Early gestation dexamethasone programs enhanced postnatal ovine coronary artery vascular reactivity

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Submitted 16 March 2004; accepted in final form 16 June 2004

A number of animal models have been developed to ascertain the intrauterine factors that presage the development of cardiovascular disease and investigate the mechanisms involved. Protein restriction models in pregnant rats demonstrated that maternal undernutrition is associated with the development of hypertension in the offspring. Excessive fetal exposure to glucocorticoids, related to inhibition of the placental enzyme responsible for inactivation of maternal corticosteroids, 11β-hydroxysteroid dehydrogenase, may play a role in this response (13, 16). Consistent with this hypothesis, inhibition of maternal corticosteroid synthesis, produced by administration of metapyrone, mitigated the effects of maternal protein restriction on the subsequent development of hypertension in the rat offspring (13, 14).

A role for excessive glucocorticoid exposure in fetal programming is supported by studies of early gestation corticosteroid administration in sheep. Dodic and colleagues (8) infused dexamethasone, which readily crosses the placenta without metabolism by 11β-hydroxysteroid dehydrogenase, to pregnant ewes for 48 h beginning at 27 days gestation (term being 145–150 days). The offspring were hypertensive at 3 mo of age and remained hypertensive at 10 and 18 mo of age, despite normal intrauterine and postnatal growth. At 7 yr of age, similarly exposed offspring displayed left ventricular hypertrophy and cardiac dysfunction (9). Further studies of this ovine model of fetal programming have demonstrated that adult offspring exposed to dexamethasone early in gestation have organ-specific alterations in renin-angiotensin system gene expression (7). There appears to be a critical window that allowed persistent programming of the hypertensive phenotype, since exposure to the same maternal dose at 64 days gestation failed to result in hypertensive offspring (8).

We recently demonstrated that acute glucocorticoid infusion to late-gestation ovine fetuses produces coronary but not mesenteric artery hyperresponsiveness to ANG II, in part related to enhanced expression of the ANG II type 1 (AT1) receptor (24). Late prenatal glucocorticoid exposure also results in fetal hypertension and enhances the contractile response of femoral arteries to endothelin and depolarizing potassium solutions, while attenuating the vasodilator effects of forskolin and bradykinin (1, 6). However, late-gestation corticosteroid exposure fails to program the development of hypertension during postnatal life (17). We postulated that early exposure to glucocorticoids, which is known to program the development of hyper-

THERE ARE EPIDEMIOLOGICAL links between poor fetal growth and the subsequent development of atherosclerosis. The concept of the fetal programming of adult disease found its inception through the work of Barker and colleagues (2). Their initial epidemiological data are supported by a number of international studies suggesting that lower birth weight, a potential marker for an adverse intrauterine environment, is an independent risk factor for the development of both hypertension and coronary artery disease (5, 15, 23).

Robert D. Roghair, Robert D., Fred S. Lamb, Francis J. Miller, Jr., Thomas D. Scholz, and Jeffrey L. Segar. Early gestation dexamethasone programs enhanced postnatal ovine coronary artery vascular reactivity. Am J Physiol Regul Integr Comp Physiol 288: R46–R53, 2005. First published June 24, 2004; doi:10.1152/ajpregu.00165.2004.—Excessive exposure of the fetus to maternally derived corticosteroids has been linked to the development of adult-onset diseases. To determine if early gestation corticosteroid exposure alters subsequent coronary artery reactivity, we administered dexamethasone (0.28 mg·kg−1·day−1) to pregnant ewes at 27–28 days gestation (term being 145 days). Vascular responsiveness was assessed in endothelium-intact coronary and mesenteric arteries isolated from steroid-exposed and age-matched control fetal sheep at 123–126 days gestation and lambs at 4 mo of age. Lambs exposed to maternal dexamethasone had higher mean arterial blood pressures than the age-matched controls (93 ± 3 vs. 83 ± 5 mmHg, P < 0.05). Mesenteric arteries from the steroid-exposed fetuses displayed diminished responses to ANG II, relative to controls. In 4-mo-old lambs, prenatal dexamethasone exposure significantly increased coronary artery vasoconstriction to ANG II, ACh, and U-46619, but not KCl. In contrast, postnatal mesenteric artery reactivity was unaltered by steroid exposure. Compared with fetal mesenteric reactivity, postnatal mesenteric reactivity to ANG II, phenylephrine, and U-46619 was diminished, whereas the response to 120 mmol/l KCl was heightened. Coro-
tension, would alter coronary vascular reactivity later in development. In particular, we sought to determine if early gestation corticosteroid exposure persistently and selectively alters coronary artery reactivity, with emphasis on coronary responsiveness to ANG II.

**METHODS**

**Tissue Collection**

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. Time-dated pregnant ewes were obtained from a local source and housed in similar environments throughout the course of study. At 27–28 days gestation, dexamethasone (0.28 mg·kg⁻¹·day⁻¹; Gensia Sicor Pharmaceuticals, Irvine, CA) was administered to the ewes by continuous intravenous infusion over 48 h. At 123–126 days gestation (term being 145 days), the first set of steroid-treated and gestational age-matched control ewes (n = 6 for each group) were anesthetized with 12 mg/kg thiopental sodium (Abbott Laboratories, Abbott Park, IL) and intubated and ventilated with a mixture of 1% halothane-33% oxygen-66% nitrous oxide. Fetuses were delivered by cesarean section and killed with intravenous pentobarbital sodium (50 mg/kg; Abbott Laboratories). Coronary and second-generation mesenteric arteries were collected from up to two fetuses per litter.

A second set of steroid-treated ewes was allowed delivery, and the offspring were raised at a remote facility in a fashion identical to that used for age-matched control lambs. The steroid-exposed lambs (n = 7) were studied concurrent with the non-dexamethasone-exposed age-matched control lambs (n = 7) at a postnatal age of 4 mo. After anesthesia was induced and maintained as previously described, a polyethylene catheter (PE-90) was inserted in the lamb’s left femoral artery and sutured in place. The catheter was tunneled subcutaneously and secured to the lamb’s back using porous elastic bandages. Ampicillin (Sigma, St. Louis, MO) was administered at the completion of surgery (2 g im), followed by intramuscular injections (1 g) every 12 h for 3 days. After surgery, the lamb was returned to an individual pen and allowed free access to food and water. After a 3-day recovery period, arterial blood pressure and heart rate were recorded using MacLab software (AD Instruments, Colorado Springs, CO) over a 1-h time period while the lambs stood comfortably supported by a sling. The transducer was thereby maintained at the level of the heart. At the completion of the study, the lambs were killed with intravenous pentobarbital sodium (50 mg/kg). Coronary, carotid, and second-generation mesenteric arteries were then collected.

**Isolated Vessel Contractile Responses**

Circumflex coronary artery segments were cleansed of adherent connective tissue and sectioned into 3-mm rings on the day of collection. The second-generation mesenteric arteries and carotid arteries were stored in chilled (4°C) bicarbonate-buffered physiological salt solution (PSS) overnight, before being sectioned into 4-mm rings. The endothelium was left intact, and the rings were mounted in individual 18-ml isolated organ chambers and connected to an isometric force transducer by 32-gauge stainless steel wire. Contractile responses were recorded with an eight-channel MacLab SE and stored on a Power Macintosh 8600 computer. The length-tension relationship was defined experimentally to 30 and 90 mmol/l KCl at varying passive stretch. Passive stretch was set at 90% of the tension required to obtain peak responses to KCl (0.7 g for both coronary and mesenteric arteries with ID 1–2 mm, 3 g for carotid arteries with ID 2–3 mm), and the rings were allowed to equilibrate in PSS at 37°C for 60 min before the start of experimentation. PSS was aerated with a mixture of 95% O₂-5% CO₂; the composition was as follows (in mmol/l): 130 NaCl, 4.7 KCl, 1.18 KH₂PO₄, 1.17 MgSO₄·7H₂O, 14.9 NaHCO₃, 1.6 CaCl₂·H₂O, 5.5 dextrose, and 0.03 CaN₂-EDTA (pH 7.30).

Initially, to allow normalization of subsequent vasoconstriction data, the contractile response of each vessel to a set concentration of KCl was recorded (120 mmol/l for coronary and mesenteric arteries, 30 mmol/l for carotid arteries). With the use of separate vascular rings for each vasoconstrictor, cumulative concentration responses to KCl (5–90 mmol/l), ANG II (10⁻¹¹ to 10⁻⁷ mol/l), ACh (postnatal coronary and carotid arteries, 10⁻¹⁰ to 10⁻⁵ mol/l), and phenylephrine (mesenteric arteries, 10⁻⁶ to 10⁻³ mol/l) were conducted with addition of increasing concentrations of the agent under study at 5-min intervals, before loss of the preceding vascular response. For the final three sets of postnatal sheep, to further investigate possible vessel-specific alterations in the ANG II signaling pathway, carotid arteries (rather than mesenteric arteries) were evaluated, and concentration responses of the coronary arteries to ANG II were determined after pretreatment with either the nitric oxide synthase inhibitor N⁵-nitro-L-arginine (L-NNA, 10⁻⁴ mol/l) or the angiotensin type 2 receptor (AT₂) antagonist PD-123319 (10⁻⁵ mol/l).

The arteries were then reequilibrated to their baseline with multiple washes of PSS over 1 h before preconstriction of each vessel with the thromboxane A₂ mimetic U-46619 (10⁻⁶ mol/l). Although time controls were not used to ensure maintenance of the U-46619 response through the ensuing relaxations, the response to U-46619 was consistently maintained over the preceding 5 min. To investigate potential alterations in cyclic nucleotide-mediated vasodilation in the coronary and mesenteric arteries, cumulative concentration responses to sodium nitroprusside (10⁻¹⁰ to 10⁻⁵ mol/l), isoproterenol (10⁻¹⁰ to 10⁻⁵ mol/l), forskolin (10⁻¹¹ to 10⁻⁶ mol/l), and Sp-cAMPS (10⁻⁶ to 10⁻⁴ mol/l) were conducted with addition of increasing concentration of the agent at 8-min intervals. Microsoft Excel 2000 was used to generate concentration-response curves for each vasoactive agent. After data analysis, Excel formatting options were used to aesthetically smooth the contour of the curves. All PSS reagents and vasoactive compounds were acquired from Sigma Chemical, with the exception of U-46619, which was supplied by Alexis (San Diego, CA).

**Immunoblotting.** Western blot analysis for AT₁ and AT₂ receptors, as well as endothelial nitric oxide synthase (eNOS), was performed as previously described (22, 24, 28). Nitrocellulose blots (20 μg protein/lane) were incubated with the primary antibody at a 1:1,000 (AT₁ and eNOS) or 1:2,000 (AT₂) dilution for 2 h at room temperature. Blots were then incubated with a 1:3,000 dilution of goat anti-rabbit or a 1:2,000 dilution of goat anti-mouse horseradish peroxidase (HRP) conjugated antibody (Sigma) at room temperature for 1 h. Binding of the secondary antibody was detected using a chemiluminescent system consisting of HRP/hydrogen peroxide oxidation of luminol (Pierce, Rockford, IL). Blots were then exposed to Kodak X-AR X-ray film for 1 min. Films were digitized, and the difference between protein signals and background was quantitated using NIH Image (National Institutes of Health, http://rsb.info.nih.gov/nih-image). Results were normalized by arbitrarily setting the densitometry of control fetal coronary arteries to 100.

**Data analysis.** Physiological parameters were compared using Student’s unpaired, two-tailed t-test (with significance at P < 0.05). Protein expression and vascular responses were compared using ANOVA, factoring for treatment and age group, thereby comparing each set of data in its entirety (dexamethasone exposed vs. control and fetal vs. postnatal). If ANOVA identified significant differences (P < 0.05), pairwise comparisons were made using the Tukey test, with P < 0.05 considered significant. All analyses were performed using SAS System 9 for Microsoft Windows (SAS Institute, Cary, NC). All values are presented as means ± SE, and n refers to the number of animals studied.
RESULTS

Physiological Parameters

Dexamethasone-exposed fetuses came from smaller litters and thus weighed more than the age-matched fetal controls (Table 1). There were no significant differences in age, body weight, or organ weights between the postnatal groups. The mean arterial blood pressure was higher in the dexamethasone-exposed than control lambs (93 ± 3 vs. 83 ± 5 mmHg, P < 0.05), whereas the heart rate was similar. Systemic hemodynamics were not measured in the fetuses.

Vascular Reactivity

Responses to voltage-dependent calcium channel activation. The vasorestrictive responses of the coronary, mesenteric, and carotid artery segments to KCl were not significantly altered by dexamethasone exposure in either the fetal or postnatal groups (Fig. 1, A–C, and Table 2). KCl responses in the coronary arteries were independent of age (Fig. 1A and Table 2). Among the mesenteric arteries, postnatal maturation was associated with heightened responses to 120 mmol/l KCl (Table 2) but diminished sensitivity to lower concentrations of KCl (Fig. 1B).

Responses to second messenger-dependent vasoconstrictors. The vasorenstrictive responses of the fetal coronary artery segments to ANG II and U-46619 were not significantly altered by dexamethasone exposure (Fig. 1, D and J). Steroid-exposed fetal mesenteric arteries were significantly less responsive to ANG II than the age-matched control arteries (Fig. 1E), with no alteration noted in their responsiveness to phenylephrine or U-46619 (Fig. 1, H and K).

Coronary artery segments from lambs exposed to antenatal dexamethasone exhibited significantly greater vasorestrictive responses to ANG II, ACh, and U-46619, relative to control vessels (Fig. 1, D, G, and J). Although the carotid arteries from the same group of dexamethasone-exposed lambs developed analogous heightened responses to U-46619 (Fig. 1L), there was no steroid-induced alteration in their responses to ANG II (Fig. 1F), and they did not exhibit any significant vasorestrictive response to ACh (Fig. 1I). The responses of the postnatal mesenteric arteries to ANG II, phenylephrine, and U-46619 were all unaltered by antenatal dexamethasone infusion and significantly diminished in comparison with the responses of the fetal mesenteric arteries (Fig. 1, E, H, and K). The latter finding was, in part, related to the heightened vasoconstrictive response to 120 mmol/l KCl noted in the postnatal compared with the fetal mesenteric arteries (given the use of that response in normalizing the responses to the aforementioned vasoconstrictors).

As with the mesenteric arteries, the coronary arteries from the 4-mo-old control lambs were significantly less responsive to U-46619 (10⁻⁸ mol/l) than their fetal counterparts (Fig. 1J). Interestingly, coronary artery responses to ANG II were enhanced by both antenatal steroid exposure and postnatal maturation (Fig. 1D), whereas those same factors were associated with diminished mesenteric artery responsiveness to ANG II (Fig. 1E). The exaggerated responses of the postnatal steroid-exposed coronary arteries to angiotensin were equally apparent after pretreatment with PD-123319 and l-NNA (Fig. 2, A and B; P = 0.09 and P < 0.05, respectively, n = 3). Tachyphylaxis to increasing concentrations of ANG II was consistently noted under all study conditions (Figs. 1 and 2).

Cyclic nucleotide-mediated pathway reactivity. There were no significant treatment group differences in fetal or postnatal vascular responses to sodium nitroprusside, isoproterenol, or Sp-cAMPS (Fig. 3). Dexamethasone exposure was associated with enhanced postnatal coronary and fetal mesenteric artery responses to forskolin (Fig. 3, E and F). That alteration was not present within the fetal coronary or postnatal mesenteric arteries (Fig. 3, E and F).

Although there were no age group-dependent differences in coronary or mesenteric artery vasodilatation to sodium nitroprusside (Fig. 3, A and B, relative to the fetal vessels, postnatal coronary arteries were less responsive to isoproterenol (Fig. 3C), and postnatal mesenteric arteries were less responsive to forskolin (Fig. 3F). Finally, in contrast to their diminished responses to isoproterenol, the postnatal coronary arteries had increased responses to Sp-cAMPS (P < 0.05 vs. fetal coronary arteries; Fig. 3G).

Immunoblotting

Western blotting consistently demonstrated the presence of AT₁ and AT₂ receptor proteins in the coronary arteries of fetal sheep and 4-mo-old lambs (Fig. 4, A and C). There was no significant difference in AT₁ receptor protein expression associated with either dexamethasone exposure or postnatal maturation (Fig. 4B). AT₂ receptor protein expression was also not altered by dexamethasone exposure, although expression was increased in the postnatal coronary arteries (P < 0.05 vs. fetal groups, Fig. 4D). There was no significant alteration in postnatal coronary artery eNOS expression after dexamethasone exposure (data not shown).

DISCUSSION

Since the initial reports by Barker and colleagues (2) showing an inverse relationship between birth weight and the development of coronary artery disease, there has been a growing interest in the programming of adult diseases. Studies in a number of animal models have demonstrated a link among...
maternal undernutrition, excessive fetal exposure to glucocorticoids, and postnatal hypertension. In contrast to the rapidly expanding body of research on the fetal origins of hypertension, there is a paucity of published work on the fetal origins of coronary artery disease. The ovine model we employed not only confirmed previous findings that early-gestation exposure to glucocorticoids elevates postnatal blood pressure (8) but also demonstrated that tissue-specific alterations in coronary artery function.

Fig. 1. Cumulative concentration-response curves for coronary (A, D, G, J), mesenteric (B, E, H, K), and carotid (C, F, I, L) artery vasoconstriction evoked by KCl (A–C), ANG II (D–F), ACh or phenylephrine (G–I), or U-46619 (10^{-6} mol/l, J–L). Responses were assessed in dexamethasone (Dex)-treated (filled circle, n = 6 animals) or control (CTL; open circle, n = 6 animals) fetuses and dexamethasone-treated (filled square, n = 7 for postnatal coronary, n = 4 for postnatal mesenteric, and n = 3 for postnatal carotid arteries) or control (open square, n = 7 for postnatal coronary, n = 4 for postnatal mesenteric, and n = 3 for postnatal carotid arteries) lambs. Values are displayed as means with vertical lines indicating SE. The † or * denotes significant differences between dexamethasone-infused and control fetuses or lambs, respectively (P < 0.05). The ‡ or † denotes significant differences between fetal and lamb responses within the control or dexamethasone-infused groups, respectively (P < 0.05).
vasoreactivity are present in these animals. To our knowledge, this is the first demonstration in an animal model of fetal programming of coronary artery responsiveness to vasoactive agents.

The major finding of this study is the observation that coronary arteries from 4-mo-old lambs that were exposed to increased maternal levels of glucocorticoids 8 mo prior displayed enhanced vasoconstriction to ANG II, U-46619, and ACh. These effects were not present in the late-gestation fetuses, evidence that these vascular changes develop after birth, a period remote from the glucocorticoid exposure. Changes in vasoreactivity were not seen in the mesenteric arteries of 4-mo-old lambs, illustrating a tissue-specific effect of early dexamethasone exposure. Taken together, these findings demonstrate that early gestation exposure to increased glucocorticoids programs postnatal coronary vascular reactivity. Of these changes, the postnatal increase in response to ANG II is most intriguing, particularly in light of the well-described role of ANG II in atherogenesis (19, 25).

The effects of ANG II are mediated by two distinct receptors, classified as type 1 (AT₁) and type 2 (AT₂) based on selective antagonism by peptide and nonpeptidic ligands (4, 29). The functions of the AT₂ receptor are unclear, although it may exert proapoptotic and vasodepressor effects (11, 32). Most of the known responses to ANG II are mediated by the AT₁ receptor, and, within the cardiovascular system, AT₁ receptor expression is acutely responsive to increased glucocorticoid exposure (18, 20, 26). The differing contractile responses to ANG II in the coronary and mesenteric arteries of glucocorticoid-exposed lambs compared with control lambs suggest fundamental differences exist among the vessels. Differential expression of the AT₁ receptor is one obvious factor that could contribute to the observed responses. We have previously shown that administration of glucocorticoids acutely increases coronary artery AT₁ receptor expression in fetal sheep (24). However, in the present study, no significant differences were seen in coronary AT₁ receptor expression between control and glucocorticoid-exposed animals. Although the present study’s power may not have been adequate to detect differential receptor protein expression, it is plausible that the physiological effects of remote steroid exposure differ from those of acute steroid infusion. Although changes in receptor expression, as quantified by immunoblotting, or localization, verified by immunohistochemistry (data not shown), do not appear to be responsible for the steroid-mediated changes in contractility, it remains possible that differences in receptor affinity, coupling to second messengers, and downstream effectors in the angiotensin signaling pathway may contribute to the observed changes.

The increased coronary artery contractile responses to ANG II after pretreatment with the AT₂ antagonist PD-123319 are consistent with the previously demonstrated divergent roles of the coronary AT₁ and AT₂ receptors (32). In particular, the AT₂ receptor appears to mediate coronary vasorelaxation. Thus it is possible that decreased coronary AT₂ receptor expression and/or function after glucocorticoid exposure contributes to the observed responses. However, the coronary artery vasoconstrictive responses to ANG II were still greater ($P = 0.09, n = 3$) in steroid-exposed lambs after pretreatment with PD-123319, suggesting alterations in AT₂ receptor function are not involved in the steroid-induced alterations in ANG II responsiveness. This is further supported by the immunoblot results showing a lack of steroid-induced alteration in the coronary expression of AT₂ receptors.

In addition to its potent vasoconstrictor properties via G protein-mediated pathways, ANG II also decreases intra-

**Table 2. Maximum vasoconstrictive force generated after stimulation of control or dexamethasone-exposed vascular rings with KCl (120 mmol/l for coronary and mesenteric arteries, 30 mmol/l for carotid arteries).**

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<th>Prenatal Groups</th>
<th>Postnatal Groups</th>
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<tr>
<td></td>
<td>Control</td>
<td>Dexamethasone</td>
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<tr>
<td>Coronary arteries</td>
<td>0.9±0.1</td>
<td>1.2±0.1</td>
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<tr>
<td>Mesenteric arteries</td>
<td>1.9±0.2</td>
<td>5.9±1.0*</td>
</tr>
<tr>
<td>Carotid arteries</td>
<td>6.0±0.7</td>
<td>7.0±0.8</td>
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Values are displayed as gram-force (means ± SE); $n = 6$ for prenatal arteries, $n = 7$ for postnatal coronary arteries, $n = 4$ for postnatal mesenteric arteries, $n = 3$ for postnatal carotid arteries. *Significantly different from corresponding prenatal values at $P < 0.05$.

![Fig. 2. Cumulative concentration-response curves for postnatal coronary artery vasoconstriction evoked by ANG II after pretreatment with PD-123319 (10⁻⁵ mol/l, A) or N-nitro-L-arginine (10⁻⁴ mol/l, B). Responses were assessed in dexamethasone-treated (■, $n = 3$) and control (○, $n = 3$) lambs. Values are displayed as means with vertical lines indicating SE. *Significant difference between dexamethasone-infused and control lambs ($P < 0.05$).](https://www.ajpregu.org)
cellular nitric oxide availability through the endothelial production of reactive oxygen species (19). In the coronary vessel segments examined, the presence of an intact and functional endothelium was suggested by the marked enhancement of coronary artery vasoconstriction to ANG II after pretreatment with the nitric oxide synthase inhibitor L-NNA. Furthermore, the persistently augmented vasoconstriction to ANG II in the steroid-exposed coronary arteries after pretreatment with L-NNA, and the eNOS immunoblot results, suggests that diminished production of nitric oxide does not play a major role in the increased responses to ANG II in these vessels.

Fig. 3. Cumulative concentration-response curves for coronary (A, C, E, G) and mesenteric (B, D, F, H) artery vasodilation (as % of preinduced tone from 10^-6 mol/l U-46619) produced by sodium nitroprusside (A and B), isoproterenol (C and D), forskolin (E and F), or Sp-cAMPS (G and H). Responses were assessed in dexamethasone-treated (●, n = 6) or control (○, n = 6) fetuses and dexamethasone-treated (■) or control (□) lambs (n = 7 for postnatal coronary and n = 4 for postnatal mesenteric arteries). Values are displayed as means with vertical lines indicating SE. The § or * denotes significant differences between dexamethasone-infused and control fetuses or lambs, respectively (P < 0.05). The † or ‡ denotes significant differences between fetal and lamb responses within the control or dexamethasone-infused groups, respectively (P < 0.05).
Alternatively, the heightened responses of the steroid-exposed coronary arteries to compounds that act, in part, through phospholipase C (e.g., ACh, angiotensin, and the thromboxane A2 mimetic U-46619) may indicate a more global effect in the programming of the coronary arteries toward increased contraction with second messenger-mediated elevation in intracellular calcium. For example, modulatory roles of prostenoids may be different in steroid-exposed compared with control coronary arteries. ANG II is known to induce the release of arachidonic acid metabolites, and cyclooxygenase and lipoxygenase inhibitors have been shown to attenuate ANG II-mediated contractile response (30). Alterations in the structure and mechanical properties of the vessels may also contribute to increased contractility. Vessel remodeling related to changes in collagen, elastin, and extracellular matrix proteins has been shown to contribute to vascular abnormalities. Finally, we cannot rule out the possibility that the observed differences in coronary artery reactivity were secondary to the development of hypertension in the glucocorticoid-exposed lambs, rather than a primary phenomenon. Regardless of the mechanisms involved, these results stress the need for further vessel-specific research in this area and highlight the possibility that the pathway connecting corticosteroids and hypertension may be different from that linking antenatal corticosteroid exposure and coronary artery disease (21, 27).

We observed no significant glucocorticoid-related differences in vascular responses to sodium nitroprusside, isoproterenol, or a membrane-permeable analog of cAMP (Sp-cAMPS). However, increased vasodilation to forskolin was present in the steroid-exposed postnatal coronary arteries and fetal mesenteric arteries, unlike the attenuated responses seen in femoral arteries after late prenatal exposure (1). Although we did not use endothelium-dependent vasodilators in the postnatal group, it is important to note that prenatal corticosteroid exposure is typically associated with paradoxically enhanced femoral artery vasodilation to ACh (1, 17). This contrasts with the impaired vasorelaxation to ACh and sodium nitroprusside viewed as a mechanism of hypertension in the offspring of undernourished rats (3, 12).

Several properties of coronary arteries make them uniquely susceptible to the long-term effects of fetal glucocorticoid exposure. Unlike the near-complete abolishment of mesenteric artery ANG II responsiveness seen with advanced age or steroid exposure, there is heightened coronary artery ANG II responsiveness with postnatal maturation and steroid exposure. Additionally, coronary arteries develop vasoconstriction, rather than endothelium-dependent vasodilation, to ACh (24, 31), a response that was exaggerated after early gestation glucocorticoid exposure. Finally, unlike other tissues like the placenta, the 11β-hydroxysteroid dehydrogenase isozyme within the coronary arteries functions largely as a reductase, rather than a dehydrogenase, regenerating active glucocorticoids from their inactive 11-dehydro derivatives (10). Taken together, these vessel-specific features would make the coronary arteries uniquely susceptible to the effects of increased circulating levels of corticosteroids within the ewe or fetus.

**Perspectives**

The present study provides novel information regarding tissue-specific glucocorticoid-induced alterations in coronary artery reactivity. If permanently programmed, the heightened contractile response of the coronary arteries to ANG II after early gestation dexamethasone exposure may contribute to accelerated atherosclerosis, possibly linking an adverse intra-
uterine environment and cardiovascular morbidity. In addition, more global effects on enhanced phospholipase C-mediated vasoconstriction may contribute to the development of coronary artery disease after undernutrition and increased intrauterine steroid exposure. Continued investigation into the effects of early gestational exposures on postnatal cardiovascular health is essential to develop methods of mitigating and ultimately preventing an important subset of cardiovascular morbidities.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Yaowen Hsu for expert assistance in performing the statistical analyses.

GRANTS

This study was supported by National Institute of Environmental Health Sciences Grant R21 ES-012268 to J. L. Segar and an Advancing Newborn Medicine fellowship grant from Forest Pharmaceuticals to R. D. Roghair.

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