Novel Roles for Nox Oxidases in Cardiac Arrhythmia and Oxidized Glutathione Export in Endothelial Function

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This issue of Circulation Research contains two articles in the area of oxidant regulation that have major implications for their novel roles in mechanisms that contribute to cardiovascular disease processes in humans. An article by Kim et al reports data showing that human cardiac myocytes isolated from right atrial appendages express NAD(P)H oxidase (Nox)-2, and that these myocytes and homogenates of the atria from patients with atrial fibrillation (AF) have increased levels of Nox-derived superoxide generation that appear to originate from increased Nox oxidase activation. Homogenates of atria from the AF patients also show evidence of nitric oxide synthase (NOS) becoming a source of superoxide generation, which is thought to originate from an uncoupling of the ability of this enzyme to efficiently synthesize NO. The article by Mueller et al reports a new important role for multidrug resistance protein-1 (MRP-1) in controlling oxidant regulation in human and animal endothelium by exporting oxidized glutathione (GSSG). In their study, this group demonstrates how the expression of this protein functions to remove increased GSSG in hypertensive rats and endothelial cells exposed to oscillatory shear stress. Interestingly, the inhibition of MRP-1 appears to restore endothelial function in the hypertensive rats and prevent shear-induced apoptosis in these models apparently through preserving endothelial cell glutathione levels. Both of these articles have major new implications for the understanding and therapeutic targeting of human disease processes.

Nox Oxidases in Cardiac Arrhythmia

There is already much evidence that oxidant processes have a major influence on the expression of AF. As discussed in the article by Kim et al, reactive oxygen species (ROS) are known to cause AF, and antioxidant and statin therapies associated with the modulation of ROS, redox and improved nitric oxide regulation modulate the expression of AF. The data in this study now demonstrate that Nox-2 is present in human cardiac myocytes, and that AF appears to be associated with increased activation, not expression of Nox oxidases. Atrial stretch and increased angiotensin II could be initiating factors for Nox activation during the early stages of AF. However, it is not yet known whether the observed Nox oxidase activation is an initial cause or a consequence of AF. It is also becoming clear that increased oxidant generation and Nox oxidase activity in endothelium can promote the uncoupling of NOS activity, which would then result in a loss of the beneficial effects of NO and a further increase in the generation of ROS and reactive NO-derived species. Many of the ion channels that are fundamental components of cardiac electrophysiology that are altered in AF are potential targets for regulation by ROS and interactions of ROS with NO through mechanisms shown in the Figure.

Oxidative stress has been implicated in the pathogenesis of ventricular tachycardia and fibrillation after reperfusion of the ischemic heart. Consistently, rapid atrial pacing has been shown to increase myocardial peroxynitrite formation and lead to a shortening of the atrial effective refractory period and AF, both of which are reversed by treatment with an antioxidant which attenuates actions of peroxynitrite, ascorbate, and with statins, drugs known to decrease Nox oxidase activation. Recent studies have shown that Nox oxidases are a major source of superoxide in the cardiovascular system. Nox oxidase activity has been detected in cardiac myocytes and myocardium. The study by Kim et al, demonstrating the presence of Nox 2 in the atrial myocytes and its apparent function as a major source of elevated oxidative stress in human AF, opens a new area of investigation for defining the role of Nox oxidase in arrhythmias. These authors have provided convincing evidence for the role of Nox oxidase activation and dysfunctional NOS as a source of increased oxidative stress in human chronic and paroxysmal AF.

Nox oxidase activation and NOS uncoupling could be important factors in the initiation of mitochondrial ROS generation, and this could contribute to the observations made in atrial tissue from AF patients. Data in the study of Kim et al also show that a mitochondrial inhibitor, rotenone, lowers the basal levels of superoxide detected in atrial homogenates from AF patients. It has been previously shown that oxidative damage in human AF alters myocardial energetics, and the oscillations in mitochondrial energetics activated by ROS have been reported to cause synchronized changes in action-potential duration, a process which potentially contributes to arrhythmias during ischemia-reperfusion injury. Thus, mitochondrial ROS could potentially be a contributing factor to persistent arrhythmias in the atria.

Studies indicate that sinus rhythm is regulated by multiple ion channels, and regulatory mechanisms could be influenced by oxidant generation. Pacemaker activity is regulated by five classes of ion channels: the hyperpolarization-activated channel (Ih), two delayed rectifier potassium channels (Ikr andIKs),...
the potassium channel activated by the muscarinic receptor (IK_{ACH}), two types of calcium channels (I_{Ca,T} and I_{Ca,L}), and the sustained inward current (I_{K}). Additionally, evidence suggests that tetrodotoxin-sensitive sodium channel Na_{i1} and/or Na_{i1,3} affect pacemaker activity. Aberrations in impulse generation, propagation, or the duration and configuration of individual cardiac action potentials form the basis of disorders of cardiac rhythm. The Na^{+} channel plays a central role in the generation of rhythm and is associated with AF. However, there is evidence that oxidative stress reduces slowly inactivating Na^{+} currents. Thus, it can be postulated that oxidative stress can potentially influence sinus node cell Na^{+} channels and induce AF. The observed reduction in expression of the α_{subunit in L-type Ca^{2+}} channels in AF patients is reversed by statin drugs, suggesting that oxidant processes may regulate the availability of functional L-type Ca^{2+} channels, thereby favoring increased atrial excitation rate and perpetuation of AF. In conjunction with electrophysiological remodeling, the amplitude of IK is also found to be increased in AF. Peroxide increases the amplitude K_{i1,5} channel currents at voltages corresponding to the action potential repolarization phase. Thus, oxidant accelerated K_{i1,5} channel opening may be a contributing factor to the increased IK_{o} seen in AF patients. Therefore, it can be suggested that Nox oxidase–derived H_{2}O_{2} could change the function of K_{i1,5} and other ion channels that could be involved in the initiation and perpetuation of AF.

Atrial remodeling has been suggested to originate from persistent arrhythmia associated with the multiple reentrant electrical wavelets seen in AF, which seem to be initiated by electrical triggers in the myocardial sleeves extending from the left atrium into the proximal regions of the pulmonary vasculature. Studies by Carnes et al have suggested that oxidative stress activates early atrial electrophysiological remodeling in chronic human AF, and Kim et al have given the first evidence for the source of oxidative stress and a connection between human AF and atrial remodeling. At this stage, how Nox oxidase–derived superoxide mediates AF-induced remodeling is unclear. However, H_{2}O_{2}-elicited activation of mitogen-activated protein kinases, including extracellular signal regulated kinase (ERK), is important in the actions of growth factors on vascular smooth muscle. Peroxide activates ERK in cardiac myocytes, and Nox oxide–derived H_{2}O_{2} activates ERK in pulmonary arterial smooth muscle. Thus, it remains to be investigated whether ROS-regulated Ras and ERK pathways have a role in remodeling of atrial and pulmonary artery myocytes associated with the initiation of persistent arrhythmias and progression of AF.

Oxidized Glutathione Export in Endothelial Function

The novel aspect of the study by Mueller et al is that it shows the importance of MRP-1 in exporting GSSG from endothelium when it is formed by oxidant-promoting conditions. Most interestingly, this study also provides evidence that the MRP-1 system contributes to endothelial dysfunction in hypertensive rats and shear-induced endothelial cell apoptosis through its role in exporting GSSG. As mentioned in the article by Mueller et al, it has been known for some time that perfused organs secrete GSSG when exposed to oxidants. In addition, the transport of GSSG by MRP-1 has been suggested as the physiological role of this class of proteins which have been characterized for their role in the transport of drugs coupled to glutathione during their metabolism by tissues. It has generally been assumed that the maintenance of low levels of oxidized GSSG by the NADPH-dependent glutathione reductase reaction and by the export of GSSG functioned to prevent adaptive and pathophysiological regulation mediated through GSSG formation and the consumption of NADPH. However, it appears this mechanism of GSSG removal can also contribute to a loss of endothelium-dependent relaxation in hypertensive rats and can promote oscillatory shear-induced endothelial cell apoptosis through depletion cellular levels of GSH. Thus, an apparent physiological function of the MRP system in protecting against the actions of oxidant stress may actually be a contributing factor to vascular pathophysiological processes.

Implications for Disease Mechanisms

Knowledge that chronic Nox oxidase activation occurs in atria from patients with AF, and that ROS generated by this class of oxidases is likely to be an important factor in promoting electrophysiological changes initiating AF and the associated atrial remodeling process, should help focus future work on defining the role of Nox oxidase in AF, and perhaps in developing treatment based on attenuation oxidase activation. The new perspective on how the export of GSSG by MRP-1 could have adverse effects on endothelial cell regulation in disease processes through depletion of glutathione, instead of its presumed protective actions by modulating glutathione-linked redox regulation, adds a new level of understanding regarding how to investigate the role of these aspects of redox in endothelial function.

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