Atrial fibrillation (AF), one of the most prevalent cardiac arrhythmias, affects >3 million people in the United States. Because the risk of developing AF increases with age, the prevalence of AF is projected to be 7.6 million in 2050, when 1 in 5 Americans will be over the age of 65. AF not only causes debilitating symptoms and functional impairments owing to worsened hemodynamics and embolic stroke, it also increases the risk of mortality up to 1.5- to 1.9-fold. Despite the significant morbidity and mortality burden incurred with AF, there are limited therapeutic options that may improve the outcomes of AF patients, and these options are associated with significant AF recurrence rates.

One possible explanation for the limitations of current therapies is that they do not address effectively or completely the underlying causes of AF. Evidence has been mounting that AF is associated with systemic and cardiac oxidation. Risk factors for AF are similar to those of atherosclerosis, a disease known to be perpetuated by oxidative stress. These risk factors, such as hypertension, aging, diabetes mellitus, and coronary artery bypass surgery, have each been associated with increases in the systemic markers of oxidation. In addition, there is also evidence of increased cardiac oxidation of myofibrillar protein and membrane lipids with AF or with risk factors linked to AF. Although it is not clear whether cardiac oxidation leads to systemic markers of oxidation or whether systemic oxidation leads to cardiac oxidation, the association of oxidative stress and AF is robust and suggests that AF is possibly a manifestation of a systemic disease.

Despite the link between oxidative stress and AF, systemic antioxidant therapy for arrhythmias has not met with much success in clinical trials. This suggests that the oxidative stress is either not in the pathogenic cascade of arrhythmogenesis, or our current antioxidant therapies are not targeting the pathogenic source of oxidative stress in arrhythmia. Consistent with the later hypothesis, a recent article by Sovari et al showed that angiotensin II (AngII)–induced ventricular arrhythmias could only be prevented by a mitochondria-targeted antioxidant rather than a general antioxidant or inhibitors of other producers of oxidative stress.

In this issue of Circulation, Purohit and colleagues present an elegant study that they conducted with the use of human tissues and mouse models to demonstrate the crucial role of oxidized Ca2+ and calmodulin-dependent protein kinase II (ox-CaMKII) in mediating AngII/pacing–induced AF. Purohit et al present strong, new evidence that oxidative stress can lead to AF. The proposed mechanistic link between the renin-angiotensin system activation–induced oxidative stress, intracellular Ca2+ handling, and the inducibility of AF also helps to explain the antiarrhythmic effects of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers.

AngII-Mediated CaMKII Oxidation Promotes Sarcoplasmic Reticulum Ca2+ Leak, Potentiating Pacing-Induced AF

Recent works by the Anderson group have identified that the oxidation of a pair of methionines (281/282) in the CaMKII regulatory domain prevents the inactivation of CaMKIIγ and that this constitutively active ox-CaMKII is responsible for AngII-induced sinoatrial node dysfunction and aldosterone-mediated cardiotoxicity. In their article, Purohit et al demonstrate that ox-CaMKII expression was increased in the atrial tissue from patients with AF in comparison with those in sinus rhythm, without affecting total CaMKII levels. Interestingly, the increase in atrial ox-CaMKII is not observed among AF patients treated with an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker. Using a mouse model of burst pacing–induced AF, they showed that 3 weeks of AngII infusion, a treatment known to increase atrial ox-CaMKII levels, significantly increased the susceptibility to AF induction. By using the CaMKIIδ oxidation-resistant knock-in mice (MM-VV), they showed that this AngII-potentiated AF induction by burst pacing requires the presence of ox-CaMKIIδ, whereas the reactive oxygen species (ROS) production, hypertrophy, and cardiac hypertrophy in response to AngII treatment were unaffected in MM-VV mice. In addition, by using mouse models lacking functional NADPH oxidase (p47−/−) or cardiac-restricted expression of methionine sulfoxide reductase, they went on to show that NADPH oxidase–dependent ROS production and elevated ox-CaMKII are essential for the proarrhythmic effects of AngII in pacing-induced AF.

To determine whether increased sarcoplasmic reticulum (SR) Ca2+ leak contributes to AngII-potentiated AF susceptibility, they measured the diastolic Ca2+ sparks in isolated atrial myocytes, where the spontaneous Ca2+ sparks and delayed afterdepolarizations (DADs) significantly increased in atrial myocytes from AngII–treated in comparison with saline-treated, wild-type mice. AngII-treated MM-VV mice
did not show an increase in Ca\(^{2+}\) sparks or DADs, suggesting the requirement of ox-CaMKII in AngII-induced SR Ca\(^{2+}\) leak and DADs. Finally, they demonstrated that the proarrhythmic effects of AngII treatment on pacing-induced AF can be abolished in the mice expressing modified RyR2 (S2814A), which is resistant to CaMKII-mediated phosphorylation, and in the mouse model with cardiac-specific expression of a CaMKII inhibitory peptide (AC3-I), as well, suggesting the critical role of CaMKII-mediated RyR2 phosphorylation at S2814 for AF inducibility in response to AngII treatment and burst pacing.

ox-CaMKII–Induced SR Ca\(^{2+}\) Leak: Another Missing Piece of the Complex Puzzle

The results by Purohit et al\(^{10}\) are consistent with previous reports showing that diastolic SR Ca\(^{2+}\) leak is increased in atrial myocytes from human and animals with AF.\(^{14}\) In a recent work by Neef et al,\(^{15}\) increased Ca\(^{2+}\) leak from SR and elevated diastolic Ca\(^{2+}\) concentration were observed in the atrial myocytes from patients with AF, which is associated with increased CaMKII expression and increased RyR2 phosphorylation at S2814. CaMKII inhibition normalizes SR Ca\(^{2+}\) leak and cytosolic Ca\(^{2+}\) levels without affecting L-type Ca\(^{2+}\) currents. Increased phosphorylation of RyR2 by CaMKII was also reported to be responsible for increased SR Ca\(^{2+}\) leak, DADs, and pacing-induced AF in mice.\(^{16}\) The findings by Purohit et al\(^{10}\) add to the existing evidence, suggesting the importance of CaMKII-induced RyR2 phosphorylation and increased SR Ca\(^{2+}\) leak in the pathogenesis of AF. Importantly, this elegant work uncovers the novel role of CaMKII as the redox sensor downstream of AngII to transduce elevated ROS production into SR Ca\(^{2+}\) dysregulation and increased AF susceptibility.

Although increased SR Ca\(^{2+}\) leak from RyR2, as shown by Purohit et al\(^{10}\) and others,\(^{14,15}\) appears to be an attractive mechanism contributing to AF by triggering DADs, this mechanism by itself will only increase diastolic Ca\(^{2+}\) concentration transiently, owing to the inevitable SR Ca\(^{2+}\) load depletion.\(^{17}\) It is possible that other Ca\(^{2+}\)-handling proteins, including SR Ca\(^{2+}\) ATPase, phospholamban, or L-type Ca\(^{2+}\) channels, are concomitantly modulated in this AngII/pacing–induced AF model to replenish SR Ca\(^{2+}\) load, thereby sustaining the SR Ca\(^{2+}\) leak and elevated diastolic Ca\(^{2+}\) levels. Indeed, phospholamban, the endogenous inhibitor of SR Ca\(^{2+}\) ATPase in its unphosphorylated state, is known to be hyperphosphorylated at Thr-17 by CaMKII in the atrial myocardium of AF patients, leading to increased SR Ca\(^{2+}\) ATPase activity and reuptake of Ca\(^{2+}\) into the SR.\(^{18}\) It would be of great interest to see if ox-CaMKII also plays a role in regulating these calcium regulatory proteins in AF patients and in the AngII/pacing-induced AF mouse model, as well.

Oxidative Stress and AF: A Complex Interplay

In the data presented by Purohit et al, inhibition of CaMKII oxidation by angiotensin-converting enzyme inhibitors or angiotensin receptor blockers did not prevent all AF. This is consistent with the idea that there are more pathways and mechanisms involved than the one outlined by Purohit. The partial success of ablation therapy focused on creating lines of electrical conduction block in humans also suggests that CaMKII-enhanced triggered activity is not the whole mechanistic answer for AF, because this therapy likely affects reentry, and arrhythmogenic foci in places such as the pulmonary veins, as well. Other effects of AngII and oxidative stress include alterations in Na\(^{+}\) current, and connexins can also contribute to forming the AF substrate. Changes in these proteins are mediated by protein kinase C (PKC)\(^{19}\) and c-Src,\(^{20}\) respectively. Mitochondrial dysfunction, associated with ROS release, enhances K\(_{ATP}\) channel activity, further inhibiting conduction and creating the substrate for reentrant arrhythmias.\(^{21}\) Mitochondria-targeting antioxidants and c-Src inhibitors, therefore, may prove to be clinically useful antiarhythmic agents in the future.

The data by Purohit et al point to the NADPH oxidase as a source of oxidative stress causing AF. Nevertheless, the results from Reilly et al\(^{22}\) show that this enzyme is downregulated over time in AF, and the Statin Therapy for the Prevention of AF (SToP AF) trial\(^{1}\) failed to show an effect of atorvastatin, a known inhibitor of the NADPH oxidase, to lower systemic oxidation or to prevent AF after cardioversion in patients with mostly persistent AF. One way to reconcile the observations of Purohit with the data above is that the NADPH oxidase may participate early in the initiation of AF, and some other process sustains the arrhythmia over time.

Although the Purohit idea provides a strong mechanistic link between ROS and AF, it does not explain the concept that AF begets AF, an idea that suggests a positive feedback loop in the maintenance of AF. In addition to structural changes with AF over time that also can be partially remediated by renin-angiotensin system inhibition, an interplay between PKC and mitochondrial ROS, where PKC activation induces mitochondrial ROS production\(^{16}\) and mitochondrial ROS activates PKC,\(^{21}\) is another candidate to explain the clinically demonstrated idea that AF perpetuates itself.

The work of Purohit adds an important piece to our understanding of AF, and each new piece suggests better therapies than we currently use. Important future directions are likely to include understanding what percentage of AF is caused by oxidative stress, what are the sources of ROS in AF, which downstream effectors such as CaMKII, c-Src, and PKC are activated by the different sources and types of ROS, what are the effects of ROS on these and other proteins, and exactly how these ROS-induced changes lead to arrhythmias. Purohit et al have done the medical community a great service by showing that oxidative stress can lead to AF and by giving us clear mechanisms to target in the future. This is an important piece of the puzzle.

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Disclosures

Dr Dudley is an inventor of the 7,550,299 Method for predicting onset/risk of atrial fibrillation (AF), 8,003,324 Modulation of sodium channels by nicotinicamide adenine dinucleotide, 11/882,627 Method for predicting onset/risk of atrial fibrillation (AF), 12/929,786 Method for Modulating or Controlling Sodium Channel Current...
by Reactive Oxygen Species, 13/032:629 Activation of the Renin-Angiotensin System (RAS) and Sudden Cardiac Death, 13/551,790 Method for Ameliorating or Preventing Arrhythmic Risk Associated with Renin-Angiotensin System Activation, and 13/507,319 Method for Modulating or Controlling Connexin 43 (Cx43) Level of a Cell. Dr Yang reports no conflicts.

References


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