Forum Minireview

New Aspects for the Treatment of Cardiac Diseases Based on the Diversity of Functional Controls on Cardiac Muscles: Mitochondrial Ion Channels and Cardioprotection

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Abstract Mitochondrial ATP-sensitive K⁺ (mitoK_{ATP}) and Ca²⁺-activated K⁺ (mitoK_{Ca}) channels exist in cardiac myocytes, and they play key roles in cardioprotection. We have recently reported that K⁺ influx through mitoK_{ATP} or mitoK_{Ca} channels occurs independently of each other and confers cardioprotection in a similar manner. Activation of mitoK_{ATP} channel is augmented by protein kinase C (PKC), whereas mitoK_{Ca} channel is activated by protein kinase A (PKA). However, phosphatidylinositol 3-kinase (PI3-K) is linked to neither mitoK_{ATP} nor mitoK_{Ca} channels. We have demonstrated that bioactive substances modulate the opening of mitoK_{ATP} channels via a PKC-dependent pathway or opening of mitoK_{Ca} channels via a PKA-dependent pathway and thereby protecting the heart from ischemia/reperfusion injury. Several endogenous substances such as adenosine and bradykinin can reduce infarct size by activation of mitoK_{ATP} channels in a PKC-dependent manner. Adrenomedullin, a potent vasodilator peptide, potentiates the opening of mitoK_{Ca} channels by PKA activation. Treatment with adrenomedullin prior to ischemia results in the reduction of infarct size via a PKA-mediated activation of mitoK_{Ca} channels. Thus, some endogenous substances confer cardioprotection via PKA- or PKC-mediated activation of mitoK_{ATP} or mitoK_{Ca} channels.

Keywords: cardioprotection, mitochondrial ATP-sensitive K⁺ channel, mitochondrial Ca²⁺-activated K⁺ channel, protein kinase A, protein kinase C, cardiac disease

Introduction

Transient episodes of nonlethal ischemia and reperfusion confer profound protection against myocardial injuries in response to a prolonged lethal episode of ischemia–reperfusion (I/R), a phenomenon that has been termed ischemic preconditioning (PC) (1). Although researchers have discovered a number of drugs that reduce I/R injury, the study of cardioprotection has been energized by the observation that PC reduces subsequent lethal ischemia–reperfusion injury (1, 2). Investigators have made considerable progress in elucidating the signaling pathways that are activated and required for

PC. Several lines of evidence support the idea that the activation of protein kinase C (PKC) and/or protein kinase A (PKA) plays an essential role in PC. Furthermore, recent findings have indicated that two kinds of potassium channels exist on the mitochondrial inner membrane, mitochondrial ATP-sensitive K⁺ (mitoK_{ATP}) channel and Ca²⁺-activated K⁺ (mitoK_{Ca}) channel; and they play critical roles in cardioprotection against I/R-induced injury (3 – 5). Activation of PKC, which figures prominently in the signal transduction cascade of PC, increases the open probability of mitoK_{ATP} channels in rabbit hearts (5), whereas activation of PKA potentiates the opening of mitoK_{Ca} channels as determined in our earlier study (3).

In this manuscript, we briefly review our recent experimental results showing that several bioactive endogenous substances modulate the opening of mitoK_{ATP}

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channels via a PKC-dependent pathway or opening of mitoK_{Ca} channels via a PKA-dependent pathway and thereby exert a cardioprotective effect in rabbit hearts. We have discussed the role of mitochondrial potassium channels in the cardioprotection against I/R-induced injury.

Mechanism of cardioprotection by mitochondrial K⁺ channels

One of the major physiological features of mitochondria is the generation of a large transmembrane potential across the mitochondrial inner membrane. This is a direct consequence of the biochemical reactions that constitute the respiratory chain. Thus, substrates supplied to mitochondria such as pyruvate, some amino acids, and products of β -oxidation of fatty acids enter the tricarboxylic acid cycle and maintain the reduced state of the NADH/NAD⁺ and FADH₂/FAD couples. These substances supply electrons to the respiratory chain, which eventually are transferred to oxygen. The process also transfers protons across the mitochondrial inner membrane, generating a proton gradient – a proton motive force that is largely expressed as a membrane potential usually estimated as 180 mV negative to the cytosol. The Ca²⁺ uptake into mitochondria is driven primarily by this large negative electrical potential of the matrix (6). Therefore, partial depolarization of the mitochondrial membrane through opening of mitochondrial K⁺ channels reduces the driving force for Ca²⁺ influx, which results in the prevention of mitochondrial Ca²⁺ overload during ischemia. Ishida et al. have reported that in rat cardiomyocytes, the selective mitoK_{ATP}-channel opener diazoxide depolarizes the mitochondrial membrane and attenuates the mitochondrial Ca²⁺ overload experimentally evoked by ouabain (7). Ouabain, a Na⁺/K⁺-ATPase inhibitor, impairs Na⁺ extrusion and consequently prevents Ca²⁺ extrusion via Na⁺/Ca²⁺ exchange. Elevation in cytosolic Ca²⁺ concentration eventually results in the accumulation of mitochondrial Ca²⁺ (8). Furthermore, we have recently demonstrated that K⁺ influx via mitochondrial K⁺ channels accelerates electron transfer by the respiratory chain and leads to net oxidation of mitochondria if uncompensated by electron donors and thereby attenuates mitochondrial Ca²⁺ overload with accompanying depolarization of the mitochondrial membrane (3, 5).

MitoK_{ATP} channel and PKC

The role of PKC as a trigger/mediator of the PC signal is clearly established with recent evidence implicating the role of PKC at the level of the mito-

chondria (9–12). Modulation of the mitoK_{ATP} channel by PKC was revealed by a previous study showing that the PKC-activating phorbol ester PMA potentiates the mitochondrial oxidation induced by diazoxide in rabbit ventricular myocytes with the use of flavoprotein fluorescence method (3). Flavoprotein oxidation was used to determine the pharmacological modulation of mitoK_{ATP} and mitoK_{Ca} channels in intact cardiac myocytes. Moreover, potentiation of mitoK_{ATP}-channel activation by adenosine via a PKC-mediated pathway was demonstrated with similar experimental methods (13). Adenosine receptors are not the only endogenous substance that triggers PC. It has been demonstrated that stimulation of various receptors can put the heart into a preconditioned state. These receptors include α_1 -adrenergic (14), bradykinin B₂ (15), angiotensin AT₁ (16), muscarinic M₂ (17), and endothelin ET_A (18) receptors. Signaling molecules for all of these receptors are released to some degree during PC ischemia and all stimulate Gi- or Gq-linked receptors and ultimately activate PKC (19). Recently, we have found that oxytocin can afford cardioprotection during I/R through the activation of mitoK_{ATP} channels (20). Oxytocin is a small peptide hormone and binds to its membrane receptors on the endometrial cells to increase the activity of phospholipase C, yielding diacylglycerol, a PKC activator. Our preliminary data indicated that oxytocin potentiates the opening of mitoK_{ATP} channels in a PKC-dependent manner and has an infarct size-limiting effect in rabbit Langendorff-perfused hearts (20).

MitoK_{Ca} channel and PKA

Activation and translocation of PKC are reportedly involved in PC (21–24). There is good evidence that the final mediators of cardioprotection are the ischemia-induced opening of the mitoK_{ATP} channels and the production of free radicals. On the other hand, a brief PC ischemia also induces a synchronized increase in the myocardial cyclic AMP (cAMP) level (25). Furthermore, several studies have demonstrated that a brief pre-ischemic exposure to β -agonists, phosphodiesterase type III inhibitors, and a cell-permeable cAMP analogue, all of which produce rapid activation of PKA, also protect the myocardium in vivo independently of PKC (25, 26). Moreover, our previous study has shown by using the flavoprotein fluorescence method that mitoK_{Ca}-channel opening can be potentiated by cAMP-dependent protein kinase (3).

To study the PKA-dependent modulation of mitoK_{Ca} channels, we examined the effects of adrenomedullin (AM) on flavoprotein oxidation in rabbit ventricular cells. AM is a potent vasodilator peptide that was origi-

Fig. 1. Effects of adrenomedullin (AM) on flavoprotein (FP) oxidation and myocardial infarct size. **A:** Representative data showing effects of AM (10 nM) on NS1619 (30 μ M)-induced FP oxidation. Bars indicate periods when the cells were exposed to each drug. FP fluorescence was calibrated by exposing the cells to 2,4-dinitrophenol (DNP, 100 μ M) at the end of experiments. **B:** Summarized data for percentage of FP oxidation. AM potentiated the NS1619-induced FP oxidation, and paxilline (PX, 2 μ M) completely inhibited the oxidative effect of NS1619 in the presence of AM. In the presence of KT5720 (KT, 200 nM), AM failed to augment NS1619-induced FP oxidation. Values are expressed as the percent relative to those obtained with DNP. * $P < 0.05$ vs. NS1619 alone. **C:** Summarized effect of AM on infarct size. AM was administered before ischemia. Pretreatment with PX or KT significantly blocked the infarct size-limiting effect of AM. Each open circle represents the infarct size in an individual heart, and closed circles with error bars are group means $\pm$ S.E.M. * $P < 0.01$ vs. control. This modified figure was reproduced with permission from Ref. 28.

nally isolated from human pheochromocytoma tissue by monitoring cAMP levels in platelets (27). Later, the peptide was shown to increase the level of cAMP in a

wide variety of cells, including smooth muscle cells, endothelial cells, and cardiac cells. Figure 1A shows the time course of changes in flavoprotein fluorescence in a cell exposed to the mitoK_{Ca}-channel opener NS1619 and AM. Opening of mitoK_{Ca} channels by NS1619 oxidized flavoprotein and increased the fluorescence intensity. Subsequent exposure to AM augmented the NS1619-induced flavoprotein oxidation. As summarized in Fig. 1B, NS1619 alone increased flavoprotein oxidation. Although AM significantly augmented the flavoprotein oxidation induced by NS1619, the peptide failed to augment the oxidative effect of NS1619 in the presence of KT5720, a potent inhibitor of PKA. Furthermore, paxilline, a mitoK_{Ca}-channel blocker, completely attenuated the oxidative effects of NS1619 in the presence of AM. Then, we examined the effect of AM on infarct size in the isolated perfused rabbit hearts subjected to 30-min global ischemia / 120-min reperfusion. As shown in Fig. 1C, administration of AM to the heart for 10 min prior to ischemia significantly reduced infarct size compared with the control group. To test whether the infarct size-limiting effect of AM was mediated by PKA-dependent activation of mitoK_{Ca} channels, we treated the hearts with paxilline or KT5720. Treatment with paxilline blocked the AM-induced reduction in infarct size. Similarly, treatment with KT5720 abolished the infarct size-limiting effect of AM. These results suggest that AM treatment prior to ischemia provides cardioprotection and that the protection is mediated by PKA-dependent activation of mitoK_{Ca} channels (28).

Mitochondrial K⁺ channels and phosphatidylinositol 3-kinase (PI3-K)

More recently, the newly developed concept of postconditioning as a way of reducing reperfusion injury has been receiving much attention. The term postconditioning refers to the phenomenon in which brief repetitive episodes of I/R at the immediate onset of reperfusion result in a reduction in infarct size (29). Importantly, evidence for the existence of postconditioning has also been obtained in humans (30). Postconditioning-induced protection seems to be mediated via the reperfusion injury salvage kinases (RISK) pathway (31). PI3-K is a component of the RISK pathway, and pharmacological activation of this kinase at reperfusion can mimic postconditioning (31). Accordingly, it would be of great interest to know the link between the activation of PI3-K/Akt and the opening of mitochondrial potassium channels.

To study the link between PI3-K and mitoK_{Ca} channels, we examined the effects of AM on flavo-

Fig. 2. Summarized effects of PI3-K on mitoK_{Ca} channels. A: AM (10 nM) augmented the oxidative effect of NS1619 (30 μ M) even in the presence of LY294002 (5 μ M). B: Erythropoietin (EPO, 10 units/ml) could not mimic the oxidative effect of AM. Values are expressed as the percent relative to those obtained with DNP. * $P < 0.05$ vs. NS1619 alone. This modified figure was reproduced with permission from Ref. 28.

protein oxidation in rabbit ventricular cells again, since Okumura et al. reported that intravenous administration of AM attenuated myocardial I/R injury through a PI3-K-dependent pathway (32). As shown in Fig. 2, AM significantly increased the oxidative effect of NS1619 even in the presence of LY294002, a potent inhibitor of PI3-K. Moreover, erythropoietin, a potent activator of PI3-K (33, 34), did not augment the NS1619-induced flavoprotein oxidation (28). These results suggest that the mitoK_{Ca} channel is not regulated by PI3-K. On the other hand, it is unclear whether activation of the PI3-K/Akt pathway modulates the opening of mitoK_{ATP} channels. Interestingly, Lee et al. have reported that BMS-180448, a prototype mitoK_{ATP}-channel opener, produces a cardioprotective effect without the phosphorylation of Akt (35). Contrary to this report, a recent study by Ahmad et al. has demonstrated that cardio-protection by mitoK_{ATP}-channel activation is dependent

on Akt translocation from the cytosol to mitochondria (36). Although the reason for this discrepancy is not clear, our preliminary unpublished data using the flavoprotein fluorescence method suggest that PI3-K activity is independent on mitoK_{ATP}-channel activation. Further studies are needed to clarify the causal relationship between the PI3-K/Akt pathway and mitoK_{ATP} channels.

Recently, it has been postulated that the activation of the RISK pathway confers its cardioprotection through the inhibition of mitochondrial permeability transition pore (PTP) (37). PTP promotes the release of pro-apoptotic signaling molecules from the mitochondria and triggers apoptosis (38). Interestingly, the mitoK_{ATP}-channel activation, either by intracellular signaling (9) or by mitoK_{ATP}-channel openers, also inhibits the opening of PTP. Taken together, these findings indicate that activation of mitochondrial potassium channels and the PI3-K/Akt pathway independently confer cardio-protection by inhibiting the opening of PTP (Fig. 3).

Functional molecules forming mitoK_{ATP} and mitoK_{Ca} channels

The molecular structure of sarcolemmal K_{ATP} (sarcK_{ATP}) channels has been clarified by cloning of the inwardly rectifying K⁺-channel subfamily Kir6.0 [Kir6.1 (48 kDa) and Kir6.2 (44 kDa)] and the receptors for sulfonylureas [SUR1 (177 kDa), SUR2A (74 kDa), and SUR2B (174 kDa)] (39, 40). From a reconstitution study, cardiac sarcK_{ATP} channels have been suggested to comprise SUR2A and Kir6.2 (41). Our functional study using Kir6.2-deficient mice has provided direct evidence that Kir6.2 forms the pore region of cardiac sarcK_{ATP} channels (42). On the other hand, molecular identification of mitoK_{ATP} channel has not been made yet.

Fig. 3. Schematic representation of the mechanism of protection mediated by mitoK_{ATP} and mitoK_{Ca} channels. PKA potentiates mitoK_{Ca}-channel activation, whereas PKC potentiates mitoK_{ATP}-channel activation. Opening of mitoK_{Ca} channels and mitoK_{ATP} channels depolarizes $\Delta\Psi_m$, which reduces the driving force for Ca²⁺ influx, thereby attenuating the mitochondrial Ca²⁺ overload. Consequently, prevention of matrix Ca²⁺ overload inhibits the opening of mitochondrial permeability transition pore (PTP) and protects against heart cell death by an antiapoptotic mechanism. It has been demonstrated that the PI3-K/Akt pathway is independently involved in cardio-protection through the direct inhibition of PTP.

However, there are many lines of evidence linking the opening of the mitoK_{ATP} channel to protection against I/R injury or apoptosis. Although the molecular identity of the mitoK_{ATP} channel remains unclear, experiments using dominant negative gene transfer have indicated that neither Kir6.1 nor Kir6.2 is a functionally important part of the mitoK_{ATP} channel in native heart cells (43). In addition, the mitoK_{ATP}-channel function, evaluated by flavoprotein oxidation, was preserved in cardiomyocytes of either Kir6.2-deficient (44) or Kir6.1-deficient mice (45). However, K⁺ uniport activity has been observed when purified mitochondrial proteins in the molecular weight range of 50–60 kDa have been reconstituted into proteoliposomes, (46, 47), and a 54-kDa protein was tentatively identified as a component of mitoK_{ATP} (48). The existence of SUR proteins in heart mitochondria is still controversial (49, 50), whilst a protein of about 28 kDa was labeled by [¹²⁵I]-glibenclamide (51). Recently, Ardehali et al. have reported that the mitoK_{ATP} channel is potentially composed of four mitochondrial proteins (mitochondrial ATP-binding cassette protein 1, phosphate carrier, adenine nucleotide translocator, and ATP synthase) and succinate dehydrogenase; and thus it is a multiprotein complex (52).

Large-conductance Ca²⁺-activated K⁺ (BK) channels are ubiquitously expressed in both excitable and non-excitable cells and contribute to diverse physiological processes. BK channels are composed of tetrameric sets consisting of a pore-forming α -subunit and an auxiliary subunit, β -subunit (53). In contrast to K_{ATP} channels, the BK channels are not functionally expressed in the sarcolemma of cardiomyocytes. However, a study from O'Rourke's laboratory (54) has suggested that a BK channel is expressed in the mitochondrial inner membrane of ventricular myocytes (mitoK_{Ca} channel). Studies of the pharmacological and biochemical profiles have suggested that mitoK_{Ca} channels belong to the Slo-1 family (54, 55); however, the molecular components of mitoK_{Ca} channels also have not been identified as mitoK_{ATP} channels. Recently Ohya et al. have demonstrated that BK channel β 1-subunit transcripts are expressed in mammalian cardiac mitochondria (56). Identification of functional molecules forming mitochondrial K⁺ channels may enable considerable progress in the search for a new type of cardioprotective drugs.

Concluding remarks

The mitochondrial K_{ATP} and K_{Ca} channels play an important role in cardioprotection. This review has focused on the compelling evidence that they can act as signal transduction elements to trigger as well as mediate the PC state. In addition, the IONA study (57)

has demonstrated a significant improvement in outcome due to a reduction in major coronary events by anti-anginal therapy with nicorandil, a mitoK_{ATP}-channel opener, in patients with stable angina. This outcome can strongly support the applicability of this class of drugs to clinical settings.

Despite unanswered questions and remaining ambiguities, many studies in this field consistently support the existence of potassium channels in the mitochondria, activation of which leads to protection against cell death and apoptosis. The pharmacological and structural profiles of these channels may be of paramount importance, and activators of these channels may provide new therapeutic strategies for ischemic heart disease.

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