restriction of fetal growth leads to elevated blood pressure in progeny of rats (18, 41) and sheep (7, 12, 15). Undernutrition during early gestation in sheep (15% reduction in maternal food intake) led to a reduced pituitary and adrenal responsiveness in late gestation (14) but switched to enhanced responsiveness postnatally (15). In these animals, exaggerated responsiveness of the HPA axis was associated with higher blood pressure (15).

Other models to study the “programming” hypothesis used prenatal GR exposure, either by elevating endogenous levels of GRs by using carbenoxolone (11β-hydroxysteroid dehydrogenase inhibitor, which blocks placental inactivation of endogenous GRs) (22) or synthetic GR dexamethasone, which is poorly metabolized by 11β-hydroxysteroid dehydrogenase (3). Both of these treatments, administered throughout rat pregnancy, reduced birth weight and resulted in hypertension in adult progeny. Adult rats exposed to carbenoxolone throughout gestation had increased basal corticosterone levels, increased corticotropin-releasing hormone levels, and reduced GR mRNA in the hypothalamic paraventricular nucleus (39). Dexamethasone administration during only the last week of pregnancy led to elevated plasma corticosterone levels as well as decreasing significantly both the mineralocorticoid (MR) and GR mRNA in the hippocampus of 16-wk-old rats (21). In addition, brief (2 days) dexamethasone treatment in guinea pigs in the second half of gestation resulted in a higher plasma F concentration in 18-day-old males (8), suggesting programming altered the HPA axis.

We were the first to show that very brief exposure of pregnant ewes to high levels of dexamethasone, for
only 2 days of the 150-day gestation, at a mean age of 27 days of gestation results in hypertensive offspring at 3–4 mo of age (9). This hypertension amplifies with age and is associated with increased cardiac output and increased insulin sensitivity of the inhibition of lipolysis (10, 13).

To study a possible role of the HPA axis in this model of hypertension, we used hemorrhage stress, which is known to be a powerful stimulus in activating several hormonal axes, including the HPA (38, 40). In the present study, we were primarily interested to see whether in animals known to be hypertensive as adults after brief prenatal exposure to dexamethasone high blood pressure is associated with an altered HPA axis. To test that hypothesis, we studied 1) basal and hemorrhage-stimulated plasma concentrations of ACTH, F, arginine vasopressin (AVP), and plasma renin concentration (PRC); and 2) gene expression of MR and GR receptors in the hippocampus and GR receptor in the hypothalamus. In addition, plasma and blood volumes were measured for more accurate estimate of a total blood loss after the hemorrhage protocol.

METHODS

Animals. All procedures were approved by the Howard Florey Institute Animal Ethics Committee. In this study, we used the same cohort of animals reported to become hypertensive after prenatal treatment with dexamethasone early in gestation (9). Briefly, pregnant ewes were treated with dexamethasone (Decadron; Merck, Sharp and Dohme, New South Wales, Australia) given intravenously (0.28 mg·kg⁻¹·days⁻¹) for 48 h between 26 and 28 days of gestation (median being 27 days of gestation) (group D). Ewes (n = 8) in group D gave birth to six female and three male lambs, including one set of twins. Only female lambs were included in further studies, for practical purposes, as there were fewer male offspring. We also had seven female (1 set of twins) lambs that were exposed to minimum stress during development as the control group of animals. Surgery (oophorectomy and carotid artery loops) was performed on all lambs at 50 days of age. Oophorectomy was performed to eliminate potential effects of estrus cycles. Sheep are seasonal breeders, and they normally spend a prolonged period in anestrus.

Hemorrhage study. The hemorrhage protocol was performed in control (n = 6) and group D (n = 5) animals at ~5 yr of age. Animals were hemorrhaged, 1 ml·kg⁻¹·min⁻¹ for 15 min or until blood pressure dropped precipitously, via a needle inserted into one carotid artery, and blood was collected into heparinized graduated cylinders. Blood lost during hemorrhage was not returned to the animal. Blood samples (10 ml) for plasma ACTH, AVP, F, PRC, ions, osmolality, glucose, and hematocrit (Hct) were taken via the contralateral carotid artery at ~60, ~30, 0, 5, 10, 30, 40, 60, 120, and 180 min. Mean arterial pressure was measured via a Tygon cannula (1.0-mm ID, 1.5-mm OD) inserted into a carotid artery and connected to a pressure transducer (TD XIII, Cobe) and was recorded only at the beginning and at the end of hemorrhage on a Gould 3000 series chart recorder (Gould, Cleveland, OH).

Sample analysis. Plasma ACTH concentration was measured on unextracted plasma [2 pg/ml sensitivity; 11% interassay coefficient of variation (CV)] using an ACTH immunoradiometric kit (DYNO test; Henning GmbH, Berlin, Germany). Plasma AVP concentration was assayed by RIA as described (40), with minor modifications. Plasma (2 ml) was extracted using C18 column and was eluted with 90% ethanol and 4% (wt/vol) acetic acid. Samples were reconstituted in 0.5 ml of assay buffer and then 150 µl were assayed in duplicate. Sensitivity of the method was 0.6 pmol/l, and the interassay CV was 10.8%. Plasma F was measured by RIA of extracted plasma (0.2 nmol/l sensitivity; 8.7% interassay CV) (36). Measurements of PRC were determined using a modification of the antibody capture technique, by measuring the generation of ANG I (0.2 pmol·h⁻¹·ml⁻¹ sensitivity; 9% interassay variation) (9). Plasma solutes (Na⁺, K⁺, glucose, and total protein) were measured on a Synchron CX5 Clinical System (Beckman, Fullerton, CA). Osmolality was measured by freezing-point depression using an Advanced Instruments Osmometer (Advanced Instruments, Needham Heights, MA). Hematocrit was determined in duplicate.

Plasma and blood volume measurements. Plasma and blood volume measurements were performed at least 2–3 mo after the hemorrhage experiment (Hct values had returned to normal). While the animal was under a local anesthesia (lidocaine 0.5% [a subcutaneous injection of 0.5 ml of lidocaine hydrochloride 5 mg/ml; ASTRA, NSW, Australia]), a cannula (1.0-mm ID, 1.5-mm OD) was placed into a jugular vein the day before experimentation. Also, an additional cannula (1.0-mm ID, 1.5-mm OD) was placed into the carotid artery at least 2 h before the experiment commenced. On the day of the experiment, 15 ml of arterial blood were withdrawn from this cannula and discarded before 4 ml of blood sample were taken into a sterile syringe. Hematocrit was taken in triplicate, and the remaining blood was spun down and plasma was obtained. One milliliter of plasma was set aside for the analysis of background counts. ¹²⁵I ovine gamma globulin (Commonwealth Serum Laboratories, Victoria, Australia) (containing 2–2.5 × 10⁶ counts in 100 µl) was added to the remaining 1–1.5 ml of plasma. One milliliter of plasma was weighed and then infused into the jugular vein, and this was followed by the infusion of 10 ml of saline to ensure all labeled plasma had entered the circulation. At t = 10 min, 12 ml of arterial blood were removed before a 3-ml blood sample was taken. The 12 ml of blood were reinjected, followed by the injection of 3 ml of saline. This was repeated at t = 20 and 30 min. From all samples, Hct was taken in triplicate immediately after withdrawal of blood. Samples were spun, and 0.5 ml of plasma were obtained. Standards were prepared using 0.2 ml of labeled plasma diluted in 20 ml saline and were taken in triplicate. All samples and standards were weighed and transferred to counting tubes for analysis on a Gamma counter (Cobra 5010 Gamma Counter; Packard Instruments, Downers Grove, IL). Counts were corrected for background and expressed as counts per minute per milliliters. An average was obtained from the three standards, and counts from the three samples were extrapolated using linear regression to t = 0. Hct was measured to one decimal place, using a microscope, and the mean of the three values was taken.

Calculations. Plasma volume was calculated using the following formula (4):

\[ PVm = \frac{Vi \times Cs \times Vc/ Ct0, where \ PVm \ is \ plasma \ volume \ measured, \ Vi \ is \ volume \ injected, \ Cs \ is \ mean \ standard \ counts, \ Vc \ is \ volume \ of \ dilution \ of \ the \ standard, \ and \ Ct0 \ is \ extrapolated \ counts \ at \ t = 0. \]

Blood volume was then calculated using the Hct and measured plasma volume: 

\[ B Vc = PVm \times 100/1 - \ Hct(m), \ where \ B Vc \ is \ blood \ volume \ calculated \ and \ Hct(m) \ is \ Hct \ measured. \]

Gene expression study. At the conclusion of all in vivo experiments, animals (~7 yr of age) were killed with an overdose (100 mg/kg) of pentobarbital sodium (Lethabarb, Arnolds of Reading, Peakhurst, Australia) for tissue collection. The hypothalamus and hippocampus were frozen in
liquid nitrogen and stored at −80°C until extraction. The method of Chirgwin et al. (5) was used to extract total RNA from the frozen hypothalamus and hippocampus, mRNA expression for the MR and GR receptors was determined using real-time PCR method.

The RNA samples were DNase treated as described previously (26). To perform the real-time PCR assay, all samples were first reverse transcribed. For each sample, 1 μg of total RNA was reverse transcribed in a 10-μl reaction containing 1× TaqMan RT buffer, 5.5 mM MgCl₂, 500 μM each 2′-deoxynucleotide 5′-triphosphate, 2.5 μM random hexamers, 0.4 U/μl RNase inhibitor, and 1.25 U/μl Multiscribe RT (PE Applied Biosystems, Forster City, CA). Control reactions for each RNA sample containing no RT enzyme were also set up to assess any genomic DNA contamination. Reverse transcription was carried out in a GeneAmp PCR system 9600 (PE Applied Biosystems) at 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min. Each reaction was then diluted 1/10 in 0.1 M EDTA, pH 8.0, and stored at −80°C.

For the relative quantitation of MR and GR and the endogenous reference 18S ribosomal RNA (18S), real-time PCR was performed using an ABI PRISM 7700 Sequence Detector (PE Applied Biosystems) (24, 26). A multiplex comparative C_T was used in this study, where a C_T value reflects the cycle number at which DNA amplification is first detected. In the multiplex reactions, either MR or GR was detected in the one tube with 18S, where primers were limited for 18S. For the comparative method, a validation experiment was performed where we demonstrated approximately equal efficiency of MR and GR amplifications together with the amplification of 18S over a range of template concentrations (50 ng–5 pg).

For real-time PCR, all primers and TaqMan probes were designed using Primer Express Version 1.0 (PE Applied Biosystems). A partial cDNA sequence of the ovine MR (Hantzis et al., unpublished data) was used to design the MR probe (GenBank/NCBI AF349768). Primer and TaqMan probe sequences for the MR and GR receptor sets are presented in Table 1. The TaqMan probe and primers for 18S were supplied by PE Applied Biosystems in a control reagent kit. PCR reactions were carried out in 25-μl volumes consisting of 1× TaqMan Universal PCR master mix (including passive reference), 50 nM TaqMan 18S probe, 20 nM 18S forward primer, 80 nM 18S reverse primer, and the appropriate concentration of primers and TaqMan probes for MR and GR receptors as described in Table 1. cDNA (50 ng) and no RT preparations were amplified using the following conditions: 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

In each assay, the C_T value of one sample from the control group of animals was determined five times. The means ± SE of these five measurements were used to determine an intraset CV. This mean ΔC_T value of the control group of animals was used as a “calibrator” to which other samples were compared. Thus comparative C_T calculations for the expression of MR and GR receptors were all relative to an internal control. First, 18S C_T values were subtracted from MR and GR receptor values for each well to get a ΔC_T value. ΔC_T values were achieved by subtracting the calibrator ΔC_T value from each ΔC_T value. The expression of the MR and GR receptors relative to the calibrator was evaluated using the expression 2^−ΔC_T. The CV for one sample of the adult hippocampus, repeated five times in the one assay, was 17% for GR and 9% for MR.

Data analysis. One-way ANOVA with the Bonferroni method (modified t-test) was used to compare blood pressure, plasma/blood volume, and gene expression data. Hormonal and plasma composition responses to hemmorhage (changes over time) were tested by using two-way repeated-measures ANOVA (Sigma Stat v2 software package; SPSS, Chicago, IL). Additional statistical analysis was performed on areas under the plasma concentration curves (AUC) for ACTH, AVP, and F. The curves were calculated for each individual sheep (Sigma Scan Pro v4 software package; Jandel Scientific Software, Chicago, IL), as the area between baseline concentration of a hormone and the concentration profile during the 4-h hemorrhage protocol. These results were then tested by using one-way ANOVA.

RESULTS

Hemorrhage study. At the time of the hemorrhage experiment, animal body weights were 47 ± 3 kg (control n = 6) and 48 ± 2 kg (group D n = 5). Even though hemorrhage ceased at the time when blood pressure dropped precipitously, the duration of hemorrhage was similar between control and group D animals (14 ± 0.4 min in control and 16 ± 1.3 min in group D).

There were no significant differences in blood loss over 15 min of the hemorrhage experiment in group D (16 ± 2 ml/kg; total blood loss of 788 ± 65 ml) compared with the control group of animals (15 ± 1 ml/kg; total blood loss of 683 ± 27 ml). In addition, the total blood loss was also similar when expressed as a percentage of the total blood volume (26 ± 1% in control and 28 ± 3% in group D).

Mean arterial pressure, measured for 1 h, immediately before the hemorrhage protocol started [control mean arterial pressure (MAPc)] was significantly higher in group D compared with the control group (83 ± 3 vs. 73 ± 1 mmHg; P < 0.01). The mean arterial pressure dropped precipitously [mean arterial pressure at the point when the hemorrhage experiment
stopped (MAPd) to similar levels in both groups of animals, although the magnitude of blood pressure fall [the difference in mean arterial pressure between MAPc and MAPd (MAPc – d)] was significantly higher in group D compared with the control group (40 ± 2 vs. 33 ± 2 mmHg; P < 0.05).

Over the 4-h period of the hemorrhage protocol, there were no significant changes in plasma sodium concentration and plasma osmolality in either the control group or group D (Table 2). There was a significant decrease in plasma potassium and total protein concentrations and a significant increase in plasma glucose concentration over time in the control group (in all, P < 0.01). These changes were of similar magnitude when compared with group D (Table 2). Hct values decreased over time in the control group, but the effect of hemorrhage on the Hct of group D was significantly different compared with the control group (Hct at 60 min; P < 0.05) (Table 2).

As shown in Fig. 1, there was a similar response in plasma concentrations of ACTH, AVP, F, and PRC during the 4-h period of the hemorrhage protocol between the control and group D animals. The values for AUC-ACTH, AUC-AVP, and AUC-F as well as ratios of AUC-ACTH/AUC-F and AUC-ACTH/AUC-APR were similar between the two groups of animals (Table 3).

**Plasma/blood volume results.** At the time of the plasma volume measurements, animal body weights were 48 ± 2 kg (control n = 6) and 49 ± 2 kg (group D n = 5). Hct values were 25 ± 0.9% (control n = 6) and 28 ± 1.6% (group D n = 5). These values were similar to those found in the hemorrhage experiment. The regression coefficients (R²) for plasma sample counts at the three time periods were 0.9 ± 0.03 in control and 0.9 ± 0.07 in group D. Measured plasma volume in group D was similar to that found in the control group (41.5 ± 1.3 vs. 40.5 ± 1.4 ml/kg). Blood volume, calculated from the measured plasma volume and Hct (4), was 54.4 ± 1.9 ml/kg in the control group, and this was similar to blood volume calculated in group D animals (57.6 ± 2.1 ml/kg).

**Gene expression study.** As shown in Fig. 2, relative to the calibrator (average ΔC_T of the control group of animals), there was no significant difference in the expression of MR and GR in the hippocampus as well as GR in the hypothalamus between control and group D animals.

**DISCUSSION**

We reported previously that offspring of sheep exposed to dexamethasone treatment for only 48 h at the end of the first month of gestation have progressively higher blood pressure with age. This form of in utero “programmed” hypertension was found to be associated with increased cardiac output and also with increased insulin inhibition of lipolysis (10, 13). Contrary to results in some other forms of in utero “programmed” hypertension, our results suggest that HPA axis does not play a major role in “programming” high blood pressure in offspring of sheep exposed briefly to dexamethasone at the end of the first month of pregnancy.

Several studies proposed that “programming” of fewer effective GR receptors, which normally mediate negative feedback of the HPA axis and therefore set the axis to operate at the higher levels, would result in chronic exposure to higher circulating concentrations of GR hormones (11). This could occur by a small change in plasma basal/resting GR levels or by an exaggerated steroid response in times of “stress.” A higher plasma F level (although within the normal range) correlated with the blood pressure in people of low birth weight (28). In addition, higher F values (basal and after a low ACTH stimulation test) have been recently reported in 20-yr-old adults (20) and in 66- to 77-yr-old men of low birth weight (30).

In the rat model, treatment with dexamethasone on days 15-20 of pregnancy caused significant decreases in the levels of the GR mRNA in the dentate gyrus and CA1 area of the hippocampus, a decrease of MR mRNA in the CA1 and CA2 regions, and elevated basal levels of plasma corticosterone at 16 wk of age (21).

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<th>TP, g/l</th>
<th>Glu, mmol/l</th>
<th>Hct, %</th>
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| 40        | 144 ± 0.9  | 4.3 ± 0.16| 294 ± 1             | 53 ± 22| 4.8 ± 0.63  | 21 ± 0.8*| 142 ± 1.6  | 4.0 ± 0.07| 293 ± 1             | 52 ± 17| 5.3 ± 0.6‡  | 23 ± 1.5*\
| 60        | 145 ± 0.8  | 4.1 ± 0.11| 294 ± 1             | 53 ± 22| 4.2 ± 0.44  | 21 ± 0.9*| 144 ± 1.8  | 3.9 ± 0.07| 292 ± 1             | 51 ± 17| 4.3 ± 0.3   | 25 ± 1.4§\
| 120       | 144 ± 0.9  | 4.0 ± 0.15†| 293 ± 1             | 53 ± 22| 3.9 ± 0.22  | 22 ± 1.0| 143 ± 0.7  | 3.7 ± 0.18*| 291 ± 1             | 55 ± 17| 3.7 ± 0.1   | 25 ± 1.0| 143 ± 1.4  | 3.5 ± 0.20†| 292 ± 1     | 53 ± 17   | 3.9 ± 0.1   | 25 ± 1.4%

Data are means ± SE. *P < 0.05; †P < 0.01; ‡P < 0.001 (effect of hemorrhage treatment within the group); and §P < 0.05 (effect of hemorrhage treatment between groups). Osm, osmolality; Glu, glucose; Hct, hematocrit; TP, total protein.
same rat offspring, dexamethasone treatment resulted in elevations of both systolic and diastolic pressures. Plasma corticosterone values were also above normal in rat pups (at days 7, 14, and 21 after birth) from mothers that had received repeated shock treatment during pregnancy (34). Prenatal exposure to dexamethasone, 4–5 wk before term, in pregnant rhesus monkeys resulted in a 20–30% reduction in size and segmental volume of the hippocampus, as determined by magnetic resonance images at 20 mo of age (37). In addition, 18-day-old male offspring of guinea pigs exposed to dexamethasone for only 2 days, between 50 and 52 days of gestation (term ≥ 110 days), have shown higher plasma resting F concentrations. In these animals, lower mRNA GR expression was found in the hippocampus (8). More recently, gender-specific effects of repeated maternal dexamethasone administration (3 courses; on days 40–41, days 50–51, and days 60–61 of gestation) on HPA regulation have been shown in 80-day-old guinea pigs (23). However, the same offspring had normal blood pressure. In addition, adult rats exposed to elevated endogenous levels of GRs throughout gestation had increased basal corticosterone levels and reduced GR mRNA in the hypothalamic paraventricular nucleus, although they had unaltered GR and MR in the hippocampus (39). Taken together, these studies (using different models in various species) demonstrated the powerful effect of prenatal exposure to either synthetic or endogenous GRs on the HPA. However, so far, we have no evidence for a major role of the HPA in our model of programmed hypertension resulted from prenatal GR exposure. Previously, we showed that, in our model, the basal values of F were similar in two groups of animals and that there was no effect of prenatal dexamethasone treatment on the pressor and plasma F concentration responses to a 5-day infusion of ACTH (9). Recently, we found no change in MR mRNA and GR mRNA expression in both the hypothalamus and hippocampus of late-gestational ovine fetuses exposed to the same early dexamethasone treatment (unpublished results). In the current study, we demonstrate that in response to hemorrhage stress, there was no change toward higher ACTH, AVP, and F responses in the hypertensive group of animals compared with the control group. In

Table 3. AUC-ACTH, AUC-AVP, AUC-F, and the ratios of AUC-ACTH/AUC-F and AUC-ACTH/AUC-AVP over the 4-h period of the hemorrhage protocol

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<th>Group D (n = 5)</th>
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Data are means ± SE. AUC, area under the curve; AVP, arginine vasopressin; F, cortisol.

Fig. 1. Plasma concentrations of ACTH, arginine vasopressin (AVP), cortisol (F), and plasma renin concentration (PRC) during the 4-h hemorrhage protocol in control (n = 6; ■) and group D (n = 5; ●) animals. Results are means ± SE.
near-term ovine fetuses of ewes treated with multiple betamethasone injections, only subtle changes were observed in basal cord plasma ACTH concentrations (32). In addition, in the current study, we were unable to demonstrate differences in the expression of the GR in the hypothalamus and both the MR and GR in the hippocampus between control and "programmed" animals.

Interestingly, in the dexamethasone-exposed animals, there was a milder effect of hemorrhage on the drop in Hct, implicating the role of the spleen, which is known to be a reservoir for the red blood cells (29), or perhaps that the restoration of the intravascular volume was different between the two groups. This observation could suggest exaggerated sympathetic activity in these animals in the time of stress, particularly because plasma/blood volumes were similar in both groups of animals. Although we do not have a direct measure of the sympathetic activity, PRC, known as a good index of the renal sympathetic activity, was similar between the two groups. It has been shown that blockade of the brain ANG II AT1 receptors in adult sheep can attenuate the stress-induced elevation in splanchnic sympathetic neural activity (17) and also attenuate the catecholamine response to restraint stress (31). Several studies in rats demonstrated altered neurotransmitters in numerous brain regions as a consequence of prenatal stress or dexamethasone exposure (25, 27, 33, 35). So, dexamethasone treatment could act via altering the activity of ANG II on neurotransmitter availability perhaps via upregulation of the AT1 receptor. In addition, ANG II receptors, predominantly of the AT1 subtype, are found in many areas of the brain known to be involved in blood pressure regulation (1). This will be of particular interest because our preliminary results have shown that in "programmed" animals, there is a significant increase in AT1 mRNA in the medulla oblongata (unpublished data). Further studies are warranted to test the role of the central/brain ANG II in this model of "programmed" hypertension.

In conclusion, neither significant changes in the HPA response to hemorrhage stress nor an altered expression of the GR in the hypothalamus and MR and GR in the hippocampus was observed in sheep with "programmed" high blood pressure. Our previous results (9) together with findings from this study gave no evidence to suggest that an altered HPA axis is the main mechanism whereby the adult sheep exposed to an excess of dexamethasone early in gestation maintain blood pressure at the higher level.

Perspectives

There is now strong epidemiologic evidence that low birth weight (small for gestational age) and postnatal catch-up growth are linked with adult risk of cardiovascular and metabolic disease. Inadequate fetal growth is thought to be only one measurable outcome of a less than optimal intrauterine environment. Certainly, there is a number of animal models in which cardiovascular and/or metabolic disease has been "programmed" by different prenatal treatments (undernutrition and steroid exposure) in some of which there is no evidence of fetal growth retardation. Permanent reset of the HPA axis has been a popular hypothesis that links adverse intrauterine environment with adult cardiovascular and metabolic disease. However, that link has not always been proven even in epidemiologic studies. In rats, prenatal exposure to dexamethasone led to adult high blood pressure associated with an altered HPA axis. In prenatally malnourished adult guinea pigs, altered HPA axis had no effect on blood pressure. And, we have the model where high blood pressure in the adult sheep, as a result of brief exposure to dexamethasone early in gestation, is not associated with changes in the HPA axis. Taken together, it is highly likely that fetal compromise to unfavorable intrauterine environment leading to increased suscep-

Fig. 2. Ratio of gene expression of mineralocorticoid (MR) and glucocorticoid (GR) receptors relative to a calibrator. Gene expression study was performed in the hippocampus (GR and MR) and hypothalamus (GR) in control (n = 6; open bars) and group D (n = 5; filled bars) animals. Values are means ± SE. As a calibrator, we used the control group of animals.
tibility to cardiovascular and/or metabolic disease occurs via a variety of mechanisms that do not invariably include the reset in the HPA axis. Another system that has been put forth by us and others is the renin-angiotensin system. Further studies are warranted to test the role of the central/brain ANG II in this model of “programmed” hypertension.

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