Calcium in atrial fibrillation – pulling the trigger or not?

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Atrial fibrillation (AF) is the most common sustained arrhythmia disease. Current drug- and surgical-based therapies are ineffective in about 40% to 50% of AF patients; therefore, there is a great need to better understand the underlying mechanisms of this disease and identify potential therapeutic targets. In this issue of the *JCI*, Greiser and coworkers discovered that atrial remodeling in response to sustained tachycardia silences Ca^{2+} signaling in isolated rabbit and human atrial myocytes. This Ca^{2+} release silencing was attributable to a failure of subcellular propagated Ca^{2+} release due to an increased cytosolic buffering strength. The results from this study challenge the current paradigm that Ca^{2+} release instability underlies AF. Instead, Ca^{2+} silencing could be protective against the massive cellular Ca^{2+} loading that occurs during chronic AF.

Atrial fibrillation: arrhythmogenic atrial remodeling and unstable Ca^{2+} handling

Atrial fibrillation (AF) is the most common sustained arrhythmia disease, affecting 1% to 2% of the US population (1). Because current therapy — either with drugs or surgical ablation — remains ineffective in about 40% to 50% of AF patients (2), there is a great need to understand the underlying mechanisms of AF and identify better therapeutic targets. Previous work has shown that a rapid atrial activation rate induces electrical remodeling of the atria, which in turn increases the risk for AF (3). The remodeling consists of L-type Ca^{2+} current reduction (4), sodium-calcium exchanger upregulation, reduced Ca^{2+} transients (5), and altered sarcoplasmic reticulum (SR) function, which is characterized by increased spontaneous Ca^{2+} sparks and waves and has been attributed to hyperactive RyR2 channels, possibly as the result of increased phosphorylation at residues Ser2808 and Ser2815 (5–7). These so-called "leaky" RyR2 channels and increased Ca^{2+} waves are capable of triggering delayed afterdepolarizations and focal atrial electrical activity (8); therefore, hyperactive RyR2 channels are thought to be important contributors to the induction and maintenance of AF in humans (9).

Rapid arterial pacing induces paradoxical Ca^{2+} signaling silencing

It remains unclear whether the Ca^{2+} signaling remodeling observed in myocytes isolated from humans with AF is a consequence of a rapid activation rate or due to concomitant heart disease. In this issue of the *JCI*, Greiser et al. (10) attempt to answer this question by characterizing subcellular Ca^{2+} signaling in atrial myocytes harvested from rabbits that underwent 5 days of rapid atrial pacing (RAP) and compared them with atrial myocytes harvested from patients with chronic AF. Surprisingly, Greiser et al. did not observe changes in Ca^{2+} sparks, Ca^{2+} waves, or Ca^{2+} release instability, but rather they found that Ca^{2+} release was strongly suppressed in the center of rabbit atrial myocytes. Greiser and colleagues have termed this suppression "Ca^{2+} signaling silencing," and a similar central Ca^{2+} release silencing also occurred in atrial myocytes harvested from patients with chronic AF. What causes the loss of central Ca^{2+} release? Because the density of transverse tubules is much lower than that of ventricular myocytes, atrial myocytes rely on Ca^{2+} diffusion to activate Ca^{2+} release in the myocyte core (11); consequently, the Ca^{2+} signal that is generated in the subsarcolemmal region from Ca^{2+} release triggered by L-type Ca^{2+} channels is propelled to the cell center by repetitive release from intracellular RyR2 clusters (Figure 1A). Greiser et al. determined that cytosolic Ca^{2+} buffering strength is markedly increased in RAP myocytes. This increased Ca^{2+} buffering capacity could be the consequence of reduced troponin I phosphorylation, which in turn would increase Ca^{2+} binding to troponin C in the myofilaments. Hence, increased Ca^{2+} binding to myofilaments may reduce the free Ca^{2+} available to activate neighboring RyR2 clusters. Together with the observed reduction in RyR2 expression, increased cytosolic buffering likely explains the failure in the centripetal Ca^{2+} propagation of RAP myocytes (Figure 1B), because SR Ca^{2+} content, RyR2 channel activity, and peripheral L-type current–induced Ca^{2+} release were all preserved.

Greiser and colleagues (10) also report that, consistent with previous studies, the remaining RyR2 clusters were hyperphosphorylated at the protein kinase A (PKA) phosphorylation site (Ser2808), which may compensate for the reduction in RyR2 protein expression and help sustain subsarcolemmal Ca^{2+} release despite reduced L-type Ca^{2+} currents (Figure 1B). However, RAP myocytes exhibited reduced RyR2 phosphorylation at the calmodulin-dependent protein kinase II (CaMKII) phosphorylation site (Ser2815) and no changes in CaMKII activity. This finding contrasts with previous studies that reported increased atrial CaMKII activity and CaMKII-dependent RyR2-Ser2815 phosphorylation in human AF (5). Moreover, other studies have shown that treatment with CaMKII inhibitors or selective disruption of the Ser2815

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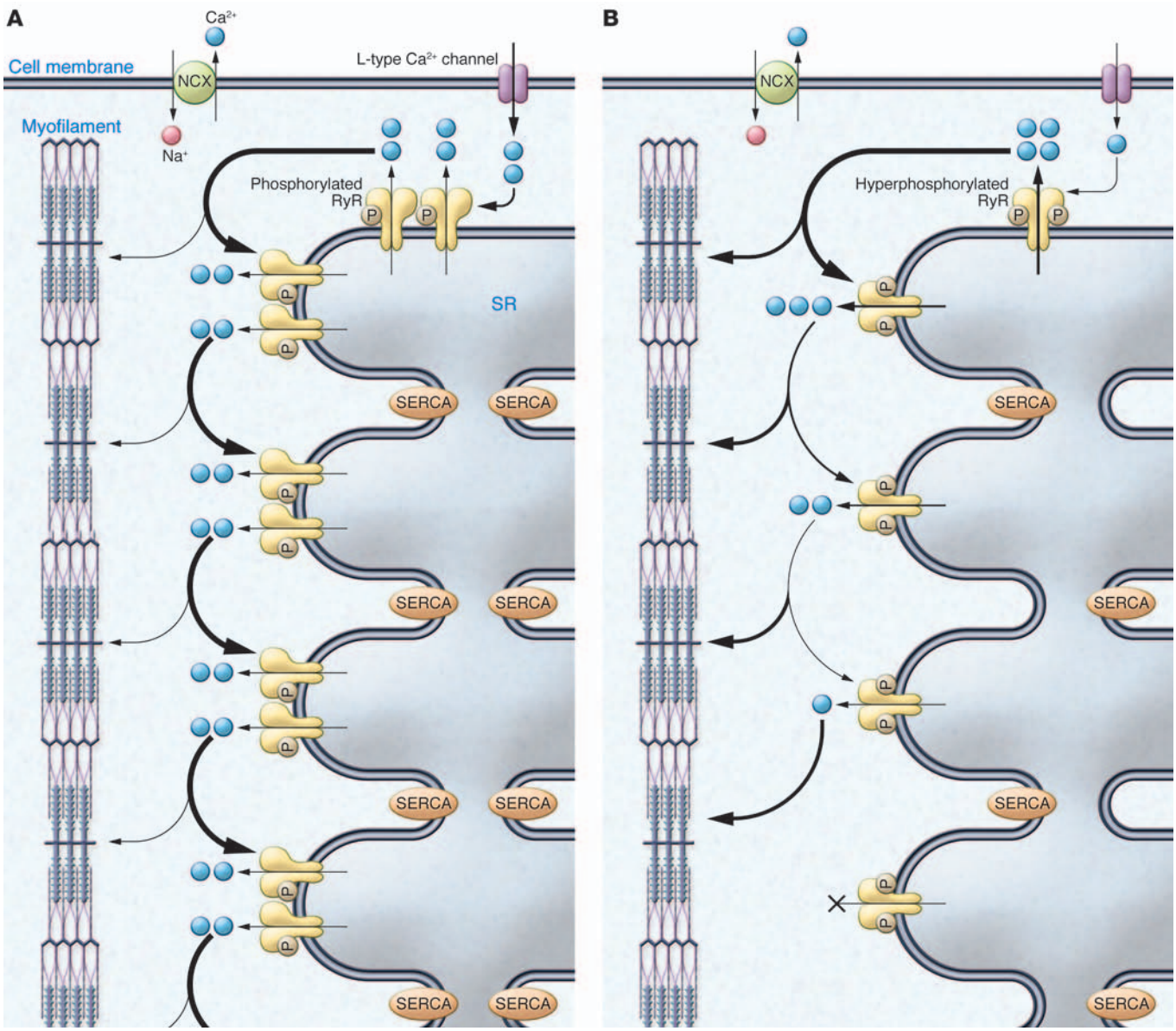

Figure 1. Ca^{2+} signaling silencing and failure of centripetal Ca^{2+} propagation in RAP atrial myocytes. Atrial myocytes rely on Ca^{2+} diffusion to neighboring RyR2 clusters to activate Ca^{2+} release from the subsarcolemmal region to the core of the myocytes (**A**). In RAP atrial myocytes, increased Ca^{2+} binding to myofilaments reduces the free Ca^{2+} available to activate neighboring RyR2 clusters. This phenomenon, together with the reduction in RyR2 expression and RyR2 cluster size, could explain the failure in centripetal wave propagation (**B**). P, PKA phosphorylation at RyR2-2808; NCX, sodium-calcium exchanger.

CaMKII phosphorylation site prevented AF in animal models through a reduction of SR Ca^{2+} leak (12). One explanation for this discrepancy could be the limited duration of pacing in the rabbit model used by Greiser and colleagues, in which none of the animals exhibited spontaneous AF after the 5 days of rapid pacing. It remains unclear whether the effects observed in response to limited atrial pacing are just transient changes due to the short period of this protocol, or whether, as suggested by Greiser et al., Ca^{2+} signaling silencing represents a maintained response of atrial myocytes to chronic rapid activation. Strikingly, but consistent with the unaltered CaMKII activity, Greiser et al. did not observe changes in phospholamban activity. Rather, they found decreased expression of the SR Ca^{2+} ATPase (SERCA), despite the fact that the Ca^{2+} load in the SR was also unchanged. This reduction in SERCA expression with no changes in SR Ca^{2+} load can be explained by the reduction in Ca^{2+} release from the SR due to Ca^{2+} release silencing; however, the role of SERCA remodeling during AF remains unclear. Recent studies suggest that in atrial muscle, SERCA activity is also regulated by sarcolipin (13, 14), and it remains to be tested whether changes in sarcolipin contribute to altered SERCA activity in the rabbit model of RAP.

Ca^{2+} signaling silencing: counteracting arrhythmogenic atrial remodeling?

The study by Greiser et al. (10) elegantly provides evidence of altered atrial Ca^{2+}

handling in response to tachycardia, but also raises new questions. It is well established that Ca^{2+}-dependent signaling affects atrial remodeling: cellular Ca^{2+} loading during rapid pacing activates calcineurin, which in turn dephosphorylates, for example, nuclear factor of activated T cells (NFAT) and promotes its translocation to the nucleus. In the nucleus, NFAT regulates several targets at the transcriptional level, including the L-type Ca^{2+} channel, which is reduced by NFAT (15). In this regard, it would be interesting to further analyze whether atrial Ca^{2+} signaling silencing reduces NFAT translocation to the nucleus and thus limits the electrical and structural remodeling that occurs after the Ca^{2+} overload in AF. This structural remodeling contributes to the reinduction of AF. Although it takes place after the electrical remodeling, the Ca^{2+} overload induced by rapid atrial activation rates is one of the main signals that triggers the remodeling process (16). Thus, while the Ca^{2+} signaling silencing discovered by Greiser et al. (10) appears to help limit the consequence of rapid pacing–induced Ca^{2+} overload, the extent of this protective effect in the intact organ during AF or in preventing AF triggering is not clear. Studies in the intact atria during and after AF will be needed to better understand the role of Ca^{2+} signaling silencing in the pathophysiology of AF.

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