

Antenatal Betamethasone Alters Vascular Reactivity in Adult Female Ovine Cerebral Arteries

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ABSTRACT: Although the use of antenatal glucocorticoids has resulted in decreased neonatal morbidity/mortality, recent animal studies have raised concerns regarding adverse effects of these medications on postnatal cardiovascular function. We hypothesized that antenatal betamethasone (Beta) exposure alters cerebral vascular reactivity in adult female sheep. We observed that K^+ -induced constriction was comparable in middle cerebral artery (MCA) from Beta-exposed animals and age-matched controls. Pressure-induced constriction was significantly attenuated in MCA from Beta-exposed compared with control sheep. Inhibition of NOS significantly augmented pressure-induced constriction in MCA from both Beta-exposed and control sheep, whereas cyclooxygenase (COX) inhibition augmented pressure-induced constriction only in MCA from Beta-exposed sheep. Furthermore, NOS and COX inhibition significantly attenuated bradykinin (BK)-induced dilation in MCA from both Beta-exposed and control sheep. However, there seemed to be a greater contribution of both NOS and COX to BK-induced dilation in Beta-exposed compared with control MCA. Our findings demonstrate that fetal exposure to a clinically relevant course of Beta alters cerebral vascular tone and reactivity in adult female sheep. (*Pediatr Res* 68: 344–348, 2010)

Glucocorticoid administration is the standard of care in mothers with preterm labor. However, little is known regarding the cardiac and vascular effects of these medications in adult life. Exposure of the ovine fetus to glucocorticoids is associated with altered kidney development and renal function (1–5). This altered kidney development/function has been linked to moderate elevation in fetal (6,7) and postnatal (1,4,8) blood pressure (BP) in animal models. Altered coronary, femoral, mesenteric, and brachial artery reactivity have been described in offspring exposed to glucocorticoids *in utero* (8–10). More recent studies have shown altered pulmonary vascular development in term rabbits after antenatal exposure to glucocorticoids (11).

After dexamethasone exposure *in utero*, fetal ovine middle cerebral arteries (MCA) develop tachyphylaxis when exposed to >10 nmol/L concentrations of endothelin-1 (12). Furthermore, Schwab *et al.* (13) have demonstrated decreased re-

gional cerebral blood flow and increased cerebral vascular resistance in fetal sheep exposed to Beta late in gestation. Thus, antenatal glucocorticoid exposure can alter cerebral vascular tone and reactivity in fetal life. Little is known regarding the effects of antenatal Beta exposure on the postnatal cerebral circulation, especially in adulthood. We hypothesized that a clinically relevant course of antenatal maternal Beta administration would alter (a) pressure-induced constriction and (b) agonist-induced dilator responses in resistance-size MCA from adult female sheep.

MATERIALS AND METHODS

Animals. All experimental protocols were performed in accordance with National Institutes of Health guidelines for the use of experimental animals. This study was approved by the Animal Care and Use Committee at Wake Forest University School of Medicine.

Pregnant sheep received two intramuscular injections of Beta (1:1 mixture of Beta acetate and Beta phosphate; Celestone Soluspan) in a manner similar to that used in the clinical setting for stimulating lung maturation, *i.e.* 0.17 mg/kg/dose 24 h apart (maximum of 12 mg/d) starting on d 80 of gestation (corresponds to about 24 weeks of human pregnancy). This GA was chosen to more closely approximate the period of human gestation at which antenatal glucocorticoids are used therapeutically. Lambs were delivered spontaneously, weaned at 3 mo of age, and placed in sex-specific enclosures. Estrous-synchronized nonpregnant Beta-exposed (Beta; $n = 7$) and age-matched control (C; $n = 6$) sheep were killed under halothane general anesthesia at 18-mo of age. Estrous synchronization was achieved by implanting sheep with Eazi-Breed CIDR Sheep (progesterone delivery device; Pfizer, Australia) for 14 d, and the sheep was killed 7 d after the implant was removed, *i.e.* at a stage of the estrous cycle where plasma estrogen levels are the lowest.

Isolation of MCA and pressurized arterial wall diameter measurements. After killing, the brain was removed and transferred into cold (4°C) physiologic saline solution [PSS; composition (in mmol/L): 118 NaCl, 4.5 KCl, 25 NaHCO_3 , 1.2 KH_2PO_4 , 1.2 MgCl_2 , 11 dextrose, 2.5 CaCl_2 , and 0.26 EDTA; pH 7.4] aerated with gas containing 21% O_2 , 74% N_2 , and 5% CO_2 . MCA segments (150–250 μm internal diameter and 2–3 mm length) were dissected, cleaned of connective tissue, and transferred to the well of a 2-mL arteriograph (Instrumentation and Model Facility, University of Vermont, Burlington, VT) fitted with a borosilicate (FHC, Bowdoinham, ME) micropipette at both ends. Both the proximal and distal micropipettes were attached to three-way Luer Stopcocks (Cole-Parmer, Vernon Hills, IL). The arteries were cannulated on the proximal pipette, secured with suture, gently flushed free of blood, and then cannulated/secured on the distal pipette. The arteriograph containing the cannulated vessel was then transferred to the stage of an inverted microscope (Nikon) where the vessel diameter was viewed, measured, and continuously recorded using video edge detection equipment and data acquisition software, respectively (IonOptix Inc., Milton, MA). After placing the arteriograph on the microscope stage, the proximal cannula was

Received May 28, 2010; accepted June 8, 2010.

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Supported by startup funds from Brenner Children's Hospital [to D.M.E.], grant HL068728 [to J.P.F.], and grant P01 HD047584 [to J.C.R.].

Abbreviations: Beta, betamethasone; C, control; COX, cyclooxygenase; Indo, indomethacin; L-NAME, N^G -omega-nitro-L-arginine methyl ester; MCA, middle cerebral artery; VSM, vascular smooth muscle

attached to the pressure reservoir and the intraluminal pressure increased to 10 mm Hg to allow a gentle flushing (~2 min) of the intraluminal contents from the vessel. The three-way stopcock on the distal cannula was then closed to pressurize the artery; thus, all experimental protocols were conducted under “no-flow” conditions. In all experiments, pressurized arteries were continuously superfused with PSS (8–10 mL/min) aerated with gas containing 21% O₂, 74% N₂, and 5% CO₂ at 37°C.

Study protocol. The vessels were equilibrated for a total of ~90 min. Arteries were initially pressurized to 10 mm Hg (as described earlier) and allowed to equilibrate for 10 min, allowing the vessel to acclimate at 37°C. The intraluminal pressure was then increased to 60 mm Hg to assess the development of pressure-induced constriction. Vessels exhibiting pressure-induced constriction at 60 mm Hg were dilated with 0.1 μM bradykinin (BK) to confirm endothelial function. After washing out BK with PSS and reestablishing a stable baseline lumen diameter, the pressurized vessels were constricted with a single dose of 50 mM KCl to confirm vascular smooth muscle (VSM) function. Vessels that did not exhibit a minimum of 20% pressure-induced constriction (at 60 mm Hg), K⁺-induced constriction, and BK-induced dilation were excluded from further study. Studies assessing the effect of antenatal Beta on pressure-induced constriction and endothelium-dependent dilation were completed as described later.

Pressure-induced vasoconstriction. In these experiments, the effect of antenatal Beta exposure on pressure-induced constriction was determined both in the absence and presence of NOS and cyclooxygenase (COX) inhibitors. Because each vessel acted as its own experimental control, each arterial segment was exposed to three sequential pressure curves; each pressure curve consisted of a step-wise increase in intraluminal pressure from 10 to 120 mm Hg. Changes in lumen diameter were recorded at each pressure.

In all experiments, the first pressure-diameter relationship acted as the “control” after which the tissues were allowed to recover for 10 min at 10 mm Hg pressure. To determine the role of NOS and COX on pressure-induced constriction, the arterial segments were then incubated with either N^ω-nitro-L-arginine methyl ester (L-NAME; 100 μM), a nonspecific NOS inhibitor, or indomethacin (Indo; 10 μM), a nonspecific COX inhibitor, for 30 min, and a second pressure curve was generated. Tissues were once again returned to 10 mm Hg, allowed to recover for 10 min, and then incubated in Ca²⁺-free PSS with diltiazem (80 μM), an L-type Ca²⁺-channel inhibitor, for 60 min to obtain the passive diameter (DP) for the pressure curves.

Pressure-induced constriction was expressed as a percent decrease of the fully dilated diameter of individual arteries at the same intravascular pressure. These values were obtained using the following equation:

$$\% \text{ Constriction} = \frac{DP - DA}{DP} \times 100$$

where DP is the “passive” diameter of the artery in Ca²⁺-free PSS with diltiazem and DA is the “active” diameter of the artery in response to the stimulus (change in intraluminal pressure) in Ca²⁺-containing PSS in the absence/presence of L-NAME or Indo. In addition, the DP value obtained at 60 mm Hg was used to assess the % pressure-induced constriction with 50 mM K⁺ reported in Figure 1.

Endothelium-dependent dilations. In these experiments, the effect of antenatal steroid exposure on endothelium-dependent dilations was determined in the absence and presence of either NOS or COX inhibitors. As described earlier, each vessel acted as its own experimental control. These experiments were performed at a single intraluminal pressure of 60 mm Hg.

After a stable lumen diameter was obtained at 60 mm Hg, control and Beta-exposed MCA were exposed to increasing concentrations of BK (0.01–10 nmol/L) and vessel diameters recorded for each concentration of BK (“control” values). After completion of the “control” curve, the arterial segments were washed with PSS and the vessel diameter allowed to return to baseline. After a 30-min recovery period, tissues were incubated with either L-NAME (100 μM) or Indo (10 μM) for 30 min to assess the contribution of NOS and COX, respectively, to BK-induced dilations. After completion of the second BK concentration-response relationship, the vessels were washed, allowed to equilibrate with a stable lumen diameter, and then incubated in Ca²⁺-free PSS with diltiazem (80 μM) for 60 min to obtain a DP at 60 mm Hg. The results from these experiments were expressed as percent reversal of constriction. These values were obtained using the following equation:

$$\% \text{ Reversal of constriction} = \frac{DV - DA}{DP - DA} \times 100$$

where DV is the diameter of the artery in response to BK in Ca²⁺-containing PSS.

Chemicals. All chemicals were purchased from Sigma Chemical Co. Chemicals (St. Louis, MO). Drug stock solutions were prepared as follows: BK was prepared daily from 1.0 mM aliquots made in an aqueous stock solution. Aliquots were diluted to 0.1 μM, and serial dilutions were made to the desired concentrations. L-NAME (100 μM) and Indo (10 μM) were prepared daily in fresh PSS at the desired concentration.

Statistical analyses. Data are expressed as mean ± SEM. In instances where multiple arterial segments from a single animal were used for a particular experimental protocol, the results obtained from all experiments were averaged to denote a single “N.” K⁺- and pressure-induced constriction were analyzed using a paired *t* test, one- or two-way ANOVA, or with a repeated measure model with within-group contrasts, as appropriate. Comparisons between groups were analyzed with a *post hoc* Newman-Keul’s multiple comparison test at the 0.05 level of significance. Differences between concentration-response curves to BK, in the absence and presence of L-NAME or Indo, were determined using GraphPad Prism’s preprogrammed nonlinear regression curve fitting model that compares the following three parameters of a dose-response curve: *bottom* (baseline response), *top* (peak response), and pD₂ (–log EC₅₀) values. Individual parameters and/or the BK dose-response curves were considered significantly different from one another if the three parameter comparison test was ≤0.05 level of significance. All statistical analysis were performed using GraphPad Prism version 5.03 (GraphPad Software, Inc., San Diego, CA), where appropriate, the level of statistical significance is indicated as follows: *, *p* < 0.05; **, *p* < 0.01; §, *p* < 0.001; and ¶, not significant (n.s.). Symbols adjacent to vertical bars (e.g. Fig. 2A and B: §) indicate differences between experimental groups.

RESULTS

VSM depolarization with KCl. At an intraluminal pressure of 60 mm Hg, MCA from control (C) and Beta sheep had similar resting (R) diameters (Fig. 1A: C-R = 155 ± 11 μm versus Beta-R = 186 ± 8 μm; *p* = n.s.) and DP (Fig. 1A: C-p = 224 ± 14 μm versus Beta-p = 242 ± 9 μm; C:n = 5,

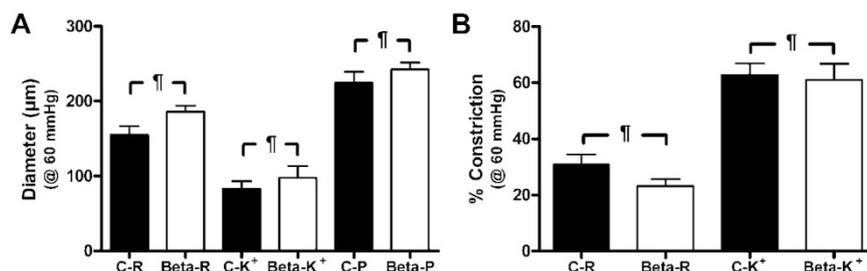


Figure 1. Effect of 50 mM KCl (K⁺) on (A) vascular diameter and (B) arterial constriction in C and Beta MCA pressurized at 60 mm Hg. (A) There was no significant difference between resting (R) (C-R vs Beta-R) and passive (P) (C-P vs Beta-P) diameters in MCA from C and Beta sheep. Furthermore, addition of 50 mM K⁺ caused a comparable decrease in arterial diameter in both C-K⁺ and Beta-K⁺ MCA (C: n = 5; Beta-exposed: n = 7; *p* = n.s.). (B) Percent pressure-induced constriction was similar in MCA from C and Beta sheep. In addition, 50 mM K⁺ resulted in a comparable degree of constriction in both C-K⁺ and Beta-K⁺ MCA (C:n = 5; Beta exposed n = 7; *p* = n.s.).

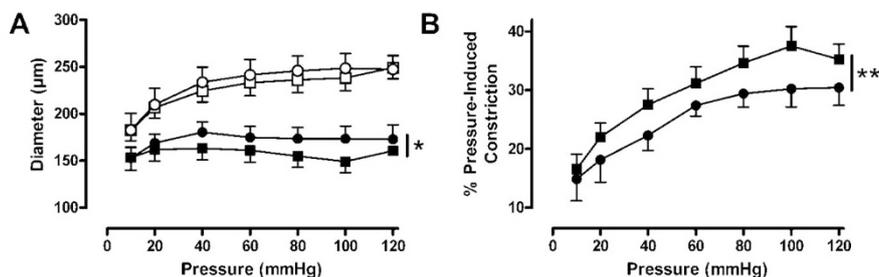


Figure 2. Effect of increasing intraluminal pressure (10–120 mm Hg) on vascular diameter (A) and arterial constriction (pressure-induced constriction; B) in C and Beta MCA. (A): MCAs from both C (□) and Beta (○) sheep had comparable DPs at all pressures studied. In contrast, C arteries (■) constricted more and thus had a smaller lumen diameter in response to a stepwise increase in intraluminal pressure when compared with Beta MCA (●) (C: $n = 5$, Beta exposed: $n = 6$; $p < 0.05$). (B) A stepwise increase in intraluminal pressure resulted in significantly greater pressure-induced constriction in C (■) compared with Beta (●) MCA. (C: $n = 5$, Beta exposed $n = 6$; $p < 0.01$)

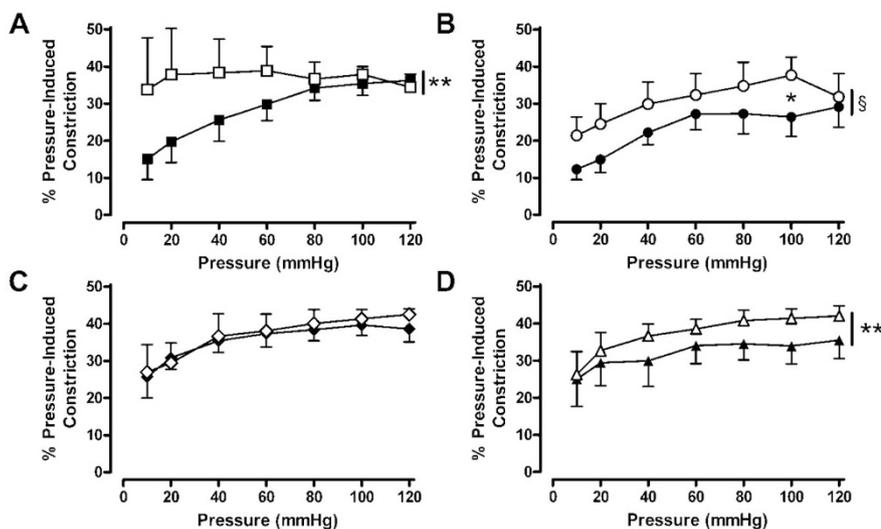


Figure 3. Effect of NOS and COX inhibition on pressure (10–120 mm Hg)-induced constriction in C and Beta MCA. (A, B) Inhibition of NOS with L-NAME (100 μM) caused an increase in pressure-induced constriction in both C (A: $n = 4$; C: ■; C + L-NAME: □; $p < 0.01$) and Beta (B: $n = 5$; Beta: ●; Beta + L-NAME: ○; $p < 0.001$) MCA. (C, D) Inhibition of COX with Indo (10 μM) had no effect on pressure-induced constriction in MCA from C sheep (C: $n = 5$; C: ◆; C + L-NAME: ◇; $p = n.s.$) but caused significant augmentation of pressure-induced constriction in Beta MCA (D: $n = 5$; Beta: ▲; Beta + L-NAME: △; $p < 0.01$).

Beta: $n = 7$; $p = n.s.$). Furthermore, addition of 50 mM KCl (K^+) resulted in a comparable decrease in arterial diameter (Fig. 1A: C- $K^+ = 83 \pm 10 \mu\text{m}$ versus Beta- $K^+ = 97 \pm 16 \mu\text{m}$; $p = n.s.$) and % arterial constriction (Fig. 1B: C- $K^+ = 62 \pm 4\%$ versus Beta- $K^+ = 61 \pm 6\%$; C: $n = 5$, Beta: $n = 7$; $p = n.s.$) in both control and beta-exposed sheep.

Contribution of NOS and COX to pressure-induced constriction. Our next series of experiments was designed to assess pressure (10–120 mm Hg)-induced constriction in MCA from control and Beta-exposed sheep. As shown in Fig. 2A, the DP of MCA from both control and Beta-exposed animals were comparable at all pressures studied, whereas the active diameters of Beta-exposed MCA were significantly greater than control MCA (control $n = 5$; Beta-exposed $n = 6$; $p < 0.05$). Pressure-induced constriction was significantly attenuated in Beta-exposed MCA compared with control MCA (Fig. 2B: control $n = 5$; Beta-exposed $n = 6$; $p < 0.01$). Inhibition of NOS with L-NAME (100 μM) caused augmentation in pressure-induced constriction in both control (Fig. 3A: $n = 4$; $p < 0.01$) and Beta-exposed (Fig. 3B: $n = 5$; $p < 0.001$) MCA. In contrast, inhibition of COX with Indo (10 μM) had no effect on pressure-induced constriction in control MCA (Figs. 3C: $n = 5$; $p = n.s.$) but significantly augmented

pressure-induced constriction in Beta-exposed MCA (Fig. 3D: $n = 5$; $p < 0.01$).

Contribution of NOS and COX to BK-induced dilations. Inhibition of NOS with L-NAME (100 μM) significantly inhibited BK-induced reversal of pressure-induced constriction (developed at 60 mm Hg) in both control (Fig. 4A: three-parameter curve fit: $p < 0.021$; $n = 4$) and Beta-exposed (Fig. 4B: pD_2 : Beta-exposed three-parameter curve fit: $p < 0.0001$; $n = 4$) MCA. However, the maximal dilation to BK was significantly reduced only in Beta-exposed MCA (Fig. 4B). Inhibition of COX with Indo (10 μM) had no effect on BK-induced reversal of pressure-induced constriction in control MCA (Fig. 4C: three parameter curve fit: $p = 0.1022$; $n = 4$) but significantly altered the BK-induced reversal of tone in Beta-exposed MCA (Fig. 4D: three parameter curve fit: $p = 0.0032$; $n = 5$). Unlike L-NAME, Indo did not affect the maximal dilation to BK in Beta-exposed animals.

DISCUSSION

To the best of our knowledge, this is the first study to evaluate alterations in adult cerebral vascular pressure-induced constriction and reactivity after fetal exposure to a

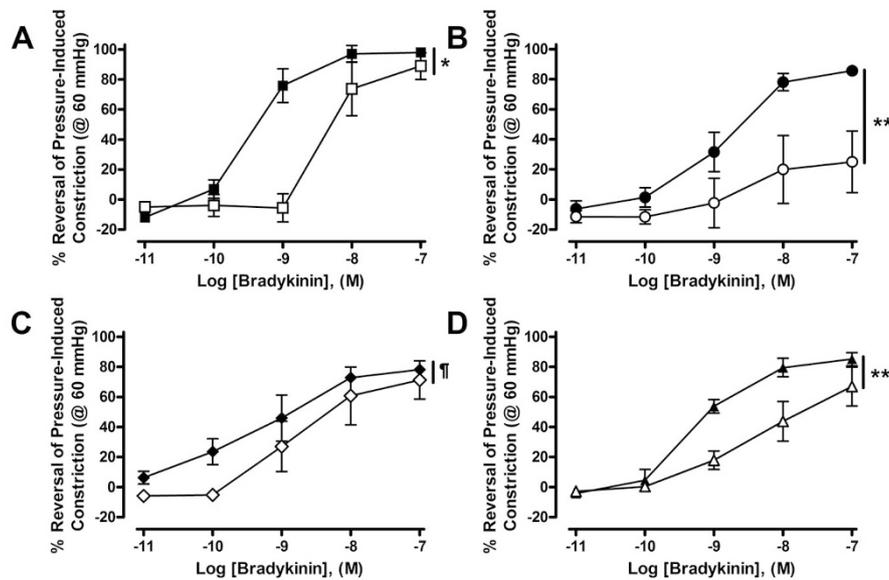


Figure 4. Effect of NOS and COX inhibition on the concentration-dependent reversal of pressure-induced constriction (at 60 mm Hg) by BK (0.01–100 nmol/L) in C and Beta MCA. (A) In C MCA, inhibition of NOS with L-NAME (100 μ M) significantly shifted the BK dose-response curve to the right without affecting maximal dilation to BK. ($n = 4$; C, \blacksquare ; C +L-NAME, \square ; pD_2 : C = -9.50 ± 0.09 vs. C +L-NAME = -8.33 ± 0.32 ; $p = 0.012$; *top*: C = 100.40 ± 3.19 vs. C +L-NAME = 98.40 ± 15.90 ; $p = 0.9005$; *bottom*: C: $n = -17.83 \pm 4.60$ vs. C +L-NAME = -10.23 ± 9.53 ; $p = 0.499$; concentration response curves were found to be significantly different using a three-parameter curve fit: $p = 0.021$). (B) In Beta MCA, inhibition of NOS with L-NAME (100 μ M) significantly shifted the BK dose-response curve to the right and reduced maximal dilation to BK. ($n = 4$; Beta: \bullet ; Beta+L-NAME: \circ ; pD_2 : Beta = -8.84 ± 0.05 vs. Beta+L-NAME = -8.59 ± 0.088 , $p = 0.049$; *top*: Beta = 88.16 ± 1.74 vs. Beta +L-NAME = 26.70 ± 1.41 ; $p < 0.0001$, *bottom*: Beta = -6.70 ± 1.582 vs. Beta +L-NAME = 12.53 ± 1.06 , $p = 0.014$; concentration response curves were found to be significantly different using a three-parameter parameter curve fit: $p < 0.0001$). (C) In C MCA, inhibition of COX with Indo (10 μ M) had no effect on the BK dose-response curve. ($n = 4$; C: \blacklozenge ; C +Indo: \diamond ; pD_2 : C: $n = -9.56 \pm 0.16$ vs. C +Indo: $n = -9.06 \pm 0.36$; $p = 0.2577$; *top*: C: $n = 76.66 \pm 3.22$ vs. C +Indo = 69.99 ± 9.86 ; $p = 0.5056$; *bottom*: C: $n = 3.74 \pm 4.96$ vs. C +Indo = -9.60 ± 10.29 ; $p = 0.3152$; concentration response curves were not different as assessed using a three-parameter curve fit: $p = 0.1022$). (D) In Beta MCA, inhibition of COX with Indo (10 μ M) shifted the BK dose-response curve significantly to the right without affecting maximal dilation to BK. ($n = 5$; Beta: \blacktriangle ; Beta+Indo: \triangle ; pD_2 : Beta: $n = -9.26 \pm 0.05$ vs. Beta +Indo = -8.39 ± 0.19 ; $p = 0.005$; *top*: Beta: $n = 85.31 \pm 1.80$ vs. Beta +Indo: $n = 67.51 \pm 5.78$; $p = 0.026$; *bottom*: Beta: $n = -7.42 \pm 2.13$ vs. Beta +Indo = -0.67 ± 3.67 ; $p = 0.1631$; concentration response curves were found to be significantly different using a three-parameter parameter curve fit: $p = 0.0032$).

clinically relevant course of Beta *via* maternal administration of the drug. Our data demonstrate the following findings in MCA from adult female sheep exposed to antenatal Beta: (1) comparable K^+ -induced constriction, (2) attenuation of pressure-induced constriction in Beta-exposed MCA, and (3) apparent alterations in the contribution of NOS and COX to pressure-induced constriction and BK-induced reversal of pressure-induced constriction in Beta-exposed MCA.

Based on the prognostic advantage to preterm infants after maternal administration of antenatal glucocorticoids, the National Institutes of Health consensus statements of 1994 and 2000 recommend a single course of antenatal glucocorticoids for pregnant women at risk for preterm delivery between 24 and 34 wk of gestation (14,15). Despite the routine use of glucocorticoids in women in preterm labor, the long-term vascular consequences of this therapy have not been well characterized. Accumulating animal data demonstrate adverse postnatal outcomes including fetal growth restriction (16,17), altered renal development (1–4), elevated BP (1), and altered vascular reactivity (8,9) when the developing fetus is exposed to glucocorticoids. Previous studies have also demonstrated altered cerebral vascular reactivity in ovine fetuses exposed antenatally to glucocorticoids (12,13). However, the effects of antenatal glucocorticoid exposure on adult cerebral vascular tone and reactivity are not well understood at present.

Here, we describe the effects of fetal exposure to Beta on pressure-induced constriction and vascular reactivity in the cerebral circulation. We first assessed VSM function by exposing MCA to 50 mM KCl. VSM depolarization with 50 mM KCl caused comparable constriction in control and Beta-exposed MCA (Fig. 1), suggesting that exposure of the female fetus to Beta does not affect voltage-dependent Ca^{2+} channel activity in adult MCA. Similarly, other investigators have demonstrated comparable K^+ -induced constriction in cerebral and femoral arteries after fetal exposure to dexamethasone (12) and coronary arteries after fetal exposure to Beta (8,18,19). Recently, Segar *et al.* (20) has demonstrated similar K^+ -induced constriction in femoral and mesenteric arteries from early dexamethasone-exposed 10- to 14-d-old lambs. In contrast, exposure of the ovine fetus to multiple courses of dexamethasone increases the maximal constriction of fetal femoral arteries to 125 mM KCl (9). This discrepancy in K^+ -induced constriction observed by Molnar *et al.* may be related to multiple courses or the particular glucocorticoid (dexamethasone) administered and/or the age of the fetus/sheep at the time of study.

We next determined the effect of antenatal Beta exposure on pressure-induced constriction and the contribution of NOS and COX signaling to pressure-induced constriction in adult female MCA. Contrary to expectations, our data demonstrate

attenuation in pressure-induced constriction in Beta-exposed compared with control MCA (Fig. 2). Schwab *et al.* (13) have previously shown increased cerebral vascular resistance at 24 and 48 h after fetal exposure to Beta. This difference in results may be related to the age of the animal at the time of study (18 mo versus 129–130 d), GA at which Beta was administered (80–81 d versus 128 d), and the route of administration (maternal versus fetal). Our data further indicate that pressure-induced constriction in MCA from control animals is modulated primarily by NOS activity with little apparent contribution by COX products. In contrast, pressure-induced constriction is modulated by both NOS and COX activity in Beta-exposed adult female sheep (Fig. 3). Thus, there seems to be an alteration in the relative contribution of endogenous NOS and COX vasodilatory products modulating pressure-induced vasoconstriction in Beta-exposed MCA. Similarly, Angeles *et al.* (21) has demonstrated that attenuation of 5-hydroxytryptamine-induced constrictions in fetal carotid arteries incubated with dexamethasone can be reversed with Indo, suggesting that there is increased contribution of COX products in the modulation of carotid artery reactivity after exposure to dexamethasone.

We next determined the effect of NOS and COX on BK-induced reversal of pressure-induced constriction in control and Beta-exposed MCA. Inhibition of NOS with L-NAME shifted the BK dose-response curve significantly to the right; however, the maximal dilation to BK was attenuated only in Beta-exposed MCA (Figs. 3A and B). Similar to our findings, Roghair *et al.* (19) has demonstrated attenuated adenosine-induced relaxation and decreased endothelial NOS staining in coronary arteries from newborn lambs exposed to dexamethasone early in gestation. In contrast, Molnar *et al.* (22) have shown enhanced acetylcholine-induced relaxation but unchanged eNOS RNA and protein levels in femoral arteries from 5-mo-old sheep exposed to dexamethasone *in utero* starting on d 103 of gestation. It is possible that the differences in eNOS protein expression observed in these two studies are related to the timing of fetal exposure to glucocorticoids and the vascular bed studied. Inhibition of COX with Indo shifted the BK response curve significantly to the right in MCA from Beta-exposed offspring (Fig. 4D) but did not affect BK-induced reversal of pressure-induced constriction in MCA from control offspring (Fig. 4C). These data indicate that COX-derived vasodilator products play a greater role in BK-induced dilation in MCA from Beta-exposed offspring compared with age-matched controls. Furthermore, these data suggest an apparent shift in the relative contribution of both NOS and COX to the modulation of BK-induced dilation in Beta-exposed compared with control MCA. We speculate that the altered contribution of NOS is associated with an increased contribution of COX vasodilatory products in MCA from Beta-exposed female sheep.

In summary, maternal administration of a clinically relevant course of Beta results in attenuated pressure-induced constriction and altered vascular reactivity in adult female MCA. Our data demonstrate comparable K^+ -induced constriction in con-

trol and Beta-exposed MCA. Fetal exposure to Beta results in a shift in the contribution of NO and prostaglandin modulation of pressure-induced constriction and BK-induced dilation in MCA. Our results provide supportive evidence for the concept of fetal programming after prenatal exposure to glucocorticoids, with subsequent development of maladaptive cardiovascular responses in adulthood.

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