

**HYPOXIC VASOCONSTRICTION IN SYSTEMIC ARTERIES
OF THE SEA LAMPREY**

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Introduction

Hypoxia is an important stimulus for the local control of mammalian vascular smooth muscle (VSM). In systemic VSM, local dilation improves flow and oxygen delivery to meet metabolic demand. Hypoxic vasoconstriction in adult pulmonary VSM (HPV) circumvents damaged or defective alveoli to match ventilation with perfusion. Dilation of systemic VSM is thought to involve closure of potassium channels (K^+_{ATP}) in response to increased intracellular ADP/ATP ratios that results in membrane hyperpolarization (Weir et al., 1995). Endothelium-derived and direct smooth muscle effects contribute to HPV, but their reported significance has varied with species examined and experimental protocol. The direct effects of HPV in mammals are thought to involve potassium channels but the exact signaling mechanism remains unknown (for reviews, see Dumas et al., 1999, Weir et al., 1995). Less is known about hypoxic vasoconstriction (HV) in non-mammals. The general trend in systemic VSM appears to be toward dilation, while HV has been noted in pulmonary tissue of the box turtle, *Trachemys scripta*, (Crossley et al., 1998), and in gill tissues of rainbow trout, *Oncorhynchus mykiss*, (Sundin and Nilsson, 1997) and cod, *Gadus morhua*, (Sundin, 1995). The present study describes *in vitro* hypoxia responses of isolated ventral and dorsal aorta (VA, DA) and coeliaco-mesenteric

artery (CMA) of the Sea lamprey, *Petromyzon marinus*. Our results show a profound HV in the post-gill (DA, CMA), but not pre-gill (VA) systemic arteries. These results indicate a phylogenetically ancient origin for HV, and suggest that lamprey may be an ideal model system for studying the HV response.

Methods

Sea lamprey (*Petromyzon marinus*) were captured by the U.S. Geological Survey, Biological Resources Division, and airlifted to Notre Dame. DA, VA, and CMA vascular rings were prepared and suspended in smooth muscle chambers as described in Olson and Meisheri, 1991. Tension was measured with Grass FTO3C force-displacement transducers and recorded on a computer-interfaced Gould or Grass polygraph. Hypoxia was administered by bubbling the chambers with 100% nitrogen gas and normoxia was restored by aeration with room air. Graded reductions in oxygen tension (P_{O_2}) were applied using a Wostoff gas-mixing pump. Agonists and drugs were applied 15 min prior to hypoxic exposure.

Treatment effects were statistically examined by paired *t*-test or repeated measures tests. Student's *t*-test and analysis of variance (ANOVA) were used for comparisons between vessels. The limit of significance was set at $p < 0.05$.

Results and Discussion

Lamprey VA either slightly contracted or were unaffected by hypoxia (Fig. 1a), whereas a profound contraction was noted in DA (Fig. 1a) and CMA (not shown). Repeated exposure to hypoxia did not affect the

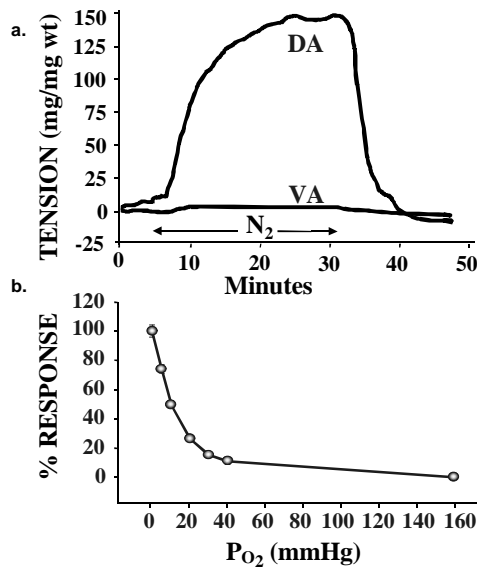


Figure 1: a) Response of lamprey DA and VA to hypoxia; b) Hypoxia Dose-response Curve for Lamprey DA

magnitude of vasoconstriction, and vessels remained viable after multiple hypoxic exposures (up to 7 times per day) over several days. HV in the lamprey DA appeared to be independent of pre-existing tonus induced by either elevated KCl (80mM), or ligand. Hypoxic vasoconstriction was dependent on oxygen tension and the magnitude of contraction could be titrated with step-wise P_{O_2} changes. The P_{O_2} at which half-maximal response was obtained (P_{50}) was calculated at 10.7 ± 1.9 mmHg for DA (n=5) (Fig. 1b). Inhibitors of cyclooxygenase (indomethacin, IND), lipoxygenase (esculetin, ESC), and cytochrome P_{450} (clotrimazole, CLOT) were ineffective against HV in the lamprey DA, as were antagonists to α -adrenergic (phentolamine, PHENT) and purinergic A_1 (8-phenyltheophylline, 8PT) receptors (Fig. 2). HV was blocked by the myosin light-chain kinase inhibitor ML-9. The present experiments show that post-gill vessels of the sea lamprey respond to hypoxia with a profound, sustained constriction. Lamprey HV and HPV are both (at least in part) intrinsic properties of VSM, can be sustained, are reproducible, are vessel-specific, and the magnitude of the response is P_{O_2} dependent.

However, HV in lamprey is independent of pre-existing tonus, is undiminished by pre-contraction with either KCl or ligand, is present in systemic vessels, does

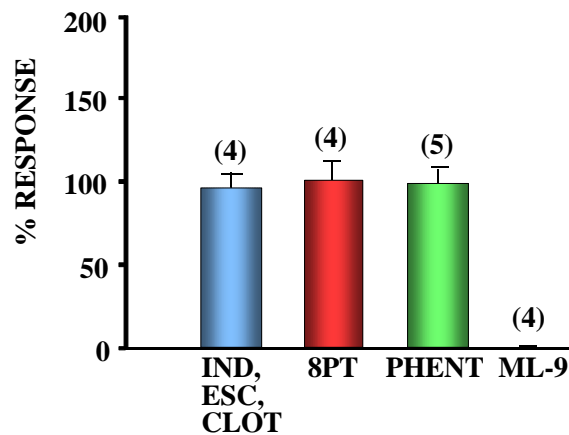


Figure 2. Effect of Inhibitors on Hypoxia in Lamprey DA. All [10^{-5} M] except [10^{-4} M] ML-9 (n= number of fish).

not appear to be endothelium dependent, and is reproducible through multiple hypoxic exposures over several days. Pre-treatment with inhibitors of enzymes that produce endothelium-derived paracrine factors or antagonists against α -adrenergic or purinergic receptors do not diminish HV. These results suggest that HV is an endothelium independent, intrinsic, and vessel-specific response of lamprey VSM.

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