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Calmodulin kinase II, sarcoplasmic reticulum Ca^{2+} leak, and atrial fibrillation

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Abstract

Although it is generally accepted that excitation-contraction coupling is defective in patients with atrial fibrillation, the underlying cellular mechanisms remain incompletely understood. Recent studies suggest that abnormal sarcoplasmic reticulum calcium 'leak' via ryanodine receptors contributes to atrial arrhythmogenesis. Increased activity of the enzyme calmodulin kinase II (CaMKII), and specifically, enhanced CaMKII phosphorylation of ryanodine receptors appears to play a critical role in the induction and perhaps maintenance of atrial fibrillation. In this review, we will summarize new insights into the role of enhanced calmodulin kinase II in sarcoplasmic reticulum calcium leak and atrial arrhythmogenesis during atrial fibrillation.

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia and is associated with an increased risk of stroke (Spence 2009). The basic mechanisms proposed to underlie AF are rapid ectopic firing and reentry (Nattel et al. 2008). Ectopic activity occurs principally by prolongation of action potential duration leading to early afterdepolarisations and by spontaneous sarcoplasmic reticulum (SR) Ca^{2+} releases causing delayed afterdepolarisations. The likelihood of reentry is primarily determined by the balance of conduction velocity and refractory period, and is favored by slow conduction and/or shorter refractoriness (Nattel et al. 2008).

Once induced, the arrhythmia itself causes changes in atrial electrophysiological properties such that AF is more easily re-induced and/or maintained (a phenomenon referred to 'AF begets AF') (Wijffels et al. 1995). Electrical remodeling has been shown to abbreviate the action potential duration and to shorten the atrial refractory period, providing an arrhythmogenic substrate for AF (Nattel 2002). In addition, atrial proteins involved in intracellular Ca^{2+} handling undergo extensive remodeling in AF (Figure 1), resulting in an increased propensity towards spontaneous SR Ca^{2+} releases (El-Armouche et al. 2006,

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Hove-Madsen et al. 2004, Neef et al. 2010.). These abnormal SR Ca^{2+} releases can act as a local trigger generator, leading to a small reentry circuit or ectopic focal activity (Mandapati et al. 2000, Mansour et al. 2001). Whereas an increasingly complete characterization of molecular modifications of Ca^{2+} -handling proteins in AF has been reported (Dobrev and Nattel 2008), it remains to be determined which of those changes are in fact causally linked to atrial arrhythmogenesis. In this review, we will discuss recent findings regarding the effects of enhanced Ca^{2+} /calmodulin-dependent protein kinase II (calmodulin kinase II, or CaMKII) activity and diastolic SR Ca^{2+} leak on atrial arrhythmogenesis.

Regulation of Intracellular Calcium Release in Atria

In atrial myocytes, excitation-contraction coupling occurs when Ca^{2+} entry ($\text{I}_{\text{Ca,L}}$) via the voltage-gated L-type Ca^{2+} channels (LTCC) triggers a much greater SR Ca^{2+} release via ryanodine receptors (RyR2), a process known as Ca^{2+} -induced Ca^{2+} release (CICR) (Bers and Guo 2005). Owing to the absence of T-tubules in atria (Dobrev et al. 2009), Ca^{2+} influx triggers a non-synchronous increase in intracellular Ca^{2+} . Ca^{2+} waves start in the myocyte periphery and then propagate to the myocyte center, activating additional Ca^{2+} -releasing sites (Dobrev and Nattel 2008). The size of the systolic Ca^{2+} transient is dynamically regulated and depends on both the RyR2 open probability and the SR Ca^{2+} content, which is indirectly a function of the Ca^{2+} reuptake through the SR Ca^{2+} -ATPase (SERCA2a).

The open probability of RyR2 is modulated by accessory binding proteins (e.g., FKBP12.6, calmodulin, sorcin, calsequestrin, junction, triadin) as well as posttranslational modifications (such as phosphorylation, oxidation and nitrosylation) (reviewed in Chelu and Wehrens 2007, Wehrens et al. 2005). For example, it has been shown that protein kinase A (PKA) and CaMKII bind to the RyR2 macromolecular complex, which enables these enzymes to dynamically phosphorylate RyR2 (Marx et al. 2001). Conversely, RyR2-bound protein phosphatases 1 (PP1) and 2A (PP2A) can dephosphorylate the channel depending on the relative kinase-phosphatase activity balance (Vest et al. 2005). The relative level of RyR2 phosphorylation, in turn, determines the open probability, and thus the amount of SR Ca^{2+} release during both systole and diastole (Marx et al. 2000, Wehrens et al. 2004b).

Regulation of SR Calcium Release by CaMKII

CaMKII is an enzyme known to decode the frequency and amplitude of intracellular Ca^{2+} transients (Schulman et al. 1992). Activated by higher average intracellular Ca^{2+} concentrations, for example at faster heart rates, CaMKII phosphorylates a variety of Ca^{2+} -handling proteins including LTCC, RyR2, and the SERCA2a-inhibitory protein phospholamban (PLN) (DeSantiago et al. 2002, Wehrens et al. 2004b, Wu et al. 1999). The effects of CaMKII phosphorylation on these proteins include greater Ca^{2+} influx, larger SR Ca^{2+} release and enhanced SERCA2a activity to prevent SR Ca^{2+} depletion by increasing SR Ca^{2+} loading (DeSantiago et al. 2002, Wehrens et al. 2004b).

Whereas multiple CaMKII isoforms are expressed in the heart (Hagemann et al. 1999), only CaMKII- δ is believed to bind to RyR2 (Wehrens et al. 2004b, Zhang et al. 2003). CaMKII- δ phosphorylates serine 2814 (Ser2814) on RyR2, which is only 6 amino acids removed from the primary PKA phosphorylation site Ser2808 (Witcher et al. 1991). Phosphorylation of Ser2814 on RyR2 by CaMKII increases the sensitivity to Ca^{2+} -dependent activation and overall RyR2 open probability (Hain et al. 1995, Wehrens et al. 2004b). CaMKII has been shown to increase both the open probability of RyR2 (Hain et al. 1995, Wehrens et al. 2004b) and the Ca^{2+} -spark frequency (Guo et al. 2006, Kohlhaas et al. 2006). CaMKII may also indirectly enhance SR Ca^{2+} release by increasing SR Ca^{2+} loading as a result of higher SERCA2a activity due to phosphorylation of PLN at threonine 17 (Thr17) that decreases PLN-dependent inhibition of SR Ca^{2+} uptake (Bluhm et al. 2000).

Defective SR Calcium Release in AF

Hove-Madsen *et al.* (2004) provided the first evidence for defective SR Ca^{2+} release in myocytes from patients with chronic AF. Similar findings were obtained by Neef *et al.* (2010), who recently demonstrated increased SR Ca^{2+} leak, which was not attributable to higher SR Ca^{2+} loading. Because the amplitude of $I_{\text{Ca,L}}$ is generally decreased in AF (Nattel *et al.* 2008), it is more likely that defects in SR Ca^{2+} release are responsible for these abnormalities. Indeed, Vest *et al.* (2005) demonstrated an increased RyR2 open probability in dogs with chronic AF. Since diastolic Ca^{2+} leak persistence is promoted if normal SR Ca^{2+} load is maintained (Eisner *et al.* 2009), PKA and CaMKII hyperphosphorylation of PLN at serine 16 (Ser16) and Thr17, respectively, may prevent SR Ca^{2+} depletion in AF (El-Armouche *et al.* 2006), explaining the preserved SR Ca^{2+} content (Hove-Madsen *et al.* 2004, Neef *et al.* 2010). The increased frequency of diastolic SR Ca^{2+} releases is believed to activate the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger (NCX), resulting in transient inward currents that may depolarize the cell membrane, causing delayed afterdepolarizations and cardiac arrhythmias (Lehnart *et al.* 2006, Schlotthauer and Bers 2000). NCX is upregulated in fibrillating atria (Schotten *et al.* 2002, El-Armouche, 2006, generating a much larger depolarizing inward current in response to the same SR Ca^{2+} release (Voigt *et al.* 2009), thus amplifying the pro-arrhythmic effects of aberrant diastolic SR Ca^{2+} leak (Figure 1). Our preliminary results show that SR Ca^{2+} depletion with caffeine produces larger depolarizing NCX current in patients with AF (Voigt *et al.* 2009), but whether the frequent spontaneous SR Ca^{2+} releases activate NCX to the critical threshold required to produce triggered action potentials and extrasystoles will require further investigation

Enhanced CaMKII Activity in AF

Tessier *et al.* (1999) were the first to report enhanced expression levels of CaMKII- δ in atrial tissue samples from patients with chronic AF. Our recent studies revealed that only the cytosolic CaMKII- δ_C but not the nuclear CaMKII- δ_B isoform is upregulated in patients with AF (Chelu *et al.* 2009). Increased CaMKII activity was associated with enhanced Ser2814 phosphorylation on RyR2 (Chelu *et al.* 2009). Like us, Neef *et al.* (2010) showed increased CaMKII expression and enhanced RyR2 phosphorylation at Ser2814 in patient with AF. Goats with sustained AF also exhibit increased autophosphorylation and thus activity of CaMKII along with enhanced CaMKII-dependent RyR2 phosphorylation (Greiser *et al.* 2009), suggesting that the high atrial rate is sufficient to cause these alterations. In addition, El-Armouche *et al.* (2006) detected substantial increase in CaMKII phosphorylation of PLN at Thr17 in patients with chronic AF. In ventricular myocytes of transgenic mice increased levels of CaMKII- δ_C enhanced fractional SR Ca^{2+} release and the resting frequency of spontaneous SR Ca^{2+} sparks (Maier *et al.* 2003). Consistent with these data, Guo *et al.* (2006) demonstrated an increase in ventricular Ca^{2+} spark activity following activation of endogenous CaMKII associated with RyR2. Moreover, CaMKII is an established contributor to RyR2-mediated SR Ca^{2+} leak linked to ventricular arrhythmias (Anderson 2005, Wu *et al.* 2006). However, until recently it remained unclear whether enhanced CaMKII activity directly relates to the abnormal diastolic SR Ca^{2+} release and arrhythmia susceptibility in the context of AF.

CaMKII Increases SR Calcium Leak Promoting Induction of AF

To dissect the specific contribution of SR Ca^{2+} leak to atrial arrhythmias, we recently studied knock-in mice with a gain-of-function mutation in RyR2 (R176Q) (Chelu *et al.* 2009). Despite the fact that the RyR2 mutation increased SR Ca^{2+} leak measured in isolated myocytes (Chelu *et al.* 2006), heterozygous R176Q/+ knock-in mice did not exhibit spontaneous AF on telemetric ECG recordings. Following rapid atrial pacing, however,

R176Q/+ mice showed an increased vulnerability to AF induction compared to wild-type littermates (Chelu et al. 2009). As expected based on prior studies, our pacing protocol increased CaMKII phosphorylation of RyR2 (Wehrens et al. 2004b). Therefore, we concluded that activation of CaMKII at faster atrial rates like in AF amplifies RyR2 Ca^{2+} leak, thereby providing the molecular basis for the arrhythmia trigger (Chelu et al. 2009). It is generally believed that diastolic SR Ca^{2+} leak activates NCX, which may cause delayed afterdepolarization and triggered activity that can induce and/or maintain cardiac arrhythmias (Pogwizd et al. 2001).

Using another knock-in mouse model in which the CaMKII phosphorylation site on RyR2 had been genetically ablated by substitution of serine by non-phosphorylatable alanine (S2814A), we demonstrated that inhibition of Ser2814 phosphorylation on RyR2 prevents induction of pacing-induced AF in S2814A mice (Chelu et al. 2009). These data suggest that enhanced phosphorylation of RyR2 represents a critical, albeit not exclusive, downstream target of activated CaMKII signaling in fibrillating atria (Chelu et al. 2009). These studies demonstrate the power of studying arrhythmia mechanisms in genetically-modified mice, as the specific contribution of a single phosphorylation event can be evaluated in the whole animal.

Surprisingly, the increased steady-state CaMKII phosphorylation of RyR2 and PLN occurs despite a globally enhanced activity of PP1 and PP2A in patients with AF (Christ et al. 2004). Phosphorylation of RyR2 and PLN at PKA-sites (S2808 and S16, respectively) is also increased in patients with AF (El-Armouche et al. 2006, Vest et al. 2005). Activity of PP1 at the SR is specifically regulated by inhibitor-1, which inhibits SR-related PP1 activity only when phosphorylated at threonine-35 (Carr et al. 2002). Phosphorylation of inhibitor-1 at threonine-35 is 10-fold higher in patients with AF (El-Armouche et al. 2006), likely preventing PP1-mediated dephosphorylation of SR-located RyR2 and PLN. Further work in suitable transgenic mouse models is needed to directly prove this hypothesis.

Potential Clinical and Therapeutic Implications

Our studies suggest that CaMKII hyperphosphorylation of RyR2 at Ser2814 increases the propensity for spontaneous SR Ca^{2+} releases, which, if occurred as a single event, might quickly lead to SR Ca^{2+} depletion and disappearance of spontaneous Ca^{2+} -release events (Eisner et al. 2009). However, the simultaneous hyperphosphorylation of PLN would promote a fast SR refilling, supporting a continuous SR Ca^{2+} leak that may cause delayed afterdepolarisations, triggered activity, and cardiac arrhythmias. Our data suggest that increased CaMKII mimics this phenotype in the context of atrial remodeling by producing two essential requirements for triggered activity and atrial arrhythmogenesis (Figure 1): a) hyperphosphorylation of PLN that indirectly enhances SR Ca^{2+} loading by increasing SERCA2a activity (El-Armouche et al. 2006) and b) hyperphosphorylation of RyR2, which increases diastolic SR Ca^{2+} leak by enhancing open probability of RyR2 channels (Chelu et al. 2009). These observations suggest that it might be possible to suppress AF by either inhibiting CaMKII enzyme activity, or by reducing RyR2 'leakiness'.

The therapeutic potential of kinase inhibitors was initially questioned because protein kinase sequences are highly conserved and kinases are often ubiquitously expressed, which would make selective and tissue-specific drug activity almost impossible. However, the success of protein kinase inhibitors as cancer therapeutics supports their potential therapeutic value for cardiac diseases (Sebolt-Leopold and English 2006). RyR2 hyperphosphorylation at Ser2808 leads to a conformational change of the channel, which is suggested to enhance dissociation of the RyR2 inhibitor FKBP12.6, promoting diastolic SR Ca^{2+} leak (Marx et al. 2000). The FKBP12.6 amount in the atrial RyR2 complex is reduced in patients with AF

(Vest et al., 2005) and FKBP12.6-deficient mice develop pacing-induced AF, confirming the role of reduced FKBP12.6 for atrial arrhythmogenesis (Sood et al. 2008). JTV519 (K201) is believed to increase FKBP12.6-RyR2 binding and to reduce diastolic SR Ca^{2+} leak and ventricular arrhythmias incidence (Wehrens et al. 2004a). However, JTV519 also blocks several atrial ion channels, which makes it less suitable for clinical use (Nakaya et al. 2000). A more specific JTV519 analogue, known as S107, is currently under investigation (Lehnart et al. 2008).

Direct RyR2 channel blockade may also fix SR Ca^{2+} leak and dysfunction. The local anaesthetic tetracaine increases the threshold SR Ca^{2+} content required for spontaneous SR Ca^{2+} releases and reduces SR Ca^{2+} leak through RyR2 (Venetucci et al. 2006). Similar effects were described for the class I antiarrhythmic agent flecainide, which also decreases RyR2 open probability (Hilliard et al. 2009). The primary action of both drugs is inhibition of Na^+ channels and this may cause malignant ventricular arrhythmias, especially in patients with coronary artery disease. Similar agents reducing SR Ca^{2+} leak without interference with Na^+ channels may prove efficient for AF treatment without collateral effects at the ventricles.

An alternative approach to inhibit the consequences of abnormal SR function would be to block NCX more efficiently in the forward (Ca^{2+} efflux) mode, thereby preventing the generation of potentially arrhythmogenic depolarizing NCX current. However, NCX inhibitors like KB-R7943 or SEA0400 are not selective and block different ion channels and transporters (Iwamoto et al. 2007), which may preclude their clinical applicability for AF.

Taken together, there is emerging evidence that CaMKII-mediated diastolic SR Ca^{2+} leak and triggered activity may support AF induction and maintenance. Although the causal relationship between local Ca^{2+} -release events and focal activity in fibrillating human atria requires direct experimental demonstration, novel therapies targeting atrial SR abnormalities promise to open new directions in the treatment of AF.

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Figure 1. Overview of alterations in atrial Ca^{2+} movements in human atrial fibrillation

Atrial remodeling during chronic atrial fibrillation (AF) leads to changes in the expression and phosphorylation level of key Ca^{2+} -handling proteins involved in atrial Ca^{2+} signaling. Whereas expression levels of the L-type Ca^{2+} channel (LTCC) typically remain unaffected, phosphorylation of the channel appears reduced leading to decreased $I_{\text{Ca,L}}$. Hypophosphorylation of this channel might be due to increased activity of protein phosphatases PP1 and PP2A. In contrast, the sarcoplasmic reticulum (SR) Ca^{2+} release channel/ryanodine receptor (RyR2) is hyperphosphorylated in AF at both protein kinase A (PKA) and Ca^{2+} /calmodulin-kinase II (CaMKII) sites. This might be caused by reduced PP1 activity, as a result of hyperphosphorylation (and thus activation) of inhibitor-1 at Thr35 that reduces SR-related PP1 activity. Similar mechanisms may also contribute to hyperphosphorylation of phospholamban (PLN). Increased cytosolic CaMKII activity also contributes to hyperphosphorylation of RyR2 (at Ser2814) and PLN (at Thr17), which might be promoted by the higher atrial rates during AF. Diastolic SR Ca^{2+} leak via RyR2 can activate the $\text{Na}^{+}/\text{Ca}^{2+}$ -exchanger, leading to generation of an inward depolarizing NCX current. This may produce delayed afterdepolarizations and triggered activity that may cause focal activity contributing to AF maintenance.