Superoxide and Chronic Hypoxia-Induced Pulmonary Hypertension in Newborn Piglets

Candice D. Fike, Judy L. Aschner, Yongmei Zhang, Daniela Salvemini and Mark R. Kaplowitz

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first, an in situ immunofluorescence assay indicated the presence of TNF-α–induced activation of cPLA2 in AECs of intact alveoli; and second, microinjections of arachidonate in saponin-permeabilized alveoli induced oscillation-damped Ca²⁺ cyt increases in ECs.⁷

An important consequence of cross-talk signaling in capillaries is the surface expression of the leukocyte adhesion receptor, P-selectin, in ECs. P-selectin is normally held in EC in intracellular vesicles. Increase of Ca²⁺ cyt or H₂O₂ causes surface expression of P-selectin, which therefore provides a marker of proinflammatory activation in EC.¹⁰ Alveolar TNF-α increased capillary P-selectin expression in adjoining capillaries within 5 min (Fig 2),⁷ attesting to the considerable rapidity of this cross-talk response. The P-selectin expression was also inhibited by intra-alveolar preinfusion of either the anti-TNF-α–induced alveolocapillary cross-talk, a role also recognized in the context of acid-induced lung inflammation,¹¹ the post-cPLA2 factor that relays the cross-talk signal remains unidentified. The possibilities are as follows: cPLA2 activation causes arachidonate release; hence, basolateral release of arachidonate from AECs might relay the signal to ECs, causing Ca²⁺ cyt-induced P-selectin expression. Also, arachidonate increases AEC reactive oxygen species formation through nicotinamide adenine dinucleotide phosphate oxidase activation¹²; hence, diffusible reactive oxygen species such as H₂O₂ could be the relay factor. H₂O₂ directly induces P-selectin expression in capillary ECs.¹⁰ Hence, H₂O₂ released basolaterally by AECs may induce proinflammatory responses in capillaries.

Although these mechanisms require further consideration, our findings provide the hitherto unrecognized role of the alveolar epithelium as an inducer of the inflammatory response of the lung. Clearly, this role is central in conveying proinflammatory signals from alveoli to adjoining blood vessels. Further research is required to elucidate the role of AECs, not only in arming the innate immune response of the lung, but also in shaping lung pathology under conditions associated with alveolar remodeling.

**References**

1. Skerrett SJ, Martin TR, Chi EY, et al. Role of the type 1 TNF receptor in lung inflammation after inhalation of endotoxin or *Pseudomonas aeruginosa*. Am J Physiol 1999; 276:L715–L727

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**Abbreviations:** RPA = resistance pulmonary artery; SOD = superoxide dismutase

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**Figure 2. Capillary P-selectin expression following alveolar TNF-α injection.** Images show an alveolarcapillary region consisting of capillaries (cap) adjoining an alveolus (alv). Solid lines are capillary walls. The dotted line indicates an alveolar wall. Arrows denote direction of blood flow. Alveolar injection of TNF-α in indicated alveolus increases fluorescence from near zero at baseline (left panel), as denoted by marked increase of gray levels (right panel).
Free radicals, including superoxide, $O_2^-$, have been implicated in the pathogenesis of certain types of pulmonary hypertension. Yet, the role of $O_2^-$ in chronic hypoxia-induced pulmonary hypertension in newborns is unknown. Moreover, there is little information regarding the influence of chronic hypoxia on the expression of $O_2^-$ enzymatic scavenging systems, such as the cytosolic copper/zinc containing enzyme, superoxide dismutase (SOD)-1 or mitochondrial manganese SOD (SOD-2). Our purpose was to determine whether $O_2^-$ is involved with the abnormal constrictor responses that develop in resistance pulmonary arteries (RPAs) of newborn pigs exposed to 3 days of hypoxia. We also evaluated whether 3 days of exposure to hypoxia alters the expression of either SOD-1 or SOD-2 in RPAs. Newborn pigs, 2 to 3 days old, were placed in normoxic (control) or hypoxic (11% $O_2$) chambers. After 3 days in the respective chambers, the piglets were euthanized and RPAs (100 to 300 μm in diameter) were dissected from the lungs. Some RPAs were cannulated and pressurized to measure the effect of the cell-permeable SOD mimetic, M40401, on responses to acetylcholine. Other RPAs were homogenized for determination of SOD-1 and SOD-2 levels by immunoblot technique. We found that untreated RPAs from hypoxic piglets constricted to acetylcholine; whereas after M40401 treatment, RPAs from hypoxic piglets dilated to acetylcholine. Although SOD-2 abundance was unaltered, SOD-1 was diminished by 20% in RPAs from hypoxic as compared to control piglets. Thus, $O_2^-$ contributes to the constrictor response to acetylcholine that develops in RPAs of piglets exposed to 3 days of hypoxia. Moreover, chronic in vivo hypoxia inhibits the expression of one of the most important intracellular antioxidant enzymes, SOD-1. $O_2^-$ generation, exaggerated by the inhibition of SOD-1, contributes to the development of chronic hypoxia-induced pulmonary hypertension in newborn pigs.

Hypoxia-induced pulmonary vasconstriction (HPV) is an important adaptive process that remains incompletely understood. In preconstricted rat pulmonary arteries (inner diameter, 250 to 400 μm), hypoxia (pO2 approximately 10 mm Hg) induces an initial transient phase and a more slowly developing sustained phase of vasoconstriction. Since the release of calcium ions ($Ca^{2+}$) from intracellular stores by redox-sensitive intracellular $Ca^{2+}$ release channels known as ryanodine receptors (RyRs) in pulmonary arterial smooth-muscle cells (PASMCs) may play a role in HPV, and considerable evidence now supports that levels of reactive oxygen species (ROS) are paradoxically increased in PASMC under hypoxia, we investigated whether redox activation of RyRs by ROS may transduce HPV. By reverse transcriptase-polymerase chain reaction, we found that all three RyR isoforms are expressed in rat pulmonary arteries and in PASMCs. The sustained phase, but not the transient phase, of HPV can be prevented by pretreating pulmonary arteries with RyR inhibitors ryanodine (200 μmol/L) or dantrolene (50 μmol/L). The addition of dantrolene, ryanodine or the thiol-reducing agent diithiothreitol (1 μmol/L) during the sustained phase of HPV reversed the hypoxic vasconstriction. However, the superoxide scavenger nitroblue tetrazolium (500 nmol/L) prevented further hypoxic pulmonary vasoconstriction during the sustained phase of HPV but did not reverse it. Taken together, our data suggest that redox activation of RyRs by ROS has an important role in transducing the sustained contraction of pulmonary arteries under hypoxia. (CHEST 2005; 128:556S–558S)

Key words: hypoxia-induced pulmonary vasconstriction; redox regulation; ryanodine receptors

Abbreviations: DTT = diithiothreitol; HPV = hypoxia-induced pulmonary vasconstriction; PASMC = pulmonary arterial smooth muscle cell; ROS = reactive oxygen species; RT-PCR = reverse transcriptase-polymerase chain reaction; RyR = ryanodine receptor

Redox Activation of Intracellular Calcium Release Channels (Ryanodine Receptors) in the Sustained Phase of Hypoxia-Induced Pulmonary Vasoconstriction*

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Hypoxia-induced pulmonary vasconstriction (HPV) is an important adaptive process that diverts blood flow away from poorly oxygenated lung regions to well-oxygenated regions, thereby optimizing oxygenation/gas exchange in patients with significant respiratory diseases. Persistent hypoxic pulmonary vasconstriction, however, can lead to the remodeling of pulmonary vasculature, debilitating pulmonary hypertension, and other untoward clinical se-

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