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Small Conductance Ca^{2+} -Activated K^+ Channels and Cardiac Arrhythmias

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Abstract

Small conductance Ca^{2+} -activated K^+ (SK, $\text{K}_{\text{Ca}2}$) channels are unique in that they are gated solely by changes in intracellular Ca^{2+} and hence, function to integrate intracellular Ca^{2+} and membrane potentials on a beat-to-beat basis. Recent studies have provided evidence for the existence and functional significance of SK channels in the heart. Indeed, our knowledge of cardiac SK channels has been greatly expanded over the past decade. Interests in cardiac SK channels are further driven by recent studies suggesting the critical roles of SK channels in human atrial fibrillation, SK channel as a possible novel therapeutic target in atrial arrhythmias and up-regulation of SK channels in heart failure (HF) in animal models and human HF. However, there remain critical gaps in our knowledge. Specifically, blockade of SK channels in cardiac arrhythmias has been shown to be both anti-arrhythmic and proarrhythmic. This contemporary review will provide an overview of the literature on the role of cardiac SK channels in cardiac arrhythmias and to serve as a discussion platform for the current clinical perspectives. At the translational level, development of SK channel blockers as a new therapeutic target in the treatment of atrial fibrillation and the possible pro-arrhythmic effects merit further considerations and investigations.

Introduction

Cardiac action potentials (APs) are shaped by the intricate interplay of inward Na^+ , Ca^{2+} and outward K^+ currents. Ca^{2+} influx through voltage-gated Ca^{2+} channels is critical not only for the initiation of cardiac excitation-contraction coupling, but also for the activation of multiple downstream molecules to couple the function of the proteins with changes in membrane potentials including Ca^{2+} -activated ion channels.

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The initial study of Ca^{2+} -activated K^+ channels in the heart dating back to 1983 did not support the functional role of the channels in the heart¹. However, Giles and Imaizumi reported a few years later that Ca^{2+} -activated K^+ currents could be observed and were larger in atria than ventricles². There were no additional reports on the functional roles of Ca^{2+} -activated K^+ channel in the heart until a decade ago when we reported the molecular identity and functional significance of small conductance Ca^{2+} -activated K^+ (SK) channels in human and mouse hearts³. Since then, our knowledge of cardiac SK channels has been greatly expanded over the past decade. Studies by our group and others have provided evidence to substantiate the important roles of SK channels in the heart^{4–15}. Indeed, interests in cardiac SK channels are further fueled by recent studies suggesting the critical roles of SK channels in human atrial fibrillation (AF)^{16, 17}, SK channel as a possible novel therapeutic target in atrial arrhythmias^{18–20} and up-regulation of SK channels in heart failure (HF) in animal models^{10,11} and human HF²¹ (see Figure 1). However, there remain major gaps in our knowledge. Conflicting studies have been reported regarding the existence of SK channels in the heart²². Moreover, blockade of SK channels in cardiac arrhythmias has been shown to be both anti-arrhythmic^{18–20} and proarrhythmic^{23–25} in various models (see Figure 1). This review attempts to provide an overview of the literature over the past decade on the role of cardiac SK channels in electrophysiology, molecular interactions, and cardiogenesis and to serve as a discussion platform for the current clinical perspectives.

Identification and functional expression of SK channels in the heart

SK channels are gated solely by intracellular Ca^{2+} and hence, provide a critical link between changes in intracellular Ca^{2+} and membrane potentials. The discovery of SK channels started more than 70 years ago when convulsions in mice were observed following injection of bee venom^{26, 27}. The active neurotoxin in bee venom is apamin, a remarkably specific blocker of SK channels^{28, 29}. Indeed, the highly selective blockade by apamin is the signature of SK channels that enables the verification of the molecular identity of SK channels in mammalian brain³⁰. The family of SK channels consists of three members with differential sensitivity to apamin: SK1 (or $\text{K}_{\text{Ca}2.1}$ encoded by *KCNN1* gene) with the least sensitivity (EC_{50} for hSK1 ~ 10 nM), SK2 (or $\text{K}_{\text{Ca}2.2}$ encoded by *KCNN2* gene) with the highest sensitivity ($\text{EC}_{50} \sim 40$ pM) and SK3 (or $\text{K}_{\text{Ca}2.3}$ encoded by *KCNN3* gene) with intermediate sensitivity ($\text{EC}_{50} \sim 1$ nM)²⁷. They have a relatively small single channel conductance (~ 10 pS in symmetrical K^+) and are activated by submicromolar concentrations of intracellular Ca^{2+} ions (apparent $\text{K}_d \sim 0.5$ μM). They are highly conserved among mammalian species and are identified in many organisms including *Drosophila*²⁷. Functional SK channels assemble to form homomeric³⁰ or heteromeric^{5, 31} tetramers. An intermediate-conductance Ca^{2+} -activated K^+ channel (IK or SK4 encoded by *KCNN4* gene) that is structurally and functionally similar to SK channels is classified to the same gene family^{27, 32}.

SK channels were first identified in brain^{30, 32}, and were later described in a variety of tissues including smooth muscle, endothelia, epithelia and blood cells³². SK4 expression is restricted to non-neuronal tissues such as muscle, epithelia and blood cells^{32, 33}. Our laboratory demonstrated that all three isoforms of SK channels are expressed in mouse and human cardiomyocytes^{3, 4}. Since then, expression of SK1 and SK3 in human heart tissues³⁴

and SK2 and SK3 in rabbit pulmonary veins has also been reported⁷. The existence of SK currents in the heart was further supported by the findings of apamin-sensitive currents in rabbit pulmonary vein^{7, 9} and ventricular myocytes^{10, 11}, human atrial myocytes^{12, 13}, and rat ventricular myocytes¹⁴. Recently, presence of SK currents has also been demonstrated in canine pulmonary vein and left atrial myocytes using a SK-specific current blocker, NS8593¹⁵. Moreover, SK channels have been identified in pacemaking cells including mouse atrioventricular nodal cells³⁵ and rabbit sinoatrial nodal cells⁹. The SK currents show inward rectification profile which may result from the pore block by intracellular divalent cations at positive membrane potential or may be mediated by the intrinsic charged residues in the sixth transmembrane domain^{27, 42}. Apamin-sensitive SK currents in atrial myocytes show the inward-rectified feature reminiscent of the hetero-expressed SK currents^{3, 30, 36}.

Cardiac SK channel interactome

Ion channels do not exist and function in isolation, instead they form part of multi-protein complexes interacting with extracellular matrix and cytosolic proteins³⁷⁻³⁹. The composition of the ion channel complex significantly affects the localization, trafficking, activation and regulation of the channel function. Gating of SK channels depends on the interplay between the pore-forming α subunits and Ca^{2+} -binding protein calmodulin (CaM)^{27, 36} through which the channels sense the intracellular Ca^{2+} leading to altered conformation. CaM binds to a highly conserved CaM-binding domain (CaMBD) residing within the C-terminus of the SK channels located immediately distal to the sixth transmembrane segment. Binding of Ca^{2+} to the EF hands of CaM results in changes in the conformation of the channels leading to channel activation. CaM is not only essential for Ca^{2+} sensitivity, but also critical to the trafficking of SK channels⁴⁰. Specifically, previous studies have demonstrated that Ca^{2+} -independent association between CaM and SK channels are necessary for cell surface expression. However, it is not known whether CaM help to anchor the SK channel or acts as a chaperone for channel trafficking.

A proteomics approach has identified protein kinase CK2 (casein kinase II) and protein phosphatase 2A (PP2A) as SK2 channel binding proteins⁴¹. CK2 and PP2A regulate SK channel's sensitivity to intracellular Ca^{2+} by phosphorylating or dephosphorylating CaM²⁷. However, CK2/PP2A modulation of SK channels has been demonstrated mainly in neurons and possible roles of CK2/PP2A in cardiac myocytes await further studies.

Using yeast two-hybrid screen against human heart library, we have identified several cytoskeletal proteins including α -actinin²^{42,43}, filamin A⁴⁴, and myosin light chain 2 (MLC2)⁴⁵ that directly interact with SK2 channels (Figure 2). Specifically, α -actinin2 and MLC2 interact *via* the C-termini and filamin A *via* the N-termini of the SK2 channel, respectively. Moreover, cardiac SK2 channels coupled with L-type calcium channels, $\text{Ca}_v1.3$ and $\text{Ca}_v1.2$, through a physical bridge, α -actinin2. SK2 channels do not physically interact with Ca^{2+} channels, instead the two channels co-localize *via* their interaction with α -actinin2 along the Z-line in atrial myocytes (Figure 2). The co-localization of SK and Ca^{2+} channels suggests the possibility that local subsarcolemmal Ca^{2+} resulting from opening of Ca^{2+} channels is sufficient to activate SK channels as was demonstrated for hippocampal neurons⁴⁶. However, two recent studies suggested that sarcoplasmic reticulum

(SR) Ca^{2+} release is required for the activation of cardiac SK channels^{47,48}. One of these studies utilized over-expressed SK channels and the regulation of the over-expressed SK channels maybe distinct compared to native cardiomyocytes. Nonetheless, it is possible that in cardiomyocytes, both Ca^{2+} entry and Ca^{2+} release from SR are required to activate SK channels.

In addition, trafficking of SK2 channels is critically dependent on the direct protein-protein interactions of the channels with α -actinin2, MLC2, and filamin A⁴³⁻⁴⁵ (see Figure 2). Knockdown of α -actinin2 or filamin A results in a decrease in SK2 channel expression on the membrane and localization of SK2 channels in endosome suggesting a reduction in recycling of SK2 channels from endosome⁴⁴⁻⁴⁵. Finally, SK2 channel trafficking is Ca^{2+} -dependent in the presence of α -actinin2. A decrease in intracellular Ca^{2+} results in a significant reduction of SK2 channel membrane localization⁴⁴. An increase in intracellular Ca^{2+} , as evident during rapid AF or atrial tachycardia, is predicted to increase SK2 channel expression leading to shortening of the atrial APs and maintenance of arrhythmias. One previous study using rapid pacing in isolated rabbit atria demonstrated a significant increase in SK2 immunostaining from a perinuclear pattern to the plasma membrane at the pulmonary vein after burst pacing suggesting an increase in forward trafficking of SK2 channels⁷.

Overall, SK channel complexes in cardiomyocytes consist at least of homomeric or heteromeric SK channel α subunits, CaM, α -actinin2, filamin A and MLC2. The interaction amongst the multi-proteins in the SK channel complexes is essential for the gating, regulation, and membrane trafficking of the channels in cardiomyocytes. With the aid of the advanced proteomics techniques, more interacting proteins may be identified as new candidates that participate in the SK channel complexes.

Roles of SK channels in cardiac repolarization

Repolarization of cardiac action potential (AP) relies on the orchestrated activity of multiple K^{+} channels and transporters. One critical question following the identification of cardiac SK channels is whether the channels contribute to cardiac repolarization. The initial study reported that inhibition of SK currents by apamin prolonged AP duration (APD) in mouse and human atrial myocytes, however, the effects were less prominent in ventricular myocytes suggesting the unique role of the channels in atrial repolarization³. Consistently, subsequent experiments using global SK2 knockout mice demonstrated that ablation of SK2 channel resulted in a significant prolongation of APD prominently in the late phase of the repolarization in atrial myocytes⁶. Importantly, the null mutant mice showed an increased susceptibility to atrial fibrillation (AF). On the other hand, there was no significant alteration in the APD in ventricular myocytes and no ventricular arrhythmias were induced in the null mutant animals⁶. In contrast, a mouse model of SK3 channel overexpression showed a significant shortening of APD in atrial myocytes⁸. These gene-targeted mouse models were not restricted to only cardiac tissues. Indeed, SK channels are abundantly expressed in a number of non-cardiomyocyte cells in the heart. Nonetheless, single isolated cardiomyocytes were used in these studies in addition to *in vivo* studies to circumvent

possible contributions from the effects of SK2 knockout or SK3 overexpression in other cell types including neuronal cells.

Consistent with these studies, a recent report using optical mapping in isolated canine left atria demonstrated that inhibition of SK channels by either apamin or UCL1684⁴⁹ prolonged APD²³. In addition, SK channel inhibitors, NS8593 and ICAGEN⁵⁰, prolonged APD in isolated human atrial myocytes¹³. The above studies supported the critical role of SK channels in the repolarization not only in mouse and canine but also human atrial myocytes⁵¹.

In atrioventricular nodal cells, SK2 channel overexpression results in shortening of spontaneous APs and an increase in the firing frequency, while ablation of SK2 channels results in the opposite effects³⁵. Recently, Chen et al studied the apamin modulation of pulmonary vein and SAN cells from rabbit heart⁹. They found that SAN cells have larger SK currents than pulmonary vein cardiomyocytes. Apamin treatment decreases the firing rate and prolongs APD₅₀ and APD₇₅ in SAN cells and pulmonary vein cardiomyocytes.

SK channels and atrial fibrillation (AF)

Significance of cardiac SK channels lies in the fact that the channels are preferentially expressed in atria compared to the ventricles³. The differential expression of SK channels in the heart may offer a unique therapeutic strategy to target atria without interfering with ventricular function. Moreover, the possible role of SK channels in human AF was recently reported using genome-wide association analysis (GWAS) revealing an association between an intronic single-nucleotide polymorphism (SNP) in *KCNN3* gene with lone AF^{16, 17}. In addition, Olesen *et al* reported one known exonic synonymous SNP in *KCNN3* that was also associated with lone AF⁵². To further test the role of SK3 channels in atrial repolarization and AF, Zhang *et al* took advantage of a SK3 transgenic mouse model to demonstrate that overexpression of SK3 results in significant shortening of APD in atrial myocytes, an abbreviation of atrial effective refractory period (AERP) and an increased susceptibility to AF⁸. A separate study using the same mouse model showed that overexpression of SK3 channels can increase the risk of sudden cardiac death associated with bradyarrhythmias, heart block and an increased susceptibility to atrial arrhythmias⁵³.

SK channel remodeling in AF

Evidence is accumulating to support SK channel remodeling in AF. The first evidence showing the AF-induced remodeling of SK channel came from a study to test the effect of a rare stimulation on the susceptibility of atrial arrhythmias⁵⁴. Apamin prevented the progressive shortening of APD near burst pacing electrode in coronary sinus and pulmonary vein (PV) regions suggesting the involvement of SK channels. Ozgen *et al* further studied the mechanism underlying the phenomenon⁷. They found that the burst pacing-induced APD shortening in pulmonary vein-atria interface in a rabbit model resulted from SK2 channel trafficking to the cell membrane, leading to increased apamin-sensitive outward currents⁷. Similarly, Qi *et al* reported that atrial-tachypacing in a canine model enhanced SK currents in PV and left atrial myocytes¹⁵. Moreover, inhibition of SK channels by a known SK channel blocker (NS8593)⁵⁵ significantly reduced AF inducibility. Of interest is the fact that

SK2 expression was found to be more abundant in pulmonary vein than left atrial myocytes. Mechanistically, our recent study has provided evidence to suggest that an increase in intracellular Ca^{2+} as seen during rapid atrial tachycardia results in an enhanced trafficking of SK channels to the membrane⁴⁴.

Contrary to the above studies, a recent report found that SK1 and SK2 channel expression as well as apamin-sensitive currents were significantly reduced in right atrial appendages recovered from chronic AF patients¹². Similarly, a recent study showed that SK3 expression was down-regulated in patients with permanent AF, which was attributed to the up-regulation of microRNA-499⁵⁶. Taken together, it is possible that AF may result in the up-regulation of SK channels as the initial response. With progression of the disease, there may be a down-regulation of the channels.

Anti-arrhythmic and proarrhythmic effects of SK channel inhibition

Recently, Diness *et al* utilized three different SK channel inhibitors including UCL1684, *N*-(pyridin-2-yl)-4-(pyridin-2-yl)thiazol-2-amine (ICA)⁵⁰ and NS8593 in *ex vivo* and *in vivo* models of AF in rat, guinea pig, and rabbit¹⁸. Inhibition of SK channels resulted in prolongation of AERP and termination of AF. Similar anti-arrhythmic effects of NS8593 and UCL1684 were observed for paroxysmal AF in a hypertensive rat model¹⁹. In an acute pacing-induced AF model in rats, it was found that intravenous application of NS8593 reduced AF duration in a dose-dependent manner and the antiarrhythmic effect is associated with increased AERP²⁰. Similar results were also obtained using UCL1684 and apamin in the same study. The results from these studies suggest that SK channels may represent a potential therapeutic target for the treatment of atrial arrhythmias.

In contrast to these studies, genetic ablation of SK2 channels in a mouse model prolonged atrial APD, increased occurrences of early after depolarization (EAD) and inducible AF⁶. In addition, a recent report found that apamin and UCL1684 promoted arrhythmia in isolated canine left atrium²³. The prolongation of APD by SK channel inhibition was accompanied by increased APD heterogeneity, occurrences of electrical alternans, and wave breaks. In addition to the previously suggested increased occurrence of EAD by ablation of SK2 channels, inhibition of SK channels may lead to more heterogeneous APD and altered dispersion of repolarization, which can promote development of reentrant arrhythmia²⁴. Moreover, SK2 channels expression in left atrial appendages was higher at the base compared to the apex. Taken together, key questions remain regarding the role of SK channels in AF, *i.e.*, whether inhibition of the channels is proarrhythmic or antiarrhythmic.

Thus, both reduced and enhanced activity of SK channels may predispose atria to AF, likely from different mechanisms depending on the heterogeneous expression, heart rate and AF-induced electrical remodeling. The discrepancy between the above studies may be related to the different AF models, varied species, different experimental techniques and conditions. Indeed, the mechanisms of AF in patients are likely to be far more complex. Further studies are necessary to address the functional roles of SK channels in AF.

Remodeling of SK channels in heart failure (HF)

Contrary to the atria, apamin does not significantly affect the APD of dog, rat, rabbit, and human ventricles under normal physiological conditions^{10, 22}. However, in a rabbit model with HF and spontaneous ventricular fibrillation, apamin prolonged the APD and eliminated the recurrent spontaneous ventricular fibrillation¹⁰. Failing ventricular myocytes showed a significant increase in SK currents compared to the normal ventricular myocytes possibly as a result of the increased sensitivity of SK channels to intracellular Ca^{2+} in failing heart. More importantly, there is a transmural gradient of SK currents with higher density in epicardial than in midmyocardial and endocardial layers in the failing heart. Similar up-regulation of SK currents was demonstrated in a post-myocardial infarction rabbit model¹¹ and failing human ventricles²¹. It was further demonstrated that a commonly used antiarrhythmic agent, amiodarone, inhibited SK2 channels and prevented post-shock APD shortening in rabbit failing heart suggesting the significance of SK channel inhibition in HF⁵⁷. A more recent study demonstrated that apamin prolonged ventricular repolarization and EAD in dog and human with end-stage HF⁵⁸. In a volume-overload HF model in rat, it was found that there was a significant increase of SK1 and SK3 expression and increased apamin-sensitive currents in the HF ventricle compared to sham control. Treatment with a β -blocker, bisoprolol, reduced the SK1 and SK3 expression, apamin-sensitive currents and their sensitivity to intracellular Ca^{2+} ¹⁴. Similarly, in an *in vivo* acute myocardial infarction model in rat, pretreatment with apamin or UCL1684 significantly increased APDs in the infarcted area of the left ventricle and inhibited spontaneous ventricular tachycardia and ventricular fibrillation⁵⁹.

The potential mechanisms underlying SK channel remodeling in HF remain unclear. Abnormal Ca^{2+} -handling in HF has been well described and may represent a significant contributor. In addition, remodeling in failing heart involves complex changes in gene expression, phosphorylation of targeted proteins and modulation of Ca^{2+} signaling pathways. One previous study has documented an increase in SK2 protein expression in failing human ventricles using total homogenates from ventricular tissues²¹. Therefore, alteration of SK channel expression, Ca^{2+} sensitivity in HF or possibly increased trafficking may be critical factors in SK channel remodeling in HF and contribute towards recurrent ventricular fibrillation and electrical storm. Additional studies are necessary to gain further mechanistic insight into the regulation of SK channels in HF. Finally, these findings provide a cautionary view for the use of SK channel blockers in the treatment of AF. AF is commonly seen associated with HF and up-regulation of SK channels in heart failure may represent an adaptive response to prevent excessive AP prolongation in the ventricles⁵⁸. Indeed, the use of SK channel blockers has been shown in an animal model to result in occurrence of torsades de pointes ventricular arrhythmias⁶⁰. The therapeutic potential for AF needs further assessment in patients with HF.

SK channels in stem cell cardiogenesis

Recent studies have demonstrated that SK channels play an important role in directed stem cell differentiation. Functional SK1, 2, 3, and 4 channels have been shown to be present in pluripotent stem cells (PSCs)^{61, 62}. Activation of SK channels by small molecule 1-ethyl-2-

benzimidazolinone (EBIO) improves the differentiation of pacemaking cardiomyocytes from mouse PSCs at two independent differentiation stages, by increasing the induction of the mesoderm lineage and the pacemaking cardiomyocytes subtype specification⁶¹. Transcriptional data demonstrated that SK4 is the dominant isoform in murine PSCs and responsible for the facilitated differentiation. The most immediate consequence of SK4 activation was membrane potential hyperpolarization. Similar to murine PSCs, SK channel activation facilitated induction of pacemaking cardiomyocytes from human PSCs, as assessed by expression of hyperpolarization-activated cyclic nucleotide-modulated (HCN)4 channel⁶². However, electrophysiological studies were not performed. Therefore, it remains to be determined if activated SK channel-induction of pacemaking cardiomyocytes is as robust in human PSCs as that in murine cells. Moreover, contrary to their murine counterpart, human PSCs have virtually no SK4 expression but SK2 as the dominant isoform with low SK1 and minimal SK3 transcripts.

Unresolved issues and future perspectives

A decade of study on cardiac SK channels has opened new and exciting fields in cardiac electrophysiology and arrhythmias. The cumulative findings so far have greatly enhanced the current understanding of the critical crosstalk between Ca²⁺ signaling and K⁺ channels in the regulation of cardiac excitability. However, challenges and important knowledge gaps remain.

First, controversies exist regarding the roles of SK channels in cardiac repolarization. Nagy *et al* recorded APs from rat, dog and human atrial and ventricular tissue preparations and found that no effects of apamin on APDs²². Diness *et al* also found that apamin does not cause significant APD prolongation in atrial myocytes from guinea pigs¹⁸. Interspecies differences and different experimental conditions may contribute to the observed discrepancies. Moreover, the heterogeneous expression of SK channels in the heart is not well defined even though a number of studies suggest that there is a transmural gradient in the expression of SK channels in failing ventricles¹⁰, and the expression of SK channels appears to be variable in the different regions of the atria²⁴. Detailed information on the region-specific expression of SK channels in the heart awaits further studies. Functional roles of SK channels in pacemaking cells and PSCs are only beginning to be recognized and need to be further explored. Finally, the role of SK channels in AF and VF is not completely defined. On the translational level, the use of SK channel blockers in the treatment of AF and the possible pro-arrhythmic effects of SK channel blockers in patients with HF require further considerations and investigations.

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References

1. Eisner DA, Vaughan-Jones RD. Do calcium-activated potassium channels exist in the heart? *Cell Calcium*. Dec.1983 4:371–386. [PubMed: 6323002]
2. Giles WR, Imaizumi Y. Comparison of potassium currents in rabbit atrial and ventricular cells. *J Physiol*. Nov.1988 405:123–145. [PubMed: 2855639]
3. Xu Y, Tuteja D, Zhang Z, et al. Molecular identification and functional roles of a Ca²⁺-activated K⁺ channel in human and mouse hearts. *J Biol Chem*. Dec 5.2003 278:49085–49094. [PubMed: 13679367]
4. Tuteja D, Xu D, Timofeyev V, Lu L, Sharma D, Zhang Z, Xu Y, Nie L, Vazquez AE, Young JN, Glatter KA, Chiamvimonvat N. Differential expression of small-conductance Ca²⁺-activated K⁺ channels SK1, SK2, and SK3 in mouse atrial and ventricular myocytes. *Am J Physiol Heart Circ Physiol*. Dec.2005 289:H2714–2723. [PubMed: 16055520]
5. Tuteja D, Rafizadeh S, Timofeyev V, Wang S, Zhang Z, Li N, Mateo RK, Singapuri A, Young JN, Knowlton AA, Chiamvimonvat N. Cardiac small conductance Ca²⁺-activated K⁺ channel subunits form heteromultimers via the coiled-coil domains in the C termini of the channels. *Circ Res*. Oct 1.2010 107:851–859. [PubMed: 20689065]
6. Li N, Timofeyev V, Tuteja D, et al. Ablation of a Ca²⁺-activated K⁺ channel (SK2 channel) results in action potential prolongation in atrial myocytes and atrial fibrillation. *J Physiol*. Mar 1.2009 587:1087–1100. [PubMed: 19139040]
7. Ozgen N, Dun W, Sosunov EA, Anyukhovskiy EP, Hirose M, Duffy HS, Boyden PA, Rosen MR. Early electrical remodeling in rabbit pulmonary vein results from trafficking of intracellular SK2 channels to membrane sites. *Cardiovasc Res*. Sep 1.2007 75:758–769. [PubMed: 17588552]
8. Zhang XD, Timofeyev V, Li N, Myers RE, Zhang DM, Singapuri A, Lau VC, Bond CT, Adelman J, Lieu DK, Chiamvimonvat N. Critical roles of a small conductance Ca²⁺-activated K⁺ channel (SK3) in the repolarization process of atrial myocytes. *Cardiovasc Res*. Feb 1.2014 101:317–325. [PubMed: 24282291]
9. Chen WT, Chen YC, Lu YY, Kao YH, Huang JH, Lin YK, Chen SA, Chen YJ. Apamin modulates electrophysiological characteristics of the pulmonary vein and the Sinoatrial Node. *European journal of clinical investigation*. Sep.2013 43:957–963. [PubMed: 23834267]
10. Chua SK, Chang PC, Maruyama M, et al. Small-conductance calcium-activated potassium channel and recurrent ventricular fibrillation in failing rabbit ventricles. *Circ Res*. Apr 15.2011 108:971–979. [PubMed: 21350217]
11. Lee YS, Chang PC, Hsueh CH, Maruyama M, Park HW, Rhee KS, Hsieh YC, Shen C, Weiss JN, Chen Z, Lin SF, Chen PS. Apamin-sensitive calcium-activated potassium currents in rabbit ventricles with chronic myocardial infarction. *J Cardiovasc Electrophysiol*. Oct.2013 24:1144–1153. [PubMed: 23718850]
12. Yu T, Deng C, Wu R, Guo H, Zheng S, Yu X, Shan Z, Kuang S, Lin Q. Decreased expression of small-conductance Ca²⁺-activated K⁺ channels SK1 and SK2 in human chronic atrial fibrillation. *Life Sci*. Jan 30.2012 90:219–227. [PubMed: 22154908]
13. Skibsbjerg L, Poulet C, Diness JG, Bentzen BH, Yuan L, Kappert U, Matschke K, Wettwer E, Ravens U, Grunnet M, Christ T, Jespersen T. Small-conductance calcium-activated potassium (SK) channels contribute to action potential repolarization in human atria. *Cardiovasc Res*. Jul 1.2014 103:156–167. [PubMed: 24817686]
14. Ni Y, Wang T, Zhuo X, Song B, Zhang J, Wei F, Bai H, Wang X, Yang D, Gao L, Ma A. Bisoprolol reversed small conductance calcium-activated potassium channel (SK) remodeling in a volume-overload rat model. *Mol Cell Biochem*. Dec.2013 384:95–103. [PubMed: 23975505]
15. Qi XY, Diness JG, Brundel BJ, Zhou XB, Naud P, Wu CT, Huang H, Harada M, Aflaki M, Dobrev D, Grunnet M, Nattel S. Role of small-conductance calcium-activated potassium channels in atrial electrophysiology and fibrillation in the dog. *Circulation*. Jan 28.2014 129:430–440. [PubMed: 24190961]
16. Ellinor PT, Lunetta KL, Glazer NL, et al. Common variants in KCNN3 are associated with lone atrial fibrillation. *Nat Genet*. Mar.2010 42:240–244. [PubMed: 20173747]

17. Ellinor PT, Lunetta KL, Albert CM, et al. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat Genet.* Jun.2012 44:670–675. [PubMed: 22544366]
18. Diness JG, Sorensen US, Nissen JD, Al-Shahib B, Jespersen T, Grunnet M, Hansen RS. Inhibition of small-conductance Ca^{2+} -activated K^{+} channels terminates and protects against atrial fibrillation. *Circ Arrhythm Electrophysiol.* Aug.2010 3:380–390. [PubMed: 20562443]
19. Diness JG, Skibsbye L, Jespersen T, Bartels ED, Sorensen US, Hansen RS, Grunnet M. Effects on atrial fibrillation in aged hypertensive rats by Ca^{2+} -activated K^{+} channel inhibition. *Hypertension.* Jun.2011 57:1129–1135. [PubMed: 21502564]
20. Skibsbye L, Diness JG, Sorensen US, Hansen RS, Grunnet M. The duration of pacing-induced atrial fibrillation is reduced in vivo by inhibition of small conductance Ca^{2+} -activated K^{+} channels. *J Cardiovasc Pharmacol.* Jun.2011 57:672–681. [PubMed: 21394037]
21. Chang PC, Turker I, Lopshire JC, Masroor S, Nguyen BL, Tao W, Rubart M, Chen PS, Chen Z, Ai T. Heterogeneous upregulation of apamin-sensitive potassium currents in failing human ventricles. *J Am Heart Assoc.* Feb.2013 2:e004713. [PubMed: 23525437]
22. Nagy N, Szuts V, Horvath Z, et al. Does small-conductance calcium-activated potassium channel contribute to cardiac repolarization? *J Mol Cell Cardiol.* Nov.2009 47:656–663. [PubMed: 19632238]
23. Hsueh CH, Chang PC, Hsieh YC, Reher T, Chen PS, Lin SF. Proarrhythmic effect of blocking the small conductance calcium activated potassium channel in isolated canine left atrium. *Heart Rhythm.* Jun.2013 10:891–898. [PubMed: 23376397]
24. Wagner S, Maier LS. Small conductance Ca-activated K channel: small but powerful proarrhythmogenic? *Heart Rhythm.* Jun.2013 10:899–900. [PubMed: 23395736]
25. Mahida S. Expanding role of SK channels in cardiac electrophysiology. *Heart Rhythm.* Jul.2014 11:1233–1238. [PubMed: 24681007]
26. Habermann E. Apamin. *Pharmacology & therapeutics.* 1984; 25:255–270. [PubMed: 6095335]
27. Adelman JP, Maylie J, Sah P. Small-conductance Ca^{2+} -activated K^{+} channels: form and function. *Annu Rev Physiol.* 2012; 74:245–269. [PubMed: 21942705]
28. Hugues M, Schmid H, Lazdunski M. Identification of a protein component of the Ca^{2+} -dependent K^{+} channel by affinity labelling with apamin. *Biochem Biophys Res Commun.* Aug 31.1982 107:1577–1582. [PubMed: 6291536]
29. Blatz AL, Magleby KL. Single apamin-blocked Ca^{2+} -activated K^{+} channels of small conductance in cultured rat skeletal muscle. *Nature.* Oct 23–29.1986 323:718–720. [PubMed: 2430185]
30. Kohler M, Hirschberg B, Bond CT, Kinzie JM, Marrion NV, Maylie J, Adelman JP. Small-conductance, calcium-activated potassium channels from mammalian brain. *Science.* Sep 20.1996 273:1709–1714. [PubMed: 8781233]
31. Strassmaier T, Bond CT, Sailer CA, Knaus HG, Maylie J, Adelman JP. A novel isoform of SK2 assembles with other SK subunits in mouse brain. *J Biol Chem.* Jun 3.2005 280:21231–21236. [PubMed: 15797870]
32. Stocker M. Ca^{2+} -activated K^{+} channels: molecular determinants and function of the SK family. *Nature reviews Neuroscience.* Oct.2004 5:758–770. [PubMed: 15378036]
33. Berkefeld H, Fakler B, Schulte U. Ca^{2+} -activated K^{+} channels: from protein complexes to function. *Physiol Rev.* Oct.2010 90:1437–1459. [PubMed: 20959620]
34. Chen MX, Gorman SA, Benson B, Singh K, Hieble JP, Michel MC, Tate SN, Trezise DJ. Small and intermediate conductance Ca^{2+} -activated K^{+} channels confer distinctive patterns of distribution in human tissues and differential cellular localisation in the colon and corpus cavernosum. *Naunyn Schmiedebergs Arch Pharmacol.* Jun.2004 369:602–615. [PubMed: 15127180]
35. Zhang Q, Timofeyev V, Lu L, Li N, Singapuri A, Long MK, Bond CT, Adelman JP, Chiamvimonvat N. Functional roles of a Ca^{2+} -activated K^{+} channel in atrioventricular nodes. *Circ Res.* Feb 29.2008 102:465–471. [PubMed: 18096820]
36. Xia XM, Fakler B, Rivard A, Wayman G, Johnson-Pais T, Keen JE, Ishii T, Hirschberg B, Bond CT, Lutsenko S, Maylie J, Adelman JP. Mechanism of calcium gating in small-conductance calcium-activated potassium channels. *Nature.* Oct 1.1998 395:503–507. [PubMed: 9774106]

37. Meadows LS, Isom LL. Sodium channels as macromolecular complexes: implications for inherited arrhythmia syndromes. *Cardiovasc Res.* Aug 15.2005 67:448–458. [PubMed: 15919069]
38. Catterall WA. Signaling complexes of voltage-gated sodium and calcium channels. *Neuroscience letters.* Dec 10.2010 486:107–116. [PubMed: 20816922]
39. Adsit GS, Vaidyanathan R, Galler CM, Kyle JW, Makielski JC. Channelopathies from mutations in the cardiac sodium channel protein complex. *J Mol Cell Cardiol.* Aug.2013 61:34–43. [PubMed: 23557754]
40. Maylie J, Bond CT, Herson PS, Lee WS, Adelman JP. Small conductance Ca^{2+} -activated K^+ channels and calmodulin. *J Physiol.* Jan 15.2004 554:255–261. [PubMed: 14500775]
41. Allen D, Fakler B, Maylie J, Adelman JP. Organization and regulation of small conductance Ca^{2+} -activated K^+ channel multiprotein complexes. *J Neurosci.* Feb 28.2007 27:2369–2376. [PubMed: 17329434]
42. Lu L, Zhang Q, Timofeyev V, Zhang Z, Young JN, Shin HS, Knowlton AA, Chiamvimonvat N. Molecular coupling of a Ca^{2+} -activated K^+ channel to L-type Ca^{2+} channels via alpha-actinin2. *Circ Res.* Jan 5.2007 100:112–120. [PubMed: 17110593]
43. Lu L, Timofeyev V, Li N, Rafizadeh S, Singapuri A, Harris TR, Chiamvimonvat N. Alpha-actinin2 cytoskeletal protein is required for the functional membrane localization of a Ca^{2+} -activated K^+ channel (SK2 channel). *Proc Natl Acad Sci U S A.* Oct 27.2009 106:18402–18407. [PubMed: 19815520]
44. Rafizadeh S, Zhang Z, Woltz RL, et al. Functional interaction with filamin A and intracellular Ca^{2+} enhance the surface membrane expression of a small-conductance Ca^{2+} -activated K^+ (SK2) channel. *Proc Natl Acad Sci U S A.* Jul 8.2014 111:9989–9994. [PubMed: 24951510]
45. Lu L, Sirish P, Zhang Z, Woltz RL, Li N, Timofeyev V, Knowlton AA, Zhang X, Yamoah EN, Chiamvimonvat N. Regulation of Gene Transcription by Voltage-Gated L-Type Calcium Channel, $\text{Ca}_v1.3$. *J Biol Chem.* Dec 23.2015
46. Marrion NV, Tavalin SJ. Selective activation of Ca^{2+} -activated K^+ channels by co-localized Ca^{2+} channels in hippocampal neurons. *Nature.* Oct 29.1998 395:900–905. [PubMed: 9804423]
47. Terentyev D, Rochira JA, Terentyeva R, Roder K, Koren G, Li W. Sarcoplasmic reticulum Ca^{2+} release is both necessary and sufficient for SK channel activation in ventricular myocytes. *Am J Physiol Heart Circ Physiol.* Mar 1.2014 306:H738–746. [PubMed: 24381116]
48. Mu YH, Zhao WC, Duan P, Chen Y, Zhao WD, Wang Q, Tu HY, Zhang Q. RyR2 modulates a Ca^{2+} -activated K^+ current in mouse cardiac myocytes. *PLoS One.* 2014; 9:e94905. [PubMed: 24747296]
49. Rosa JC, Galanakis D, Ganellin CR, Dunn PM, Jenkinson DH. Bis-quinolinium cyclophanes: 6,10-diaza-3(1,3),8(1,4)-dibenzena-1,5(1,4)-diquinolincyclodecaphane (UCL 1684), the first nanomolar, non-peptidic blocker of the apamin-sensitive Ca^{2+} -activated K^+ channel. *J Med Chem.* Jan 1.1998 41:2–5. [PubMed: 9438015]
50. Gentles RG, Grant-Young K, Hu S, Huang Y, Poss MA, Andres C, Fiedler T, Knox R, Lodge N, Weaver CD, Harden DG. Initial SAR studies on apamin-displacing 2-aminothiazole blockers of calcium-activated small conductance potassium channels. *Bioorganic & medicinal chemistry letters.* Oct 1.2008 18:5316–5319. [PubMed: 18774291]
51. Nattel S, Qi XY. Calcium-dependent potassium channels in the heart: clarity and confusion. *Cardiovasc Res.* Feb 1.2014 101:185–186. [PubMed: 24385343]
52. Olesen MS, Jabbari J, Holst AG, Nielsen JB, Steinbrüchel DA, Jespersen T, Haunso S, Svendsen JH. Screening of *KCNN3* in patients with early-onset lone atrial fibrillation. *Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology.* Jul.2011 13:963–967.
53. Mahida S, Mills RW, Tucker NR, Simonson B, Macri V, Lemoine MD, Das S, Milan DJ, Ellinor PT. Overexpression of *KCNN3* results in sudden cardiac death. *Cardiovasc Res.* Feb 1.2014 101:326–334. [PubMed: 24296650]
54. Sosunov EA, Anyukhovskiy EP, Hefer D, Rosen TS, Danilo P Jr, Janse MJ, Rosen MR. Region-specific, pacing-induced changes in repolarization in rabbit atrium: an example of sensitivity to the rare. *Cardiovasc Res.* Aug 1.2005 67:274–282. [PubMed: 15950206]

55. Strobaek D, Hougaard C, Johansen TH, Sorensen US, Nielsen EO, Nielsen KS, Taylor RD, Pedarzani P, Christophersen P. Inhibitory gating modulation of small conductance Ca^{2+} -activated K^+ channels by the synthetic compound (R)-N-(benzimidazol-2-yl)-1,2,3,4-tetrahydro-1-naphthylamine (NS8593) reduces afterhyperpolarizing current in hippocampal CA1 neurons. *Mol Pharmacol*. Nov.2006 70:1771–1782. [PubMed: 16926279]
56. Ling TY, Wang XL, Chai Q, et al. Regulation of the SK3 channel by microRNA-499--potential role in atrial fibrillation. *Heart Rhythm*. Jul.2013 10:1001–1009. [PubMed: 23499625]
57. Turker I, Yu CC, Chang PC, Chen Z, Sohma Y, Lin SF, Chen PS, Ai T. Amiodarone inhibits apamin-sensitive potassium currents. *PLoS One*. 2013; 8:e70450. [PubMed: 23922993]
58. Bonilla IM, Long VP 3rd, Vargas-Pinto P, et al. Calcium-activated potassium current modulates ventricular repolarization in chronic heart failure. *PLoS One*. 2014; 9:e108824. [PubMed: 25271970]
59. Gui L, Bao Z, Jia Y, Qin X, Cheng ZJ, Zhu J, Chen QH. Ventricular tachyarrhythmias in rats with acute myocardial infarction involves activation of small-conductance Ca^{2+} -activated K^+ channels. *Am J Physiol Heart Circ Physiol*. Jan 1.2013 304:H118–130. [PubMed: 23086994]
60. Chang PC, Hsieh YC, Hsueh CH, Weiss JN, Lin SF, Chen PS. Apamin induces early afterdepolarizations and torsades de pointes ventricular arrhythmia from failing rabbit ventricles exhibiting secondary rises in intracellular calcium. *Heart Rhythm*. Oct.2013 10:1516–1524. [PubMed: 23835258]
61. Kleger A, Seufferlein T, Malan D, et al. Modulation of calcium-activated potassium channels induces cardiogenesis of pluripotent stem cells and enrichment of pacemaker-like cells. *Circulation*. Nov 2.2010 122:1823–1836. [PubMed: 20956206]
62. Muller M, Stockmann M, Malan D, et al. Ca^{2+} activated K^+ channels-new tools to induce cardiac commitment from pluripotent stem cells in mice and men. *Stem Cell Rev*. Sep.2012 8:720–740. [PubMed: 22038332]

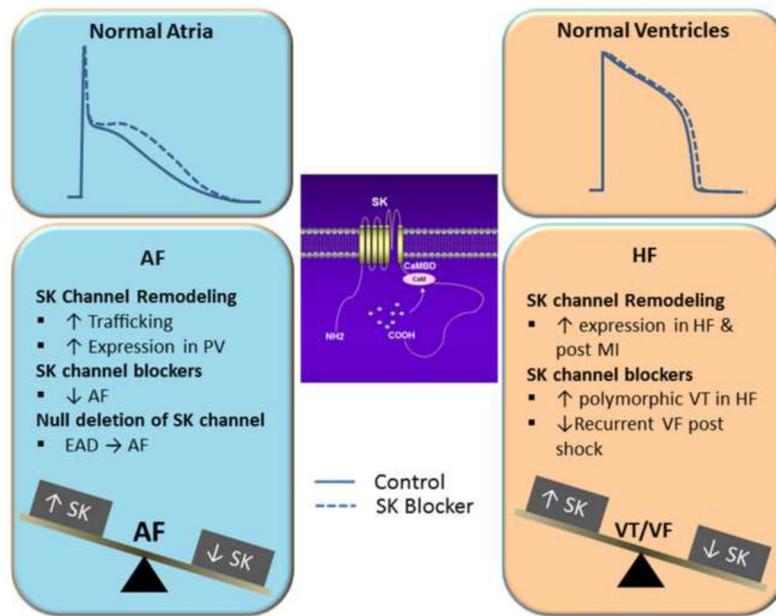


Figure 1. Functional roles of SK channels in normal and diseased hearts
 Distinct roles of SK channels in atria and ventricles are depicted together with remodeling in AF, HF and post MI. EAD, early afterdepolarization; PV, pulmonary veins, AF, atrial fibrillation; VT/VF, ventricular tachycardia and fibrillation.

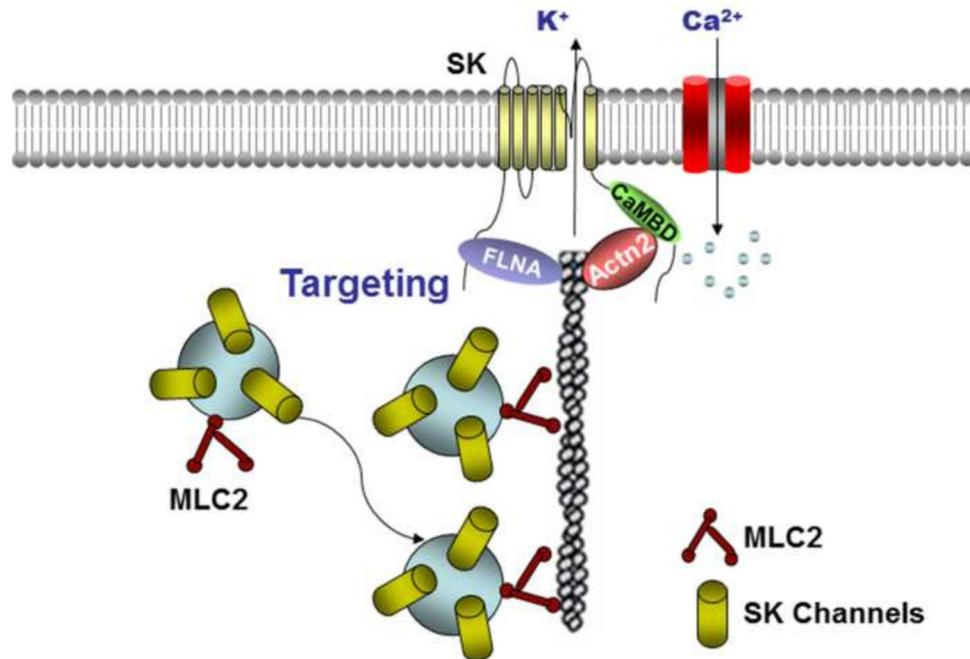


Figure 2. Localization, trafficking and molecular partners of cardiac SK channels

SK channels interactome includes α-actinin2 (Actin2), filaminA (FLNA), and myosin light chain 2 (MLC2). Cardiac SK channels have been shown to couple to L-type calcium channels through a physical bridge, α-actinin2. SK2 channels do not physically interact with the Ca²⁺ channels, instead the two channels co-localize *via* their interaction with α-actinin2 along the Z-line in atrial myocytes. An increase in intracellular Ca²⁺, as evident during rapid AF or atrial tachycardia, is predicted to increase SK2 channel expression leading to shortening of the atrial APs and maintenance of arrhythmias.