Journal of Molecular and Cellular Cardiology 58 (2013) 182–187

Contents lists available at SciVerse ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Review article

The Ca-calmodulin dependent kinase II: A promising target for future antiarrhythmic therapies?

Thomas H. Fischer, Stefan Neef, Lars S. Maier *

Abt. Kardiologie und Pneumologie/Herzzentrum, Deutsches Zentrum für Herzkreislaufforschung, Georg-August-Universität, Göttingen, Germany

ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form 2 November 2012
Accepted 5 November 2012
Available online 13 November 2012

Keywords:
Calcium cycling
Ion channels
Arrhythmias
CaMKII
Phosphorylation
Afterdepolarizations

ABSTRACT

Treating arrhythmias is a challenge for clinicians because pharmacological therapies are often ineffective or have severe side effects. Patients with heart failure frequently present with supreventricular and ventricular arrhythmias. New antiarrhythmic therapies are needed that modulate the specific pathomechanisms underlying the development of cardiac arrhythmias and may have a better safety-profile. The Ca-calmodulin dependent kinase II (CaMKII) seems to be involved in the development of heart failure and arrhythmias and may therefore be a promising target for the development of antiarrhythmic therapies. The current review aims at discussing some novel as well as known cytosolic and sarcolemmal mechanisms involved in CaMKII-dependent arrhythmias without being able to cover all aspects known in the field. This article is part of a Special Issue entitled "Calcium Signaling in Heart".

Contents

1. Introduction

The treatment of cardiac arrhythmias remains a major challenge for clinicians due to their high prevalence and the limitations of currently available pharmacological therapies. Arrhythmias can be subdivided into supra-ventricular and ventricular arrhythmias, both of which potentially have severe consequences. Atrial fibrillation (AF), for example, is the most common sustained arrhythmia and confers a 5-fold risk of stroke [1]. As pharmacological therapies often are ineffective in preventing AF recurrence or even exert severe side effects (*e.g.* amiodarone or flecainide), invasive procedures like pulmonary vein isolations are frequently needed, bringing about further risks for patients. Additionally, the bulk of patients with AF has to be treated with anticoagulants and is thus subjected to a higher risk of hemorrhage. Better medical control of this supra-ventricular arrhythmia would be of great benefit. Furthermore, heart failure is associated with a strongly increased risk of sudden cardiac death. In fact, it is well known that approximately 40% of heart failure patients die due to malignant ventricular arrhythmias [2]. The over-all prevalence of heart failure is estimated to be around 2% [3] and will further increase due to demographic changes. There are estimated survival rates of only 50% for

* Corresponding author at: Abt. Kardiologie und Pneumologie/Herzzentrum, Deutsches Zentrum für Herzkreislaufforschung, Georg-August-Universität Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany. Tel.: +49 551 39 6372; fax: +49 551 39 14131.
E-mail address: lmaier@med.uni-goettingen.de (L.S. Maier).

0022-2828
http://dx.doi.org/10.1016/j.yjmcc.2012.11.003

five years and 10% for ten years after diagnosis [4–6]. The tight association between heart failure and arrhythmias confronts clinicians with a demanding task because many inotropic agents used in the setting of decompensated acute heart failure were shown to increase the likelihood of sudden death [7] and current antiarrhythmic drugs acting on sarcolemmal ion channels often confer proarrhythmic side effects on functionally impaired myocardium [8]. Thus, new antiarrhythmic therapies are needed that can directly modulate the specific pathomechanisms underlying the development of cardiac arrhythmias in the respective clinical context (*e.g.* atrial fibrillation/atrial remodeling or heart failure) and hence harbor a more effective and safer action-profile. Huge efforts have been made in the last decade to further elucidate this issue. Increasing evidence indicates that the Ca-calmodulin dependent kinase II (CaMKII) could be a promising new target for such future antiarrhythmic therapies [9].

1.1. The physiological role of CaMKII in Ca cycling

CaMKII, a serine/threonine protein kinase, is a key regulator of Ca cycling in cardiomyocytes. It has been known for a long time that CaMKII modulates Ca influx, SR Ca release and SR Ca reuptake during excitation–contraction coupling. These effects are mainly exerted by phosphorylation of several key Ca handling proteins. CaMKII was shown to functionally modulate ryanodine receptor type 2 (RyR2) function in the sarcoplasmatic reticulum (SR) [10]. CaMKII-dependent RyR2 phosphorylation (at Ser2815) results in a pronounced RyR2-activation [11]. Another target of CaMKII is phospholamban (PLN, at Thr17). PLN is a physiological inhibitor of the SR Ca ATPase 2a (SERCA2a) and one of the major mediators of the cardiac contractility response upon β-adrenergic stimulation [12]. The inhibition of SERCA2a by PLN is dependent on the phosphorylation status of PLN and is most pronounced when PLN is dephosphorylated. Phosphorylation of PLN by CaMKII activates SERCA2a leading to enhanced SR Ca-reuptake. Furthermore, CaMKII has been shown to regulate sarcolemmal L-type Ca-currents [13] as well as Na-currents [14].

Physiologic regulatory roles of CaMKII have been demonstrated as to the adaptation of Ca cycling parameters to cellular demands in the context of the force-frequency-relationship [15] and the fight or flight response [16]. Also, CaMKII has been shown to be centrally involved in cardiac recovery from acidosis [17,18].

There is however convincing evidence that chronic overactivation of CaMKII is associated with deterioration of myocardial function and is also causally linked to cardiac arrhythmias in different cardiac pathologies. In fact, transgenic overexpression of the CaMKII isoform δ_c induces severe hypertrophy and heart failure [19] and CaMKIIδ_c overexpressing mice are more prone to arrhythmias [20] (see Fig. 1). The fact that CaMKII was shown to be activated by autophosphorylation at Thr287 [21] as well as oxidation at Met281/282 [22,23] enables a disproportionate CaMKII activation to set in during conditions of diastolic Ca overload and shortage of energy supply. Interestingly, CaMKII was found to be upregulated in heart failure [24] as well as in atrial fibrillation [25,26]. Up to date, several ion channels have been identified as CaMKII targets and may possibly contribute to the initiation of arrhythmias by triggering early and delayed afterdepolarizations.

1.2. The ryanodine receptor type 2

Several groups have demonstrated the pathologically relevant impact of CaMKII on RyR2 function in animal models as well as in human disease [19,20,27–30]. Hyperphosphorylation of RyR2 by CaMKII at Ser2815 leads to an impaired diastolic RyR2 closure, resulting in an increased frequency of short and tightly localized SR Ca release events during diastole (Ca sparks) [19,27] (see Fig. 2). One Ca spark is generated from a limited number of clustered RyR2s that form a functional SR Ca release unit. The spontaneous opening of one receptor can consecutively trigger the activation of neighboring RyR2s of the same Ca release unit in the dyadic cleft *via* Ca induced Ca release (CICR) [31]. This mechanism on the one hand can contribute to a depletion of SR Ca storage, which leads to impaired contractility [19,27] and also constitutes an arrhythmic trigger on the other hand. Ca ions, once released, are partly removed from the cytosol *via* the Na/Ca exchanger (NCX) that eliminates one Ca ion in exchange for three Na ions and thus evokes a transient inward current (I_{Ti}). In this manner, the elimination of leaking Ca from the SR can lead to a depolarization of the cell membrane that possibly reaches the threshold potential and triggers an action potential causing delayed afterdepolarizations [32]. It could be shown in atrial cardiomyocytes from guinea pigs that cellular Ca overload induces delayed afterdepolarizations and sustained triggered activity [33] in an NCX-dependent manner, thus confirming the hypothesis of a Ca induced transient inward current *via* NCX. This mechanism is aggravated in heart failure and atrial fibrillation by the fact that NCX is higher expressed in these pathologies [24,34,35]. Furthermore, transgenic mice overexpressing CaMKII showed an increased diastolic SR Ca leak triggering a larger number of delayed afterdepolarizations through I_{Ti} and spontaneous action potentials compared to wild type mice [20].

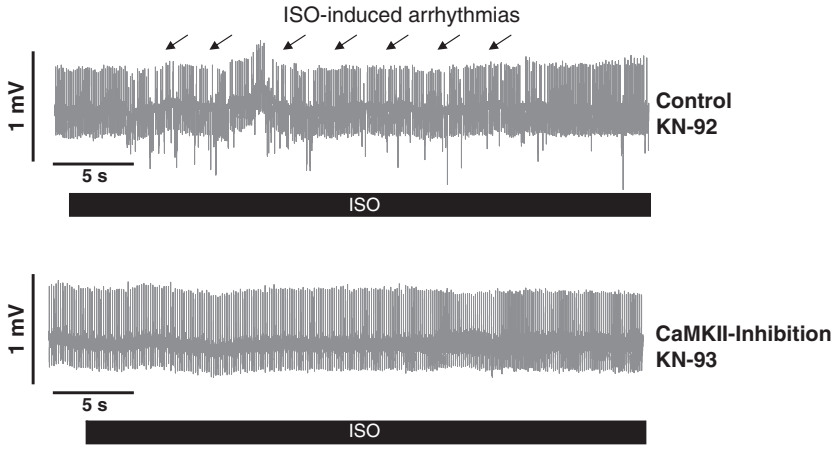

Fig. 1. Arrhythmogenic effects of CaMKII *in vivo*: ECG recordings from CaMKIIδc-TG mice showing arrhythmias after isoproterenol stress (2 mg/kg body weight), which could be inhibited by inhibiting CaMKII with KN-93.
Modified from Sag et al., Circ Heart Fail, 2009.

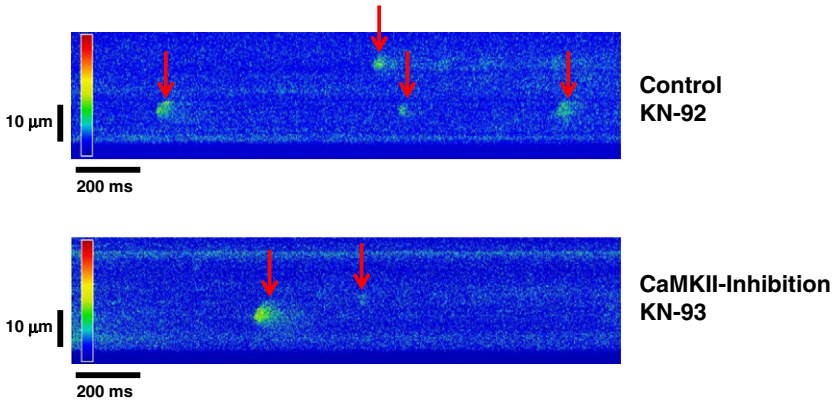

Fig. 2. CaMKII-dependent arrhythmogenic mechanisms: SR Ca leak: SR Ca leak in atrial myocytes from AF patients, as measured by Ca sparks (red arrows) showing enhanced SR Ca leak under control conditions (KN-92) and reduction of SR Ca leak upon CaMKII inhibition (KN-93).
Modified from Neef et al., Circ Res, 2010.

Conclusively, CaMKII inhibition could reduce arrhythmic events in these mice *in vitro* as well as *in vivo*. Additionally, knock-in mice with a point mutation mimicking constitutive RyR2 phosphorylation at the CaMKII dependent site Ser2814 (equals Ser2815 in human) showed higher diastolic spark frequencies and an increased susceptibility to pacing-induced AF [26]. Also, mice known to have catecholaminergic polymorphic ventricular arrhythmias due to the RyR2 point mutation R4496C show strongly increased mortality when crossbred with CaMKII transgenic mice [36]. The observed clinical signs of heart failure are most likely due to severely altered intracellular Ca handling and arrhythmogenic events *in vitro* and *in vivo*. In rabbit cardiomyocytes isolated from healthy or failing hearts respectively, spontaneously occurring Ca waves could be suppressed by CaMKII inhibition. CaMKII inhibition also effectively antagonized the arrhythmic effects of isoproterenol treatment [37]. Furthermore, it has recently been shown that digitalis induced arrhythmias are at least in part CaMKII mediated [38]. Treatment of rat cardiomyocytes with ouabain increased CaMKII autophosphorylation and provoked spontaneous electric activity and Ca waves. These arrhythmic events could, in turn, be reduced by CaMKII inhibition.

Similar results could be obtained from human cardiac tissue. We have recently shown that CaMKII activity is increased in human AF compared to sinus rhythm, associated with increased RyR phosphorylation at the CaMKII site [25]. In line with this, we observed an increased SR Ca leak in AF, which was due to CaMKII mediated effects as it could be completely normalized by CaMKII inhibition. Additionally, CaMKII inhibition could effectively normalize elevated diastolic Ca levels in AF, which consecutively reduced the transient inward current *via* NCX and suggests that the CaMKII-dependent SR Ca leak could play an important role in the initiation and maintenance of human AF. These data were recently confirmed by a study of another group, which also demonstrated increased CaMKII-dependent SR Ca leak in AF and verified increased open probability of RyR2 in AF by single channel recordings [26]. This important role of CaMKII mediated SR Ca leak is further corroborated by the finding that mice with mutated RyR2 that cannot be phosphorylated by CaMKII (S2814A mutation) are protected from AF [39,40].

1.3. L-type Ca channels

L-type Ca channels (LTCC) are voltage dependent Ca channels located in the plasma membrane that open up rapidly during systole upon the onset of an action potential. The LTCC protein complex includes a pore forming α-subunit ($Ca_v1.2$) and accessory β-subunits [41]. The Ca ions entering the cell *via* LTCC (I_{Ca}) then bind to RyR2 in the membrane of the SR and initiate Ca induced Ca release (CICR). LTCCs and clusters of RyR2 are physiologically situated in close proximity due to invaginations of the plasma-membrane (T-tubuli) forming the dyadic cleft. These subcellular ultrastructures facilitate CICR but have been found to be impaired in heart failure [42]. As CaMKII is thought to be attached to the RyR2-LTCC microdomain, it is not surprising that it functionally modulates not only RyR2 but also LTCC. It was shown that CaMKII can phosphorylate the β_{2a}-subunit of the LTCC at Thr498 [43] and promotes the entry of the LTCC into a highly active gating mode (mode 2) [13]. This mode is characterized by frequent and long openings of the LTCC and most likely underlies I_{Ca} facilitation, which is a positive feedback mechanism augmenting L-type Ca currents and slowing down I_{Ca} inactivation in response to increased intracellular Ca concentrations. Models that prevent mode 2 gating also interrupted I_{Ca} facilitation [35].

Mode 2 gating altogether leads to an increase in Ca influx *via* the LTCC and is known to promote early afterdepolarizations under certain conditions [44,45]. These are membrane potential oscillations taking place during the plateau or late repolarization phase of an action potential [46]. Furthermore, an increased Ca influx likewise leads to a cytoplasmic Ca overload and hence promotes NCX-dependent transient inward currents and consecutively delayed afterdepolarizations. Early as well as delayed afterdepolarizations, as discussed earlier, constitute potent triggers for arrhythmias. It was shown in a rabbit model of long-QT-syndrome that a drug-induced switch of LTCC into mode 2 gating can effectively induce polymorphic ventricular tachycardia [47]. It could also be shown that a sole overexpression of WT β_{2a}-subunits in paced adult rabbit cardiomyocytes causes cellular Ca overload, membrane potential oscillations and even premature death by favoring mode 2 gating [43]. This effect could be counteracted by CaMKII inhibition and was attenuated when mutant β_{2a}-subunits lacking the CaMKII dependent phosphorylation site Thr498 were overexpressed. Thus, these data confirm the fundamental role of CaMKII for LTCC mode switch and I_{Ca} facilitation. Interestingly, the β_{2a}-subunit of LTCC was found to be overexpressed in human heart failure [48]. This, alongside with an increased CaMKII activity, leads to elevated $Ca_v1.2$ currents and can be assumed to contribute to the high arrhythmogenity.

Importantly, although the shortening of action potential duration in AF due to reduced L-type Ca current is believed to contribute to the perpetualization of AF and CaMKII inhibition might be expected to further reduce this current, this appears not to be the case: studies from our group [25] as well as from another group have shown that CaMKII inhibition does not further decrease the L-type Ca current in AF. So in this pathology, CaMKII inhibition could specifically target

one arrhythmogenic mechanism (SR Ca leak) while not further aggravating the other (reduced L-type Ca current).

1.4. Na channels

Influx of Na ions *via* voltage-dependent Na channels during an action potential has a depolarizing effect on the membrane potential. Two major types of Na currents have been identified in cardiomyocytes [49]. First, there is a rapid and quickly inactivating (1–10 ms) Na current (peak Na current, mostly $Na_v1.5$) leading to the steep upstroke of the action potential. Second, there is a background Na conductance that is rather small in amplitude but persists throughout the whole action potential (late Na current, $I_{Na,late}$). This late Na current has been found to be increased under various pathological conditions [50–52]. Due to its long duration, the integral of I_{Na} late may exceed that of peak I_{Na} and contribute to cellular Na overload and prolongation of the AP duration (APD). Accordingly, it has been shown that an increased Na influx contributes to action potential prolongation in heart failure [51]. Increased action potential duration translates into a prolonged QT interval and constitutes a major risk factor for sudden cardiac death caused by ventricular tachycardia or fibrillation.

It could be shown that calmodulin can bind to cardiac Na channels slowing down their inactivation and consecutively triggering ventricular arrhythmias [53]. This effect may be partly attributed to CaMKII activation. We could show that acute and chronic overexpression of CaMKII in rabbit and mouse ventricular cardiomyocytes leads to a robust increase of late Na current and intracellular Na concentration [14] (see Fig. 3). Additionally, we demonstrated that reactive oxygen species (ROS) can effectively enhance CaMKII autophosphorylation and oxidation and consecutively increase late Na current [54]. This mechanism possibly partly underlies late I_{Na} activation in human heart failure as the generation of ROS is increased due to ischemia. The effect was abolished in $CaMKII^{(-/-)}$ mice [54]. Furthermore, CaMKII overexpression also slowed fast I_{Na} inactivation, enhanced the accumulation of intermediate I_{Na} inactivation and shifted steady-state inactivation of Na channels to a more negative membrane potential [14]. Interestingly, these alterations in Na gating resemble the phenotype seen in genetic disorders of cardiac excitability, such as the long QT syndrome type 3 and Brugada syndrome [55,56], both of which are known for the increased risk of sudden cardiac death [57]. Accordingly, isolated papillary muscles of transgenic mice overexpressing CaMKII exhibited premature arrhythmogenic contractions, which could be effectively suppressed with the late I_{Na} inhibitor ranolazine [58]. *In vivo*, these mice showed prolonged QT intervals and QRS durations and a raised propensity for ventricular arrhythmias [14]. These studies hence confirm the proarrhythmic potential of a CaMKII dependent modulation of Na gating. Additionally, it was also shown that an increased late Na current can, in turn, activate CaMKII [59]. Treatment of neonatal rat cardiomyocytes with the late I_{Na} enhancer sea anemone toxin II (ATX II) increased CaMKII autophosphorylation resulting in CaMKII dependent hyperphosphorylation of target proteins like PLN and RyR2. Thus, a vicious circle could take root in the diseased heart that maintains CaMKII activation and late I_{Na} augmentation and thus aggravates the propensity for arrhythmias.

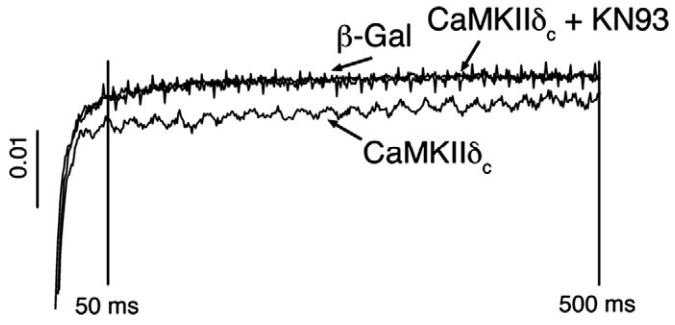

Fig. 3. CaMKII-dependent arrhythmogenic mechanisms: late Na current: original registrations illustrating increased late Na current in cardiomyocytes overexpressing CaMKIIδc compared to control (β-gal). Late Na current could be normalized in CaMKIIδc overexpressing cells by CaMKII-inhibition with KN-93.
Modified from Wagner et al., JCI, 2006.

1.5. K channels

In contrast to Na and Ca ions, the highest potassium concentration is found inside cardiomyocytes under steady-state conditions leading to K outward currents upon opening of K-channels. The concentration gradient is maintained *via* the Na/K pump in the plasma membrane. During an action potential, voltage-gated K outward currents (I_K) are the major driving force for membrane repolarization. I_K thus modulates action potential duration. A reduction of I_K is a frequent finding in cardiac disease leading to a prolongation of the QT interval. Furthermore, the long QT syndrome type 1 (LQT1) can be attributed to mutations in cardiac K channels, which result in a decreased repolarizing K current and exhibit a high risk for live threatening arrhythmic events [60]. I_K consists of the short transient outward K current (I_{to}) and the inwardly rectifying K current (I_{K1}). I_{to}, in turn, comprises a fast and a slow component, $I_{to,fast}$ ($K_v4.2/K_v4.3$) and $I_{to,slow}$ ($K_v1.4$). We demonstrated that acute and chronic overexpression of CaMKII increases $I_{to,slow}$ current and the expression of $K_v1.4$ protein [61]. The threonine residue at position 602 of the $K_v1.4$ protein was identified as a potential target for CaMKII dependent phosphorylation [62]. $I_{to,fast}$ currents however were found to be reduced upon chronic overexpression of CaMKII in mice, with the corresponding channel-protein ($K_v4.2$) being downregulated [61]. As these alterations of K currents could not be reversed by drug-based CaMKII inhibition, one has to assume a CaMKII-dependent regulation of channel expression or trafficking rather than direct I_{to} modification at the channel protein. In contrast to that, the recovery from inactivation was accelerated by an overexpression of CaMKII and could acutely be reversed by CaMKII inhibition. Furthermore, chronic overexpression of CaMKII leads to downregulation of the inward rectifying current $I_{K1,}$ whereas acute overexpression of CaMKII increased I_{K1}. Accordingly, chronic overexpression of CaMKII in mice prolonged APD *via* reduction of $I_{to,fast}$ and I_{K1} as well as an induction of Na currents (see above) whereas an acute overexpression of CaMKII in rabbit cardiomyocytes shortened APD (consistent with enhanced I_{K1} and $I_{to,slow}$ and faster I_{to} recovery) [61]. Furthermore, it was shown that the chronic inhibition of CaMKII by transgenic expression of an inhibitory peptide in mice leads to an upregulation of $I_{to,fast}$ and I_{K1} and consecutively shortens the QT-interval [63]. This study therefore confirms the hypothesis that the reduction of K-currents might be another mechanism of a CaMKII dependent QT-prolongation in the context of chronically elevated CaMKII activity, as it is present in various human cardiac diseases [25,29]. Indeed, it could be shown in human cardiac tissue that the expression of $K_v4.2/K_v4.3$ is reduced in heart failure [64]. The effects of CaMKII activity on K-gating in cardiomyocytes, however, seem to be crucially dependent on the timeframe (acute *vs.* chronic) and obviously involve direct modulations of the gating properties of channel proteins as well as effects on channel protein expression.

2. Conclusion

The current literature thus provides compelling evidence for the arrhythmogenic potency of CaMKII overactivity in cardiac disease. All major classes of proteins involved in the formation of an action potential (voltage gated Na and K channels, L-type Ca channels) as well as key SR Ca handling proteins (RyR2, PLN) have been shown to be modulated by CaMKII — with potentially proarrhythmic effects. This has also been verified by computational models [65]. Taken together, these data thus underline the therapeutic potential of CaMKII

inhibition in human cardiac pathologies such as heart failure. The possibility to elegantly combine inotropic and antiarrhythmic therapies by targeting a cellular mechanism that is responsible for both would thereby be a unique feature of this approach. First studies have already demonstrated antiarrhythmic effects of CaMKII inhibition. Further research will allow us to more clearly define conditions where CaMKII inhibition would be most potent for antiarrhythmic application. The organ specific application of CaMKII inhibitors, however, might be a major hurdle still to be overcome as CaMKII has been shown to be of major importance for memory consolidation and neuronal excitability. Consequently, the safety of CaMKII inhibition as a therapeutic strategy has to be further validated before being considered for human use.

Author disclosure statement

L.S.M. acknowledges research grants and funding from CVT, GILEAD, and MENARINI/Berlin-Chemie. L.S.M. received honoraria from Berlin-Chemie, Astra-Zeneca, Daiichi-Sankyo, and Böhringer-Ingelheim.

Acknowledgments

L.S.M. is funded by the Deutsche Forschungsgemeinschaft (DFG) grant MA 1982/4-2, and MA 1982/2-2, as well as TPA03 SFB 1002. L.S.M. is also funded by grants from the Fondation Leducq 'Alliance for CaMKII Signaling in Heart' as well as 'Redox and Nitrosative Regulation of Cardiac Remodeling'.

References

[1] Camm AJ, Kirchhof P, Lip GY, Schotten U, Savelieva I, Ernst S, et al. Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). Europace 2010;12:1360-420.
[2] Packer M. Sudden unexpected death in patients with congestive heart failure: a second frontier. Circulation 1985;72:681-5.
[3] Davies M, Hobbs F, Davis R, Kenkre J, Roalfe AK, Hare R, et al. Prevalence of left-ventricular systolic dysfunction and heart failure in the Echocardiographic Heart of England Screening study: a population based study. Lancet 2001;358:439-44.
[4] Mosterd A, Cost B, Hoes AW, de Bruijne MC, Deckers JW, Hofman A, et al. The prognosis of heart failure in the general population: The Rotterdam Study. Eur Heart J 2001;22:1318-27.
[5] MacIntyre K, Capewell S, Stewart S, Chalmers JW, Boyd J, Finlayson A, et al. Evidence of improving prognosis in heart failure: trends in case fatality in 66,547 patients hospitalized between 1986 and 1995. Circulation 2000;102:1126-31.
[6] Cowie MR, Wood DA, Coats AJ, Thompson SG, Suresh V, Poole-Wilson PA, et al. Survival of patients with a new diagnosis of heart failure: a population based study. Heart 2000;83:505-10.
[7] Krell MJ, Kline EM, Bates ER, Hodgson JM, Dilworth LR, Laufer N, et al. Intermittent, ambulatory dobutamine infusions in patients with severe congestive heart failure. Am Heart J 1986;112:787-91.
[8] Greenberg HM, Dwyer Jr EM, Hochman JS, Steinberg JS, Echt DS, Peters RW. Interaction of ischaemia and encainide/flecainide treatment: a proposed mechanism for the increased mortality in CAST I. Br Heart J 1995;74:631-5.
[9] Rokita AG, Anderson ME. New therapeutic targets in cardiology: arrhythmias and Ca2+/calmodulin-dependent kinase II (CaMKII). Circulation 2012;126:2125-39.
[10] Witcher DR, Kovacs RJ, Schulman H, Cefali DC, Jones LR. Unique phosphorylation site on the cardiac ryanodine receptor regulates calcium channel activity. J Biol Chem 1991;266:11144-52.
[11] Wehrens XH, Lehnart SE, Reiken SR, Marks AR. Ca2+/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. Circ Res 2004;94:e61-70.
[12] Mattiazzi A, Kranias EG. CaMKII regulation of phospholamban and SR Ca2+ load. Heart Rhythm 2011;8:784-7.
[13] Dzhura I, Wu Y, Colbran RJ, Balser JR, Anderson ME. Calmodulin kinase determines calcium-dependent facilitation of L-type calcium channels. Nat Cell Biol 2000;2:173-7.
[14] Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof P, et al. Ca2+/calmodulin-dependent protein kinase II regulates cardiac Na+ channels. J Clin Invest 2006;116:3127-38.
[15] Kushnir A, Shan J, Betzenhauser MJ, Reiken S, Marks AR. Role of CaMKIIdelta phosphorylation of the cardiac ryanodine receptor in the force frequency relationship and heart failure. Proc Natl Acad Sci U S A 2010;107:10274-9.
[16] Wu Y, Gao Z, Chen B, Koval OM, Singh MV, Guan X, et al. Calmodulin kinase II is required for fight or flight sinoatrial node physiology. Proc Natl Acad Sci U S A 2009;106:5972-7.
[17] DeSantiago J, Maier LS, Bers DM. Phospholamban is required for CaMKII-dependent recovery of Ca transients and SR Ca reuptake during acidosis in cardiac myocytes. J Mol Cell Cardiol 2004;36:67-74.
[18] Sag CM, Dybkova N, Neef S, Maier LS. Effects on recovery during acidosis in cardiac myocytes overexpressing CaMKII. J Mol Cell Cardiol 2007;43:696-709.
[19] Maier LS, Zhang T, Chen L, DeSantiago J, Brown JH, Bers DM. Transgenic CaMKIIdeltaC overexpression uniquely alters cardiac myocyte Ca2+ handling: reduced SR Ca2+ load and activated SR Ca2+ release. Circ Res 2003;92:904-11.
[20] Sag CM, Wadsack DP, Khabbazzadeh S, Abesser M, Grefe C, Neumann K, et al. Calcium/calmodulin-dependent protein kinase II contributes to cardiac arrhythmogenesis in heart failure. Circ Heart Fail 2009;2:664-75.
[21] Meyer T, Hanson PI, Stryer L, Schulman H. Calmodulin trapping by calcium-calmodulin-dependent protein kinase. Science 1992;256:1199-202.
[22] He BJ, Joiner ML, Singh MV, Luczak ED, Swaminathan PD, Koval OM, et al. Oxidation of CaMKII determines the cardiotoxic effects of aldosterone. Nat Med 2011;17:1610-8.
[23] Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, et al. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. Cell 2008;133:462-74.
[24] Hasenfuss G. Alterations of calcium-regulatory proteins in heart failure. Cardiovasc Res 1998;37:279-89.
[25] Neef S, Dybkova N, Sossalla S, Ort KR, Fluschnik N, Neumann K, et al. CaMKII-dependent diastolic SR Ca2+ leak and elevated diastolic Ca2+ levels in right atrial myocardium of patients with atrial fibrillation. Circ Res 2010;106:1134-44.
[26] Voigt N, Li N, Wang Q, Wang W, Trafford AW, Abu-Taha I, et al. Enhanced sarcoplasmic reticulum Ca2+ leak and increased Na+–Ca2+ exchanger function underlie delayed afterdepolarizations in patients with chronic atrial fibrillation. Circulation 2012;125:2059-70.
[27] Zhang T, Maier LS, Dalton ND, Miyamoto S, Ross Jr J, Bers DM, et al. The deltaC isoform of CaMKII is activated in cardiac hypertrophy and induces dilated cardiomyopathy and heart failure. Circ Res 2003;92:912-9.
[28] Kohlhaas M, Zhang T, Seidler T, Zibrova D, Dybkova N, Steen A, et al. Increased sarcoplasmic reticulum calcium leak but unaltered contractility by acute CaMKII overexpression in isolated rabbit cardiac myocytes. Circ Res 2006;98:235-44.
[29] Sossalla S, Fluschnik N, Schotola H, Ort KR, Neef S, Schulte T, et al. Inhibition of elevated Ca2+/calmodulin-dependent protein kinase II improves contractility in human failing myocardium. Circ Res 2010;107:1150-61.
[30] Toischer K, Rokita AG, Unsold B, Zhu W, Kararigas G, Sossalla S, et al. Differential cardiac remodeling in preload versus afterload. Circulation 2010;122:993–1003.
[31] Endo M. Calcium release from the sarcoplasmic reticulum. Physiol Rev 1977;57:71–108.
[32] Bers DM. Cardiac excitation–contraction coupling. Nature 2002;415:198-205.
[33] Song Y, Shryock JC, Belardinelli L. An increase of late sodium current induces delayed afterdepolarizations and sustained triggered activity in atrial myocytes. Am J Physiol Heart Circ Physiol 2008;294:H2031-9.
[34] Pieske B, Maier LS, Bers DM, Hasenfuss G. Ca2+ handling and sarcoplasmic reticulum Ca2+ content in isolated failing and nonfailing human myocardium. Circ Res 1999;85:38-46.
[35] Hasenfuss G, Schillinger W, Lehnart SE, Preuss M, Pieske B, Maier LS, et al. Relationship between Na+–Ca2+-exchanger protein levels and diastolic function of failing human myocardium. Circulation 1999;99:641-8.
[36] Dybkova N, Sedej S, Napolitano C, Neef S, Rokita AG, Hunlich M, et al. Overexpression of CaMKIIdeltac in RyR2R4496C+/− knock-in mice leads to altered intracellular Ca2+ handling and increased mortality. J Am Coll Cardiol 2011;57:469-79.
[37] Curran J, Brown KH, Santiago DJ, Pogwizd S, Bers DM, Shannon TR. Spontaneous Ca waves in ventricular myocytes from failing hearts depend on Ca(2+)-calmodulin-dependent protein kinase II. J Mol Cell Cardiol 2010;49:25-32.
[38] Gonano LA, Sepulveda M, Rico Y, Kaetzel M, Valverde CA, Dedman J, et al. Calcium-calmodulin kinase II mediates digitalis-induced arrhythmias. Circ Arrhythm Electrophysiol 2011;4:947-57.
[39] Chelu MG, Sarma S, Sood S, Wang S, van Oort RJ, Skapura DG, et al. Calmodulin kinase II-mediated sarcoplasmic reticulum Ca2+ leak promotes atrial fibrillation in mice. J Clin Invest 2009;119:1940-51.
[40] Li N, Wang T, Wang W, Cutler MJ, Wang Q, Voigt N, et al. Inhibition of CaMKII phosphorylation of RyR2 prevents induction of atrial fibrillation in FKBP12.6 knockout mice. Circ Res 2012;110:465-70.
[41] Catterall WA. Structure and regulation of voltage-gated Ca2+ channels. Annu Rev Cell Dev Biol 2000;16:521-55.
[42] Song LS, Sobie EA, McCulle S, Lederer WJ, Balke CW, Cheng H. Orphaned ryanodine receptors in the failing heart. Proc Natl Acad Sci U S A 2006;103:4305-10.
[43] Koval OM, Guan X, Wu Y, Joiner ML, Gao Z, Chen B, et al. CaV1.2 beta-subunit coordinates CaMKII-triggered cardiomyocyte death and afterdepolarizations. Proc Natl Acad Sci U S A 2010;107:4996-5000.
[44] Wu Y, MacMillan LB, McNeill RB, Colbran RJ, Anderson ME. CaM kinase augments cardiac L-type Ca2+ current: a cellular mechanism for long Q-T arrhythmias. Am J Physiol 1999;276:H2168-78.
[45] Anderson ME, Braun AP, Wu Y, Lu T, Schulman H, Sung RJ. KN-93, an inhibitor of multifunctional Ca++/calmodulin-dependent protein kinase, decreases early afterdepolarizations in rabbit heart. J Pharmacol Exp Ther 1998;287:996–1006.
[46] Wit AL, Rosen MR. Pathophysiologic mechanisms of cardiac arrhythmias. Am Heart J 1983;106:798-811.
[47] Mazur A, Roden DM, Anderson ME. Systemic administration of calmodulin antagonist W-7 or protein kinase A inhibitor H-8 prevents torsade de pointes in rabbits. Circulation 1999;100:2437-42.

[48] Hullin R, Matthes J, von Vietinghoff S, Bodi I, Rubio M, D'Souza K, et al. Increased expression of the auxiliary beta(2)-subunit of ventricular L-type Ca(2)+ channels leads to single-channel activity characteristic of heart failure. PLoS One 2007;2:e292.
[49] Coraboeuf E, Deroubaix E, Coulombe A. Effect of tetrodotoxin on action potentials of the conducting system in the dog heart. Am J Physiol 1979;236:H561-7.
[50] Undrovinas AI, Fleidervish IA, Makielski JC. Inward sodium current at resting potentials in single cardiac myocytes induced by the ischemic metabolite lysophosphatidylcholine. Circ Res 1992;71:1231-41.
[51] Undrovinas AI, Maltsev VA, Sabbah HN. Repolarization abnormalities in cardiomyocytes of dogs with chronic heart failure: role of sustained inward current. Cell Mol Life Sci 1999;55:494-505.
[52] Ward CA, Giles WR. Ionic mechanism of the effects of hydrogen peroxide in rat ventricular myocytes. J Physiol 1997;500(Pt 3):631-42.
[53] Tan HL, Kupershmidt S, Zhang R, Stepanovic S, Roden DM, Wilde AA, et al. A calcium sensor in the sodium channel modulates cardiac excitability. Nature 2002;415:442-7.
[54] Wagner S, Ruff HM, Weber SL, Bellmann S, Sowa T, Schulte T, et al. Reactive oxygen species-activated Ca/calmodulin kinase IIdelta is required for late I(Na) augmentation leading to cellular Na and Ca overload. Circ Res 2011;108:555-65.
[55] Veldkamp MW, Viswanathan PC, Bezzina C, Baartscheer A, Wilde AA, Balser JR. Two distinct congenital arrhythmias evoked by a multidysfunctional Na(+) channel. Circ Res 2000;86:E91-7.
[56] Bezzina C, Veldkamp MW, van Den Berg MP, Postma AV, Rook MB, Viersma JW, et al. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. Circ Res 1999;85:1206-13.
[57] Vohra J. Diagnosis and management of Brugada syndrome. Heart Lung Circ 2011;20:751-6.
[58] Sossalla S, Maurer U, Schotola H, Hartmann N, Didie M, Zimmermann WH, et al. Diastolic dysfunction and arrhythmias caused by overexpression of CaMKIIdelta(C) can be reversed by inhibition of late Na(+) current. Basic Res Cardiol 2011;106:263-72.
[59] Yao L, Fan P, Jiang Z, Viatchenko-Karpinski S, Wu Y, Kornyeyev D, et al. Nav1.5-dependent persistent Na+ influx activates CaMKII in rat ventricular myocytes and N1325S mice. Am J Physiol Cell Physiol 2011;301:C577-86.
[60] Barsheshet A, Goldenberg I, J.O.U., Moss AJ, Jons C, Shimizu W, et al. Mutations in cytoplasmic loops of the KCNQ1 channel and the risk of life-threatening events: implications for mutation-specific response to beta-blocker therapy in type 1 long-QT syndrome. Circulation 2012;125:1988-96.
[61] Wagner S, Hacker E, Grandi E, Weber SL, Dybkova N, Sossalla S, et al. Ca/calmodulin kinase II differentially modulates potassium currents. Circ Arrhythm Electrophysiol 2009;2:285-94.
[62] Hagiwara K, Nunoki K, Ishii K, Abe T, Yanagisawa T. Differential inhibition of transient outward currents of Kv1.4 and Kv4.3 by endothelin. Biochem Biophys Res Commun 2003;310:634-40.
[63] Li J, Marionneau C, Zhang R, Shah V, Hell JW, Nerbonne JM, et al. Calmodulin kinase II inhibition shortens action potential duration by upregulation of K+ currents. Circ Res 2006;99:1092-9.
[64] Beuckelmann DJ, Nabauer M, Erdmann E. Alterations of K+ currents in isolated human ventricular myocytes from patients with terminal heart failure. Circ Res 1993;73:379-85.
[65] Grandi E, Puglisi JL, Wagner S, Maier LS, Severi S, Bers DM. Simulation of Ca-calmodulin-dependent protein kinase II on rabbit ventricular myocyte ion currents and action potentials. Biophys J 2007;93:3835-47.