EARLY GESTATION DEXAMETHASONE ALTERS BAROREFLEX AND VASCULAR
RESPONSES IN NEWBORN LAMBS PRIOR TO HYPERTENSION

Jeffrey L. Segar, Robert D. Roghair, Emily M. Segar, Melissa C. Bailey,
Thomas D. Scholz, Fred S. Lamb

Department of Pediatrics, University of Iowa Carver College of Medicine,
Iowa City, IA 52242

Correspondence:
Jeffrey L. Segar, M.D.
Professor of Pediatrics, Division of Neonatology
Children’s Hospital of Iowa
200 Hawkins Dr.
Iowa City, IA 52242
319-356-7244 (phone)
319-356-4685 (fax)
jeffrey-segar@uiowa.edu

Running title: Fetal programming of autonomic function
ABSTRACT

Exposure of the early gestation ovine fetus to exogenous glucocorticoids induces alterations in postnatal cardiovascular physiology, including hypertension. To determine if autonomic function and systemic vascular reactivity are altered by in utero programming prior to the development of systemic hypertension, we examined arterial baroreflex function and in vivo hemodynamic and in vitro vascular responses to vasoactive agents in 10-14 day old newborn lambs exposed to early gestation glucocorticoids. Dexamethasone (dex, 0.28 mg/kg/day) or saline was administered to pregnant ewes by intravenous infusion over 48 hours beginning at 27 days gestation (term 145 days) and lambs were allowed to deliver (n = 6 in each group). Resting mean arterial blood pressure (MABP), (77 ± 1 vs. 74 ± 3 mmHg) and heart rate (HR), (249 ± 9 vs. 226 ± 21 bpm) were similar in dexamethasone-exposed and control animals, respectively. The arterial baroreflex curve, relating changes in HR to MABP, was significantly shifted toward higher pressure in the dex-exposed lambs although no change in the sensitivity (gain) of the response was seen. In vivo changes in blood pressure in response to bolus doses of angiotensin II (20, 50, 100 ng/kg) and phenylephrine (2, 5, 10 ug/kg) were similar in the two groups. However, dex-lambs displayed greater decreases in MABP in response to ganglionic blockade with tetraethylammonium bromide (10 mg/kg) (-30 ± 3 vs. –20 ± 3 mmHg, p<0.05) and greater increases in MABP following nitric oxide synthase blockade with L-NNA (25 mg/kg) (23 ± 3 vs. 13 ± 2 mmHg, p<0.05) compared to control lambs. By in vitro wire myography, mesenteric and femoral artery microvessel contractile responses to KCl were similar whereas responses to endothelin (in mesenteric) and norepinephrine (in femoral) were significantly attenuated in dex-lambs compared to controls. Femoral vasodilatory responses to forskolin and sodium nitroprusside were similar in the two groups (n = 4). These findings suggest that resetting of the baroreflex, accompanied by increased sympathetic activity and
altered nitric oxide-mediated compensatory vasodilatory function may be important contributors to programming of hypertension.

Key words: autonomic nervous system, cardiovascular, fetal programming, glucocorticoids, nitric oxide
INTRODUCTION

Over the past decade, there has been increasing evidence supporting the concept that adverse factors in the perinatal environment predispose an individual to disease later in life (1, 2). Central to this concept is the well substantiated link between birth weight and the development of a number of adult diseases, including hypertension, coronary artery disease and insulin resistance (7, 8, 23, 40, 41). Importantly, this association between birth weight and cardiovascular disease is independent of established cardiovascular risk factors (7, 8, 40, 41).

A number of animal studies suggest that exposure to increased levels of glucocorticoids early in development may mediate programming of hypertension later in life. In rats, administration of synthetic glucocorticoids during the last week of pregnancy results in elevated blood pressure in the offspring (3, 22). Similarly, increased exposure of the fetus to maternal glucocorticoids by inhibiting 11β-hydroxysteroid dehydrogenase (11βHSD), a placental enzyme that converts active glucocorticoids to inactive metabolites, results in the postnatal development of hypertension and hyperglycemia (24). Moderate protein restriction in pregnant rats leads to a decrease in 11βHSD activity with a consequent increase in blood pressure in the offspring (21). Finally, maternal administration of metyrapone, an inhibitor of glucocorticoid synthesis, inhibits the development of hypertension in offspring following intrauterine protein restriction (3, 21, 22).

Studies by Dodic et al. (later confirmed by our group), demonstrate that the offspring of ewes treated with dexamethasone (~12mg/day) for 48 hours at 26 to 28 days gestation (term being 145-150 d) results in hypertensive offspring at 3-4 mo of age (13, 42). With increasing age and in the presence of underlying hypertension, resetting of the heart rate (HR) – blood pressure baroreflex relationship, increased cardiac output and left ventricular hypertrophy have been observed (11, 12).
We have shown in this same model that coronary artery but not mesenteric artery vascular reactivity is altered at two weeks and 4 mo of age (42, 43).

An important concern when studying the mechanisms for programming of hypertension relates to distinguishing factors that are a cause, rather than a consequence of the hypertension. As such, it is vital to investigate cardiovascular function prior to the emergence of hypertension. Therefore, the present studies were undertaken in an ovine model of fetal programming to examine the hypothesis that autonomic dysfunction is present prior to the development of hypertension. Specifically, we examined in 10-14 day old offspring of ewes administered dexamethasone early in gestation in vivo baroreflex function and hemodynamic responses to a number of vasoreactive agents as well as in vitro vascular responses of isolated mesenteric and femoral artery microvessels. These data suggest a primary role for the sympathetic nervous system in driving the developmental of fetally programmed high blood pressure. Hypertension may be masked by compensatory increases in endothelial nitric oxide production early in life.
METHODS

Animals and surgical preparations Time-dated pregnant ewes of Dorset and Suffolk mixed breeding were obtained from Iowa State University (ISU) and housed at the ISU Agricultural Station throughout the course of study. At 27-28 days gestation (term being ~145 days), a 16 gauge, single lumen polyurethane catheter (Cook Critical Care, Bloomington, IN) was placed into left jugular vein the pregnant ewe using a modified Seldinger technique and dexamethasone (0.28 mg/kg/day; Gensia Sicor Pharmaceuticals, Irvine, CA) or an equivalent volume of vehicle (0.9% NaCl) was continuously infused over 48 hours. The catheter was then removed and the ewes allowed to complete gestation. The ewes delivered naturally with 2 twin and 4 single deliveries within both the dexamethasone-exposed group and the control group. A single lamb from each twin group was used for the studies, thus a total of 6 lambs of either sex were included in each group. Prior to the infusion period, pregnant ewes were allowed to pasture graze and were provided with 1 pound/day of shelled corn with a mineral supplement. After the infusion period, ewes were fed an alfalfa/grass mix hay diet along with 1-3 lbs/d of shelled corn with mineral and protein supplementation. Lambs were allowed to nurse ad libitum. Within the first week of life, the lambs and ewes were transferred from the ISU Agricultural Station to the University of Iowa Animal Care Unit. All procedures were performed within the regulations of the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Iowa Animal Care and Use Committee.

Lambs were anesthetized with 12 mg/kg of thiopental sodium (Abbott Laboratories, Abbott Park, IL), intubated and ventilated with a mixture of halothane (1-2%), oxygen (33%) and nitrous oxide (66%). Polyethylene catheters (PE-90, ID = 0.86 mm, OD = 1.27 mm, Intramedic, Franklin
Lakes, NJ) were inserted into the lamb’s left femoral artery and vein and sutured in place. The catheter was tunneled subcutaneously and secured to the back of the lamb using porous elastic bandages. Ampicillin (Sigma, St. Louis, MO) was administered at the completion of surgery (2 g intramuscular), and every 12 hours for 48 hours (1 g intramuscular). After surgery, the lamb was returned to an individual pen with its mother.

**Experimental protocol.** The physiological studies were begun 3 d after surgical preparation. The morning of the experiment, the lamb was transferred to the laboratory, weighed and placed in a sling-frame assembly, allowing the lamb to stand. Lambs received a continuous infusion of 5% dextrose in 0.2% NaCl at 4 cc/kg/h during the experiment. A 90 min equilibration period was allowed before the start of the experiment. During each experiment, mean arterial pressure was recorded using Statham P23 Db pressure transducers (Spectramed, Critical Care Division, Oxnard, CA) placed at the level of the heart, MacLab hardware and software (ADInstruments, Inc. Colorado Springs, CO) and a G4 Powerbook (Apple Computers, Cupertino CA). Heart rate was monitored with a cardiotachometer triggered from the arterial pressure pulse wave. Arterial blood (1 ml) for pH, PCO₂ and PO₂ and Hgb was collected anaerobically in a heparinized syringe at the beginning of the experiment and measurements immediately determined at 39.5°C using a 1302 BGM pH/blood gas analyzer (Instrumentation Laboratory; Lexington MA). Hematocrit was determined using centrifugation and a micrometer caliper.

Baseline MABP and HR were initially obtained for 30 min followed by measurement of changes in response to three sequentially increasing doses of ANG II (20, 50 100 ng/kg, i.v. bolus). A 20 min recovery period was allowed for MABP and HR to return to baseline before administration of the next dose. After a one-hour recovery period, resting MABP and HR were again recorded, and baroreflex curves determined by producing ramp changes in MABP with
continuous i.v. infusion of increasing does of phenylephrine (5 - 40 ug/kg/min) or nitroprusside (5 - 40 ug/kg/min) over a 3 min period, using a Harvard infusion pump. A 30 min recovery period was allowed before the alternative drug was administered. Upon completing baroreflex curves, and allowing a one hour recovery period, the ganglionic blocking agent tetraethylammonium bromide (TEA, 10 mg/kg i.v., Sigma) was administered. MABP and HR, which were notable for a lack of spontaneous variability, were averaged over 5 min beginning 3 min after the injection. Finally, after a 2 h recovery period to allow hemodynamic parameters to return to baseline values, MABP and HR were again recorded before and 1 h following administration of the angiotensin II type 1 receptor antagonist, losartan (10 mg/kg i.v.).

Lambs were allowed to recover for 2 days before undergoing a second day of experiments, consisting of baseline MABP and HR measurements, and dose responses to phenylephrine (2, 5 and 10 ug/kg iv), performed in a similar manner to that for angiotensin II. Baroreflex curves using phenylephrine and nitroprusside were again performed as previously described. After a one hour recovery period, lambs then received the nitric oxide synthase inhibitor N(G)-nitro-L-arginine (L-NNA, 25 mg/kg iv). MABP and HR were again recorded for 15 min, beginning 15 min after administration of L-NNA.

**Femoral and mesenteric artery contractile responses.** Following another 2 d recovery period, the lambs were euthanized with intravenous pentobarbital sodium (50mg/kg; Abbott Laboratories, Abbott Park, IL) and femoral and mesenteric arteries collected. Branch femoral and mesenteric artery segments with internal diameters of 100-150 μm were cleansed of adherent connective tissue and sectioned into rings on the day of collection. The endothelium was left intact and the rings were mounted within a small vessel myography apparatus (Model 610, Danish Myotechnology, Aarhus, Denmark) using 40 μm stainless steel wires. Contractile responses were
recorded with an eight-channel MacLab 8E and stored on a Power Macintosh G3 computer. The length-tension relationship was defined experimentally to 90 mmol/L KCl at varying passive stretch. Passive stretch was set at 80% of the tension required to obtain peak responses to KCl (1.2 mN) for both vessel types, and the rings were allowed to equilibrate in bicarbonate-buffered physiological salt solution (PSS, composition as follows (in mmol/L): 130 NaCl, 4.7 KCl, 1.18 KH_{2}PO_{4}, 1.17 MgSO_{4} .7 H_{2}O, 14.9 NaHCO_{3}, 1.6 CaCl_{2} .H_{2}O, 5.5 dextrose and 0.03 CaNa_{2}-EDTA (pH 7.30)) aerated with a mixture of 95% O_{2} - 5% CO_{2} at 37°C for 60 min before the start of experimentation.

The contractile response of each vessel to 120 mmol/L KCl was first recorded to allow normalization of subsequent vascular responses. The vessels were then re-equilibrated to their baseline with multiple washes of PSS prior to measurement of vasoconstrictor responsiveness. Cumulative concentration-responses to KCl (4 –100 mmol/L), norepinephrine (NE, 10^{-9} to 10^{-5} mol/L) and endothelin-1 (ET-1, 10^{-11} to 10^{-7} mol/L) were conducted with addition of increasing concentrations of the agent under study prior to loss of the preceding vascular response. Arteries were re-equilibrated to their baseline with multiple washes of PSS between vasoconstrictor agents. To investigate potential alterations in cyclic nucleotide-mediated vasodilation, separate baths were used to assess cumulative concentration vasorelaxant responses of femoral microvessels to sodium nitroprusside (10^{-9} to 10^{-5} mmol/L) or forskolin (10^{-10} to 10^{-7} mmol/L) following preconstriction with norepinephrine (10^{-5} mmol/L). All PSS reagents and vasoactive compounds were acquired from Sigma Chemical (St. Louis, MO) with the exception of ET-1 (Alexis Corporation, San Diego, CA). Microsoft Excel 2000 was used to generate concentration-response curves for each vasoactive agent.
Computation and data analysis. The number of animals examined was based on power calculations assuming a normal distribution with equal variances, limiting the alpha error to 0.05 and a sample size sufficient to identify a 33% increase in the response to a given intervention (for which we used the blood pressure response to TEA). In order to achieve a power of 0.80, a sample size of 6 animals per group was needed.

The changes in HR in response to alterations in MABP were used to generate the baroreflex curves. Data points sampled every 5 sec were analyzed with a logistic sigmoid function (GraphPad Prism version 4.0, GraphPad Software, Inc., San Diego CA) according to the following equation:

\[ HR = P_4 + P_1 / (1 + \exp(P_2(MABP - P_3))) \]

where \( P_1 \) is the range between the upper and lower plateaus, \( P_2 \) is a coefficient used to calculate the gain as a function of pressure, \( P_3 \) is the MABP at the midrange of the curve and \( P_4 \) is the lower plateau (18). The gain (maximal slope) of the curve was calculated from the first derivative of the above equation. Threshold pressure (lowest pressure that produces a decline in HR) and saturation pressure (pressure necessary to achieve maximal inhibition of HR) were calculated from the third derivative of the equation (18). For an individual animal, the parameters describing the HR-MABP baroreflex relationship where calculated for both days tested, then averaged together prior to the final analysis. Physiologic parameters, including baroreflex curve parameters, and maximal hemodynamic and vascular responses were compared using Student’s unpaired, two-tailed t-test (with significance at \( P < 0.05 \)). Concentration-responses to the vasoactive agents were compared using analysis of variance (ANOVA), factoring for treatment group and dose. If the F-statistic identified significant differences (\( P < 0.05 \)), pairwise comparisons were made using the Tukey test, with \( P < 0.05 \) considered significant. All statistical analyses were performed using SAS System 9 for Microsoft Windows (SAS Institute Inc., Cary, NC). All values are presented as mean ± S.E.M.
RESULTS

Age, weight, arterial pH, blood gases and hematocrit were similar between the two groups at the start of the experiments (Table 1). Baseline MABP and HR were similar between the groups and did not differ between the first and second experimental day (Table 1).

The peak change in MABP in response to 20, 50 and 100 ng/kg angiotensin II were similar in the two groups, although the maximal decrease in HR to 50 and 100 ng/kg angiotensin II was greater in dex-exposed compared to control lambs (Figure 1). Maximal changes in MABP and HR to phenylephrine (2, 5 and 10 ug/kg) were similar in control and dex-exposed lambs (Figure 2).

While resting MABP and HR were not different between groups, early gestation exposure to dexamethasone resulted in a significant shift in the baroreflex curve towards the right, or higher pressure (Figure 3 and Table 2). In particular, the baroreflex curve midpoint ($P_3$, $75 \pm 2$ vs. $84 \pm 3$ mmHg) and threshold pressure ($55 \pm 3$ vs. $68 \pm 4$ mmHg) were significantly increased in the dex-exposed group, indicating a shift of the baroreflex curve relating MABP and HR to the right. Upper and lower plateaus of HR, as well as the gain and saturation pressure of the baroreflex curves were not statistically different.

The changes in MABP and HR in response to intravenous injections of TEA, L-NNA and losartan are depicted in Figure 4. Following ganglionic blockade with TEA, the magnitude of the decrease in MABP and HR was significantly ($P<0.05$) greater in dex-exposed lambs than in control animals. Dex-exposed lambs also displayed a greater increase in MABP with L-NNA compared to control lambs, although no differences in the HR responses were detected. Finally, blockade of AT$_1$ receptors with losartan resulted in similar decreases in MABP but a greater decrease in HR in the dex-exposed lambs.
Vascular Reactivity

Responses to voltage-dependent calcium channel activation: No effect of treatment group was identified on the maximal responses of femoral and mesenteric artery segments to KCl (120 mM); femoral: 6.43 ± 2.29 vs. 6.99 ± 2.36 mN, mesentery: 7.59 ± 1.25 vs. 6.80 ± 0.85 mN for control and dex-exposed, respectively. The mesenteric and femoral artery cumulative concentration vasoconstrictive responses to KCl were also not significantly altered by dexamethasone exposure (Figure 5).

Responses to second messenger-dependent vasoconstrictors: Dex-exposure tended to attenuate vasoconstrictive responses of systemic microvessels. Specifically, compared to control responses, femoral and mesenteric arteries from dex-exposed lambs displayed decreased responsiveness to norepinephrine and endothelin-1, respectively (Figure 5). Femoral artery response to endothelin-1 and mesenteric artery response to norepinephrine were similar in both groups.

Responses to vasodilators: There was no significant effect of early gestation dexamethasone exposure on femoral arterial responses to sodium nitroprusside or forskolin (Figure 6).
DISCUSSION

Numerous studies have documented adult-onset blood pressure elevation following fetal nutrient deprivation or exposure to excess glucocorticoids (29). Potential contributions of renal, vascular and autonomic mechanisms to the programming of postnatal blood pressure have previously been suggested, although consensus is lacking (29). The major findings of this study include the observations that in newborn lambs exposed to exogenous glucocorticoids early in gestation, the MABP-HR baroreflex relationship is shifted toward higher pressure and that these animals display heightened blood pressure responses to ganglionic and nitric oxide synthase blockade. These altered cardiovascular responses are present prior to the development of systemic hypertension and therefore are not a consequence of programmed hypertension but may be important contributors to the development of the phenotype.

While it is well established that the baroreflex provides an important buffering mechanism to counteract short-term fluctuations in blood pressure, more recent evidence also supports a role for baroreceptors in long-term control of blood pressure via regulation of sympathetic activity and sodium excretion (25). Alterations in baroreflex function have previously been demonstrated in animal models of fetal programming, suggesting the arterial baroreflex may impact the development or maintenance of in utero programmed hypertension. In the offspring of rats fed a low protein diet, the HR baroreflex response curve is significantly shifted toward high pressure (37). The HR baroreflex is also shifted to the right in 40 mo old sheep exposed to dexamethasone at the end of the first month of gestation (11). However in both these studies, the programmed animals were already hypertensive and resetting of the baroreflex could have been a consequence of hypertension. In contrast, our findings demonstrate that the HR baroreflex is reset prior to the development of hypertension and suggests abnormalities in autonomic function may contribute,
rather than result from, the hypertension. Resetting of the baroreflex prior to changes in blood pressure has previously been shown in several hypertensive rat models (14, 15). The mechanisms and significance of resetting of the baroreflex remain to be defined. The known role of the renin-angiotensin system in regulating baroreflex function and the concurrent finding that AT$_1$ receptor expression within select brain cardiovascular centers is increased fetuses and adults exposed to dexamethasone early in gestation suggest central ANG II and AT$_1$ receptors may participate in the resetting of the baroreflex (37, 39). Future studies will also need to determine if baroreflex control of sympathetic outflow is altered with \textit{in utero} programmed hypertension.

There are reasons to believe altered sympathetic function may contribute to the programming of hypertension resulting from an adverse intrauterine environment. Environmental exposures at crucial points in development have been shown to permanently alter sympathetic function (31, 51). For example, mammals reared at elevated temperatures have altered sympathetic innervation of sweat glands, improved thermoregulatory responses and are more tolerant of extreme temperatures as adults (6, 9). In the chick, mild hypoxia during embryonic development results in increased basal sympathetic tone and sympathetic hyperinnervation of resistance arteries (45). Studies in humans examining autonomic nervous activity suggest low birth weight, a surrogate marker for a poor intrauterine environment, is associated with altered heart rate variability increased muscle sympathetic activity (5, 16, 28).

Mechanisms responsible for the increase in sympathetic tone remain to be explored. Extensive animal studies detail that exposure to glucocorticoids early in development enhance maturation of catecholaminergic and cholinergic cells, receptor mechanisms and synaptogenesis in brain (4, 30, 47, 48, 52). Alterations in the brain renin-angiotensin system, which plays an important role in the regulation of blood pressure, in part by its modulation of sympathetic outflow, may also participate
in programmed hypertension. As previously noted, angiotensin II type 1 (AT\textsubscript{1}) receptor gene expression is increased in the medulla oblongata of fetal and adult sheep exposed to dexamethasone early in gestation (10), whereas AT\textsubscript{1} receptor expression is increased in the subfornical organ and the vascular organ of the lamina terminalis (OVLT) in offspring of protein restricted rats. The accentuated changes in heart rate in response to angiotensin II receptor stimulation or blockade in the dex-exposed lambs relative to controls, in the absence of differences in changes in blood pressure, further support the concept that alterations in the renin-angiotensin system contribute to changes in baroreflex function and programming of hypertension. The finding that both stimulation and inhibition of angiotensin receptors resulted in greater slowing of the heart rate in dex-infused compared to control animals may be related to a number of factors including 1) altered distribution or signaling of AT\textsubscript{1} and AT\textsubscript{2} receptors within cardiovascular centers within the brain, 2) accessibility of the agents to cardiovascular centers with and without a blood-brain barrier and 3) differences in subpopulations of neurons and other neurotransmitter pathways. Ultimately, these differences likely result in distinct effects on sympathetic and parasympathetic drive to the heart.

We cannot rule out the contribution of a renal mechanism contributing to increased sympathetic tone. Previous studies in rats and sheep demonstrate that perinatal environments which ultimately result in hypertension in the offspring are associated with abnormal kidney morphology, including reduced nephron number (49, 50). We did not examine the kidneys of the animals in our study. However, it is possible that underlying renal abnormalities may have contributed to the apparent increase in sympathetic tone in the dex-exposed lambs (17).

Vascular dysfunction has been hypothesized to explain the relationship between fetal growth retardation and the future development of hypertension. Studies of hypertensive offspring of rats
fed a caloric or protein restricted diet demonstrate increased contractile responses and impaired
impaired small artery endothelium-dependent and -independent responses (20, 34, 35). In
particular, these animals display reduced vascular smooth muscle activity of the NO-cGMP
pathway (19, 36). Fetuses of dietary restricted pregnant ewes display blunted femoral vasodilatory
responses to acetylcholine and bradykinin, as well as nitroprusside, suggesting impaired smooth
muscle sensitivity to NO (32, 33). Finally, in human infants and children, low birth weight has
been shown to be associated with impaired vascular responsiveness to acetylcholine, but not
nitroprusside, findings reflective of endothelial dysfunction (26, 27).

In view of these previous studies, we were surprised to find that blockade of nitric oxide
production with L-NNA produced a greater increase in blood pressure in the dex-exposed lambs.
In vivo, blockade of NO synthesis may promote increased blood pressure by two mechanisms: loss
of peripheral vasodilator tone and enhanced central sympathetic drive. With regard to peripheral
mechanisms, the greater pressor response observed in dex-exposed lambs following L-NNA
suggests either increased endogenous NO production or enhanced smooth muscle sensitivity to NO
in these animals. The attenuated contractile responses to NE and endothelin-1 seen in femoral and
mesenteric artery from dex-exposed lambs are also consistent with enhanced NO-mediated
vasorelaxation. Although the current study did not examine NO dependent vasodilation in isolated
vessels, we previously reported early gestation dexamethasone exposure had no effect on ovine
coronary or mesenteric artery vasodilatory responses to nitroprusside or the cGMP analog 8-Br-
cGMP (42). Taken together, these findings suggest that early in life, peripheral NO production is
enhanced following early gestation exposure to glucocorticoids. Differences in species, underlying
adverse intrauterine influence, timing of the events, vessels studied and methodologies may
contribute to the differences in findings between our study and those of other investigators.
It should also be recognized that nitric oxide modulates autonomic function at several sites within the brain and exerts tonic central constraint on sympathetic outflow (38, 46). Given that L-NNA, as used in this study, is known to cross the blood-brain barrier, central inhibition of NO synthase may contribute, in part, to the increases in blood pressure, following L-NNA, particularly in dex-exposed animals.

There are several limitations of the study. Maternal food intake, which could be influenced by dexamethasone treatment and/or zygosity was not measured. Animals of both genders and from single and twin gestations and both genders were also utilized for the studies. Work by a number of investigators have found sex-specific differences in the programming of hypertension and glucose intolerance (29). Twin gestation may also predispose to physiological programming (44). These effects may be related to differences in nutritional status and/or the development of the hypothalamic-pituitary adrenal axis (for review see McMillen and Robinson (29). Finally, lambs were gently restrained in a sling-frame assembly for the study. Differences in the stress response to this intervention may also have contributed to our findings.

**Perspectives**

We have demonstrated that in this sheep model, “programming” resets the baroreflex and likely modifies sympathetic efferent tone. Furthermore, “programming” by exposure to early gestation glucocorticoids appears augments nitric oxide mediated vasodilatation early in life. The interaction of these two systems in long-term blood pressure regulation requires further investigation. Alterations in either the “buffering” capability of NO mediated vasodilation or autonomic control of circulatory function may ultimately contribute to the development of “programmed” hypertension. Further understanding of how the perinatal environment effects physiological
systems is necessary to enable the development of interventions aimed at alleviating the deleterious consequences of intrauterine programming.
ACKNOWLEDGEMENTS

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LITERATURE CITED


FIGURE LEGENDS

Figure 1. Effect of intravenous bolus doses of angiotensin II (20, 50, 100 ng/kg) on mean arterial blood pressure (MABP) and heart rate in control and dexamethasone-exposed lambs (n=6 for each group). Values represent means ± SE. * P < 0.05 compared to control.

Figure 2. Effect of intravenous bolus doses of phenylephrine (2, 5, 10 ug/kg) on mean arterial blood pressure (MABP) and heart rate in control and dexamethasone-exposed lambs (n=6 for each group). Values represent means ± SE.

Figure 3. Mean baroreflex curves relating heart rate and mean arterial blood pressure in control ( ) and early gestation dexamethasone exposed lambs ( ) (n = 6 for each group). Individual values for different baroreflex function parameters are presented in Table 2.

Figure 4. Effect of the ganglionic blocking agent tetraethylammonium bromide (TEA, 10 mg/kg iv), the nitric oxide synthase inhibitor N(G)-nitro-L-arginine (L-NNA, 25 mg/kg iv), and the angiotensin II type 1 receptor antagonist losartan (10 mg/kg iv) on mean arterial blood pressure (MABP) and heart rate in control and dexamethasone-exposed lambs (n=6 for each group). Values represent means ± SE. * P < 0.05 compared to control.

Figure 5. Mesenteric and femoral artery microvessel contractile responsiveness to cumulative doses of KCl, norepinephrine and endothelin-1 (n = 6 for each group). Values are displayed as means ± SE. * P <0.05 compared to control group (ANOVA, effect for treatment group).
Figure 6. Femoral artery vasorelaxation responses to cumulative doses of forskolin and sodium nitroprusside (n = 4 for each group). Values are displayed as means ± SE. Vessels were preconstricted with norepinephrine prior to addition of forskolin or sodium nitroprusside.
Table 1. Growth, arterial blood and hemodynamic parameters for control and early gestation
dexamethasone exposed lambs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 6)</th>
<th>Dex-exposed (n = 6)</th>
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</thead>
<tbody>
<tr>
<td>Age, days</td>
<td>11 ± 1</td>
<td>12 ± 1</td>
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<tr>
<td>Weight, kg</td>
<td>7.05 ± 0.72</td>
<td>7.61 ± 0.74</td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Twin gestation*</td>
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<tr>
<td>pH</td>
<td>7.44 ± 0.01</td>
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<td>PCO₂, mmHg</td>
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<td>33 ± 1</td>
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<tr>
<td>PO₂, mmHg</td>
<td>96 ± 3</td>
<td>98 ± 3</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>30 ± 2</td>
<td>31 ± 3</td>
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<td>Heart rate1, bpm</td>
<td>226 ± 21</td>
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<td>SBP, mmHg₁</td>
<td>98 ± 4</td>
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<td>DBP, mmHg₁</td>
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<td>59 ± 4</td>
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<td>MABP₁, mmHg</td>
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<td>Heart rate2, bpm</td>
<td>208 ± 20</td>
<td>221 ± 10</td>
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<tr>
<td>MABP², mmHg</td>
<td>75 ± 4</td>
<td>77 ± 4</td>
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</table>

Values are means ± SE. Age and weight reflect values from first day of the study. 1, 2 represent
values obtained on first (1) and second (2) day of the study. SBP, systolic blood pressure; DBP,
diastolic blood pressure; MABP, mean arterial blood pressure. *Only one lamb from each twin
gestation was used for the study.
Table 2. Parameter values describing baroreflex control of heart rate in control and dexamethasone exposed newborn lambs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 6)</th>
<th>Dex-exposed (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper plateau (P₄ + P₁), beats/min</td>
<td>279 ± 16</td>
<td>289 ± 6</td>
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<tr>
<td>Lower plateau (P₄), beats/min</td>
<td>107 ± 11</td>
<td>113 ± 7</td>
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<td>Gain, bpm/mmHg</td>
<td>-5.28 ± 0.42</td>
<td>-6.28 ± 0.76</td>
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<td>Curve midpoint (P₃), mmHg</td>
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<td>84 ± 3*</td>
</tr>
<tr>
<td>Threshold, mmHg</td>
<td>55 ± 3</td>
<td>68 ± 4*</td>
</tr>
<tr>
<td>Saturation, mmHg</td>
<td>96 ± 4</td>
<td>105 ± 4</td>
</tr>
</tbody>
</table>

Values were obtained after averaging two separate determinations of baroreflex for each animal.

Values are means ± SE. n = no. of animals. * P < 0.05 compared to control.
Figure 1

The figure shows a bar graph comparing the change in Mean Arterial Blood Pressure (MABP) and change in heart rate between control and dexamethasone-exposed groups at different doses (20 ng/kg, 50 ng/kg, 100 ng/kg). The error bars indicate the standard error of the mean. Significant differences are marked with an asterisk (*).
Figure 2

![Graph showing the change in MABP (mmHg) and heart rate (bpm) for control and dexamethasone-exposed groups at 2 μg/kg, 5 μg/kg, and 10 μg/kg. The data is represented as bars with error bars indicating the standard error.](image-url)
Figure 3

- TEA
- Losartan
- L-NNA

Heart Rate (bpm) and MABP (mmHg) comparisons between Control and Dexamethasone-exposed groups.
Figure 4

Mesenteric artery vs. Femoral artery

- Control
- Dex treated

% of response vs. [KCl] (mM)

% of response vs. log [Norepinephrine], (M)

% of response vs. log [Endothelin-1], (M)
Figure 5
Figure 6

The graph shows the relationship between mean arterial blood pressure and heart rate for control and Dex-exposed subjects. The y-axis represents heart rate (bpm) ranging from 0 to 350, and the x-axis represents mean arterial blood pressure ranging from 0 to 125. Two lines are depicted, one for control subjects (square symbols) and one for Dex-exposed subjects (circle symbols). The graph indicates that as the mean arterial blood pressure increases, the heart rate decreases.