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Endogenous and agonist-induced opening of mitochondrial big *vs*. small Ca^{2+}-sensitive K^+ channels on cardiac cell and mitochondrial protection

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Abstract

Both big (BK_{Ca}) and small (SK_{Ca}) conductance Ca^{2+}-sensitive K^+ channels are present in mammalian cardiac cell mitochondria (m). We used pharmacological agonists and antagonists of BK_{Ca} and SK_{Ca} channels to examine the importance of endogenous opening of these channels and the relative contribution of either or both of these channels to protect against contractile dysfunction and reduce infarct size after ischemia reperfusion (IR) injury through a mitochondrial protective mechanism. Following global cardiac IR injury of *ex vivo* perfused guinea pig hearts we found the following: both agonists NS1619 (for BK_{Ca}) and DCEB (for SK_{Ca}) improved contractility; BK_{Ca} antagonist paxilline (PAX) alone or with SK_{Ca} antagonist NS8593 worsened contractility and enhanced infarct size; both antagonists PAX and NS8593 obliterated protection by their respective agonists; BK_{Ca} and SK_{Ca} antagonists did not block protection afforded by SK_{Ca} and BK_{Ca} agonists, respectively; and all protective effects by the agonists were blocked by scavenging superoxide anions ($O_2^{\bullet-}$) with TBAP. Contractile function was inversely associated with global infarct size. In *in vivo* rats, infusion of NS8593, PAX, or both antagonists enhanced regional infarct size while infusion of either NS1619 or DCEB reduced infarct size. In cardiac mitochondria isolated from *ex vivo* hearts after IR, combined SK_{Ca} and BK_{Ca} agonists improved respiratory control index (RCI) and Ca^{2+} retention capacity (CRC) compared to IR alone, whereas the combined antagonists did not alter RCI but worsened CRC. Although the differential protective bioenergetics effects of endogenous or exogenous BK_{Ca} and SK_{Ca} channel opening remain unclear, each channel likely responds to different sensing Ca^{2+} concentrations and voltage gradients over time during oxidative stress-induced injury to individually or together protect cardiac mitochondria and myocytes.

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Keywords

Cardiac mitochondria; inner mitochondrial membrane; cell signaling; ischemia reperfusion injury; oxidant stress; large and small conductance Ca^{2+} -sensitive K^+ channels

Introduction

Altered mitochondrial (m) bioenergetics, excess reactive oxygen species (ROS) emission, and excess mCa^{2+} overload, are major factors that underlie ischemia and reperfusion (IR) injury.[1] Prophylactic measures targeted to mitochondria that reduce cardiac IR injury[2, 3] include ischemic preconditioning (IPC, i.e., brief pulses of ischemia and reperfusion before longer index ischemia) and pharmacologic pre-conditioning (PPC), i.e., cardiac protection elicited some time after the drug is washed out before index IR. Drugs that reduce cardiac metabolism or mitochondrial bioenergetics when given before and or after IR also afford protection.

The induction of mitochondrial (m) K^+ influx is now recognized as an important underlying mechanism to reduce IR injury. We reported previously that activation by NS1619 of the large (big) conductance Ca^{2+}-sensitive K^+ channel ($BK_{Ca,}$ $K_{Ca}1.1_{,}$ maxi-K), that is found in the cardiac myocyte inner mitochondrial membrane (IMM), induced pharmacologic preconditioning (PPC) and that this protection was blocked by the mitochondrial targeted superoxide anion ($O_2^{\bullet -}$) scavenger TBAP.[4] We subsequently reported that NS1619 had biphasic effects on mitochondrial respiration, membrane potential ($\Delta\Psi_m$), and $O_2^{\bullet -}$ emission in isolated mitochondria.[5, 6] Due to our findings and many other reports on the protective effects of BK_{Ca} and putative K_{ATP} channel agonists in mitochondria, it is likely that opening of other mK^+ channels might also be protective but rely on different ligands and conditions for activation.

K_{Ca} channels of intermediate or small conductance were first identified in non-cardiac cells[7–10] as membrane bound, calmodulin (CaM)-dependent and activated by Ca^{2+}. These channels have smaller unit conductances of 3–30 (small, SK_{Ca}) and 20–90 (intermediate IK_{Ca}) pS.[11] The opening of SK_{Ca} channels is initiated by Ca^{2+} binding to calmodulin within the C terminus of the channel to form a dimer.[12, 13] Of the four known genes encoding SK_{Ca} channel proteins, one of these, the $K_{Ca}2.3$ (aka SK3) isoform, was found in vascular endothelial cell membranes where it exerted a potent, tonic hyperpolarization that promoted reduced vascular smooth muscle tone.[14] Later, the isoform $K_{Ca}2.2$ (aka SK2) was found in rat and human heart cell sarcolemma membranes by Western blot and RT-PCR.[15] Amplification of the channel from total atrial and ventricular RNA showed much greater expression in atria than in ventricles, and electrophysiological recordings exhibited much greater atrial than ventricular sensitivity to AP repolarization by apamin, a selective SK_{Ca} antagonist.[15, 16]

Dolga et al.[17] found that the SK2 channel was expressed also in mitochondria of neuronal HT-22 cells. At the same time, we furnished the first evidence for the presence of SK2 and SK3 isoforms in the IMM of guinea pig cardiac mitochondria.[18] We tested the functionality of SK_{Ca} channel opening[18] by perfusing isolated hearts transiently before ischemia with the

$K_{Ca}3.1$ ($IK_{Ca}1$)[7, 9, 19] and $K_{Ca}2.2$ and $K_{Ca}2.3$[20–23] opener DCEBIO (DCEB). We found[18] that DCEB elicited PPC in a manner similar to that of the BK_{Ca} channel agonist NS1619.[4] Either the SK_{Ca} antagonist NS8593[24, 25] or TBAP, which dismutates $O_2^{\bullet-}$ to H_2O_2, abolished the cardioprotection.[18] DCEB also reduced the deleterious effects of IR injury on mitochondrial bioenergetics by attenuating cardiac mitochondrial Ca^{2+} overload and $O_2^{\bullet-}$ levels, and by preserving (more reduced redox state) cardiac mitochondrial NADH and FAD levels after IR.[18] These studies established that K_{Ca} channel opening initiates a cascade of $O_2^{\bullet-}$-dependent cardiac protective mechanisms that are based primarily in cardiac myocyte mitochondria. In a recent extension of this research,[26] we: a) confirmed SK_{Ca} protein localization in cardiac ventricular mitochondria of rats and humans, in addition to guinea pigs; b) identified mitochondrial splice variants of SK3 in guinea pig and human cardiac mitochondria; and c) found that overexpression or silencing of SK3 reduced or enhanced, respectively, stress induced damage in two cardiac cell lines, HL-1 and H9c2.

Our aim in the present study was to determine if endogenous opening of the BK_{Ca} and or SK_{Ca} channel protected hearts and mitochondria from IR injury and if there were differential or additive cardioprotective effects of drug-induced opening of BK_{Ca} and SK_{Ca} channels. To test this we: a) conducted a pharmacological comparison of SK_{Ca} and BK_{Ca} agonists and antagonists given before and after ischemia on global ventricular infarct size and developed left ventricular pressure (LVP) in isolated *ex vivo* perfused guinea pig hearts; b) examined the effect of SK_{Ca} and BK_{Ca} agonists and antagonists on regional infarct size when infused in rats *in vivo*; c) assessed the role of mitochondrial-targeted $O_2^{\bullet-}$ scavenging by TBAP to block protection by the two agonists; and d) measured respiratory control index (RCI) and Ca^{2+} retention capacity (CRC) after IR injury to further assess the likely role of mitochondria in protecting or impairing cardiac function and damage by treating *ex vivo* perfused hearts with SK_{Ca} and BK_{Ca} agonists or antagonists before and after IR injury.

Materials and methods

Function and Infarction in Ex Vivo Guinea Pig Hearts after Global IR Injury

The investigation conformed to the Guide for the Care and Use of Laboratory Animals (NIH Publication 85-23, revised 1996); all animal protocols were reviewed and approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee. The hearts of adult guinea pigs of either sex were isolated and prepared as described previously in detail.[4, 18, 27–33] Guinea pig hearts were chosen because of their more human-like cardiac action potentials and ion currents compared to rats.[34] In brief, animals were injected intraperitoneally with heparin (1000 units) and ketamine (50 mg/kg). Once anesthetized, thoracotomy was performed, hearts were excised, and their aortas immediately perfused in retrograde manner with a cold modified Krebs-Ringer's (KR) perfusate. After aortic cannulation, hearts were transferred to a Langendorff perfusion setup and instrumented with a saline filled balloon and transducer to measure isovolumetric, developed (systolic-diastolic) LVP and perfused at a constant aortic root pressure of 55 mmHg with the KR solution at 37°C. Heart rate was measured from right atrial and ventricular recording electrodes, and coronary (aortic) flow was measured with a flow transducer. Hearts were subjected to 35 min global ischemia and 120 min reperfusion; hearts not used for

mitochondrial isolation were stained with 2,3,5-triphenyltetrazolium chloride (TTC) and infarct size was determined as a percentage of total ventricular heart weight.[4, 18, 28, 32] Infarct size measurements were blinded to the drug administered.

Isolated guinea pig hearts (18 groups of 6–8 hearts each) were perfused with no drugs (control) or test drugs, i.e. BK_{Ca} or SK_{Ca} agonists or antagonists, or TBAP alone or in combination, before and after the 35 min period of global ischemia. Time control (TC) experiments were also conducted, i.e., perfusion of isolated hearts with vehicle (DMSO) but without drugs or IR for the duration of the IR protocol. Time control (TC, no IR) functional variables decreased less than 5% over a 2.5 h period. To assess endogenous cardioprotection mediated by IR-induced BK_{Ca} and SK_{Ca} channel activation, antagonists were given beginning 20 min before ischemia. To assess exogenous BK_{Ca} and or SK_{Ca} activation, agonists were given beginning 15 min before ischemia; all drugs were discontinued 20 min after the onset of reperfusion. Functional variables for display in figures were recorded at 25 min before ischemia (baseline) and at 120 min reperfusion. Concentrations of drugs were gauged to elicit maximal effects as reported in the literature.[35–38] Drugs were dissolved in less than 0.1% DMSO in KR buffer (vehicle) and perfused into isolated hearts. They were: 3 μM DCEBIO (DCEB), a non-selective $K_{Ca}2.2$ and $K_{Ca}2.3$ channel agonist;[7, 20–23] 10 μM NS8593, a specific antagonist of SK_{Ca} channels;[24, 25] 3 μM NS1619, an agonist of BK_{Ca} channels; 40 μM paxilline (PAX), an antagonist of BK_{Ca} channels,[39] and 20 μM TBAP, a chemical dismutator of $O_2^{\bullet-}$ that preferentially enters the mitochondrial matrix to convert $O_2^{\bullet-}$ to H_2O_2, which is eventually converted to H_2O. NS8593 was selected as a specific antagonist of SK_{Ca} channels because DCEB can also open IK_{Ca} channels.[7, 9, 19, 23] Generally, combinations of drugs would include an agonist with an antagonist, both agonists, both antagonists, or TBAP with either or both agonists. The protocols were designed to expose any protection afforded by endogenous BK_{Ca} *vs.* SK_{Ca} channel activation and any potential additive or potentiating cardiac protective or anti-protective effects of drug-induced opening or blocking of BK_{Ca} and SK_{Ca} channels.

Infarction in In Vivo Rat Hearts after Regional IR Injury

Adult rats (250–300 g) of either sex were anesthetized with inactin 150 mg/kg i.p., intubated, and artificial respiration was initiated. The right carotid artery was cannulated to monitor blood pressure continuously. After thoracotomy a suture was placed around the proximal LAD to induce reversible coronary artery occlusion verified by regional epicardial cyanosis. Rats were chosen because the area-at-risk for cardiac infarction is more uniform than in guinea pigs.[40] Hearts (6 groups of 4 each) were subjected to 35 min LAD occlusion by tightening the suture; releasing the suture was followed by 120 min reperfusion. Other hearts had the suture placed around the LAD but not tightened (sham, time control, (TC)). Inactin, 25 mg/kg i.p., was supplemented as needed. Infarct size was assessed after reperfusion using the Evan's Blue area-at-risk and TTC staining techniques and expressed as percent ventricular area-at-risk. Drugs, dissolved in 0.1% DMSO and infused at 0.35 mL/h via the left jugular vein, were saline + 0.1% DMSO (vehicle control) or DCEB (30 μg/kg/min), NS1619 (30 μg/kg/min), NS8593 (100 μg/kg/min), or PAX (20 μg/kg/min). Total DMSO infused was less than 250 μg/h. Drug concentrations for infusion were obtained from the literature.[35–38] PAX and NS8593, the antagonists for the BK_{Ca} and SK_{Ca} channels,

respectively, were also perfused together to assess for any additive damaging effects. Drugs were infused 20 min before regional ischemia and for 20 min after release of the LAD occlusion. Infarct size measurements were blinded to the drug administered.

Respiration and Ca^{2+} Retention Capacity in Mitochondria Isolated after Global IR Injury

Ex vivo guinea pig hearts were subjected to global ischemia with or without drug treatment as described above. At 20 min of reperfusion some hearts (6 groups of approximately 5 each) that were not used to measure infarct size were removed from the perfusion apparatus and the ventricles were minced in cold mitochondrial isolation buffer on ice. Mitochondria were isolated by differential centrifugation as described previously[5, 6, 41–46] and the mitochondria (0.5 mg protein/mL) were suspended in respiration buffer containing 130 mM KCl, 5 mM K_2HPO_4, 20 mM MOPS, 40 μM EGTA (carried over from the isolation buffer), 1 μM $Na_4P_2O_7$, 0.1% BSA, pH 7.15 adjusted with KOH. To test mitochondrial viability and function, the respiratory control index (RCI, state 3/state 4) was determined under different substrate conditions: Na-pyruvate (P, 10 mM) or Na-succinate (S, 10 mM) or S + rotenone (R, 4 μM). State 3 respiration was determined after adding 250 μM ADP. In other experiments using the same mitochondrial pellet, Ca^{2+} retention capacity (CRC) was evaluated by measuring the decline in buffer [Ca^{2+}] with Fura-4 penta-K^+ using fluorescence spectrophotometry (QM-8, Horiba/Photon Technology International, PTI) as described previously[44–48] during bolus additions of 20 μM $CaCl_2$ every 90 s. Based on the similar improvement in cardiac function by individual or combined agonists, only the mitochondrial effects of the combined agonists NS1619 and DCEB were examined in the respiration and CRC experiments.

Statistical Analyses of Heart and Mitochondrial Data

Data obtained from hearts and mitochondria were expressed as means ± standard error of means. Appropriate comparisons were made among groups that differed by a variable at a given condition or time. Statistical differences were measured across groups using data comparisons at 25 min before global or regional ischemia *vs.* 120 of reperfusion (*ex vivo* and *in vivo* hearts). Mitochondria were isolated 20 min after reperfusion and data were collected within 2 h of isolation. Differences among variables were determined by two-way multiple ANOVA for repeated measures (Statview® and CLR anova® software programs for Macintosh®); if F tests were significant, appropriate post-hoc tests (e.g., Student-Newman-Keul's, SNK) were used to compare means. Mean values were considered significant at P values (two-tailed) <0.05.

Results

Global Protection by BK_{Ca} and SK_{Ca} Agonists and Enhanced Damage by BK_{Ca} plus SK_{Ca} Antagonists after Cardiac IR Injury in Ex Vivo Guinea Pig Isolated Hearts

We conducted an extensive analyses of the interactions of SK_{Ca} and BK_{Ca} agonists and antagonists on cardiac function and tissue damage based on assessment in changes in developed LVP (Fig. 1A–F) and % infarct size (Fig. 2A–F) after perfusion of hearts with single or combined drugs. Developed LVP was markedly decreased after 35 min global ischemia and 120 min reperfusion compared to TC (Fig. 1, IR) whereas % infarct size was

markedly increased (Fig. 2, IR). After perfusion of hearts with the agonists and antagonists we found that: a) DCEB or NS1619 alone was equally protective as was combining NS1619 + DCEB on developed LVP (Fig. 1A) and infarct size (Fig. 2A); b) NS8593 alone did not worsen cardiac function (Fig. 1B) or infarct size (Fig. 2B) when compared to IR injury alone, whereas PAX alone and PAX + NS8593 further worsened cardiac function and infarct size; c) protection by NS1619 (Figs. 1C, 2C) was blocked by PAX and PAX+NS8593 but not by NS8593 alone; d) protection by DCEB (Fig. 1D, Fig. 2D) was blocked by NS8593 but not altered by PAX alone or by NS1619 + PAX; e) TBAP completely blocked protection induced either by NS1619 or DCEB or combined NS1619 + DCEB (Figs. 1E, 2E). In general, the effects of the BK_{Ca} and SK_{Ca} agonists and antagonists on developed LVP (Fig. 1) were approximately inversely proportional to their effects on infarct size (Fig. 2). Spontaneous heart rate before ischemia was approximately 240 beats/min in TCs and before ischemia. Heart rate, LVP and infarct size were not affected by perfusion of any of the drugs before IR injury; heart rate and coronary flow returned to control values during the reperfusion phase in all groups.

Tissue Protection by BK_{Ca} and SK_{Ca} Agonists and Enhanced Tissue Damage by BK_{Ca} plus SK_{Ca} Antagonists after Regional IR Injury in in Vivo Rat Hearts

We then tested if the BK_{Ca} and SK_{Ca} agonists and antagonists had any effects on the heart when delivered intravenously before ischemia and during reperfusion. Rats were infused with vehicle, PAX, NS8593, or PAX+NS8593, or with NS1619 or DCEB, before and after regional ischemia (LAD occlusion). Infarct size (% area-at-risk) was assessed after 120 min reperfusion (Fig. 3). There was no significant infarction in the sham animals and the infusion of drugs did not alter LVP or heart rate (data not shown) before IR. NS8593 or PAX infused alone enhanced infarct size whereas simultaneous infusion of both NS8593 and PAX additionally enhanced infarct size; infusion of either NS1619 or DCEB reduced infarct size.

Overall, these heart experiments demonstrated endogenous SK_{Ca} and BK_{Ca} channel activation *in vivo* rat hearts based on their antagonists' effects to enhance regional infarct size as well as endogenous BK_{Ca} channel activation in *ex vivo* guinea pig hearts based on an additive effect of inhibiting both channels on contractile function. The experiments also established the selectivity of the respective antagonists to block their respective agonists, and the role of ROS in mediating the exogenous protection afforded by both BK_{Ca} and SK_{Ca} channel agonists.

Mitochondrial Respiration and mCa^{2+} Uptake after IR injury in Guinea Pig Ex Vivo Hearts Treated with BK_{Ca} and SK_{Ca} Agonists or Antagonists

Cardiac protection due to BK_{Ca} and SK_{Ca} channel opening may be mediated largely by effects on mitochondrial bioenergetics. Thus we tested if combined BK_{Ca} and SK_{Ca} agonists improved oxidative-phosphorylation assessed by respiration rates and RCI in cardiac mitochondria isolated 20 min after reperfusion and 35 min global ischemia (Table 1 and Fig. 4). The agonists were given together because we observed equivalent effects with the single agonists in the hearts and because the agonists given together did not additionally improve cardiac function or reduce infarct size when compared to IR control (Figs. 1–3). Representative traces of O_2 consumption (respiratory rate) in mitochondria energized with

pyruvate (Fig. 4A upper left) are shown with mean RCI's under the three substrate conditions (Fig. 4A). Under each substrate condition, states 2 and 4 respiration (Table 1) were increased, and state 3 respiration was decreased in mitochondria isolated after 20 min reperfusion and 35 min of ischemia (red tracing/bars) compared to no IR (TC, black tracing/bars) (Fig. 4A); thus RCI was markedly reduced by 2 to 6 fold indicating significant uncoupling of phosphorylation from oxidation (Table 1). In hearts treated during IR with the combined BK_{Ca} and SK_{Ca} agonists, NS1619 and DCEB (blue tracing/bars), RCI's (state 3/state 4) improved under each substrate condition compared to IR alone (Fig. 4).

By using the same mitochondrial pellets, we also monitored mitochondrial structural integrity by challenging isolated mitochondria with boluses of 20 μM $CaCl_2$ added to the respiration buffer (Fig. 4B) until the capability to take up and sequester $CaCl_2$ (CRC) was exceeded so that mitochondria extrude Ca^{2+} through the so-called mitochondrial permeability transition pore (mPTP). Representative traces of CRC in mitochondria energized under succinate + rotenone conditions are displayed (Fig. 4B, top left) with mean CRC's under the 3 substrate conditions during state 2 respiration (Fig. 4B); cumulative mitochondrial Ca^{2+} uptake was reduced after IR injury because of early mPTP opening but was greater after hearts were treated with NS1619 and DCEB (Fig. 4B, red and blue tracings). Total average mitochondrial Ca^{2+} uptake before mPTP activation was greater when *ex vivo* hearts were perfused with NS1619 + DCEB before and after ischemia in mitochondria energized with any of the three substrate conditions (columns Fig. 4B). This indicated that relatively uncoupled mitochondria have elevated mitochondrial Ca^{2+} levels during and after IR as we have shown previously in intact hearts,[27, 31, 49] or have less capacity to sequester the added $CaCl_2$. The higher RCIs with BK_{Ca} and SK_{Ca} agonist treatment during IR coincided with greater mitochondrial CRCs.

We also determined if BK_{Ca} and/or SK_{Ca} antagonism worsened oxidative-phosphorylation assessed by respiration rates and RCI in cardiac mitochondria isolated after 20 min reperfusion and 35 min global ischemia (Table 2, Fig. 5A). The antagonists were given separately and together because we observed different effects of NS8593 when given alone or with PAX on cardiac function and infarct size (Figs. 1–3). Representative traces of O_2 consumption in mitochondria energized with succinate (Fig 5A upper left) are displayed with mean RCI's under the three substrate conditions (Fig. 5A). Similar to data in Table 1 and Fig. 4A for the agonist experiments, states 2 and 4 respiratory rates were increased, and state 3 respiration was decreased in mitochondria isolated after ischemia and 20 min reperfusion (red tracing/bars) compared to no IR (TC, black tracing/bars) (Table 2, Fig. 5A). Treatment of hearts before and after IR with BK_{Ca} and or SK_{Ca} antagonists PAX and NS8593 (green tracing/bar) did not worsen average RCI's (state 3/state 4) compared to IR alone under any substrate condition, which is different from the cardiac functional and tissue infarction results. This is not unexpected because the respiratory data is derived from an aggregate of functional and dysfunctional mitochondria retrieved during the isolation process and the variability of respiratory measurements may be insufficient to distinguish differences.

Using the same mitochondrial pellets isolated after IR injury, we again monitored mitochondrial structural integrity by challenging isolated mitochondria with boluses of 20

μM $CaCl_2$ (Fig. 5B). Treatment with the combination of PAX and NS8593 slightly reduced CRC in mitochondria energized with pyruvate or succinate, but not with succinate + rotenone, when compared to IR alone. The reduced CRC after combined BK_{Ca} and SK_{Ca} antagonist treatment during IR corroborates the results on cardiac functional and tissue infarction.

Discussion

Our results demonstrate that intrinsic activation of BK_{Ca} and SK_{Ca} channels provides endogenous cardiac functional protection (Fig. 1B) in the *ex vivo* cardiac global IR model on the basis of lowered developed LVP in the presence of PAX alone, and more so in the presence of PAX+NS8593 *vs.* IR alone; however, only the presence of PAX enhanced global infarct size (Fig. 2B). In contrast, in the *in vivo* model of regional IR injury (Fig. 3), regional infarct size was larger in the presence of either PAX or NS8593 and even larger with infusion of both PAX and NS8593. The specific agonists for the BK_{Ca} and the SK_{Ca} channels, NS1619 and DCEB, provided equivalent protection against IR injury without additive effects. Our protocol for the isolated heart experiments was different from that used in our prior studies in which the BK_{Ca} and SK_{Ca} agonists were given only briefly to trigger pharmacologic preconditioning (PPC) and the antagonists were given to prevent PPC.[4,18] The finding that NS8593 alone did not enhance infarct size in the *ex vivo* global ischemia model as it did in the *in vivo* LAD occlusion model could be related to many factors, including differences in species, anesthetics given, collateral flow, potential for ischemic or anesthetic preconditioning, methods of inducing ischemia and drug delivery. Nevertheless, the present results suggest that opening or preventing the opening of these K^+ channels leads to protective or anti-protective effects, respectively, via a common downstream mediator that is likely mitochondrial generated superoxide ($O_2^{\bullet-}$) as observed by the loss of protection in the presence of TBAP (Figs. 1E, 2E).

In combination, the BK_{Ca} and the SK_{Ca} channel agonists NS1619 and DCEB did not provide additional protection, unlike the combined antagonists that worsened function *ex vivo,* and enhanced infarct size *in vivo*. This may indicate that providing either agonist exogenously provides maximal protection, whereas both channels are endogenously important in reducing IR injury. Because the mitochondrial targeted $O_2^{\bullet-}$ scavenger, TBAP, given before and after ischemia, abrogated the functional recovery and the magnitude of infarction by both agonists alone or in combination, this suggested that the protection was mediated in large part by a mitochondrial effect to stimulate $O_2^{\bullet-}$ generation. This was further supported not only by effects of BK_{Ca} and SK_{Ca} agonists to improve oxidative phosphorylation (RCI) and retain excess mCa^{2+} (CRC) (Fig. 4) but also by effects of BK_{Ca} and SK_{Ca} antagonists to reduce Ca^{2+} loading after IR injury (Fig. 5). Overall, our novel observations suggest an important role for IR-induced BK_{Ca} and SK_{Ca} channel activation in cardiomyocyte mitochondria in providing endogenous protection against IR injury as suggested by treatment of hearts with either or both channel antagonists.

BK_{Ca} and SK_{Ca} Channel Agonists Reduce and Antagonists Enhance Cardiac IR Injury via a Mitochondrial Mechanism

Individually, BK_{Ca} and SK_{Ca} channels appear to be important for protecting against cardiac myocyte damage during oxidative stress.[4, 18, 26, 50–53] Allowing for possible non-specific effects of the agonists and antagonists, our data furnish support that pharmacologic opening of either BK_{Ca} or SK_{Ca} channels is cardioprotective. Moreover, intrinsic activation of BK_{Ca} or SK_{Ca} channels, and especially when activated together, may be essential for endogenous protection against IR injury. In contrast, because there were no additive effects of the two agonists (Figs. 1A, 2A), protection by either of these agonists may have provided maximal protection via a mK^+ uptake mechanism.

In our experiments the antagonists were given before, during and after ischemia to test for possible endogenous cardioprotection. Due to different K^+ channel characteristics and endogenous protective effects we speculate that mSK_{Ca} channels open gradually under conditions in which $\Delta\Psi_m$ becomes reduced, pH_m becomes progressively lower, and matrix Ca^{2+} progressively rises, as occurs during ischemia. In contrast, mBK_{Ca} channel opening may occur when $\Delta\Psi_m$ rebounds, cytosolic Ca^{2+} surges, and pH_m is high, as during initial reperfusion. This purported timing of opening of these channels, although likely over-simplified, is based on our studies of changes in redox state (NAHD, FAD), diastolic, systolic, and mitochondrial matrix [Ca^{2+}], and cytosolic [Na^+] during the time course of IR injury.[27, 54–58]

Importantly, the BK_{Ca} and SK_{Ca} channel agonists and antagonists were bioactive when infused intravenously, as evidenced by the differences in cardiac infarct size after LAD occlusion in the *in vivo* rat model (Fig. 3). This suggests a translational value for potential intravenous treatment of K_{Ca} channel agonists during cardiac IR injury in humans. In fact, we have recently identified splice variants of the SK_{Ca} channel in human ventricular myocytes,[26] which warrants development of SK_{Ca}-targeted therapeutic strategies for human cardiac IR injury.

The isolated heart and animal studies could not indicate specifically where the anti- and pro-cardioprotective effects of BK_{Ca} and SK_{Ca} antagonist and agonists, respectively, occur, i.e. in mitochondria *vs.* other cellular sites such as the sarcolemma. However, pharmacologic evidence for a role of mitochondrial BK_{Ca} and SK_{Ca} channels is given by the improved or worsened RCI's and CRC's (Fig. 4A, B *vs.* Fig. 5A, B) in isolated guinea pig mitochondria after *ex vivo* IR injury in the presence of SK_{Ca} and BK_{Ca} agonists/antagonists. That the protection is mediated at the mitochondrial level is further supported by the effect of the free radical scavenger TBAP to abolish cardioprotection (Figs. 1E, 2E). This is because the respiratory complexes of the mitochondrial electron transport chain are most likely the major source of signaling ROS for cytoprotection and deleterious excess ROS production during IR injury.[1] Indeed, both BK_{Ca}[50] and SK_{Ca}[18, 26] channels are located in cardiac cell IMM rather than in the sarcolemmal membrane, and drug-induced opening of the BK_{Ca} channel in isolated mitochondria leads to mild ROS production without significantly affecting $\Delta\Psi_m$.[6] We believe that this small amount of $O_2^{\bullet-}$ emitted is important for signaling mechanisms that confer protection during treatment with a BK_{Ca} or SK_{Ca} agonist.

Distribution and Function of Ca^{2+}-Sensitive K^+ Channels

The cell membranes of vascular smooth muscle, neural, and secretory cells contain large conductance (200–300 pS, i.e. Big Ca^{2+}-sensitive K^+ (BK_{Ca}, aka maxi-K_{Ca}) channels that when activated produce vasodilation, hyperpolarization, and secretion in these cells, respectively. BK_{Ca} channel opening is activated by increased cytosolic [Ca^{2+}] and by a large voltage gradient.[59] Activation of BK_{Ca} channels over a range of [Ca^{2+}] is mediated at several binding sites within the channel[60] so that there is a wide range of [Ca^{2+}] responsiveness (K_a 10–1000 μM).[61] As K^+ exits the cell with BK_{Ca} channel opening, this elicits cell membrane repolarization or hyperpolarization, which in turn reduces cell Ca^{2+} entry by closing voltage-dependent Ca^{2+} channels. The finding of altered redox potential in smooth muscle cells[62] suggested there was a mitochondrial involvement. Indeed, Xu et al.[50] provided the first evidence that BK_{Ca} channels are prominently located in cardiac mitochondria. In contrast, SK_{Ca} channels are not voltage dependent and their agonists interact with their Ca^{2+} sensor calmodulin in the C-terminus region; however, at least for NS8593, the SK_{Ca}-mediated gating modulation occurs via interaction with gating structures near the selectivity filter within the inner pore vestibule.[24]

Studies of effects of BK_{Ca} and SK_{Ca} channels in cell membranes might help to assess the mechanisms of protection by their counterparts that reside in the IMM. For example, BK_{Ca} channels are proposed to regulate cell membrane potential in neurons.[64] SK_{Ca} channels in neurons lie adjacent to Ca^{2+} stores and Ca^{2+} channels to sense Ca^{2+} activated K^+ flux.[65] Neuronal SK_{Ca} channels appear to play a role in setting the intrinsic firing frequency, whereas neuronal BK_{Ca} channels may regulate action potential shape.[15] SK_{Ca} channels are also located in neuronal (hippocampus-derived cells) mitochondria where they appear to provide protection against excitotoxicity.[17]

BK_{Ca} and SK_{Ca}-mediated K^+ flux in mitochondria may be differentiated by the number of channels/surface area, sensitivity to Ca^{2+}, and dependence on $\Delta\Psi_m$ during mitochondrial respiration. Because there are several differences in the triggers for activating these channels, the functional effects of opening these channel may be different; e.g., unlike for BK_{Ca}, SK_{Ca} channel opening may “fine tune” matrix K^+ influx due to changing matrix Ca^{2+} levels independent of changes in $\Delta\Psi_m$ during the variable rate of oxidative phosphorylation. The presence of the SK_{Ca} channels specifically in the IMM[18] probably indicate that they have an important function in modulating mitochondrial bioenergetics in response to high matrix [Ca^{2+}], perhaps via modulation of K^+-mediated mitochondrial volume that leads to an uncoupling effect[63] due to K^+/H^+ exchange (KHE) (cartoon, Fig. 6). In contrast, the voltage and Ca^{2+}-dependent BK_{Ca} channels may open primarily when $\Delta\Psi_m$ and pH_m are restored and excess cytosolic [Ca^{2+}] leads to high matrix [Ca^{2+}] during reperfusion after ischemia.

Protection by mK^+ Channel Types Likely Requires Superoxide Release

There is ample evidence that $O_2^{\bullet-}$ is necessary to trigger protection by a variety of mK^+ channels;[1, 3] however, the mechanism of $O_2^{\bullet-}$, and its downstream mediators, in mediating protection remain unresolved. An increase in redox state (increased NADH, decreased FAD) at a given [O_2] can result in a small increase in signaling $O_2^{\bullet-}$ generation.[66] This is different

than the much larger amount of deleterious $O_2^{\bullet-}$ "bursts" that occur during reperfusion when excess O_2 is available but mitochondrial respiration is compromised. Our group[4, 28, 29, 67] and others[68, 69, 70] have shown, moreover, that $O_2^{\bullet-}$ and its products are also formed in excess during ischemia despite lower tissue O_2 tension.

In the present study, evidence that the protective effects of DCEB and NS1619 are mediated by signaling $O_2^{\bullet-}$ is again indicated by reversal of the protection in the presence of TBAP as shown previously for SK_{Ca}[18, 26] and BK_{Ca}[4] channel agonists. Enhanced transfer of electrons by uncoupling before ischemia may minimize respiratory inefficiency; i.e., by reducing matrix contraction and improving respiration on reperfusion. mSK_{Ca} channel opening, like opening of any mK^+ channel, could induce protection by a small increase in $O_2^{\bullet-}$ generation to stimulate enzymatic pathways that help to protect cells from IR injury. In any case, we propose that TBAP accelerates $O_2^{\bullet-}$ dismutation to H_2O_2 and thus blocks the signaling $O_2^{\bullet-}$ that leads to protection by either NS1619 or DCEB. We have reported in isolated cardiac mitochondria that low, but not high, concentrations of the BK_{Ca} channel opener NS1619 increased resting state 4 respiration and mildly enhanced H_2O_2 levels while maintaining $\Delta\Psi_m$.[6]

We have suggested, moreover,[63] that an increase in intra-matrix K^+ is replaced over time with H^+ via mKHE and that at low concentrations, these openers produce a transient increase in matrix acidity, i.e., in which the proton leak stimulates respiration but maintains $\Delta\Psi_m$ so that signaling $O_2^{\bullet-}$ is generated at mitochondrial respiratory complexes due to accelerated electron transport. Therefore, during the periods of ischemia and reperfusion, mK^+ influx, due to condition-dependent activation of SK_{Ca} and BK_{Ca} channels, and, in turn, mKHE may trigger a signaling amount of $O_2^{\bullet-}$, due to enhanced respiration and electron leak, that then stimulates downstream signaling pathways. The net effect of improved mitochondrial bioenergetics during late ischemia and reperfusion, i.e., higher redox state, lower mCa^{2+}, and reduced deleterious $O_2^{\bullet-}$ emission contributes to improved contractile and relaxant function.[29, 31, 57, 71]

Ca^{2+}-induced mK^+ Influx Causes Mitochondrial Swelling and Changes in Respiration

We have reported that cytosolic and mCa^{2+} both increase steadily during the period of cardiac ischemia and that cytosolic Ca^{2+} peaks on initial reperfusion.[27, 49] During IR injury the rise in cytosolic and in mCa^{2+} likely triggers mK^+ influx via mBK_{Ca} and mSK_{Ca} channel activation to initially induce matrix swelling as a potential protective mechanism. Alternatively, or in addition, H^+ influx via mKHE may enhance respiration to maintain $\Delta\Psi_m$ but also induce signaling levels of $O_2^{\bullet-}$ (cartoon, Fig. 6). We observed that PAX, NS1619 and PAX+NS8593 treatments reduced the increase in pyruvate-mediated state two respiration found after IR alone, but increased the succinate-mediated state 2 respiration compared to IR alone (Table 2). This implies that endogenous mK_{Ca} channel activation does lead to mild uncoupling via mKHE during succinate but not during pyruvate consumption. It is also possible that K^+ is required for optimal functioning of oxidative phosphorylation because mK^+ flux largely regulates mitochondrial volume that in turn may modulate bioenergetics.[72–74] Thus mSK_{Ca} channels, like mBK_{Ca} channels,[50, 75] may also act to modulate mitochondrial volume during the increase in matrix Ca^{2+} loading that occurs

during ischemia and more so on reperfusion.[27, 31] Xu et al.[50] suggested that opening mBK_{Ca} channels to enhance matrix K^+ influx mitigates IR injury in a manner similar to putative mK_{ATP} channel opening. They proposed[50] that the function of mBK_{Ca} channels was to improve the efficiency of mitochondrial energy production. In addition to mBK_{Ca} and mSK_{Ca} channels, and the putative mK_{ATP} channels,[72, 75–79] another K^+ channel, the Na^+-activated K^+ channel, SLO2,[80, 81] has been reported to be located in mitochondria; this channel is also cytoprotective, at least in part due to activation of mK^+ influx. Thus, although the mK^+ channel sensing conditions may be different, the net effect of mK^+ influx, increases in mitochondrial volume and or mH^+ via mKHE, appears to be the same.

Because mK_{Ca} channels likely play a major role in regulating mitochondrial bioenergetics by modulating ion flux, it is unclear exactly how opening of these channels leads to higher NADH/FAD ratios and decreases in excess ROS and in Ca^{2+} overload during IR. For the presumptive mK_{ATP} channel, it was proposed that its opening depolarizes the IMM and decreases mCa^{2+} overload.[75, 76, 82] It should be noted that Garlid's group[72, 83] has proposed that the physiological role of mK^+ channel opening is control of mitochondrial volume rather than dissipation of $\Delta\Psi_m$ and uncoupling, because swelling induced by mK^+ uptake (without mKHE) would occur with concomitant uptake of Cl^- and subsequently water by osmosis. They suggested that subsequent activation of mKHE would only slightly dissipate the proton motive gradient ($\Delta\mu H$) by increasing matrix acidity (H^+ leak) without significantly altering $\Delta\Psi_m$.[73, 83]

We found that NS1619 resulted in an increase in mK^+ uptake, but only in the presence of quinine, a non-specific KHE blocker;[63] this suggested that mKHE promoted mK^+ efflux over time. However, our results also indicated that mpH was lower when quinine was present; this led to the suggestion that mH^+ influxy induced by NS1619 was not exclusively via mKHE and that NS1619 may also directly or indirectly act as a protonophore. Moreover, valinomycin-induced mK^+ influx exerted a biphasic effect, i.e. to alkalinize and then to acidify the matrix.[63] Thus the mechanism and timing remain unclear. What does appear apparent is that either agonist of mK_{Ca} channels leads to improved mitochondrial bioenergetics, with reduced mCa^{2+} overload and ROS production, which thereby provides overall substantial mitochondrial and myocyte protective effects during cardiac IR injury *ex vivo* and reduced infarct size *ex vivo* and *in vivo*.

Based on limited experimental observations and some speculation, there are several possible scenarios of potential mechanisms for mBK_{Ca} and mSK_{Ca} activation and effectors that lead to cardiac protection (cartoon, Fig. 7). In scenario A, as reperfusion restores cytosolic pH, this promotes excess cytosolic Ca^{2+} loading by sarcolemal NHE and NCE, which then causes excess mCa^{2+} loading with $\Delta\Psi_m$ repolarization; in turn this activates mK^+ influx predominately via mBK_{Ca} but also via mSK_{Ca} channels due to increased mCa^{2+}. In scenario B, as ischemia ensues, an increase in the IMM chemical gradient for Ca^{2+} enhances mCa^{2+} uptake when $\Delta\Psi_m$ and cell pH are lower; this induces mK^+ influx predominately via SK_{Ca} channels but also via BK_{Ca} channels due to increased cyctosolic Ca^{2+}; via either pathway mK^+ influx is countered by activation of mKHE that acidifies the matrix and promotes uncoupling (higher respiration). In scenario C, independent increases of either cell or mCa^{2+}, induced under conditions of ischemia or reperfusion, stimulates mK^+ influx

independently via mBK_{Ca} and mSK_{Ca} channels. In each scenario, uncoupling via mKHE leads to reversal of the increase in mitochondrial volume and generation of small amounts of signaling $O_2^{\bullet-}$. In each scenario, if the matrix signaling $O_2^{\bullet-}$ generated by uncoupling were rapidly dismutated to H_2O_2 by TBAP, the protection would be lost because downstream ROS-dependent protective pathways would not be activated. We suggest that scenario B may best explain the utility of independent activation of either type of mK_{Ca} channel during ischemia and during reperfusion that together protect against cardiac IR injury. This is because we observed that the antagonists together exerted some additive endogenous anti-protective effects that likely depend on the different biochemical conditions that occur during ischemia and during reperfusion.

Summary and Limitations

We have furnished comparative evidence for cardiac protective effects of BK_{Ca} and SK_{Ca} channel agonists and counter protective effects of BK_{Ca} and SK_{Ca} channel antagonists with demonstrated specificity of the antagonists to hinder protection by the agonists. Moreover, systemic application of the agonists and antagonists appears efficacious and suggests therapeutic potential in humans. The end effect of mK^+ channel opening in cardiac mitochondria appears to be a respiration dependent pathway that stimulates a small amount of signaling mitochondrial $O_2^{\bullet-}$ that leads to cardioprotection by downstream and local mitochondrial mechanisms as shown by drug treatments on mitochondrial respiration and mCa^{2+} uptake. However, there is as yet little delineation of the individual stages and timing of triggering, activation, and end-effects of mK^+ channel opening. Unraveling this apparently complicated, and potentially redundant, mechanism that culminates in cardiac protection could lead to therapeutic approaches for protection, specifically via a mitochondrial-targeted mechanism. However, an efficacious therapy would likely require targeting of specific cardiac mitochondrial splice variants of mK_{Ca} channels, so that the function of other BK_{Ca} and SK_{Ca} channels in other organs and subcellular sites would not be impinged.

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Abbreviations

IR	ischemia reperfusion
SK_{Ca}	small conductance Ca^{2+} -sensitive K^+ channel
BK_{Ca}	big conductance Ca^{2+} -sensitive K^+ channel
FAD	flavin adenine dinucleotide

KHE	K^+/H^+ exchange
NCE	Na^+/Ca^{2+} exchange
NHE	N^+/H^+ exchange
K_{ATP}	ATP -sensitive K^+ channel
DCEBIO	DCEB, 5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazol-2-one
NADH	nicotinamide adenine dinucleotide
NS1619	(1-(2′-hydroxy-5′-trifluoromethylphenyl)-5-trifluoromethyl-2(3H) benzimid-axolone)
IMM	inner mitochondrial membrane
$\Delta\Psi_m$	mitochondrial membrane potential
TBAP	Mn(III) tetrakis (4-benzoic acid) porphyrin
IPC	ischemic preconditioning
PPC	pharmacological preconditioning
PAX	paxilline
NS8593	N-[(1R)-1,2,3,4-tetrahydro-1-naphthalenyl]-1H-benzimidazol-2-amine hydrochloride
RT-PCR	reverse transcription -polymerase chain reaction
RCI	respiratory control index
TTC	2,3,5-triphenyltetrazolium chloride
CRC	Ca^{2+} retention capacity
CaM	calmodulin

References

1. Stowe DF, Camara AK. Mitochondrial Reactive Oxygen Species Production in Excitable Cells: Modulators of Mitochondrial and Cell Function. Antioxid Redox Signal. 2009; 11:1373–1414. [review]. [PubMed: 19187004]
2. Camara AK, Lesnefsky EJ, Stowe DF. Potential Therapeutic Benefits of Strategies Directed to Mitochondria. Antioxid Redox Signal. 2010; 13:279–347. [review]. [PubMed: 20001744]
3. Camara AK, Bienengraeber M, Stowe DF. Mitochondrial Approaches to Protect against Cardiac Ischemia and Reperfusion Injury. Front Physiol. 2011; 2:1–34. [review]. [PubMed: 21423411]
4. Stowe DF, Aldakkak M, Camara AK, Riess ML, Heinen A, Varadarajan SG, Jiang MT. Cardiac Mitochondrial Preconditioning by Big Ca^{2+}-Sensitive K^+ Channel Opening Requires Superoxide Radical Generation. Am J Physiol Heart Circ Physiol. 2006; 290:H434–440. [PubMed: 16126810]
5. Heinen A, Aldakkak M, Stowe DF, Rhodes SS, Riess ML, Varadarajan SG, Camara AK. Reverse Electron Flow-Induced ROS Production Is Attenuated by Activation of Mitochondrial Ca^{2+}-

Sensitive K^+ Channels. Am J Physiol Heart Circ Physiol. 2007; 293:H1400–1407. [PubMed: 17513497]

6. Heinen A, Camara AK, Aldakkak M, Rhodes SS, Riess ML, Stowe DF. Mitochondrial Ca^{2+}-Induced K^+ Influx Increases Respiration and Enhances ROS Production While Maintaining Membrane Potential. Am J Physiol Cell Physiol. 2007; 292:C148–156. [PubMed: 16870831]
7. Singh S, Syme CA, Singh AK, Devor DC, Bridges RJ. Benzimidazolone Activators of Chloride Secretion: Potential Therapeutics for Cystic Fibrosis and Chronic Obstructive Pulmonary Disease. J Pharmacol Exp Ther. 2001; 296:600–611. [PubMed: 11160649]
8. Syme CA, Gerlach AC, Singh AK, Devor DC. Pharmacological Activation of Cloned Intermediate- and Small- Conductance Ca^{2+}-Activated K^+ Channels. Am J Physiol Cell Physiol. 2000; 278:C570–581. [PubMed: 10712246]
9. Wulff H, Miller MJ, Haensel W, Grissmer S, Cahalan MD, Chandy KG. Design of a Potent and Selective Inhibitor of the Intermediate -Conductance Ca^{2+}-Activated K^+ Channel, $IK_{Ca}1$: A Potential Immunosupressant. Proc Natl Acad Sci U S A. 2000; 97:8151–8156. [PubMed: 10884437]
10. Stocker M. Ca^{2+}-Activated K^+ Channels: Molecular Determinants and Function of the SK Family. Nat Rev Neurosci. 2004; 5:758–770. [PubMed: 15378036]
11. Hille, B. Ion Channels in Excitable Membranes. Sunderland: Sinauer Asociates, Inc; 2001. p. 814
12. Schumacher MA, Rivard AF, Bachinger HP, Adelman JP. Structure of the Gating Domain of a Ca^{2+}-Activated K^+ Channel Complexed with Ca^{2+}/Calmodulin. Nature. 2001; 410:1120–1124. [PubMed: 11323678]
13. Bruening-Wright A, Schumacher MA, Adelman JP, Maylie J. Localization of the Activation Gate for Small Conductance Ca^{2+}-Activated K^+ Channels. J Neurosci. 2002; 22:6499–6506. [PubMed: 12151529]
14. Taylor MS, Bonev AD, Gross TP, Eckman DM, Brayden JE, Bond CT, Adelman JP, Nelson MT. Altered Expression of Small-Conductance Ca^{2+}-Activated K^+ (SK3) Channels Modulates Arterial Tone and Blood Pressure. Circ Res. 2003; 93:124–131. [PubMed: 12805243]
15. Xu Y, Tuteja D, Zhang Z, Xu D, Zhang Y, Rodriguez J, Nie L, Tuxson HR, Young JN, Glatter KA, Vazquez AE, Yamoah EN, Chiamvimonvat N. Molecular Identification and Functional Roles of a Ca^{2+}-Activated K^+ Channel in Human and Mouse Hearts. J Biol Chem. 2003; 278:49085–49094. [PubMed: 13679367]
16. Weatherall KL, Seutin V, Liegeois JF, Marrion NV. Crucial Role of a Shared Extracellular Loop in Apamin Sensitivity and Maintenance of Pore Shape of Small-Conductance Calcium-Activated Potassium (SK) Channels. Proc Natl Acad Sci U S A. 2011; 108:18494–18499. [PubMed: 22025703]
17. Dolga AM, Netter MF, Perocchi F, Doti N, Meissner L, Tobaben S, Grohm J, Zischka H, Plesnila N, Decher N, Culmsee C. Mitochondrial Small Conductance SK2 Channels Prevent Glutamate-Induced Oxytosis and Mitochondrial Dysfunction. J Biol Chem. 2013; 288:10792–10804. [PubMed: 23430260]
18. Stowe DF, Gadicherla AK, Zhou Y, Aldakkak M, Cheng Q, Kwok WM, Jiang MT, Heisner JS, Yang M, Camara AK. Protection against Cardiac Injury by Small Ca^{2+}-Sensitive K^+ Channels Identified in Guinea Pig Cardiac Inner Mitochondrial Membrane. Biochim Biophys Acta. 2013; 1828:427–442. [PubMed: 22982251]
19. Sheng JZ, Ella S, Davis MJ, Hill MA, Braun AP. Openers of SK_{Ca} and IK_{Ca} Channels Enhance Agonist-Evoked Endothelial Nitric Oxide Synthesis and Arteriolar Vasodilation. FASEB J. 2009; 23:1138–1145. [PubMed: 19074509]
20. Strobaek D, Teuber L, Jorgensen TD, Ahring PK, Kjaer K, Hansen RS, Olesen SP, Christophersen P, Skaaning-Jensen B. Activation of Human IK and SK Ca^{2+}-Activated K^+ Channels by NS309 (6,7-Dichloro-1h-Indole-2,3-Dione 3-Oxime). Biochim Biophys Acta. 2004; 1665:1–5. [PubMed: 15471565]
21. Pedarzani P, McCutcheon JE, Rogge G, Jensen BS, Christophersen P, Hougaard C, Strobaek D, Stocker M. Specific Enhancement of Sk Channel Activity Selectively Potentiates the Afterhyperpolarizing Current I_{AHP} and Modulates the Firing Properties of Hippocampal Pyramidal Neurons. J Biol Chem. 2005; 280:41404–41411. [PubMed: 16239218]

22. Wulff H, Kolski-Andreaco A, Sankaranarayanan A, Sabatier JM, Shakkottai V. Modulators of Small- and Intermediate-Conductance Calcium-Activated Potassium Channels and Their Therapeutic Indications. Curr Med Chem. 2007; 14:1437–1457. [PubMed: 17584055]
23. Pedarzani P, Stocker M. Molecular and Cellular Basis of Small--and Intermediate-Conductance, Calcium-Activated Potassium Channel Function in the Brain. Cell Mol Life Sci. 2008; 65:3196–3217. [PubMed: 18597044]
24. Jenkins DP, Strobaek D, Hougaard C, Jensen ML, Hummel R, Sorensen US, Christophersen P, Wulff H. Negative Gating Modulation by (R)-N-(Benzimidazol-2-Yl)-1,2,3,4-Tetrahydro-1-Naphthylamine (NS8593) Depends on Residues in the Inner Pore Vestibule: Pharmacological Evidence of Deep-Pore Gating of $K_{Ca}2$ Channels. Mol Pharmacol. 2011; 79:899–909. [PubMed: 21363929]
25. 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. 2010; 3:380–390. [PubMed: 20562443]
26. Yang M, Camara AK, Aldakkak M, Kwok WM, Stowe DF. Identity and Function of a Cardiac Mitochondrial Small Conductance Ca^{2+}-Activated K^+ Channel Splice Variant. Biochim Biophys Acta. 2017; 1858:442–458. [PubMed: 28342809]
27. Varadarajan SG, An JZ, Novalija E, Smart SC, Stowe DF. Changes in $[Na^+]_I$, Compartmental $[Ca^{2+}]$, and NADH with Dysfunction after Global Ischemia in Intact Hearts. Am J Physiol Heart Circ Physiol. 2001; 280:H280–293. [PubMed: 11123243]
28. Kevin L, Camara AKS, Riess MR, Novalija E, Stowe DF. Ischemic Preconditioning Alters Real-Time Measure of O_2 Radicals in Intact Hearts with Ischemia and Reperfusion. Am J Physiol Heart Circ Physiol. 2003; 284:H566–H574. [PubMed: 12414448]
29. Kevin L, Novalija E, Riess MR, Camara AKS, Rhodes SS, Stowe DF. Sevoflurane Exposure Generates Superoxide but Leads to Decreased Superoxide During Ischemia and Reperfusion in Isolated Hearts. Anesth Analg. 2003; 96:945–959.
30. Riess ML, Camara AKS, Chen Q, Novalija E, Rhodes SS, Stowe DF. Altered NADH and Improved Function by Anesthetic and Ischemic Preconditioning in Guinea Pig Intact Hearts. Am J Physiol Heart Circ Physiol. 2002; 283:H53–60. [PubMed: 12063274]
31. Riess ML, Camara AK, Novalija E, Chen Q, Rhodes SS, Stowe DF. Anesthetic Preconditioning Attenuates Mitochondrial Ca^{2+} Overload During Ischemia in Guinea Pig Intact Hearts: Reversal by 5- Hydroxydecanoic Acid. Anesth Analg. 2002; 95:1540–1546. [PubMed: 12456413]
32. An JZ, Camara AK, Rhodes SS, Riess ML, Stowe DF. Warm Ischemic Preconditioning Improves Mitochondrial Redox Balance During and after Mild Hypothermic Ischemia in Guinea Pig Isolated Hearts. Am J Physiol Heart Circ Physiol. 2005; 288:H2620–2627. [PubMed: 15653757]
33. Camara AK, Riess ML, Kevin LG, Novalija E, Stowe DF. Hypothermia Augments Reactive Oxygen Species Detected in the Guinea Pig Isolated Perfused Heart. Am J Physiol Heart Circ Physiol. 2004; 286:H1289–1299. [PubMed: 14644763]
34. Attwell D, Cohen I, Eisner DA. The Effects of Heart Rate on the Action Potential of Guinea-Pig and Human Ventricular Muscle. J Physiol. 1981; 313:439–461. [PubMed: 7277229]
35. Cai RP, Xue YX, Huang J, Wang JH, Wang JH, Zhao SY, Guan TT, Zhang Z, Gu YT. Ns1619 Regulates the Expression of Caveolin-1 Protein in a Time-Dependent Manner Via ROS/PI3K/PKB/FOXO1 Signaling Pathway in Brain Tumor Microvascular Endothelial Cells. J Neurol Sci. 2016; 369:109–118. [PubMed: 27653874]
36. Criado-Marrero M, Santini E, Porter JT. Modulating Fear Extinction Memory by Manipulating Sk Potassium Channels in the Infralimbic Cortex. Front Behav Neurosci. 2014; 8:96. [PubMed: 24715857]
37. Haugaard MM, Hesselkilde EZ, Pehrson S, Carstensen H, Flethoj M, Praestegaard KF, Sorensen US, Diness JG, Grunnet M, Buhl R, Jespersen T. Pharmacologic Inhibition of Small-Conductance Calcium-Activated Potassium (SK) Channels by NS8593 Reveals Atrial Antiarrhythmic Potential in Horses. Heart Rhythm. 2015; 12:825–835. [PubMed: 25542425]
38. Matthews EA, Weible AP, Shah S, Disterhoft JF. The BK-Mediated F_{AHP} Is Modulated by Learning a Hippocampus-Dependent Task. Proc Natl Acad Sci U S A. 2008; 105:15154–15159. [PubMed: 18799739]

39. Sanchez M, McManus OB. Paxilline Inhibition of the Alpha-Subunit of the High-Conductance Calcium-Activated Potassium Channel. Neuropharmacology. 1996; 35:963–968. [PubMed: 8938726]
40. Maxwell MP, Hearse DJ, Yellon DM. Species Variation in the Coronary Collateral Circulation During Regional Myocardial Ischaemia: A Critical Determinant of the Rate of Evolution and Extent of Myocardial Infarction. Cardiovasc Res. 1987; 21:737–746. [PubMed: 3440266]
41. Riess ML, Camara AK, Heinen A, Eells JT, Henry MM, Stowe DF. K_{ATP} Channel Openers Have Opposite Effects on Mitochondrial Respiration under Different Energetic Conditions. J Cardiovasc Pharmacol. 2008; 51:483–491. [PubMed: 18437094]
42. Riess ML, Eells JT, Kevin LG, Camara AKS, Henry MM, Stowe DF. Attenuation of Mitochondrial Respiration by Sevoflurane in Isolated Cardiac Mitochondrial Is Mediated in Part by Reactive Oxygen Species. Anesthesiology. 2004; 100:498–505. [PubMed: 15108961]
43. Huang M, Camara AK, Stowe DF, Qi F, Beard DA. Mitochondrial Inner Membrane Electrophysiology Assessed by Rhodamine-123 Transport and Fluorescence. Ann Biomed Eng. 2007; 35:1276–1285. [PubMed: 17372838]
44. Blomeyer CA, Bazil JN, Stowe DF, Pradhan RK, Dash RK, Camara AK. Dynamic Buffering of Mitochondrial Ca^{2+} During Ca^{2+} Uptake and Na^{+}-Induced Ca^{2+} Release. J Bioenerg Biomembr. 2013; 45:189–202. [PubMed: 23225099]
45. Boelens AD, Pradhan RK, Blomeyer CA, Camara AK, Dash RK, Stowe DF. Extra-Matrix Mg^{2+} Limits Ca^{2+} Uptake and Modulates Ca^{2+} Uptake-Independent Respiration and Redox State in Cardiac Isolated Mitochondria. J Bioenerg Biomembr. 2013; 45:203–218. [PubMed: 23456198]
46. Blomeyer CA, Bazil JN, Stowe DF, Dash RK, Camara AK. Mg^{2+} Differentially Regulates Two Modes of Mitochondrial Ca^{2+} Uptake in Isolated Cardiac Mitochondria: Implications for Mitochondrial Ca^{2+} Sequestration. J Bioenerg Biomembr. 2016; 48:175–188. [PubMed: 26815005]
47. Lindsay DP, Camara AK, Stowe DF, Lubbe R, Aldakkak M. Differential Effects of Buffer pH on Ca^{2+}-Induced ROS Emission with Inhibited Mitochondrial Complexes I and III. Front Physiol. 2015; 6:58. [PubMed: 25805998]
48. Aldakkak M, Stowe DF, Dash RK, Camara AK. Mitochondrial Handling of Excess Ca^{2+} Is Substrate-Dependent with Implications for Reactive Oxygen Species Generation. Free Radic Biol Med. 2013; 56:193–203. [PubMed: 23010495]
49. Rhodes SS, Camara AK, Heisner JS, Riess ML, Aldakkak M, Stowe DF. Reduced Mitochondrial Ca^{2+} Loading and Improved Functional Recovery after Ischemia-Reperfusion Injury in Old vs. Young Guinea Pig Hearts. Am J Physiol Heart Circ Physiol. 2012; 302:H855–863. [PubMed: 22140052]
50. Xu W, Liu Y, Wang S, McDonald T, Van Eyk JE, Sidor A, O'Rourke B. Cytoprotective Role of Ca^{2+}- Activated K^{+} Channels in the Cardiac Inner Mitochondrial Membrane. Science. 2002; 298:1029–1033. [PubMed: 12411707]
51. Clements RT, Terentyev D, Sellke FW. Ca^{2+}-Activated K^{+} Channels as Therapeutic Targets for Myocardial and Vascular Protection. Circ J. 2015; 79:455–462. [PubMed: 25746520]
52. Borchert GH, Yang C, Kolar F. Mitochondrial Bk_{ca} Channels Contribute to Protection of Cardiomyocytes Isolated from Chronically Hypoxic Rats. Am J Physiol Heart Circ Physiol. 2011; 300:H507–513. [PubMed: 21112945]
53. Kang SH, Park WS, Kim N, Youm JB, Warda M, Ko JH, Ko EA, Han J. Mitochondrial Ca^{2+}-Activated K^{+} Channels More Efficiently Reduce Mitochondrial Ca^{2+} Overload in Rat Ventricular Myocytes. Am J Physiol Heart Circ Physiol. 2007; 293:H307–313. [PubMed: 17351070]
54. Riess M, Rhodes SS, Novalija E, Jiang MT, Stowe DF. Reduced Cytosolic [Ca^{2+}] During Preconditioning with Sevoflurane Is Associated with Improved Function and Reduced Infarct Size after Global Ischemia in Intact Hearts. Anesthesiology. 2001; 95:A497.
55. An JZ, Varadarajan SG, Camara A, Chen Q, Novalija E, Gross GJ, Stowe DF. Blocking Na^{+}/H^{+} Exchange Reduces $[Na^{+}]_I$ and $[Ca^{2+}]_I$ Load after Ischemia and Improves Function in Intact Hearts. Am J Physiol Heart Circ Physiol. 2001; 281:H2398–H2409. [PubMed: 11709405]

56. Riess ML, Varadarajan SG, Chen Q, Camara AKS, Novalija E, Rhodes SS, Stowe DF. Anesthetic Preconditioning Decreases Mitochondrial [Ca^{2+}] Overload During Global Ischemia in Isolated Guinea Pig Hearts. FASEB J. 2002; 16:A488.
57. Camara AK, Chen Q, An J, Novalija E, Riess ML, Rhodes SS, Stowe DF. Comparison of Hyperkalemic Cardioplegia with Altered [$CaCl_2$] and [$MgCl_2$] on [Ca^{2+}]$_I$ Transients and Function after Warm Global Ischemia in Isolated Hearts. J Cardiovasc Surg (Torino). 2004; 45:1–13.
58. Aldakkak M, Camara AK, Heisner JS, Yang M, Stowe DF. Ranolazine Reduces Ca^{2+} Overload and Oxidative Stress and Improves Mitochondrial Integrity to Protect against Ischemia Reperfusion Injury in Isolated Hearts. Pharmacol Res. 2011; 64:381–392. [PubMed: 21741479]
59. Stefani E, Ottolia M, Noceti F, Olcese R, Wallner M, Latorre R, Toro L. Voltage-Controlled Gating in a Large Conductance Ca^{2+}-Sensitive K^+ Channel (H_{slo}). Proc Natl Acad Sci U S A. 1997; 94:5427–5431. [PubMed: 9144254]
60. Xia XM, Zeng X, Lingle CJ. Multiple Regulatory Sites in Large-Conductance Calcium-Activated Potassium Channels. Nature. 2002; 418:880–884. [PubMed: 12192411]
61. Sheng JZ, Weljie A, Sy L, Ling S, Vogel HJ, Braun AP. Homology Modeling Identifies C-Terminal Residues That Contribute to the Ca^{2+} Sensitivity of a BK_{Ca} Channel. Biophys J. 2005; 89:3079–3092. [PubMed: 16100257]
62. Wang ZW, Nara M, Wang YX, Kotlikoff MI. Redox Regulation of Large Conductance Ca^{2+}-Activated K^+ Channels in Smooth Muscle Cells. J Gen Physiol. 1997; 110:35–44. [PubMed: 9234169]
63. Aldakkak M, Stowe DF, Cheng Q, Kwok WM, Camara AK. Mitochondrial Matrix K^+ Flux Independent of Large-Conductance Ca^{2+}-Activated K^+ Channel Opening. Am J Physiol Cell Physiol. 2010; 298:C530–541. [PubMed: 20053924]
64. Barrett JN, Magleby KL, Pallotta BS. Properties of Single Calcium-Activated Potassium Channels in Cultured Rat Muscle. J Physiol. 1982; 331:211–230. [PubMed: 6296366]
65. Millar ID, Bruce J, Brown PD. Ion Channel Diversity, Channel Expression and Function in the Choroid Plexuses. Cerebrospinal Fluid Res. 2007; 4:8. [PubMed: 17883837]
66. Rumsey WL, Schlosser C, Nuutinen EM, Robiolio M, Wilson DF. Cellular Energetics and the Oxygen Dependence of Respiration in Cardiac Myocytes Isolated from Adult Rat. J Biol Chem. 1990; 265:15392–15402. [PubMed: 2394731]
67. Riess ML, Camara AK, Kevin LG, An J, Stowe DF. Reduced Reactive O_2 Species Formation and Preserved Mitochondrial NADH and [Ca^{2+}] Levels During Short-Term 17°C Ischemia in Intact Hearts. Cardiovasc Res. 2004; 61:580–590. [PubMed: 14962488]
68. Becker LB, Vanden Hoek TL, Shao ZH, Li CQ, Schumacker PT. Generation of Superoxide in Cardiomyocytes During Ischemia before Reperfusion. Am J Physiol Heart Circ Physiol. 1999; 277:H2240–2246.
69. Vanden Hoek TL, Li C, Shao Z, Schumacker PT, Becker LB. Significant Levels of Oxidants Are Generated by Isolated Cardiomyocytes During Ischemia Prior to Reperfusion. J Mol Cell Cardiol. 1997; 29:2571–2583. [PubMed: 9299379]
70. Duranteau J, Chandel NS, Kulisz A, Shao Z, Schumacker PT. Intracellular Signaling by Reactive Oxygen Species During Hypoxia in Cardiomyocytes. J Biol Chem. 1998; 273:11619–11624. [PubMed: 9565580]
71. Camara AK, An JZ, Chen Q, Novalija E, Varadarajan SG, Schelling P, Stowe DF. Na^+/H^+ Exchange Inhibition with Cardioplegia Reduces Cytosolic [Ca^{2+}] and Damage after 4 Hour Cold Ischemia. J Cardiovasc Pharmacol. 2003; 41:689–698.
72. Kowaltowski AJ, Seetharaman S, Paucek P, Garlid KD. Bioenergetic Consequences of Opening the ATP-Sensitive K^+ Channel of Heart Mitochondria. Am J Physiol Heart Circ Physiol. 2001; 280:H649–657. [PubMed: 11158963]
73. Dos Santos P, Kowaltowski AJ, Laclau MN, Seetharaman S, Paucek P, Boudina S, Thambo JB, Tariosse L, Garlid KD. Mechanisms by Which Opening the Mitochondrial ATP-Sensitive K^+ Channel Protects the Ischemic Heart. Am J Physiol Heart Circ Physiol. 2002; 283:H284–295. [PubMed: 12063301]

74. Korge P, Honda HM, Weiss JN. K^+-Dependent Regulation of Matrix Volume Improves Mitochondrial Function under Conditions Mimicking Ischemia-Reperfusion. Am J Physiol Heart Circ Physiol. 2005; 289:H66–77. [PubMed: 15764674]

75. O'Rourke B. Pathophysiological and Protective Roles of Mitochondrial Ion Channels. [review]. J Physiol. 2000; 529:23–36. [PubMed: 11080248]

76. Akao M, O'Rourke B, Teshima Y, Seharaseyon J, Marban E. Mechanistically Distinct Steps in the Mitochondrial Death Pathway Triggered by Oxidative Stress in Cardiac Myocytes. Circ Res. 2003; 92:186–194. [PubMed: 12574146]

77. Das M, Parker JE, Halestrap AP. Matrix Volume Measurements Challenge the Existence of Diazoxide/Glibencamide-Sensitive K_{ATP} Channels in Rat Mitochondria. J Physiol. 2003; 547:893–902. [PubMed: 12562892]

78. Murata M, Akao M, O'Rourke B, Marban E. Mitochondrial ATP-Sensitive Potassium Channels Attenuate Matrix Ca^{2+} Overload During Simulated Ischemia and Reperfusion: Possible Mechanism of Cardioprotection. Circ Res. 2001; 89:891–898. [PubMed: 11701616]

79. Drose S, Brandt U, Hanley PJ. K^+-Independent Actions of Diazoxide Question the Role of Inner Membrane K_{ATP} Channels in Mitochondrial Cytoprotective Signaling. J Biol Chem. 2006; 281:23733–23739. [PubMed: 16709571]

80. Wojtovich AP, Smith CO, Urciuoli WR, Wang YT, Xia XM, Brookes PS, Nehrke K. Cardiac Slo2.1 Is Required for Volatile Anesthetic Stimulation of K^+ Transport and Anesthetic Preconditioning. Anesthesiology. 2016; 124:1065–1076. [PubMed: 26845140]

81. Wojtovich AP, Sherman TA, Nadtochiy SM, Urciuoli WR, Brookes PS, Nehrke K. Slo-2 Is Cytoprotective and Contributes to Mitochondrial Potassium Transport. Plos One. 2011:6.

82. Holmuhamedov EL, Jovanovic S, Dzeja PP, Jovanovic A, Terzic A. Mitochondrial ATP-Sensitive K^+ Channels Modulate Cardiac Mitochondrial Function. Am J Physiol. 1998; 275:H1567–1576. [PubMed: 9815062]

83. Garlid KD. Opening Mitochondrial K_{ATP} in the Heart--What Happens, and What Does Not Happen. Basic Res Cardiol. 2000; 95:275–279. [PubMed: 11005581]

84. Halestrap AP. Regulation of Mitochondrial Metabolism through Changes in Matrix Volume. Biochem Soc Trans. 1994; 22:522–529. [review]. [PubMed: 7958359]

85. Hanley PJ, Mickel M, Loffler M, Brandt U, Daut J. K_{ATP} Channel-Independent Targets of Diazoxide and 5-Hydroxydecanoate in the Heart. J Physiol. 2002; 542:735–741. [PubMed: 12154175]

Summary

Big and small conductance Ca^{2+} -sensitive K^{+} channel isoforms are present in the inner mitochondrial membrane of cardiac ventricular mitochondria. These channels appear to have a differential protective role against cardiac ischemia reperfusion injury.

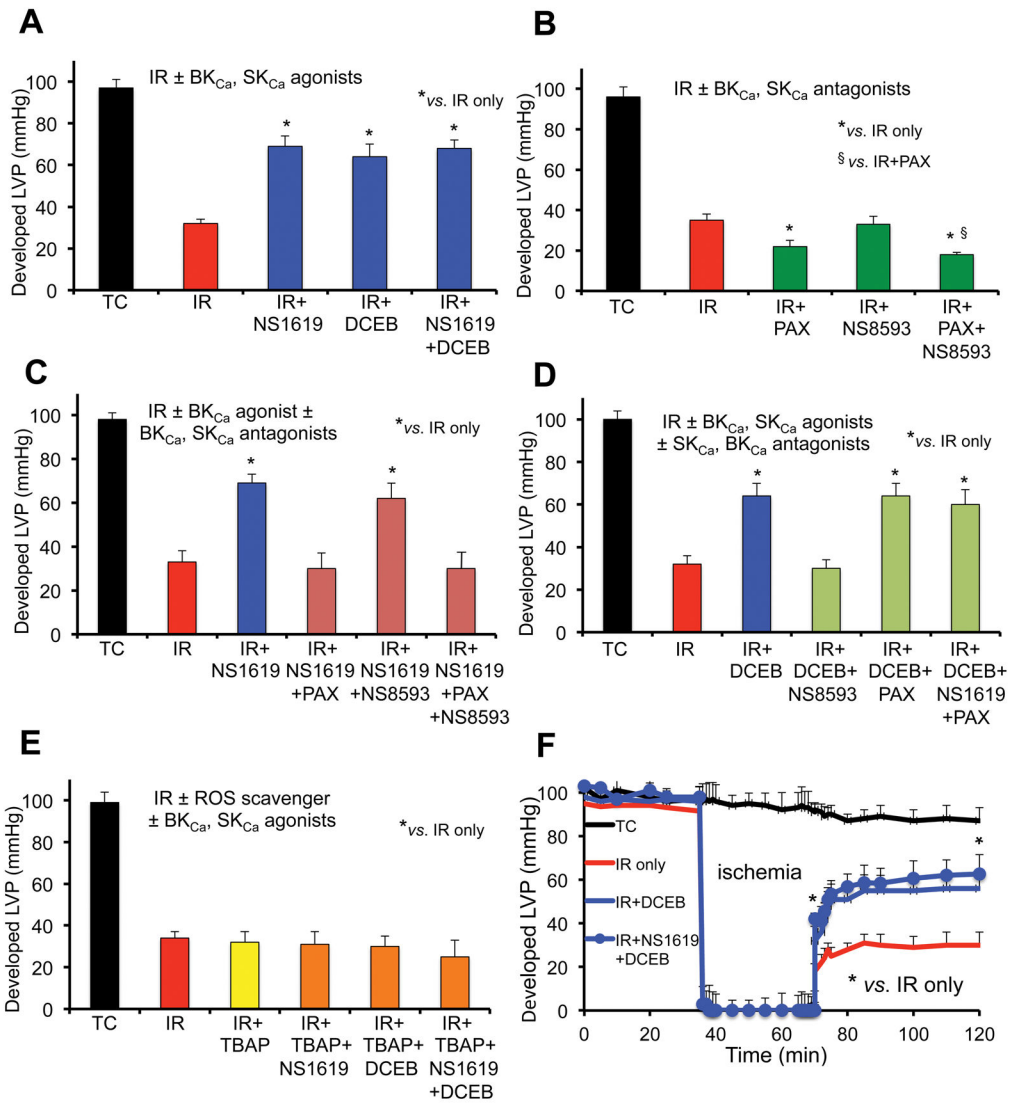

Fig. 1.

Average developed (systolic-diastolic) left ventricular pressure (LVP) 120 min after global IR injury when isolated guinea pig hearts were perfused without ischemia (Time Controls, TC); with 35 min ischemia and 120 min reperfusion (IR); or with IR + BK_{Ca} and or SK_{Ca} channel agonists (**A**); IR + BK_{Ca} and or SK_{Ca} channel antagonists (**B**); IR + BK_{Ca} agonist and or BK_{Ca}, SK_{Ca} antagonists (**C**); IR + BK_{Ca} agonist and or SK_{Ca} or BK_{Ca} antagonist (**D**); and SOD dismutator + BK_{Ca} and or SK_{Ca} agonist (**E**). Developed LVP over time for the IR only, IR + SK_{Ca} agonist and SK_{Ca} + BK_{Ca} agonist groups are displayed (**F**). Note the worsened effects on developed LVP of BK_{Ca} and BK_{Ca} + SK_{Ca} antagonists (green bars) *vs.* IR only (red bars); the block of protection by the BK_{Ca} or SK_{Ca} agonists when the BK_{Ca} or SK_{Ca} antagonists, respectively, was present (green bars); the maintained protection by the BK_{Ca} and or SK_{Ca} agonists in the presence of either the SK_{Ca} or the BK_{Ca} antagonist, respectively, (blue bars); and the loss of protection by the SK_{Ca} and or BK_{Ca} agonists in the presence of SOD inhibition by TBAP (orange/green bars). For each treatment group n = 5–6

hearts; note that for 32 of these hearts' mitochondria were isolated after 20 min reperfusion to assess RCI and CRC (Figs. 4,5). Data expressed as mean ± sem. *,§ $P<0.05$.

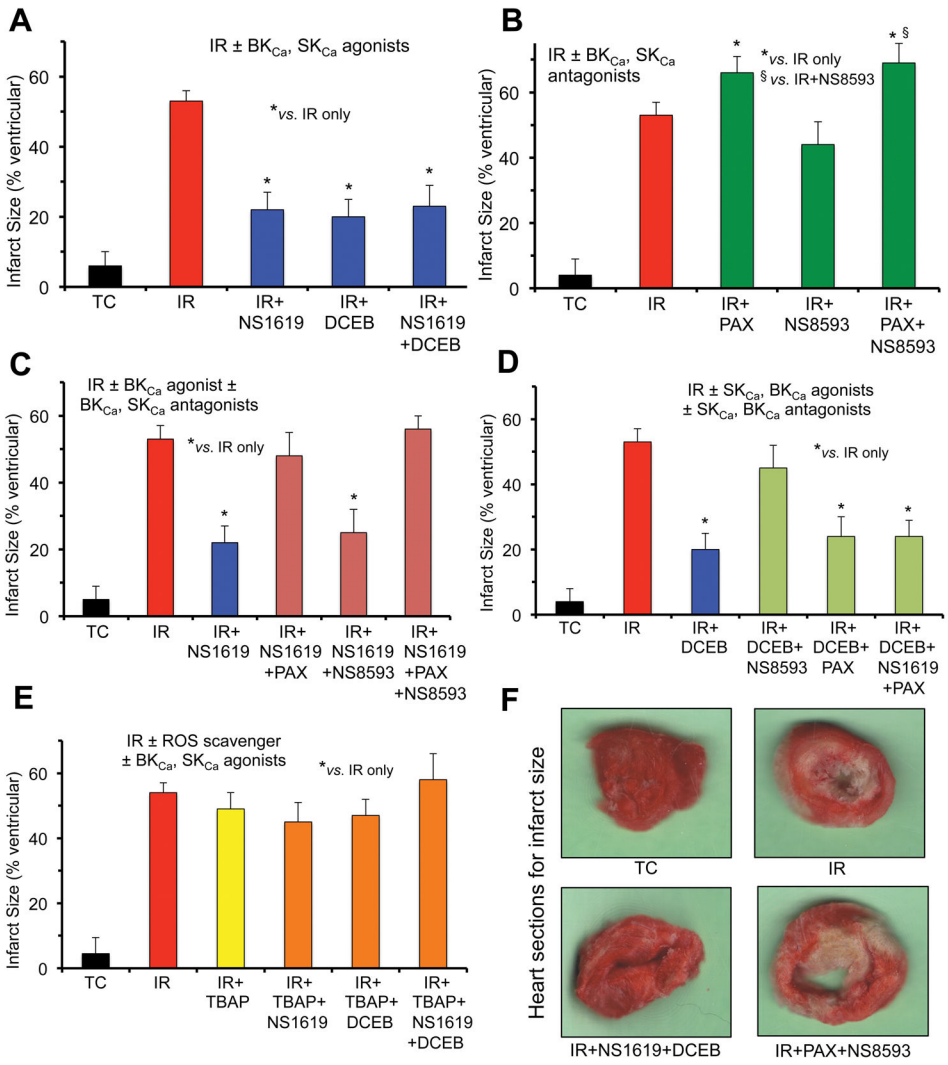

Fig. 2.

Percent infarct size assessed 120 min after global IR injury when the same (Fig. 1) isolated guinea pig hearts were perfused without ischemia (Time Controls, TC); with 35 min ischemia and 120 min reperfusion (IR); or with IR + BK_{Ca} and or SK_{Ca} channel agonists (**A**); IR + BK_{Ca} and or SK_{Ca} channel antagonists (**B**); IR + BK_{Ca} agonist and or BK_{Ca}, SK_{Ca} antagonists (**C**); IR + BK_{Ca} agonist and or SK_{Ca} or BK_{Ca} antagonist (**D**); and SOD dismutator + BK_{Ca} and or SK_{Ca} agonist (**E**). Representative hearts from 4 groups displaying normal and infarcted tissue are displayed (**F**). Note the enhanced infarct size after treatment with BK_{Ca} or BK_{Ca} + SK_{Ca} antagonists (green bars) *vs.* IR only (red bars); the block of tissue protection by the BK_{Ca} or SK_{Ca} agonists when the BK_{Ca} or SK_{Ca} antagonists, respectively, was present (green bars); the maintained reduction in infarct size by the BK_{Ca} and or SK_{Ca} agonists in the presence of either the SK_{Ca} or the BK_{Ca} antagonist, respectively, (blue bars); and the loss of protection by the SK_{Ca} and or BK_{Ca} agonists in the presence of SOD inhibition by TBAP (orange/green bars). For each treatment and control group n=4. *,§$P<0.05$.

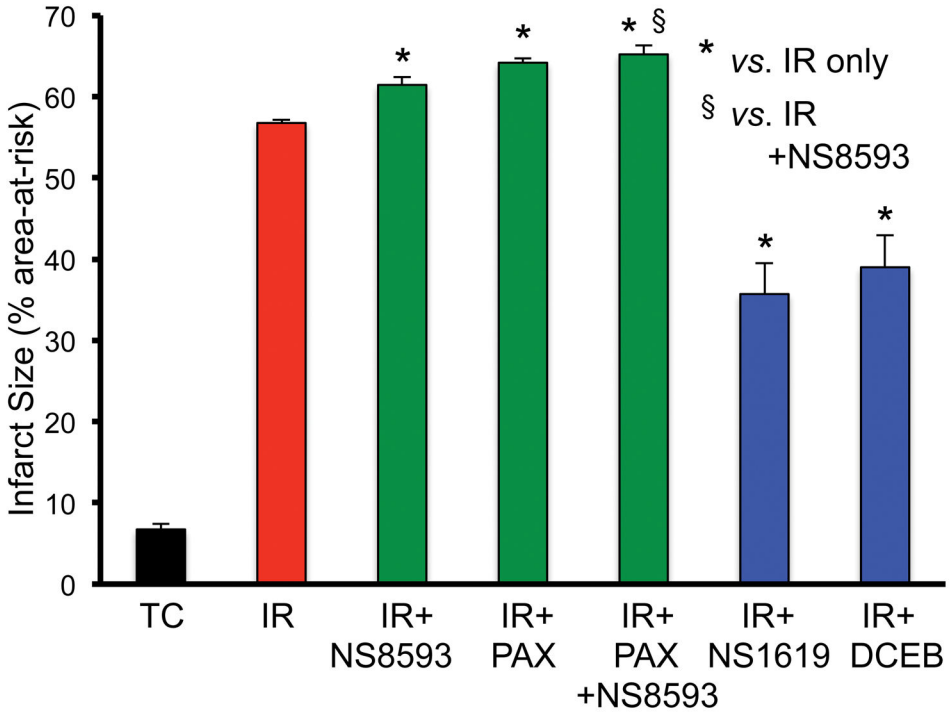

Fig. 3.

Infarct size (% area-at-risk) without (time control, TC) or after regional ischemia (LAD occlusion, IR) when 28 intact rats were infused i.v. with no drug (IR only), or with IR plus BK_{Ca} and or SK_{Ca} channel antagonists, or with the BK_{Ca} or SK_{Ca} channel agonist. Note that treatment either with the SK_{Ca} or the BK_{Ca} channel antagonist (green bars), or both together, increased infarct size *vs.* IR only (red bar), and that treatment with either the BK_{Ca} or SK_{Ca} agonist reduced infarct size (blue bars) vs. IR only. Average left ventricular area-at-risk was 39.2±2.1. For each treatment and control (sham) group n=4. $*,§P<0.05$.

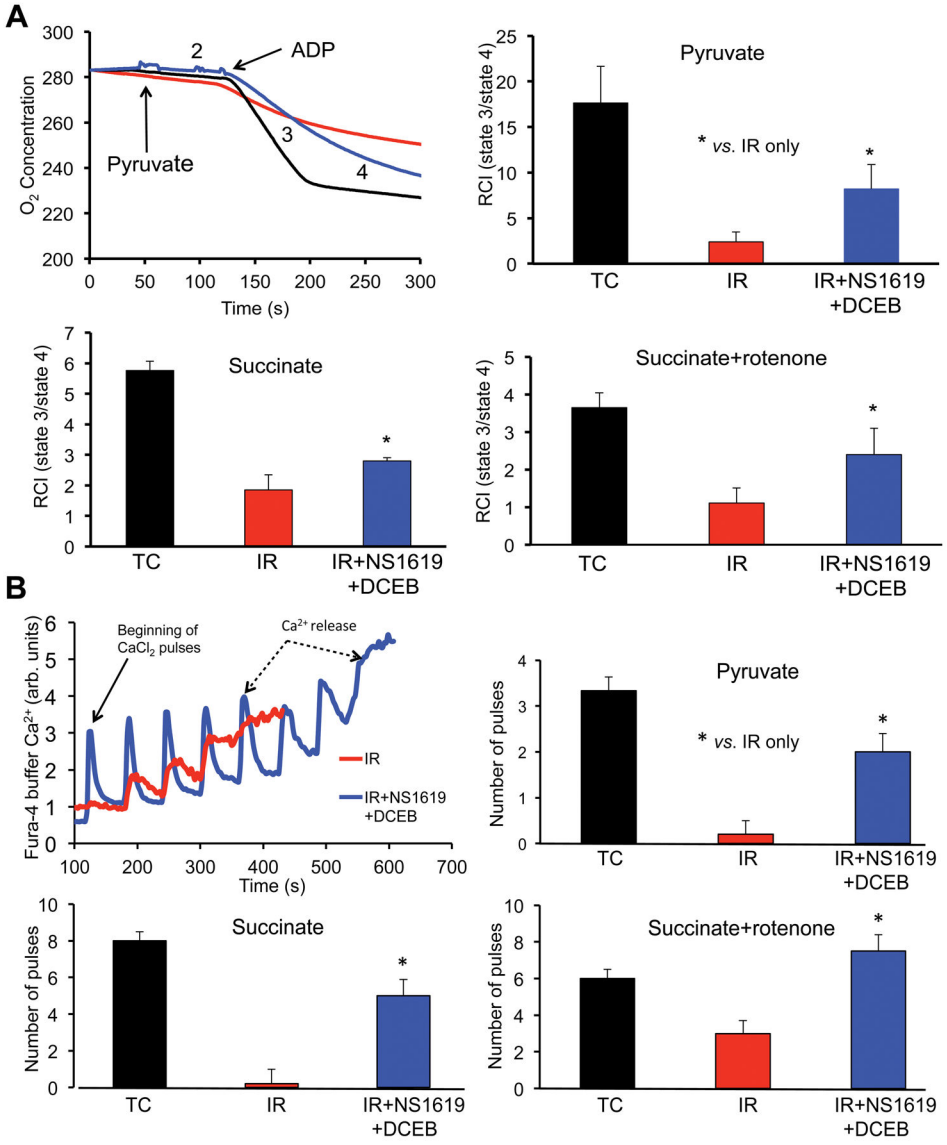

Fig. 4.

Effect of no treatment (TC, no I/R), IR only, or IR + BK_{Ca} channel agonist NS1619 + SK_{Ca} channel agonist DCEB, in 12 isolated guinea pig hearts on respiration (**A**) and Ca^{2+} retention capacity (CRC) (**B**) in mitochondria isolated after 20 min reperfusion from groups described in Figs. 1,2. The rate of O_2 consumption (respiration) was measured when substrate was added (state 2), followed by ADP (state 3) and after the added ADP was consumed (state 4); substrates added were pyruvate, succinate, or succinate + rotenone (see also Table 1); state 3/state 4 ratio is defined as the respiratory control index (RCI). Uptake of external Ca^{2+} was assessed (Fura-4 fluorescence) in mitochondria energized under the same 3 substrate conditions; increased number of 20 μM $CaCl_2$ injections (pulses) = increased CRC. Note increased RCI and retained Ca^{2+} (CRC) with combined BK_{Ca} and SK_{Ca} agonist treatment *vs.* IR only. For each group n = 4; RCI and CRC were measured from same mitochondrial pellet from each heart. *,§ $P<0.05$.

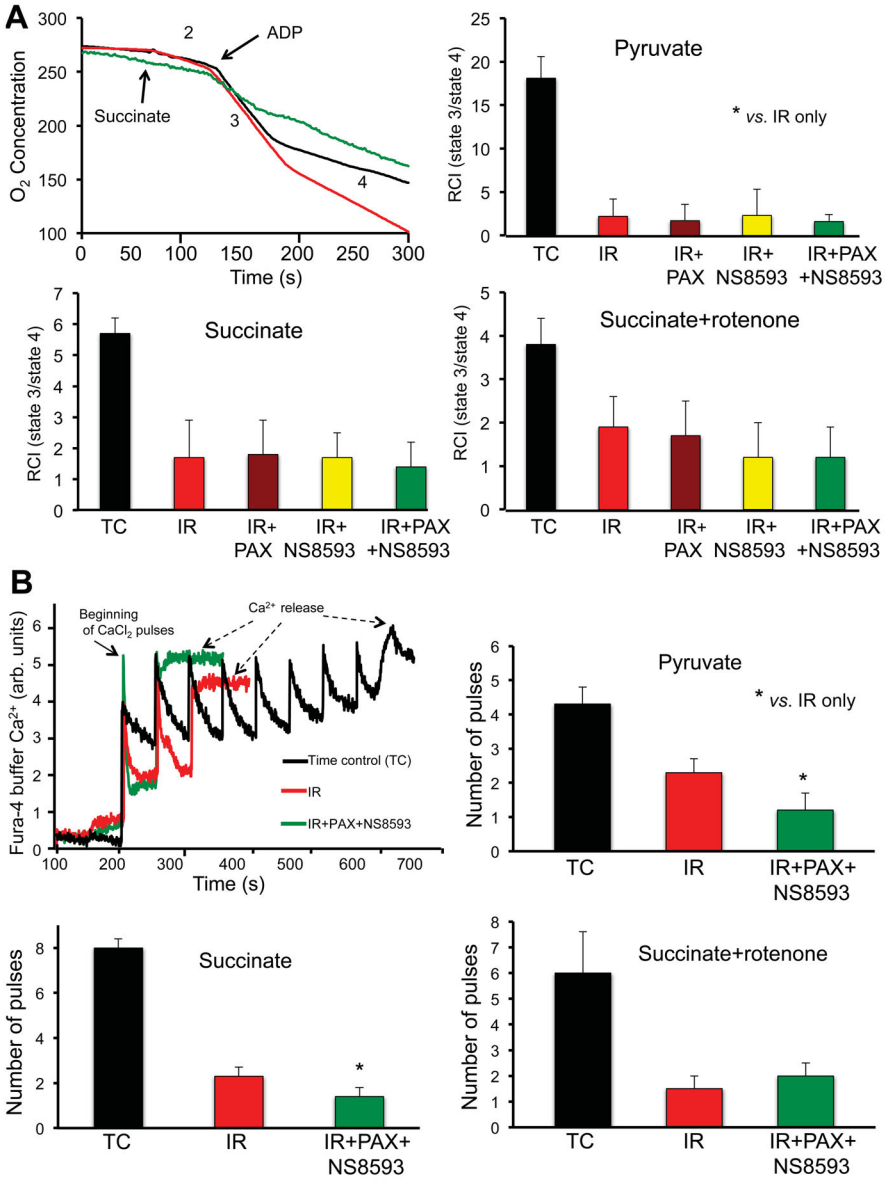

Fig. 5.

Effect of no treatment (TC, no I/R), IR only, or IR + BK_{Ca} channel antagonist PAX ± SK_{Ca} channel antagonist NS8593, in 20 isolated guinea pig hearts on respiration (**A**) and Ca^{2+} retention capacity (CRC) (**B**) in mitochondria isolated after 20 min reperfusion from groups described in Figs. 1,2. The rate of O_2 consumption (respiration) was measured when substrate was added (state 2), followed by ADP (state 3) and after the added ADP was consumed (state 4); substrates added were pyruvate, succinate, or succinate + rotenone (see also Table 2); state 3/state 4 ratio is defined as the respiratory control index (RCI). Uptake of external Ca^{2+} was assessed (Fura-4 fluorescence) in mitochondria energized under the same 3 substrate conditions; increased number of 20 μM $CaCl_2$ injections (pulses) = increased CRC. Note hat the decreased RCI with IR only was not different after treatment with the BK_{Ca} and or SK_{Ca} antagonists; in contrast, CRC was reduced after treatment with the BK_{Ca}

and or SK_{Ca} antagonists in the presence of pyruvate or succinate. For each group n = 4; RCI and CRC were measured from same mitochondrial pellet from each heart. * $P<0.05$.

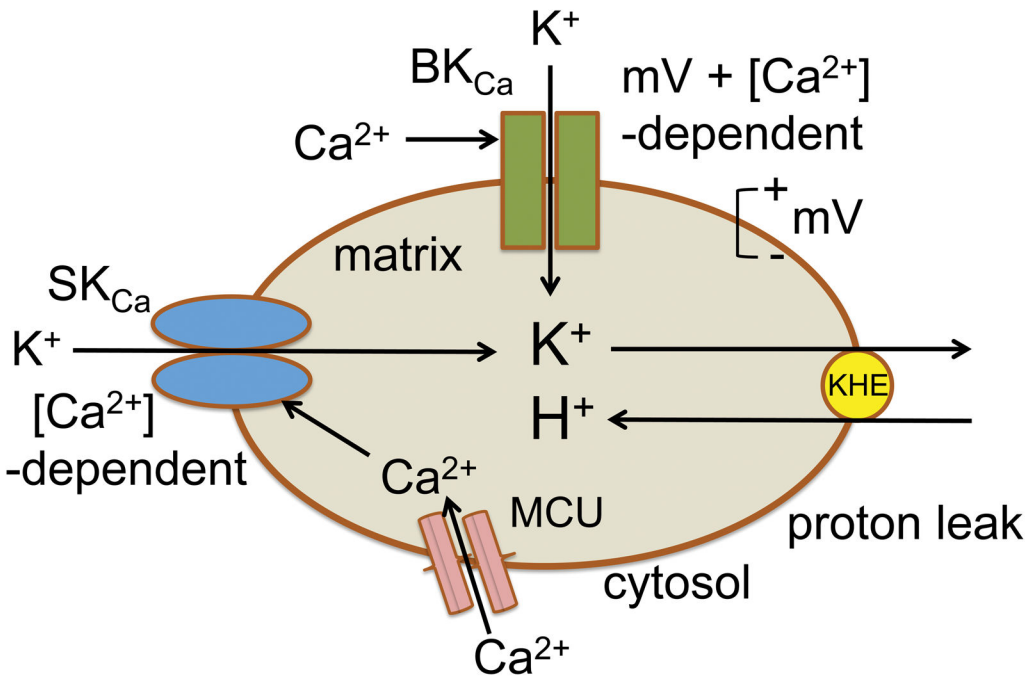

Fig. 6.

Schema of proposed interaction of BK_{Ca} and SK_{Ca} channels on regulation of mitochondrial K^+ influx stimulated by increases in cytosolic and mitochondrial $[Ca^{2+}]$ and on reciprocal mitochondrial H^+ influx with K^+ influx via mitochondrial K^+/H^+ exchange (KHE).

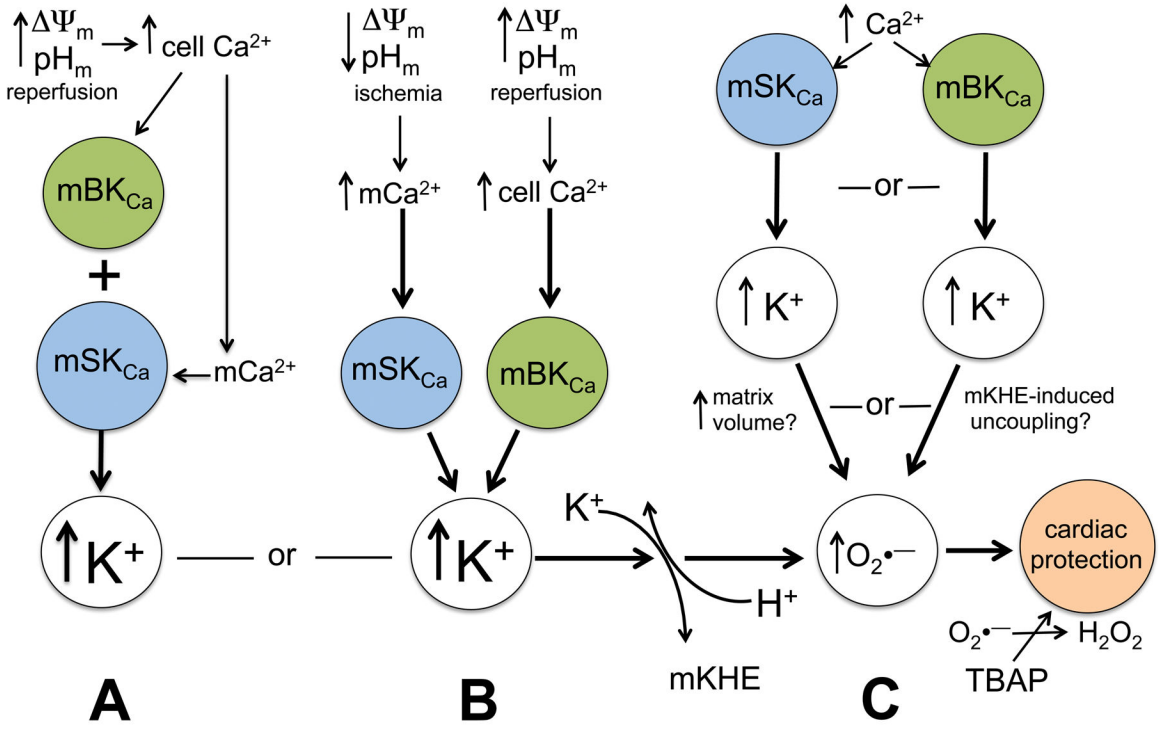

Fig. 7.

Cartoon summarizes possible scenarios for the interactions of mSK_{Ca} and mBK_{Ca} channels and the effects in mediating protection against IR injury. BK_{Ca} channels might be rapidly activated during reperfusion just after ischemia when $\Delta\Psi_m$ rapidly becomes fully charged and there is a surge in cytosolic and concomitantly an increase in mitochondrial $[Ca^{2+}]$ (**A**). Independently, SK_{Ca} channels might be activated more slowly during ischemia when mitochondrial matrix $[Ca^{2+}]$ progressively rises in association to the lower $\Delta\Psi_m$ and pH_m during ischemia (**B**); in this scenario, BK_{Ca} and SK_{Ca} channels might function rather independently to enhance K^+ influx and trigger K^+/H^+ exchange (KHE), cause mild uncoupling, and induce small amounts of signaling ROS. In another alternative pathway, net mK^+ influx due to SK_{Ca} channel opening might be affected by simultaneous BK_{Ca} channel opening to alter either the matrix volume or the degree of mKHE-induced uncoupling (**C**). In all possibilities, signaling levels of ROS would need to be produced as a result of mK^+ influx to initiate cardiac protection. **A** = additive or single pathway, **B** = parallel pathways with convergence, **C** = parallel pathways with different mechanisms.

Table 1

Modulation of respiration rate in isolated cardiac mitochondria after cardiac IR injury ±BK_{Ca} + SK_{Ca} channel agonists.

Pyruvate	**state 2**	**state 3**	**state 4**	**RCI**
TC	6.7 ±0.7	222.4 ±23.5	11.6 ±3.8	17.6 ±2.6
IR	37.6 ±0.7§	125.3 ±53.2§	69.0 ±17.1§	2.4 ±1.1§
IR+NS1619+DCEB	7.3 ±4.2	221.8 ±31.3*	33.4 ±4.4*§	8.2 ±2.7*§
Succinate	state 2	state 3	state 4	RCI
TC	65.0 ±0.7	457.6 ±38.6	79.7 ±15.4	5.8 ±0.4
IR	73.7 ±9.4	203.7 ±49.0§	114.6 ±7.7§	1.9 ±0.6§
IR+NS1619+DCEB	96.9 ±9.1*§	456.5 ±6.8*	181.5 ±10.3*§	2.8 ±0.2*§
Succinate+rotenone	state 2	state 3	state 4	RCI
TC	63.0 ±10.7	281.4 ±57.0	77.1 ±0.7	3.7 ±0.5
IR	63.0 ±5.4	113.2 ±67.0§	100.5 ±17.7§	1.1 ±0.6§
IR+ NS1619+DCEB	82.7 ±13.7	312.0 ±72*§	138.0 ±4.9§	2.4 ±0.5*§

Respiration rate (O_2 consumption, nmol/min/mg protein) was measured in cardiac mitochondria isolated at 20 min perfusion after 35 min global ischemia ± BK_{Ca} and SK_{Ca} channel agonists NS1619 and DCEB given 15 min before and for 20 min after ischemia. RCI (respiratory control index) = state 3/state 4. Time controls (TC) were not subject to ischemia but 75 min perfusion. Note that RCI's were higher after hearts were treated with NS1619 + DCEB before IR *vs.* IR alone with pyruvate and succinate + rotenone, but not with succinate. N = hearts/group with 3–4 replicates within each mitochondrial pellet. $P<0.05$;

*IR + drugs *vs.* IR only;

§IR ± drugs *vs.* TC.

See also Fig. 3A.

Table 2

Modulation of respiration rate in isolated cardiac mitochondria after cardiac IR injury ±BK_{Ca} + SK_{Ca} and/or SK_{Ca} channel antagonists.

Pyruvate	**state 2**	**state 3**	**state 4**	**RCI**
TC	6.8 ±0.7	210.2 ±22.4	11.6 ±3.8	18.1 ±2.5
IR	35.7 ±2.3§	112.0 ±33.9§	59.4 ±3.4§	2.2 ±2.0§
IR+PAX	12.0 ±2.6*§	92.3 ±25.2§	53.4 ±3.6§	1.7 ±1.9§
IR+NS8593	30.9 ±2.1*§	145.1 ±37.0§	58.6 ±6.2§	2.3 ±3.0§
IR+PAX+NS8593	20.6 ±1.1*§	96.0 ±20.1§	59.4 ±3.7§	1.6 ±0.8§
Succinate	state 2	state 3	state 4	RCI
TC	64.5 ±0.8	450.4 ±37.5	79.5 ±10.3	5.7 ±0.5
IR	72.0 ±3.2§	220.2 ±32.9§	129.0 ±12.0§	1.7 ±1.2§
IR+PAX	90.1 ±6.1*§	286.2 ±38.8§	154.1 ±14.2§	1.8 ±1.1§
IR+NS8593	168.1 ±8.2*§	280.0 ±53.3§	161.1 ±18.2§	1.7 ±0.8§
IR+PAX+NS8593	116.1 ±9.5*§	246.0 ±38.5§	178.2 ±15.2*§	1.4 ±0.8§
Succinate+rotenone	state 2	state 3	state 4	RCI
TC	62.4 ± 9.8	290.5 ±56.2	76.8 ±0.8	3.8 ±0.6
IR	87.2 ± 6.4	202.0 ±32.9	105.2 ±16.4§	1.9 ±0.7§
IR+PAX	77.4 ±11.6	234.2 ±55.1	136.0 ±33.7§	1.7 ±0.8§
IR+NS8593	96.2 ±14.2§	312.0 ±48.6*	261.3 ±38.2*§	1.2 ±0.8§
IR+PAX+NS8593	108.2 ±26.0§	305.3 ±45.7*	262.3 ±31.6*§	1.2 ±0.7§

Respiration rate (O_2 consumption, nmol/min/mg protein) was measured in cardiac mitochondria isolated at 20 min perfusion after 35 min global ischemia ± BK_{Ca} and SK_{Ca} channel antagonists NS8593 ± PAX given 20 min before and for 20 min after ischemia. RCI (respiratory control index) = state 3/state 4. Time controls (TC) were not subject to ischemia but 75 min perfusion. Note that the lower RCI's after IR were not significantly lower after hearts were treated with PAX ± NS8593 compared to IR alone with any substrate. N = hearts/group with 3–4 replicates within each mitochondrial pellet. $P<0.05$;

*IR + drugs *vs.* IR only;

§IR ± drugs *vs.* TC.

See also Fig. 4A.