Development of fetal vascular responses to endothelin-1 and acetylcholine in the sheep

CHERYL C. DOCHERTY, JUDIT KALMAR-NAGY, MARC ENGELEN, AND PETER W. NATHANIELSZ

Laboratory for Pregnancy and Newborn Research, Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853

Received 19 September 2000; accepted in final form 5 October 2000

The fetal circulation is designed to meet the needs of a rapidly growing organism existing at a low PO2 relative to eututcreine life. Blood flow is distributed within the fetus according to local requirements via a circulation anatomically and functionally distinct from the adult. Because of its unique environment and physiology, the fetal circulation must be studied directly, and regulation of its different components cannot be extrapolated from information gained from investigations in adult or even newborn (NB) animals.

Several studies have demonstrated that vascular reactivity varies dramatically with postnatal developmental age. This observation holds for a variety of vascular beds, including cerebral (19, 40, 41), renal (18, 28), femoral (36), and pulmonary (14, 15, 42) circulations. These data have been obtained in various species such as sheep (1, 41), rat (12), pig (36, 42), dog (37), rabbit (14, 18), baboon (19, 20), and human (5).

In addition to effects of developmental age, vessel diameter also influences vascular responsiveness (1). Considerable information is available on the reactivity of large-diameter conduit vessels. However, marked structural and functional differences exist throughout the vascular tree. As a result, extrapolation of data from larger vessels to smaller resistance arteries is inappropriate. Examination of developmental changes in ovine cerebral artery composition and reactivity, from fetal to NB life and maturation to adulthood, showed that the effects of aging varied considerably along the cerebrovascular tree, with more pronounced effects in larger arteries (35). These studies confirm the presence of diversity in cell function depending on location within the vascular tree. Diversity in cell function is important in physiology and pathophysiology to allow the response(s) to vasoactive agents to vary between or within vascular beds.

Blood pressure measured chronically in vitro in fetal sheep shows increments of ~1 mmHg each day over the final 30 days of gestation (38). This physiological rise may be due to changes in the periphery, i.e., reduced effects of peripheral vasodilators and/or increasing effects of peripheral vasoconstrictors in various vascular beds and/or altered cardiac output. Endothelin-1 (ET-1), produced by endothelial cells, is a potent vasoconstrictor peptide that probably has an important role in the maintenance of basal vasomotor tone, interacting with other vasoactive agents, such as catecholamines, potentiating their vasoactive effect (21). Concentrations of ET-1 in human amniotic fluid increase approximately fivefold at term compared with...
mid-trimester values. As a result, it has been suggested that ET-1 may contribute to fetal hemodynamic changes (32). In vivo administration of ET-1 has been shown to produce systemic and pulmonary hypertension in the late gestation fetal sheep (24). Evidence exists for postnatal age-related changes in ET-1-induced responses in pulmonary (14) and systemic (23) arteries.

The endothelial-derived agonists ET-1 and nitric oxide (NO) influence the effects mediated by one another. For example, NO inhibits the production of ET-1 in vivo and in cultured vascular endothelial cells (8). ET-1 can stimulate the release of NO via activation of endothelial ET_B receptors. However, ET-1 was shown to inhibit NO synthesis via activation of ET_A receptors in rat vascular smooth muscle cells (22).

Because altered vascular responsiveness with fetal development may reflect changes in the endothelium, we also examined responses of preconstricted arteries to the endothelium-dependent relaxatory agent acetylcholine (ACh). Numerous reports indicate a role of NO during fetal life in various vascular beds. For example, basal production of NO was shown to maintain baseline renal blood flow in third trimester fetal sheep (6). As with studies of vascular contractility, most previous studies examining development of relaxatory responses compare only postnatal age points (3, 33, 36).

There is a lack of information regarding development of vascular responsiveness during the final third of gestation, a critical period of fetal development, when fetal blood pressure is increasing. In the present study, we sought to determine the in vitro functional response of fetal [110–145 days of gestational age (dGA)] and NB (<24 h) sheep resistance arteries from adrenal, femoral, renal, and cerebral vascular beds to the known vasoconstrictor agents K^+ and ET-1 and the endothelium-dependent relaxatory agent ACh. We chose these specific fetal vascular beds because the cerebral and adrenal circulations are “protected” at times of oxygen deficiency and undergo vasodilation and increased flow (10). In contrast, the renal and femoral beds are unprotected, acting as reservoirs of peripheral vascular resistance to maintain perfusion pressure in protected vascular beds. In addition, to date no longitudinal prenatal ontogenic study has been performed in several vascular beds from the same fetuses. Therefore, in addition to examining ontogenic changes in arteries from a given vascular bed, we also aimed to compare effects in these functionally different fetal circulations at particular fetal age points.

**MATERIALS AND METHODS**

**Animal Preparation**

Mature Rambouillet-Columbia cross-bred ewes (*Ovis aries*) carrying a fetus of known GA after exposure to the ram at a single estrus were studied. Fetal sheep were removed from ewes at cesarean section under halothane general anesthesia. Fetuses at 110 (n = 4), 125 (n = 5), 138 (n = 6), and 145 (n = 6) dGA (term ~148 dGA) and 0- to 24-h NB (n = 7) lambs were euthanized by exanguination under halothane anesthesia. Fetal or neonatal brain, left kidney, left adrenal, and femoral muscle from the left side were collected in ice-cold physiological salt solution (PSS). The experimental protocol was approved by Institutional Animal Care and Use Committee at Cornell University for College of Veterinary Medicine. All facilities were approved by the American Association for the Accreditation of Laboratory Animal Care.

**Wire Myography**

Second-order middle cerebral, renal arcuate, adrenal cortical, and femoral arteries were immediately dissected from the collected tissue. Isolated arteries (~2 mm in length) were mounted on two 40-μm tungsten wires in a small vessel wire myograph as previously described (2). Vessels were equilibrated in PSS at 37°C and continuously aerated with 95% O_2-5% CO_2 (pH 7.4) for 30 min. Vessel dimensions were then normalized individually by using their resting wall tension internal circumference curve. This determined the point on the fitted exponential curve corresponding to the internal circumference that the artery would have attained in situ when relaxed and exposed to a transmural pressure of 13.3 kPa (100 mmHg), termed \( L_{100} \). Preliminary studies showed that the active tension development is maximal in ovine fetal resistance arteries when set to 0.9 \( L_{100} \) (Fig. 1). Thus experiments described in this study were performed at 0.9 \( L_{100} \).

After several washes and a further 30-min equilibration, vessel responsiveness was tested by stimulation with NEK [5 μM noradrenaline (NE) in 125 mM potassium-substituted PSS (KPSS)], KPSS alone, 5 μM NE alone, and a second exposure to NEK. Cumulative concentration response curves were performed to KCl (2–125 mM) and ET-1 (10 pM-0.1 μM). The relaxing effects of the cumulative addition of ACh (1 pM-10 μM) were assessed after preconstriction with 5 μM NE for femoral and renal arteries. Because NE-induced vasoconstriction was not maintained in adrenal and middle cerebral arteries, 1 μM 5-hydroxytryptamine and 50 mM KCl were used to constrict these arteries, respectively. Additions were performed after a plateau in the response had been attained to the preceding concentration.

**Drugs and Solutions**

PSS was of the following composition (in mM): 119 NaCl, 4.7 KCl, 1 KH_2PO_4, 0.9 MgSO_4, 2.5 CaCl_2, 11.1 glucose, 2.5 NaHCO_3, and 0.021 EDTA. KPSS was prepared by equimolar substitution of NaCl by KCl in PSS. Chemicals for PSS and NE bitartrate salt, 5-hydroxytryptamine, and ACh were...
purchased from Sigma (St. Louis, MO). ET-1 was obtained from Bachem (Torrance, CA).

Data Analysis

Tension is expressed as millinewtons per millimeter artery length or as a percentage of the maximal response to K\(^+\). Relaxation is expressed as a percentage of the preinduced tension. Concentration-response curves were constructed by fitting data to the logistic sigmoid equation (log agonist concentration vs. effect) using the GraphPad Prism program (San Diego, CA). Sensitivity (pEC\(_{50}\)) to the agonists is expressed as the negative log of the effective molar concentration of the agonist required to elicit 50% of the maximum response.

Statistical tests were performed using the SigmaStat program (Jandel, San Rafael, CA). Magnitude of responses and pEC\(_{50}\) values were compared between groups by one-way ANOVA, followed by Tukey’s post hoc test. To examine the possibility of correlations in the responses with GA, the data were first subjected to a normality test. Data sets for which the residuals were normally distributed were subjected to the Pearson product moment correlation test. The Spearman correlation test was used to analyze data that failed the normality test. Correlation tests were performed on scatter plots of individual values obtained in each artery. Differences were considered statistically significant at P < 0.05. Power analysis tests were performed on statistical results. All results are presented as means ± SE, and n refers to the number of animals studied.

RESULTS

Normalized Internal Diameters

All arteries were normalized to 0.9 \(L_{100}\). The calculated internal diameters for all arteries within each age group are shown in Table 1. Arteries within each vascular bed were of a similar internal diameter regardless of the age point of study. For each particular age point, the only significant difference was seen between renal and middle cerebral arteries from 138 dGA fetuses (Table 1).

Responses to KCl

Affect of GA. Sensitivity to K\(^+\) was similar in middle cerebral arteries at all age points studied (Fig. 2C). Renal (Fig. 2D) and femoral (Fig. 2B) arteries from NB

Table 1. Calculated internal diameter of small arteries from fetal and NB sheep

<table>
<thead>
<tr>
<th>Developmental Age, dGA</th>
<th>110</th>
<th>125</th>
<th>138</th>
<th>145</th>
<th>NB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal</td>
<td>312.5 ± 56.6</td>
<td>(5)</td>
<td>344 ± 45.7</td>
<td>(5)</td>
<td>338.7 ± 28</td>
</tr>
<tr>
<td>Femoral</td>
<td>359.2 ± 39.8</td>
<td>(4)</td>
<td>349.2 ± 22.5</td>
<td>(4)</td>
<td>324.3 ± 27.1</td>
</tr>
<tr>
<td>MCA</td>
<td>341.2 ± 18.4</td>
<td>(4)</td>
<td>418.1 ± 20.3</td>
<td>(5)</td>
<td>406.3 ± 20.8*</td>
</tr>
<tr>
<td>Renal</td>
<td>327.2 ± 24.2</td>
<td>(4)</td>
<td>282 ± 39.8</td>
<td>(5)</td>
<td>282.3 ± 22.3</td>
</tr>
</tbody>
</table>

Values are means ± SE in µm; numbers in parentheses are no. of sheep studied. NB, newborn; dGA, days gestational age; MCA, middle cerebral arteries. Statistical significance by ANOVA followed by Tukey’s post hoc test: *P < 0.05 vs. renal arteries from 138 dGA.
lambs were significantly less sensitive than those from 110, 125, and 138 dGA fetal sheep. Adrenal and renal arteries from NB lambs were less sensitive to K⁺ than those from 145 dGA (Fig. 2, A and D, respectively).

Analyzing individual values for each artery showed that there was a significant positive correlation of the magnitude of the response to 125 mM K⁺ with dGA in femoral (Fig. 3B, $r = 0.5, P = 0.02$) and middle cerebral arteries (Fig. 3C, $r = 0.00005$) but not in adrenal (Fig. 3A, $P = 0.08$) or renal (Fig. 3D, $P = 0.33$) arteries.

Comparing K⁺-induced vasoconstriction in NB vs. fetal arteries, the response in femoral and renal arteries from NB lambs was not significantly different from those in femoral and renal arteries from fetal sheep. Vasoconstriction to K⁺ was greater in middle cerebral arteries from NB lambs (2.28 ± 0.56 mN/mm) compared with 110 dGA fetuses (0.61 ± 0.12, $P < 0.05$). NB lamb adrenal arteries had a markedly attenuated K⁺ response compared with those isolated from 145 dGA fetuses (0.86 ± 0.15 vs. 1.78 ± 0.22, $P < 0.05$).

Comparison of vascular beds. Data in Fig. 2 allow comparison of K⁺ sensitivity in arteries from the different vascular beds for each developmental age. Potency of K⁺ was similar in all vascular beds at 110 and 125 dGA. Middle cerebral arteries were more sensitive compared with adrenal and renal arteries at 138 dGA and adrenal and femoral arteries at 145 dGA. K⁺ sensitivity was greater in middle cerebral arteries from NB lambs compared with all others. In isolated vessels from 145 dGA fetuses, renal arteries were more sensitive to K⁺ than femoral arteries. At <24 h after birth, renal arteries were significantly less sensitive than all others (Fig. 2A). The magnitude of the vasoconstriction to 125 mM K⁺ was similar in all isolated vessels at each age point examined.

Responses to ET-1

Affect of GA. Sensitivity of adrenal arteries to ET-1 was greater at 145 dGA compared with 125 and 138 dGA and in NB adrenal arteries compared with those from 125 dGA fetuses (Fig. 4A). Femoral arteries from 138 dGA fetuses were markedly more sensitive than those from 110 and 125 dGA fetuses and NB lambs; sensitivity was also greater at 145 compared with 110 dGA (Fig. 4B). Middle cerebral arteries from NB lambs were less sensitive to ET-1 compared with arteries from 138 and 145 dGA fetuses. The potency of ET-1 was also reduced at 110 and 125 dGA compared with 145 and 138 dGA, respectively (Fig. 4C). Renal arteries from 125 dGA fetuses were significantly less sensitive to ET-1 compared with those examined from 138 and 145 dGA and NB lambs (Fig. 4D).

There was a significant positive correlation of the magnitude of the maximum ET-1-induced response (in mN/mm) with dGA in femoral (Fig. 5A, $P = 0.002$), middle cerebral (Fig. 5C, $P = 0.0004$), and renal arteries (Fig. 5D, $P = 0.03$) but not in adrenal arteries (Fig. 5A, $P = 0.12$). The maximal ET-1 response in NB arteries was similar to those in term fetal arteries from all vascular beds.

Comparison of vascular beds. At all stages of fetal development studied, middle cerebral arteries (Fig. 4C) were significantly more sensitive to ET-1 compared with adrenal, renal, and femoral arteries. ET-1 potency was greater in femoral arteries compared with renal and adrenal arteries at 125 and 138 dGA and renal arteries only at 145 dGA. Adrenal artery ET-1 sensitivity was greater compared with that of renal and femoral arteries at 125 and 145 dGA, respectively. ET-1 sensitivity was similar in all arteries examined from NB lambs (Fig. 4).
The maximum ET-1-induced vasoconstriction was significantly greater in renal (1.46 ± 0.2 mN/mm) compared with femoral (0.76 ± 0.2 mN/mm) and middle cerebral (0.61 ± 0.1 mN/mm) arteries at 110 dGA (P < 0.05). At all other GAs there was no significant difference in ET-1-induced maximum contractions, expressed as millinewtons per millimeter, between artery types.

Analyzing the ET-1 maximum as a percentage of K+ maximum for each artery showed a significantly greater vasoconstriction in adrenal and renal arteries compared with middle cerebral arteries at 125 dGA (adrenal 149.2 ± 11.4% and renal 151.1 ± 8.2% vs. middle cerebral 90.8 ± 7%, P < 0.01) and 24 h after birth (adrenal 159.8 ± 3.1% and renal 142.7 ± 16.1% vs. middle cerebral 86.8 ± 8.1%, P < 0.05). The mag-

Fig. 4. Endothelin-1 (ET-1) sensitivity (pEC\textsubscript{50}) in adrenal (A; n = 4–6), femoral (B; n = 3–6), middle cerebral (C; n = 3–6), and renal arteries (D; n = 4–6) from 110, 125, 138, and 145 dGA fetal and NB sheep. Values are means ± SE. Statistical significance by ANOVA (followed by Tukey’s post hoc test), P < 0.05 or better. Comparisons of GA vs. 138 dGA (a) vs. 145 dGA (b) vs. NB (c). Comparison of vascular beds vs. adrenal arteries (1) vs. femoral arteries (2) vs. middle cerebral arteries (3).

Fig. 5. Significant positive correlation between magnitude of maximum ET-1-induced vasoconstriction (mN/mm) and dGA in femoral (B; r = 0.63, P = 0.002), middle cerebral (C; r = 0.68, P = 0.0004), and renal (D; r = 0.48, P = 0.03) arteries but not adrenal arteries (A; P = 0.12). Statistical significance by Pearson product moment correlation for A, C, and D and Spearman correlation for B; data did not pass normality test.
The magnitude of the ET-1 maximum was also greater in femoral compared with middle cerebral arteries from NB lambs (168.9 ± 15.2% vs. 86.8 ± 8.1%, P < 0.01).

Responses to ACh

Affect of GA. Sensitivity of adrenal (pEC_{50} = 7.1) and femoral (pEC_{50} = 7.2) arteries to ACh-induced relaxation did not alter with developmental age. Maximal relaxatory responses were comparable in adrenal arteries at all GAs, being ~65% of the induced tone (Fig. 6A). However, in femoral arteries the maximal relaxatory response was greater at 125 (16.2 ± 0.7% of induced tone) compared with 145 dGA (66.7 ± 13.2%, P < 0.05). A marked ACh-induced relaxation was restored 0–24 h after birth, as the maximal relaxation of femoral arteries from NB lambs (16.4 ± 6.6%) was greater than that noted at 138 (60.9 ± 15.1%) and 145 dGA (P < 0.05, Fig. 6B).

ACh did not evoke a relaxatory response in renal arteries from 125-dGA fetuses and 0- to 24-h NB lambs (Fig. 6D). Renal arteries from 138 and 145 dGA fetuses relaxed ~50% of the induced tone (Fig. 6D). ACh concentrations higher than 0.3 μM evoked a contractile response. No relaxatory response was noted in middle cerebral arteries from fetal or NB sheep. Indeed, increasing ACh concentrations tended to augment the vascular tone (Fig. 6C).

Comparison of Vascular Beds

The maximal ACh-induced response was similar in adrenal, femoral, and renal arteries examined from 138 and 145 dGA fetuses. Because of the small or negligible relaxation of renal and middle cerebral arteries, the maximal vasorelaxation of femoral arteries was greater than that of renal and middle cerebral arteries at 125 dGA (P < 0.01) and 0–24 h after birth (P < 0.001). Relaxatory response of adrenal arteries was greater than that of 1) middle cerebral arteries from 125 dGA (P < 0.05) and NB (P < 0.001) preparations and 2) renal arteries from NB lambs (P < 0.001).

DISCUSSION

K⁺-Induced Responses

Adrenal, femoral, and renal arteries from NB lambs were less sensitive to K⁺ compared with fetal arteries, whereas NB lamb middle cerebral arteries remained unchanged. Other investigators suggest that vascular maturation involves important shifts in the mechanisms mediating vascular pharmamechanical coupling. In particular, in the cerebral vasculature it has been proposed that normal development may involve a reduction in the Ca^{2+}-sensitizing effects of agonists (1).

Furthermore, under pathophysiological conditions it is possible that failure of this proposed shift may lead to maintained vascular hyperreactivity in adult vessels. Although K⁺ sensitivity of middle cerebral arteries was unaltered in the transition from fetal to NB life, the magnitude of the maximal K⁺-induced vasoconstriction was augmented. An earlier study on isolated ovine common carotid, basilar, posterior communicating, and middle cerebral arteries also showed an increase in maximum contractile tension during the transition from fetal to NB life (35). The increase in tension with age ranged from 18% in middle cerebral arteries to 82% in the common carotid. In contrast, Wagerle et al. (41) reported a reduction in the magnitude of NE response in ovine middle cerebral arteries.
of a larger diameter than we examined with GA (105 dGA, 7 days postnatal). Akopov et al. (1) reported that K+ evoked a similar contractile tension and cytosolic Ca2+ response in basilar arteries from 138–141 dGA fetal and adult sheep. Hence a possible explanation for differing findings between studies with regard to the effect on maximal tension generated may be related to methodological factors such as differences in diameter of arteries under investigation and set-up procedures. Baseline Ca2+ sensitivity of ovine cerebral arteries was shown to vary with artery size and age, being greater in immature than in mature arteries, correlating inversely with artery size (1). Inositol 1,4,5-trisphosphate [Ins(1,4,5)P3] receptor density increased from 140 dGA to NB and adults in ovine common carotid but not in posterior, middle, or anterior cerebral arteries (43).

Sensitivity to K+ was unaltered (manifested by similar pEC50 values) in all arteries over the GA period we examined. However, the magnitude of the maximal vasoconstriction was positively correlated with GA in femoral and middle cerebral arteries, possibly because of increase in vascular smooth muscle mass. Differences in responsiveness with GA and between arteries of different origin could reflect dissimilarities in vascular smooth muscle phenotype. Expression of smooth muscle proteins and myosin heavy chain isoforms have been shown to be developmentally regulated and tissue dependent in the sheep (9). Studies in perinatal and adult sheep arterial smooth muscle show that significant maturational changes occur in the isotonic and isometric mechanical properties of vascular pulmonary and systemic smooth muscle (4).

**ET-1-Induced Responses**

ET-1 evoked a potent concentration-dependent vasoconstriction in isolated resistance arteries from several ovine systemic beds, including renal arcuate arteries. A contractile effect of ET-1 has been shown in isolated renal arteries from several species, including adult rat (13) and human (26). However, in vivo ET-1 has a vasodilatory effect in the renal circulation of the fetal sheep. ET-1 activates two different receptors, ET_A and ET_B. In this earlier study (7), ET-1 was shown to act primarily via ET_B receptors, producing vasodilation; however, ET_A receptors, mediating vasoconstriction, were shown to contribute to renal vascular tone. Such disparities in findings may be due to differences in experimental conditions, such as in vivo vs. in vitro, vessel size investigated, and/or species variability, factors that have been shown to alter ET receptor populations (30).

In adrenal, femoral, renal, and middle cerebral arteries cumulative addition of ET-1 resulted in tachyphylaxis at concentrations exceeding 0.1–0.3 μM. This desensitization was specific for ET-1 because it was not observed with K+. Such a tachyphylactic phenomenon has been reported previously for ET-1-induced responses (29) and is also commonly observed in studies involving angiotensin II (25).

In contrast to our finding with K+, sensitivity to ET-1 increased with fetal age in all vascular beds. ET-1-induced maximal vasoconstriction increased with GA in femoral, middle cerebral, and renal arteries but not adrenals. Human fetal ET plasma levels are significantly higher than maternal (27), and developmental changes also occur in fetal plasma ET-1 levels (16). Endo et al. (16) measured serum NO metabolites and ET-1 in healthy human neonates at birth, at 12 and 24 h, and at 5 days postnatally. The lowest concentration of NO metabolites was observed at birth and increased with age, whereas the highest concentration of ET-1 was observed at birth and decreased with age (16). These findings together with our own indicate an important role for ET-1 as a vasoactive agent involved in the transition from fetal to extraterrestrial life.

Middle cerebral arteries from ovine fetuses showed the greatest sensitivity to K+ and ET-1 compared with the other systemic arteries we investigated. Porcine cerebral arteries also have been shown to be more sensitive to ET-1 compared with coronary and renal arteries (17). Enhanced responses of canine cerebral and coronary arteries to ET-1 compared with mesenteric arteries was shown to be dependent on Ca2+ influx through voltage-dependent Ca2+ channels (34). ET-1 also induced greater accumulation of inositol bisphosphate (InsP2) in rat mesenteric arteries than in thoracic aorta, suggesting that turnover of Ins(1,4,5)P3 may be faster in the former than in the latter (34).

The ability to regulate blood supply to the fetal and neonatal brain very precisely according to prevailing conditions is critical for survival of the challenges of perinatal life. Cerebrovascular homeostasis is dependent on the responsiveness of the cerebral arteries to vasoactive agents. The importance of precise control of cerebral blood flow is reflected in our finding of significantly enhanced contractile responsiveness of middle cerebral compared with adrenal, femoral, and renal arteries.

**ACh-Induced Responses**

We utilized the endothelial-dependent agent ACh to examine the role of NO in fetal and NB arterial responsiveness. Concentration-dependent relaxations were noted in all femoral and adrenal arteries. A notable relaxation of renal arcuate arteries was only evident in vessels from 138 and 145 dGA fetuses when tension was reduced to ~50% of the preconstricted tone. Similarly, ACh infusion in third trimester fetal sheep caused a 60% reduction in baseline tension (6). Hence in vitro and in vivo studies indicate a regulatory role of NO (basal and/or agonist induced) in renal blood flow.

In contrast to the other arteries, second-order middle cerebral arteries from fetal and NB sheep did not relax to ACh. Similarly, examination of endothelium-dependent relaxations in isolated arteries from adult rabbits and dogs demonstrated a reduced effect of ACh in cerebral compared with extracerebral (e.g., femoral) arteries (31). Hayashi et al. (19) reported that isolated cerebral artery strips from premature and NB baboons
showed a marked contractile response to ACh, whereas arteries from adult baboons showed little response. In contrast, in vivo studies of cerebral arterioles of 90–111 dGA, 128–143 dGA, and NB sheep showed an increase in pial arteriolar diameter in response to ACh, thus demonstrating a sensitive dilator response in immature fetuses (39). Such discrepancies may be related to the specific cerebral arterial branch being examined. For example, vascular segments in the fetal sheep lung have been shown to differ anatomically and functionally from one another. Immunostaining for soluble guanylate cyclase showed very weak to no staining in large arteries but intense staining in distal arteries, implying a greater role for NO-mediated vasodilation in smaller arteries (11). Because vessels were precontracted with K+ depolarization, we are not able to comment on endothelium-derived hyperpolarizing factor-dependent mechanisms.

Sensitivity of femoral and adrenal arteries to ACh did not change with developmental age. However, the mean maximum relaxation in femoral arteries was greater at 125 compared with 145 dGA. The reduction in relaxation with increasing GA may indicate a relative attenuation in NO generation. Such a change could contribute to the physiological rise in fetal blood pressure of ~1 mmHg/day, which occurs from ~125 dGA (38). ACh-induced relaxation was restored in femoral arteries 0–24 h after birth. The increased ACh responsiveness may be indicative of a greater role of basal and/or agonist-induced NO in femoral artery tone at 125 dGA and <24 h after birth compared with the other GA examined. Interestingly, the ACh relaxation was greatest in femoral arteries in the groups that corresponded to the age points in femoral arteries, which showed the lowest ET-1 sensitivity. Moreover evidence exists for the interaction of these two endothelium-derived agents (8, 22).

Other investigators have examined postnatal changes in ACh-induced relaxation of femoral arteries. The relaxatory response to ACh was shown to be similar in femoral arteries from NB and 7-day-old piglets. Interestingly, ACh response was not influenced by N^G^-monomethyl-L-arginine (NOS inhibitor) in NB arteries but was inhibited in 7-day-old arteries, indicating differences in the underlying mechanism(s) with postnatal development (36).

In summary, previous studies examining ontogeny of vascular responsiveness have focused on postnatal changes and those occurring with adult maturation. To our knowledge, this is the first study that examines reactivity of resistance arteries from several vascular beds, both protected and unprotected, of the same species across the last third of gestation and the 24 h after birth. The overall findings are that the responsiveness of isolated ovine resistance arteries varies 1) with fetal developmental age and 2) between four distinct and critical vascular beds, both protected and unprotected, and 3) these effects are agonist specific.

Information on such fundamental differences in vascular reactivity to both vasoconstrictor and vasodilator agents between vascular beds and the variation with fetal developmental age is essential to our knowledge of regulation of vascular function at this critical stage of development during which correct vascularization of developmental tissue is vital to normal tissue growth and development.

C. C. Docherty is supported by a Wellcome Trust Travelling Research Fellowship. This research was supported by grants from Wellcome Trust UK and National Heart, Lung, and Blood Institute (HL-55416).

REFERENCES