Age-dependent modulation of endothelium-dependent vasodilatation by chronic hypoxia in ovine cranial arteries

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Williams, James M., and William J. Pearce. Age-dependent modulation of endothelium-dependent vasodilatation by chronic hypoxia in ovine cranial arteries. J Appl Physiol 100: 225–232, 2006. First published September 22, 2005; doi:10.1152/japplphysiol.00221.2005.—Although abundant evidence indicates that chronic hypoxia can induce pulmonary vascular remodeling, very little is known of the effects of chronic hypoxia on cerebrovascular structure and function, particularly in the fetus. Thus the present study explored the hypothesis that chronic hypoxemia also influences the size and shape of cerebrovascular smooth muscle and endothelial cells, with parallel changes in the reactivity of these cells to endothelium-dependent vasodilator stimuli. To test this hypothesis, measurements of endothelial and vascular smooth muscle cell size and density were made in silver-stained common carotid and middle cerebral arteries from term fetal and nonpregnant adult sheep maintained at an altitude of 3,820 m for 110 days. Chronic hypoxia induced an age-dependent remodeling that led to smooth muscle cells that were larger in fetal arteries but smaller in adult arteries. Chronic hypoxia also increased endothelial cell density in fetal arteries but reduced it in adult arteries. These combined effects resulted in an increased (adult carotid), decreased (adult middle cerebral), or unchanged (fetal arteries) per cell serosal volume of distribution for endothelial factors. Despite this heterogeneity, the magnitude of endothelium-dependent vasodilatation to A23187, measured in vitro, was largely preserved, although sensitivity to this relaxant was uniformly depressed. N\textsuperscript{G}-nitro-l-arginine methyl ester, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, and endothelium denudation each independently blocked A23187-induced vasodilation without unmasking any residual vasoconstrictor effect. Indomethacin did not significantly attenuate A23187-induced relaxation except in the hypoxic adult middle cerebral, where a small contribution of prostanooids was evident. Vascular sensitivity to exogenous nitric oxide (NO) was uniformly increased by chronic hypoxia. From these results, we conclude that chronic hypoxia reduced endothelial NO release while also upregulating some component of the NO-cGMP-PKG vasodilator pathway. These offsetting effects appear to preserve endothelium-dependent vasodilation after adaptation to chronic hypoxia. A23187; cerebral arteries; fetus; indomethacin; N\textsuperscript{G}-nitro-l-arginine methyl ester; ontogeny; vascular morphometry

POSTNATAL MATURATION INVOLVES numerous changes in both vascular structure and functional reactivity (10) that enable the growing neonate to match tissue perfusion to increasing metabolic demand. Correspondingly, environmental influences that alter the ability of the neonatal circulation to deliver adequate support for metabolism commonly stimulate adaptive changes in the pace and character of neonatal vascular remodeling. For example, chronic hypoxia alters both the reactivity and morphology of smooth muscle and endothelial cells, particularly in the pulmonary circulation where this effect has been studied most extensively (5, 12, 16, 20). Interestingly, such responses to chronic hypoxia are highly heterogeneous among different vascular beds (7, 27). Given the unique susceptibility of the developing brain to environmental challenges such as chronic hypoxia (26), the potential importance of cerebrovascular adaptations to these challenges (19), and the fact that very little is known of the effects of chronic hypoxia on cerebral artery structure and vasodilator capacity, the present study explored the hypothesis that chronic hypoxemia influences the size and shape of cerebrovascular smooth muscle and endothelial cells, with parallel changes in the reactivity of these cells to vasodilator stimuli.

To facilitate comparison of cerebral and extra-cerebral adaptations to chronic hypoxia, these studies examined both common carotid (COM) and middle cerebral arteries (MCAs) taken from sheep maintained at an altitude of 3,820 m for 110 days. As shown in numerous previous studies from our laboratory (19), this altitude produces an average arterial oxygen tension of ~60 Torr in the adult and 19 Torr in the fetus. To assess the influence of age on vascular adaptations to chronic hypoxia, arteries were taken from both nonpregnant adult sheep as well as term fetal sheep (138–141 days) raised at altitude for the final 110 days of gestation. Computer-assisted morphometry of silver-stained specimens enabled assessments of hypoxia-induced changes in cell sizes. In vitro vasoreactivity to endothelium-dependent (A23187) and endothelium-independent [nitric oxide (NO)] vasodilators in the presence and absence of cyclo-oxygenase, NO synthase, and guanylate cyclase inhibitors defined the effects of hypoxia on vasodilator capacity. Together, these studies offer a unique and original view of cerebrovascular adaptations to chronic hypoxia.

MATERIALS AND METHODS

All procedures used in these studies were approved by the Animal Research Committee of Loma Linda University and adhered to the policies and practices outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All protocols utilized segments of COM and MCAs from fetal (139–142 days of gestation) and young nonpregnant adult sheep (18–24 mo old). Chronically hypoxic animals were maintained for ~110 days at the Barcroft Laboratory, White Mountain Research Station (Bishop, CA, altitude 3,820 m). After the vessels were cleaned of extraneous connective and adipose tissue, the arteries were cut into segments 3–5 mm in length and mounted on paired wires between a low-compliance force transducer (Kulite BG-10) and a post attached to a micrometer used to vary resting tension. The segments then equilibrated at least...
1 h at 38.5°C (normal ovine core temperature) in a bicarbonate Krebs solution containing (in mM) 122 NaCl, 25.6 NaHCO3, 5.56 dextrose, 1.17 KCl, 2.49 MgSO4, 1.60 CaCl2, 0.114 ascorbic acid, and 0.027 disodium EDTA, continuously bubbled with 5% O2-5% CO2. During equilibration, segments were maintained at optimum resting tensions, as previously described in detail (24).

Endothelial and vascular smooth muscle cell staining and morphometry. To determine the effects of chronic hypoxia on endothelial and vascular smooth muscle morphology, unmounted arterial segments were stained en face using silver nitrate. Artery circumference and smooth muscle cell dimensions were measured in adjacent segments of the same arteries used for the endothelial cell measurements, with the exception that the vascular endothelium was first removed by mild mechanical abrasion. For this treatment, each segment was carefully opened longitudinally with fine scissors, washed with 5% dextrose in saline, and then incubated for 3 min in 0.25% AgNO3. After silver nitrate exposure, the segments were again washed with 5% dextrose in saline, and then incubated in 3% CoBr2 in 1% NH4Br for 10 min. The segments were again washed with 5% dextrose in saline and then fixed in 4% formaldehyde. The fixed segments were mounted in glycerin and examined using a computer-aided imaging system attached to a charge-coupled device camera and light microscope using Image Pro software (version 3, Media Cybernetics, Silver Spring, MD) and calibrated with a slide micrometer. From each segment examined, the widths and lengths of at least 10 individual endothelial and vascular smooth muscle cells were recorded, and these data were averaged to produce a single average measurement of cell length and width per segment. Multiple segments of the same artery type from the same animal were routinely examined, and these data were averaged across segments to produce a single average measurement of cell length and width for each artery type from each individual animal. All methods used for measurements of cell dimensions have been previously described (4).

For each artery examined, the average cellular cross-sectional area was calculated as $\pi/4 \times$ the product of cell width and length, assuming that the cells were all longitudinally ellipsoidal in shape. In an adjacent segment, measurements of wall thickness and luminal area were obtained by examining vascular coronal sections using our computer-aided imaging system attached to a charge-coupled device camera and microscope. All measurements were calibrated using a stage micrometer. For each vessel segment studied, wall thickness was expressed as the mean of at least 10 measurements.

Measurements of wall volume per endothelial cell were calculated using:

$$A \cdot T \cdot (D + T) / D$$

as previously described (31). In this expression, $A$ is the average cross-sectional area per endothelial cell in units of square micrometers, $T$ is the artery wall thickness, and $D$ is the luminal diameter in micrometers. Similarly, the number of endothelial cells per millimeter of artery length were calculated as the ratio of luminal area to endothelial cell area for a 1-mm length of artery.

A23187 dose-response studies. During all dose-response experiments, an online computer continuously digitized, recorded, and normalized vessel contractile tensions. Arteries initially were contracted by exposure to an isotonic potassium Krebs solution containing 122 mM KCl and 31 mM Na+. After peak tensions were obtained, we washed the vessels with normal sodium Krebs solution and allowed them to return to baseline levels of tension for 30 min. The arteries were then contracted a second time with 3 μM serotonin following incubation with 1 μM prazosin to inhibit α1-adrenergic receptor activation and 0.2 μM cocaine to inhibit neuronal reuptake. After attainment of a stable level of initial tone, the capacity of the endothelium to produce vasorelaxation was tested via the receptor independent calcium ionophore A23187. Cumulative log doses of A23187 were added to the baths to produce concentrations ranging from $10^{-10}$ to $10^{-3}$ M. A23187 was dissolved in DMSO, and the maximal bath concentration of DMSO attained during any experiment was 0.1%, which had no independent effect on vessel tension.

Effects of indomethacin, L-arginine, Nω-nitro-ω-arginine methyl ester, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, and endothelium removal on A23187-induced relaxation responses. To assess the contribution of eicosanoids to endothelium-dependent relaxation in these arteries, responses to 10 μM A23187 were obtained following incubation for 30 min in 10 μM indomethacin (8). Validation experiments have shown this duration of treatment with this concentration of indomethacin sufficient to completely block responses to 1 μM arachidonic acid. The role of NO-mediated vasorelaxation was assessed by responses to A23187 following incubation for 30 min in 100 μM Nω-nitro-ω-arginine methyl ester (l-NAME) and 100 μM 1- nitroarginine. To assess any role of soluble guanylate cyclase (sGC) in A23187-mediated relaxation, responses were also measured in artery segments treated with 10 μM 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a specific soluble guanylate cyclase inhibitor (9). In separate segments, the endothelium was removed by gentle mechanical abrasion before mounting the arteries for contractility measurements. Denudation was verified morphologically by the complete absence of endothelial cells following silver staining and microscopic examination. All relaxant responses to 1 μM ADP or bradykinin were absent (<10%) in denuded arteries.

S-nitroso-N-acetyl-penicillamine dose-response studies and effects of ODQ on relaxation responses. To assess the capacity of NO to elicit vasorelaxation, responses to the NO donor S-nitroso-N-acetyl-penicillamine (SNAP) were measured in normoxic and hypoxic fetal and adult arteries. Endothelium was removed from all artery segments as previously described. After contraction with 3 μM 5-hydroxytryptamine, cumulative log doses of SNAP were added to the baths to produce concentrations ranging from $10^{-10}$ to $10^{-3}$ M. To verify that the responses to SNAP were mediated through sGC, separate artery segments were incubated with the sGC inhibitor ODQ at 10 μM, followed by 10 μM SNAP. SNAP and ODQ were dissolved in DMSO, and the maximal bath concentration of DMSO attained during any experiment was 0.1%.

Data analysis and statistics. Maximum relaxation responses were calculated as percentage of relaxations of the maximum initial contractile tension. For determination of apparent affinity (pD2) ($-\log$ half-maximal effective dose), relaxation data were normalized relative to maximum percentage relaxation and then fitted to the logistic equation using nonlinear regression as previously described (31). Percent maximum relaxation and pD2 values were each independently analyzed using a two-way ANOVA with oxygen level as one factor (normoxic vs. hypoxic) and maturation as the other (fetal vs. adult). Homoscedasticity was verified for all ANOVA comparisons using Bartlett’s test. The effects of hypoxia within each artery type were determined by post hoc Duncan’s tests. Endothelial cell cross-sectional area was analyzed using a Behren’s-Fisher analysis.

RESULTS

In total, 782 segments were obtained from 25 normoxic and 30 hypoxic adults and 26 normoxic and 28 hypoxic fetal sheep. When the same protocol was run using multiple segments of the same artery type from the same animal, the results were averaged by animal. Throughout the text, the given values of $N$ refer to the number of animals studied and not the number of arterial segments. The results are organized into four main sections addressing the effects of chronic hypoxia on 1) artery cell dimensions and morphology, as determined by silver staining; 2) endothelial vasodilator capacity, as indicated by responses to A23187; 3) the mechanisms of endothelium-dependent vasodilatation, as determined through the use of
specific enzyme inhibitors; and 4) the vasodilator responses to exogenous NO.

Endothelial and vascular smooth muscle morphology. Across both age groups and artery types, chronic hypoxia reduced endothelial cell widths but did not affect endothelial cell lengths except in adult COM where hypoxic endothelial cells were longer than in normoxic arteries (Fig. 1, middle). Chronic hypoxia also significantly increased the respective length-to-width ratios for endothelial cells in the fetal COM (normoxic: 2.5 ± 0.2; hypoxic: 4.7 ± 0.3), adult MCA (normoxic: 5.1 ± 0.04; hypoxic: 6.1 ± 0.4), and adult COM (normoxic: 3.0 ± 0.02; hypoxic: 7.7 ± 0.5) but not in the fetal MCA (normoxic: 5.6 ± 0.4; hypoxic: 6.0 ± 0.5). As indicated in Table 1, chronic hypoxia increased apparent endothelial cell density in both fetal artery types but decreased density in both adult artery types.

In vascular smooth muscle cells, chronic hypoxia had opposite effects on size and shape in fetal and adult arteries. In both adult artery types, hypoxia significantly reduced cell widths without affecting cell lengths (Fig. 1, top). In contrast, chronic hypoxia significantly increased cell width and length in both fetal artery types. Corresponding length-to-width ratios were unchanged by hypoxia in fetal MCA (normoxic: 10.8 ± 0.6; hypoxic: 10.6 ± 1.0), fetal COM (normoxic: 10.9 ± 0.9; hypoxic: 13.1 ± 1.1), and adult MCA (normoxic: 17.5 ± 2.1; hypoxic: 17.8 ± 1.3) segments but were significantly increased in adult COM (normoxic: 18.5 ± 0.6; hypoxic: 27.9 ± 1.5) segments.

Regarding the vascular wall volume associated with each endothelial cell, this volume was significantly less in fetal than adult arteries of the same artery type, and this age difference persisted following acclimatization to chronic hypoxia. Chronic hypoxia did not significantly alter this ratio in fetal arteries (Fig. 1, bottom) but significantly decreased it in adult MCA segments and increased it in adult COM segments. Altogether, the adult morphology results revealed that chronic hypoxia reduced endothelial density, reduced vascular smooth muscle size, and reduced the wall volume per endothelial cell in the MCA but increased this ratio in the COM. In sharp contrast, in fetal arteries, chronic hypoxia increased endothelial density, increased vascular smooth muscle cell size, and had no overall effect on the wall volume per endothelial cell.

**A23187 dose-response relations.** The calcium ionophore A23187 is a receptor-independent activator of the endothelium and maximal responses to this drug are a reasonable estimate of maximum endothelium-dependent vasodilator capacity (11, 30). A23187 elicited dose-dependent relaxation in all arteries (Fig. 2). Because these responses were absent in denuded arteries, we attribute them to enhanced calcium entry into endothelial cells. Chronic hypoxia did not influence the maximum efficacy of A23187 except in fetal MCA where efficacy was enhanced. Age-related differences in maximum efficacy (adult > fetal) were observed in normoxic, but not hypoxic, MCA. Across all arteries (ANOVA), sensitivity to A23187 was significantly less in hypoxic than normoxic arteries. Post hoc analyses revealed significant individual hypoxic pD2 differences only in the fetal and adult MCA. No age-related differences in pD2 values were observed. Together, the A23187 results suggest that chronic hypoxia did not generally affect endothelial maximum vasodilator capacity but depressed the sensitivity of the endothelium to activation.

**Effects inhibitors and endothelial removal on A23187-induced relaxation.** Vasorelaxation responses to A23187 at the near-maximally effective concentration of 10 μM were unaffected by chronic hypoxia in MCA segments (Fig. 3) but were significantly depressed by chronic hypoxia across COM segments of both age groups (ANOVA). Individually, this effect was significant only in fetal COM segments (Fig. 3, control group). Pretreatment of the artery segments with 10 μM...
indomethacin, a nonspecific cyclooxygenase inhibitor, did not affect the magnitude of A23187-induced vasodilatation except in the hypoxic adult MCA where relaxation was reduced a modest 12%. Treatment with 100 μM l-NAME/l-nitroarginine [endothelial NO synthase (eNOS) inhibitors], ODQ (a sGC inhibitor), and endothelium removal significantly attenuated responses to A23187 in all arteries (Fig. 3). The magnitudes of the inhibitions produced by l-NAME/l-nitroarginine, ODQ, and endothelium removal averaged 84% and did not vary significantly among the different treatments. Together, these results strongly suggest that NO is the primary mediator of endothelium-dependent vasodilatation in all artery types and ages examined.

**SNAP dose-response relations.** The exogenous NO donor SNAP elicited dose-dependent relaxation in all arteries studied (Fig. 4). Chronic hypoxia significantly enhanced the maximum efficacy to SNAP in all arteries studied (ANOVA). Sensitivity to SNAP was significantly enhanced only in the fetal COM. Vasorelaxation responses to 10 μM SNAP were significantly attenuated by the use of 10 μM ODQ, and the magnitudes of this inhibition were near maximal in all artery groups examined (94.7 and 93.9% in fetal MCA, 93.3 and 97.4% in fetal COM, 94.6 and 98.5% in adult MCA, and 95.9 and 92.5% in adult COM normoxic and hypoxic segments, respectively). These ODQ results strongly suggest that soluble guanylate mediated the responses to NO in the artery groups examined.

**DISCUSSION**

Owing to the critical role of chronic hypoxia in the clinically important pathologies underlying obstructive lung disease and neonatal pulmonary hypertension, numerous previous studies of chronic hypoxia have focused on hypoxia-induced remodeling in the pulmonary vasculature (12). These studies have demonstrated that chronic hypoxia induces medial thickening (16) and impairs endothelium-dependent relaxation (20) in pulmonary arteries. Carotid body studies have further shown that such changes are not restricted to the pulmonary vasculature and that, at least in rats, chronic hypoxia increases total vascular volume (2). Aside from these studies, virtually nothing is known of the effects of chronic hypoxia on the remodeling of cerebral arteries. Such effects may be quite relevant clinically given that numerous complications during pregnancy culminate in chronic fetal hypoxia, which, in turn, is associated with a broad spectrum of hypoxic-ischemic cerebral pathologies in the neonate (17).

Postnatal maturation is typically associated with major increases in both the length and width of cerebrovascular smooth muscle cells (4), and the present results demonstrate that this process accelerates in the presence of chronic hypoxia (Fig. 1, top). In contrast, in adult arteries, chronic hypoxia significantly

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**Table 1. Effects of chronic hypoxia on age-related changes in artery circumference and endothelial cell density**

<table>
<thead>
<tr>
<th>Artery</th>
<th>Age Group</th>
<th>Circumference, μm</th>
<th>Cells/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>F N</td>
<td>5.75±0.30†</td>
<td>32.68±2.250†</td>
</tr>
<tr>
<td>C</td>
<td>F H</td>
<td>5.71±0.70†</td>
<td>43.98±1.200†</td>
</tr>
<tr>
<td>C</td>
<td>A N</td>
<td>9.28±0.270</td>
<td>46.87±0.790</td>
</tr>
<tr>
<td>C</td>
<td>A H</td>
<td>9.12±0.120</td>
<td>31.98±1.740*</td>
</tr>
<tr>
<td>M</td>
<td>F N</td>
<td>1.03±0.80†</td>
<td>4.43±0.390†</td>
</tr>
<tr>
<td>M</td>
<td>F H</td>
<td>1.30±0.70†*</td>
<td>7.42±0.520†*</td>
</tr>
<tr>
<td>M</td>
<td>A N</td>
<td>2.00±0.90</td>
<td>5.69±0.160</td>
</tr>
<tr>
<td>M</td>
<td>A H</td>
<td>1.69±0.100*</td>
<td>4.80±0.170*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6–8 arteries in each group. Measurements from common carotid (C) and middle cerebral (M) arteries taken from term fetal (F) and adult (A) sheep maintained at altitude (H) or at sea level (N) for the final 110 days of gestation are summarized. Cells/mm, number of endothelial cells per mm of circumference. †Significant effects of hypoxia between arteries of corresponding age and type (P < 0.05). *Significant effects of age between arteries of the same type and treatment (P < 0.05). Note that chronic hypoxia increased endothelial cell density in fetal arteries but had the opposite effect in adult arteries.

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**Fig. 2. Effects of chronic hypoxia on A23187 concentration-response relations.** A23187 is an endothelium-dependent but receptor-independent vasodilator that enables a reasonable measure of maximal endothelial vasodilator capacity. Shown are the A23187 concentration-relaxation relations observed in normoxic and hypoxic fetal and adult middle cerebral and common carotid arteries. Relaxation magnitudes are expressed as percent reductions in the contractile tone produced by 3 μM serotonin. Solid lines indicate curves of best fit, as determined by nonlinear regression with the logistic equation. Insets: A23187 apparent affinity (pD2) values, also obtained by nonlinear regression. Hypoxia enhanced the maximum %relaxation (efficacy) but only in fetal middle cerebral arteries. Maturation also enhanced efficacy but only in normoxic middle cerebral arteries, as indicated by ●. Across all arteries, chronic hypoxia significantly reduced A23187 pD2 values (factorial ANOVA), suggesting that hypoxia depressed the sensitivity of the endothelium to activation. Effects of hypoxia on pD2 were individually significant by post hoc Duncan’s analyses in middle cerebral arteries. Age had no significant effects on pD2. Vertical error bars indicate standard errors for 6–16 animals in each case.

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**Fig. 4.** Chronic hypoxia significantly enhanced the maximum efficacy to SNAP in all arteries studied (ANOVA). Sensitivity to SNAP was significantly enhanced only in the fetal COM. Vasorelaxation responses to 10 μM SNAP were significantly attenuated by the use of 10 μM ODQ, and the magnitudes of this inhibition were near maximal in all artery groups examined (94.7 and 93.9% in fetal MCA, 93.3 and 97.4% in fetal COM, 94.6 and 98.5% in adult MCA, and 95.9 and 92.5% in adult COM normoxic and hypoxic segments, respectively). These ODQ results strongly suggest that soluble guanylate mediated the responses to NO in the artery groups examined.
reduced cell width, demonstrating that hypoxic acclimatization leads to vascular remodeling in adult cerebral and carotid arteries as reported in other vascular beds (25). However, the results also emphasize that the remodeling effects of chronic hypoxia must be fundamentally different in neonatal and adult cranial arteries and may reflect the well-established idea that maternal mechanisms “buffer” the influence on hypoxia on the developing fetus (19). Key differences in the respective mixtures of cell types and phenotypes between well-differentiated adult arteries and immature arteries undergoing proliferation (33) are very likely involved.

Hypoxic acclimatization also changed the size and shape of endothelial cells in the arteries examined (Fig. 1, middle). In contrast to vascular smooth muscle, however, hypoxia decreased endothelial cell widths in both age groups, with few effects on cell length. Hypoxic acclimatization also produced several artery-specific changes in internal circumferences (Table 1) that were combined with changes in endothelial cell size to calculate endothelial cell density. These calculations revealed that chronic hypoxia significantly increased cell density per unit length of artery in both fetal artery types but decreased endothelial density in adult arteries. Again, these findings suggest that hypoxia has a pro-proliferative general cerebrovascular effect in the fetus but an anti-proliferative effect in the adult. In light of abundant evidence that hypoxia induces VEGF production in both endothelial and vascular smooth muscle cells (21, 23), further studies of the potential involvement of VEGF in these effects appear both promising and well justified. Independent of the mechanisms involved, the present findings reveal that the processes governing hypoxic vascular and endothelial remodeling are independent and reinforce the view that hypoxia may influence not only endothelial phenotype and shape but also the phenotype and structure of the adjacent smooth muscle (21, 22).

From a functional perspective, one possible consequence of hypoxic remodeling is that the signaling between cerebrovascular endothelial and smooth muscle cells could be influenced. To explore this possibility, we calculated the wall volume served by each endothelial cell to estimate the artery wall volume of distribution for endothelial factors released from the endothelium in the serosal direction. As shown in Fig. 1, bottom, the volume of distribution was significantly less in fetal than adult arteries of the same type, suggesting that effective wall concentrations of vasoactive factors released from the endothelium may be reached by a smaller total release in fetal than adult arteries. In addition, this volume of distribution was unchanged by hypoxia in fetal arteries but was increased and decreased in large and small adult arteries, respectively. These findings predict that, for a given rate of release of endothelial vasoactive factors, the artery wall concentrations attained would be unaffected by hypoxia in fetal arteries, would be augmented by hypoxia in adult MCA, and
would be attenuated by hypoxia in adult COM. Assuming a concentration-dependent effect of vasoactive factors on vascular tone, these results further predict that, in the adult only, chronic hypoxia would promote peripheral vasodilatation but attenuate conduit artery vasodilatation in response to endothelial activation, thus shifting a greater fraction of cerebrovascular conductance to the cerebrovascular periphery.

The idea that hypoxic remodeling should alter endothelium-dependent vasodilatation was supported by the concentration response experiments conducted with the endothelium-dependent vasodilator A23187 (Fig. 2). As a calcium ionophore, A23187 is receptor independent and thus avoids complications due to possible hypoxia-induced changes in receptor density (11) but is still capable of yielding maximal activation of endothelium-dependent calcium-mediated vasorelaxation pathways (30). Across all arteries, chronic hypoxia had few effects on A23187 efficacy but significantly and consistently depressed sensitivity to A23187. This pattern can be explained by at least three different mechanisms. First, hypoxia may alter the ability of A23187 to insert into the endothelial cell membrane due to changes in membrane lipid composition (15) that can be affected by chronic hypoxia (13). Alternatively, hypoxia may alter endothelial sensitivity to calcium, which in turn may reflect either reduced eNOS abundance or intrinsic activity (6). In addition, hypoxia may also alter the relative contributions from NO-dependent and NO-independent vasodilator pathways as well as endothelium-dependent vasoconstrictor pathways (1). To explore possible effects of hypoxia on the mix of vasoactive factors released in response to endothelial activation, we compared the effects of endothelium denudation, eNOS inhibition, sGC inhibition, and cyclooxygenase inhibition on A23187-dependent relaxation (Fig. 3). In all arteries, endothelium denudation eliminated virtually all relaxation responses to A23187, thus verifying the endothelium-dependence of these responses. The small component of relaxation persisting after denudation suggests that the direct vascular effects of A23187 (34), together with the effects of any remaining endothelial cells not removed by denudation, were negligible in this preparation. In turn, across all arteries, inhibition of eNOS via treatment with 1-NAME also eliminated responses to A23187, as did treatment with ODQ to inhibit soluble guanylate cyclase. These effects argue strongly that the vasorelaxation produced by A23187 was almost exclusively dependent on endothelial release of NO. Supporting this interpretation was the parallel finding that inhibition of cyclooxygenase with a concentration of indomethacin sufficient to inhibit responses to 1 μM arachidonic acid (31) had no significant effect on A23187-induced relaxation in all but the hypoxic adult MCA. In this artery, indomethacin modestly but significantly reduced relaxation responses to A23187, suggesting that chronic hypoxia upregulated endothelial capacity for vasodilator prostanooid synthesis and release (28), but only in the adult MCA. Aside from this singular effect, chronic hypoxia did not influence the mix of vasoactive factors released in response to A23187. Together with previous studies (31), the present results emphasize that A23187-mediated vasorelaxation in both normoxic and hypoxic ovine cranial arteries is mediated primarily by endothelial NO release.

In light of reports that chronic hypoxia can influence smooth muscle content of sGC in pulmonary arteries (18), we also examined the effects of chronic hypoxia on the ability of exogenous NO to induce relaxation. Consistent with previous reports, the magnitude of NO-induced relaxation was significantly greater in hypoxic than in normoxic arteries (Fig. 4). This effect was observed in all artery groups independent of age and was associated with significantly increased sensitivity to NO in fetal arteries. In addition, all responses to exogenous NO were eliminated by pretreatment with ODQ, thus identifying sGC as the mediator of vasorelaxant responses to NO in these preparations. Together, these findings predict that responses to endothelium-dependent vasodilators should be en-
hanced by chronic hypoxia, but this pattern was not observed; chronic hypoxia had few effects on vasorelaxation responses to A23187 (Figs. 2 and 3), suggesting at least two possibilities. First, chronic hypoxia may also enhance the release of endothelial contractile factors, as has been reported in other preparations (14). Arguing against this is the finding that responses to A23187 were almost completely NO and sGC dependent (Fig. 3). Alternatively, chronic hypoxia may attenuate endothelial release of NO through effects on eNOS abundance or specific activity. Such effects would be consistent with the reduction in sensitivity to A23187 observed in these experiments (Fig. 1). Inhibition of endothelial NO release by chronic hypoxia would also be consistent with reported effects of chronic hypoxia on NO release in pulmonary arteries (3).

Overall, the present results offer novel and original evidence that chronic hypoxia promotes remodeling of cerebral arteries. This remodeling is age dependent and leads to smooth muscle cells that are larger in fetal arteries but smaller in adult arteries. In parallel, chronic hypoxia also increased endothelial cell density in fetal arteries but reduced it in adult arteries. These opposite effects yielded changes in the geometric relations between smooth muscle and endothelial cells that increased (adult carotid), decreased (adult MCA), or left unchanged (all fetal arteries) the serosal volume of distribution for endothelial factors. Despite this heterogeneity of effects, the magnitude of endothelial-dependent vasodilatation to A23187 was largely preserved, although sensitivity to this relaxant was uniformly depressed. Because iNOS, eNOS, and endothelium denudation each independently blocked A23187-induced vasodilation without unmasking any residual vasoconstrictor effect of A23187, hypoxic attenuation of sensitivity to A23187 was attributable to either reduced endothelial NO output or reduced vascular smooth muscle NO sensitivity. Consistent with this interpretation, indomethacin did not significantly attenuate A23187-induced relaxation except in the hypoxic adult MCA, where a small contribution of prostanoids was evident. Given that vascular smooth muscle sensitivity to NO was uniformly increased by chronic hypoxia, the combined data suggest that hypoxia reduced endothelial NO release through effects on eNOS abundance, its specific activity, or its sensitivity to calcium. Whereas further investigation of these possible mechanisms seems promising, it is clear from the present data that chronic hypoxia has multiple age-dependent effects on the endothelium-dependent vasodilator pathway. In many ways, these effects appear to offset one another, thus preserving endothelium-dependent relaxation. Failure to make these adjustments could easily result in significant endothelial dysregulation and contribute to the cerebrovascular morbidity often associated with neonates surviving chronic perinatal hypoxia (17). Conversely, the finding that chronic hypoxia consistently upregulated some component of the NO-cGMP-PKG vasodilator pathway suggests that neonates exposed to chronic prenatal hypoxia will be preferentially sensitive to either inhaled NO or phosphodiesterase inhibition. Because both of these treatments are used clinically to treat neonates with persistent pulmonary hypertension (29, 32), the effects of these treatments on hypoxic nonpulmonary vessels appear worthy of further investigation.

GRANTS

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