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EDITORIAL COMMENT

Ca^{2+} Leak in Atrial Fibrillation

Junctophilin-2 Stabilizes Ryanodine Receptor*

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Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, poses a formidable challenge to modern-day medicine. AF affects as many as 5 million Americans (1), and responses to therapy are highly variable among patients. Some therapies are fraught with substantial risk of proarrhythmia. As our population ages, the incidence of this rhythm disturbance, which can be associated with markedly increased morbidity and mortality, will continue to increase. Although strides have been made in elucidating underlying mechanisms, key molecular events remain elusive (2).

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Cytosolic Ca^{2+} governs a wide range of events in cardiac myocytes, including excitation-contraction coupling, signaling events, and activation of enzymes. As such, intracellular Ca^{2+} levels are tightly regulated in a context wherein they fluctuate 50-fold with each contraction. Ca^{2+} influx occurs through plasma-membrane localized L-type Ca^{2+} channels (LTCCs), which trigger much greater Ca^{2+} release from the sarcoplasmic reticulum (SR) via opening of the SR Ca^{2+} release channel (ryanodine receptor type 2 [RYR2]) (3). With repolarization, cytosolic Ca^{2+} is rapidly returned to the SR by the SR ATPase or extruded from the cell via the plasma membrane–localized sodium-calcium exchanger (NCX) (3).

Elevated cytosolic Ca^{2+} levels during diastole, as well as transient increases in Ca^{2+} ("sparks"), are capable of triggering delayed afterdepolarizations (DADs) and focal ectopic electrical activity, each of which is implicated in atrial and ventricular arrhythmogenesis (2). Spontaneous Ca^{2+} release events from the SR have also been observed in atrial myocytes from patients with chronic AF (4–7). Intriguingly, similar events occur in catecholaminergic polymorphic ventricular tachycardia, in which genetic mutations in RYR2 are implicated (8).

RYR2, a large tetrameric membrane protein (>500 kDa), is localized to the junctional membrane complex (JMC), a point of apposition between plasma membrane and the endoplasmic reticulum/SR (9). JMCs are a feature common to all excitable cell types, serving to facilitate cross-talk between the cell surface (specifically, its invaginations as transverse (T) tubules) and intracellular ion channels. Junctophilin-2, the dominant cardiac-expressed junctophilin isoform (10), is an essential component of JMCs.

In this issue of the *Journal*, Beavers et al. (11) report a previously undescribed mutation in the gene coding for junctophilin-2 in an individual afflicted with paroxysmal AF and hypertrophic cardiomyopathy. The authors go on to dissect mechanisms by which mutations in this protein participate in dysregulation of SR Ca^{2+} release in patients with paroxysmal AF and structural heart disease.

Previous work from this group focusing on nonischemic cardiomyopathy has demonstrated that hyperphosphorylation of RYR2, mediated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), provokes spontaneous channel opening with persistent Ca^{2+} leak from the SR (12). These same authors (7,13) and others (5) have reported this phenomenon in atrial cardiomyocytes from patients with chronic AF. Again, elevated cytosolic Ca^{2+} levels and spontaneous SR Ca^{2+} release events (i.e., sparks) that provoke DADs were implicated in the arrhythmogenic state. The study reported here suggests that a similar mechanism may predispose to atrial arrhythmias (11).

This report makes a significant contribution in its careful elucidation of the role of junctophilin-2 in the molecular pathogenesis of RYR2 Ca^{2+} leak (11). The work was informed by the observation that adult-onset deficiency of junctophilin-2 in mice results in disruption of the JMCs with mislocalization of RYR2s and LTCCs, increased diastolic Ca^{2+} leak from the SR, and Ca^{2+} sparks (14). The authors were also guided by previous reports of missense mutations in *JPH2*, the gene coding for junctophilin-2, in patients with hypertrophic cardiomyopathy (15,16), which were observed to alter Ca^{2+} signaling in myocytes in vitro (15,17).

Here, the authors discovered 2 novel missense mutations in the *JPH2* coding region on screening a population of patients with hypertrophic cardiomyopathy, one of which was associated with paroxysmal AF in the proband and supraventricular arrhythmias in his father (11). They modeled these 2 mutations in mice using a clever strategy of crossing a transgenic mouse expressing mutant *JPH2* with cardiomyocyte-specific *JPH2* knockdown mice. The resultant "pseudo-knockin" mice manifested myocardial junctophilin-2 expression at levels equivalent to those of wild type, while harboring a mix of both mutant and wild-type protein (11) akin to the situation in

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patients heterozygous for these mutations. Remarkably, only the mice carrying the glutamate-to-lysine mutation at position 169 in human junctophilin-2 (E169K, detected in the proband with atrial fibrillation), manifested increased susceptibility to pacing-induced AF, spontaneous SR Ca^{2+} release events, and increased RYR2 open probability (11). Additionally, only the E169K mutant protein demonstrated diminished interaction with RYR2, implicating this region of junctophilin-2 in stabilizing RYR2 in its closed state.

Beavers et al. (11) map critical aspects of junctophilin-2 interaction with RYR2 to a specific region of the junctophilin-2 protein. Seizing this observation, they find that overexpression of a short peptide mimicking this region attenuated Ca^{2+} spark frequency and RYR2 open probability. Whether a similar mechanism provokes alterations in Ca^{2+} signaling observed with expression of the S165F and Y141H mutants (17,18) remains to be determined.

Previous studies have implicated increased CaMKII-mediated phosphorylation of RYR2 in AF pathogenesis (5,7,13). Indeed, CaMKII-mediated RYR2 phosphorylation at S2814 was noted to be essential for conferring arrhythmia susceptibility in a mouse model of RYR2 gain-of-function (13). Further, knock-in of S2814A (nonphosphorylatable) and S2814D (phosphomimetic) RYR2 mutants attenuated and exacerbated AF susceptibility, respectively (7,19). Also, loss of FKBP12.6, a RYR2-stabilizing protein, provokes SR Ca^{2+} leak and predisposes to atrial arrhythmias (6,19).

As with all discoveries, this elegant study raises new questions. Given the small sample size studied here and the lack of a matched control group, the association of the reported *JPH2* mutations with hypertrophic cardiomyopathy remains to be confirmed. Notwithstanding this limitation, earlier studies have suggested that mutations resulting in alteration in junctophilin-2 function are associated with hypertrophic cardiomyopathy (15,16). Indeed, reduced junctophilin-2 abundance is observed in left ventricular tissue from patients with hypertrophic cardiomyopathy harboring diverse mutations in sarcomeric and other genes, and junctophilin-2 knockdown provokes cardiomyocyte hypertrophy in vitro (17).

A recent study modeled hypertrophic cardiomyopathy in induced pluripotent stem cells generated from a family of individuals harboring a mutation in the β-myosin heavy chain gene *(MYH7)* (20). Cardiomyocytes transdifferentiated from these cells manifested elevations in cytosolic Ca^{2+} and frequent small depolarizations with increased propensity to generate DADs, preceding spontaneous development of hypertrophy (20). Conversely, elevations of cytosolic Ca^{2+}, as observed with the E169K junctophilin-2 mutant, may be speculated to drive expression of prohypertrophic transcription factors such as MEF-2, which is activated in a mouse model of hypertrophic cardiomyopathy provoked by a distinct human *MYH7* mutation (21).

Interestingly, the current report did not describe hypertrophy in mice expressing the junctophilin-2 mutants (11), and earlier studies with cardiomyocyte-specific junctophilin-2 knockdown in vivo did not report hypertrophy (14). Thus, further work is needed to establish a putative cause-and-effect relationship between reduced junctophilin-2 function and hypertrophy. One might speculate that reduced junctophilin-2 function may be sufficient to drive the pathogenesis of hypertrophy via mechanisms currently not defined, as with the alanine-to-serine mutation at position 405, which was detected in an individual with hypertrophic cardiomyopathy but did not alter Ca^{2+} cycling (11). It also plausible that the primary effect of reduced junctophilin-2 function may be to confer susceptibility to arrhythmogenesis, with elevated diastolic Ca^{2+} levels provoking accelerated prohypertrophic signaling as a disease-modifying event.

Another interesting observation from this study is a reduction in the ratio of junctophilin-2 to RYR2 abundance in atrial tissue from patients with paroxysmal AF and structural heart disease, eliciting relative junctophilin-2 deficiency driven primarily by up-regulation of RYR2 abundance (11). The authors speculate that this relative deficiency fosters destabilization of RYR2 with resultant Ca^{2+} leak from the SR (7). Indeed, an inverse correlation between myocardial junctophilin-2 levels and susceptibility to AF induction in the Beavers et al. (11) study lends support to this notion. Although gene expression changes in Ca^{2+} cycling proteins were not observed in a previous study of right atrial tissue from a comparable patient population with paroxysmal AF (22), junctophilin-2 expression was not evaluated, and interindividual variability with small sample sizes precludes direct comparisons with the current study. Whether a relative or absolute reduction in junctophilin-2 expression and/or function occurs in atrial myocytes from patients with chronic AF remains to be determined. It is also intriguing to note that genomewide association studies in AF patients have not uncovered mutations or polymorphisms in *JPH2,* which may indicate that deficient junctophilin-2 function or expression may be rare and/or not the primary driver of pathogenesis.

Accumulating evidence from preclinical studies points to JMC remodeling in post-myocardial infarction heart failure (23) and pressure overload–induced hypertrophy (24). These changes are associated with reduced junctophilin-2 abundance and decreased coupling of LTCC with RYR2 in the ventricular myocardium, suggesting that deficiency of junctophilin-2 may confer susceptibility to ventricular arrhythmogenesis across several common forms of cardiac pathology. Reductions in junctophilin-2 expression may be the primary event in provoking T-tubule (and JMC) dysfunction, as suggested by the observation of miR-24–induced suppression of junctophilin-2 transcription in pressure overload–induced hypertrophy (25). Indeed, restoring junctophilin-2 expression in vivo with a miR-24–targeting antagomir preserved T-tubule architecture without affecting the hypertrophic response (25). Reductions in junctophilin-2 expression may also occur via calpain-mediated proteolysis (26), conceivably provoking JMC disruption and Ca^{2+} cycling abnormalities.

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The current study raises additional questions. What is the role of junctophilin-2 in the pathogenesis of ventricular arrhythmias? Notably, the 2 individuals carrying the E169K mutation demonstrated prolonged QTc, and the proband required pacemaker implantation for sinoatrial exit block, suggesting effects of the mutation beyond the atrial myocyte (11). Also, the mechanistic relevance of these findings to human AF is perhaps better explored in large animal models, which have a well-developed T-tubule network in atrial myocytes versus the rudimentary T-tubule system in rodents (27).

Notwithstanding our present lack of understanding as to whether junctophilin-2 deficiency is an initiator or propagator of arrhythmias, the current study along with previous work supports a role for junctophilin-2 deficiency in fostering Ca^{2+} leak from the SR and consequent proarrhythmia. These data suggest that preventing junctophilin-2 dysfunction or deficiency and, by corollary, restoring the structure of the JMC, may be a meaningful therapeutic target in abrogating certain rhythm disturbances. Indeed, demonstration here of efficacy of a small junctophilin-2 peptide in stabilizing RYR2 and preventing SR Ca^{2+} leak is a first step toward translating this notion into practice.

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