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Commentary

Commentary on "Hypoxia, hypoxic signaling, tissue damage, and detection of reactive oxygen species (ROS)"

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In patients, severe or profound hypoxia (anoxia; 0–1% oxygen) can occur during cardiopulmonary arrest. Unfortunately, even in medically sophisticated communities, survival from such events remains disappointingly low (<30%) [1,2]. During resuscitation, reoxygenation with reperfusion markedly augments generation of ROS, increasing oxidant stress and exacerbating tissue injury. Most recently, this issue has been highlighted in controversies related to oxygen use during resuscitation of the asphyxiated newborn, in which infant outcomes and biochemical markers of oxidant stress after resuscitation with room air levels of supplemental oxygen (21% oxygen) are reduced in comparison with the use of 100% oxygen [3,4]. Although low oxygen tension in the normal fetus is critical for normal organ development in utero [5], chronic reductions in oxygen tension impair growth in the developing fetus [6], illustrating the complex mechanisms linking hypoxia, reperfusion, and ROS production during development. During postnatal life, moderate persistent or intermittent hypoxia (5–10% oxygen or greater) occurs in diverse clinical settings, such as during acute and chronic adaptation to high altitude, obstructive sleep apnea, acute and chronic lung diseases, and others. Importantly, regional hypoxic microenvironments also contribute to abnormal cell growth and metastatic disease in cancer. Hypoxia, with or without ischemia, occurs during circulatory shock, especially in association with acute respiratory distress syndrome, systemic inflammatory response, and/or multiple organ failure. Hypoxia with ischemia or reperfusion injury plays a prominent role in the setting of vascular diseases, such as sickle cell anemia, organ transplantation, myocardial infarction, and stroke. However, the mechanisms through which ROS contribute to the pathophysiology of organ injury in these diverse clinical settings remain incompletely understood.

In recent years, controversies with regard to these questions about hypoxia and ROS have arisen which include the following: (1) Is ROS production increased or decreased during tissue hypoxia without reoxygenation, and, if produced in excess, what is the source? (2) Do ROS contribute to signaling responses as the host adapts to hypoxia? (3) Do ROS contribute to tissue damage that can result from hypoxia?

Since ROS generation is more commonly considered in the setting of hyperoxia, excess production of ROS during hypoxia seems counterintuitive and, if this contributes to tissue damage, paradoxical. Success in answering these important questions depends on rigorous measurement of ROS[1] (recently reviewed in [7,8]), which can be technically challenging and, at times, difficult to interpret. Take home messages have included the following: (1) accurate ROS detection remains challenging, (2) if fluorescent dyes are used, reactions with these indicators which suggest the presence of ROS, but not due to the presence of ROS, can occur [9] and precautions must be taken to avoid such artifacts [10–12], and (3) appropriate controls must be included. A number of similar concerns and precautions pertain to commonly used chemiluminescent probes [13–15]. Electron paramagnetic resonance (EPR) and electron spin resonance (ESR) can add an important dimension to ROS detection [16] given the specificity of signals that can be obtained. Use of

Abbreviations: ROS, reactive oxygen species; XOR, xanthine oxidoreductase; HIFs, hypoxia-inducible factor; PHDs, prolyl-4-hydroxylases; FIH, factor inhibiting HIF; GAGs, glycosoaminoglycans.

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[1] "The Rigorous Measurement of ROS" was the topic of a workshop that initiated the 12th annual meeting of the Society in November, 2005 in Austin. The workshop was organized by Dr. Ronald P. Mason, with teaching contributions by Society President Garry R. Buettner, Lifetime Achievement Awardee Balaraman Kalyanaraman, and an outstanding faculty. As the field moves forward and we continue to seek answers to these critical questions, a great benefit to our understanding will be provided by increased incorporation of the techniques for ROS detection detailed in this course. The syllabus for the course, along with the lectures from the Society's Sunrise Free Radical School provided each year at the Annual Meeting, are now available to the public at the Society's website: www.strbm.org.

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complementary techniques can provide assurance that conclusions are accurate.

Studies suggesting that excess ROS are produced during hypoxia often have been conducted in contractile cells and tissues, including vascular smooth muscle, cardiomyocytes, and striated, skeletal muscles such as the diaphragm, all of which are rich in mitochondria. These measurements often have relied on use of fluorescent dyes. Evidence favoring both increased and decreased ROS production in hypoxia has been found (reviewed in [17] and [18]). Various sources for ROS production in hypoxia, with or without reoxygenation, including mitochondria [19], NADPH oxidases [20–22], and xanthine oxidoreductase (XOR), have been cited.

In studies that have identified excessive ROS production during hypoxia, fewer have been conducted in vascular endothelium, a cell considerably less rich in mitochondria. Among these, few have used the most rigorous techniques such as EPR. In those, anoxia and/or anoxia–reoxygenation generally have been evaluated. In general, superoxide formation has been detected following anoxia–reoxygenation [23–27] but not anoxia alone. One study examining the effect of severe hypoxia (4% oxygen) in porcine distal pulmonary arteries using EPR inconsistently found "spectra consistent with hydroxyl and alkyl radicals" [28]. In addition, studies by others have indicated that ischemia without hypoxia or reperfusion can promptly result in pulmonary microvascular endothelial cell lipid peroxidation [29]. However, in a number of endothelial and nonendothelial cells or tissues, ROS production is either unchanged or decreased in hypoxia [30]. In the setting of organ preservation, endothelial damage can be decreased by storage in hypoxia [31,32], implying decreased ROS production in hypoxia.

Among signaling responses contributing to adaptation in hypoxia are (1) global changes in stability of the hypoxia-inducible factors (HIFs) and secondary gene expression, (2) rapid responses of membrane potassium channels (limitation of K^+ efflux), membrane depolarization, and activation of voltage-gated (L-type) calcium channels (Ca^{2+} influx) in pulmonary arteries (reviewed in [17]), and (3) alterations in purinergic signaling related both to accumulation of adenosine [33] and the paradoxical release of ATP into the extracellular space [34]. Additional changes in cellular oxidation–reduction state occur in hypoxia such that NADH/NAD^+ [17] and NADPH/$NADP^+$ [35] ratios increase, and these redox changes may modulate signaling responses. Each of these rapid hypoxic sensing/signaling responses may communicate to a plethora of downstream effectors [36–38].

Among the activators of hypoxic adaptive responses, the hypoxia-inducible factors, especially HIF-1alpha [39], have emerged as master regulators. HIF-1alpha can alter expression of more than 500 genes [40], many of which pertain to enhancement of processes such as glucose utilization, red blood cell production, angiogenesis, and cell survival and/or growth. The role of HIF-specific prolyl-4-hydroxylases (PHDs) in degrading HIFs at normal oxygen tensions has been discovered in recent years [41–43], and the role of the asparaginyl hydroxylase factor inhibiting HIF (FIH) [44,45] in transcriptional regulation has been described. Modulation of these systems through genetic or pharmacologic manipulation can have profound effects resembling hypoxic adaptive responses. In particular, stabilizing HIFs through the actions of peptides or small molecules which interfere with continuous degradation of HIFs can recapitulate complex actions of hypoxia such as erythropoiesis [46], enhanced glucose transport and utilization and angiogenesis [41,47–49]. By contrast, genetic inhibition of HIFs can prevent important biologic responses to hypoxia such as polycythemia, pulmonary hypertension and vascular remodeling, and electrophysiologic changes [50–52].

Despite the critical role of the PHDs, new data using genetic approaches suggesting a contributory or modulating role for ROS in stabilizing HIFs in hypoxia at all but the lowest oxygen tensions [53,54] and in secondary gene regulation continue to emerge [55]. Interestingly, moderate overexpression of MnSOD also can diminish HIF-1alpha accumulation under marked hypoxia [56]. Some have suggested that ROS produced in ischemia–reperfusion also could trigger angiogenesis [57]. It is not altogether clear how mechanisms dependent on ROS production in hypoxia can be integrated into the critical regulatory role of the PHDs and FIH and their modulation of HIFs by proline and asparagine hydroxylation, respectively. However, it is notable that reactive nitrogen species such as NO also can have potent effects in modulating HIFs [58,59], and at least some of these interactions involve mitochondria [60]. At lower NO concentrations (<400 nM), NO can inhibit mitochondrial respiration which may, paradoxically, make oxygen more available, even under hypoxic conditions, leading to decreased HIF stability [61]. Nonetheless, during sustained hypoxia, NO production in some tissues, such as the lung, can be downregulated [62]. By contrast, other tissues, such as myocardium, can show increased NO production with chronic hypoxia [63]. Developmental and/or organ-specific factors could play a role in these differing adaptive responses.

In this issue of the Journal, Kelley et al, demonstrate that moderate, physiologic hypoxia (10% oxygen) can upregulate XOR in primary endothelial cells. Upregulation of XOR appears to occur through multiple mechanisms since only modest increases in mRNA and protein occur, but a much greater enhancement of XOR activity occurs via an adenosine-receptor-mediated mechanism. The latter is suggested by the findings of (1) similar actions of adenosine, relative to hypoxia, upon XOR activity, (2) potent enhancement of XOR activity in endothelial cells by the nonspecific adenosine receptor agonist NECA, and (3) inhibition of hypoxic enhancement of endothelial XOR activity by the adenosine receptor antagonist 8PST. Further, purified XOR enzyme activity was enhanced during hypoxia.

The activation and release of XOR during tissue hypoxia [64,65], with or without reoxygenation and/or ischemia followed by reperfusion, could contribute to inflammation [66] and tissue damage in disease or disease models [67–69]. When XOR is released into the circulation it can bind avidly to vascular cell glycosaminoglycans (GAGs) [70] where secondary ROS can alter NO-mediated vasodilatation [67,71]. In addition, XOR bound to GAGs appears to be resistant to inhibitors such as oxypurinol [72] and superoxide formed by XOR unavailable to exogenous SOD [73].