REVIEW

WILEY

Mitochondrial ion channels as targets for cardioprotection

Derek J. Hausenloy[1,2,3,4,5] | Rainer Schulz[6] | Henrique Girao[7,8,9] (iD) | Brenda R. Kwak[10] |
Diego De Stefani[11] | Rosario Rizzuto[11] | Paolo Bernardi[11,12] | Fabio Di Lisa[11,12] (iD)

[1]Cardiovascular & Metabolic Disorders Program, Duke-National University of Singapore Medical School, Singapore, Singapore

[2]National Heart Research Institute Singapore, National Heart Centre, Singapore, Singapore

[3]Yong Loo Lin School of Medicine, National University Singapore, Singapore, Singapore

[4]The Hatter Cardiovascular Institute, University College London, London, UK

[5]Cardiovascular Research Center, College of Medical and Health Sciences, Asia University, Taichung City, Taiwan

[6]Institute of Physiology, Justus-Liebig University Giessen, Giessen, Germany

[7]Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal

[8]Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Coimbra, Portugal

[9]Clinical Academic Centre of Coimbra, CACC, Coimbra, Portugal

[10]Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland

[11]Department of Biomedical Sciences, University of Padova, Padova, Italy

[12]CNR Neuroscience Institute, Padova, Italy

Correspondence
Fabio Di Lisa, Department of Biomedical Sciences, University of Padova, Via Ugo Bassi 58/B, 35131 Padova, Italy.
Email: dilisa@bio.unipd.it

Abstract

Acute myocardial infarction (AMI) and the heart failure (HF) that often result remain the leading causes of death and disability worldwide. As such, new therapeutic targets need to be discovered to protect the myocardium against acute ischaemia/reperfusion (I/R) injury in order to reduce myocardial infarct (MI) size, preserve left ventricular function and prevent the onset of HF. Mitochondrial dysfunction during acute I/R injury is a critical determinant of cell death following AMI, and therefore, ion channels in the inner mitochondrial membrane, which are known to influence cell death and survival, provide potential therapeutic targets for cardioprotection. In this article, we review the role of mitochondrial ion channels, which are known to modulate susceptibility to acute myocardial I/R injury, and we explore their potential roles as therapeutic targets for reducing MI size and preventing HF following AMI.

KEYWORDS
acute ischaemia/reperfusion injury, cardioprotection, Mitochondria, mitochondrial permeability transition pore

1 | INTRODUCTION

Acute myocardial infarction (AMI) and the heart failure (HF) that often result remain the leading causes of death and disability worldwide. As such, new therapeutic targets are needed to protect the myocardium against the detrimental effects of acute ischaemia/reperfusion (I/R) injury in order to reduce myocardial infarct (MI) size, preserve left ventricular (LV) function and prevent the onset of HF. Mitochondrial dysfunction during acute I/R injury is a critical determinant of cell death following AMI, and therefore, ion channels in the inner mitochondrial membrane (IMM), which are known to influence cell death and survival, provide potential therapeutic targets for cardioprotection. In this article, we review the role of mitochondrial ion channels (including the mitochondrial permeability transition pore [PTP], mitochondrial calcium uniporter, mitochondrial potassium channels, connexin-43 and mitochondrial uncoupling proteins [UCPs]), which have been shown to modulate susceptibility to acute myocardial I/R injury, and we highlight their potential roles as therapeutic targets for reducing MI size and preventing HF following AMI. However, recent attempts to translate cardioprotective strategies that target some of these mitochondrial ion channels have been hugely disappointing, and the translational issues facing mitochondrial protection are also discussed in this article, and in other articles of this special issue of JCMM.

2 | THE MITOCHONDRIAL PTP

The mitochondrial permeability transition (PT) defines a sudden increase in the permeability of the IMM to solutes with molecular masses up to 1500 Da. This process is attributed to the opening of a voltage- and Ca^{2+}-dependent high-conductance channel known as the mitochondrial PT pore (PTP).[1] Besides Ca^{2+}, PTP opening is favoured by reactive oxygen species (ROS), and a decrease in mitochondrial membrane potential ($\Delta\Psi_m$), and it is antagonized by Mg^{2+}, adenine nucleotides and especially matrix pH values <7.[1,2] The immediate and obligatory consequence of PTP opening is the dissipation of the proton gradient across the IMM that, in an uncoupling-like effect, hampers adenosine triphosphate (ATP) synthesis and favours ATP hydrolysis. As the proton gradient is also utilized for mitochondrial Ca^{2+} uptake, PTP opening, which is stimulated by matrix Ca^{2+} accumulation, inhibits further Ca^{2+} entry while causing its release. This process appears to represent a physiological aspect of PT that might act as a pathway to avoid a potentially detrimental rise in intramitochondrial Ca^{2+} levels.[3] Indeed, pharmacological and genetic inhibition of PTP opening results in increased matrix [Ca^{2+}].[4,5] It is worth pointing out that PTP opening and the consequent matrix swelling are reversible, as initially documented in isolated mitochondria.[6] In isolated cells, openings of short duration (ie, <1 sec) are apparently not associated with changes in $\Delta\Psi_m$, simply because intracellular redistribution of fluorescent probes used for monitoring changes in mitochondrial membrane potential is slower. Nevertheless, changes in mitochondrial calcein

fluorescence provided evidence that in untreated cells, transient PTP openings occur without affecting cell viability.[7] However, at present, especially in intact hearts or in vivo, specific interventions are not available to distinguish between short- and long-duration openings. This methodological limitation does not exclude the hypothesis that transient PTP openings contribute to cardioprotection induced by ischaemic pre-conditioning (IPC).[8]

This limited, possibly problematic approach, results from the combination of the cardioprotective effects elicited by drugs interacting with cyclophilin D (CyPD), such as cyclosporin A (CsA),[1,9] with the lack of definitive knowledge on the molecular identity of the PTP.[10] In this latter respect, genetic studies have ruled out the possibility that the PTP is formed by proteins located to the IMM, such as the adenine nucleotide translocase (ANT) and the phosphate carrier, or to the outer mitochondrial membrane, such as the voltage-dependent anion channel (VDAC) or the benzodiazepine receptor or TSPO.[11-13] More recently, data from different laboratories concur in suggesting that F-ATP synthase, namely the enzyme that couples the proton gradient with ATP synthesis (or hydrolysis), is directly involved in PTP formation.[10,14] Two major models are now being validated by multiple approaches. In one model, the c subunit is suggested to act as a pore, although several features of the PTP, especially the Ca^{2+} sensitivity, are not displayed by patch clamp studies of purified c subunits.[15] Conversely, all known features of the PTP are observed in electrophysiological studies carried out in purified F-ATP synthase dimers, as opposed to the lack of response to Ca^{2+} displayed by monomers.[16,17] Further support to the role of F-ATP synthase in PTP formation is provided by showing that major features of the PTP, such as Ca^{2+}- and voltage dependence, as well as pH modulation, are selectively lost in mutants generated by means of site-directed mutagenesis.[10] In particular, the mutation of a single histidyl residue in the oligomycin sensitivity conferral protein subunit abolishes the pH-dependence making the H112Q mutant more susceptible to cell death induced by simulated ischaemia. This finding supports the notion that PTP opening is inhibited by acidosis during ischaemia.[18]

The formation of the PTP from F-ATP synthase has been questioned by deleting various subunits of this enzyme. Those deletions prevented assembly of functional F-ATPase, yet Ca^{2+}-dependent Ca^{2+} release apparently remained. However, Ca^{2+}-induced swelling occurred after an abnormal lag with a slower rate and to a reduced extent. These features are not characteristic of the large conductance PTP, but they could suggest opening of a smaller channel (Figure 1). Interestingly, mutants lacking c subunits display small conductance properties inhibited not only by CsA, but also by the selective ANT inhibitor bongkrekic acid.[19] Whereas this finding might explain previous and recent results on ANT contribution to PTP formation,[20,21] mitochondria appear to contain pores with different conductances formed by conformational changes of various proteins, and F-ATP synthase is required for formation of the large conductance PTP. Whereas opening of the large conductance pore is most likely to contribute to pathological processes, small conductance PTP channels could play physiological roles, including self-defence
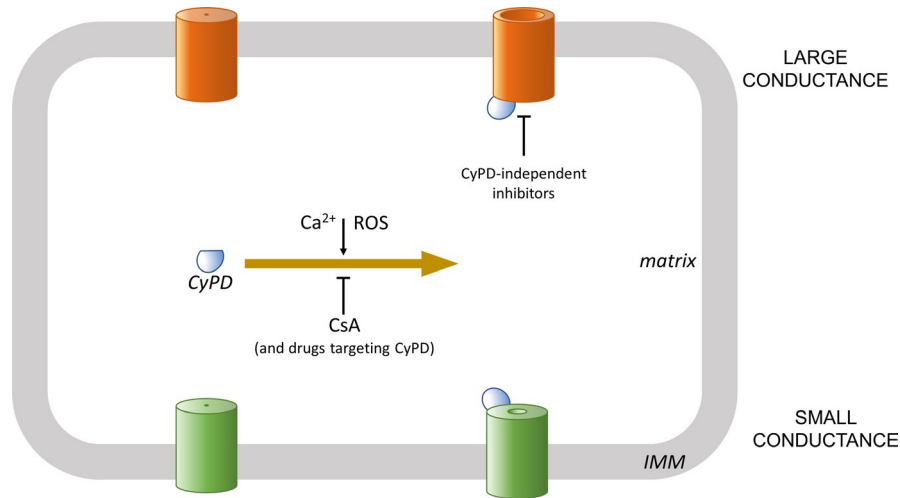
FIGURE 1 Current hypotheses on PTP opening. As illustrated in the upper part of figure, an increase in matrix levels of Ca^{2+} and/or ROS promotes opening of the large conductance PTP. This process is contributed by binding of CyPD to the PTP. Therefore, drugs, such as CsA, or genetic interventions targeting CyPD decrease, but do not abolish the open probability of the PTP. More recently, Ca^{2+}-induced PTP opening with small conductance has been reported that can still be inhibited by CsA. Although the proteins involved have not yet been conclusively identified, as discussed in Section 2, available information supports the involvement of F-ATP synthase in forming the large conductance PTP. The small conductance could be due to other proteins, such as ANT, explaining the use of a different colour in lower part of the scheme. However, different conformations of the same protein might form pores with increasing sizes. CsA, cyclosporin A; CyPD, cyclophylin D; IMM, inner mitochondrial membrane; ROS, reactive oxygen species

mechanisms. Besides testing this hypothesis by genetic approaches, for translational approaches pharmacological studies should also be implemented using PTP inhibitors that do not target CyPD. Recently, isoxazole and triazole compounds have been reported to afford a remarkable cardioprotective efficacy independent of CyPD.[22] Besides identifying the molecular target(s), future studies should investigate whether these novel PTP inhibitors are devoid of the detrimental effects of a prolonged CyPD deletion or targeting, such as worsening of adverse LV remodelling in hypertrophied hearts.[5]

At variance from our current limited knowledge on possible physiological roles, a large body of evidence supports a pathological role for PTP opening,[1,23-25] especially in the case of the loss of cardiomyocyte viability associated with I/R injury.[26-31] The evidence supporting this hypothesis is indirect, as it is largely based upon the protective effects afforded by pharmacological or genetic approaches that prevent PTP opening. Although many small animal studies have shown that the administration of CsA at the time of reperfusion to target PTP opening, reduces MI size (reviewed in 32), the studies in large animal pig AMI models have been mixed.[33,34] Endogenous cardioprotective strategies such as IPC and ischaemic post-conditioning have also been shown to mediate their protective effects by inhibiting PTP opening at reperfusion.[35,36]

Other than in vitro techniques for detecting PTP opening in isolated mitochondria or cells,[37] methods or biomarkers for detection of PTP opening in vivo are not available. Thus, not only the precise time and the mechanisms driving PTP opening have hardly been characterized in vivo, but also the PTP contribution to any given process still relies solely on interventions that in most cases target the PTP modulator CyPD and not the pore itself.[1,38] This indirect approach has serious shortcomings that complicate the possibility to translate experimental data into clinical settings, as well as the interpretation of data obtained in clinical trials,[38] as discussed in Section 8.

In summary, whereas PTP opening is of crucial relevance in acute myocardial I/R injury, the conclusive elucidation of its molecular nature is necessary to solve the current uncertainties on functional modalities and their involvement in pathophysiology. This information will allow the development of direct and specific drugs to be tested in clinical trials for the proper re-evaluation of PTP inhibition in human cardioprotection.

3 | THE MITOCHONDRIAL CALCIUM UNIPORTER

Apart from many crucial roles played in excitation-contracting coupling and signalling pathways in the cytosol,[39-41] Ca^{2+} represents a pivotal factor in both mitochondrial physiology and mitochondria-dependent pathological processes.[42-47] Within the matrix, Ca^{2+} contributes to the activation of key enzymes in substrate oxidation that fuel the respiratory chain with reducing equivalents resulting in an increased ATP synthesis to match the increased demand dictated by a rise in cytosolic [Ca^{2+}]. The contribution of mitochondrial Ca^{2+} uptake to cytosolic Ca^{2+} oscillations related to myocardial contractile activity remains a controversial issue.[48] However, as mentioned in Section 2, a large rise in mitochondrial Ca^{2+} is a major factor in promoting PTP opening. Unfortunately, despite major advancements in techniques for monitoring Ca^{2+} levels in various cellular compartments, a threshold between physiological and pathological [Ca^{2+}] cannot yet be established even in vitro. Nevertheless, influx and efflux pathways operate

continuously to regulate the intramitochondrial (ie, matrix) $[Ca^{2+}]$ with respect to Ca^{2+} levels in the cytosol. The electrochemical gradient ($\Delta\mu_H$) across IMM generated by mitochondrial respiration drives both Ca^{2+} uptake and release. Indeed, the electrical component of $\Delta\mu_H$, namely Dy, drives Ca^{2+} entry into the matrix by means of the mitochondrial calcium uniporter (MCU), whereas the chemical component of $\Delta\mu_H$, namely ΔpH, is utilized for mitochondrial Ca^{2+} release catalysed mostly by the mitochondrial Na^+/Ca^{2+} exchanger (or more precisely, Na^+/Ca^{2+} Li^+-permeable exchanger, abbreviated as mNCLX).[49] In fact, mNCLX is functionally coupled with the mitochondrial Na^+/H^+ exchanger, so that eventually mitochondrial Ca^{2+} release is paralleled by H^+ uptake. In addition, PTP opening might represent a fast pathway for mitochondrial Ca^{2+} release (see Section 2).

Our current understanding of mitochondrial Ca^{2+} homoeostasis has been greatly advanced by the elucidation of the molecular nature of MCU and mNCX.[50,51] MCU is a 40 kD protein with two transmembrane domains separated by a short loop containing acidic residues that are critical for Ca^{2+} channelling function. The discovery of the MCU was preceded by the identification of the mitochondrial calcium uptake protein 1[52] that acts as a gatekeeper preventing mitochondrial Ca^{2+} uptake at low cytosolic $[Ca^{2+}]$.[47,53-55] More recently other proteins have been identified that control MCU activity and are grouped under the term MCU complex (reviewed in 47,53,56). Therefore, the net mitochondrial Ca^{2+} uptake results from the balance between the activities of several proteins. This structural complexity hinders the development of genetic or pharmacological strategies aimed at controlling mitochondrial Ca^{2+} levels, as well as the interpretation of results from loss-of-function approaches. Indeed, this appears to be the case with studies investigating MCU role in acute myocardial I/R injury. As upon reperfusion the large increase in mitochondrial $[Ca^{2+}]$ occurring upon reperfusion favours PTP opening, MCU deletion should confer protection against I/R injury. However, mice models have generated controversial results.[57] Although MCU deletion was embryonically lethal in a pure genetic background (ie, C57BL/6), mice devoid of MCU were generated in a mixed genetic background (ie, CD1). Not only was basal cardiac function not affected, but also, when hearts were isolated and subjected to I/R injury, infarct size was not different from WT littermates and CsA-induced protection was abolished.[58] Also lack of protection resulted from MCU down-regulation obtained by means of transgenic expression of a dominant negative (MCU).[59] Conversely, the specific deletion of cardiac MCU in adult mice elicited a significant protection against in vivo I/R injury.[60,61] The discrepancy between results in total body deletion and conditional knockout has not been resolved. Besides differences between in vitro and in vivo acute myocardial I/R injury models, timing and duration of MCU deletion are likely to explain the difference in sensitivity to I/R injury. The lack of MCU throughout development might trigger compensatory mechanisms involved in death pathways.[57]

In summary, whereas results from a prolonged MCU deletion would suggest that cardiomyocyte death can be obtained independently of mitochondrial Ca^{2+} overload and PTP opening, findings in conditional MCU KO mice lend support to the relevance of mitochondrial Ca^{2+} overload as a determining factor in acute myocardial I/R injury. This concept is further supported by genetic manipulations of mNCLX. Its deletion was shown to cause a severe mitochondrial Ca^{2+} overload resulting in lethal HF, whereas cardiac specific overexpression prevented I/R-induced PTP opening and cardiomyocyte death.[62] Drugs specifically targeting the MCU complex are necessary to validate or dispute the occurrence and the role of mitochondrial Ca^{2+} increase in I/R injury models in large animals and clinical settings.

4 | THE MITOCHONDRIAL K_{ATP} CHANNEL

Several K^+ channels have been described in mitochondria.[63-65] However, due to its role in cardioprotection most of the attention has been focused on the mitochondrial ATP-sensitive (ie, inhibited by physiological levels of ATP) K^+ channel (mitoK_{ATP}).[66-70] The maintenance of matrix volume depends on the net balance between K^+ uptake and release.[64,71] A K^+ cycle across the IMM exists whereby the electrophoretic influx driven by $\Delta\psi_m$ is balanced by the electroneutral K^+/H^+ antiporter.[71] An uncontrolled increase in K^+ influx, such as that induced by valinomycin, results in mitochondrial depolarization and large matrix swelling, so that K^+ channels have to be tightly regulated. This is the case with mitoK_{ATP} that was originally described in liver mitoplasts.[72] Its function was initially attributed to the regulation of mitochondrial volume[73] with no precise role in pathophysiology in any organ, in contrast from various functions attributed to plasma membrane K_{ATP} channels.[74,75] The mitoK_{ATP} channels are hetero-octameric complexes of 4 transmembrane inward-rectifying K^+ (KIR) channel subunits and four sulfonylurea receptor (SUR) subunits. Two KIR isoforms (KIR6.1/6.2) and 3 SUR isoforms (SUR1/2A/2B) have been described.[75] For instance, in the heart the sarcolemmal K_{ATP} channel (surfaceK_{ATP}) comprises KIR6.2 and SUR2A subunits.

As its initial discovery, the cardiac surfaceK_{ATP} has been suggested to be associated with cardioprotection.[76] Activation occurs in response to ischaemia-induced [ATP] decrease. The consequent shortening of action potential reduces Ca^{2+} entry and contractility favouring the maintenance of ATP levels and cell viability during acute I/R injury (reviewed in 77). This hypothesis appeared to be supported by reports showing that K_{ATP} openers mimicked the cardioprotective effects of IPC, with IPC-induced protection being abolished by K_{ATP} antagonists.[69] However, subsequent reports argued against a major surfaceK_{ATP} involvement by showing that: (a) K_{ATP} channel activators, including diazoxide (DZX), protected non-contracting cardiomyocytes[78]; (b) surfaceK_{ATP} channel activity was hardly affected by DZX, whereas drugs specifically targeting the surfaceK_{ATP} channel were not protective.[78,79] On the other hand, DZX was shown to restore ATP-inhibited K^+ flux in isolated mitochondria[80] that also displayed 5-hydroxydecanoate (5HD) inhibition.[81] Therefore, the attention shifted towards mitochondria to

both elucidate $mitoK_{ATP}$ structure and clarify the protective mechanisms triggered by its opening.

The fact that DZX and 5HD also affected mitochondrial processes other than the K_{ATP} channel[63] renders the identification of its molecular identity especially important. Initial attempts provided evidence that electrophysiological properties of K^+ channels can be observed in lipid bilayers containing partially purified mitochondrial proteins.[73,82] More recently, an unbiased proteomic approach carried out in cardiac IMM led to the identification of *Kir1.1*, another member of the *Kir* gene family, also known as the renal outer medullary K^+ channel (ROMK).[68] This protein has been attributed the role of $mitoK_{ATP}$ channel based upon results obtained in cardiomyoblasts whereby ROMK overexpression protected against death stimuli. These results should be validated by in vivo approaches of loss- and gain of function. Of note, the pharmacologic properties of ROMK do not completely match those reported for $mitoK_{ATP}$ and ROMK interaction with SUR components has not yet been demonstrated. Lately, $mitoK_{ATP}$ identity has been attributed to a protein complex of the IMM that, similar to surfaceK_{ATP} channels, is composed of pore forming and ATP-binding subunits, termed MITOK and MITO SUR, respectively.[70] Whereas overexpression of only MITOK generated an excessive K^+ influx resulting in large matrix swelling, the in vitro reconstitution of MITOK together with MITOSUR displayed the main electrophysiological and pharmacologic properties of $mitoK_{ATP}$. In addition, MITOK deletion abolished DZX-induced cardioprotection in isolated hearts.[70] Regarding the protective mechanisms, $mitoK_{ATP}$ opening causes a mild increase in mitochondrial levels of ROS that appears to be required for cardioprotection.[83] Actually, a feedback loop has been proposed whereby ROS would result in PKC-ε activation that would prolong $mitoK_{ATP}$ activation and prevent PTP opening.[83] This loop might also involve connexin43 (Cx43).[84]

In summary, whereas the involvement of $mitoK_{ATP}$ in cardioprotection is generally accepted mostly based upon pharmacological evidence, the protective mechanisms are far from being conclusively defined. The recent identification of the molecular nature of the $mitoK_{ATP}$ will provide the molecular tools to investigate its interaction with signalling pathways and determining factors of acute myocardial I/R injury, such as proteins involved in mitochondrial ROS formation, Ca^{2+} transport and PT.

5 | THE MITOCHONDRIAL BKCa CHANNEL

In addition to the $mitoK_{ATP}$ channel, there is also a large or "big" conductance calcium-sensitive and voltage-activated K^+ channel (termed the BKCa channel) present in the IMM.[85] A number of experimental studies have shown that opening of the BKCa channel, using the pharmacological agonist NS1619, reduced MI size in animal models of acute myocardial I/R injury and that this cardioprotective effect was abrogated in the presence of the BKCa channel antagonist paxilline.[85,86] In addition, mice overexpressing BKCa channel were found to be protected against acute I/R injury, confirming

the cardioprotective role of this mitochondrial potassium channel. The mechanisms underlying this cardioprotective effect have been attributed to its effect on mitochondrial membrane depolarization, attenuation of mitochondrial Ca^{2+} overload,[87] production of mitochondrial signalling ROS,[88] and reduced oxidative stress at reperfusion.[89]

Interestingly, in common with the $mitoK_{ATP}$ channel, it has also been shown that the infarct-limiting effects of both classical and delayed IPC,[86,90] ischaemic post-conditioning (IPost),[91] and limb remote ischaemic conditioning (RIC),[92,93] were abrogated in the presence of a pharmacological BKCa blocker, suggesting that opening of the BKCa channel is also required for ischaemic conditioning cardioprotection. Furthermore, it has been shown that protein kinase A (PKA), which is known to be activated by IPC, can increase opening of the BKCa channel, providing a potential link between IPC and BKCa channel opening.[87]

In summary, in a similar manner to the $mitoK_{ATP}$ channel, the opening of the mitochondrial BKCa is cardioprotective and contributes to ischaemic conditioning protection. How the two mitochondrial potassium channels interaction to confer cardioprotection needs further investigation.

6 | CONNEXIN43

Cx43 is a protein well known for the formation of gap junctions. It is the main connexin isoform expressed in cardiomyocytes, but it is also found in other cell types of the heart such as endothelial cells and fibroblasts (for review, see [94,95]). Six Cx43 molecules assemble into hemichannels, and gap junctions are formed by docking of hemichannels from adjacent cells, which connect the cytoplasm of cells. Gap junctions are regulated by post-translational modifications of Cx43, amongst them phosphorylation mediated by several protein kinases (for review, see [95,96]). In addition to its localization at the plasma membrane, Cx43 is also detected at other cellular compartments including the nucleus,[97,98] subsarcolemmal mitochondria,[99-103] and exosomes,[104,105] which attribute to Cx43 other biological functions that extend beyond intercellular communication (for review, see [105,106]). Interestingly, gap junctions form intimate associations with mitochondria, and the contact between annular gap junction vesicles and mitochondria may facilitate Cx43 delivery to the mitochondria (Figure 2).[107]

Within mitochondria, Cx43 regulates central functions—all of them being important for I/R injury and protection from it—including oxygen consumption and ATP production,[108] ROS formation,[109,110] uptake of K^+[111] and Ca^{2+},[112] PTP opening[112,113] and apoptosis.[114] The amount of mitochondrial Cx43 increases with ischaemia,[115] and also rises by treatment with DZX or fibroblast growth factor 2, compounds known to reduce irreversible I/R injury.[100,116] Despite these above-described functions of mitochondrial Cx43, the exact contribution of the protein to mitochondrial function in I/R injury and cardioprotection remains unclear. Cx43 interacts with a number of other proteins and the analysis of the
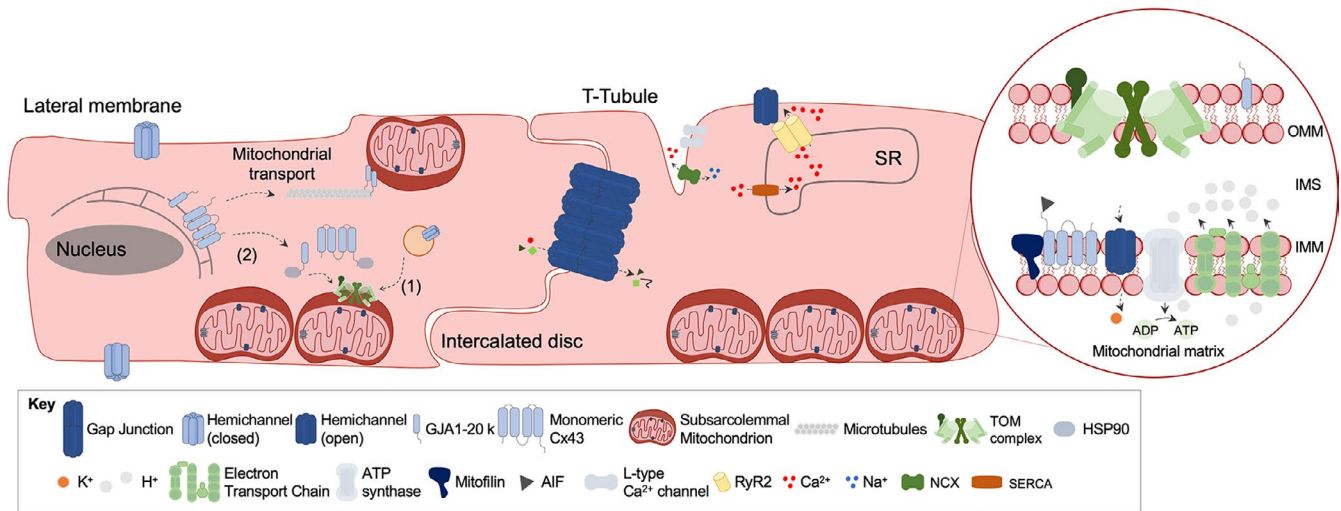
FIGURE 2 Mitochondrial Cx43 in cardiomyocytes. In cardiomyocytes, the accumulation of Cx43 in mitochondria occurs primarily in the inner membrane of subsarcolemmal mitochondria, where the close proximity to the plasma membrane and internalized annular gap junctions likely favour the transfer of Cx43 to the mitochondria. At mitochondria, Cx43 interacts with apoptosis-inducing factor, beta-subunit of the electron-transfer protein, ATP synthase alpha and beta, cytochrome c oxidase subunit 4, voltage-dependent anion channel protein 1, mitochondrial stress protein 70, mitofilin and subunits of the Kir6.1 potassium channels. Cx43-hemichannels can also interact with ryanodine receptors at the sarcoplasmic reticulum. Besides the full length, a shorter NT-truncated form of Cx43, the GJA1-20 kD, is present in the outer mitochondrial membrane of cardiomyocytes, where it mediates the microtubule-dependent mitochondrial transport

interactome of whole cell Cx43 under normoxic conditions and after myocardial I/R injury by mass spectrometry identified proteins involved in metabolism, signalling, trafficking, but also in gap junction-independent processes.[117] Mass spectrometry analysis of native immunoprecipitated mitochondrial extracts showed that Cx43 interacts with the apoptosis-inducing factor and the beta-subunit of the electron-transfer protein,[118] the F-ATP synthase α and β subunits, cytochrome c oxidase subunit 4, VDAC protein 1, mitochondrial stress protein 70 and mitofilin,[119] a protein important for cristae structure and morphology.[120] Mitochondrial Cx43 also interacts with subunits of the Kir6.1 potassium channels[121] and it regulates the expression of mitochondrial nitric oxide synthase isoforms and thereby the mitochondrial nitric oxide formation.[122] The importance of such protein-protein interactions for mitochondrial function and I/R injury requires further investigation. The presence of Cx43 at the mitochondria is also important to regulate Ca^{2+} homoeostasis.[112]

A contribution of Cx43 to the cardioprotection by IPC is shown in heterozygous Cx43-deficient mice,[123] which have no longer MI size reduction after a classical IPC protocol.[123] The first evidence that mitochondrial Cx43 is involved in such protection came from a study in isolated cardiomyocytes—apparently lacking gap junctions—in which cytoprotection by pre-conditioning was achieved in wild-type, but not in Cx43-deficient cells.[124] Inhibition of the mitochondrial import of Cx43 abolished MI size reduction by pharmacological pre-conditioning.[125] Although mitochondrial Cx43 increased after I/R, such an increase was absent in hearts undergoing a IPC protocol,[126] and the phosphorylation status of mitochondrial Cx43 was preserved.[127]

Although isolated mitochondria can be protected by IPC, such beneficial effects depend on the presence of Cx43.[128] The mechanism by which Cx43 contributes to cardioprotection by IPC seems to involve ROS signalling.[84] In addition, S-nitrosylation—a post-translational modification of general importance for cardioprotective signalling—involves mitochondrial Cx43.[129] Studies on the role of mitochondrial Cx43 in pre-conditioning have recently been reviewed.[129]

Data on the contribution of mitochondrial Cx43 to RIC or IPost are limited. Although mitochondrial Cx43 increases after I/R, such an increase is absent in hearts undergoing an IPost protocol.[126] Depending on the species, IPost is associated with either decreased[126] or increased[130] phosphorylation of Cx43. In H9C2 cells, hypoxic post-conditioning enhanced the amount of mitochondrial Cx43 and the protein contributed to cytoprotection via decreasing ROS formation.[131] In addition, phosphorylation of mitochondrial Cx43 is also involved in pharmacological post-conditioning with sphingosine-1-phosphate (S1P).[132] The S1P-induced reduction of MI size after ex vivo I/R was lost in hearts of mice with a truncated C-terminus of Cx43 (Cx43K258/KO) or in which the S368 is mutated to a non-phosphorylatable alanine (Cx43S368A/S368A), illustrating the importance of this site in cardioprotection.[132] Moreover, infusion of short peptides (αCT1 and αCT11), known to increase S368 phosphorylation of Cx43 in cardiomyocytes, preserved LV function when applied before or after ischaemia.[133] However, it should be kept in mind that alterations in Cx43 expression/phosphorylation induced by ischaemic or pharmacological post-conditioning may be involved but are likely not causal for such cardioprotection, as heterozygous Cx43-deficient mice also show an effective infarct size reduction by IPost.[134] Studies on the role of mitochondria in post-conditioning have recently been reviewed.[129]

TABLE 1 Compounds that have been shown to modulate mitochondrial channels and have been tested for reduction of myocardial infarct size

Channel	Effect	Compound	Species	Ex vivo	In vivo	Notes	References
PTP	Inhibition	CsA	Mouse		x		163,164
			Rat		x		165-168
				x			30,169
					x	No protection	153
			Rabbit		x		170-174
			Pig		x		33
					x	No protection	175,176
		Debio-025	Mouse		x		177
		Sanglifehrin A	Mouse		x		178
			Rat	x			31
		NIM 811	Rabbit		x		172
		Compound 22	Rabbit		x		179
K_{ATP}	Activation	Diazoxide	Rat	x			66
					x		180-182
			Rabbit		x		183-186
					x	No protection	187
BKCa	Activation	NS1619	Rat	x			188
			Rabbit	x			85
			Dog		x		86

Connexin genes are commonly classified according to sequence homology and alphabetically divided into five subfamilies[135] and the Cx43 gene is therefore called *GJA1*. Cx43 is subject to internal (alternative) translation, *that is* the translation of the coding region of *GJA1* mRNA produces not only full-length Cx43 but at least 4 additional NT-truncated forms with different molecular weight, of which the 20 kD form is the most abundant.[136-140] Functionally, it was first shown that GJA1-20k interacts with full-length Cx43 thereby regulating its subcellular distribution and the formation of gap junctions. This mechanism appeared of particular importance to limit gap junction formation during epithelial-mesenchymal transition.[136,138] In cardiomyocytes, GJA1-20k stabilizes actin filaments to guide Cx43 delivery to the intercalated discs[140] and it facilitates microtubule-dependent mitochondrial transport important for mitochondrial network integrity during cellular stress.[139]

Recent studies showed an up-regulation of GJA1-20k upon hypoxia, ischaemia or I/R.[141-143] GJA1-20k targets to the outer mitochondrial membrane of cardiomyocytes.[143] Furthermore, AAV9-mediated gene transfer of GJA1-20k in mouse hearts reduced I/R injury in vivo and ex vivo. Similar to what has been observed during IPC, GJA1-20k promotes mitochondrial biogenesis and a protective mitochondrial phenotype, with a reduction of membrane potential, respiration and ROS production.[143] Interestingly, Cx43 has also been observed in mitochondria of endothelial cells.[113] However, the role of mitochondrial Cx43 in endothelial cells and its possible involvement in cardiac I/R remains to be investigated.

In summary, Cx43 not only forms gap junctions but is also present in mitochondria, where it is important for several aspects of mitochondrial function. Mitochondrial Cx43 is clearly involved in the cardioprotection by IPC.

7 | MITOCHONDRIAL UCPs

Mitochondrial production of ATP to fuel cardiac contraction and relaxation is critically dependent on maintaining a large electrochemical gradient for protons across the mitochondrial inner membrane. Prior experimental studies have shown that mild chemical mitochondrial uncoupling can confer IPC-like cardioprotection, suggesting that modifying mitochondrial respiratory function can mediate cardioprotection.[144,145] The mitochondrial UCPs are inner membrane carrier proteins that are able to induce proton leak and dissipate mitochondrial membrane potential, thereby reducing mitochondrial ROS production under conditions of stress, such as acute myocardial I/R injury (reviewed in 146). Overexpression of UCP1 has been shown to protect H9C2 heart-derived cells, and the adult murine heart against acute I/R injury, by reducing oxidative stress at reperfusion.[147,148] Reperfusion has been shown to increase myocardial gene and protein expression of UCP2, and the overexpression of UCP2 has been reported to reduce susceptibility to acute myocardial I/R injury in the rat heart.[149] IPC was shown to increase UCP2 expression, with siRNA knockdown of

UCP2 abolishing IPC cardioprotection.[149] Interestingly, delayed pre-conditioning has also been reported to up-regulate both UCP2 and UCP3 in the rat heart and reduce oxidative stress at reperfusion, with abrogation of UCP2 but not UCP3 blocking the cardioprotection elicited by delayed pre-conditioning, suggesting a role of UCP2 in mediating endogenous cardioprotection due to classical and delayed IPC.[150] A cardioprotective role for UCP3 has also been demonstrated, with mice deficient in UCP3 being more susceptible to acute myocardial I/R injury,[151,152] and cardiac arrhythmias.[152] Interestingly, the cardioprotective effect of IPC was attenuated in these mice, suggesting a role for UCP3 as a mediator of IPC.[152]

In summary, overexpression of UCPs protects the myocardium against acute I/R injury, with UCP2 and UCP3 mediating the cardioprotection elicited by endogenous cardioprotection strategies such as IPC.

8 | TRANSLATION OF MITOCHONDRIAL PROTECTION

Although a large number of experimental studies have demonstrated MI size reduction with pharmacological agents that target mitochondrial channels in experimental small and large animal models of myocardial I/R injury, the translation of mitochondrial protective strategies into the clinical setting for patient benefit has been hugely disappointing. Amongst these, the most promising mitochondrial target, had been the PTP, given the substantial experimental data demonstrating MI size reduction using CsA to target PTP opening at reperfusion—although not all studies had been positive.[34,153] Despite, an initial small proof-of-concept clinical study in AMI patients demonstrating a reduction in MI size with CsA administered at time of reperfusion compared to control,[29,154] two subsequent large randomized controlled trials, CIRCUS and CYCLE, failed to report any benefit with CsA on either MI size reduction or clinical outcomes.[155-157] The reasons for this failure to translate PTP inhibition for clinical benefit in AMI patients are not clear but have been attributed to: insufficient delivery of CsA to ischaemic cardiomyocytes and the presence of factors which are known to confound cardioprotection, such as co-morbidities (eg, age, diabetes), and co-medications (eg, platelet P2Y12 inhibitors). Interestingly, experimental data has shown that PTP inhibition using CsA to reduce MI size was ineffective in pre-diabetic Zucker obese rats compared to Zucker lean rats.[158] CsA has also been tested as a cardioprotective agent in the setting of cardiac surgery, where it has been shown to reduce the extent of peri-operative myocardial injury.[159,160] Other therapeutic strategies that target mitochondria to reduce MI size have also failed in AMI patients—these include elamipretide (a cell-permeable peptide suggested to preserve the integrity of cardiolipin),[161] and TRO40303 (suggested to inhibit the TSPO).[162]

In summary, despite there being substantial experimental data supporting the targeting of mitochondria as a therapeutic strategy to reduce MI size, the translation to the clinical setting for patient benefit has been disappointing. It may well be that other more potent PTP inhibitors that are CypD-independent,[22] may provide alternative cardioprotective strategies in future studies.

9 | CONCLUSIONS

A number of mitochondrial ion channels in the IMM have been implicated as therapeutic targets for cardioprotection. The compounds used are summarized in Table 1. The mechanisms underlying the protective effects of these channels have not been fully elucidated and need further study. Of these channels, the mitochondrial PTP has been extensively investigated as a cardioprotective target in the pre-clinical setting using CsA to inhibit PTP opening at reperfusion, but the results in the clinical setting of AMI have been disappointing. Recent advances in the molecular identification of some of these mitochondrial ion channels (eg, MCU, PTP, MitoK$_{ATP}$) will be helpful in this regard and allow the design of new more specific modulators of these mitochondrial ion channels for translating cardioprotection into the clinical setting for patient benefit.

CONFLICTS OF INTERESTS
All authors have no conflicts or disclosures.

AUTHOR CONTRIBUTION
We confirm that all the authors are experts in this research topic and have contributed to the planning, writing and reviewing of this paper.

ORCID
Henrique Girao (iD) https://orcid.org/0000-0002-5786-8447
Fabio Di Lisa (iD) https://orcid.org/0000-0003-0705-7539

REFERENCES
1. Bernardi P, Di Lisa F. The mitochondrial permeability transition pore: molecular nature and role as a target in cardioprotection. *J Mol Cell Cardiol*. 2015;78C:100-106.
2. Di Lisa F, Bernardi P. A CaPful of mechanisms regulating the mitochondrial permeability transition. *J Mol Cell Cardiol*. 2009;46:775-780.
3. Bernardi P, Petronilli V. The permeability transition pore as a mitochondrial calcium release channel: a critical appraisal. *J Bioenerg Biomembr*. 1996;28:131-138.
4. Altschuld RA, Hohl CM, Castillo LC, et al. Cyclosporin inhibits mitochondrial calcium efflux in isolated adult rat ventricular cardiomyocytes. *Am J Physiol Heart Circ Physiol*. 1992;262:H1699-H1704.
5. Elrod JW, Wong R, Mishra S, et al. Cyclophilin D controls mitochondrial pore-dependent Ca(2+) exchange, metabolic flexibility, and propensity for heart failure in mice. *J Clin Invest*. 2010;120:3680-3687.
6. Petronilli V, Nicolli A, Costantini P, et al. Regulation of the permeability transition pore, a voltage- dependent mitochondrial channel inhibited by cyclosporin A. *Biochim Biophys Acta*. 1994;1187:255-259.
7. Petronilli V, Penzo D, Scorrano L, et al. The mitochondrial permeability transition, release of cytochrome *c* and cell death.

Correlation with the duration of pore openings *in situ*. *J Biol Chem*. 2001;276:12030-12034.

8. Hausenloy D, Wynne A, Duchen M, et al. Transient mitochondrial permeability transition pore opening mediates preconditioning-induced protection. *Circulation*. 2004;109:1714-1717.

9. Di Lisa F, Carpi A, Giorgio V, et al. The mitochondrial permeability transition pore and cyclophilin D in cardioprotection. *Biochim Biophys Acta*. 2011;1813:1316-1322.

10. Carraro M, Checchetto V, Szabo I, et al. F-ATP synthase and the permeability transition pore: fewer doubts, more certainties. *FEBS Lett*. 2019;593:1542-1553.

11. Kokoszka JE, Waymire KG, Levy SE, et al. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature*. 2004;427:461-465.

12. Baines CP, Kaiser RA, Sheiko T, et al. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat Cell Biol*. 2007;9:550-555.

13. Sileikyte J, Blachly-Dyson E, Sewell R, et al. Regulation of the mitochondrial permeability transition pore by the outer membrane does not involve the peripheral benzodiazepine receptor (TSPO). *J Biol Chem*. 2014;289(20):13769-13781.

14. Bernardi P, Di Lisa F, Fogolari F, et al. From ATP to PTP and back: a dual function for the mitochondrial ATP synthase. *Circ Res*. 2015;116:1850-1862.

15. Alavian KN, Beutner G, Lazrove E, et al. An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore. *Proc Natl Acad Sci USA*. 2014;111:10580-10585.

16. Urbani A, Giorgio V, Carrer A, et al. Purified F-ATP synthase forms a Ca(2+)-dependent high-conductance channel matching the mitochondrial permeability transition pore. *Nat Commun*. 2019;10:4341.

17. Giorgio V, von Stockum S, Antoniel M, et al. Dimers of mitochondrial ATP synthase form the permeability transition pore. *Proc Natl Acad Sci USA*. 2013;110:5887-5892.

18. Antoniel M, Jones K, Antonucci S, et al. The unique histidine in OSCP subunit of F-ATP synthase mediates inhibition of the permeability transition pore by acidic pH. *EMBO Rep*. 2017;19:257-268.

19. Neginskaya MA, Solesio ME, Berezhnaya EV, et al. ATP synthase C-subunit-deficient mitochondria have a small cyclosporine A-sensitive channel, but lack the permeability transition pore. *Cell Rep*. 2019;26:11-17.e2.

20. Brustovetsky N, Klingenberg M. Mitochondrial ADP/ATP carrier can be reversibly converted into a large channel by Ca^{2+}. *Biochemistry*. 1996;35:8483-8488.

21. Karch J, Bround MJ, Khalil H, et al. Inhibition of mitochondrial permeability transition by deletion of the ANT family and CypD. *Sci Adv*. 2019;5:eaaw4597.

22. Antonucci S, Di Sante M, Sileikyte J, et al. A novel class of cardioprotective small-molecule PTP inhibitors. *Pharmacol Res*. 2019;151:104548.

23. Di Lisa F, Bernardi P. Mitochondria and ischemia-reperfusion injury of the heart: fixing a hole. *Cardiovasc Res*. 2006;70:191-199.

24. Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion-a target for cardioprotection. *Cardiovasc Res*. 2004;61:372-385.

25. Elrod JW, Molkentin JD. Physiologic functions of cyclophilin D and the mitochondrial permeability transition pore. *Circ J*. 2013;77:1111-1122.

26. Di Lisa F, Ziegler M. Pathophysiological relevance of mitochondria in NAD^+ metabolism. *FEBS Lett*. 2001;492:4-8.

27. Griffiths EJ, Halestrap AP. Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. *Biochem J*. 1995;307:93-98.

28. Baines CP, Kaiser RA, Purcell NH, et al. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature*. 2005;434:658-662.

29. Piot C, Croisille P, Staat P, et al. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med*. 2008;359:473-481.

30. Hausenloy DJ, Maddock HL, Baxter GF, et al. Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? *Cardiovasc Res*. 2002;55:534-543.

31. Hausenloy DJ, Duchen MR, Yellon DM. Inhibiting mitochondrial permeability transition pore opening at reperfusion protects against ischaemia-reperfusion injury. *Cardiovasc Res*. 2003;60:617-625.

32. Hausenloy DJ, Boston-Griffiths EA, Yellon DM. Cyclosporin A and cardioprotection: from investigative tool to therapeutic agent. *Br J Pharmacol*. 2012;165:1235-1245.

33. Skyschally A, Schulz R, Heusch G. Cyclosporine A at reperfusion reduces infarct size in pigs. *Cardiovasc Drugs Ther*. 2010;24:85-87.

34. Karlsson LO, Bergh N, Cyclosporine GL. Cyclosporine A, 2.5 mg/kg, does not reduce myocardial infarct size in a porcine model of ischemia and reperfusion. *J Cardiovasc Pharmacol Ther*. 2012;17:159-163.

35. Argaud L, Gateau-Roesch O, Raisky O, et al. Postconditioning inhibits mitochondrial permeability transition. *Circulation*. 2005;111:194-197.

36. Hausenloy DJ, Yellon DM, Mani-Babu S, et al. Preconditioning protects by inhibiting the mitochondrial permeability transition. *Am J Physiol Heart Circ Physiol*. 2004;287:H841-H849.

37. Bøtker HE, Hausenloy D, Andreadou I, et al. Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. *Basic Res Cardiol*. 2018;113:39.

38. Bernardi P, Di Lisa F. Cyclosporine before PCI in acute myocardial infarction. *N Engl J Med*. 2016;374:89-90.

39. Carafoli E. Intracellular calcium homeostasis. *Annu Rev Biochem*. 1987;56:395-433.

40. Bers DM. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol*. 2008;70:23-49.

41. Eisner DA, Caldwell JL, Kistamás K, et al. Calcium and excitation-contraction coupling in the heart. *Circ Res*. 2017;121:181-195.

42. Williams GS, Boyman L, Lederer WJ. Mitochondrial calcium and the regulation of metabolism in the heart. *J Mol Cell Cardiol*. 2015;78:35-45.

43. Maack C, O'Rourke B. Excitation-contraction coupling and mitochondrial energetics. *Basic Res Cardiol*. 2007;102:369-392.

44. Zhou B, Tian R. Mitochondrial dysfunction in pathophysiology of heart failure. *J Clin Investig*. 2018;128:3716-3726.

45. Bertero E, Maack C. Calcium signaling and reactive oxygen species in mitochondria. *Circ Res*. 2018;122:1460-1478.

46. Carafoli E. Historical review: mitochondria and calcium: ups and downs of an unusual relationship. *Trends Biochem Sci*. 2003;28:175-181.

47. De Stefani D, Rizzuto R, Pozzan T. Enjoy the trip: calcium in mitochondria back and forth. *Annu Rev Biochem*. 2016;85:161-192.

48. Williams GSB, Boyman L, Chikando AC, et al. Mitochondrial calcium uptake. *Proc Natl Acad Sci USA*. 2013;110:10479-10486.

49. Palty R, Silverman WF, Hershfinkel M, et al. NCLX is an essential component of mitochondrial Na+/Ca^{2+} exchange. *Proc Natl Acad Sci USA*. 2010;107:436-441.

50. Baughman JM, Perocchi F, Girgis HS, et al. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature*. 2011;476:341-345.

51. De Stefani D, Raffaello A, Teardo E, et al. A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. *Nature*. 2011;476:336-340.

52. Perocchi F, Gohil VM, Girgis HS, et al. MICU1 encodes a mito-chondrial EF hand protein required for Ca(2+) uptake. *Nature*. 2010;467:291-296.

53. Penna E, Espino J, De Stefani D, et al. The MCU complex in cell death. *Cell Calcium*. 2018;69:73-80.

54. Mallilankaraman K, Doonan P, Cárdenas C, et al. MICU1 is an es-sential gatekeeper for MCU-mediated mitochondrial Ca(2+) up-take that regulates cell survival. *Cell*. 2012;151:630-644.

55. Csordás G, Golenár T, Seifert E, et al. MICU1 controls both the threshold and cooperative activation of the mitochondrial Ca(2)(+) uniporter. *Cell Metab*. 2013;17:976-987.

56. Joseph SK, Booth DM, Young MP, et al. Redox regulation of ER and mitochondrial Ca(2+) signaling in cell survival and death. *Cell Calcium*. 2019;79:89-97.

57. Liu JC, Parks RJ, Liu J, et al. The in vivo biology of the mitochon-drial calcium uniporter. *Adv Exp Med Biol*. 2017;982:49-63.

58. Pan X, Liu J, Nguyen T, et al. The physiological role of mitochon-drial calcium revealed by mice lacking the mitochondrial calcium uniporter. *Nat Cell Biol*. 2013;15:1464-1472.

59. Rasmussen TP, Wu Y, Joiner M-L, et al. Inhibition of MCU forces extramitochondrial adaptations governing physiological and pathological stress responses in heart. *Proc Natl Acad Sci*. 2015;112(29):9129-9134.

60. Luongo T, Lambert J, Yuan A, et al. The mitochondrial calcium uniporter matches energetic supply with cardiac workload during stress and modulates permeability transition. *Cell Rep*. 2015;12:23-34.

61. Kwong J, Lu X, Correll R, et al. The mitochondrial calcium uniporter selectively matches metabolic output to acute contractile stress in the heart. *Cell Rep*. 2015;12:15-22.

62. Luongo TS, Lambert JP, Gross P, et al. The mitochondrial Na(+)/Ca(2+) exchanger is essential for Ca(2+) homeostasis and viability. *Nature*. 2017;545:93-97.

63. Smith CO, Nehrke K, Brookes PS. The Slo(w) path to identifying the mitochondrial channels responsible for ischemic protection. *Biochem J*. 2017;474:2067-2094.

64. Szabo I, Zoratti M. Mitochondrial channels: ion fluxes and more. *Physiol Rev*. 2014;94:519-608.

65. O'Rourke B . Mitochondrial ion channels. *Annu Rev Physiol*. 2006;69:19-49.

66. Garlid KD, Paucek P, Yarov-Yarovoy V, et al. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K+ channels. Possible mechanism of cardioprotection. *Circ Res*. 1997;81:1072-1082.

67. Murata M, Akao M, O'Rourke B, et al. Mitochondrial ATP-sensitive potassium channels attenuate matrix Ca(2+) overload during simu-lated ischemia and reperfusion: possible mechanism of cardiopro-tection. *Circ Res*. 2001;89:891-898.

68. Foster DB, Ho AS, Rucker J, et al. Mitochondrial ROMK channel is a molecular component of mitoK(ATP). *Circ Res*. 2012;111:446-454.

69. Gross GJ, Fryer RM. Sarcolemmal versus mitochondrial ATP-sensitive K+ channels and myocardial preconditioning. *Circ Res*. 1999;84:973-979.

70. Paggio A, Checchetto V, Campo A, et al. Identification of an ATP-sensitive potassium channel in mitochondria. *Nature*. 2019;572:609-613.

71. Garlid KD. Cation transport in mitochondria–the potassium cycle. *Biochem Biophys Acta*. 1996;1275:123-126.

72. Inoue I, Nagase H, Kishi K, et al. ATP-sensitive K+ channel in the mitochondrial inner membrane. *Nature*. 1991;352:244-247.

73. Paucek P, Mironova G, Mahdi F, et al. Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K+ channel from rat liver and beef heart mitochondria. *J Biol Chem*. 1992;267:26062-26069.

74. Ashcroft FM. Adenosine 5'-triphosphate-sensitive potassium channels. *Annu Rev Neurosci*. 1988;11:97-118.

75. Nichols CG. KATP channels as molecular sensors of cellular metab-olism. *Nature*. 2006;440:470-476.

76. Noma A. ATP-regulated K+ channels in cardiac muscle. *Nature*. 1983;305:147-148.

77. Nichols CG. Adenosine triphosphate-sensitive potassium currents in heart disease and cardioprotection. *Card Electrophysiol Clin*. 2016;8:323-335.

78. Liu Y, Sato T, O'Rourke B, et al. Mitochondrial ATP-dependent po-tassium channels: novel effectors of cardioprotection? *Circulation*. 1998;97:2463-2469.

79. Sato T, Sasaki N, Seharaseyon J, et al. Selective pharmaco-logical agents implicate mitochondrial but not sarcolemmal K(ATP) channels in ischemic cardioprotection. *Circulation*. 2000;101:2418-2423.

80. Garlid KD, Paucek P, Yarov-Yarovoy V, et al. The mitochondrial KATP channel as a receptor for potassium channel openers. *J Biol Chem*. 1996;271:8796-8799.

81. Grover GJ, Garlid KD. ATP-sensitive potassium channels: a re-view of their cardioprotective pharmacology. *J Mol Cell Cardiol*. 2000;32:677-695.

82. Ardehali H, Chen Z, Ko Y, et al. Multiprotein complex containing succinate dehydrogenase confers mitochondrial ATP-sensitive K+ channel activity. *Proc Natl Acad Sci USA*. 2004;101:11880-11885.

83. Costa AD, Jakob R, Costa CL, et al. The mechanism by which the mitochondrial ATP-sensitive K^+ channel opening and H_2O_2 inhibit the mitochondrial permeability transition. *J Biol Chem*. 2006;281:20801-20808.

84. Heinzel FR, Luo Y, Li X, et al. Impairment of diazoxide-induced for-mation of reactive oxygen species and loss of cardioprotection in connexin 43 deficient mice. *Circ Res*. 2005;97:583-586.

85. Xu W, Liu Y, Wang S, et al. Cytoprotective role of Ca^{2+}- activated K^+ channels in the cardiac inner mitochondrial membrane. *Science*. 2002;298:1029-1033.

86. Shintani Y, Node K, Asanuma H, et al. Opening of Ca^{2+}-activated K+ channels is involved in ischemic preconditioning in canine hearts. *J Mol Cell Cardiol*. 2004;37:1213-1218.

87. Sato T, Saito T, Saegusa N, et al. Mitochondrial Ca^{2+}-activated K+ channels in cardiac myocytes: a mechanism of the cardiopro-tective effect and modulation by protein kinase A. *Circulation*. 2005;111:198-203.

88. Stowe DF, Aldakkak M, Camara AK, et al. Cardiac mitochondrial preconditioning by Big Ca^{2+}-sensitive K+ channel opening requires superoxide radical generation. *Am J Physiol Heart Circ Physiol*. 2006;290:H434-H440.

89. Goswami SK, Ponnalagu D, Hussain AT, et al. Expression and acti-vation of BKCa channels in mice protects against ischemia-reper-fusion injury of isolated hearts by modulating mitochondrial function. *Front Cardiovasc Med*. 2018;5:194.

90. Wang X, Yin C, Xi L, et al. Opening of Ca^{2+}-activated K+ chan-nels triggers early and delayed preconditioning against I/R in-jury independent of NOS in mice. *Am J Physiol Heart Circ Physiol*. 2004;287:H2070-H2077.

91. Huhn R, Heinen A, Weber NC, et al. Ischaemic and morphine-in-duced post-conditioning: impact of mK(Ca) channels. *Br J Anaesth*. 2010;105:589-595.

92. Gao Q, Hu J, Hu J, et al. Calcium activated potassium channel and protein kinase C participate in the cardiac protection of remote post conditioning. *Pak J Pharm Sci*. 2013;26:285-290.

93. Cao Y, Zhang S-Z, Zhao S-Q, et al. The mitochondrial Ca(2+)-activated K(+) channel contributes to cardioprotection by limb remote ischemic preconditioning in rat. *Life Sci*. 2011;88:1026-1030.

94. Meens MJ, Kwak BR, Duffy HS. Role of connexins and pannexins in cardiovascular physiology. *Cell Mol Life Sci.* 2015;72:2779-2792.

95. Leybaert L, Lampe PD, Dhein S, et al. Connexins in cardiovascular and neurovascular health and disease: pharmacological implications. *Pharmacol Rev.* 2017;69:396-478.

96. Schulz R, Görge PM, Görbe A, et al. Connexin 43 is an emerging therapeutic target in ischemia/reperfusion injury, cardioprotection and neuroprotection. *Pharmacol Ther.* 2015;153:90-106.

97. Dang X, Doble BW, Kardami E. The carboxy-tail of connexin-43 localizes to the nucleus and inhibits cell growth. *Mol Cell Biochem.* 2003;242:35-38.

98. Zhao X, Tang X, Ma T, et al. Levonorgestrel inhibits human endometrial cell proliferation through the upregulation of gap junctional intercellular communication via the nuclear translocation of Ser255 phosphorylated Cx43. *Biomed Res Int.* 2015;2015:758684.

99. Sun J, Nguyen T, Aponte AM, et al. Ischaemic preconditioning preferentially increases protein S-nitrosylation in subsarcolemmal mitochondria. *Cardiovasc Res.* 2015;106:227-236.

100. Srisakuldee W, Makazan Z, Nickel BE, et al. The FGF-2-triggered protection of cardiac subsarcolemmal mitochondria from calcium overload is mitochondrial connexin 43-dependent. *Cardiovasc Res.* 2014;103:72-80.

101. Koncsos G, Varga ZV, Baranyai T, et al. Nagarse treatment of cardiac subsarcolemmal and interfibrillar mitochondria leads to artefacts in mitochondrial protein quantification. *J Pharmacol Toxicol Methods.* 2018;91:50-58.

102. García-Niño WR, Correa F, Rodríguez-Barrena JI, et al. Cardioprotective kinase signaling to subsarcolemmal and interfibrillar mitochondria is mediated by caveolar structures. *Basic Res Cardiol.* 2017;112:15.

103. Boengler K, Stahlhofen S, Sand A, et al. Presence of connexin 43 in subsarcolemmal, but not in interfibrillar cardiomyocyte mitochondria. *Basic Res Cardiol.* 2009;104:141-147.

104. Soares AR, Martins-Marques T, Ribeiro-Rodrigues T, et al. Gap junctional protein Cx43 is involved in the communication between extracellular vesicles and mammalian cells. *Sci Rep.* 2015;5:13243.

105. Martins-Marques T, Ribeiro-Rodrigues T, Batista-Almeida D, et al. Biological functions of Connexin43 beyond intercellular communication. *Trends Cell Biol.* 2019;29:835-847.

106. Ribeiro-Rodrigues TM, Martins-Marques T, Morel S, et al. Role of connexin 43 in different forms of intercellular communication - gap junctions, extracellular vesicles and tunnelling nanotubes. *J Cell Sci.* 2017;130:3619-3630.

107. Bell CL, Shakespeare TI, Smith AR, et al. Visualization of annular gap junction vesicle processing: the interplay between annular gap junctions and mitochondria. *Int J Mol Sci.* 2018;20:44.

108. Boengler K, Ruiz-Meana M, Gent S, et al. Mitochondrial connexin 43 impacts on respiratory complex I activity and mitochondrial oxygen consumption. *J Cell Mol Med.* 2012;16:1649-1655.

109. Soetkamp D, Nguyen TT, Menazza S, et al. S-nitrosation of mitochondrial connexin 43 regulates mitochondrial function. *Basic Res Cardiol.* 2014;109:433.

110. Pecoraro M, Pinto A, Popolo A. Inhibition of Connexin 43 translocation on mitochondria accelerates CoCl2-induced apoptotic response in a chemical model of hypoxia. *Toxicol In Vitro.* 2018;47:120-128.

111. Boengler K, Ungefug E, Heusch G, et al. Connexin 43 impacts on mitochondrial potassium uptake. *Front Pharmacol.* 2013;4:73.

112. Gadicherla AK, Wang N, Bulic M, et al. Mitochondrial Cx43 hemichannels contribute to mitochondrial calcium entry and cell death in the heart. *Basic Res Cardiol.* 2017;112:27.

113. Boengler K, Schulz R. Connexin 43 and mitochondria in cardiovascular health and disease. *Adv Exp Med Biol.* 2017;982:227-246.

114. Goubaeva F, Mikami M, Giardina S, et al. Cardiac mitochondrial connexin 43 regulates apoptosis. *Biochem Biophys Res Comm.* 2007;352:97-103.

115. Chung YW, Lagranha C, Chen Y, et al. Targeted disruption of PDE3B, but not PDE3A, protects murine heart from ischemia/reperfusion injury. *Proc Natl Acad Sci USA.* 2015;112(17):E2253-E2262.

116. Yang F, Chen WL, Zheng MZ, et al. Heat shock protein 90 mediates anti-apoptotic effect of diazoxide by preventing the cleavage of Bid in hypothermic preservation rat hearts. *J Heart Lung Transplant.* 2011;30:928-934.

117. Martins-Marques T, Anjo SI, Pereira P, et al. Interacting network of the gap junction (GJ) protein connexin43 (Cx43) is modulated by ischemia and reperfusion in the heart. *Mol Cell Proteomics.* 2015;14:3040-3055.

118. Denuc A, Núñez E, Calvo E, et al. New protein-protein interactions of mitochondrial connexin 43 in mouse heart. *J Cell Mol Med.* 2016;20:794-803.

119. Boengler K, Lochnit G, Schulz R. Mitochondria, "THE" target of myocardial conditioning. *Am J Physiol Heart Circ Physiol.* 2018;315:H1215-H1231.

120. Zerbes RM, van der Klei IJ, Veenhuis M, et al. Mitofilin complexes: conserved organizers of mitochondrial membrane architecture. *Biol Chem.* 2012;393:1247-1261.

121. Waza AA, Andrabi K, Hussain MU. Protein kinase C (PKC) mediated interaction between conexin43 (Cx43) and K(+)(ATP) channel subunit (Kir6.1) in cardiomyocyte mitochondria: Implications in cytoprotection against hypoxia induced cell apoptosis. *Cell Signal.* 2014;26:1909-1917.

122. Kirca M, Kleinbongard P, Soetkamp D, et al. Interaction between connexin 43 and nitric oxide synthase in mice heart mitochondria. *J Cell Mol Med.* 2015;19:815-825.

123. Schwanke U, Konietzka I, Duschin A, et al. No ischemic preconditioning in heterozygous connexin43-deficient mice. *Am J Physiol Heart Circ Physiol.* 2002;283:H1740-H1742.

124. Li X, Heinzel FR, Boengler K, et al. Role of connexin 43 in ischemic preconditioning does not involve intercellular communication through gap junctions. *J Mol Cell Cardiol.* 2004;36:161-163.

125. Rodriguez-Sinovas A, Boengler K, Cabestrero A, et al. Translocation of connexin 43 to the inner mitochondrial membrane of cardiomyocytes through the heat shock protein 90-dependent TOM pathway and its importance for cardioprotection. *Circ Res.* 2006;99:93-101.

126. Penna C, Perrelli M-G, Raimondo S, et al. Postconditioning induces an anti-apoptotic effect and preserves mitochondrial integrity in isolated rat hearts. *Biochem Biophys Acta.* 2009;1787:794-801.

127. Totzeck A, Boengler K, van de Sand A, et al. No impact of protein phosphatases on connexin 43 phosphorylation in ischemic preconditioning. *Am J Physiol Heart Circ Physiol.* 2008;295:H2106-H2112.

128. Ruiz-Meana M, Núñez E, Miro-Casas E, et al. Ischemic preconditioning protects cardiomyocyte mitochondria through mechanisms independent of cytosol. *J Mol Cell Cardiol.* 2014;68:79-88.

129. Rodríguez-Sinovas A, Ruiz-Meana M, Denuc A, et al. Mitochondrial Cx43, an important component of cardiac preconditioning. *Biochem Biophys Acta.* 2018;1860:174-181.

130. Boengler K, Heusch G, Schulz R. Nuclear-encoded mitochondrial proteins and their role in cardioprotection. *Biochem Biophys Acta.* 2011;1813:1286-1294.

131. Tu R-H, Li Q-J, Huang Z, et al. Novel functional role of heat shock protein 90 in mitochondrial connexin 43-mediated hypoxic postconditioning. *Cell Physiol Biochem.* 2017;44:982-997.

132. Morel S, Christoffersen C, Axelsen LN, et al. Sphingosine-1-phosphate reduces ischaemia-reperfusion injury by phosphorylating the gap junction protein Connexin43. *Cardiovasc Res.* 2016;109:385-396.

133. Jiang J, Hoagland D, Palatinus JA, et al. Interaction of alpha carboxyl terminus 1 peptide with the connexin 43 carboxyl terminus preserves left ventricular function after ischemia-reperfusion injury. *J Am Heart Assoc*. 2019;8:e012385.

134. Heusch G, Büchert A, Feldhaus S, et al. No loss of cardioprotection by postconditioning in connexin 43-deficient mice. *Basic Res Cardiol*. 2006;101:354-356.

135. Abascal F, Zardoya R. Evolutionary analyses of gap junction protein families. *Biochem Biophys Acta*. 2013;1828:4-14.

136. Smyth JW, Shaw RM. Autoregulation of connexin43 gap junction formation by internally translated isoforms. *Cell Rep*. 2013;5:611-618.

137. Salat-Canela C, Sesé M, Peula C, et al. Internal translation of the connexin 43 transcript. *Cell Commun Signal*. 2014;12:31.

138. James CC, Zeitz MJ, Calhoun PJ, et al. Altered translation initiation of Gja1 limits gap junction formation during epithelial-mesenchymal transition. *Mol Biol Cell*. 2018.29(7):797-808.

139. Fu Y, Zhang S-S, Xiao S, et al. Cx43 isoform GJA1-20k promotes microtubule dependent mitochondrial transport. *Front Physiol*. 2017;8:905.

140. Basheer WA, Xiao S, Epifantseva I, et al. GJA1-20k arranges actin to guide Cx43 delivery to cardiac intercalated discs. *Circ Res*. 2017;121:1069-1080.

141. Zeitz MJ, Calhoun PJ, James CC, et al. Dynamic UTR usage regulates alternative translation to modulate gap junction formation during stress and aging. *Cell Rep*. 2019;27:2737-2747.e5.

142. Ul-Hussain M, Olk S, Schoenebeck B, et al. Internal ribosomal entry site (IRES) activity generates endogenous carboxyl-terminal domains of Cx43 and is responsive to hypoxic conditions. *J Biol Chem*. 2014;289:20979-20990.

143. Basheer WA, Fu Y, Shimura D, et al. Stress response protein GJA1-20k promotes mitochondrial biogenesis, metabolic quiescence, and cardioprotection against ischemia/reperfusion injury. *JCI Insight*. 2018;3(20):1-20.

144. Brennan J, Southworth R, Medina R, et al. Mitochondrial uncoupling, with low concentration FCCP, induces ROS-dependent cardioprotection independent of KATP channel activation. *Cardiovasc Res*. 2006;72:313-321.

145. Minners J, van den Bos EJ, Yellon DM, et al. Dinitrophenol, cyclosporin A, and trimetazidine modulate preconditioning in the isolated rat heart: support for a mitochondrial role in cardioprotection. *Cardiovasc Res*. 2000;47:68-73.

146. Cadenas S. Mitochondrial uncoupling, ROS generation and cardioprotection. *Biochim Biophys Acta Bioenerg*. 2018;1859:940-950.

147. Hoerter J, Gonzalez-Barroso M-D-M, Couplan E, et al. Mitochondrial uncoupling protein 1 expressed in the heart of transgenic mice protects against ischemic-reperfusion damage. *Circulation*. 2004;110:528-533.

148. Bienengraeber M, Ozcan C, Terzic A. Stable transfection of UCP1 confers resistance to hypoxia/reoxygenation in a heart-derived cell line. *J Mol Cell Cardiol*. 2003;35:861-865.

149. Wu H, Ye M, Liu DI, et al. UCP2 protect the heart from myocardial ischemia/reperfusion injury via induction of mitochondrial autophagy. *J Cell Biochem*. 2019;120:15455-15466.

150. McLeod CJ, Aziz A, Hoyt RF, et al. Uncoupling proteins 2 and 3 function in concert to augment tolerance to cardiac ischemia. *J Biol Chem*. 2005;280:33470-33476.

151. Perrino C, Schiattarella GG, Sannino A, et al. Genetic deletion of uncoupling protein 3 exaggerates apoptotic cell death in the ischemic heart leading to heart failure. *J Am Heart Assoc*. 2013;2:e000086.

152. Ozcan C, Palmeri M, Horvath TL, et al. Role of uncoupling protein 3 in ischemia-reperfusion injury, arrhythmias, and preconditioning. *Am J Physiol Heart Circ Physiol*. 2013;304:H1192-H1200.

153. Paulis D, Chiari P, Teixeira G, et al. Cyclosporine A at reperfusion fails to reduce infarct size in the in vivo rat heart. *Basic Res Cardiol*. 2013;108:379.

154. Hausenloy DJ, Yellon DM. Time to take myocardial reperfusion injury seriously. *N Engl J Med*. 2008;359:518-520.

155. Hausenloy DJ, Yellon DM. Targeting myocardial reperfusion injury-the search continues. *N Engl J Med*. 2015;373:1073-1075.

156. Ottani F, Latini R, Staszewsky L, et al. Cyclosporine A in reperfused myocardial infarction: the multicenter, controlled, open-label CYCLE trial. *J Am Coll Cardiol*. 2016;67:365-374.

157. Cung T-T, Morel O, Cayla G, et al. Cyclosporine before PCI in patients with acute myocardial infarction. *N Engl J Med*. 2015;373:1021-1031.

158. Huhn R, Heinen A, Hollmann MW, et al. Cyclosporine A administered during reperfusion fails to restore cardioprotection in prediabetic Zucker obese rats in vivo. *Nutr Metab Cardiovasc Dis*. 2010;20:706-712.

159. Chiari P, Angoulvant D, Mewton N, et al. Cyclosporine protects the heart during aortic valve surgery. *Anesthesiology*. 2014;121:232-238.

160. Hausenloy DJ, Kunst G, Boston-Griffiths E, et al. The effect of cyclosporin-A on peri-operative myocardial injury in adult patients undergoing coronary artery bypass graft surgery: a randomised controlled clinical trial. *Heart*. 2014;100:544-549.

161. Gibson CM, Giugliano RP, Kloner RA, et al. EMBRACE STEMI study: a Phase 2a trial to evaluate the safety, tolerability, and efficacy of intravenous MTP-131 on reperfusion injury in patients undergoing primary percutaneous coronary intervention. *Eur Heart J*. 2016;37:1296-1303.

162. Atar D, Arheden H, Berdeaux A, et al. Effect of intravenous TRO40303 as an adjunct to primary percutaneous coronary intervention for acute ST-elevation myocardial infarction: MITOCARE study results. *Eur Heart J*. 2015;36:112-119.

163. Boengler K, Hilfiker-Kleiner D, Heusch G, et al. Inhibition of permeability transition pore opening by mitochondrial STAT3 and its role in myocardial ischemia/reperfusion. *Basic Res Cardiol*. 2010;105:771-785.

164. Gomez L, Paillard M, Thibault H, et al. Inhibition of GSK3beta by postconditioning is required to prevent opening of the mitochondrial permeability transition pore during reperfusion. *Circulation*. 2008;117:2761-2768.

165. Xie JR, Yu LN. Cardioprotective effects of cyclosporine A in an in vivo model of myocardial ischemia and reperfusion. *Acta Anaesthesiol Scand*. 2007;51:909-913.

166. Squadrito F, Altavilla D, Squadrito G, et al. Cyclosporin-A reduces leukocyte accumulation and protects against myocardial ischaemia reperfusion injury in rats. *Eur J Pharmacol*. 1999;364:159-168.

167. Ikeda Y, Miura T, Sakamoto J, et al. Activation of ERK and suppression of calcineurin are interacting mechanisms of cardioprotection afforded by delta-opioid receptor activation. *Basic Res Cardiol*. 2006;101:418-426.

168. Niemann CU, Saeed M, Akbari H, et al. Close association between the reduction in myocardial energy metabolism and infarct size: dose-response assessment of cyclosporine. *J Pharmacol Exp Ther*. 2002;302:1123-1128.

169. Fang J, Wu L, Chen L. Postconditioning attenuates cardiocyte ultrastructure injury and apoptosis by blocking mitochondrial permeability transition in rats. *Acta Cardiol*. 2008;63:377-387.

170. Leshnower BG, Kanemoto S, Matsubara M, et al. Cyclosporine preserves mitochondrial morphology after myocardial ischemia/reperfusion independent of calcineurin inhibition. *Ann Thorac Surg*. 2008;86:1286-1292.

171. Wang C, Neff DA, Krolikowski JG, et al. The influence of B-cell lymphoma 2 protein, an antiapoptotic regulator of mitochondrial permeability transition, on isoflurane-induced and ischemic postconditioning in rabbits. *Anesth Analg*. 2006;102:1355-1360.

172. Argaud L, Gateauroesch O, Muntean D, et al. Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury. *J Mol Cell Cardiol*. 2005;38:367-374.

173. Krolikowski JG, Bienengraeber M, Weihrauch D, et al. Inhibition of mitochondrial permeability transition enhances isoflurane-induced cardioprotection during early reperfusion: the role of mitochondrial KATP channels. *Anesth Analg*. 2005;101:1590-1596.

174. Argaud L, Gateau-Roesch O, Chalabreysse L, et al. Preconditioning delays Ca^{2+}-induced mitochondrial permeability transition. *Cardiovasc Res*. 2004;61:115-122.

175. Lie RH, Stoettrup N, Sloth E, et al. Post-conditioning with cyclosporine A fails to reduce the infarct size in an in vivo porcine model. *Acta Anaesthesiol Scand*. 2010;54:804-813.

176. Karlsson LO, Zhou A-X, Larsson E, et al. Cyclosporine does not reduce myocardial infarct size in a porcine ischemia-reperfusion model. *J Cardiovasc Pharmacol Ther*. 2010;15:182-189.

177. Gomez L, Thibault H, Gharib A, et al. Inhibition of mitochondrial permeability transition improves functional recovery and reduces mortality following acute myocardial infarction in mice. *Am J Physiol Heart Circ Physiol*. 2007;293:H1654-H1661.

178. Lim S, Davidson S, Hausenloy D, et al. Preconditioning and post-conditioning: the essential role of the mitochondrial permeability transition pore. *Cardiovasc Res*. 2007;75:530-535.

179. Fancelli D, Abate A, Amici R, et al. Cinnamic anilides as new mitochondrial permeability transition pore inhibitors endowed with ischemia-reperfusion injury protective effect in vivo. *J Med Chem*. 2014;57:5333-5347.

180. Zhang K, Zhao J, Yang Y, et al. Cardioprotective effects of diazoxide on myocardial ischemia/reperfusion injury in rats. *J Huazhong Univ Sci Technolog Med Sci*. 2006;26:690-692.

181. Takashi E, Wang Y, Ashraf M. Activation of mitochondrial K(ATP) channel elicits late preconditioning against myocardial infarction via protein kinase C signaling pathway. *Circ Res*. 1999;85:1146-1153.

182. Fryer RM, Eells JT, Hsu AK, et al. Ischemic preconditioning in rats: role of mitochondrial K(ATP) channel in preservation of mitochondrial function. *Am J Physiol Heart Circ Physiol*. 2000;278:H305-H312.

183. Naitoh K, Ichikawa Y, Miura T, et al. MitoKATP channel activation suppresses gap junction permeability in the ischemic myocardium by an ERK-dependent mechanism. *Cardiovasc Res*. 2006;70:374-383.

184. Takano H, Tang XL, Bolli R. Differential role of K(ATP) channels in late preconditioning against myocardial stunning and infarction in rabbits. *Am J Physiol Heart Circ Physiol*. 2000;279:H2350-H2359.

185. Ockaili R, Emani VR, Okubo S, et al. Opening of mitochondrial KATP channel induces early and delayed cardioprotective effect: role of nitric oxide. *Am J Physiol*. 1999;277:H2425-H2434.

186. Baines CP, Liu GS, Birincioglu M, et al. Ischemic preconditioning depends on interaction between mitochondrial KATP channels and actin cytoskeleton. *Am J Physiol*. 1999;276:H1361-H1368.

187. Hale SL, Kloner RA. Effect of combined K(ATP) channel activation and Na(+)/H(+) exchange inhibition on infarct size in rabbits. *Am J Physiol Heart Circ Physiol*. 2000;279:H2673-H2677.

188. Cao C-M, Xia Q, Gao Q, et al. Calcium-activated potassium channel triggers cardioprotection of ischemic preconditioning. *J Pharmacol Exp Ther*. 2005;312:644-650.