

Title: Vulnerability of the fetal cerebral vasculature to hypoxic stress: effects on smooth muscle differentiation and contractile phenotype

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"wpearce" <wpearce@llu.edu>Objectives: Hypoxic-ischemic insults produce a broad spectrum of injury that depends on insult severity and duration, as well as the tissue types involved. Whereas most investigations of fetal cerebral hypoxic-ischemic insults have focused on neuronal and glial injury, fetal cerebral vascular tissues are also highly vulnerable to these insults. The present studies explore the hypothesis that hypoxic-ischemic insults dramatically influence fetal cerebrovascular tissues resulting in altered patterns of fetal vascular differentiation and contractile phenotypes in arteries supplying the immature brain. The corollary hypothesis addressed by these studies is that hypoxic-ischemic injury to fetal cerebral arteries compromises contractile function and impairs coupling between cerebral metabolism and blood flow, which in turn can exacerbate hypoxic-ischemic neuronal/glial injury in the immature brain.

Methods: Cerebral arteries were harvested from term fetal and adult sheep maintained at either sea level, or an altitude of 3820m (White Mountain Research Station, Bishop, CA) for the last 110 days of gestation. Arterial PO₂ values in the hypoxic fetuses averaged 19 Torr, compared to 26 Torr in normoxic fetuses. In situ specific activities and abundances of Soluble Guanylate Cyclase (sGC), Protein Kinase G (PKG), and Myosin Light-Chain Kinase (MLCK) were measured in smooth muscle using computer-controlled rapid-freeze techniques. Column chromatography and Electrospray Ionization Mass Spectrometry were used to purify and microsequence Myosin Light Chain (MLC) to examine possible stress-related shifts in MLC isoform. Visible and fluorescent immunohistochemistry were used to examine the transmural distribution of all proteins of interest including phenotype specific markers (Non-Muscle Myosin, Myosin Heavy Chain B, and α -Smooth Muscle Actin). Contractile phenotypes were defined by simultaneous measurements of contractile force and intracellular calcium (via Fura-2) measured in response to high extracellular K⁺ or 10 μ M 5-HT. To assess endothelial function we examined eNOS abundance and specific activity, as well as the effects of graded shear and pharmacological stimulation on NO release. Perivascular nerve function was examined using HPLC to measure norepinephrine content, release, and reuptake in response to electrical field stimulation. Contractile responses to nerve stimulation were also measured, as was the density and distribution of adrenergic innervation via fluorescent histochemistry.

Results: Hypoxia dramatically altered the distribution and activity of each enzyme examined; sGC activity decreased precipitously through an 8-fold drop in enzyme abundance that was absent in adult arteries. Protein Kinase G activity was upregulated in fetal arteries, but the inhibitory effects of this enzyme on agonist affinity, calcium extrusion, and myofilament calcium sensitivity were attenuated by hypoxia. Myosin Light-Chain Kinase activity was dramatically upregulated in fetal arteries, as was expression of Non-Muscle Myosin. Hypoxia greatly enhanced expression of α -Smooth Muscle Actin. The mass spec sequencing results were consistent with the hypothesis that MLC isoform expression is related to smooth muscle phenotype. Hypoxia enhanced endothelial sensitivity to shear stress and capacity for NO release through alteration of eNOS abundance and specific activity. The contractile efficacy of fetal perivascular nerves was upregulated 5-fold by hypoxia, this effect was absent in adult arteries, and did not involve changes in NE content, release, or reuptake; changes in synaptic ultrastructure appear to have been involved.

Conclusions: Taken together, these results demonstrate that the endothelial, neuronal, and smooth muscle cell types of fetal cerebral arteries are all dramatically altered, both functionally and structurally, by hypoxic stress in a highly age-dependent manner. These results thus support the hypothesis that blood vessels are a major target of hypoxic or ischemic injury, which also suggests that compromised vascular function may reduce coupling efficiency between flow and metabolism, which may aggravate the extent of neuronal injury in the post-ischemic fetal brain. Based on the pronounced differences between fetal and adult vascular responses to hypoxia, these results further suggest that age-related differences in cerebrovascular phenotype, structure, and function may help explain why vulnerability to hypoxic-ischemic stress varies so markedly between the immature and mature brain.