Point: Counterpoint: Hypoxic pulmonary vasoconstriction is/is not mediated by increased production of reactive oxygen species

**Purpose and Scope of the Point:Counterpoint Debates**

This series of debates was initiated for the Journal of Applied Physiology because we believe an important means of searching for truth is through debate where contradictory viewpoints are put forward. This dialectic process whereby a thesis is advanced, then opposed by an antithesis, with a synthesis subsequently arrived at, is a powerful and often entertaining method for gaining knowledge and for understanding the source of a controversy.

Before reading these Point:Counterpoint manuscripts or preparing a brief commentary on their content, the reader should understand that authors on each side of the debate are expected to advance a polarized viewpoint and to select the most convincing data to support their position. This approach differs markedly from the review article where the reader expects the author to present balanced coverage of the topic. Each of the authors has been strictly limited in the lengths of both the manuscript (1,200 words) and the rebuttal (400). The number of references to publications is also limited to 30, and citation of unpublished findings is prohibited.

**Point: Hypoxic Pulmonary Vasoconstriction is Mediated by Increased Production of Reactive Oxygen Species**

Hypoxic pulmonary vasoconstriction (HPV) diverts blood away from poorly ventilated regions of the lung and so optimizes gas exchange. Great strides have been made in recent years in the search for the mechanisms of HPV, although not necessarily in the same direction. Although there is agreement that these mechanisms reside within the pulmonary artery (PA) and include elevation of smooth muscle intracellular [Ca\(^{2+}\)] (\([\text{Ca}^{2+}]_i\)) and increased Ca\(^{2+}\) sensitivity (4, 16, 21, 24), the identity of the transduction pathways is somewhat controversial—discussions concerning the oxygen sensor and its distal signaling moieties often end in wailing and gnashing of teeth. The most significant disagreement concerns the role of reactive oxygen species (ROS) and whether an increase (e.g., Refs. 14, 29) or decrease (e.g., Refs. 19, 20) in ROS production acts as the initiating stimulus for HPV. And let us not forget the "Who cares?" constituency, who oppose any role for ROS in HPV whatsoever (e.g., Refs. 4, 6). We believe the evidence is strongly in favor of the first option and have no qualms about going mano e mano with our friends in the North.

Most agree that mitochondria function as the oxygen sensor for HPV (but see Ref. 29). Oxidation of NADH by complex I of the electron transport chain (ETC), and FADH\(_2\) and succinate by complex II, results in transfer of electrons by ubiquinol of the electron transport chain (ETC) by univalent donation of electrons to O\(_2\) to form superoxide, primarily at complexes I and III; ubisemiquinone is the donor in complex III, but only when formed at the Qo site can superoxide enter the mitochondrial intermembrane space (Fig. 1; Refs. 18, 19). Superoxide is converted by superoxide dismutate (SOD) to H\(_2\)O\(_2\), the ROS signaling moiety of choice. A simplistic view would predict that because O\(_2\) is the substrate for ROS production, then clearly as [O\(_2\)] falls so should ROS. However, the rate of ROS formation is proportional to [O\(_2\)] \times [electron donor], the latter being increased if electron transport is slowed and the proximal ETC becomes more reduced (18). Thus ROS production would increase if [electron donor] (e.g., ubisemiquinone) increases more than [O\(_2\)] falls—in antimycin A, which inhibits cyt b\(_1\) and increases ubisemiquinone lifetime, promotes ROS generation (19). The above relationship could explain the apparent paradox that both hypoxia and hyperoxia (5) increase ROS; in normoxia, [O\(_2\)] is maximal for COX, so hyperoxia could not affect electron transport or [electron donor].

So if ROS production can increase in hypoxia, does it, in fact, do so? Here those unlucky readers not in the field ask “Why not just measure it and leave us in peace?” Therein lies the rub. It has been, and we are not at peace. Various indicators, including dichlorofluorescein (DCF), have been used to show increased ROS production in live cells during hypoxia, often suppressed as predicted by antioxidants and/or catalase (9, 11, 12, 22, 23). However, many believe that DCF is an unreliable witness that should be consigned to the dustbin of history. But luminol and lucigenin also have shortcomings and, especially in complex preparations such as perfused lungs, are likely to detect primarily extracellular ROS. These are largely derived from NAD(P)H oxidases and may well fall in hypoxia (29), effectively obfuscating concomitant increases in cytosolic ROS. Notably, the Schumacker group (7) has demonstrated an increase in ROS during hypoxia using a novel fluorescence resonance energy transfer-based intracellular sensor, with fewer shortcomings. Importantly, hypoxic signaling is associated with DNA base oxidation products in PA endothelial and smooth muscle cells (SMC) (30), strongly implying an increase in cytosolic oxidant stress.

If HPV is mediated by an increase in ROS then we can make certain predictions. At the most basic level, antioxidants should suppress HPV without mimicking hypoxia, whereas exogenous ROS should do the opposite. A significant body of evidence supports this prediction. For example, superoxide scavengers (26, 27), SOD inhibitors (which prevent breakdown to H\(_2\)O\(_2\)) (22, 28), catalase (which breaks down H\(_2\)O\(_2\)) (11, 17, 23), and agents that enhance or mimic the glutathione antioxidant axis (22, 23) all suppress HPV and/or the associated elevation in [Ca\(^{2+}\)] without mimicking hypoxia. Rather more damning for
the opposition are reports that exogenous H2O2 causes PA vasoconstriction in normoxia (8, 22), and we have recently shown that low concentrations (1–100 μM) of H2O2 or menadione (a promoter of intracellular ROS generation) cause sustained constrictions and elevations of [Ca2+]i in intrapulmonary arteries (IPA) with similarities to those induced by hypoxia (3). Conversely the same concentrations of H2O2 generally vasodilate systemic arteries (e.g., Refs. 3, 15). High concentrations of ROS may constrict or relax PA (8), presumably in part by frying the preparation. At the very least, these studies would seem to predicate against the hypothesis that HPV is due to a fall in ROS.

Indirect studies are also convincing. All agree that ETC inhibitors acting proximally to ubisemiquinone in complex III (e.g., rotenone: complex I; myxothiazol: complex III Rieske Fe-S center), caused distal oxidation, reduced availability of ubisemiquinone, and decreased ROS production. Thus if a rise in ROS does underlie HPV, proximal inhibition of the ETC should suppress HPV without mimicking hypoxia. Consistent with this, we and others have reported that proximal inhibitors not only suppress HPV without significantly affecting other vasoconstrictor mechanisms, but also do not elicit constriction or elevations in [Ca2+]i, in normoxia (10, 22, 25); this is critical, because if a fall in ROS mediates HPV, then proximal inhibition should always mimic hypoxia. In addition, we have shown that the complex II substrate succinate, which bypasses complex I by providing electrons to complex III, restores HPV in the presence of rotenone (but not myxothiazol) (10, 21). As succinate restores electron transport in the presence of rotenone and thus ROS production at complex III, even with the wildest stretch of imagination it is extremely difficult to see how these data could be compatible with a decreased production of ROS being the stimulus for HPV; au contraire, they are entirely consistent with an increase.

Finally, inhibition of COX (cyanide) and complex III distal to ubisemiquinone (antimycin A) might be expected to mimic hypoxia, by decreasing electron flux and increasing [ubisemiquinone], respectively. Indeed, cyanide has been shown to increase ROS, induce constriction in normoxia and/or enhance HPV (1, 10, 20, 22, 23), and antimycin A shows similar responses (1, 23).

Doubtless our honorable opponents will try to convince you otherwise, perhaps using a different “spin” for interpretation of the above data, but we believe that even in this limited space we have provided sufficient evidence (summarized in the figure) for an almost insurmountable case for the defense, and that you the jury will now shout “Yes! HPV is indeed mediated by increased production of ROS (probably).”

ACKNOWLEDGMENTS

Thanks are due for helpful comments (and otherwise) from other interested parties.

GRANTS

Our work was supported by the Wellcome Trust and British Heart Foundation.
REFERENCES


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COUNTERPOINT: HYPOXIC PULMONARY VASOCONSTRICTION IS NOT MEDIATED BY INCREASED PRODUCTION OF REACTIVE OXYGEN SPECIES

The acute hypoxic pulmonary vasoconstriction (HPV) that we discuss in this debate involves rapid constriction of small pulmonary arteries in response to physiological levels of alveolar hypoxia. HPV starts within seconds of the onset of hypoxic ventilation and we focus on the early events before changes in gene expression are involved. Hypoxia causes contraction of pulmonary artery smooth muscle cells (PASMCs) directly (12), although this contraction is modulated by the endothelium.

Most scientists working in the field of HPV would probably agree on three components of the executive part of HPV. These are: hypoxic inhibition of potassium channels with consequent membrane depolarization and calcium entry through L-type calcium channels, release of calcium from the sarcoplasmic reticulum with subsequent entry of calcium through store-operated channels (SOC), and increased sensitivity of actin/myosin to any particular level of calcium, mediated by increased activity of rho kinase (23). Different researchers will emphasize the importance of particular components differently but the element involving potassium channels is present not only in the pulmonary vasculature but also in the other oxygen-sensing tissues that comprise the mammalian “specialized oxygen homeostatic system” (the carotid body, the neuroepithelial body, and fetal adrenomedullary chromaffin cells). If we agree, more or less, on the executive arm, what is the disagreement on the sensing mechanism that initiates executive action? Our