Focus on oxidative stress in the cardiovascular and renal systems

Oxidative stress remains an elusive phenomenon. It is difficult to define. It represents an excess of reactive oxygen species (ROS) in the tissues under consideration or in the whole body. This implies either an increased production of ROS, for example, by specific oxidase such as NADPH oxidase, xanthine/xanthine oxidase, various arachidonic acid monoxygenases, or the mitochondrial respiratory chain. Alternatively, it may derive from a failure to metabolize ROS. The major pathways for metabolism are superoxide dismutase (SOD), which is expressed as extracellular (EC), intracellular, and mitochondrial isoforms that metabolize superoxide anion (O$_2^-$) to H$_2$O$_2$. Peroxidases such as catalase and glutathione peroxidase (predominantly intracellular) further metabolize H$_2$O$_2$ to O$_2$ and water. However, such a definition is highly simplistic because of the other, biologically important, ROS such as hydroxyl anion (-OH) formed from H$_2$O$_2$, peroxynitrate (ONOO$^-$) formed principally by the interaction of nitric oxide (NO) and O$_2^-$ or -OH, or hypochlorous acid formed by myeloperoxidase. There are multiple other reaction products with other mediators. Important interactions occur between ROS and NO where O$_2^-$ not only reduces NO bioactivity by shortening its half-life but also generates highly reactive species such as ONOO$^-$ that are themselves implicated in oxidative and nitrating reactions.

No less problematic is the quantitative assessment of oxidative stress (10). At the level of the whole animal, use is often made of the appearance of oxidized end products of ROS metabolism. These end-products include lipid peroxidation products such as isoprostanes, which are formed predominantly nonenzymically by the interaction of O$_2^-$ with arachidonate or malondialdehyde. More stable and long-lasting products include oxidative modifications of DNA. ONOO$^-$ can be assessed semiquantitatively from nitrosation of tyrosine epitopes in proteins, but the reaction products are difficult to quantify by Western blot analysis. Moreover, this reaction also is influenced by nitrate, which generates NO$_2^-$ and reverses the tyrosine nitration. At the level of the isolated vessels or single cells, fluorescent probes to detect O$_2^-$, H$_2$O$_2$, ONOO$^-$, and NO are receiving increasing attention. A particularly promising technique is to assess the redox ratio of biologically determined products such as oxidized:reduced thiols (e.g., cysteine or homocysteine) or oxidized:reduced nucleotides (e.g., flavin adenine dinucleotide) within cells. Because the thiols are in redox equilibrium throughout the body, this is an attractive technique to sample the redox chemistry of the body or a particular tissue. However, this approach may break down where the thiol-redox pair is determined by specific enzyme regulations, for example, the oxidized:reduced ratio of glutathione is under the control of glutathione peroxidase and reductase that may be as important as the redox state in governing the thiol pair ratio. Nevertheless, assessment of appropriate redox pairs gets more directly at the problem of quantitating the redox state within the cells and tissues under study.

Initial interest in ROS as vasoactive mediators focused particularly on O$_2^-$ and its interaction with NO as a mechanism to explain endothelial dysfunction (12, 16, 31) and renal vasoconstriction (2, 3, 31, 42), for example, in ANG II-induced hypertension (1, 27). Mori and Cowley (24) had shown that O$_2^-$ generated by NADPH oxidase under the influence of ANG II modifies tubulovascular NO cross-talk in the outer medulla. In this issue, Pallone and colleagues (48) report their study of microvascular O$_2^-$ and NO generation assessed directly in vasa recta dissected from the rat kidney medulla. They show that, unlike renal afferent arterioles (38), chronic ANG II does not stimulate O$_2^-$ generation in vasa recta and indeed enhances NO generation leading to a diminished ANG II constriction.

Increasing interest focuses on the role of H$_2$O$_2$ (19–22). This can be active itself (20) or be further metabolized to highly reactive species such as -OH. In this issue, Hatoum et al. (11) report their studies of human submucosal intestinal vessels where H$_2$O$_2$ is a vasoactive mediator. One pathway is for H$_2$O$_2$ to act as an endogenous endothelium-derived hyperpolarizing factor (EDHF). H$_2$O$_2$ was considered to be generated in endothelial cells and to activate potassium channels in vascular smooth muscle cells, thereby causing hyperpolarization and diminished reactivity. The source of H$_2$O$_2$ in the endothelial cells, its transmembrane passage, and the details of its interaction with specific channels on vascular smooth muscle cells were unclear. The new findings of Hatoum et al. (11) are that endothelial production of H$_2$O$_2$ does indeed occur in response to acetylcholine but that H$_2$O$_2$ is not simply on EDHF in their vessels. They provide evidence that H$_2$O$_2$ functions rather to release an EDHF. Increasing evidence suggests that EDHF may be at least as important as endothelium-derived relaxing factor-NO in the regulation of tone and contractility of the resistance vessels, whereas NO is generally of predominant importance in the large conduit vessels.

Of special importance is the regulation of NADPH oxidase activity during prolonged ANG II exposure or other physiological stimuli. Gupta et al. (9) compare NADPH-dependent O$_2^-$ generation in bovine pulmonary and coronary arteries during adaptation to hypoxia. An important finding is that differences in oxidase activities relate not only to expression of the components of NADH oxidase but rather to the availability of the substrate NADPH, which is generated by glucose.
6-phosphate dehydrogenase (8, 25). In this special section, Welch et al. (41) report their studies of NADPH oxidase activity, p22phox, and EC-SOD expression in the rat renal cortex. They confirm that prolonged ANG II infusion increases NADPH oxidase activity and p22phox and reduces EC-SOD. Remarkably, concurrent infusion of a permeant SOD mimetic to obviate oxidative stress prevents all these changes. They conclude that ROS themselves are autocatalytic and induce further ROS generation in the kidney via increased p22phox and reduced EC-SOD, thereby contributing to the sustained effects of ANG II on ROS accumulation during a slow pressor response.

In other articles on this issue, Weber et al. (39) and Laude et al. (17) report their studies of transgenic mice overexpressing p22phox. These mice have a twofold increased vascular expression of p22phox and H2O2, accompanied by increased NOX-1 (13). Yet, despite this, the blood pressure and endothelium-dependent relaxation responses are intact. This is related to enhanced endothelial NO synthase (eNOS) and EC-SOD expression, leading to an enhanced capacity to generate NO. They conclude that ROS evoke a compensatory increase in eNOS and SOD that can maintain normal vascular function and hemodynamics (17). Moreover, their studies of vascular smooth muscle cell hypertrophy in this model show that ANG II causes an exaggerated structural response in the blood vessels of the p22phox overexpressing mice. Weber et al. (39) conclude that p22phox and NADPH oxidase potentiate smooth muscle cell hypertrophy. Their findings raise important questions concerning the signaling by ROS. In this issue, Yang et al. (47) report their study of the role of phospholipase D (PLD) in ROS generation in the mouse kidney. Their studies in the dopamine 5 (D5) receptor knockout mouse associate the hypertension in this model with increased PLD expression that engages NADPH oxidase activity. The potential relevance of NO and ROS in explaining gender differences in renal injury are explored in the study by Ji et al. (14). Their studies in the renal-wrap model of hypertension show similar degrees of hypertension, yet enhanced indexes of renal damage in male rats that these authors relate to gender-specific regional differences in NOS expression within the kidneys.

Many new avenues of study of ROS are emerging. These include competition of NO with O2 in setting the level of cellular respiration and oxygen usage and hence the oxygen tension of the tissues. Consequent activation of the hypoxia inducible factor may engage a further set of translational events that could dictate altered gene expression and a new physiological and pathophysiological environment. Welch et al. (41) report their studies which confirm (40) that the kidneys of chronically ANG II-infused rats use O2 inefficiently for Na+ reabsorption and consequently are hypoxic. They relate the hypoxia to the generation of O2 because hypoxia is reversed by confusion of an SOD mimetic (41). In this issue, Fitzpatrick et al. (5) report that a period of adaptation to hypoxia after birth confers prolonged protection against cardiac ischemia in the rabbit. They relate this to expression of eNOS, leading to NO-dependent activation of adenosine triphosphate potassium channels (28, 29, 33).

Finally, an area of great interest is the hypothesis that ROS are proximate in multiple signaling pathways implicated in organ dysfunction or damage. Downstream from ROS are activation of proinflammatory pathways such as nuclear factor-κB that link ROS to cellular adhesion, inflammation, and cytokine production, which are key events in the pathophysiology of chronic cardiovascular and kidney diseases (Fig. 1). A hierarchy of regulation could be envisaged whereby initial damage within the heart, blood vessels, or kidneys, with secondary pathophysiological changes in neurohormones, pressures, and flows engages oxidative stress that recruits a wide range of aggressive genes, cytokines, or hormones that perpetuate the organ damage. Such a schema suggests new mechanisms whereby primary disease of one system, for example, the kidneys, can lead to secondary dysfunction of another, such as the heart (Fig. 1).

The Cardiovascular Kidney Investigators Meeting at Amelia Island covered many of these evolving issues with focused

Fig. 1. Flow diagram of some pathophysiological interaction coordinated by reactive oxygen species (ROS).
presentations that form a special, fully refereed addition of the American Journal of Physiology-Heart & Circulatory Physiology. The meeting was jointly sponsored by the National Heart, Lung, and Blood Institute Program Project Grants (PPGs) PO1-HL-68686 (Georgetown University) and P50-HL-68769 (Medical College of Wisconsin), and a Specialized Center of Research in Myocardial Ischemia in African-Americans Grant P50-HL-065203 (Medical College of Wisconsin). Additional funds were provided by the George E. Schreiner Chair of Nephrology at Georgetown University and by unrestricted educational grants from AstraZeneca, Boehringer Ingelheim Pharmaceutical, Merck, and GlaxoSmithKline.

The principle of the National Heart, Lung, and Blood Institute-sponsored PPGs and SCOR grants is to foster collaboration and share new ideas between investigators working in different disciplines who have committed to study a central theme. We expanded this vision to include collaboration between three competing PPG or SCOR grants that had a focus on oxidative stress. Participants included both investigators and member of the external advisory committees of these projects. Invited and informal “hot topic” presentations were made by the group during a 3-day meeting at Amelia Island Plantation, Florida.

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REFERENCES


