Ca\textsuperscript{2+}-Activated K\textsuperscript{+} Channels as Therapeutic Targets for Myocardial and Vascular Protection

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Small- and large-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (SKCa and BKCa, respectively) may be important targets for therapeutic interventions in a variety of cardiac conditions. In cardiomyocytes, BKCa channels are localized to mitochondria where they beneficially modulate reactive oxygen species, mitochondrial Ca\textsuperscript{2+}, and respiration. In vascular smooth muscle cells, BKCa channels regulate vascular tone and promote vasodilation. Activation of BKCa channels has demonstrated significant cardioprotection following ischemic injury, including improved function and reduced infarct size. SKCa channels are expressed in both the membrane and mitochondria of cardiomyocytes. Modulation of cardiomyocyte SKCa channels may be beneficial for arrhythmia, heart failure, and ischemia. Mitochondrial SKCa channels may provide similar benefit to BKCa channels. In addition, activation of SKCa channels on the endothelium promotes vasodilation. This mini-review focuses on the modulation of cardiomyocyte BKCa and SKCa channels for cardioprotection and briefly addresses associated potential therapeutic benefits in the coronary circulation. (Circ J 2015; 79: 455–462)

Key Words: Arrhythmia; Coronary circulation; Ion channels; Mitochondria; Myocardial ischemia
Expression in the Cardiovascular System

In cardiomyocytes, multiple lines of evidence indicate that BKCa channels are expressed in the inner membrane of mitochondria (Figure 1). Mitochondrial BKCa channel expression has been controversial, presumably because of the low levels of expression and less than optimal available antibodies. BKCa-like currents in mitochondria were first described by Xu et al, who also showed significant cardioprotection using the BKCa activator NS1619 in guinea pig hearts. Recently, BKCa-like currents were detected in mouse cardiac mitoplast preparations in 2 independent studies. BKCa-dependence of these currents was confirmed, as similar currents were absent in mitoplasts from BKCa knockout (KO) mice. In addition, Singh et al recently demonstrated that cardiomyocyte BKCa channels contain a novel C-terminal splice variant (termed DEC), which when expressed localizes the channel exclusively to cardiomyocyte mitochondria. Importantly, there is no known expression of BKCa channels on the cardiomyocyte plasma membrane. Activation of plasma membrane K⁺ currents could lead to altered action potential and arrhythmia, as seen with KATP modulating compounds.

In coronary VSMC, BKCa channel expression is more straightforward. They are expressed on the plasma membrane (Figure 2) where they modulate vascular tone and relaxation (see later). However, mitochondrial localization has not been investigated. VSMC BKCa channels are predominantly associated with the β1 subunit. Endothelial cell BKCa expression is controversial, but may include both plasmalemmal and mitochondrial expression. The remainder of the review will focus on cardiomyocyte and VSMC BKCa channels and we refer the reader to recent reviews concerning endothelial BKCa channels.

Pharmacologic Modulation

The field of BKCa-mediated cardioprotection and clinical translation has been significantly hampered by the lack of highly specific and potent pharmacological tools. The majority of studies examining BKCa activation have relied on the agents NS1619 and NS11021. Both drugs have documented BKCa-activating properties, with NS11021 showing considerably greater potency. Unfortunately, both are known to have a number of off-target effects that need to be considered carefully in interpretation of results (for review). Specificity is a problem with both drugs, with NS11021 displaying a narrow effective dose range and NS1619 capable of inhibiting L-type Ca²⁺ channels. Another potent BKCa activator is the polyphenol, rotterlerin (also known as mallotoxin (MTX)). Rotterlerin/MTX can shift the voltage-dependent activation of the channel (~70mV) and is effective in submicromolar concentrations. Importantly, rotterlerin acts in a distinct manner from other activators, as it is ineffective when LRRC26 is coexpressed with the channel, and is insensitive to the presence of particular splice inserts required for NS1619-dependent activation. Nevertheless, there are a number of specificity issues and off-
BKCa and SKCa Channels in Cardioprotection

BKCa and SKCa Channels

Structure/Function

The SKCa channels are not sensitive to membrane potential (in contrast to the BK channels), but are exclusively activated by intracellular [Ca\(^{2+}\)]. The SK channel family consists of 3 members: SK1 (KCa2.1, KCNN1), SK2 (KCa2.2, KCNN2), and SK3 (KCa2.3, KCNN3). The intermediate conductance Ca\(^{2+}\)-activated K\(^+\) channel (SK4, KCa3.1, KCNN4) is often considered a 4th member of the family because of its structural/functional similarities to SK1–3. A functional single SKCa channel that displays conductance of 5–20 pS (20–90 pS for SK4) is formed by 4 \(\alpha\) subunits of the same or different SK subtypes. Highly homologous \(\alpha\) subunits of all 4 SK subtypes have 6 transmembrane segments (S1–S6), with the pore region located between segments 5 and 6. The Ca\(^{2+}\) sensitivity of SKCa channels is conferred by a calmodulin (CaM) constitutively associated with the C-terminus of each \(\alpha\) subunit.

Apparent affinity of SK channels to Ca\(^{2+}\) is approximately 500 nmol/L, which is substantially higher than that of BKCa channels and it can be increased 5-fold when SK-bound CaM is dephosphorylated at threonine 79/80. Phosphorylation of SK-bound CaM is controlled by casein kinase 2 (CK2) and serine/threonine protein phosphatase 2 (PP2) localized to the channel.

Expression in the Cardiovascular System

SKCa channels are widely expressed in different tissues, including neuronal tissue, smooth and skeletal muscles, blood cells, cardiac myocytes, and vascular endothelium (Figure 2). SKCa subtypes exhibit differential distribution in the heart. Tuteja et al showed that in the mouse heart expression levels of SK1 and SK2 are approximately 50% higher in the atria than in the ventricles, while SK3 levels are similar. SK channels in mouse atria demonstrate the following mRNA expres-

Figure 2. Large- and small-conductance (BKCa and SKCa, respectively) channels in the vasculature. SKCa channels reside in the endothelium where they cause hyperpolarization, which can promote microdomain increases in Ca\(^{2+}\) to activate eNOS and other vasodilating agents or directly through intercellular communication. BKCa channels are predominantly on smooth muscle cells and respond to Ca\(^{2+}\) sparks from RyRs and are targets of numerous dilatory signals to limit depolarization and intracellular Ca\(^{2+}\). BKCa channels are also inhibited by vasoconstrictors. COX, cyclooxygenase; eNOS, endothelial nitric oxide synthase; IP3R, IP3 receptors; MLC-P, phosphorylated myosin light chain; PKG, protein kinase G; RyR, ryanodine receptor; TP, thromboxane prostanoid receptor; TXA2, thromboxane A2; VDCC, voltage dependent Ca\(^{2+}\) channels; Vm, membrane voltage. Note: for clarity, numerous intermediate steps and additional signals have not been shown.
sion pattern SK1>SK2>SK3. However, in humans, mRNA levels of SK2 and 3 were reported to be higher than that of SK1. The KCa current in vascular endothelium is mediated by SK3 and the intermediate conductance KCa channel SK4.

**Intracellular SKCa** It is important for ion channels to be precisely localized to specific subcellular microdomains to perform specific tasks. Precise function of the KCa channels that translate changes in local [Ca^{2+}] into changes in membrane potential largely depends on the source of Ca^{2+}. Lu et al demonstrated that in atrial myocytes SK2 is coupled with the plasmalemmal voltage-gated Ca^{2+} channel Cav1.3 and to a lesser degree to Cav1.2 via the cytoskeletal protein α-actinin. Importantly, in cultured rat ventricular myocytes overexpressing SK2 we recently demonstrated that Ca^{2+} release from the sarcoplasmic reticulum (SR) Ca^{2+} channels’ ryanodine receptors (RyR2’s) is not only necessary but also sufficient to evoke hyperpolarizing Isk. In parallel, Mu et al showed that RyR2-mediated SR Ca^{2+} release is at least in part responsible for activation of atrial plasmalemmal SKs. Colocalization of SKCa channels with voltage-dependent Ca^{2+} channels points to their involvement in repolarization of the action potential. However, responsiveness of SKs to SR Ca^{2+} release suggests that they can play an additional important role counteracting the depolarizing force of the Na+/Ca^{2+} exchanger. This is essential not only for modulating the late phase of the action potential, but in diastole as well; attenuating adverse effects of spontaneous activation of RyR2’s that can promote arrhythmia.

In addition to plasmalemmal localization, the presence of functional SKs was shown in the inner mitochondria membrane (msKCa) of cardiomyocytes (Figure 1). In theory, depending on “sidedness”, the msKCa channel may sense fluctuations of intramitochondrial [Ca^{2+}] or cyclical elevations of cytosolic [Ca^{2+}] during Ca^{2+} transients that initiate energy-demanding contractions. Although the exact role of msKs in healthy myocytes is currently unknown, it is conceivable to propose that by linking intracellular [Ca^{2+}] to mitochondrial K+ flux they provide additional positive feedback regulating mitochondrial bioenergetics to finely tune ATP production with metabolic demand.

**Endothelial SKCa** In the arterial endothelium, SK3 and SK4 are considered to be the key components of “endothelium-derived hyperpolarizing factor (EDHF)” activation. Activation of either of these channels is thought to play an important role in initializing EDHF-dilator responses in resistance-sized and conduit arteries because of electrical coupling of endothelial and smooth muscle cells or via changes in near-membrane [K+]. Despite apparent overlap in function, SK3 and SK4 are thought to contribute to mechanistically different EDHF-signaling pathways, because of their distinct subcellular localization. SK3 channels are found predominantly in caveolin-1 rich compartments, which suggests that they may participate in NO synthesis by endothelial NO synthase (eNOS) localized to these regions by generating the driving force to Ca^{2+} entry, which is important for activation of eNOS. Ca^{2+} for activation of endothelial SK3 most probably originates from adjacent Ca^{2+}-influx channels of the TRP gene family regulated by G-protein coupled receptors. In contrast, SK4 channels localized in caveolin-free regions are thought to be activated by Ca^{2+} released from the endoplasmic reticulum through IP3Rs.

Interestingly, endothelial SK channels appear to play an important role in pulmonary vein (PV) physiology. In non-denuded PVs, inhibition of SK channels increased PV spontaneous activity and tension, whereas in denuded PVs spontaneous activity was lower and tension remained unchanged in the presence of SK blocker.

**BKCa Channels as Therapeutic Targets for Cardiomyocyte and Vascular Protection**

Activation of BKCa channels in cardiomyocyte mitochondria promotes enhanced survival and contractile function without electrophysiologic alterations in the cardiomyocyte. In VSMC, BKCa channels modulate basal vascular tone and promote vasodilation. Therefore, agents that activate BKCa channels may be ideal therapeutics for mitigation of myocardial ischemic injury, associated with impaired cardiomyocyte contraction and survival, as well as vascular perturbations including enhanced vasoconstriction and propensity for coronary vasospasm.

**BKCa Channels and Myocardial Protection**

Activation of BKCa channels in the myocardium has been repeatedly associated with ischemic cardioprotection. The most used activator, NS1619, has been used in multiple studies and species to reduce infarct size and improve myocardial function in ischemia-reperfusion (IR) models (reviewed by Bentzen et al). In whole hearts, Bentzen et al. demonstrated that activation of BKCa channels with NS11021 dose-dependently reduced infarct size and improved left ventricular developed pressure (LVDP) following 35 min global ischemia when given before or after reperfusion. Importantly, BKCa activation reduced left ventricular end-diastolic pressure (LVEDP) and ischemic contracture, possibly indicating improved energy reserve/utilization during ischemia. In addition, Solysinska et al recently demonstrated that IPC (ischemic preconditioning)-dependent reductions in infarct size are completely blocked in BKCa KO animals. Multiple lines of evidence indicate that BKCa channels are likely an important end effector of IPC signals (additional review in Walters et al). Given the pharmacological concerns just described, some of the best recent evidence supporting BKCa-dependent cardioprotection comes from studies using BKCa KO mice. Two independent groups examining myocardial infarction (MI) in the whole heart using global BKCa KO mice with and without the established BKCa activators, NS11021 and NS1619, reported reduced infarct size and improved cardiac function that was absent in BKCa KO mice. Singh et al demonstrated exclusive expression of BKCa channels in cardiac mitochondria owing to a specific splice insert, and attributed cardioprotection to improved mitochondrial function. However Wojtovich et al showed that some of the cardiac mitochondrial effects of both NS1619 and NS11021 were independent of BKCa channels, but that whole hearts were protected potentially through BKCa activation in cardiac neurons. Both studies highlight the potential beneficial effects of BKCa activation; however the study by Wojtovich et al clearly demonstrates the less than optimal specificity of the most used activators NS11021 and NS1619 and a current need for tissue-specific BKCa deletion.

Our group recently demonstrated in rats that BKCa activation with rottlerin, significantly improved myocardial functional recovery following 2h of ischemic hypothermic cardioplegic arrest. Although, not associated with significant infarct development, this is a model of mild ischemic injury that develops myocardial stunning. BKCa activation with rottlerin significantly improved LVDP and dp/dt, as well as promoted enhanced coronary flow. We have also confirmed specificity of rottlerin’s effects using wildtype and BKCa KO mice, as well as verified significant cardioprotection with rottlerin-supplemented cardioplegia in a pig model of cardioplegia and cardiopulmonary bypass (unpublished observations). Myocardial stunning associated with mild ischemic insults is an additional...
area where BKCa activators may have significant therapeutic value.

**Mechanism of Mitochondrial BKCa-Channel-Mediated Cardioprotection**

Activation of mitochondrial BKCa channels results in K⁺ influx from the cytosol to the matrix given the purported large K⁺ gradient. The exact mechanism of BKCa-mediated ischemic cardioprotection is unknown, but may involve reductions in mitochondrial Ca²⁺ overload, reductions in reactive oxygen species (ROS), modulation of mitochondrial matrix volume, and beneficial modulation of respiration. These may all have the effect of reducing mitochondria permeability transition pore (MPTP) opening and subsequent cell death.  

**Mitochondrial Ca²⁺** BKCa-mediated reductions in mitochondrial Ca²⁺ are thought to occur through limitation of Ca²⁺ influx. During ischemia, cytosolic Ca²⁺ increases, which can lead to mitochondrial Ca²⁺ overload. Increased matrix Ca²⁺ is a potent stimulator of MPTP opening. Activation of the BKCa channel and increased matrix K⁺ is thought to lead to limited depolarization of the mitochondria, which subsequently limits the electrochemical driving force for Ca²⁺. 8 Stowe et al. showed reduced Ca²⁺ influx with IR following NS1619 pretreatment.40 In addition, Singh et al. showed that mitochondria treated with NS1619 had increased resistance to Ca²⁺-induced MPTP opening.7 Interestingly, both the BKCa activators, NS11021 and NS1619, elicited minor if any detectable depolarization of mitochondrial membrane potential when used at appropriate BKCa-activating concentrations. 15,41 Therefore, charge-mediated limitations of Ca²⁺ influx would likely be equally mild. It is possible that reductions in mitochondrial Ca²⁺ and MPTP opening may be mediated by other as yet unidentified K⁺-dependent mechanisms.

**Modulation of ROS** ROS are generated during ischemia and large increases in mitochondrial ROS are generated during early reperfusion. The majority of ROS is generated by aberrant electron transfer in complexes I and III during IR. Importantly, increased ROS is thought to further damage the electron transport chain (ETC), leading to even greater generation of ROS following IR.42 ROS can lead to oxidant-dependent damage of the Ca²⁺ handling machinery, contractile apparatus, inefficient respiration and promote MPTP opening. Multiple studies have demonstrated that activation of mitochondrial BKCa channels can cause significant reductions in ROS generation. In brain isolated mitochondria, Kuliwiar et al demonstrated reduced ROS following CGS7184 (another BKCa activator) and NS1619 treatment.43 Soltysinska et al. recently showed that cardiac mitochondria from BKCa KO mice have increased ROS production post anoxia.8 Our group has demonstrated reduced mitochondrial ROS following in vitro hypoxia/reoxygenation in H9c2 cells following treatment with rottlerin.37 In isolated working guinea pig hearts, Stowe et al. demonstrated significant reductions in ROS production following global IR injury with the BKCa activator NS1619.40 NS1619 was also shown to attenuate ROS production in isolated mitochondria under conditions to stimulate reverse electron flow.44 The overall consensus is that activation of the BKCa channel limits ROS following IR, and that this may be one of the major cardioprotective benefits of channel activation.

**Mitochondrial Volume Homeostasis** Activation of mitochondrial K⁺ channels is known to alter mitochondrial volume and induce mitochondrial swelling.45 The BKCa channel activator NS11021 has been shown to induce swelling.15 It is currently unclear what potential cardioprotective role mitochondrial swelling may impart; however, possibilities include beneficial modulation of mitochondrial/cristae architecture and stimulation of respiration.15,45

**Mitochondrial Respiration** Recent studies have established a clear connection between mitochondrial respiration and BKCa activation. Following IR injury Heinen et al demonstrated more efficient electron flow with NS1619 treatment.46 In addition, multiple groups have documented increased respiration following BKCa activation using NS1619 and NS11021.41,46 However, in endothelial cells, ibetoxin-sensitive NS1619-induced respiration was associated with increased non-phosphorylating respiration or proton leak.47 In addition, deletion of the channel was shown to impair mitochondrial respiration and reduce respiratory control ratios in mitochondria isolated from BKCa KO mice.8 Our group has recently demonstrated enhanced respiration by rottlerin following in vitro hypoxic CP/R using H9c2 cardiomyoblasts (unpublished observations). Importantly, Bednarzyk et al showed in astrocytoma cells that BKCa channel subunits associate with mitochondrial ETC complexes and that enhanced mitochondrial respiration inhibits BKCa channel opening.48 These findings raise the interesting possibility that BKCa channels promote increased ETC activity and are in turn negatively regulated by the ETC. Generally, enhanced respiration is thought to significantly contribute to enhanced ROS generation. However, BKCa channel activation is paradoxically associated with enhanced respiration while decreasing mitochondrial-derived ROS production. Elucidation of the responsible mechanism will have significant implications for cardioprotection as well as metabolism in general.

**BKCa Channels and Vascular Protection** As opposed to cardiomyocytes, BKCa channels in VSMC reside on the plasma membrane (Figure 2). In this section, we will provide a brief overview of relevant VSMC BKCa channel regulation. For more in depth analysis we direct the reader to some excellent reviews.1,2 BKCa channels facilitate eflux of K⁺, which counteracts depolarization thus promoting vasodilation. In VSMC, BKCa are in close proximity to rymodine receptors, which can activate the channel through microdomain increases in Ca²⁺.1 As they are also voltage sensitive, increased depolarization of the membrane promotes activation of the channel to counteract contraction via inhibition of voltage dependent Ca²⁺ channels and regulate vascular tone.1

Consistent with the important role in modulating vascular tone and dilation, BKCa α-subunit KO mice exhibit elevated blood pressure; however, some of these effects may be related to additional BKCa-dependent processes leading to hypoaldosteronism.47 Nevertheless, those researchers noted diminished dilation to cGMP and elevated myogenic tone in isolated vessels. In addition, BKCa KO animals demonstrate increased frequency of spontaneous contraction in both bladder and aortic vascular smooth muscle.48,49 Deletion of the BKCa β1 subunit was also demonstrated to increase blood pressure, but this result has been questioned in subsequent studies.50-52 It is interesting to note that genetic deletion of the BKCa α subunit results in only mild increases in blood pressure. Therefore, it is quite probable that these mice may have compensatory increases of additional vascular K⁺ channels. This view is supported by the observation that BKCa KO mice have enhanced responses to hydrogen sulfide, (known to activate additional vascular K⁺ channels).48 Evidence indicates that BKCa activation is a general mechanism to promote dilation; however, recent studies have demonstrated a more complex role of BKCa in the regulation of
specific vasoreactive signaling cascades. Li et al have shown that the thromboxane receptor (TP receptor) physically interacts with and antagonizes activation of BKCa.53 BKCa inhibition appears to be a common signaling consequence of numerous vasoconstrictors (reviewed by Hu and Zhang). Similarly, BKCa activation can blunt thromboxane-induced constrictive responses, but these effects are specific to thromboxane, as phenylephrine-induced constriction is unaffected by BKCa channel activation.88,54 Therefore, BKCa activation may selectively modulate specific vasoreactive cascades based on the particular mediator and intracellular binding partners.

Overall, activation of the channel would be highly beneficial in the vasculature following ischemic insults to help improve flow to damaged tissues. Vascular abnormalities following IR injury include enhanced contraction and a propensity for vaso-spasm (following periods of enhanced dilation because of reactive hyperemia).55 Indeed, BKCa activation during hypothermic IR significantly increases coronary flow, which is associated with improved function.56 In addition, precipitating conditions of ischemic heart disease (hypercholesterolemia, diabetes, and metabolic syndrome) are all associated with impaired vasodilation and altered vasoactivity. A large body of work (outside the scope of the current review) demonstrates that vascular perturbations in these diseases are all associated with altered expression, activity, and upstream signaling associated with BKCa channel subunits (for review54,55). Therefore, activation of BKCa channels may be an important strategy to normalize vascular impairments in acute IR injury and chronic conditions associated with ischemic heart disease.

**SKCa and Cardiac Pathology**

**SKCa, Atrial Fibrillation (AF), and Conduction Disturbances**

AF is the most common form of arrhythmia and the potential pathophysiological role of SKCa channels in AF is a subject of extensive research.21 Two recent genome-wide association studies demonstrated a link of 2 variations of the gene encoding SK3 channels (KCNN3) with AF.21 Reduced expression levels of SKCa channels were demonstrated in human patients with chronic AF, suggestive of the important role of SK channels in maintaining normal atrial function.26,57 Furthermore, in mice, ablation of SK2 resulted in prolongation of the atrial action potential duration (APD) and enhanced inducibility of AF. Optical mapping experiments in the atra from healthy canine hearts also showed that inhibition of SKCa channels prolongs and enhances heterogeneity of the APD, thereby promoting arrhythmia by inducing alternans and wavebreaks (for review39).

Paradoxically, several recent studies demonstrated that inhibition of I-sk can be protective against atrial arrhythmia (for review21). In healthy hearts from guinea pigs, rats, and rabbits, blockade of SKCa channels was shown to terminate or prevent AF induced by burst pacing and/or acetylcholine infusion. Similar results were obtained in aging hypertensive rats. These effects were attributed to increases in the atrial effective refractory period caused by SKCa-inhibition. In addition, upregulation of SKCa channels is thought to contribute to early atrial remodeling. In rabbits, intermittent burst pacing for 3h induced enhanced trafficking of SK2 towards the plasma membrane, which resulted in APD shortening localized to the PV-atria interface, thus providing a basis for the evolution of arrhythmogenic substrate. In dog atria, 7-day tachypacing induced upregulation of SK1 channels in both the atria and PVs.24 In vivo experiments showed that in this model SKCa channels contribute to AF maintenance and their inhibition significantly prolongs atrial refractoriness and reduces AF duration. However, these studies need to be interpreted cautiously because pharmalogic SKCa channel modulators are subject to numerous off-target effects,21 as described for BKCa channels.

Malignant effects of SKCa upregulation in the conduction system were confirmed in transgenic mice overexpressing SKs.39 Mice overexpressing SK3 exhibited an enhanced propensity to sudden cardiac death from conduction abnormalities resulting in bradycardia and atioventricular (AV) block. Interestingly, SK2 overexpressing mice did not show AV block, but exhibited significantly increased firing rate of AV nodal cells resulting in shortening of the PR interval. The underlying causes of such discrepancy are currently unknown.

**SKCa and Ventricular Arrhythmia/HF**

SKCa channels, expressed in ventricular myocytes from healthy hearts, are purported to be functionally dormant.21 However, in HF, the contribution of I-sk to repolarization during AP was demonstrated; and both an increase in expression levels and sensitivity to [Ca2+] of SKCa channels in animal models and human HF have been established.31,56 Enhanced propensity to ventricular tachyarrhythmia is a major contributor to the death of patients with HF. Experiments using a rabbit model of tachypacing-induced cardiomyopathy demonstrated heterogeneous upregulation of SK channels, which can contribute to shortening of the APD and the development of ventricular fibrillation at fast rates.21,60 Inhibition of SK Ca channels was thought to be protective, reducing the substrate for reentrant arrhythmias by prolongation and increased uniformity of APD. However, at slow stimulation rates in optically mapped hearts the SKCa channels were shown to play an important positive role by attenuating HF-induced reduction in repolarization reserve. In addition to the major loss in repolarizing K+ currents, HF is accompanied by profound changes in intracellular Ca2+ homeostasis that lead to enhanced propensity to spontaneous SR Ca2+ release mediated by hyperactive RyR2s.61 We recently showed that SKCa channels overexpressed in rat ventricular myocytes are responsive to spontaneous Ca2+ waves and can effectively diminish the amplitude of pro-arrhythmic afterdepolarizations.29 In line with our findings, single-cell electrophysiology studies of ventricular myocytes from a chronic canine model of HF demonstrated that SKCa inhibition effectively evokes AP instability.59 Underscoring a protective role of SKCa channels in diseased hearts, blockade of SKCa channels in rabbit failing hearts was shown to enhance predisposition to Ca2+-dependent triggers exacerbating arrhythmic potential.60

**IR Injury/MI**

Recent findings from a rat model of acute MI indicate that SKCa channels in ventricular myocytes can be functionally recruited nearly instantaneously and subsequently evoke APD shortening in the MI zone within 10 min after ligation of the left anterior descending coronary artery.62 APD shortening in the MI zone was abolished by administration of SKCa inhibitors, resulting in attenuation of spontaneous sustained ventricular tachycardia/fibrillation and inducibility of tachyarrhythmias in acute MI rat hearts. Importantly, SKCa inhibition did not produce any changes in the remote myocardium. The authors propose that reduction in the dispersion of repolarization induced by SK-inhibition may contribute to decreased reentry and thereby the substrate for tachyarrhythmia in acute MI hearts. The role of SKCa channels in healed MI is currently not known.

**Mitochondrial SKCa**

Recently, SKCa channels were found in the inner mitochondria membrane of ventricular myocytes.31
It has been proposed that activation of SKCa channels may be protective against IR injury, similar to mBKCa and KATP (see earlier). Indeed, pharmacological preconditioning with a SK agonist resulted in a 2-fold increase in LV pressure on reperfusion in guinea pig hearts and a dramatic decrease in infarct size vs. non-treated hearts associated with reduced superoxide and mito[Ca\(^{2+}\)], and less severe changes in NADH and FAD during IR. These effects were completely abolished by co-administration of SKCa inhibitor.

**SKCa and Vascular Disease Perturbations**

The information about possible perturbations in vascular endothelium SK expression/function in cardiac disease remains limited. In general, hypertension is associated with a substantial decrease in SKCa channels, contributing to reduced dilatory responses. Further studies are needed to determine whether use of activators of SKCa channels is a viable option to enhance circulation in injured or diseased hearts.

**Conclusions**

In conclusion, K\(_{Ca}\) channels may be important targets for therapeutic interventions in a variety of cardiac conditions. Mitochondrial BKCa channels promote improved survival and cardiac function after ischemic insults. BKCa-mediated protection appears to be mediated by improved mitochondrial function, reduction in ROS, and modulation of respiration. BKCa activation during ischemic insults and/or chronic vascular disturbances would also be beneficial for improved vascular dilation. The situation with SKCa channels is less clear. For cardiomyocyte membrane SKCa channels, depending on disease and specific tissue (e.g., atria vs. ventricles), both activation and inhibition may provide protection from arrhythmic disturbances. Activation of the membrane channels may reduce pro-arrhythmic triggers, whereas inhibition is thought to beneficially limit maintenance of arrhythmia. On the other hand, endothelial and mitochondrial SKCa channels may provide cardioprotective benefit similar to BKCa channels. Unfortunately, specificity of the available pharmacological agents to modulate both BKCa and SKCa channels is suboptimal. Development and use of more specific activators/inhibitors and increased use of genetic mouse models will help settle controversies and verify the potentially important therapeutic value of K\(_{Ca}\) channels.

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