

Atrial remodelling in atrial fibrillation: CaMKII as a nodal proarrhythmic signal

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Abstract

CaMKII is a serine–threonine protein kinase that is abundant in myocardium. Emergent evidence suggests that CaMKII may play an important role in promoting atrial fibrillation (AF) by targeting a diverse array of proteins involved in membrane excitability, cell survival, calcium homeostasis, matrix remodelling, inflammation, and metabolism. Furthermore, CaMKII inhibition appears to protect against AF in animal models and correct proarrhythmic, defective intracellular Ca²⁺ homeostasis in fibrillating human atrial cells. This review considers current concepts and evidence from animal and human studies on the role of CaMKII in AF.

Keywords

CaMKII • Atrial fibrillation • Atrial remodelling • Arrhythmias

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1. Introduction

Atrial fibrillation (AF) is the most common sustained clinical arrhythmia, affecting approximately 33.5 million people annually worldwide.¹ Age is a major risk factor for AF, and the disease incidence and prevalence have increased with the ageing of the general population.^{2,3} AF is associated with increased morbidity, primarily from stroke and heart failure, and increased mortality.^{4,5}

AF is a progressive disease, and the current clinical classification of AF is based on its pattern and duration.⁶ AF is classified as paroxysmal AF (episodes that last <7 days with or without intervention), persistent AF (episodes lasting ≥7 days), long-standing persistent AF (continuous AF >12 months in duration), or permanent AF (when no further attempts are made to achieve normal sinus rhythm). Affected individuals do not necessarily progress through these various forms of AF in a linear or predictable fashion. The pathophysiology of AF consists of mechanisms involved in the initiation of the arrhythmia, maintenance of the arrhythmia, and progression to long-lasting forms of AF.⁷ The progression of AF is associated with the concept of 'AF begets AF', in which changes in atrial structure and function promote and perpetuate atrial arrhythmias,⁸ and these changes are referred to as atrial arrhythmogenic remodelling.⁹

An understanding of the molecular basis for these AF-related changes and how they pertain to the initiation and progression of the disease is essential to the development of effective therapeutic strategies. A conceptual framework for a mechanistic understanding of

these mechanisms can be divided into three components.¹⁰ The first component is the basic arrhythmia mechanism responsible for the initiation and maintenance of AF. This refers to triggered (ectopic) and reentrant activities. AF initiation fundamentally requires a trigger in the form of either triggered (ectopic) activity or reentrant activity usually in the presence of a vulnerable substrate that may be anatomic or functional. An exception will be in lone AF in which AF occurs in the absence of structural heart disease. Both focal ectopic firing and reentrant mechanisms can maintain AF; however, the latter is the likely mechanism in the majority of cases. The second mechanistic component is atrial arrhythmogenic remodelling that promotes AF and comprises electrical, structural, and autonomic nervous system and Ca²⁺-handling changes observed in AF.⁹ The last component consists of the aetiological factors that predispose to AF. These include concurrent cardiovascular diseases such as heart failure, sinus node dysfunction (SND), valvular heart disease, hypertension, and ageing; genetic factors such as single gene mutations, gene polymorphisms, and gene variants; and other extrinsic factors such as sleep apnoea, obesity, and thyroid disease. Atrial remodelling can occur either as a consequence of these aetiological factors or as a direct consequence of AF itself.

The Ca²⁺- and calmodulin-dependent protein kinase II (CaMKII) is a multifunctional serine–threonine kinase that is now recognized as a critical Ca²⁺ and reactive oxygen species (ROS) sensor that mechanistically links several of the observed cellular perturbations and mechanisms in AF with proarrhythmic atrial remodelling.

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The goal of this review is to highlight new and emerging evidence highlighting the role of CaMKII in AF and how CaMKII participates in the complex interplay of the various mechanistic components of AF. We start with an overview of CaMKII and relevant downstream targets and then proceed to discuss known and potential effects of CaMKII in the spectrum of AF disease. We conclude with a discussion of potential therapeutic implications. Most of our knowledge regarding CaMKII comes from studies performed in ventricular cardiomyocytes. Although discussing a wide range of studies, we have tried to emphasize a growing body of work in humans and animals using atrial myocardium.

2. CaMKII—biology, activation, and downstream targets

2.1 Biology and structure

CaMKII is a multifunctional serine–threonine protein kinase that is abundantly expressed in various tissues, including the heart. There are four CaMKII gene products resulting in four homologous isoforms of CaMKII— α , β , δ , and γ , and these share activation mechanisms.^{11–14} These isoforms are thought to co-assemble as heteromultimeric holoenzymes and are known to have varied expression levels in different tissues¹² (Figure 1). For instance, CaMKII α and CaMKII β are preferentially enriched in neuronal tissues, whereas CaMKII δ and CaMKII γ are the predominant isoforms in the heart. There is a hypervariable region in CaMKII located between the association domain and the C-terminus of the regulatory domain that gives rise to various splice variants. There is a CaMKII δ splice variant with a nuclear localization signal (NLS; CaMKII δ_B) and another lacking an NLS sequence (CaMKII δ_C).¹⁵ The presence or absence of the NLS does not absolutely determine localization, perhaps because these isoforms co-assemble as heteromultimers and the ratio of the NLS-containing isoforms biases cellular targeting.¹⁵ Nevertheless, the specific role of these splice variants remains unclear, and both CaMKII δ_B and CaMKII δ_C are present in nuclear and cytosolic compartments.¹⁶

Structurally, CaMKII exists as a holoenzyme that consists of two stacked ring-shaped hexamers.¹⁴ Each monomer consists of an

N-terminal catalytic domain, a central regulatory domain, and a C-terminal association domain.¹⁷ The catalytic domain harbours the adenosine triphosphate (ATP) and substrate binding sites and is responsible for the catalytic activity of the protein. The regulatory domain contains an inhibitory pseudosubstrate sequence, several amino acid residues susceptible to post-translational modification, and the calmodulin (CaM) binding region. The association domain is responsible for binding with other subunits to form the holoenzyme (Figure 1).¹⁴

2.2 Activation

In the inactive state of CaMKII, the pseudosubstrate region of the regulatory domain inhibits the catalytic domain of each CaMKII subunit by sterically blocking the substrate and ATP-binding pocket.^{18,19} With increases in intracellular $[Ca^{2+}]$, Ca^{2+} binds to CaM and the calcium-bound CaM (Ca^{2+}/CaM) activates CaMKII by binding to the C-terminal region of the regulatory domain. This causes a conformational change with allosteric displacement of the pseudosubstrate region, allowing the substrate and ATP access to the catalytic domain.²⁰ Sustained increases in Ca^{2+}/CaM in the presence of ATP result in autophosphorylation at Thr 287 in the autoinhibitory region of the regulatory domain. Autophosphorylation promotes a conformational change that prevents the association of the catalytic and regulatory domains, resulting in sustained Ca^{2+} -independent activation.^{21–23}

Our group discovered that oxidation of a pair of methionine residues at positions 281/282 results in a similar form of Ca^{2+}/CaM -independent persistent activation compared with autophosphorylation.²⁴ Other modes of post-translational modification that result in autonomous CaMKII activity include O-linked glycosylation of serine at position 280 by O-linked N-acetylglucosamine²⁵ and NO-dependent nitrosylation of cysteine residues at putative positions 116, 273, or 290.²⁶ Interestingly, phosphorylation of Thr 306/307 causes CaMKII inactivation by decreasing the binding to Ca/CaM complexes.²⁷ Very recently, it was discovered that NO modification of Ser 273, a position outside of the autoinhibitory domain, inactivates CaMKII.²⁸ Thus, CaMKII may be activated or inactivated by post-translational modifications, but post-translational modifications to the CaMKII autoinhibitory domain

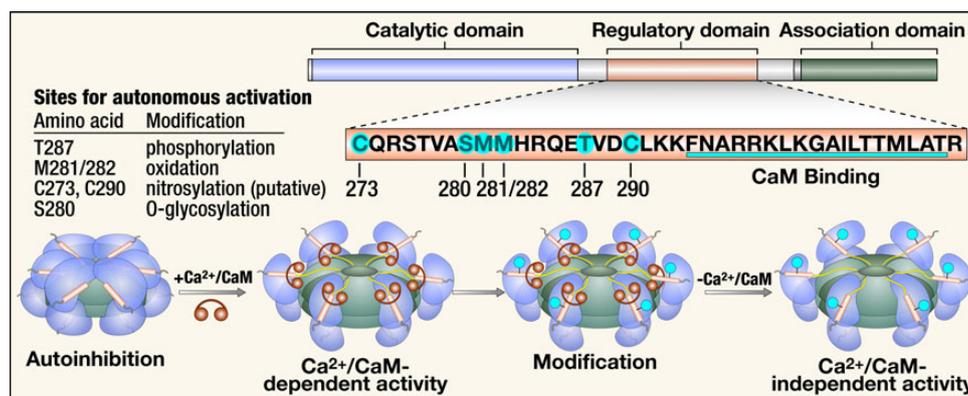


Figure 1 Structure, activation, and post-translational modifications of CaMKII. CaMKII monomers consist of a catalytic domain, a regulatory domain, and an association domain. The regulatory domain has an autoinhibitory binding region with several sites for post-translational modification and a CaM binding region. Co-assembly of monomers through the association domain forms the CaMKII holoenzyme. In the presence of Ca^{2+} , Ca^{2+}/CaM binds to the regulatory site, which causes a conformational change and activation of the autoinhibited inactive enzyme with exposure of the catalytic domain. Post-translational modification of the autoinhibitory region at any of the sites highlighted results in constitutive activity of CaMKII that is autonomous of Ca^{2+}/CaM . CaM, calmodulin.

provide a path for CaMKII to become persistently active under the influence of upstream signals that increase intracellular $[Ca^{2+}]$ and ROS.^{24,29}

The capacity of CaMKII to transduce the effects of a multitude of upstream messengers raises the question of the existence of a central mechanism that regulates the activation of CaMKII similar to the protein kinase A (PKA) signalling pathway. Such a central mechanism does not appear to exist; rather what is clear is that initiation of CaMKII activation requires Ca^{2+}/CaM binding, and sustained elevation in intracellular $[Ca^{2+}]$ can promote Ca^{2+}/CaM autonomous activity by 'autophosphorylation' at Thr 287. However, Ca^{2+}/CaM -independent activity can also follow increased ROS, even in the presence of physiological (diastolic) intracellular $[Ca^{2+}]$.³⁰ Additionally, similar to PKA, β -adrenergic stimulation can activate CaMKII through the cAMP-Epac pathway (discussed subsequently). Changes in global cytosolic $[Ca^{2+}]$, such as those occurring in excitation–contraction coupling (ECC) and local $[Ca^{2+}]$, for example, in the dyadic space can activate CaMKII. CaMKII achieves signal specificity through a variety of mechanisms, as discussed subsequently.

2.3 Localization and intracellular targeting

Cellular signalling systems generally have mechanisms that facilitate the interaction of effector molecules with their targets often through close proximity to achieve signal specificity and efficiency. CaMKII achieves subcellular targeting in a heterogeneous fashion such as compartmentalization, employing anchoring proteins and also identifying target protein motifs with homology to the CaMKII regulatory domain autoinhibitory region.^{11,31} CaMKII is enriched in specific subcellular domains such as those in the transverse tubules adjacent to L-type Ca^{2+} channels (LTCC) and ryanodine receptors.³² Earlier we briefly mentioned the existence of CaMKII δ splice variants—CaMKII δ_B and δ_C . It was initially thought that CaMKII δ_B preferentially localized to the nucleus and CaMKII δ_C to the cytosol; however, it has subsequently been shown that these isoforms are not exclusively limited to these cellular compartments.¹⁶ In fact, these and other isoforms of CaMKII co-assemble as heteromultimers, and the relative composition of the heteromultimer biases cellular targeting. The study of Mishra *et al.*¹⁶ showed that there is compartmentalized activation of CaMKII δ independent of the isoform with the location of activation determined by the stimulus and the Ca^{2+} release site. Phenylephrine preferentially activated both CaMKII δ isoforms in the nuclear compartment, whereas caffeine preferentially activated these isoforms in the sarcoplasmic reticulum (SR). Compartmentalization also had functional significance, with phenylephrine selectively phosphorylating nuclear transcriptional regulator HDAC5 at a specific CaMKII site (Ser 498) and caffeine selectively phosphorylating phospholamban (PLN) at the CaMKII-specific phosphorylation site (Thr 17).

Unlike other signalling molecules such as PKA that have extensively validated anchoring proteins that act as adaptors, there is less known about how CaMKII achieves target specificity. However, a few anchoring proteins have been described that regulate coupling of CaMKII to some target proteins, thus enhancing signalling specificity. The first of these was the *N*-methyl-D-aspartate subunit NR2B in the nervous system, which caused activation-dependent subcellular targeting through translocation.³³ Subsequently, other anchoring proteins have been described in the heart. Alpha-kinase anchoring protein is an example of a protein that anchors sarcoplasmic endoplasmic reticulum ATPase (SERCA2a) and CaMKII, and through this interaction modulates CaMKII-dependent PLN phosphorylation.³⁴ Another is β -arrestin

that interacts with CaMKII δ ³⁵ and functions as an anchoring protein that links β 1-adrenergic receptors to the cAMP-Epac-CaMKII signalling pathway.³⁶ It forms a multimeric complex with CaMKII and Epac in the cytoplasm, and this complex then translocates to the plasma membrane after β -adrenergic stimulation to bring CaMKII and Epac in close proximity with cAMP. A membrane-associated guanylate kinase protein, SAP97, has also been shown in rat atrial myocytes to anchor CaMKII to the C-terminus of $K_v4.3$ orchestrating CaMKII-mediated increases in the transient outward current (I_{to}).³⁷

CaMKII uses recognition and targeting of CaMKII-binding motifs in its interaction with LTCC by binding to a CaMKII binding site on the β_{2a} subunit of LTCC.³⁸ Similarly, CaMKII uses this mechanism to bind to β_{IV} -spectrin in targeting voltage-gated Na^+ channels.³⁹ Taken together, CaMKII employs different mechanisms as described to achieve target specificity and efficiency in its role as a master regulator of several cellular processes.

2.4 Downstream targets

CaMKII acts on diverse downstream targets in cardiomyocytes by lowering the free energy required to phosphorylate a target serine or threonine residue. Phosphorylation can lead to conformational changes that affect protein function. A full discussion of CaMKII target proteins is beyond the scope of this review, but CaMKII targets related to myocardial ECC may be particularly important for understanding the role of CaMKII in AF. CaMKII appears to affect myocardial cell membrane excitability through its action on most voltage-gated ion channels such as LTCCs,^{40–42} K^+ channels,^{37,43,44} and Na^+ channels.^{45–47} Other CaMKII targets include Ca^{2+} cycling proteins such as the type 2 ryanodine receptor (RyR2)⁴⁸ and phospholamban (PLN).⁴⁹ The net effect of CaMKII phosphorylation on these targets, in the context of pathological stress, is to promote a proarrhythmic circumstance by favouring cell membrane hyperexcitability and afterdepolarizations. Collectively, early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs) are thought to be triggers for initiating arrhythmias, including AF (see the section on Mechanisms and experimental models of AF).

2.4.1 L-type Ca^{2+} channels

The LTCC is the major portal for extracellular Ca^{2+} to enter the cytoplasm.⁵⁰ CaMKII-catalysed phosphorylation of LTCCs produces high-activity mode-2 gating, resulting in increased open probability of I_{CaL} channels.³⁸ This increases the influx of Ca^{2+} into atrial myocytes. CaMKII also results in ' Ca^{2+} -dependent I_{CaL} -facilitation'⁴⁰ in which there is increased peak I_{CaL} and slowed I_{CaL} inactivation,⁵¹ a signature LTCC response to repeated depolarizing pulses.¹¹ Mode-2 gating, and I_{Ca} facilitation are thought to be proarrhythmic by predisposing to action potential (AP) prolongation, EADs, SR Ca^{2+} -leak, inward Na^+/Ca^{2+} exchanger (NCX) current, and DADs.^{32,38,52,53}

2.4.2 Voltage-gated Na^+ channels

The α isoform of the cardiac sodium (Na) channel ($Na_v1.5$) is phosphorylated by CaMKII.⁴⁵ This leads to Na^+ gating modification with increased steady-state inactivation of the Na^{2+} channel with high heart rates. CaMKII-dependent phosphorylation of $Na_v1.5$ also slows Na^{2+} current (I_{Na}) inactivation and augments the non-inactivating 'late' component of I_{Na} .^{39,54} Sustained late I_{Na} increases subsarcolemmal $[Na^+]$ and indirectly increases intracellular $[Ca^{2+}]$ by prolonging AP duration (APD) and by reducing the efficacy of the NCX to extrude Ca^{2+} from the cytoplasm to the extracellular space.⁵⁵ These effects contribute to EADs and DADs. CaMKII inhibition by autamide-2-related inhibitory

peptide in murine and human atrial myocytes or genetic knockout of CaMKII δ c (CaMKII δ c-knockout mice) prevents late I_{Na} -dependent SR Ca^{2+} -leak.⁵⁶

2.4.3 Voltage-gated K^+ channels

Voltage-gated K^+ (I_K) currents are primarily responsible for membrane repolarization. I_K is a composite of the effect of several ion channels. Here, we discuss the I_K channels that are best understood to be directly affected by CaMKII. The transient outward K^+ current (I_{to}) is responsible for early repolarization and termination of the plateau phase of the AP, and CaMKII activates I_{to} in atrial myocytes.^{43,57} This results in slowing of I_{to} inactivation and acceleration of its recovery, with the overall effect of shortening the AP and decreasing the refractory period. The $K_V4.3$ pore-forming subunit of I_{to} is regulated by CaMKII-dependent phosphorylation through the accessory protein SAP97, and this also results in increased I_{to} .³⁷ $K_V1.4$ is postulated to have a CaMKII phosphorylation target motif.⁵⁸ The inward rectifier current (I_{K1}) is another component of I_K that is activated by CaMKII.⁴⁶ This is the primary cardiac inward rectifier current, and the protein Kir2.1 subunit is the essential pore-forming unit for this channel. I_{K1} is important for maintaining the resting cell membrane potential and sculpting terminal phase 3 repolarization. I_{K1} is increased in chronic AF,^{59,60} and this helps sustain high-frequency reentrant sources called rotors that are characteristic of reentrant arrhythmias.⁶¹ I_{K1} and the ultrarapid delayed rectifier K^+ current (I_{KUR}) both appear to be augmented by CaMKII,^{43,57} which offsets the APD-prolonging effects of CaMKII-dependent I_{CaL} and I_{Na} phosphorylation. Contrary to earlier studies, a study of atrial myocytes from human subjects in SR and AF showed that CaMKII inhibition with KN-93 had no effect on I_{K1} current in sinus rhythm or chronic AF patients, whereas protein kinase C (PKC) decreased I_{K1} current in chronic AF.⁶² Further, PKC-dependent phosphorylation also increased constitutive acetylcholine-sensitive K^+ current ($I_{K,ACh}$) activity in chronic AF.⁶² Thus, complex actions at multiple voltage-gated ion channels can contribute to arrhythmias by promoting EADs and DADs (i.e. arrhythmia triggers) and by augmenting dispersion of repolarization that may be a substrate for reentry.

2.4.4 Type 2 ryanodine receptor

The RyR2 is an intracellular Ca^{2+} channel that controls release of myofilament activating Ca^{2+} from the SR lumen to the cytoplasm. This Ca^{2+} initiates the mechanical phase of ECC, but can also influence Ca^{2+} -sensitive cell membrane conductances (i.e. by actions on ion channels, exchangers, and transporters), transcription, and Ca^{2+} -regulated mitochondrial processes. A mostly LTCC-mediated trigger of intracellular Ca^{2+} is the fundamental signal for activating RyR2, but its opening probability is biased by phosphorylation. CaMKII-dependent hyperphosphorylation of Ser 2814 on RyR2 increases open probability, augmenting dyssynchronous SR Ca^{2+} release⁴⁸ and proarrhythmic DADs,⁶³ which are mostly due to inward NCX current (I_{NCX}). There is increased NCX expression in persistent AF, which magnifies the size of DAD-generating inward I_{NCX} for any given amount of aberrant Ca^{2+} release. RyR2 dysfunction that destabilizes the closed state increases AF susceptibility in genetic mouse models.^{64–66} Inhibition of CaMKII-dependent RyR2 phosphorylation reduces the incidence of rapid pacing-induced AF in mice.^{66,67}

2.4.5 Phospholamban

Phospholamban (PLN) is a negative regulator of the SERCA2a.^{68,69} Phosphorylation of PLN by PKA (at Ser 16) or CaMKII (Thr 17) promotes

PLN aggregation and disengagement from SERCA2a and SERCA2a disinhibition, resulting in increased SR Ca^{2+} -reuptake from the cytoplasm.^{70,71}

Activation of these target proteins, by CaMKII-catalysed phosphorylation, can explain the acute proarrhythmic role of CaMKII in AF and other arrhythmias, by enhancing the probability of afterdepolarizations and reentry.^{72,73} Chronic CaMKII activation favours cardiomyocyte death and fibrosis, myocardial hypertrophy, and extracellular matrix remodelling—processes that contribute to proarrhythmic remodelling of the tissue substrate. In the case of AF, tissue substrate remodelling is an important factor in the transition of AF from sporadic or paroxysmal forms to chronic and permanent forms.^{72,74}

Regulation of phosphorylation, and thus activity of the various Ca^{2+} -handling proteins discussed earlier, does not depend solely on the actions of CaMKII. Other protein kinases such as PKA and PKC also play significant roles in the regulation of these proteins. For the purposes of this review, we briefly highlight the PKA signalling pathway and how this interacts with CaMKII signalling in the context of AF. Both PKA and CaMKII act at different sites on these proteins with similar consequences in most, but not all, cases. Some of the known PKA phosphorylation sites for these proteins include Ser 1700 for LTCC, Ser 2030 and Ser 2808 for RyR2, and Ser 16 for PLN. Classic PKA signalling involves cyclic adenosine monophosphate (cAMP) activation of PKA in response to β -adrenergic signalling. PKA activation under physiological conditions occurs through β -adrenergic signalling and causes intracellular influx of Ca^{2+} and increased SR Ca^{2+} uptake and storage that culminates in increased systolic Ca^{2+} transients.⁷⁵ CaMKII activation occurs either directly through increased intracellular Ca^{2+} (as discussed earlier) or alternatively through a cAMP-dependent mechanism. The latter occurs via activation of adenylyl cyclases with subsequent increase in cytosolic cAMP that then binds and activates a guanine nucleotide exchange protein activated by cAMP (Epac). Epac modulates cardiac Ca^{2+} -handling in various ways such as increasing the frequency of Ca^{2+} oscillations and inducing SR Ca^{2+} release independent of PKA through effectors such as phospholipase C and CaMKII.^{76–78} PKC also may affect CaMKII through this mechanism.⁷⁸ Epac increases CaMKII-mediated diastolic Ca^{2+} -leak through RyR2 activation.⁷⁷ This is considered the cAMP- and Epac-induced SR Ca^{2+} -leak pathway. RyR2 PKA hyperphosphorylation is present in atrial tissue from a canine model of atrial pacing-induced AF and in atrial tissue from humans with AF when compared with sinus rhythm,⁷⁹ and this results in increased open probability of the channel.⁸⁰

β -adrenergic stimulation also induces PKA-dependent and PKA-independent CaMKII-mediated diastolic SR Ca^{2+} -leak.^{80,81} Late I_{Na} also increases diastolic SR Ca^{2+} -leak in atrial myocytes/myocardium from mice and humans with permanent AF by CaMKII- and PKA-dependent phosphorylation of PLN (Thr 17 and Ser 16, respectively) and RyR2 (Ser 2814 and Ser 2808, respectively).⁵⁶ Both kinases appeared to be necessary for late I_{Na} -dependent SR Ca^{2+} -leak induction.

It is clear that several signalling pathways including cAMP/PKA, cAMP/Epac, and direct CaMKII activation are involved in AF; however, the relative contribution of PKA and CaMKII and the relevant signalling pathways to the initiation, maintenance, or progression of AF are unclear. Previous studies suggest differential contribution of both kinases. For instance, PKA activity significantly activated I_{Ca} and SR Ca^{2+} uptake with modest effects on RyR2.^{82,83} CaMKII, in contrast, had more robust RyR2 effects with modest I_{Ca} activation and SR Ca^{2+} uptake.⁸⁴ Historically, there has been some controversy on the effects of PKA and CaMKII on RyR2, with CaMKII considered to be downstream of PKA.

However, more recent studies demonstrate that these kinases act independently but synergistically on β -adrenergic stimulation.^{56,81,85} There is ample evidence of crosstalk between PKA and CaMKII-dependent signalling at different levels. With regard to Ca^{2+} -signalling, CaMKII affects cAMP levels in a negative-feedback loop fashion through its action on phosphodiesterase 4D, thus indirectly affecting PKA activity.⁸⁶ PKA also facilitates CaMKII-dependent SR Ca^{2+} -leak induction by maintaining SR Ca^{2+} -load.⁵⁶

3. CaMKII in AF

CaMKII appears to be a myocardial stress response modulator because its activity and expression are increased in diverse forms of structural heart disease. Furthermore, CaMKII activity seems to augment classical fight or flight heart rate responses.⁸⁷ Tables 1 and 2 show a summary of various studies evaluating CaMKII protein expression and activity in animal models (Table 1) and human tissue (Table 2) with atrial tachyarrhythmias and AF.

4. Mechanisms and experimental models of AF

AF and atrial tachycardia-related remodelling are the consequences of a complex interplay of pathophysiological mechanisms. An understanding of the fundamental processes that constitute the molecular basis of AF is necessary to highlight the particular steps now thought to be affected by CaMKII. The primary mechanisms of AF are focal ectopic or triggered activity and reentrant activity.⁹⁷ Triggered activity can occur after full repolarization of the AP and these are called DADs.

Alternatively, EADs are triggered activities that occur during phase 2 or 3 of the AP. Ca^{2+} overload with associated shortening of the APD favours generation of DADs and these are rate-dependent, whereas EADs are favoured by APD prolongation, persistent inward $I_{\text{Ca,L}}$,³² I_{Na} ,³⁹ and spontaneous SR Ca^{2+} release.^{98–100} EADs and DADs can also be driven by adrenergic stimulation, at least in part because of their action to activate CaMKII and its downstream targets. Reentrant activity occurs in the presence of suitable substrate, which can be either anatomic (e.g. fibrosis) or functional (e.g. electrical refractoriness) that allows for the perpetuation of an excitable circuit. The substrate can be the consequence of AF-related atrial remodelling or the result of other conditions such as genetic mutations, ageing, structural heart disease, or other systemic disease.⁷ Reentrant activity is the primary mode for AF maintenance. Persistent AF is likely maintained by the combination of complex multiple circuit reentry and ectopic activity.¹⁰¹

Importantly, as discussed in the previous section, excessive CaMKII activation has been implicated in AF triggering and reentry mechanisms through its downstream effects on ion channels and proarrhythmic remodelling that results from cell death, fibrosis, and hypertrophy.

Various experimental paradigms have been developed to study the mechanisms of AF.⁹⁷ Clinical AF is a heterogeneous mix of phenotypes. AF often exists in association with structural heart disease such as congestive heart failure, valvular disease, or hypertension; however, there are times that AF occurs in the absence of any detectable structural heart disease ('lone AF'). Also there are different clinical forms of AF: paroxysmal, persistent, and permanent AF. The dominant paradigm for the natural progression of the disease is from paroxysmal to permanent AF. The rapid atrial pacing (RAP) model^{8,102} of experimental AF aims to mimic 'lone AF'. Of note, earlier studies used a single atrial burst

Table 1 CaMKII in AF: summary of animal studies

Author	Year	Species	Arrhythmia model	CaMKII	Effect
Greiser <i>et al.</i> ⁸⁸	2009	Goat	Rapid atrial pacing	CaMKII-dependent hyperphosphorylation of RyR2	Atrial hypocontractility Decreased SR Ca^{2+} load
Chelu <i>et al.</i> ⁸⁹	2009	Mouse	Genetic mouse model (CREM-Ib Δ C-X transgenic mice)	Increased expression and autophosphorylation of CaMKII δ	Hyperphosphorylation of RyR2, arrhythmia suppression by CaMKII inhibition.
Chelu <i>et al.</i> ⁸⁹	2009	Goat	Rapid atrial pacing	Increased expression and autophosphorylation of CaMKII δ	Hyperphosphorylation of RyR2
Wakili <i>et al.</i> ⁹⁰	2010	Canine	Rapid atrial pacing	Increased expression and autophosphorylation of CaMKII δ	Atrial hypocontractility Decreased APD Decreased Ca^{2+} transient
Purohit <i>et al.</i> ⁶⁶	2013	Mouse	Angiotensin-II	Increased ox-CaMKII	Increased AF susceptibility dependent on ox-CaMKII δ and RyR2 S2814 phosphorylation, arrhythmia suppression by CaMKII inhibition
Greiser <i>et al.</i> ⁹¹	2014	Rabbit	Atrial myocytes	No change in CaMKII-dependent RyR2 phosphorylation or CaMKII activity	Ca^{2+} signalling silencing with increased intracellular Ca^{2+} and no changes in Ca^{2+} sparks, Ca^{2+} waves, Ca^{2+} release, and Ca^{2+} release instability
Fischer <i>et al.</i> ⁵⁶	2015	Mouse	Atrial cardiomyocytes treated with ATX-II	Increased p-CaMKII	Enhanced late I_{Na} Increased SR Ca^{2+} -leak Hyperphosphorylation of CaMKII target sites: RyR2 (Ser2815) and PLN (Thr17)
Lenski <i>et al.</i> ⁹²	2015	NVRM	Electrical stimulation	Increased p-CaMKII/CaMKII	Increased p-AMPK/AMPK Increased FA uptake Decreased glucose uptake

AP, action potential; AMPK, AMP-activated protein kinase; ATX-II, Anemonia-sulcata toxin-II; FA, fatty acid; NVRM, neonatal ventricular rat myocytes; ox-CaMKII, oxidized CaMKII; p-CaMKII, phosphorylated CaMKII; p-AMPK, phosphorylated AMPK; PLN, phospholamban; SR, sarcoplasmic reticulum; RyR2, ryanodine type 2 receptor.

Table 2 CaMKII in AF: summary of human studies

Author	Year	Species	Type of AF	Tissue	CaMKII	Effect
Tessier <i>et al.</i> ⁴³	1999	Human	Chronic AF	RA	Increased CaMKII δ expression	CaMKII slowed I_{to} inactivation; CaMKII inhibition hastened I_{to} inactivation
Christ <i>et al.</i> ⁹³	2004	Human	Chronic AF	RAA	Increased protein expression of the catalytic subunit of protein phosphatase 2A and phosphatase activity	Decreased basal I_{CaL} density in chronic AF
Chelu <i>et al.</i> ⁸⁹	2009	Human	Chronic AF	RAA	Increased CaMKII δ expression and autophosphorylation	Hyperphosphorylation of RyR2
Neef <i>et al.</i> ⁹⁴	2010	Human	Unclassified	RA	Increased CaMKII expression	Increased SR Ca^{2+} -leak and diastolic Ca Increased RyR2 phosphorylation, reversal of SR Ca^{2+} -leak with CaMKII inhibition
Voigt <i>et al.</i> ⁹⁵	2012	Human	Chronic AF	RAA	Increased autophosphorylation of CaMKII	Hyperphosphorylation of RyR2 Increased SR Ca^{2+} -leak, diastolic Ca^{2+} , frequency of spontaneous Ca^{2+} release events Reversal of SR Ca^{2+} -leak and afterdepolarizations with CaMKII inhibition
Purohit <i>et al.</i> ⁶⁶	2013	Human	Mixed (paroxysmal, persistent/permanent, and unclassified)	RA	Increased ox-CaMKII	AF patients had more ox-CaMKII compared with patients in sinus rhythm
Greiser <i>et al.</i> ⁹¹	2014	Human	Persistent AF	RA	No change in CaMKII-dependent RyR2 phosphorylation or CaMKII activity	Ca^{2+} signalling silencing with increased intracellular Ca^{2+} and no changes in Ca^{2+} sparks, Ca^{2+} waves, Ca^{2+} release, and Ca^{2+} release instability
Christ <i>et al.</i> ⁹⁶	2014	Human	Chronic AF	RAA	Reduced CaMKII-dependent phosphorylation of PLN but not RyR2	Blunting of agonist-evoked arrhythmias in atrial myocytes from patients with AF compared with those in sinus rhythm
Lenski <i>et al.</i> ⁹²	2015	Human	Permanent AF	LAA	Increased p-CaMKII/CaMKII	Increased FA uptake Decreased glucose uptake

I_{to} , transient outward K^+ current; RA, right atrium; RAA, right atrial appendage; LAA, left atrial appendage; FA, fatty acid; ox-CaMKII, oxidized CaMKII; p-CaMKII, phosphorylated CaMKII; SR, sarcoplasmic reticulum; PLN, phospholamban; RyR2, ryanodine type 2 receptor.

pacemaker,¹⁰³ and these were associated with high ventricular rates that likely caused mild tachycardia-mediated cardiomyopathy that potentially complicated the observations in these studies. More recent studies combine RAP with complete atrioventricular block^{104,105} to avoid or minimize this effect. Both models are relevant to human disease as the former simulates AF with no rate control and the latter AF with adequate rate control.⁹⁷ The RAP model is also of clinical significance as a model for studying the transition from paroxysmal to chronic AF. Another AF experimental model to study the relationship between heart failure and AF exists, in which rapid ventricular pacing induces heart failure.¹⁰⁴ There are differences in the electrical and structural remodelling observed in RAP model when compared with the heart failure model. Other forms of experimental AF in animals include AF combined with structural heart disease,¹⁰⁶ atrial dilatation without heart failure,¹⁰⁷ and sterile pericarditis to mimic post-operative AF.¹⁰⁸ Increasingly, transgenic mice are being used as AF experimental models.^{66,89,109,110} The main advantage of mouse models is their genetic tractability and ability to generate mutant genes to mimic human diseases and to dissect candidate AF molecular mechanisms. For the purposes of this review, we will focus primarily on the RAP model in 'large' animals, as a model to study AF-related atrial remodelling and AF progression, and on various genetically modified mouse models with the power to dissect defined molecular components of AF pathways.

The transition from paroxysmal to the more chronic forms of AF is poorly understood, and the role that CaMKII plays in this process is an

area of on-going interest in the field. The most established role for CaMKII in AF *in vivo* (animal) systems relates to mechanisms of arrhythmia triggering, by contributing to RyR2 hyperphosphorylation and Ca^{2+} -leak, which triggers afterdepolarizations.^{56,67,89,111} Importantly, this mechanism pertains to fibrillating human atrium.^{94,95} Although triggering may be important for paroxysmal or more permanent forms of AF,⁹⁵ it appears to be required for paroxysmal AF in the absence of structural heart disease (i.e. lone AF). This is consistent with reports in which CaMKII contributes to triggering in pulmonary vein cardiomyocytes.¹¹² Taken together, these data align with the idea that CaMKII contributes to early stage AF. However, CaMKII could also contribute to the transition from paroxysmal to more permanent forms of AF by several mechanisms: loss of normal intracellular [Ca^{2+}] homeostasis, increased cardiomyocyte death and fibrosis, matrix remodelling by elaboration of matrix metalloproteinase-9 (MMP-9), and inflammatory gene transcription. Although intriguing and of potential clinical relevance, the role of CaMKII in late stage AF pathophysiology is less completely understood.

5. Components of atrial remodelling in AF

Atrial remodelling is characterized by several atrial structural and functional alterations that occur either as a result of AF or underlying structural heart disease that predates or coexists with AF. These changes

can be classified as electrical remodelling, tissue (structural) remodelling, Ca^{2+} -handling abnormalities, and neurohormonal.⁹ Although tissue, Ca^{2+} , and electrical remodelling are complex and involve diverse signalling pathways, CaMKII has emerged as a nodal signal with the potential to orchestrate many of these changes. Furthermore, CaMKII activity is activated downstream to neurohumoral agonists, suggesting that CaMKII is a transduction signal linking neurohumoral signalling and ROS with proarrhythmic mechanisms in AF. Here, we will focus on the components that are known or postulated to be directly modulated by CaMKII and also discuss several CaMKII-dependent molecular changes that occur in AF.

6. Electrical remodelling

Electrical remodelling entails changes in the electrophysiological properties of the atria. This is a composite of the alterations that occur in ion channels, pumps, and exchangers in atrial cells.

APD shortening and enhanced variability of APD are central features of AF-related electrical remodelling and are accompanied by loss of rate adaptation of the atrial effective refractory period (AERP), which facilitates the maintenance of reentrant circuits in both animal AF models and human AF.^{8,113–115} Reduced AP duration is a result of reduced $I_{\text{Ca,L}}$.^{113,116,117} The reduced $I_{\text{Ca,L}}$ measured in atrial myocytes isolated from chronic AF patients may indicate an adaptive response to arrhythmia-induced intracellular Ca^{2+} overload.¹¹⁷ The reduction in $I_{\text{Ca,L}}$ is due to decreased mRNA and protein expression of the α subunit of $I_{\text{Ca,L}}$ ($\text{Ca}_v1.2$) in AF through the negative feedback action of Ca^{2+} -dependent phosphatase calcineurin/nuclear factor of activated T-cells (NFAT).¹¹⁸ Translocation of NFAT into the nucleus results in transcriptional downregulation of the $\text{Ca}_v1.2$ gene with consequent AP duration shortening.¹¹⁸ Although most of the available data show increased mRNA and protein levels of $\text{Ca}_v1.2$, others have found no alteration in protein levels in AF.^{93,119} An intriguing finding in one of these studies was that CaMKII inhibition with KN-93 resulted in about a 40% reduction in basal $I_{\text{Ca,L}}$ in patients with sinus rhythm, but had no effect on basal $I_{\text{Ca,L}}$ in patients with chronic AF.⁹³ This was associated with increased phosphatase activity (especially protein phosphatase 2A) in chronic AF, suggesting that an imbalance in the kinase-phosphatase activity in favour of increased phosphatase activity is responsible for the reduced basal $I_{\text{Ca,L}}$ in patients with chronic AF. The local CaMKII activity was not measured, but hypothetically there could be a local reduction in CaMKII activity due to several factors, such as cytoskeletal protein abnormalities due to AF that affect CaMKII activity, medications, or differences in experimental models.^{93,119} Also, increased activation of the Ca^{2+} -dependent protease calpain promotes breakdown of $I_{\text{Ca,L}}$ channels.¹²⁰ Taken together, this suggests that the net effect on $I_{\text{Ca,L}}$ may depend more on post-translational modification of the channel by phosphorylation rather than by expression.

A recent study by Greiser *et al.*⁹¹ challenges the current paradigm in the field that atrial remodelling results from dysfunctional Ca^{2+} handling that results in increased triggered activity and the role that CaMKII plays in tachycardia-mediated atrial remodelling. They observed a paradoxical suppression of central Ca^{2+} release with no changes in Ca^{2+} sparks, Ca^{2+} waves, SR Ca^{2+} release, and Ca^{2+} release instability in atrial myocytes from rabbits exposed to RAP for 5 days and in atrial myocytes from chronic AF patients. This was termed 'Ca²⁺ signalling silencing'. It was attributed to increased Ca^{2+} buffering capacity of the atrial myocytes through binding to myofilaments and reduction in RyR2 expression with smaller RyR2 clusters in the SR. Additionally,

there was hyperphosphorylation of RyR2 at the PKA phosphorylation site (Ser 2808), but reduced CaMKII-dependent RyR2 phosphorylation (Ser 2815) with no changes in CaMKII and PLN activity. It should be noted that the animal model used was a short-lived duration (5 days) of atrial pacing, with no evidence of AF in the rabbits. Further studies are needed to determine whether Ca^{2+} signalling silencing is a transient response to tachycardia or a more sustained response to chronic rapid activation with potential relevance to the pathophysiology of AF. Another group has reported a marked reduction in agonist-induced arrhythmias in the atria of patients with AF when compared with those in sinus rhythm with a corresponding reduction in maximal $I_{\text{Ca,L}}$ responses.⁹⁶ The agonists used were norepinephrine, epinephrine, serotonin, and forskolin (a cAMP activator). CaMKII inhibition with KN-93 reduced norepinephrine-evoked $I_{\text{Ca,L}}$ responses in the atria from myocytes from patients with sinus rhythm but not in atrial myocytes from patients with AF. Also, adrenergic stimulation evoked reduced CaMKII-dependent phosphorylation of PLN (Thr 17), but no changes in PKA-dependent PLN phosphorylation (Ser 16) and no changes in RyR2 phosphorylation either by CaMKII or by PKA. Taken together, this was suggestive of loss of CaMKII-dependent phosphorylation of LTCC and PLN in response to adrenergic stimulation in chronic AF. It is not clear to us how to reconcile these results from Christ with the report by Voigt *et al.*,⁹⁵ showing that CaMKII is critical for agonist-evoked RyR2 Ca^{2+} -leak and arrhythmia triggering. These studies highlight the complexity of the mechanisms involved in AF and more specifically raise a question about the role of CaMKII in chronic AF. Further investigations to definitively understand these issues better are needed.

Decreased atrial conduction velocity may contribute to reentry and maintenance of AF. This occurs via a reduction in I_{Na} or direct inhibition of gap-junction channels in atrial cardiomyocytes.¹²¹ In murine experiments, decreased I_{Na} was shown to be due to an acute Ca^{2+} -dependent inhibition of I_{Na} and reduced $\text{Na}_v1.5$ subunit expression under chronic conditions.¹²² This Ca^{2+} -dependent reduction in I_{Na} promotes the stability of AF primarily by a reentrant mechanism, but may also reduce ectopic activity.¹²³ Oxidized CaMKII (ox-CaMKII) slows conduction in the infarct border zone of ventricular myocardium,¹²⁴ but it is currently unknown whether CaMKII modulates atrial conduction velocity. However, it has been suggested that CaMKII-dependent phosphorylation could also reduce I_{Na} , especially at fast heart rates as seen in AF.⁴⁵

7. Structural remodelling

The hallmark of structural atrial remodelling is atrial fibrosis.^{104,125} It is a common final pathway for AF promoting conditions and a negative predictor of treatment outcomes.^{9,126} Atrial fibrosis results from interaction between atrial cardiomyocytes and fibroblasts. Ca^{2+} influx into atrial fibroblasts induces proliferation and differentiation into collagen-secreting myofibroblasts, resulting in increased fibrosis and consequently heterogeneous conduction slowing and reentry.¹²⁷ These changes are brought about by influx of Ca^{2+} into atrial fibroblasts via multiple ion channels. In human atrial fibroblasts, transient-receptor potential (TRP) channels are major participants in this process, especially TRP melastatin-related-7 (TRPM7) and canonical-3 (TRPC3) channels.^{128,129} CaMKII putatively can act both upstream and downstream of these TRP channels.¹³⁰ AF patients have larger TRPM7 currents in their atrial fibroblasts, and knockdown of TRPM7 expression reduces basal differentiation of fibroblasts from chronic AF patients.¹²⁸ It is unknown whether CaMKII interacts with

TRPM7 in atrial myocytes, although CaMKII was shown to inhibit TRPM7-like channels in hepatocytes.¹³¹ Influx of Ca^{2+} through TRPC3 channel promotes CaMKII activation and NADPH-oxidase-mediated production of ROS in a genetic mouse model of dilated cardiomyopathy.¹³²

CaMKII is involved in profibrotic signalling in the myocardium. Aldosterone stimulation leads to increased ox-CaMKII and activation of myocyte enhancer factor 2 that causes increased myocardial MMP-9 synthesis.²⁹ Angiotensin II increases ox-CaMKII that causes sinoatrial cell death, SND, and atrial fibrosis, whereas CaMKII inhibition is protective against death and fibrosis in sinoatrial cells.¹³³ Further investigation is needed to determine whether CaMKII contributes to atrial fibrosis in AF by these or other pathways, but CaMKII inhibition does reduce fibrosis in ventricular myocardium.²⁹

Excessive CaMKII activity triggers cell death, and this results in reparative fibrosis and adverse remodelling in ventricular myocytes in a pressure overload model of heart failure,¹³⁴ ventricular myocytes, and sinoatrial cells following Ang II infusion.^{24,133} CaMKII inhibition is also protective against beta-adrenergic-receptor agonist-induced apoptosis, and this anti-apoptotic mechanism involves PLN regulation of SR Ca^{2+} content^{135,136} and mitochondria.¹³⁷ Mitochondrial-targeted CaMKII inhibition prevents myocyte death following myocardial infarction and catecholamine infusion¹³⁷ (discussed subsequently). These various CaMKII-mediated mechanisms of cell death have not been directly tested in AF.

There is an established link between inflammation and AF,^{138,139} and several inflammatory markers such as cytokines, C-reactive protein, and complementary factors are elevated in AF. Inflammation leads to structural remodelling, primarily in the form of fibrosis. We previously showed that CaMKII induces local inflammatory response through the nuclear factor- κB pathway in cardiomyocytes when exposed to bacterial endotoxin or myocardial infarction and CaMKII inhibition suppressed this response.¹⁴⁰ CaMKII is also oxidatively activated during myocardial infarction or by endotoxin as part of a toll-like receptor/myeloid differentiation protein 88 pathway.¹⁴¹ All these findings suggest that CaMKII may play an important role in proarrhythmic atrial remodelling favouring AF.

Atrial enlargement is another component of structural remodelling. Mice with transgenic overexpression of the transcriptional repressor CREM-Ib $\Delta\text{C-X}$ in cardiomyocytes (CREM mice) develop age-dependent progression from spontaneous atrial ectopy to paroxysmal and long-lasting AF episodes.¹⁴² Ca^{2+} -handling abnormalities and atrial enlargement precede the spontaneous development of AF. Genetic inhibition of CaMKII-dependent RyR2 phosphorylation, by knockin replacement of a validated CaMKII-targeted phosphorylation site (RyR2-Ser 2814), in CREM mice prevents Ca^{2+} -handling abnormalities and spontaneous AF, atrial dilatation, and conduction abnormalities.¹⁴² Thus CaMKII-dependent RyR2 hyperphosphorylation leads to both triggered activity and progressive development of an AF substrate, promoting atrial hypertrophy and dilatation, and AF progression. These mechanistic insights suggest that targeted treatment of CaMKII or SR Ca^{2+} -leak via RyR2 has the potential to slow or arrest the progression of AF.

8. Role of CaMKII in ROS/redox sensing and signalling

ROS are involved in modulating the action of various cellular targets with functional consequences under physiological and pathological conditions in the cardiovascular system. AF is associated with increased oxidative stress, and this has been linked to AF-related atrial

remodelling.^{143–145} There is increasing interests in the role of ROS and CaMKII in cardiac disease, with significant progress made in recent years in our understanding of links between ROS and CaMKII in the pathophysiological mechanisms of AF.¹⁴⁶

ROS directly activates CaMKII by oxidation with subsequent persistent constitutive CaMKII activity, which is proarrhythmic in its effect on downstream targets.^{24,66,147} The acute proarrhythmic actions of ox-CaMKII are due to increased EADs and DADs^{54,148} and reentry.¹²⁴

NADPH oxidases (Nox) are important sources of ROS in AF.¹⁴⁹ Increased Nox4 expression in cardiomyocytes is associated with mitochondrial damage and apoptosis.¹⁵⁰ In a zebrafish model, overexpression of Nox is associated with an arrhythmogenic phenotype, and this effect is mediated by ROS-induced activation of CaMKII.¹⁵¹ Mice that lack functional Nox ($p47^{-/-}$ mice) are resistant to angiotensin II-dependent increases in ROS and oxidation of CaMKII and are also resistant to angiotensin-II-mediated AF.⁶⁶ Inhibition of Nox4 by apocynin attenuates electrical remodelling and AF inducibility in a rabbit model of RAP.¹⁵² As the duration of AF increases, sources of intracellular ROS shift from Nox to other cellular sources, including mitochondrial oxidases (xanthine oxidase), monoamine oxidase, and uncoupled eNOS.¹⁵³

In a computational model,¹⁵⁴ the arrhythmogenic pattern of EADs observed during oxidative stress was dependent on ox-CaMKII activation and pacing-cycle length. Oxidative stress is also associated with reduced AERP.¹⁴³

Despite the link between oxidative stress and AF, attempts at utilizing antioxidants to prevent AF and AF-related atrial remodelling have been largely disappointing.¹⁵⁵ One idea is that general antioxidants lack potency, pharmacokinetic attributes, or access to key subcellular domains necessary to exert beneficial actions. The shift from Nox as a source of cellular ROS to other cellular sources may explain why statins, which decrease ROS production by Nox via inhibition of Rac1, are only effective in acute AF models such as post-operative AF but ineffective in ameliorating ROS production in chronic AF models such as permanent AF.¹⁵³ There was a recent comprehensive review of this topic.¹⁵³ The finding that genetically modified mice with ROS-resistant CaMKII were protected from AF triggered by rapid pacing and primed by angiotensin II infusion⁶⁶ suggests that CaMKII could be part of a concise ROS-responsive molecular pathway and a candidate therapeutic antioxidant target for AF.

9. CaMKII and mitochondria, energy expenditure, and its implications for AF mechanism

Mitochondria are essential for ATP production, the primary form of myocardial cellular energy, and they are the primary sources of ROS. They are an integral component of intracellular Ca^{2+} and ROS signalling. In cardiomyocytes, excessive mitochondrial Ca^{2+} and ROS cause opening of the mitochondrial permeability transition pore (mPTP), which leads to dissipation of the mitochondrial inner membrane potential and decreased ATP synthesis and this results in myocardial cell death via apoptotic mechanisms.^{156–158} CaMKII through its dual action of increasing ROS production and intracellular Ca^{2+} via SR Ca^{2+} -leak results in mPTP opening.¹⁵⁹ We previously showed that CaMKII is present in the mitochondria and that cardiomyocytes from transgenic mice with mitochondrial-targeted CaMKII inhibitory protein¹³⁷ had a blunted mPTP response to Ca^{2+} . These mice were protected from programmed cell death with exposure to different myocardial

stressors such as ischaemia/reperfusion, myocardial infarction, and adrenergic stimulation.¹³⁷ At this time, the constituents of mPTP are unknown and this pathway has not been directly tested in AF models. CaMKII also increases mitochondrial Ca^{2+} by phosphorylating the mitochondrial Ca^{2+} uniporter,¹³⁷ although this is controversial.¹⁶⁰ This Ca^{2+} -sensitive channel is a major Ca^{2+} entry portal through the mitochondrial inner membrane into the mitochondria.^{161–163} Thus, mitochondrial CaMKII may contribute to a feed-forward mechanism by which Ca^{2+} overload triggers mPTP opening and increases ROS production, thereby causing more CaMKII activation and activity.

Increased oxidative stress and increased ox-CaMKII have been demonstrated in the atria of AF patients,^{66,145,164} and this was associated with mitochondrial DNA damage and reduction in ATP synthesis in these patients.¹⁶⁴ Additionally, mitochondria undergo structural and morphological changes such as swelling and disturbance of cristae structure during RAP and AF.^{164–166} Functionally, impaired oxidative phosphorylation and respiration are observed in atrial tissue in response to RAP.^{166,167} These changes were Ca^{2+} -dependent and were prevented by blocking LTCCs.

Ca^{2+} thus functions as a second messenger that links AF to structural and functional mitochondrial changes. Also, CaMKII plays an important role in the pathological mitochondrial changes that result in mitochondrial dysfunction and myocardial cell death in AF and likely contributes to atrial remodelling. The potential role of mitochondrial CaMKII represents an interesting and potentially important frontier in CaMKII research in AF.

10. CaMKII and atrial metabolic remodelling in AF

There is interplay between CaMKII and AMP-activated protein kinase (AMPK). AMPK is a serine/threonine kinase that regulates cellular energy metabolism and protein synthesis.¹⁶⁸ CaMKII, Ca^{2+} /calmodulin-dependent protein kinase kinase (CaMKKII), or Ca^{2+} alone can modulate the activity of AMPK in its role as a cellular energy sensor.^{169,170} The net effect of AMPK activation is enhancing energy-generating pathways and reducing energy demand processes.^{168,171,172} Lenski *et al.*⁹² recently demonstrated both *in vitro* and *in vivo* that arrhythmia induces metabolic changes in atrial myocardium characterized by lipid accumulation and reduced glucose uptake. They postulated that activation of AMPK causes these changes and CaMKII can activate AMPK. Inhibition of CaMKII and AMPK independently prevented these changes *in vitro*. This pathway involves elevated fatty acid translocase (FAT/CD36) membrane expression with resultant increased fatty acid uptake and lipid accumulation. Also, there is decreased membrane expression of synaptosomal-associated protein 23 in the plasma membrane, which results in reduced AMPK-induced glucose transporter subtype 4 membrane translocation and glucose uptake.⁹² These changes correlated with activation of proapoptotic pathways and atrial remodelling.

AMPK activation in AF was recently shown in canine and human atrial myocytes to be protective against AF-induced metabolic stress.¹⁷³ High atrial rate and associated metabolic stress resulted in decreased $I_{\text{Ca,L}}$, intracellular Ca^{2+} transients, and cell shortening. The increased energy demand caused AMPK phosphorylation and subsequent regulation of Ca^{2+} handling through activation of and interaction with intracellular targets such as $\text{Ca}_v1.2$ and NCX, to counteract the effects of the metabolic stress. There was no evidence of interaction with other Ca^{2+} -handling proteins such as RyR2, PLN, and SERCA2a. AMPK

phosphorylation was increased in canine left atrial tissue after 1 week of tachypacing model of AF. Similarly, fractional AMPK phosphorylation was increased in human right atrial appendage tissue from subjects with paroxysmal AF compared with SR; however, there was a significant decrease in chronic AF subjects. This finding is interesting as it may be an indication that the AMPK system protects against paroxysmal or short bouts of AF, but does not necessarily play a role in persistent AF. It also buttresses the idea that there are likely different mechanisms underlying the different clinical forms of AF.

11. Atrial mechanical dysfunction

Atrial mechanical dysfunction, which manifests as atrial hypocontractility, is another feature of AF-related atrial remodelling and is a major cause of stroke and thromboembolism associated with AF.^{174,175} It coexists with on-going AF and can persist for variable periods after conversion to sinus rhythm in patients with long-standing AF.¹⁷⁶ The latter is known as 'atrial stunning', and the degree of hypocontractility correlates with the duration of AF.¹⁷⁶ In a canine atrial tachycardia model, there was reduced fractional cell shortening in atrial myocytes, and this was explained, in part, by abnormal intracellular Ca^{2+} handling and decreased systolic Ca^{2+} transients.^{175,177} In humans with prolonged AF, atrial contractility is reduced by as much as 75%.¹⁷⁷ Others have shown that AP duration shortening could be responsible for the observed atrial hypocontractility.¹⁷⁸ Additionally, in a canine model of tachypacing, there is altered myofilament function due to abnormal phosphorylation of myosin and myosin-binding protein C (MyBP-C).⁹⁰ In this study, there was increased autophosphorylation and expression of CaMKII δ and protein phosphatase I activity with downregulation of PKA. However, a direct link between CaMKII and altered myofilament function was not shown. Several studies show that MyBP-C is phosphorylated by CaMKII, which leads to hypocontractility and impaired relaxation in heart failure.^{179–181} Acute β -adrenergic stimulation leads to CaMKII phosphorylation of MyBP-C,¹⁷⁹ but the effect of chronic β -adrenergic stimulation on this and the functional significance is unclear.¹⁸² Titin, which is a large myofilament protein, contributes to myocardial diastolic stiffness, and its mechanical properties are changed in heart failure.^{183–185} Specific CaMKII phosphorylation sites were recently identified on titin.¹⁸⁶ CaMKII-dependent titin phosphorylation resulted in decreased myocardial passive force and altered diastolic stress in murine and human failing hearts,¹⁸⁶ potentially linking diastolic dysfunction and AF via CaMKII. Thus, CaMKII acts through diverse mechanisms with the potential to reduce mechanical function. However, more definitive studies and more thorough identification of key CaMKII target sites will be necessary to test these concepts definitively.

12. AF and heart failure

AF commonly coexists with structural heart disease such as heart failure in patients.^{187,188} Atrial CaMKII activity is increased in a ventricular tachypacing-induced canine model of heart failure¹⁸⁹ and in goats with atrial dilatation.⁸⁸ In heart failure, there is chronic activation of neurohormonal agonist signalling, which begins as an adaptive response but subsequently becomes pathological and contributes to disease progression. This chronic pathological response results from increased intracellular Ca^{2+} and ROS.¹¹ The agonists involved in this pathway are β -adrenergic receptors agonists, angiotensin II, and aldosterone, and all activate CaMKII.^{24,29,190} Indeed, CaMKII transcription, protein expression, and activity are increased in the failing heart.^{29,191–193} In addition to the neurohormonal system activation, other mechanisms

by which CaMKII is increased in heart failure include increased calcineurin activity.^{194,195} The mechanism of increased CaMKII activity in heart failure appears to hinge on autophosphorylation of Thr 287 and/or oxidation of methionines 281 and 282.¹¹ We found that ox-CaMKII was increased after myocardial infarction and was further enhanced by angiotensin II,²⁴ aldosterone,²⁹ or hyperglycaemia.¹⁹⁶ Pathological β -adrenergic receptor stimulation in heart failure could activate CaMKII by cAMP-EPAC-dependent and cAMP-independent mechanisms to hyperphosphorylate RyR, causing SR Ca^{2+} -leak and arrhythmias.^{77,78,85} As described earlier, the downstream effects of CaMKII activation likely result in AF-related atrial remodelling changes that promote AF initiation and progression. This may explain, in part, the association between heart failure and AF.

13. SND and AF

AF frequently coexists with SND.¹⁹⁷ In the MOST trial, among patients with SND who required pacemakers, AF was the most frequent arrhythmia. About 45–50% of the subjects had a history of AF at the time of pacemaker implantation.¹⁹⁸ SND is associated with changes in the sinoatrial pacemaker complex that are similar to AF-related atrial remodelling.^{199,200} AF has been shown to cause SND²⁰¹ and conversely, SND predisposes to AF.^{199,202,203} The mechanisms for these observations are due to atrial remodelling—structural remodelling especially fibrosis,²⁰⁴ electrical remodelling, and neurohormonal stimulation, as described earlier in this review.

CaMKII through its effect on intracellular Ca^{2+} dynamics plays a key role in pacemaker activity.²⁰⁵ In this study, inhibition of CaMKII either by autacamide-2-inhibitory peptide (AIP), a specific peptide inhibitor, or by KN-93 resulted in decreased I_{CaL} amplitude and complete arrest of sinoatrial cells. Work from our laboratory indicated that CaMKII contributes approximately half of the dynamic range of fight or flight heart rate increases by actions in sinoatrial cells³² and that angiotensin II infusion in mice caused increased atrial and sinoatrial node ox-CaMKII. Ox-CaMKII triggered a critical threshold of sinoatrial cell apoptosis, atrial fibrosis, and conduction slowing that contributed to SND.¹³³ Targeted CaMKII inhibition in the heart or sinoatrial node of mice protected against the deleterious effects of angiotensin II: sinoatrial cell apoptosis, fibrosis, and SND. We also found elevated levels of ox-CaMKII in the right atria from human patients with SND¹³³ and AF when compared with those in sinus rhythm and also in the atria of Ang-II-infused mice.⁶⁶ These mice had increased ROS, SR Ca^{2+} sparks, DADs, and heightened AF susceptibility compared with saline-infused mice. Mice with genetically engineered resistance to CaMKII oxidation and mice lacking functional NADPH oxidase ($p47^{-/-}$) were resistant to angiotensin-II-mediated AF. These data show that ox-CaMKII is involved in the pathophysiology of both AF and SND and may be important in the clinical coexistence and synergy between both diseases.

14. Genetics and CaMKII in AF

It is recognized that there is a genetic contribution to AF, and certain familial and inheritable forms of AF have been described. Additionally, common genetic variants and single nucleotide polymorphisms have been identified in relation to AF. This was recently comprehensively reviewed.²⁰⁶ Although CaMKII has been implicated in some inherited arrhythmias such as catecholaminergic polymorphic ventricular tachycardia (CPVT), long QT type 3, ankyrin-B syndrome (long QT type 4),^{207,208} and Timothy syndrome (long QT type 8),²⁰⁹ we are

unaware of a specific CaMKII genetic mutation or variation that has been identified in relation to AF. A loss-of-function mutation in ANK2 (a gene that encodes for ankyrin-B) was noted in some patients with early onset AF.²¹⁰ In this study, mice with ankyrin-B deficiency manifested electrophysiological properties consistent with human AF, and DeGrande *et al.*²⁰⁷ showed that CaMKII inhibition rescued the proarrhythmic features of ankyrin-B deficiency in models of human ankyrin-B syndrome. Also, gain-of-function mutations in RyR2 have been shown to predispose to CPVT and AF.^{65,89,211,212}

15. Potential as a therapeutic target in AF

Current treatment strategies for the management of AF include anti-arrhythmic drugs, ablation, and surgical procedures. Although useful, these oftentimes have suboptimal efficacy, and the anti-arrhythmic drugs have potential adverse effects and toxicities, including life-threatening proarrhythmia.²¹³ The central role of Ca^{2+} -dysregulation and CaMKII in the pathophysiology of AF makes CaMKII an attractive therapeutic target.

15.1 Pharmacological approaches to modulation of CaMKII activity

Several CaMKII-specific inhibitors exist, but none possesses drug-like properties necessary for therapeutic purposes. This topic was recently reviewed in detail.²¹⁴ Here we discuss the use of relevant CaMKII inhibitors and highlight their use in relation to AF. KN-93 is a relatively specific CaMKII inhibitor²¹⁵ that is widely employed for experimental purposes²¹⁴ and is the best CaMKII inhibitor available. It competitively and selectively binds to the CaM-binding site of CaMKII, allosterically blocking activation. It does not compete with ATP and so does not have an effect on the activity of autophosphorylated CaMKII. Thus, once CaMKII is activated and autophosphorylated, KN-93 is ineffective in CaMKII inhibition, irrespective of the mode of activation. It selectively inhibits CaMKII relative to other kinases such as PKA and PKC, but has been shown to also effectively inhibit CaMKI and CaMKIV,^{216,217} and a few other kinase targets.²¹⁸ A drawback to the use of KN-93 is its off-target effects. It affects LTCC^{52,219} and several voltage-gated potassium channels.²²⁰ Unfortunately, these off-target actions occur at concentrations necessary for CaMKII inhibition (e.g. IC₅₀ in myocardial lysates of ~ 2.5 mM)⁵² and are not shared by the control drug, KN-92. Thus, it is our view that it is best to use a variety of complementary approaches to CaMKII inhibition, such as gene deletion and inhibitory peptides, in order to be confident that experimental outcomes are not due to off-target actions. This is particularly true for studies of arrhythmias, in which ion channel antagonist actions of KN-93 have the potential to achieve anti-arrhythmic actions. However, these compounds have been useful and remain a key tool in our understanding of CaMKII signalling. KN-93 has been shown in both *in vitro* and *in vivo* mouse models to prevent pacing-induced AF.⁸⁹ It also decreases SR Ca^{2+} -leak in atrial myocytes from AF patients.^{94,95} KN-62 is another CaMKII inhibitor that is structurally similar to KN-93 and has the same mechanism of action, but has largely been replaced by KN-93.

CaMKII inhibitory peptides, which mimic the autoinhibitory region of the CaMKII regulatory domain, include AIP and autacamide-3 inhibitor (AC3-I). These are highly selective inhibitors of CaMKII relative to PKA, PKC, and CaMKIV^{221,222} and have been used experimentally to study CaMKII function in cardiovascular physiology and disease. *In vivo* murine

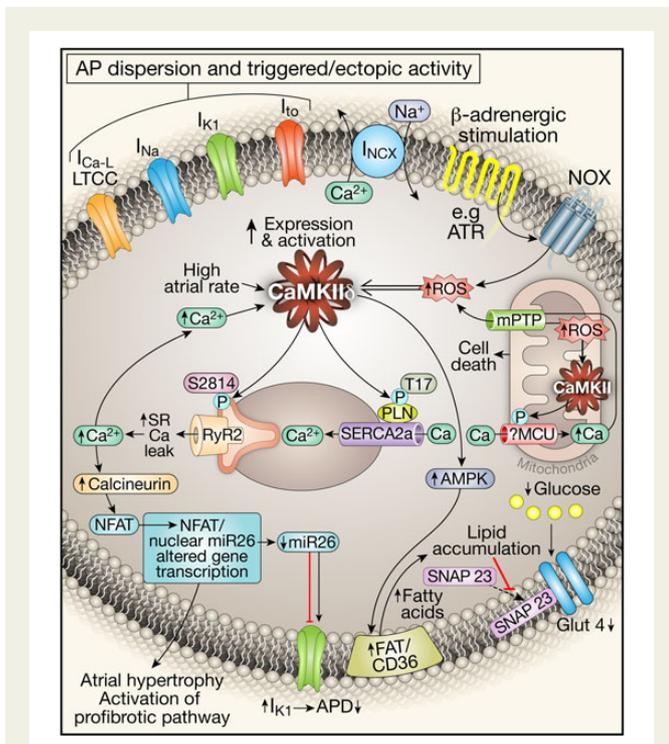


Figure 2 CaMKII-mediated intracellular mechanisms of AF-related atrial remodelling. Stress signals result in increased intracellular Ca^{2+} and ROS that lead to increased CaMKII expression and activity. CaMKII has diverse downstream targets through which it promotes arrhythmogenesis. Its cumulative effect on ionic channels (I_{Ca-L} , I_{Na} , I_{K1} , I_{to} , and I_{NCX}) is inhomogeneous AP propagation and repolarization dispersion that leads to triggered activity. It also results in increased SR Ca^{2+} , further increasing intracellular $[\text{Ca}^{2+}]$. This acts via the calcineurin/NFAT pathway to activate profibrotic pathways and induce atrial hypertrophy. Increased intracellular $[\text{Ca}^{2+}]$ also leads to increased mitochondrial $[\text{Ca}^{2+}]$, which through its action on mPTP opening causes increased ROS and CaMKII activation in the mitochondria that can cause cell death. CaMKII also acts through AMPK activation to increase lipid accumulation and to decrease glucose uptake. β -adrenergic stimulation acts through angiotensin receptors to activate NADPH oxidase (NOX) to increase intracellular ROS and to activate CaMKII via oxidation.

studies have used internalization sequences or transgenic expression. Mice with transgenic myocardial expression of AC3-I were protected from pacing-induced AF, following angiotensin II infusion.⁶⁶ There is the potential for off-target effects and altered selectivity with modification to these peptides either from fusion with green fluorescent protein, myristoylation or from changes to internalization sequences to enhance specific attributes of the peptide, such as increased expression, metabolic stability, or cell permeation.^{87,214,223} AC3-I has the potential to inhibit protein kinase D,²²³ but this potential has not been validated in other studies.²²⁴

CaMKIIN is a small protein endogenous to neurons but not to myocardium that inhibits CaMKII with high specificity and potency. We have used CaMKIIN as an experimental tool that can be delivered to specific target sites through local application of adenoviral constructs, targeted transgenic expression to specific tissues, and/or intracellular compartments.^{87,133,137} Sinoatrial node-targeted CaMKII inhibition by local application of adenoviral construct of CaMKIIN was protective against

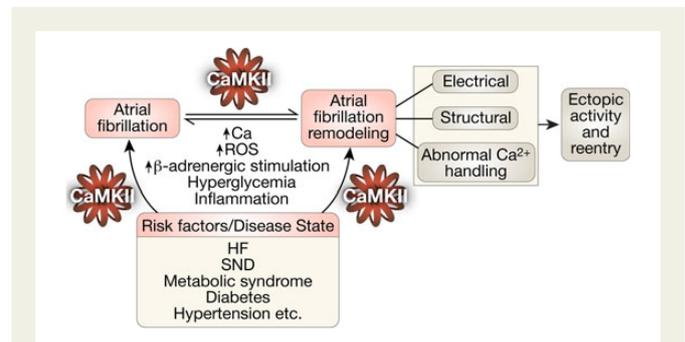


Figure 3 CaMKII transduces proarrhythmic signalling in atrial remodelling and AF in a feed-forward manner. Increased intracellular Ca^{2+} and ROS induce autonomous CaMKII activity. Excessive CaMKII activity through its action on multiple downstream targets (ion channels, proteins, etc.) results in triggered and reentrant activities that can initiate and perpetuate AF. It also results in several changes that are associated with atrial remodelling in AF. Underlying disease states or risk factors also cause excessive CaMKII activity with similar proarrhythmic downstream consequences. HF, heart failure; SND, sinus node dysfunction.

Ang-II-induced SND in mice.¹³³ There are no studies of these peptides in AF models to our knowledge.

The CaMKII inhibitors described earlier have all been in the context of experimental models, but highlight the potential for CaMKII-targeted therapeutics. Although there are currently no CaMKII inhibitors available clinically, several potential small molecule inhibitors for cardiovascular application are in early stages of development.

15.2 Potential therapeutic application of CaMKII inhibition

CaMKII inhibition or elimination of CaMKII-dependent RyR2 phosphorylation has been shown to protect from AF in mouse models and is also beneficial in atrial cardiomyocytes of chronic AF patients.^{66,67,95} Furthermore, CaMKII is a truly nodal signal, so that CaMKII inhibition has the potential to counter AF by actions relevant to its progression from paroxysmal to permanent. However, given the ubiquitous nature of CaMKII and its multifunctional role in several physiological processes, it will be important to prove that systemic CaMKII inhibition does not contribute to undesirable side effects such as reduced fertility and impaired memory.^{225,226} Despite these potential issues, CaMKII is dispensable, as demonstrated by the viability of CaMKII knockout mice, and is likely present in heart to maximize heart rate and mechanical performance as part of the fight or flight response. Arguably, these attributes are not of central importance to most AF patients. A clinically useful CaMKII inhibitor likely should not cross the blood–brain barrier and should possess a sufficiently wide therapeutic window to avoid potential toxicities. Allosteric or isoform-specific CaMKII inhibitors could also enhance selectivity and reduce the potential for off-target effects.²²⁷

MicroRNAs have emerged as another potential therapeutic tool in the management of human disease.²²⁸ Some microRNAs have been identified that are involved in the regulation of CaMKII δ expression such as microRNA-145²²⁹ and microRNA-30b-5p,²³⁰ whereas several others have been identified in the transcriptional changes that occur in AF.²³¹ Targeting these microRNAs in the heart may emerge as a unique tool managing arrhythmias and other cardiac diseases. The

pathophysiological mechanisms and importance of CaMKII may vary based on experimental AF models and in different forms of clinical AF, and the effect of CaMKII inhibition in these various conditions is unclear. Pacing-induced AF in the setting of angiotensin II infusion⁶⁶ may mirror post-operative AF because of the shared circumstance of neurohumoral activation and elevated ROS. Based on the link between CaMKII activation and tissue remodelling, it is possible that a CaMKII inhibitor drug could reduce progression of AF from paroxysmal to chronic or permanent forms.

Given the landscape of potential therapeutic targets, our view is that CaMKII inhibition is a promising approach.

16. Conclusion

AF and atrial remodelling in the context of AF occur through a complex interplay of several molecular and cellular mechanisms, and these are implicated in the mechanisms for the initiation, maintenance, and progression of AF. Available evidence shows that there is excessive CaMKII activity in the setting of AF. CaMKII by its actions as a Ca²⁺ and ROS sensor modulates several of the pathways involved in AF and atrial remodelling to promote a proarrhythmic milieu in the atrium (Figures 2 and 3). Animal studies show that inhibition of CaMKII is protective against AF. This suggests that CaMKII inhibition is a potential therapeutic target for the management of AF. Further studies are however needed to understand the mechanistic role of CaMKII in atrial remodelling and AF.

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Conflict of interest: M.E.A. is a named inventor on intellectual property claiming to treat arrhythmias by CaMKII inhibition and is a co-founder of Allosteros Therapeutics, a company aiming to develop CaMKII inhibitor therapeutics. O.O.M. reports no conflict of interest.

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