Could Plugging the Oxidation-Mediated Ca^{2+} Leak Stem the Tide of the Atrial Fibrillation Epidemic?

Peter H. Backx

As the most common sustained arrhythmia, atrial fibrillation (AF) afflicts >2 million patients in the United States alone, and this number is predicted to double by the year 2050.[1] Although AF is not life-threatening, it is linked to a 2-fold increase in mortality when present as a comorbidity in heart disease patients.[2,3] AF can have devastating consequences, such as stroke, impaired cardiac performance, and promotion of cardiomyopathy. The treatment of AF can also produce life-threatening side effects, such as ventricular arrhythmias and hemorrhage. AF patients are generally classified as paroxysmal (episodes lasting <7 days), persistent (lasting >7 days but treated to restore sinus rhythm), or permanent (no attempt to restore sinus rhythm).[2,3] AF is usually secondary to other conditions such as hypertension, heart failure, valvular disease, sleep apnea, hyperthyroidism, and diabetes. Most of these AF-inducing conditions are associated with elevated atrial pressure and atrial stretch, as well as increased oxidative stress and inflammation, which lead to structural and electric changes (remodeling) that create a substrate for supporting AF induction and maintenance.[4,5]

Article, see p 708

Although the majority of AF patients have preexisting cardiovascular disease, an important subpopulation do not and are therefore referred to as "lone AF" patients. Besides the obvious increase in stroke risk, a major concern with lone AF patients is their predisposition to develop persistent or permanent AF, because bouts of AF appear to promote more AF (ie, AF begets AF), which leads to chronic AF and its associated complications. It has long been suspected that many lone AF patients, particularly those afflicted at a relatively young age, have underlying genetic factors predisposing to AF. Indeed, many single-gene mutations and some polymorphisms are linked to electric and structural changes in atria that are predicted to promote electric ectopy and reentry.[6]

Recent human studies have identified a link between lone AF and mutations of the sarcoplasmic reticulum (SR) Ca^{2+} release channels (ie, ryanodine receptor 2 [RYR2]),[7–12] which, along with mutations in calsequestrin2 (CASQ2), a regulator of RYR2 activity, cause a deadly ventricular arrhythmia called catecholaminergic polymorphic ventricular tachycardia (CPVT). These CPVT mutations of RYR2 and CASQ2 are associated with enhanced RYR2 activity and spontaneous Ca^{2+} leak from the SR.[13] The link between RYR2 channel mutations and AF is of particular interest because abnormal atrial Ca^{2+} homeostasis is a hallmark of chronic AF.[4,5] Specifically, atrial myocardium from chronic AF patients shows increases in RYR2 open probability leading to SR Ca^{2+} sparks and leak, which underlie delayed afterdepolarizations and triggered electric activity, 2 key ingredients in the initiation and maintenance of AF. Involvement of RYR2 channels in AF is supported by the observation that RYR2 channels in AF patients, as well as in paced dogs, show elevated phosphorylation at Ser2808 (protein kinase A and Ca^{2+}/calmodulin-dependent protein kinase II [CaMKII] site) and Ser2814 (CaMKII site),[14–16] which both increase RYR2 open probabilities, possibly as a result of the dissociation of calstabin2 (FKBP12.6) from the SR Ca^{2+} release channel complex.[15] Moreover, several mouse studies demonstrated that AF can be prevented by inhibiting CaMKII-dependent phosphorylation of RYR2 at position S2814 in mice either expressing CPVT-causing RYR2 mutations or lacking FKBP12.6,[16–19] as well as by treatment with drugs that stabilize calstabin2 binding to the RYR2 complex.[19–22]

In this issue of *Circulation Research*, Shan et al[23] report on 3 CPVT-causing RYR2 mutations (R2474S, N2386I, and L443P) that enhance SR Ca^{2+} leak from atrial myocytes and increase susceptibility to AF induction by rapid atrial pacing. Moreover, despite the activation of CaMKII and phosphorylation of RYR2 at position S2814, the enhanced sensitivity of these CPVT mice to pacing-induced AF required RYR2 oxidation, leading to calstabin2 dissociation from RYR2 and enhanced spontaneous SR Ca^{2+} release. These observations are potentially of great importance because elevated oxidative stress is a common feature of both chronic AF and a number of cardiovascular conditions (such as hypertension and diabetes) that predispose to AF.[6] The authors further reported that treatment with S107, a drug that enhances the binding of calstabin2 to the RYR2 channel complex,[24] prevented AF inducibility of CPVT mice and reduced SR Ca^{2+} channel leakiness in CPVT myocytes. Because S107 treatment did not prevent RYR2 oxidation in CPVT atrial myocardium, the authors argue that enhanced RYR2 oxidation was the primary event responsible for increased vulnerability of these mice to leaky SR Ca^{2+} channels and AF. Accordingly, reducing agents eliminated differences in RYR2 oxidation and restored the calstabin2 association with RYR2 in atrial myocardium between the groups. The observation by Shan et al[23] that oxidation of RYR2 channels is central to AF induction in CPVT is intriguing, because dietary and pharmacological antioxidants have often been advocated for treatment of AF, with mixed results.[6,25,26] More important, the results of Shan et al[23] suggest that prevention of the dissociation

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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(*Circ Res*. 2012;111:662-665.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.112.275453

of calstabin2 from RYR2 channels may be sufficient to treat the oxidation-mediated induction of AF, opening up exciting new approaches for treating AF.

Shan et al[23] also concluded that neither protein kinase A nor CaMKII activation plays a notable role in the enhanced sensitivity of CPVT mice to pacing-induced AF susceptibility. Their conclusion is supported by the absence of differences between CVPT mice and wild-type mice in phosphorylation of RYR2 channels at either position S2808 (the protein kinase A/CaMKII site) under baseline conditions or position S2814 (the CaMKII site) after rapid pacing. Furthermore, treatment with β-blockers did not reduce AF incidence in the 3 CPVT mouse models. At first glance, these observations are somewhat unexpected, because ventricular arrhythmias in humans and mice with CPVT are usually initiated by exercise or acute emotional stress, which causes β-adrenergic stimulation that leads to RYR2 phosphorylation at positions S2808 and S2814.[13] Accordingly, β-blocker therapies are generally effective in treating CPVT patients. Although several competing mechanisms have been proposed to account for the β-adrenergic–mediated ventricular tachycardia observed in CPVT, none incorporates RYR2 oxidation as a critical component, consistent with the absence of elevated RYR2 oxidation in the ventricular myocardium of the CPVT mice.[23] These results suggest that the mechanism for arrhythmia induction by CPVT-causing RYR2 mutations in atria could be distinct from the ventricle and could help explain why AF is not commonly observed in CPVT patients.[13] However, the conclusion that β-adrenergic activation and RYR2 phosphorylation do not play a role is at odds with the fact that 3 of the 5 case reports linking AF to CPVT found that AF was induced by exercise or emotional stress.[7–11] Because the CPVT-causing RYR2 mutations identified in the case reports were distinct from those studied by Shan et al,[23] it is conceivable that different CPVT-causing RYR2 mutations induce AF by different mechanisms. Consistent with this possibility, CPVT mice expressing both R176Q-RYR2 via one allele and RYR2 channels lacking the CaMKII phosphorylation site (S2418A-RYR2) via the other allele were resistant to pacing-induced AF.[18] It is, therefore, unfortunate that detailed mechanistic studies by Shan et al[23] were largely limited to R2474S-RYR2 mice and not extended to the N2381I-RYR2 and L443P-RYR2 mice.

Several other observations further suggest that RYR2 phosphorylation does not contribute to pacing-induced AF in CPVT mice. Specifically, although Shan et al[23] found that RYR2 phosphorylation at S2808 did not differ between wild-type and CPVT mice under baseline conditions, it is conceivable that rapid atrial pacing causes hemodynamic changes that cause reflex sympathetic activation (and thereby protein kinase A–dependent, or CaMKII-dependent, RYR2 phosphorylation at S2808) and that are unlikely to be prevented by β-blockers. In addition, Shan et al[23] argue that CaMKII phosphorylation is unimportant for AF in CPVT mice, because mutant mice lacking the RYR2 CaMKII phosphorylation site (S2814A) showed the same susceptibility to AF induction as wild-type mice after myocardial infarction. Although this interesting result is clearly relevant to a common cardiovascular condition that predisposes to AF, its relationship to pacing-induced AF in CPVT patients is less obvious. In addition, AF in CVPT mice with R176Q-RYR2 channels was prevented by pharmacological or genetic inhibition of CaMKII,[16] as well as when 50% of the RYR2 channels lacked the CaMKII phosphorylation site (ie, S2814-RYR2).[18]

It is important to appreciate that the absence of differences in RYR2 phosphorylation (at position S2808 under baseline conditions and at position S2814 after atrial pacing) between wild-type and R2474S-CPVT mice does not necessarily rule out a role for RYR2 phosphorylation in AF inducibility, because phosphorylation can work synergistically with oxidation to increase RYR2 activity (a 2-hit mechanism).[22,27] For example, constitutively phosphorylated RYR2 channels (ie, S2808D-RYR2) are more sensitive to oxidation than wild-type RYR2 channels,[22] and both phosphorylation and thiol oxidation were reported to be required for ventricular arrhythmogenesis and contractile dysfunction in canine heart disease.[27] Synergy between RYR2 oxidation and phosphorylation in CPVT mice deserves further investigation for several reasons. First, β-adrenergic activation, which is high even at baseline in mice, not only activates protein kinase A and CaMKII but also drives the generation of reactive oxygen species by mitochondria, which can oxidize RYR2 channels and increase spontaneous SR Ca^{2+} release.[28,29] Second, elevations in reactive oxygen species (ROS) activate CaMKII and vice versa. Finally, sympathetic-mediated elevations in cardiac output involve venous pressure (ie, the Frank-Starling mechanism), leading to atrial stretch, which increases oxidative stress, possibly via mechanisms described in ventricular myocytes.[30] Stretch-mediated elevations in RYR2 oxidation might also be relevant for sustaining AF, because Ca^{2+} transients are blunted in AF, resulting in reduced atrial contractility and atrial stretch. Clearly, more studies will be required to assess whether multiple modifications of mutant CPVT RYR2 channels are necessary to enhance AF susceptibility and whether these modifications differ between different RYR2 mutations.

The observation by Shan et al[23] that RYR2 oxidation is a necessary ingredient for the generation of lone AF in CPVT mice with R2474S-RYR2 channels is also interesting, because elevated oxidative stress is associated with intense exercise, and lone AF is extremely common in endurance athletes.[31] On the other hand, as already stated, AF in CPVT patients appears to be linked to exercise or emotional stress,[13,21,24] thus raising concerns about the physiological utility of pacing-induced AF in CPVT mice. It would clearly be of interest to examine whether atrial RYR2 channels in CPVT mice become oxidized under conditions of β-adrenergic stimulation or during exercise, as documented in ventricular myocardium.[32] It is also critical for future studies to validate the essential role played by RYR2 oxidation in CPVT. In this regard, although Shan et al[23] observed much higher Ca^{2+} spark frequencies in atrial than in ventricular cardiomyocytes isolated from R2474S-CVPT mice, the ability of antioxidants to prevent AF and normalize the Ca^{2+} spark frequencies between the atrial and ventricular myocytes was not examined, nor was the basis for the enhanced RYR2 oxidation in the atria versus ventricles investigated. Does the

presence of R2474S-RYR2 channels create a subclinical elevation in oxidative stress (presumably in atria only), and could structural changes induced by the R2474S mutation increase the susceptibility of RYR2 channels to oxidation (as seen in S2808D-RYR2 channels)?[22] Do both wild-type and mutant RYR2 channels become oxidized in CPVT patients, who typically express both mutant and wild-type RYR2 channels? As already mentioned, results from R176Q-RYR2/S2814A mice suggest increased phosphorylation of both mutant and wild-type channels can occur in CPVT.[18] Definitive answers to these questions will require biochemical methods for separating R2474S-RYR2 channels from wild-type RYR2 channels in the CPVT atria, possibly by incorporation of an antigenic tag into the mutant channels. Alternatively, measuring susceptibility to oxidation by use of heterologous expression of R2474S or other CPVT channels might also be informative.

The observation that CPVT mutations of RYR2 cause AF could provide unique opportunities for further investigation of the cellular and ionic mechanisms underlying AF, especially the contribution of abnormal Ca^{2+} handling in the setting of lone AF. These types of studies are sorely needed, because most previous mechanistic studies have relied on tissues and myocytes isolated from atria that have already undergone electric and structural remodeling. It will be interesting to explore whether AF shares features of ventricular arrhythmias seen in CPVT mutations. For example, bidirectional and polymorphic ventricular tachycardia could be explained by the appearance of regular delayed afterdepolarizations after pacing.[33] Previous studies have also shown that enhanced Ca^{2+} release properties of RYR2 channels predispose to the development of pacing-induced Ca^{2+} alternans, which leads to the appearance of electric alternans and increases dispersion of repolarization.[34] Ca^{2+} alternans might be particularly important in atrial myocytes because of their distinct Ca^{2+}-handling ultrastructure compared with ventricular myocytes.[35] Taking advantage of these CPVT models for understanding AF mechanisms will require detailed analyses of surface and intracardiac ECGs during bouts of AF, as well as optical mapping of voltage and Ca^{2+} in isolated atria (as has been performed previously).[16] These types of studies could prove extremely helpful in the testing and optimization of existing therapies for AF, as well as the identification of new and more refined approaches for the treatment of this condition. In CPVT patients, aggressive treatment of AF may prove to be important for reducing the frequency and consequences of ventricular arrhythmias by preventing the rapid, irregular ventricular pacing that results from AF. Further studies are clearly required to assess the potential causal link between atrial and ventricular arrhythmias in CPVT patients.

Sources of Funding

Dr Backx is supported by grants from the Canadian Institutes of Health Research (MOP 260107) and the Heart & Stroke Foundation of Ontario (T6485) and is a Career Investigator of the Heart & Stroke Foundation of Ontario.

Disclosures

None.

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KEY WORDS: Editorials ■ arrhythmia ■ atrial fibrillation ■ myocytes, cardiac ■ mutation

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Circ Res. 2012;111:662-665
doi: 10.1161/CIRCRESAHA.112.275453
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571