

Induction of vascular endothelial growth factor in the late gestation fetal brain following intrauterine hypoxia produced by umbilical cord occlusion.

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The developing brain is vulnerable to hypoxia, which can result in damage due to cerebral edema, hemorrhage and vascular leakage. Hypoxia induces up-regulation of specific hypoxia-sensitive genes, including that for vascular endothelial growth factor (VEGF). Originally described as a vascular permeability factor, VEGF is also an endothelial cell-specific mitogen inducing angiogenesis, and exerting trophic effects on neurons and glia. Thus, VEGF may be important for both normal brain development and repair responses that follow insults causing cerebral damage. While VEGF up-regulation has been shown in the neonatal brain after hypoxic challenge, it is not known if this occurs in the fetal brain following intrauterine hypoxia.

Objective: We sought to investigate the expression and regional distribution of VEGF immunoreactivity (IR) in the late gestation fetal sheep brain following severe systemic hypoxia produced by umbilical cord occlusion (UCO).

Methods: At 124-127 days gestation (term 147), singleton fetal sheep underwent surgery for implantation of catheters and placing an inflatable cuff around the umbilical cord. A 10 minute UCO or sham UCO occurred at 132 days gestational age. Post mortem (PM) occurred at 24 hours or 48 hours after UCO when the fetal brain was collected for immunohistochemistry using 10µm paraffin embedded sections. Sections were incubated with primary antibody mouse monoclonal anti-VEGF (1:200) overnight at 4°C. Following rinses in PBS-Tween 20, sections were also incubated with a second primary antibody to either anti-GFAP (1:100) to label astrocytes, or anti-MAP2 (1:100) to label neurons. Immunoreactivity was subsequently visualised with fluorescent labelled secondary antibodies; Alexa Fluor A488 goat anti-mouse (1:1000, green) and Alexa Fluor 594 goat anti-mouse (1:1000, red). Sections were then examined using an Axioplan 2 microscope (Zeiss, 400x magnification) using an Axiocam-2 camera.

Results: Low to moderate levels of VEGF-IR was present in all regions examined of control fetal brains; this constitutive expression was mainly (but not exclusively) astrocytic. VEGF expression, both astrocytic and neuronal, was increased markedly at 24 h post-UCO in the corpus callosum, caudate nucleus, internal capsule, putamen, germinal matrix, cortex (layers I-VI), periventricular and subcortical white matter. At 48 h after UCO, the VEGF-IR was still present at high levels in neurons, but it was also strongly associated with reactive astrocytes, particularly in the cortical white matter, external granule, molecular, Purkinje and internal granular layers of the cerebellum compared to both control and 24 h post-UCO fetuses.

Conclusion: Neuronal VEGF expression was strongly induced by systemic hypoxia throughout the late gestation fetal sheep brain. VEGF-IR was also seen in the cerebellar external granule cell layer which did not label with either MAP2 or GFAP; we speculate that this occurred in progenitor cells in this proliferative zone of the developing cerebellum. The increased VEGF expression in the fetal brain following UCO may be involved in the induction of angiogenesis and/or neurogenesis as it has been shown to occur in the adult and neonatal brain. Whether the increased VEGF expression is a response to local injury, an adaptive response that limits further cell death, and is involved in increased angiogenesis and/or neurogenesis following hypoxia awaits further investigation.