We used optical microscopy approaches in combination with fluorescent reporter molecules to investigate a role for NO-induced zinc release in pulmonary vasoregulation. An isolated, perfused mouse lung (IPL) model was adapted for intravital imaging, allowing us to study the integrative nature of signaling events in the intact, living tissue. Confocal microscopy was used to show that acute hypoxia caused a reversible vasoconstriction of small subpleural blood vessels (<40 µM) of the mouse IPL. We then focused on pulmonary endothelium and noted that hypoxia caused an NO-dependent increase in labile zinc (as detected by zinc-specific fluorophore, FluoZin3) in these small arterioles. The latter observation was critically dependent upon the zinc binding protein, metallothionein (MT) as it was not apparent in IPL of MT-/- mice. We adopted fluorescence resonance energy (FRET) approaches to further study NO-based signaling events during hypoxia. We used tail vein injection of DOTAP: cholesterol liposomes followed by either adenovirus or plasmid containing cDNA to achieve endothelial expression of the FRET-based reporters in mouse lung. Data obtained using full spectral laser scanning confocal imaging of these NO-sensitive FRET reporters (cygnet-2 that reports activation of soluble guanylyl cyclase; FRET-MT that reports S-nitrosation of MT) supported hypoxia induced NO production in pulmonary endothelium. Lastly, we found that hypoxic vasoconstriction was blunted in IPL of MT-/- mice or in wild-type mice treated with the zinc chelator, N,N,N',N'-Tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN), suggesting a role for chelatable zinc in hypoxic pulmonary vasoconstriction. Collectively, these data suggest that zinc thiolate signaling is a component of the effects of acute hypoxia mediated NO biosynthesis and this pathway may contribute to constriction in pulmonary resistance vessels. Furthermore, this work illustrates the utility and importance of combining an integrated physiological model with sophisticated imaging technologies to study intracellular signaling pathways in a biologically relevant context.

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