Primary Effect of Reactive Oxygen Species on Electrical Remodeling of the Heart

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R eactive oxygen species (ROS), including superoxide (O$_2^•$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals (OH), are highly reactive molecules that are thought to exert both physiological and pathophysiological effects on the heart. ROS have been implicated in the pathogenesis of ischemia-reperfusion injury, heart failure (HF), hypertension, cardiac hypertrophy, and arrhythmias.1-3 Given the importance of ROS in cardiovascular (patho)physiology, this topic has been intensively studied, but many controversial issues remain.4 In this issue of the Journal, Kurokawa et al5 add new, supportive evidence to this database by showing how oxidative stress primarily affects the progression of electrical remodeling without apparent structural heart disease.

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**Generation and Elimination of ROS in the Heart**
The main source of ROS in cardiomyocytes comes from O$_2^•$− as a byproduct during ATP synthesis. In addition, O$_2^•$− can be generated via other enzymes, including xanthine oxidase and NAD(P)H oxidase (Nox). Under normal conditions, ROS are kept at physiological levels by a number of antioxidative enzymes. For example, superoxide dismutase (SOD) catalyzes the degradation of O$_2^•$− to form less active H$_2$O$_2$ and O$_2$. There are 3 types of SODs, two of which are Cu-Zn type (cytoplasmic SOD1 and extracellular SOD3) and the other is Mn type (mitochondrial SOD2). The resulting H$_2$O$_2$ is in turn reduced to H$_2$O and O$_2$ by other complementary enzymatic scavenging systems such as catalase, glutathione peroxidase, and peroxidredoxin (Figure). When the balance between the production of oxidants and antioxidant defenses is disturbed, intracellular ROS levels can increase, leading to oxidative stress, which in turn exerts numerous pathologic effects on the heart by affecting ion channel and transporter properties.2-5

**Acute Modification of Ion Channel and Transporter Activities by ROS in the Heart**
The function (gating or activity) of ion channels and transporters can be modified acutely by ROS via direct protein oxidation or phosphorylation by ROS-activated CaMKII (Figure and refer to recent review articles for details2-4).

**L-Type Voltage-Gated Calcium Current (I_{Ca,L})** Song et al6 and our group7 have demonstrated that H$_2$O$_2$ increased peak I_{Ca,L} and promoted its re-opening during the action potential in a CaMKII-dependent way. However, a decrease in I_{Ca,L} may occur with ROS exposure, because of direct oxidation of the pore-forming aC subunit. These 2 effects are opposite and may play roles in different settings of (patho)physiologic conditions.

**Voltage-Gated Sodium Current (I_{Na})** Both direct oxidation8 and CaMKII-mediated phosphorylation9 under ROS (eg, H$_2$O$_2$) exposure increases the late phase I_{Na} by disrupting its inactivation. This effect is also proarrhythmic by promoting action potential duration (APD) prolongation, early afterdepolarization (EAD) generation, and enhancement of the reverse mode of the sodium-calcium exchange current (I_{SCX}), leading to Ca$^{2+}$ overload.

**Transient Outward Potassium Current (I_{to})** We have recently shown that I_{to} is activated by acute H$_2$O$_2$ treatment. The recovery from inactivation of I_{to} was also facilitated by H$_2$O$_2$.10 These results fit well with previous studies by Tessier et al on the CaMKII-dependent regulation of I_{to}.11 We suggest that ROS-dependent activation of I_{to} favors EAD generation by facilitating I_{Ca,L} reactivation, thus likely promoting arrhythmogene-
sis.10

**Other Calcium-Handling Proteins** Direct oxidative modification of sarcoplasmic reticulum (SR) calcium ATPase (SERCA2a) inhibits its Ca reuptake function. However, ROS-activated CaMKII may phosphorylate phospholamban (PLB) and subsequently increase SERCA2a function by disrupting PLB inhibition. It seems likely the inhibition outweighs the activation because depletion of SR Ca$^{2+}$ content is always observed under oxidative stress conditions.

Oxidative stress results in both direct redox modification and CaMKII-dependent phosphorylation (at Ser2814) on RyR2, both of which in turn promote spontaneous Ca release from RyR2 during diastole. This further reduces the SR Ca$^{2+}$ content and eventually reduces Ca$^{2+}$ transient amplitude and causes contractile dysfunction.

ROS also enhance the activity of I_{SCX}, probably by direct oxidation. I_{SCX} is the major contributor to the transient inward current, which accounts for the generation of delayed afterdepolarization (DADs) and EADs under cytosolic Ca$^{2+}$ overload conditions.

Conversely, the cytosolic Ca$^{2+}$ overload condition may activate several Ca$^{2+}$-dependent signaling molecules, such as CaMKII, calcineurin and the transcriptional factor NFAT. These may be involved in the underlying mechanism(s) of regulation...
ROS and Electrical Remodeling in the Heart

ROS and a higher level of mitochondrial superoxide and hydrogen peroxide formation in the myocardium, indicating a primary higher oxidative stress state. Prolongation of monophasic APD and effective refractory period were detected in BSO-treated H/M-Sod2+/– hearts. Real-time RT-PCR and Western blotting data demonstrated reduced expression of Kv4.2 and K+ channel-interacting protein-2 (KChIP2) in these hearts, suggesting that a decrease of Ito fast component accounts for the electrophysiological remodeling (Figure).

There are limitations to this study. The authors did not carry out patch-clamp experiments. The possible involvement of CaMKII was not evaluated either. It remains possible that Ica,L and/or INa might exhibit higher activities in the H/M-Sod2+/–+BSO group, although their mRNA/expression levels remain unchanged.

Relevance to Cardiac Pathology
Downregulation of Ito is a central and consistent electrophysiological change in cardiac diseases. For example, decreased expression of Kv4.2, Kv4.3, and/or KChIP and the function of Ito have been demonstrated in human failing hearts and numerous HF animal models. The consequent prolongation of the APD leads to larger Ca2+ and Na+ entry. In addition, ROS-induced activation of CaMKII enhances Ica,L, late INa, and RyR2 activities and reduces SERCA function. It is likely that under chron-

Figure. (A) Generation and elimination of reactive oxygen species (ROS) in the heart. (B) Modification of ion channel and transporter functions in cardiomyocytes by acute ROS treatment. (C) Electrical remodeling in the H/M-Sod2+/– mouse heart treated with L-buthionine-sulfoximine (BSO). See text for details.
ic oxidative stress conditions, all these modifications play a concerted role in the development of HF by generating cytosolic Ca\(^{2+}\) overload and impaired diastolic contractile function. This condition is also proarrhythmic, because EADs will be promoted by the increased late \(I_{\text{Na}}\) and \(I_{\text{Ca,L}}\), whereas DADs will be facilitated by SR Ca leak and consequent cytosolic Ca\(^{2+}\) overload (Figure).

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