

## ASPHYXIA ACTIVATES P65 AND INDUCES VEGF-A GENE EXPRESSION IN RETINA AND CHOROID FROM NEWBORN PIGLETS

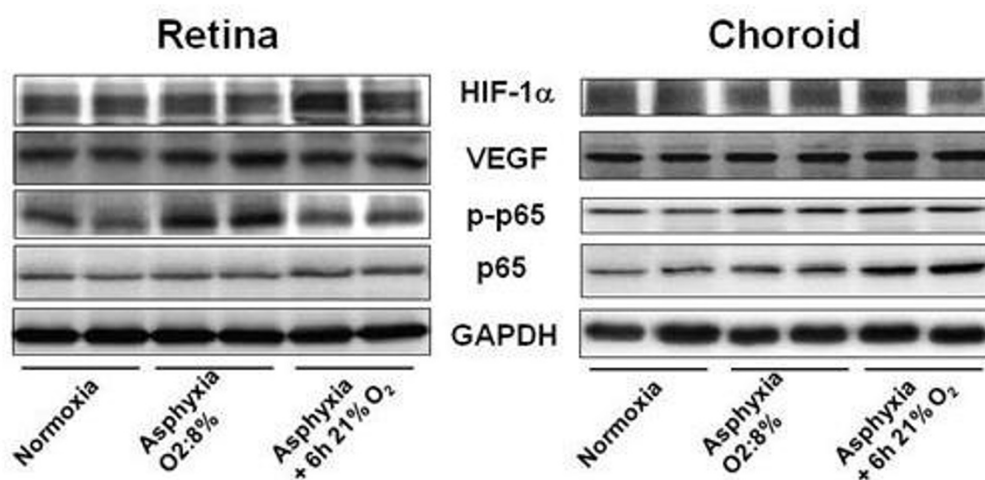
R. Solberg<sup>1</sup>, A. Arduini<sup>2</sup>, J. Escobar<sup>3</sup>, O. Saugstad<sup>1</sup>, V. Maximo<sup>4</sup>

<sup>1</sup>*Pediatric Research Institute, University of Oslo, Oslo, Norway*, <sup>2</sup>*Physiology, University of Valencia*, <sup>3</sup>*Health Research Institute (I.I.S. La Fe)*, <sup>4</sup>*Division of Neonatology, University and Polytechnic Hospital La Fe, Valencia, Spain*

**Objective:** Exposure to lower oxygen causes oxidative stress and promotes angiogenesis. Asphyctic neonates have shown higher cord-blood vascular endothelial growth factor (VEGF). We hypothesized that retina and choroid having a different circulatory regulation (choroid lacks vascular auto-regulation) would acutely stimulate angiogenesis in response to short and severe hypoxemia.

**Methods:** Retina/choroid were obtained from hypoxic (FiO<sub>2</sub>:8% x 30 min) newborn piglets resuscitated with 21% O<sub>2</sub> and controls (ventilated with 21% O<sub>2</sub>) at 6h after birth. Vegfa mRNA expression was determined by real-time PCR. Protein level of hypoxia inducible factor-1alpha (HIF-1alpha), Vegf, phosphorylated p65 (S539), p65, and Gapdh (housekeeping gene) were studied by western blotting.

**Results:** Vegfa mRNA expression in retina and choroid significantly increased during hypoxia (p < 0.01 vs. control). At protein level, Hif-1 $\alpha$  did not change significantly with hypoxia or re-oxygenation. Vegf protein did not increase in retina or choroid with hypoxia or re-oxygenation. P65 was significantly more phosphorylated in response to hypoxia (p < 0.05 vs. control), and restored to baseline after re-oxygenation just in retina. Total levels of p65 increased in choroid after 6h of resuscitation (p < 0.05 vs. control).



[RETINA AND CHOROID WESTERN BLOTTING]

### Conclusions:

- (i) Short-term hypoxemia promotes p65 activation in retina and choroid.
- (ii) Vegf-mRNA expression is not Hif-1 $\alpha$ -dependent but secondary to p65-activation.
- (iii) Angiogenic factors' synthesis has not been achieved due to time elapsed between hypoxemia and tissue extraction.