MITOCHONDRIAL POTASSIUM CHANNELS AS PHARMACOLOGICAL TARGET FOR CARDIOPROTECTIVE DRUGS

L. Testai, S. Rapposelli, A. Martelli, M.C. Breschi, V. Calderone*

Department of Pharmacy, University of Pisa

Via Bonanno, 6-56120

Pisa

* **Corresponding author**

Dr. Vincenzo Calderone

Via Bonanno, 6

56120-Pisa

e-mail:calderone@farm.unipi.it

phone +39-50-2219589

fax +39-50-2219589

Abstract

Brief periods of ischemia are known to confer to the myocardium an increased resistance to the injury due to a later and more prolonged ischemic episode. This phenomenon, known as ischemic pre-conditioning (IPC), is ensured by different biological mechanisms. Although an exhaustive comprehension of them has not been reached yet, it is widely accepted that mitochondria are pivotally involved in controlling cell life and death, and thus in IPC. Among the several signaling pathways involved, as triggers and/or end-effectors, in the mitochondrial mechanisms of cardioprotection, an important role is played by the activation of potassium channels located in the mitochondrial inner membrane (mitoK) of cardiomyocytes. Presently, different types of mitoK channels have been recognized in the heart, such as ATP-sensitive ($mitoK_{ATP}$) and calcium-activated ($mitoBK_{Ca}$ and $mitoSK_{Ca}$) potassium channels. Consistently, drugs modulating mitochondrial KCs, on one hand, have been employed as useful experimental tools for early basic studies on IPC. On the other hand, activators of mitochondrial KCs are promising and innovative therapeutic agents for limiting the myocardial injury due to ischemic episodes.

In this review, we report the experimental evidence supporting the role of mitoK channels in signaling pathways in the mechanisms of cardioprotection and an overview on the most important molecules acting as modulators of these channels, with their profiles of selectivity. Some innovative pharmaceutical strategies for mitochondriotropic drugs have been also reported. Finally, an appendix describing the main experimental approaches usually employed to study mitoK channels in isolated mitochondria or in intact cells, has been added.

Keywords

Ischemia-reperfusion, mitochondrial potassium channels, myocardial injury, potassium channel activators, cardioprotective drugs.

1- ISCHEMIA/REPERFUSION INJURY

Acute myocardial ischemia is one of the major causes of morbidity in western society and despite the recent advances in therapy, it remains a major cause of mortality. Indeed, the reduced coronary blood supply leads to cell death and loss of cardiomyocytes population, resulting in serious and often irreversible consequences on myocardial functionality [1]. The myocardial cell death during an ischemic episode is caused by necrosis, mostly due to the irreversible opening of the mitochondrial permeability transition pore (MPTP) and by the stimulation of the death receptor-induced necrosis, a process known as necroptosis [2-3]. Currently, the most effective strategy to reduce ischemic damage is an early reperfusion, but paradoxically the reperfusion in itself is responsible for an additional damage. Indeed, the global myocardial damage is referred to as ischemia-reperfusion (I/R) injury [4].

A Ischemia-induced cell death

During ischemia, the rate of glycolysis in the myocardial cell rises, in order to produce energy by mitochondrion-independent pathways; this causes a metabolic rise of lactic acid and acidification of the intracellular pH. Besides, the Na^+/K^+ ATPase is inhibited, because of the decline of ATP concentration and the intracellular $[Na^+]$ increase, then the Na^+/H^+ antiporter is inhibited, with further lowering of the pH. The Na^+/Ca^{++} antiporter, that usually pumps Ca^{++} out of the cell and Na^+ into the cell, is inhibited or reversed, leading to an overload of Ca^{++}, responsible for hypercontracture, that contributes to the irreversible opening of MPTP [5] ATP is rapidly converted to ADP and AMP, which leak out of the cell and contribute to reduced cardiac performance. During the ischemia episode, the cytosol concentration of ROS exhibits a biphasic behaviour: in the first minutes, a small amount of ROS is produced; while after 20-25 minutes, a dramatically higher ROS concentration is formed. The depletion of

ATP, together with elevated intracellular [Ca^{++}] and ROS, leads the cell to a gradual and irreversible decline of its integrity [5-7] (Figure 1A).

B Reperfusion-induced cell death

The beginning of the reperfusion is associated with a burst of ROS production, primarily formed by complex 1, 2 and complex 3 of the respiratory chain, but also by other mitochondrial oxidative enzymes, such as xanthine oxidase, NAPH oxidase, monoamine oxidase and aconitase [8-10]. The mitochondrial proteins are particularly susceptible to ROS-induced damage: ROS have direct effects on the respiration and play a critical role in the opening of the MPTP [5-7, 11, 12]. MPTP is a high conductance mega-channel, anchored between the mitochondrial outer and inner membrane. When it is assembled, it allows the connection between the cytoplasm and mitochondrial matrix [13]. It is formed by an arrangement of the voltage-dependent anion channel (VDAC), located in the outer mitochondrial membrane, with the adenine nucleotide transporter (ANT) and the proteic translocator (TSPO) , located in the inner mitochondrial membrane, and cyclophilin D in the matrix [14-16].

It is thought that during ischemia the MPTP is closed, since the pore is powerfully inhibited by low pH (<7) [17]. Indeed the rapid energization of mitochondria at reperfusion leads to electrogenic uptake of Ca^{++}, previously accumulated into the cytosol during ischemia, that supports a “destructive” hypercontracture. This factor, together with the rise of ROS production and the recovery of neutral pH, promotes the opening of MPTP [18-20]. The opening of just a single pore in one mitochondrion is sufficient to cause its immediate depolarization [21], and then enlisting further MPTP opening, since the MPTP activation is triggered by depolarization. As a consequence of MPTP opening, all small molecular weight solutes (< 1.4 kDa) equilibrate across the inner membrane; in contrast, the largest molecules

(i.e. proteins) remain entrapped in the matrix, exerting an osmotic pressure that leads to the uptake of water and matrix swelling [22, 23]. Although the unfolding of the cristae allows the matrix to expand without rupture of the inter-membrane, the outer one breaks and leads to the release of pro-apoptotic proteins, confined in the inter-membrane space, such as cytochrome c [24-26]. There is an increasing evidence that the time of MPTP opening is closely correlated with the extent of damage. Indeed inhibitors of the pore opening (such as cyclosporine A) protect the heart from injury and many preconditioning strategies, aim at inhibiting opening of MPTP [27-30] (Figure 1B).

2- MECHANISMS OF CARDIOPROTECTION

A Ischemic pre-conditioning (IPreC)

Transient brief episodes of I/R (typically of 2-5 minutes)confer on myocardium an increased resistance against a subsequent prolonged severe episode of I/R. This phenomenon is known as ischemic pre-conditioning (IPreC) and is recognized as one of the most powerful endogenous cardioprotective mechanisms. IPreC was first described in dog hearts [31], and thereafter confirmed in many mammalian species, including humans [32-35].

In particular, two distinct phases of IPreC are recognized: an early one, the “classic IPreC”, that lasts for 3 h after the stimulus, and a delayed one, the “second window of IPreC”, starting about 24 h after the triggering stimulus and lasting for up to 3 days following the stimulus [32, 36-38].

Early IPreC is due to the involvement of already existing biomolecules acting as effectors; whereas the delayed one is mediated by newly synthesized cardioprotective proteins [39] (Figure 2).

B Ischemic post-conditioning (IPostC)

More recently, Zhao and colleagues demonstrated that brief intermittent cycles of coronary re-occlusion (typically 30 seconds) and reperfusion (30 seconds) during the first minute of reperfusion after a severe ischemic event, reduce the infarct size by about 40% in canine hearts [40]. As observed for the IPreC, also the IPostC has been confirmed in many other mammalian species [41-42]. Presently, the mechanisms involved in IPostC have not been fully understood, but reduction of ROS production, mitochondrial Ca^{++} overload and inflammation have been observed [35,40, 43] (Figure 2).

C Remote ischemic pre- and post-conditioning (ReIPreC, ReIPostC)

Schmidt et al. demonstrated that brief intermittent cycles of limb ischemia afforded a significant protection during myocardial infarction, preserving the cardiac function and reducing the arrhythmias typical of reperfusion [44]. Similarly, Li et al. also observed that limb brief ischemia periods reduced the myocardial damage after an ischemic period by inhibiting oxidative stress [45]. Again, renal ischemia episodes, in the first minute of reperfusion, reduced the myocardial infarct size in rats [46].

These phenomena are called remote ischemic preconditioning (ReIPreC) and remote ischemic postconditioning (ReIPostC). They consist in brief episodes of I/R applied to an organ distant from the heart, before or after prolonged ischemia, and afford cardioprotection [46-48]. Although the molecular mechanisms accounting for ReIPreC and ReIPost are not well understood (most studies are largely observational), these phenomena have been clearly observed in many mammalian species, including rat, rabbit and pigs, and are likely to require the transfer of protective factors or signals through humoral and/or neural pathways, such as some kinases [49].

D Pharmacological pre-conditioning (Ph-PreC)

A clinical procedure of IPreC, anticipating a myocardial infarct, is almost impossible to be applied, since the infarct onset cannot be predicted. In contrast, a clinical cardioprotective strategy based on IPostC is conceptually feasible in patients with acute myocardial infarct, but it shows many difficulties, risks and disadvantages [47, 50]. The definition of the signaling pathways of IPreC and IPostC, paves the way to develop pharmacologic strategies, to mimic the protection with drugs able to trigger the same mitochondrial pathways (Figure 2). The IPreC, as well as IPostC which recruits analogous signaling pathways, is mediated by numerous endogenous factors, including adenosine [51], acetylcholine [52], bradykinin [53] and opioids [54-59] and gaseous molecules, such as NO [60-63] and H_2S [64].

Moreover, the IPreC and IPostC involve the activation of protein kinase C (PKC), isozymes which sometimes exert opposing roles in both normal signaling and disease states [65], and possibly other kinases, such as reperfusion injury salvage kinase (RISK), glycogen synthase kinase 3 beta (GSK3B), signaling transducer and activator of transcription 3 (STAT3) [63].

As regard the isoforms of PKC, the ε and δ isoforms play opposite roles in cardioprotection. In particular, PKCε activation is cardioprotective, while PKCδ activation mediates the most part of the injury induced by myocardial ischemia [66]. Recent evidence shows that PKCε activates the mitochondrial aldehyde dehydrogenase 2 (ALDH2), which removes products of lipid peroxidation [67] thus protecting the mitochondrial functions. Moreover, Baines et al. reported that the translocation of PKCε can be considered as an additional mechanism for protection: the direct phosphorylation of the MPTP components inhibits the pore opening [38, 68, 69]. In contrast, PKCδ inhibition during the reperfusion phase is cardioprotective. Actually, the PKCδ activation triggers the mitochondrial pyruvate dehydrogenase kinase [70], thus inhibiting both pyruvate dehydrogenase and ATP regeneration. Moreover, PKCδ activation induces impaired perfusion of myocytes after the ischemic event, thus leading to further tissue injury [71]. However, the role of PKC for cardioprotection in mammalian is still

controversial. Heusch et al. reported that the PKC inhibitor staurosporine did not prevent IPreC in swine [72]. Moreover, after the first encouraging evidence of Mochly-Rosen that inhibition of PKCδ at the onset of reperfusion reduced the tissue injury, the clinical trial failed to show any significant benefit [73].

In addition to PKC isozymes, other different protein kinases have been proposed as mediators of cardioprotective pathway. For example, STAT3 has been recently identified in mitochondria and its expression is correlated with the reduction of myocardial injury by IPreC and IPostC. consistently, the deletion of STAT3 or its inhibition abrogated the cardioprotection mediated by conditioning strategies [74, 75].

Again, several types of potassium channels, present in the inner mitochondrial membranes and similar to those present in the plasma membrane of several cell types, have been suggested to be triggers and end-effectors in cardioprotection. Presently, ATP-sensitive [76] and calcium-activated [77-80] potassium channels have been recognized and has been proved to be involved in the regulation of mitochondrial volume, membrane potential, pH-regulation and apoptosis.

On the other hand, connexin 43, a transmembrane protein which allow direct communication between cytoplasm of adjacent cells, by the formation of gap junctions, has been described in mitochondria and demonstrated to participate in the IPreC [81]. Interestingly, mitochondrial connexin 43 contributes to mitochondrial potassium uptake, forming hemichannel-like structures or modulating existing ion transporters [81-83]. Noteworthy, connexin 43 is hypothesized to be involved in the cardioprotection induced by the K_{ATP} activator diazoxide, because, in connexin 43 deficient mice, diazoxide is devoid of beneficial effects [84].

3- MITOCHONDRIAL POTASSIUM CHANNELS INVOLVED IN CARDIOPROTECTION

The critical role of mitochondria and the involvement of mitochondrial pathways activated by IPreC or IPostC are crucial in the mechanism of cardioprotection. In this context, the mitochondrial potassium channels (mitoK) represent an attractive pharmacological target. In normal conditions, the mitochondrial inner membrane is almost impermeable to K^+. Hence, when mitoK channels are closed, the entry of K^+ ions is negligible and easily buffered by a K^+/H^+ antiporter, that pumps K^+ out of the matrix; therefore the influence of such a K^+ leakage on the mitochondrial membrane potential is almost negligible, although it can regulate the matrix volume.

By contrast, in stress or ischemia conditions, the opening of mitoK channels causes a significant influx of K^+ ions, with diffusion of water and uptake of anions, resulting in matrix swelling. This effect ensures the preservation of low permeability of the outer membrane for nucleotides and then the creation of a favorable gradient for ATP synthesis and transfer to cytoplasm [85]. Then, the activation of mitoK channels controls the matrix volume, preserving a narrow intermembrane space, necessary to preserve an effective oxidative phosphorylation. Moreover, the opening of mitoK channels produces a mild depolarization of membrane potential, responsible for a reduced uptake of Ca^{++} into the mitochondrial matrix, preserving it from the Ca^{++}-overload and the subsequent MPTP opening [86].

Finally, the cardioprotection offered by mitoK channel activation can be also linked to a mild uncoupling of mitochondrial respiration [87] (Figure 3).

Effects of mitoK channel activators is summarized in table 1.

A MitoK$_{ATP}$ channels

The first mitoK channel recognized as an effective target for Ph-PreC was the ATP-sensitive one (mitoK$_{ATP}$). K$_{ATP}$-openers, such as bimakalim and cromakalim, were reported to produce

cardioprotective effects at doses devoid of any influence on action potential duration, suggesting the existence of an intracellular site of action, independent of the sarcolemmal channels [88-91].

Later, the first direct evidence about a role of mitoK_{ATP} channels was the observation of the effect of the K_{ATP} opener diazoxide on bovine heart mitochondria, at a concentration clearly lower than that necessary for opening the sarcolemmal channel [92]. Although for several years there has been a debate on which type of K_{ATP} channel (sarcolemmal or mitochondrial) was involved in cardioprotection, presently, it is generally accepted that the mitochondria play a crucial role in the I/R event and the role of the mitoK_{ATP} channel is likely to be prevalent [93].

Indeed, numerous experimental reports in support of this hypothesis have been collected in the last years, demonstrating that the IPreC, and most recently even the IPostC, can be mimicked by several selective mitoK_{ATP} channel activators [94-98].

To date, no definitive molecular identity has been assigned to the mitoK_{ATP} channel; nevertheless, a resemblance with the sarcolemmal one has been proposed. In fact, mitoK_{ATP} is likely to be an hetero-octameric complex, containing K_{IR}6.1 or K_{IR}6.2 subunits and SUR2 sulphonylurea receptor subunits, while the SUR1 subunit seems to be excluded (for a more complete and authoritative examination of the molecular composition of mitoK_{ATP} channels: [99-102]). Recently, a mitochondrial-specific SUR2 variant (mitoSUR2), generated by an intraexonic splicing of the classical SUR2 subunit, has been described; noteworthy, in SUR2 knock-out mice, in which the mitoSUR2 form remained expressed, the IPreC strategy was still effective in reducing ischemic injury [103].

Moreover, the pharmacological cross-talk between the mitoK_{ATP} channel and mitochondrial respiratory complex II (succinate dehydrogenase) led to the hypothesis that complex II could be a further component of the mitochondrial channel [104]. Interestingly, a step forward in

understanding the molecular structure of the mitoK$_{ATP}$ channel was achieved by the O'Rourke group: they demonstrated that the ROMK (renal outer medullary potassium) channel, normally expressed on the surface membrane in the kidney cells, is localized on the inner membrane of cardiac mitochondria and mediates ATP-sensitive K^+ flux, giving protection against cell death stimuli. This observation led to speculation that mitoROMK may be a pore-forming subunit of the mitoK$_{ATP}$ channel [105].

B MitoK$_{ATP}$ activators

The first pioneering experiments probing the cardioprotective properties of K$_{ATP}$ channel activators were carried out with cromakalim or its analogs. It is a benzopyran compound completely devoid of selectivity for the mitoK$_{ATP}$ channels, thus the protective effects are endowed with vasodilatation and action potential duration shortening, due to the activation of the sarcolemmal K$_{ATP}$ channels present in vascular smooth muscle cells and cardiomyocytes, respectively. The benzopyran nucleus represents the scaffold most widely subjected to chemical manipulation in order to develop novel mitoK$_{ATP}$ activators [106].

Diazoxide, a prototype of the benzothiadiazine class, has been reported to open mitoK$_{ATP}$ channels with a good, but not absolute, selectivity. At moderate doses it can activate mitoK$_{ATP}$ channels, producing Ph-PreC, without showing non-selective effects. Nevertheless, at higher doses, an activation of sarcolemmal K$_{ATP}$ channels cannot be excluded [107-108]. Although the role of ROS in the IPreC is controversial, Heusch and colleagues demonstrated that diazoxide conferred protection through the generation of free radicals [84, 109]. MitoK$_{ATP}$-independent mechanisms of diazoxide have also been hypothesized and a possible target is likely to be the mitochondrial succinate dehydrogenase [110-112]. Indeed, Wojkovich and his colleagues proved that the complex II is an important regulator or component of the mitoK$_{ATP}$ channel, since diazoxide is able to activate the channel, but also to inhibit the complex II;

conversely, a complex II inhibitor atpenin A5, is able to open at lower concentration the mitoK$_{ATP}$ channel [113-114].

Unfortunatly, diazoxide is also responsible for undesired vasodilator and hyperglicaemic effects [95, 115-116].

Attempting to ameliorate the selectivity versus the mitoK$_{ATP}$ channel, the Bristol Mayer Squibb Company has developed original hybrids, conjugating the benzopyran moiety of cromakalim with the cyanoguanidine nucleus (another important scaffold of non-selective K$_{ATP}$ openers, such as pinacidil) [106, 117].

This research led to two interesting compounds: BMS 180448, endowed with a good cardioprotective potency, but still conserving a poor vasorelaxing potency and BMS191095, a 4-N aryl-substituted benzopyran, were synthesized. The latter molecule resulted about 30-fold more selective than BMS 180448 and its pharmacological effect was antagonized by 5-hydroxy decanoic acid (5-HD), a selective mitoK$_{ATP}$ blocker. Nevertheless, BMS 191095 is not suitable for clinical use, because of its neuronal toxicity; it remains only a useful tool for research purposes [118]. Afterwards, Lee and his colleagues developed new molecules based on a benzopyran scaffold bearing a 2'-carboxyalkyl-indole or 2'-carboxyalkyl-indoline nucleus in 4-position. In particular two 2-ethyl-esters showed the best cardioprotective activity in vivo and in vitro, and a weak vasorelaxing potency. The cardioprotective effect of these novel compounds was completely blocked by 5-HD, suggesting the involvement of the mitoK$_{ATP}$ channels [119]. Moreover, the benzopyranyl-indole analog KR-31466 reduces hypoxic injury in heart-derived H9c2 cells through mitoK$_{ATP}$ opening. Jung et al. suggested that this protection from hypoxia-induced death could also involve PKC activation [120].

More recently, a novel series of spiro-morpholine and spiro-morpholone benzopyran derivatives has been projected and synthetized by us [95, 121-123]. The insertion of spiro-cyclic substitution on the carbon 4 of the benzopyran group had the objective to confer a

structural rigidity in this molecular portion, in order to improve the selectivity *vs* the mitoK$_{ATP}$ channel.

Some compounds of this series exhibited a good cardioprotective profile in ex vivo models of I/R and modest effects on vascular smooth muscle. In particular, the best of the series, the N-acetyl spiro-morpholone derivative (F163) has been submitted to further studies. The resolution of the racemic mixture, according to the results previously obtained with BMS 180448, showed the enantioselectivity of the cardioprotective profile; in fact only the levorotatory enantiomer was effective [124]. An in depth pharmacological investigation confirmed the cardioprotection on cardiomyoblasts culture submitted to anoxia/reperfusion and in vivo on acute myocardial infarct; moreover the mitochondrial target of F163 has been demonstrated [95]. Actually, it was observed that the novel compound was able to produce all the typical effects of a mitoK channel activation: mild depolarization, reduction of Ca^{++}-uptake, increase of Ca^{++}-release and mild mitochondrial swelling, reversed by selective mitoK$_{ATP}$ blockers. According to the literature, a close correlation between drug-induced depolarization and Ca^{++}-release from the matrix of Ca^{++}-preloaded mitochondria was also reported, leading to the observation that a small depolarization is sufficient to greatly affect the movement of Ca^{++}. This study suggested that the novel derivative could represent an interesting prototype for the development of innovative mitoK$_{ATP}$ openers, that are more potent than diazoxide and devoid of significant influences on blood pressure and glucose metabolism [95] (in figure 4 are shown the chemical structures of K$_{ATP}$ activators).

Presently, no specific study attempted to identify the binding site of K$_{ATP}$ activators on the mitoK$_{ATP}$ channels; however, in analogy with the sarcolemmal channels, the interaction with the SUR subunit seems to be more probable hypothesis.

C Mitochondrial calcium-sensitive potassium channels

C.1 MitoBK$_{Ca}$ channels and their activators

In 2002, O'Rourke's laboratory for the first time found the presence of a large conductance calcium-activated potassium channel (BK_{Ca}, or $K_{Ca}1.1$) on the guinea-pig cardiac inner membrane of mitochondria (mitoBK_{Ca}) [79], previously described only on mitochondria of glioma cells without assigning a specific function [77]. In this work, they employed patch-clamp mitoplasts and identified the K^+ current across this channel as a component of background K^+ conductance. Moreover, they identified a role for the mitoBK_{Ca} channel in the protection against ischemic injury. Indeed, the BK_{Ca} activator, NS1619, decreased the infarct size following an I/R period, and this effect was antagonized by the BK_{Ca} blocker paxilline [79, 104]. Other reports have also confirmed that the pharmacological opening of mitoBK_{Ca} channels mimicked both early and delayed preconditioning [125-127], as well as postconditioning [94].

Noteworthy, cardiopreventive effects of NS1619 seemed to be also mediated by an anti-inflammatory activity that prevented leukocyte-endotelial cells adhesion and preserved mitochondrial functionality [128-130].

However, micromolar concentrations of NS1619 cause also anomalous effects on mitochondria, such as decreased respiratory control, insensitivity to selective BK_{Ca} blockers, and a deep membrane potential drop even in the absence of K^+ [131-132]. Such a profile of NS1619, similar to diazoxide, is due to off-target effects on mitochondria, including inhibition of the respiratory chain and uncoupling [133]. Notably, at higher concentrations NS1619 can also inhibit L-type Ca^{++}-channels, calcium-activated Cl^- currents, voltage-gated calcium channels and K^+ and Na^+ channels; these aspecific effects complicate the interpretation of the role of mitoBK_{Ca} channels in cardioprotection [134-137].

Another novel BK_{Ca} activator, chemically unrelated to, but more potent and specific than NS1619, is NS11021 [138]. It exerts protective effects through activation of BK_{Ca} channels; these effects were completely inhibited by paxilline. Nanomolar concentrations of NS11021 improved the bioenergetic performance of heart mitochondria, through enhanced K^+-uptake and mild swelling, without large changes in mitochondrial membrane potential ($\Delta\Psi$). Interestingly, the improvement of mitochondrial respiration observed with NS11021 by Aon et al. was due to a decrease in state 4 respiration (related to the phase in which all of the ADP is converted to ATP), while the state 3 respiration (corresponding to the phase in which ADP is converting to ATP) was unchanged. Such an unexplained behavior of NS11021 is different from $mitoK_{ATP}$ openers, which dose-dependently improve respiration through an increase of state 3 [139-140].

Besides the most extensively studied NS-series, the more potent compound CGS7184 (ethyl 1-[(4-chlorophenyl)-amino]oxo]-2-hydroxy-6-trifluoromethyl-1H-indole-3-carboxylate) has been developed [141]. CGS7184 reduced the ROS production in mitochondria isolated from rat brain [142], and depolarized the $\Delta\Psi$ in the EAhy926 endothelial cell line [143]. These effects, that could be predictive of protective properties, seem to be related to modulation of intracellular Ca^{++} homeostasis, probably by interaction with the sarcoplasmic reticulum [144].

The molecular composition of $mitoBK_{Ca}$ channels is yet unknown; although an auxiliary beta1 subunit, necessary for the IPreC and identical to the sarcolemmal one, was found [145-146]; however some splice variants at the mitochondrial level cannot be excluded. Presently, it is thought that the mitochondrial and sarcolemmal structures are conserved, and then the $mitoBK_{Ca}$ is likely to be composed of four alpha subunits, forming the pore, and four auxiliary beta-subunits, which modulate calcium sensitivity and activity [147].

Very recently, the Toro group unequivocally demonstrated the existence of the KCNMA1 (Slo1) gene at the mitochondrial level, encoding for BK_{Ca} channel. Their hypothesis was

supported with experiments on Kcnma1 knockout mice, in which NS1619 failed to produce cardioprotection [148].

Notably, the combined application of sub-maximally effective concentrations of two different mitoK activators NS1619 and diazoxide on cardiac mitochondria, produced additive effects, reducing flavoprotein oxidation and ouabain-induced mitochondrial Ca^{++} overload; thus contributing to cardioprotection [149] (in figure 4 are shown the chemical structures of BK_{Ca} activators).

C.2 MitoIK$_{Ca}$ channels

Besides the mitoBK$_{Ca}$ channels, an intermediate conductance calcium-sensitive potassium channel (IK$_{Ca}$, or K$_{Ca}$3.1) has been detected in purified mitochondria of human colon carcinoma cells [99, 150], of HeLa cells and embryonic fibroblasts [151]. Its molecular weight is the same of IK$_{Ca}$ channel localized on plasma membrane, suggesting equivalent biophysical and pharmacological properties [151]. The physiological role of mitoIK$_{Ca}$ has not been studied in detail so far, but TRAM-34 (an IK$_{Ca}$ inhibitor) induced hyperpolarization of the mitochondrial membrane, in agreement with the typical profile of mitochondrial potassium channel activators [151]. At the best of our knowledge, mitoIK$_{Ca}$ channels have not been presently recognized in the heart.

C.3 MitoSK$_{Ca}$ channels

Very recently, a small conductance calcium-sensitive potassium (SK$_{Ca}$, or K$_{Ca}$2) channel has been identified and localized thanks to immune-electron microscopy and purified by isoelectric focusing on the inner membrane of guinea-pig cardiac mitochondria and in mitochondria of neuronal cells [152]. The authors furnished convincing evidence of the presence of mitoSK$_{Ca}$ channels, and also demonstrated that their activation triggers the Ph-

PreC and then confers cardioprotection, as shown by metabolic and functional improvement recorded during the reperfusion on isolated and perfused guinea-pig hearts. Finally, the activation of mitoSK_{Ca} channels appears to converge, similarly to mitoBK_{Ca} and mitoK_{ATP} channel opening, on the control of intra-mitochondrial ROS generation [80, 125, 133].

Within the compounds tested as activators of mitoSK_{Ca}, DCEBIO was shown to induce cardioprotection which was antagonized by both NS8593 [153] an antagonist of SK_{Ca} isoforms and TBAP, a scavenger of peroxynitrite (SOD mimetic), thus indicating that the mitoSK_{Ca} channel opening is mediated by SOD [80] (in figure 4 is shown the SK_{Ca} activator).

D MitoKv channels

In 2005, Szabò and colleagues found the presence of voltage-gated 1.3 potassium (Kv1.3) channels on the inner membrane of mitochondria in T lymphocytes [154]. Later, Kv1.3 channels were also reported in the mitochondria of macrophages [155] and hippocampal neurons [156].

Although the Kv1.3 channels described in mitochondria of hippocampal neurons displayed a different conductance with respect to the Kv1.3 channels on lymphocytes, both channels are sensible to margatoxin. Interestingly, a hyperpolarization of mitochondrial membrane potential could be recorded upon inhibition of mitoKv1.3, indicating that in energized mitochondria these channels are normally active [154], and their inactivation is important in the initiation of apoptosis [157].

The Zoratti/Szabò group further reported that J774 macrophages express Kv1.3 and Kv1.5 channels in their mitochondria and that inhibition of both channels with specific blockers could efficiently induce apoptosis in this macrophage cell line. Thus, these results indicate that the mechanism proposed for Kv1.3 can be extended to other Kv channels in

macrophages, important players in the immune system [158-159]. Noteworthy, Leanza et al. have recently shown that the inhibition of mitoKv1.3 induces a drastic effect on tumor cell apoptosis by means a Bax/Bak-independent pathway [160-161].

At the best of our knowledge, mitoIK$_{Ca}$ channels have not been presently recognized in the heart.

E MitoTASK-3 channels

Recently, TASK-3 (KCNK9), one of the twin-pore domain potassium channels, was described in mitochondria of melanoma, keratocyte cells [162] and embryonic rat hippocampus [163], while its functional properties remain to be largely investigated [164]. In 2014, the Szewczyk group demonstrated that this channel has a protective role in human keratinocytes after UVB radiation exposure [165]. At the best of our knowledge, mitoTASK-3 channels have not been presently recognized in the heart.

F "Dirty" drugs able to activate mitoK$_{ATP}$ and mitoBK$_{Ca}$ channels.

Levosimendan, a cardiovascular drug used to treat acute and decompensated heart failure, owes its action to a calcium sensitization of the contractile proteins and to a vasorelaxing effect mediated by the opening of sarcolemmal K$_{ATP}$ channels [166-167]. Recently, levosimendan has been observed to activate also the mitoK$_{ATP}$ channels [168-169] and has been demonstrated to protect myocardium against the I/R injury [170].

Moreover, it has been speculated that the cardioprotective effects of numerous polyphenols (such as theaflavin and epigallocatechin) are due to an activation of mitoK$_{ATP}$ channels [171].

Volatile anesthetics activate some of the same pathways that lead to protection through IPreC or IPostC. Zaugg et al. verified that exposing cardiomyocytes to the volatile anesthetics, sevoflurane and isoflurane, before myocardial ischemia, the damage decreased in a dose-dependent manner [172]. These effects were antagonized by the mitoK$_{ATP}$ channel blocker 5-HD, demonstrating that they prime the mitoK$_{ATP}$ channels [173-175].

In addition, Stumpner at al. reported the involvement of the BK$_{Ca}$ channel in the desflurane-induced post-conditioning in mice subjected to a coronary artery occlusion [176].

Other molecules, not described as BK$_{Ca}$ openers, belonging to different therapeutic class, such as phosphodiesterase inhibitors (cilostazol and sildenafil), can mimic the Ph-PreC by an activation of mitoBK$_{Ca}$ [177, 146].

Very recently it has been demonstrated that naringenin, a flavanone abundant in the Citrus genus, possesses cardioprotective activity by opening the mitoBK$_{Ca}$ channels. Indeed, naringenin treatment before the onset of in vivo acute infarct or a global ischemia halved I/R injury sizes. Moreover, on isolated cardiac mitochondria, naringenin was able to produce all the typical effects of the mitoK channel openings, and the involvement of mitoBK$_{Ca}$ was suggested by the inhibitory effect of selective blockers [178]. Similarly, Gao et al., published a work on the cardioprotective effect of the isoflavone puerarin. They observed the involvement of mitoBK$_{Ca}$ channels, since the pretreatment with paxilline prevented its beneficial effect [179] (in figure 5 are shown the chemical structures of the dirty drugs).

G Innovative mito-delivered drugs

Ideal mitoK channel openers should have the ability to easily penetrate into the cell and be effectively delivered to their mitochondrial target. The lack of this pharmacokinetic property is a serious limitation for the clinical application of mitoK channel openers. Therefore a

timely and challenging issue is to obtain anti-ischemic drugs highly selective for the mitoK channels and able to be selectively delivered into mitochondria (in figure 6 are shown the chemical structures of the mito-delivered drugs). For this purpose, the most widely used synthetic strategy is the combination in a single molecule of the pharmacophore moiety and a "mitochondria-addressed" cation, such as tetraphenyl phosphonium (TPP^+), throughout an appropriate aliphatic linker. Generally, the length of the spacer influences the degree of hydrophobicity of such mitochondriotropic agents and consequently it could affect their efficacy both in *in vitro* and *in vivo* models [180-181]. These compounds are able to rapidly and extensively accumulate into mitochondria, driven by the mitochondrial membrane potential (about -180 mV)[182-183]. The main advantages in the use of lipophilic cations are their low reactivity and poor interaction with cell components. From a medicinal chemistry point of view, an example of synthetic pathway to obtain mitochondriotropic compounds is depicted in figure 7. In this instance, in order to deliver resveratrol into mitochondria, this polyphenol has been combined with TPP^+ [184] (Figure 7A). In particular, resveratrol has been submitted to an O-alkylation by the use of an appropriate bis-halide-alkyl chain and then converted to the desired TPP^+ derivative by means of two consecutive nucleophilic substitution steps: $Cl^- \rightarrow I^- \rightarrow TPP^+I^-$. A most recent paper [185] reports the synthesis and the pharmacological profile of SkBQ (10-(*p*-benzoquinonyl)decyltriphenylphosphonium bromide). As regards the synthetic pathway, also in this case the *p*-benzoquinone was alkylated with the appropriate alkylcarboxylic acid by the use of a radical substitution reaction with simultaneous decarboxylation catalyzed by silver nitrate in the presence of ammonium persulfate. The product was then converted to the desired TPP^+ derivative by means of a nucleofilic substitution ($Br^- \rightarrow TPP^+I^-$) (figure 7B). The above examples of synthetic pathways can be usefully applied to a plethora of different compounds in order to target them to mitochondrion. Many TPP^+-based antioxidants have been tested for their

selectivity to mitochondria, including MitoVitE, MitoQ and MitoL. To date, a TPP^+ derivative of MitoQ, an ubiquinol antioxidant, has received great attention, since it is endowed with cardioprotective effects in the I/R injury model [186-188]. In a recent study Mito-Tempol (a piperidine nitroxide conjugated to the TPP^+) exhibited cardioprotective effects in an in vivo rat model [189].

Besides the lipophilic cations, some peptides can also be used to direct molecules to mitochondria. Particularly the Szeto-Schiller (SS) peptides are able to selectively bind to the inner membrane, independently of potential. This feature is an advantage, because they can penetrate even into the diseased organelles with reduced potential [190]. An important difference between lipophilic cations and the SS-peptides is that the latter have an intrinsic activity on mitochondria. In particular, SS-02 and SS-31 were able to scavenge hydrogen peroxide, hydroxyl radical, and peroxynitrite in vitro [191] and the observed decrease of myocardial I/R injury in ex vivo and in vivo studies was attributed to their ability to promote mitochondrial respiration and ATP synthesis, reduce ROS generation, and inhibit mitochondrial swelling [192]

Noteworthy, new penetrating cations have been very recently developed. They are rhodamine-19, berberine and palmatine conjugated through aliphatic linkers with plastoquinone (SKQR1, SKQBerb, SKQPalm, respectively), endowed with antioxidant effects; they have been shown to penetrate into mitochondrial matrix, both in isolated mitochondria and in living cells in culture [193].

4- LOSS OF EFFICACY OF MITOCHONDRIAL POTASSIUM CHANNEL ACTIVATORS IN AGEING

Presently, the number of people over the age of 65 is growing, especially in western society. The incidence and the prevalence of cardiovascular diseases, such as coronary artery disease, hypertension and acute myocardial infarct, increases proportionally with age. At the same time the IPreC and IPostC self-defence mechanisms reduce their efficacy and the vulnerability of myocardium increases with ageing [194].Therefore an amplification of the damage is observed when aged hearts are exposed to various kinds of stress. The loss of intrinsic myocardial tolerance to ischemia in mouse myocardium begins during middle-age (12 months) and becomes more evident with ageing (18 months and 24-28 months). Similar results have been observed in rats (6 vs 24 months) [195-198]. The fundamental processes that lead to cardiac ageing and loss of myocardial resistance are not yet understood, although several mitochondrial defects have been detected, including a reduced activation of STAT3 observed in old mice [199] or an impaired connexin 43 translocation in the mitochondria of aged heart, that may contribute to the loss of efficacy of cardioprotective strategies [200] It is thought that the mitochondrial dysfunction in electron transport or oxidative phosphorylation, typical of ageing, is closely implicated in the increased susceptibility to ischemic injury. These age-related mitochondrial defects are reflected by partial depolarization of membrane potential and greater probability of MPTP opening [11, 201].

In this respect, the mitoK_{ATP} channel activation, that is generally considered an effector in the protection pathway, can lead to ineffective cardioprotection in ageing hearts [202]. Several observations suggested that cardioprotective signaling pathways, acting through mitoK_{ATP} channels, are compromised with ageing; although it is unclear whether the attenuated cardiac mitoK_{ATP} channel function is due to changes in channel density, responsiveness to stress stimuli or a defective respiratory chain [203-204]. Similar data have been also obtained when mitoBK_{Ca} channel openers were used on aged hearts. In aged rat heart mitochondria, Heinen and his colleagues have observed that the opening of mitoBK_{Ca} channels by NS1619 was

ineffective on respiratory parameters and then was unable to influence the respiratory efficacy, suggesting the possibility of a decreased sensitivity of mitoBK$_{Ca}$ channels due to ageing [205].

This result is ageing-dependent [206-207], but also gender-dependent. In fact, the infarct-limiting efficacy of diazoxide is less evident in aged male hearts, and entirely absent in aged female hearts, suggesting a more severe defective function of mitoK$_{ATP}$ channels in aged female hearts [208]. Such evidence leads to hypothesize the implication of estrogens in the ageing; in fact, these hormons may stimulate directly (acting on specific receptors localized in the organelle) and indirectly (regulating the mitochondrial transcription) the mitochondrial function [209]. Moreover, a recent paper highlighted an altered expression of six proteins in cardiac mitochondria of aged female rats [210].

Since human life expectancy increases due to effective treatment of cardiovascular and other diseases, there is a stringent need for the development of strategies designed to preserve the efficiency of cardioprotective mechanisms also in aged hearts or to slow ageing.

Although numerous drugs produce Ph-PreC in experimental models, none has been translated to clinical use, with the consequence that currently there is no FDA approved drug for reducing I/R-induced myocardial injury [211].

Clearly, the blockage or the reversal of the dysfunction of mitochondrial ion channels may improve the function of the organelle, and may also be beneficial in slowing down the process of mitochondria ageing, and the related pathologies .

5 CONCLUSIONS

Mitochondria play a unique and crucial role in the cardiac protection against I/R injury, then emerging therapeutic approaches are focused on the development of new molecules acting on specific mitochondrial sites. In this respect, the modulation of the flow of different ion species

across the mitochondrial membrane is considered as a greatly promising pharmacological approach. Since the accumulation of Ca^{++} into the matrix is a crucial step, in the dramatic chain of events leading to I/R-induced cell death, new intriguing strategies are addressed at reducing Ca^{++} accumulation by direct pharmacological inhibition of the Ca^{++} uniporter and of the MPTP [212-215]. Together with these newest pharmacological targets, mitochondrial potassium channels represent a reliable and well tried field of research. Indeed, extensive research has been performed in the last years, in order to highlight the exact role of these mitochondrial ion channels and innovative drugs have been developed in order to mimic conditioning phenomena. Nevertheless, to date, there are still many dark sides: 1) the exact molecular composition of many mitochondrial channels remains elusive; 2) the mitochondrial pathways triggered by the activation of these channels have not been clearly understood [216]; 3) possible changes in expression/activity of mitochondrial potassium channel in elderly patients should be better clarified; 4) similarity between sarcolemmal and mitochondrial potassium channels may compromise the development of selective drugs.

Many difficulties can be correlated, at least in part, with the internal localization of these channels, hardly accessible with the usual experimental techniques, and data should be carefully interpreted.

Below we have inserted an appendix in which we attempted to describe the most used experimental approaches aimed at studying mitochondrial potassium channels and their physio-pathological roles, on intact cell or in isolated organelles.

6- APPENDIX

Techniques used to characterize mitochondrial potassium channels

Approaches on isolated mitochondria are better for mechanistic studies, in order to highlight a specific mitochondrial target. The investigation is clearer, lacking the interference with cytosolic factors; however, sound protocols of isolation have to be carry out, in order to avoid damage of mitochondria [217], since they may be unavoidably aggregated or damaged by unreliable procedures.

Conversely, working on intact cells, shows the advantage to leave the organelles in their cellular environment, where the interactions with the cell are preserved, then obtaining results with a greater physiological relevance, without artifacts due to mitochondria isolation [218]. Nevertheless, the results can be more complex and difficult to understand, mostly if a mitochondrial channel has to be investigated.

However, the main obstacle in studying the mitoK channels is the availability of reliable assays. As above described, the opening of a mitoK channel is responsible for cardioprotection through a mild depolarization and consequent reduction of Ca^{++}-uptake and increase of Ca^{++}-release, thus reducing the probability of MPTP opening and apoptotic death. Therefore the possible strategies of study are two: to directly record the potassium movements, or to evaluate the mitochondrial downstream events consequent to the K^+ movements.

A Direct measurements of potassium ions movements

In order to achieve a direct measurement of the mitoK current the work on isolated mitochondria is fundamental, since undesired recording of sarcolemmal K^+ currents cannot be excluded in intact cells. An important contribution to understand the pharmacological properties of the mitoK channels comes from the use of electrophysiological techniques, where mitochondria are patched by micro-electrode and the current of the single potassium channel can be recorded. For this purpose, two methods can be used: voltage-clamp on planar

lipid bilayer and patch-clamp on mitochondria or mitoplasts [219]. The first one is based on the incorporation of purified inner or outer mitochondrial membrane fragments, containing the mitochondrial channel of interest, with liposome to form bilayer vesicles. This method allows the study of channels, which are not easily accessible for the patch-clamp technique [220].

On the other hand, the patch-clamp method on intact mitochondria has the advantage of holding the channels in their natural environment, because the isolation process for the bilayer method may be harmful for the channel. Disadvantages of the patch-clamp technique are: a) mitochondria are in close contact with membranes of the endoplasmic reticulum and it might be difficult to completely remove these membranes [221]; b) some channels might not be accessible for the glass pipette; thus, the patch clamp technique might fail to detect some important ion channel currents [222].

Even mitoplasts, prepared from the mitochondrial fraction by a swelling procedure or with French press technique [223] have been successfully employed for electrophysiological measurements of K^+ channel currents. In particular, Inoue et al., used this technique to demonstrate for the first time the activity of mitoK_{ATP} channels [76].

The major advantage of this technique is the possibility to work only with the K^+ channel of the inner membrane; in fact it is well known that most preparations of isolated mitochondria can be contaminated with plasma membranes, unless further purified by density gradient centrifugation and even then it is difficult to achieve complete removal of contaminating membranes [224].

In isolated mitochondria, potassium movements can be also directly measured using K^+-sensitive mini-electrodes or fluorescent indicators.

K^+-sensitive mini-electrodes are available, however they are not satisfactorily sensitive and reliable [225]. Instead, potassium binding fluorescence indicators are more sensitive and

allow the recording of changes of potassium ions in the mitochondrial matrix, as reported by Costa et al., 2006 [80, 133, 226].

Alternatively, fluorescent probes sensitive to thallium (Tl^+), a potassium-mimetic cation, are either commercially available or can be easily homemade. The ionic radii of Tl^+ and K^+ are similar, and thus heavy metal is widely and originally used to study sarcolemmal trans-membrane K^+ flow. Such an experimental approach has been recently applied to evaluate mitoK currents and published by Wojtovich and his colleagues, and used also by us [113, 178].

B Indirect measurements of potassium ions movements

Most studies on the mitoK channels to date use indirect approaches on isolated mitochondria or on intact cells, by measuring downstream effects the mitochondrial K^+ uptake, such as changes in respiration, matrix alkalinization, flavoprotein fluorescence, and swelling-induced light scatter [227]. Nevertheless, the reliability of these methods is limited by the ability of other mitochondrial phenomena (e.g, electron transport chain activity, volume changes, membrane potential) to interfere with the measured parameters.

As regards flavoproteins, they are able to emit autofluorescence in the oxidated, but not reduced state [228], hence this can reflect the electronic trafficking of mitochondria and can be used to monitor the metabolic state of cells. Indeed, the autofluorescence of flavoproteins in intact cardiac myocytes is used as an indirect indicator of mitochondrial K^+ channel activity. Marban and colleagues reported that diazoxide caused an oxidation of mitochondrial flavoproteins, reversed by 5-HD. Similar effects have been reported in guinea-pig myocytes using NS1619. However, this experimental approach has many limitations, and other researchers have failed to reproduce these results, speculating that non-specific actions of diazoxide or NS1619 could influence the assay [229-230]. In the case of diazoxide, the

increase in flavoprotein fluorescence can be attributed to the inhibition of succinate dehydrogenase, protonophoric uncoupling, or even transient opening of the MPTP.

The swelling assay is based on the fact that the K^+ uptake into isolated mitochondria is followed by osmotically-obliged water, leading to mild swelling, thus the ion movements lead to changes in matrix volume, that can be measured at a wavelength of 520 nm. In particular, the swelling causes a decrease of refractive index. This assay has been criticized as irreproducible by some laboratories, because the mitochondrial isolation timing appears to be a critical factor.

Another approach used to investigate the activity of mitoK channels either in cardiac myocytes and in isolated mitochondria, is based on the recording of the depolarization of the mitochondrial membrane potential. Lipophilic cationic compounds are often used for the determination of $\Delta\Psi$, in fact these compounds permeate the mitochondrial membranes and distribute in accordance with the Nernst equation; thus the accumulation in the mitochondrial membrane matrix is driven by $\Delta\Psi$.

These lipophilic ions include fluorescent, radiolabeled and unlabeled probes.

As regards the unlabeled probes, they can be determined by ion-selective electrodes, that allow the continuous monitoring of changes in mitochondrial membrane potential. The lipophilic cation most used is the tetraphenylphosphonium (TPP^+), with its selective electrode [95, 178, 231, 232].

Direct measurements of membrane potential can be also carried out with fluorescent probes, monochromatic lipophilic cations, such as dihexyloxacarbocyanine (DiOC6(3)) and rhodamines, or JC-1 (5,5',6,6'-tetrachloro1,1',3,3'-tetramethylbenzimidazolylcarbocyanine iodide), whose emission spectrum shifts from red, when it forms into mitochondria J-aggregations, to a green emission reflecting the monomeric form of JC-1, due to the exit from mitochondria after membrane depolarization. The ratio of this green/red fluorescence is

independent of mitochondrial shape, density, or size, but is influenced only by the membrane potential. Fluorescence probes are largely used on intact cells, because if it has at its disposal isolated mitochondria, easier techniques are available. The choice of the probe is related largely to their equilibration characteristics and also to their toxicity versus the electron transport chain.

A net K^+ uptake results necessarily in matrix alkalinization, because overall transport must be electroneutral, then the K^+ influx will be balanced exactly by the electrogenic H^+ efflux driven by the electron transport. In other words, the K^+ for H^+ exchange would increase matrix pH by an amount determined by the buffering power of the matrix. Costa et al. [233] reported that the increase in matrix K^+, mediated either by mitoK_{ATP} or by potassium ionophor valinomycin, was accompanied by matrix alkalinization. This effect accounts for the role of mitoK_{ATP} in cardioprotection and can be measured by means of pH-sensitive fluorescence probes (such as 2',7'-biscarboxyethyl-5(6)-carboxyfluorescein) [133, 234].

Finally, the mitoK opening influences calcium movements across the mitochondrial membrane. Changes of Ca^{++} movements can be recorded by means of specific calcium-sensitive mini-electrodes. Using this approach, Holmuhamedov and colleagues observed that diazoxide and pinacidil reduced mitochondrial Ca^{++}uptake, in agreement with the evidence that the Ca^{++}-accumulation through the Ca^{++} uniporter is driven by the difference in membrane potential across the inner mitochondrial membrane. Conversely, this approach allowed to demonstrate also that mitoK channel openers can promote Ca^{++} discharge from Ca^{++} pre-loaded mitochondria, phenomenon at the basis of cardiac protection [226, 235].

The primary mitochondrial Ca^{++} release pathway includes the MPTP, which can be activated by mitochondrial membrane depolarization and swelling [236-238]. Activation of mitoK_{ATP} channels has been associated with mitochondrial membrane depolarization and can prime

swelling [107, 231], transiently activating the MPTP, and thus causing Ca^{++} discharge from preloaded mitochondria.

7- ACKNOWNLEDGEMENT

The authors wish thank Prof. Robert Learmonth for the helpful assistance in the English restyling of the manuscript.

8- REFERENCES

[1] Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosamond W, Sacco R, Sorlie P, Roger VL, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2010 update: a report from the American Heart Association. Circulation 2010;121:e46-215.

[2] Vanlangenkker N, Vanden BT, Krysko DV, Festjens N, Vandenabeele P. Molecular mechanisms and pathophysiologicy of necrotic cell death. Curr Mol Med 2008;8:207-220.

[3] Vanlangenakker N, Vanden BT, Vandenabeele P. Many stimuli pull the necrotic trigger, an overview. Cell Death Differ 2012;19:75-86.

[4] Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. N Engl J Med 2007;357:1121-1135.

[5] Halestrap AP, Kerr PM, Javadov S, Woodfield KY. Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart. Biochim Biophys Acta 1998; 1366:79-94.

[6] Halestrap AP, Clarke SJ, Javadov S. Mitochondrial permeability transition pore opening during myocardial reperfusion- A target for cardioproteciton. Cardiovasc Res 2004;61:372-385.

[7] Solaini G, Harris DA. Biochemical dysfunction in heart mitochondria exposed to ischemia and reperfusion. Biochem J 2005;390:377-394.

[8] Kevin LG, Camara AKS, Riess ML, Novalija E, Stowe DF. Ischemic preconditioning alters real-time measure of O2-radicals in intact hearts with ischemia and reperfusion. Am J Physiol 2003;284:H566-574.

[9] Kalogeris T, Baines CP, Krenz , Korthius RJ. Cell Biology of ischemia/reperfusion injury. Intl Rev Cell MolBiol 2012;298:229-317.

[10] Chen YR, Zweier JL. Cardiac mitochondria and reactive oxygen species generation. Circ Res 2014;114:524-37.

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

[12] Pacher P, Nivorozhkin A, Szabo C. Therapeutic effects of wanthine oxidase inhibitors: renaissance half a century after the discovery of alluopurinol. Pharmacol Rev 2006;58:87-114.

[13] Hunter DR, Haworth RA, Southard JH. Relationship between configuration, function, and permeability in calcium-treated mitochondria. J Biol Chem 1976;251:5069-5077.

[14] Crompton M, Andreeva L. On the involvement of a mitochondrial pore in reperfusion injury. Basic Res Cardiol 1993;88:513-23.

[15] Griffithsn EJ, Halestrap AP. Protection by Cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts. Cardiovasc Res 1993;25:1461-9.

[16] Heusch G, Boegler K, Schulz R. Inhibition of mitochondrial permeability transition pore opening: the holy grail of cardioprotection. Basic Res Cardiol 2010; 105:151-4.

[17] Halestrap AP. Calcium-dependent opening of a non-specific pore in the mitochondrial inner membrane is inhibited at pH values below 7-Implications for the protective effect of low pH against chemical and hypoxic cell damage. Biochem J 1991;278:715-719.

[18] Skyschally A, Schulz R, Heusch G. Pathophysiology of myocardial infarction. Herz 2008; 33:88-100.

[19] Kim JS, Jin YG, Lemasters JJ. Reactive oxygen species, but not Ca++ overloading trigger pH- and mitochondrial permeability transition-dependent death of adult rat myocytes after ischemia-reperfusion. Am J Physiol 2006;290:H2024-2034.

[20] Kimura Y, Iyengar R, Subramanian R, Cordis GA, Das DK. Preconditioning of the heart by repeated stunning: attenuation of post-ischemic dysfunction. Basic Res Cardiol 1992;87:128-138.

[21] Scorrano L, Petronilli V, Bernardi P. On the voltage dependence of the mitochondrial permeability transition pore. A critical appraisal. J Biol Chem 1997;272:12295-12299.

[22] Ryu SY, Peixoto PM, Teijido O, Dejean LM, Kinnally KW. Role of mitochondrial ion channels in cell death. Biofactors 2010;36:255-63.

[23] Zoratti M, Szabò I, De Marchi U. Mitochondrial permeability transitions: how many doors to the house? Biochim Biophys Acta. 2005;1706:40-52.

[24] Bernardi P. Mitochondrial transport of cations: channels, exchangers and permeability transition. Physiol Rev 1999;79:1127-1155.

[25] Doran E, Halestrap AP. Cytochrome c release from isolated rat liver mitochondria can occur independently of outer-membrane rupture: possible role of contact sites. Biochem J 2000;348:343-350.

[26] Martinou JC, Green DR. Breaking the mitochondrial barrier. Nat Rev Mol Cell Biol 2001;2:63-67.

[27] Javadov SA, Clarke S., Das M, Griffiths EJ, Lim KHH, Halestrap AP. Ischemic preconditioning inhibits opening of mitochondrial permeability transition pores in the reperfused rat heart. J Physiol 2003;549:513-524.

[28] Griffiths EJ, Halestrap AP. Protection by cyclosporine A of ischemia-reperfusion-induced damage in isolated rat hearts. J Mol Cell Cardiol 1993;25:1461-1469.

[29] Skyschally A, Schulz R, Heusch G. Cyclosporine A at reperfusion reduces infarct size in pigs. Cardiovascular Drugs Therap. 2010; 24:85-7.

[30] Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mawton N, Elbelghiti R, Cung T, Bonnefoy E, Angoulvant D, Macia C, Raczka F, Sportouch C, Gahide G, Finet G, Andre-Fouet X, revel D, Kirkorian G, Monassier JP, Derumeaux G, Ovize M. effect of cyclosporine on reperfusion injury in acute myocardial infarction. New England Journal of Medicine 2008; 359:473-81.

[31] Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986,74:1124-1136.

[32] Edwards RJ, Saurin AT, Rakhit RD, Marber MS. Therapeutic potential of ischemic preconditioning. Br J Clin Pharmacol 2000;50:87-97.

[33] Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. Physiol Rev 2003;83:1113-1151.

[34] Kloner RA, Rezkalla SH. Preconditioning, postconditioning and their application to clinical cardiology. Cardiovasc Res 2006;70:297-307.

[35] Heusch G. cardioprotection: chances and challenges of its translation to the clinic. Lancet 2013; 381:166-175.

[36] Millar CG, Baxter GF, Thiemermann C. Protection of the myocardium by ischemic preconditioning: mechanisms and therapeutic implications. Pharmacol Ther 1996;69:143-151.

[37] Schwartz LM, Jennings RB, Reimer KA. Premedication with the opioid analgesic butorphanol raises the threshold for ischemic preconditioning in dogs. Basic Res Cardiol 1997;92:106-114.

[38] Bianes CP, Song CX, Zheng YT, Wang GW, Zhang J, Wang OL, Guo Y, Bolli R, Cardwell EM, Ping P. Protein kinase C epsilon interacts with and inhibits the permeability transition pore in cardiac mitochondria. Circ Res 2003; 92:873-880.

[39] Minamino T. Cardioprotection from ischemia-reperfusion injury. Basic Transl Res. Circ J 2012;76:1074-1082.

[40] Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: Comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol 2003,285:H579-H588.

[41] Skyschally A, van Caster P, Iliodromtis EK, Schulz R, Kremastinos DT, Heusch G. Ischemic postconditioning: experimental models and protocol algorithms. Basic Res Cardiol 2009;104:469-483.

[42] Staat P, Rioufol G, Piot C Cottin Y, Cung TT L'huillir I,Aupetit JF, Bonnefoy E, Finet G, André-Fouët X, Ovize M. Postconditioning the human heart. Circ 2005;112:2143-2148.

[43] Sun HY, Wang NP, Kerendi F, Halkos M, Kin H, Guyton RA, Vinte-Johansen J, Zhao ZQ. Hypoxic postconditioning reduces cardiomyocyte loss by inhibiting ROS generation and intracellular Ca++ overload. Am J Physiol Heart Circ Physiol 2005;288:H1900-H1908.

[44] Schmidt MR, Smerup M, Konstantinov IE, Shimizu M, Li J, Cheung M, White PA, Kristiansen SB, Sorensen K, Dzavik V, Redington AN, Kharbanda RK. Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a K_{ATP}-dependent mechanism: first demonstration of remote ischemic perconditioning. Am J Physiol Heart Circ Physiol 2007;292:H1883-H1890.

[45] Li CM, Zhang XH, Ma XJ, Luo M. Limb ischemic postconditioning protects myocardium from ischemia-reperfusion injury. Scand Cardiovasc J 2006;40:312-317.

[46] Kerendi F, Kin H, Halkos ME, Jiang R, Zattas AJ, Zhao ZQ, Guyton RA, Vinten-Johansen J. Remote postconditioning. Brief renal ischemia and reperfusion applied before coronary artery reperfusion reduces myocardial infarct size via endogenous activation of adenosine receptors. Basic Res Cardiol 2005;100:404-412.

[47] Zhao ZQ. Postconditioning in reperfusion injury: a status report. Cardiovasc Drugs Ther 2010;24:265-279.

[48] Thielmann M, Kottenberg E, Kleinbongard P, Wendt D, Gedik N, Pasa S, Price V, Tsagakis K, Neuhaused M, Peters J, Jakob H, Heusch G. Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single randomized, double-blind, controlled trial. Lancet 2013; 382:597-604.

[49] Vinten-Johansen, Shi W. The science and clinical translation of remote postconditioning. J Cardiovasc Med 2013;14:206-213.

[50] Yang XC, Liu Y, Wang LF, Cui L, Wang T, Ge YG, Wang HS, Li WM, Xu L, Ni ZH, Liu SH, Zhang L, Jia HM, Vinten-Johansen J, Zhao ZQ. Reduction in myocardial infarct size by postconditioning in patients after percutaneous coronary intervention. J Invasive Cardiol 2007;19:424-430.

[51] Liu G, Thornton J, Van Winkle D, Stanley A, Olsson R, Downey J. Protection again infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. Circ 1991;84:350-356.

[52] Yao Z, Gross GJ. Acetylcholine mimics ischemic preconditioning via a glybenclamide-sensitive mechanism in dogs. Am J Physiol Heart Circ Physiol 1993;264:H2221-H2225.

[53] Schulz R, Post H, Vahlhaus C, Heusch G. Ischemic preconditioning in pigs: a graded phenomenon: its relation to adenosine and bradykinin. Circ 1998;98:1022-1029.

[54] Zhang WM, Jin WQ, Wong TM. Multiplicity of kappa opioid receptor binding in the rat cardiac sarcolemma. J Mol Cell Cardiol 1996;28:1547-1554.

[55] Weil J, Eschenhagen T, Fleige G, Mittmann C, Orthey E, Scholz H. Localization of preproenkephalin mRNA in rat heart: selective gene expression in left ventricular myocardium. Am J Physiol 1998;275:H378-H384.

[56] Schulz R, Rose J, Post H, Heusch G. Regional short-term myocardial hibernation in swine does not involve endogenous adenosine or K_{ATP} channels. Am J Physiol 1995;268:H2294-H2301.

[57] Schwarz ER, Whyte WS, Kloner RA. Ischemic preconditioning. Curr Opin Cardiol 1997;12:475-481.

[58] Fryer RM, Schultz JE, Hsu AK, Gross GJ. Importance of PKC and tyrosine kinase in single or multiple cycles of preconditioning in rat hearts. Am J Physiol 1999;276:H1229-H1235.

[59] Wang GY, Wu S, Pei JM, Yu XC, Wong TM. Kappa- but not delta-opioid receptors mediate effects of ischemic preconditioning on both infarct and arrhythmia in rats. Am J Physiol Heart Circ Physiol 2001;280:H384-H391.

[60] Lochner A, Marais E, Genade S, Moolman JA. Nitric oxide: a trigger for classic preconditioning? Am J Physiol Heart Circ Physiol 2000;279:H2752-H2765.

[61] Jones SP, Bolli R. The ubiquitous role of nitric oxide in cardioprotection. J Mol Cell Cardiol 2006;40:16-23.

[62] Bolli R. Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. J Mol Cell Cardiol 2001;33:1897-1918.

[63] Heusch G, Boegler K, Schulz R. Cardioprotection, Nitric oxide, protein kinases, and mitochondria. Circulation 2008;118:1915-19.

[64] Zhang Z, Huang H, Liu P, Tang C, Wang J. Hydrogen sulfide contributes to cardioprotection during ischemia-reperfusion injury by opening K_{ATP} channels. Can J Physiol Pharmacol 2007;85:1248-1253.

[65] Murriel CL, Mochly-Rosen D. Opposing roles of delta and epsilon PKC in cardiac ischemia and reperfusion: targeting the apoptotic machinery. Arch Biochem Biophys 2003;420:246–254.

[66] Chen L, Hahn H, Wu G, Chen CH, Liron T, Schechtman D, Cavallaro G, Banci L, Guo Y, Bolli R, Dorn GW, Mochly-Rosen D. Opposing cardioprotective actions and parallel hypertrophic effects of delta PKC and epsilon PKC. Proc Natl Acad Sci U S A 2001;98:11114–11119.

[67] Chen CH, Budas GR, Churchill EN, Disatnik MH, Hurley TD, Mochly-Rosen D. Activation of aldehyde dehydrogenase-2 reduces ischemic damage to the heart. Science 2008;321:1493–1495.

[68] Ping P, Zhang J, Qui Y, Tang XL, Manchikalapuldi S, Cao X, Bolli R. Ischemic preconditioning induces selective translocation of protein kinase C isoforms and in the heart of conscious rabbits without subcellular redistribution of total protein kinase C activity. Circ Res 1997;81:404-414.

[69] Baines CP, Zhang J, Wang GW, Zheng YT, Xiu JX, Cardwell EM, Bolli R, Ping P. Mitochondrial PKC epsilon and MAPK form signaling modules in the murine heart. Enhanced mitochondrial PKC-epsilon MAPK interactions and differential MAPK activation in PKC epsilon-induced cardioprotection. Circ Res 2002;90:390-397.

[70] Churchill EN, Murriel CL, Chen CH, Mochly-Rosen D, Szweda LI. Reperfusion-induced translocation of deltaPKC to cardiac mitochondria prevents pyruvate dehydrogenase reactivation. Circ Res 2005;97:78–85.

[71] Ikeno F, Inagaki K, Rezaee M, Mochly-Rosen D. Impaired perfusion after myocardial infarctionis due to reperfusion-induced deltaPKC-mediated myocardial damage. Cardiovasc Res 2007;73:699–709.

[72] Vahlhaus C, Schulz R, Post H, Onallah R, Heusch G. No prevention of ischemic preconditioning by the protein kinase C inhibitor staurosporine in swine. Circ Res. 1996;79:407-14.

[73] Mochly-Rosen D, Grimes K. Myocardial salvage in acute myocardial infarction: challenges in clinical translation. Journal of Molecular and Cellular Cardiology 2011;51:451-3.

[74] Boengler K, Hilfiker-Kleiner D, Heusch G, Schulz R. Inhibition of permeability transition pore opening by mitochondrial STAT3 and its role in myocardial ischemia/reperfusion. Basic Research Cardiol. 2010;105:771-85.

[75] Heusch G, Musiolik J, Gedik N, Skyschally A. Mitochondrial STAT3 activation and cardioprotection by ischemic preconditioning in pigs with regiona myocardial ischemia/reperfusion. Circulation Research 2011;109:1302-8.

[76] Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K+ channel in the mitochondrial inner membrane. Nature 1991;352:244-247.

[77] Siemen D, Loupatatzis C, Borecky J, Gulbins E, Lang F. Ca2+-activated K channel of the BK_{Ca}-type in the inner mitochondrial membrane of a human glioma cell line. Biochem Biophys Res Commun 1999;257:549-554.

[78] De Marchi U, Sassi N, Fioretti B, Catacuzzeno L, Cereghetti GM, Szabò I, Zoratti M. Intermediate conductance Ca^{2+}-activated potassium channel (KCa3.1) in the inner mitochondrial membrane of human colon cancer cells. Cell Calcium 2009;45:509-516.

[79] Xu WH, Liu YG, Wang S, McDonald T, VanEyk JE, Sidor A, O'Rourke B. Cytoprotective role of Ca^{++}-activated K^+ channels in cardiac inner mitochondrial membrane. Science 2002;298:1029-1033.

[80] Stowe DF, Gadicherla AK, Zhou Y, Aldakkak M, Cheng Q, Kwok WM, Jiang MT, Heisner JS, Yang MY, Camara AK. Protection against injury by small Ca^{++}-sensitive K^+

channels identified in guinea-pig cardiac inner mitochondrial membrane. Biochim Biophys Acta 2013;828:427-442.

[81] Boengler K, Dodoni G, Rodriguez-Sinovas A, Cabestrero A, Ruiz-Meana M, Gres P, Konietzka I, Lopez-Iglesias C, Garcia-Dorado D, Di Lisa F, Heusch G, Schulz R. Connexin 43 in cardiomyocyte mitochondria and its increase by ischemic preconditioning. Cardioascular Research 2005; 67: 234-44.

[82] Miro-Casas E, Ruiz-Meana M, Agullo E, Stahlofen S, Rodriguez-Sinovas A, Cabestrero A, Jorge I, Torre I, Vazquez J, Boenlger K, Schulz R, Heusch G, Garcia-Dorado D. Connexin 43 in cardiomyocyte mitochondria contributes to mitochondrial potassium uptake. Cardiovascular Research 2009;83:747-56.

[83] Boengler K, Ungefug E, Heusch G, Leybaert L, Schulz R. Connexin 43 impacts on mitochondrial potassium uptake. Frontiers in Pharmacology 2013; 73:1-5.

[84] Heinzel F, Luo Y, Li X, Boengler K, Buechert A, Garcia-Dorado D, Di Lisa F, Schulz R, Heusch G. Impairement of diazoxide-induced formation of reactive oxygen species and loss of cardioprotection in connexin 43 deficient mice. Circulation Research 2005; 97:583-6.

[85] Garlid KD. Opening mitochondrial K(ATP) in the heart-what happens, and what does not happen. Basic Res Cardiol 2000;95:275-279.

[86] Hausenloy DJ, Tsang A, Mocanu MM, Yellon DM. Ischemic preconditioning protects by activating prosurvival kinases at reperfusion. Am J Physiol Heart Circ Physiol 2005;288:H971-H976.

[87] Vanden Hoek TL, Becker LB, Shao Z, Li C, Schumacker PT. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. J Biol Chem 1998;273:18092-18098.

[88] Yao Z, Gross GJ. Effects of the K_{ATP} channel opener bimakalim on coronary blood flow, monophasic action potential duration, and infarct size in dogs. Circ 1994;89:1769-1775.

[89] Grover GJ, D'Alonzo AJ, Parham CS, Darbenzio RB. Cardioprotection with the K_{ATP} opener cromakalim is not correlated with ischemic myocardial action potential duration. J Cardiovasc Pharmacol 1995;26:145-152.

[90] Grover GJ, McCullough JR, D'Alonzo AJ, Sargent CA, Atwal KS. Cardioprotective profile of the cardiac-selective ATP-sensitive potassium channel opener BMS-180448. J Cardiovasc Pharmacol 1995;25:40-50.

[91] Grover GJ, D'Alonzo AJ, Dzwonczyk S, Parham CS, Darbenzio RB. Preconditioning is not abolished by the delayed rectifier K+ blocker dofetilide. Am J Physiol 1996;271:H1207-H1214.

[92] Garlid KD, Paucek P, Yarov-Yarovoy V, Murray HN, Darbenzio RB, D'Alonzo AJ, Lodge NJ, Smith MA, Grover GJ. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K+ channels. Possible mechanism of cardioprotection. Circ Res 1997;81:1072-1082.

[93] Garlid KD, Halestrap AP. The mitochondrial K_{ATP} channel. Fact or finction? J Mol Cell Cardiol 2012;52:578-583.

[94] Jin C, Wu J, Watanabe M, Okada T, Iesaki T. Mitochondrial K+ channels are involved in ischemic postconditioning in rat hearts. J Physiol Sci 2012;62:325-332.

[95] Calderone V, Testai L, Martelli A, Rapposelli S, Digiacomo M, Balsamo A, Breschi MC. Anti-ischemic properties of a new spiro-cyclic benzopyran activator of the cardiac mito-K_{ATP} channel. Biochem Pharmacol 2010;79:39-47.

[96] Hsu CY, Fang SY, Chen YZ, Roan JN, Chang SW, Huang CC, Liu YC, Lam CF, Tsai YC. Cardiovascular protection of activating K_{ATP} channel during ischemia-reperfusion acidosis. Shock 2012;37:653-658.

[97] Nuñez IP, Fantinelli J, Arbeláez LF, Mosca SM. Mitochondrial K_{ATP} channels participate in the limitation of infarct size by cariporide. Naunyn Schmiedebergs Arch Pharmacol 2011;383:563-571.

[98] Du Toit EF, Genis A, Opie LH, Pollesello P, Lochner A. A role for the RISK pathway and K_{ATP} channels in pre- and post-conditioning induced by levosimendan in the isolated guinea pig heart. Br J Pharmacol 2008;154:41–50.

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

[100] Lacza Z, Snipes JA, Miller AW, Szabó C, Grover G, Busija DW. Heart mitochondria contain functional ATP-dependent K+ channels. J Mol Cell Cardiol 2003;35:1339-1347.

[101] Mitchell MB, Meng X, Ao L, Brown JM, Harken AH, Banerjee A. preconditioning of isolated rat heart is mediated by protein kinase C. Circ Res 1995;76:73-81.

[102] Szewezyk A, Skaòska M, Glab B, Kulawiak B, Malinska D, Koskela-Piotrowska I, Kunz WS. Mitochondrial potassium channels: from pharmacology to function. Biochim Biophys Acta 2006;1757:715-720.

[103] Ye B, Kroboth SL, Pu JL, Sims JJ, Aggarwal NT, McNally EM, Makielski JC, Shi NQ. Molecular identification and functional characterization of a mitochondrial sulfonylurea receptor 2 splice variant generated by intraexonic splicing. Circ Res 2009;105:1083-1093.

[104] Ardehali H, O'Rourke B. Mitochondrial K_{ATP} channels in cell survival and death. J Mol Cell Cardiol 2005; 39:7-16.

[105] Foster DB, Ho AS, Rucker J, Garlid AO, Chen L, Sidor A, Garlid KD, O'Rourke B. Mitochondrial ROMK channel is a molecular component of mitoK(ATP). Circ Res 2012;111:446-454.

[106] Mannhold R. K_{ATP} channel openers: structure-activity relationships and therapeutic potential. Med Res Rev 2004;24:213-266.

[107] Garlid KD, Paucek P, Yarov-Yarovoy V, Sun X, Schindler PA. The mitochondrial K_{ATP} channel as a receptor for potassium channel openers. J Biol Chem 1996;271:8796-8799.

[108] D'hahan N, Moreau C, Prost AL, Jacquet H, Alekseev AE, Terzic A, Vivaudou M. Pharmacological plasticity of cardiac ATP-sensitive potassium channels toward diazoxide revealed by ADP. Proc Natl Acad Sci U S A 1999;96:12162-12167.

[109] Pain T, Yang X, Critz S, Yue Y, Nakano A, Liu GS, Heusch G, Cohen MV, Downey JM. Opening of mitochondrial KATP channels tiggers the preconditioned state by generating free radicals. Circulation Research 2000;87:460-6.

[110] Rodrigo GC, Davies NW, Standen NB. Diazoxide causes early activation of cardiac sarcolemmal K_{ATP} channels during metabolic inhibition by an indirect mechanism. Cardiovasc Res 2004;61:570-579.

[111] Holmuhamedov EL, Jahangir A, Oberlin A, Komarov A, Colombini M, Terzic A. Potassium channel openers are uncoupling protonophores: implication in cardioprotection. FEBS Lett 2004;568:167-170.

[112] Lim KH, Javadov SA, Das M, Clarke SJ, Suleiman MS, Halestrap AP. The effects of ischaemic preconditioning, diazoxide and 5-hydroxydecanoate on rat heart mitochondrial volume and respiration. J Physiol 2002;545:961-974.

[113] Wojtovich AP, Nehrke KW, Brookes PS. The mitochondrial complex II and ATP-sensitive potassium channel interaction: quantitation of the channel in heart mitochondria. Acta Biochim Pol 2010;57:431-434.

[114] Wojtovich AP, Brookes PS. The complex II inhibitor atpenin A5 protects against cardiac ischemia-reperfusion injury via activation of mitochondrial K_{ATP} channels. Basic Res Cardiol 2009;104:121-129.

[115] Antoine MH, Berkenboom G, Fang ZY, Fontaine J, Herchuelz A, Lebrun P. Mechanical and ionic response of rat aorta to diazoxide. Eur J Pharmacol 1992;216:299-306.

[116] Pirotte B, de Tullio P, Lebrun P, Antoine MH, Fontaine J, Masereel B, Schynts M, Dupont L, Herchuelz A, Delarge J. 3-(Alkylamino)-4H-pyrido[4,3-e]-1,2,4-thiadiazine 1,1-dioxides as powerful inhibitors of insulin release from rat pancreatic B-cells: a new class of potassium channel openers? J Med Chem 1993;36:3211-3213.

[117] Atwal KS, Grover GJ, Ahmed SZ, Ferrara FN, Harper TW, Kim KS, Sleph PG, Dzwonczyk S, Russell AD, Moreland S, McCullough JR, Russell AD, Normandin DE. Cardioselective anti-ischemic ATP-sensitive potassium channel openers. J Med Chem 1993;36:3971-3974.

[118] Grover GJ, Atwal KS. Pharmacologic profile of the selective mitochondrial-K(ATP) opener BMS-191095 for treatment of acute myocardial ischemia. Cardiovasc Drug Rev 2002;20:121-136.

[119] Lee S, Yi KY, Kim SK, Suh J, Kim NJ, Yoo SE, Lee BH, Seo HW, Kim SO, Lim H. Cardioselective anti-ischemic ATP-sensitive potassium channel (K_{ATP}) openers: benzopyranyl indoline and indole analogues. Eur J Med Chem 2003;38:459-471.

[120] Jung YS, Jung YS, Kim MY, Kim MH, Lee S, Yi KY, Yoo SE, Lee SH, Baik EJ, Moon CH, Cho JP. KR-31466, a benzopyranylindol analog, attenuates hypoxic injury through mitochondrial K(ATP) channel and protein kinase C activation in heart-derived H9c2 cells. J Pharmacol Sci 2003;92:13-18.

[121] Breschi MC, Calderone V, Digiacomo M, Manganaro M, Martelli A, Minutolo F, Rapposelli S, Testai L, Tonelli F, Balsamo A. Spirocyclic benzopyran-based derivatives as new anti-ischemic activators of mitochondrial ATP-sensitive potassium channel. J Med Chem 2008;51:6945-6954.

[122] Breschi MC, Calderone V, Martelli A, Minutolo F, Rapposelli S, Testai L, Tonelli F, Balsamo A. New benzopyran-based openers of the mitochondrial ATP-sensitive potassium channel with potent anti-ischemic properties. J Med Chem 2006;49:7600-7602.

[123] Rapposelli S, Breschi MC, Calderone V, Digiacomo M, Martelli A, Testai L, Vanni M, Balsamo A. Synthesis and biological evaluation of 5-membered spiro heterocycle-benzopyran derivatives against myocardial ischemia. Eur J Med Chem 2011;46:966-973.

[124] Rapposelli S, Calderone V, Cirilli R, Digiacomo M, Faggi C, La Torre F, Manganaro M, Martelli A, Testai L. Enantioselectivity in cardioprotection induced by (S)- (-)-2,2-dimethyl-N-(4'-acetamido-benzyl)-4-spiromorpholone-chromane. J Med Chem 2009;52:1477-1480.

[125] Stowe DF, Aldakkak M, Camara AK, Riess ML, Heinen A, Varadarajan SG, Jiang MT. Cardiac mitochondrial preconditioning by Big Ca^{2+}-sensitive K^+ channel opening requires superoxide radical generation. Am J Physiol Heart Circ Physiol 2006;290:H434-40.

[126] Wang X, Yin C, Xi L, Kukreja RC. Opening of Ca++-activated K+ channels triggers early and delayed preconditioning against I/R injury independent of NOS in mice. Am J Physiol Heart Circ Physiol 2004;287:H2070-H2077.

[127] Cao CM, Xia Q, Gao Q, Chen M, Wong TM. Calcium-activated potassium channel triggers cardioprotection of ischemic preconditioning. J Pharmacol Exp Ther 2005;312:644-650.

[128] Liu Y, Kalogeris T, Wang M, Zuidema MY, Wang Q, Dai H, Davis MJ, Hill MA, Korthius RJ. Hydrogen sulfide preconditioning or neutrophil depletion attenuates ischemia-reperfusion-induced mitochondrial dysfunction in rat small intestine. Am. J. Physiol. Gastrointest. Liver hysiol. 2012;302:G44-54.

[129] Zuidema MY, Yang Y, Wang M, Kalogeris T, Liu Y, Meininger CJ, Hill MA, Davis MJ, Korthius RJ. Antecedent hydrogen sulfide elicits an anti-inflammatory phenotype in postischemic murine small intestine: role of BK channels. Am. J. Physiol. Heart Circ. Physiol. 2010;299:H1554-67.

[130] Wang Q, Kalogeris T, Wang M, Jones AW, Korthuis RJ. Antecedent ethanol attenuates cerebral ischemia/reperfusion-induced leukocyte-endothelial adhesive interactions and delayed neuronal death: role of large conductance, Ca^{++}-activated K^{+} channels. Microcirulation 2010;17:427-38.

[131] Bednarczyk P, Barker GD, Halestrap AP. Determination of the rate of K(+) movement through potassium channels in isolated rat heart and liver mitochondria. Biochim Biophys Acta 2008;1777:540-548.

[132] Bentzen BH, Osadchii O, Jespersen T, Hansen RS, Olesen SP, Grunnet M. Activation of big conductance Ca(2+)-activated K (+) channels (BK) protects the heart against ischemia-reperfusion injury. Pflugers Arch 2009;457:979-988.

[133] Aldakkak M, Stowe DF, Cheng Q, Kwok WM, Camara AK. Mitochondrial matrix K^{+} flux independent of large-conductance Ca^{2+}-activated K^{+} channel opening. Am J Physiol Cell Physiol 2010;298:C530-C541.

[134] Park WS, Kang SH, Son YK, Kim N, Ko JH, Kim HK, Han J. The mitochondrial Ca++-activated K+ channel activator, NS 1619 inhibits L-type Ca++ channels in rat ventricular myocytes. Biochem Biophys Res Comm 2007;326:31-36.

[135] Saleh SN, Angermann JE, Sones WR, Leblanc N, Greenwood IA. Stimulation of Ca++-gated Cl- currents by the calcium dependent K+ channel modulators NS 1619 [1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one] and isopimaric acid. J Pharmacol Exp Ther 2007;321:1075-1084.

[136] Edwards G, Niederste-Hollenberg A, Schneider J, Noack T, Weston AH. Ion channel modulation by NS1619, the putative BKCa channel opener, in vascular smooth muscle. Br J Pharmacol 1994;113:1538-1547.

[137] Olesen SP, Munch E, Moldt P, Drejer J. Selective activation of Ca++-dependent K+ channels by novel benzimidazolone. Eur J Pharmacol 1994;251:53-59.

[138] Bantzen BH, Nardi A, Calloe K, Madsen LS, Olesen SP, Grunnet M. The small molecule NS11021 is a potent and specific activator of Ca++-activated big-conductance K+ channels. Mol Pharmacol 2007;72:1033-1044.

[139] Debska G, Kicinska A, Skalska J, Szewczyk A, May R, Elger CE, Kunz WS. Opening of potassium channels modulates mitochondrial function in rat skeletal muscle. Biochim Biophys Acta 2002;1556:97-105.

[140] Aon MA, Cortassa S, Wei AC, Grunnet M, O'Rourke B. Energetic performance is improved by specific activation of K+ fluxes through K(Ca) channels in heart mitochondria. Biochim Biophys Acta 2010;1797:71-80.

[141] MacPherson LJ, Bayburt EK, Capparelli MP, Carroll BJ, Goldstein R, Justice MR, Zhu L, Hu S, Melton RA, Fryer L, Goldberg RL, Doughty JR, Spirito S, Blancuzzi V, Wilson D, O'Byrne EM, Ganu V, Parker DT. Discovery of CGS 27023A, a non-peptidic, potent, and orally active stromelysin inhibitor that blocks cartilage degradation in rabbits. J Med Chem 1997;40:2525-2532.

[142] Kulawiak B, Kudin AP, Szewczyk A, Kunz WS. BK channel openers inhibit ROS production of isolated rat brain mitochondria. Exp Neurol 2008;212:543-547.

[143] Wrzosek A, Łukasiak A, Gwóźdź P, Malińska D, Kozlovski VI, Szewczyk A, Chlopicki S, Dołowy K. Large-conductance K+ channel opener CGS7184 as a regulator of endothelial cell function. Eur J Pharmacol 2009;602:105-111.

[144] Wrzosek A, Tomaskova Z, Ondrias K, Lukasiak A, Szewczyk A. The potassium channel opener CGS7184 activates Ca^{2+} release from the endoplasmic reticulum. Eur J Pharmacol 2012;690:60-67.

[145] Ohya S, Kuwata Y, Sakamopto K, Muraki K, Imaizumi Y. Cardioprotective effects of estradiol include the activation of large-conductance Ca++-activated K+ channels in cardiac mitochondria. Am J Physiol Heart Circ Physiol 2005;289:H1635-H1642.

[146] Wang X, Fisher PW, Xi L, Kukreja RC. Essential role of mitochondrial Ca++-activated and ATP-sensitive K+ channels in sildenafil-induced late cardioprotection. J Mol Cell Cardiol 2008;44:105-113.

[147] Meera P, Wallner M, Jiang Z, Toro L. A calcium switch for the functional coupling between alpha (hslo) and beta subunits (K,V,Ca beta) of maxi K channels. FEBS Lett. 1996;382:84-88.

[148] Singh H, Lu R, Bopassa JC, Meredith AL, Stefani E, Toro L. MitoBK(Ca) is encoded by the Kcnma1 gene, and a splicing sequence defines its mitochondrial location. Proc Natl Acad Sci USA 2013;110:10836-10841.

[149] Sato T, Saito T, Saegusa N, Nakaya H. Mitochondrial Ca2+-activated K+ channels in cardiac myocytes: a mechanism of the cardioprotective effect and modulation by protein kinase A. Circ 2005;111:198-203.

[150] De Marchi U, Sassi N, Fioretti B, Catacuzzeno L, Cereghetti GM, Szabò I, Zoratti M. Intermediate conductance Ca2+-activated potassium channel (KCa3.1) in the innermitochondrial membrane of human colon cancer cells. Cell Calcium 2009;45:509-16.

[151] Sassi N, De Marchi U, Fioretti B, Biasutto L, Gulbins E, Francolini F, Szabò I, Zoratti M. An investigation of the occurrence and properties of the mitochondrial intermediate conductance Ca2+ activated K+ channel mtKCa3.1. *Biochim Biophys Acta Bioenergetics* 2010;1797:260–1267.

[152] Dolga AM, Netter MF, Perocchi F, Doti N, Meissner L, Tobaben S, Grohm J, Zischka H, Plesnila N, Decher N, Culmsee C. Mitochondrial small conductance SK2 channels prevent glutamate-induced oxytosis and mitochondrial dysfunction. Journal of Biological Chemistry 2013:288:10792-804.

[153] Sørensen US, Strøbaek D, Christophersen P, Hougaard C, Jensen ML, Nielsen EØ, Peters D, Teuber L. Synthesis and structure-activity relationship studies of 2-(N-substituted)-

aminobenzimidazoles as potent negative gating modulators of small conductance Ca2+-activated K+ channels. J Med Chem 2008;51:7625-7634.

[154] Szabò I, Bock J, Jekle A, Soddemann M, Adams C, Lang F, Zoratti M, Gulbins E. A novel potassium channel in lymphocyte mitochondria. J Biol Chem 2005;280:12790–12798.

[155] Vicente R, Escalada A, Villalonga N, Texidó L, Roura-Ferrer M, Martín-Satué M, López-Iglesias C, Soler C, Solsona C, Tamkun MM, Felipe A. Association of Kv1.5 and Kv1.3 contributes to the major voltage-dependent K+channel in macrophages. J Biol Chem 2006;281:37675–37685.

[156] Bednarczyk P, Kowalczyk JE, Beresewicz M, Dołowy K, Szewczyk A, Zabłocka B. Identification of a voltage gated potassium channel in gerbil hippocampal mitochondria. Biochem Biophys Res Commun 2010;397:614–620.

[157] Gulbins E, Szabo I, Baltzer K, Lang F. Ceramide-induced inhibition of T lymphocyte voltage-gated potassium channel is mediated by tyrosine kinases. Proc Natl Acad Sci USA 1997;94:7661–7666.

[158] Leanza L, Zoratti M, Gulbins E, Szabò I. Induction of apoptosis in macrophages via Kv1.3 and Kv1.5 potassium channels. Curr Med Chem 2012;19:5394-5404.

[159] Szabò I, Zoratti M, Gulbins E. Contribution of voltage-gated potassium channels to the regulation of apoptosis. FEBS Lett 2010;584:2049-2056.

[160] Leanza L, Henry B, sassi N, Zoratti M, Chandy G, Gulbins E, Szabo I. Inhibitors of mitochondrial Kv1.3 channels induce Bax/Bak-indepenent death of cancer cells. EMBO Molecular Medicine 2012;4:1-17.

[161] Leanza L, Trentin L, Becker KA, Frezzato F, Zoratti M, Semenzato G, Gulbins E, Szabo I. Clofazimine, Psora-4 andPAP-1, inhibitors of the potassium channel Kv1.3, as a new and selective therapeutic strategy in chronic lymphocyteic leukemia. Leukemia 2013;1-4.

[162] Szewczyk A, Jarmuszkiewicz W, Kunz WS. Mitochondrial potassium channels. IUBMB Life. 2009;61:134-43.

[163] Kajma A, Szewczyk A. A new pH-sensitive rectifying potassium channel in mitochondria from the embryonic rat hippocampus. Biochim Biophys Acta 2012;1817:1867-78.

[164] Rusznák Z, Bakondi G, Kosztka L, Pocsai K, Dienes B, Fodor J, Telek A, Gönczi M, Szucs G, Csernoch L. Mitochondrial expression of the two-pore domain TASK-3 channels in malignantly transformed and non-malignant human cells. Virchows Arch 2008;452:415-426.

[165] Toczylowska-Maminska r, Olszewska A, Laskowski M, Bednarczyk P, Skowronek K, Szewczyk A. Potassium channel in the mitochondria of human keratinocytes. J Invest Dermatology 2014;134:764-72.

[166] Haikala H, Kaivola J, Nissinen E, Wall P, Levijoki J, Linden IB. Cardiac troponin C as a target protein for a novel calcium sensitizing drug, levosimendan. J Mol Cell Cardiol 1995;27:1859–1866.

[167] Jamali IN, Kersten JR, Pagel PS, Hettrick DA, Warltier DC. Intracoronary levosimendan enhances contractile function of stunned myocardium Anesth Analg 1997;85:23–29.

[168] Kopustinskiene DM, Pollesello P, Saris NE. Levosimendan is a mitochondrial K_{ATP} channel opener Eur J Pharmacol 2001;428:311–314.

[169] Kopustinskiene DM, Pollesello P, Saris NE. Potassium-specific effects of levosimendan on heart mitochondria. Biochem Pharmacol 2004;68:807-812.

[170] Terzic A, Dzeja PP, Holmuhamedov EL. Mitochondrial K_{ATP} channels: probing molecular identity and pharmacology J Mol Cell Cardiol 2000;32:1911–1915.

[171] Ma H, Huang X, Li Q, Guan Y, Yuan F, Zhang Y. ATP-dependent potassium channels and mitochondrial permeability transition pores play roles in the cardioprotection of theaflavin in young rat. J Physiol Sci 2011;61:337-342.

[172] Zaugg M, Lucchinetti E, Spahn DR, Pasch T, Schaub MC. Volatile anesthetics mimic cardiac preconditioning by priming the activation of mitochondrial K(ATP) channels via multiple signaling pathways. Anesthesiology 2002;97:4-14.

[173] Van Allen NR, Krafft PR, Leitzke AS, Applegate RL, Tang J, Zhang JH. The role of Volatile Anesthetics in Cardioprotection: a systematic review. Med Gas Res 2012;2:22doi: 10.1186/2045-9912-2-22.

[174] Riess ML, Camara AK, Novalija E, Chen Q, Rhodes SS, Stowe DF. Anesthetic preconditioning attenuates mitochondrial Ca2+ overload during ischemia in Guinea pig intact hearts: reversal by 5-hydroxydecanoic acid. Anesth Analg 2002;95:1540-6.

[175] Riess ML, Novalija E, Camara AK, Eells JT, Chen Q, Stowe DF. Preconditioning with sevoflurane reduces changes in nicotinamide adenine dinucleotide during ischemia-reperfusion in isolated hearts: reversal by 5-hydroxydecanoic acid. Anesthesiology 2003;98:387-95.

[176] Stumpner J, Lange M, Beck A, Smul TM, Lotz CA, Kehl F, Roewer N, Redel A. Desflurane-induced post-conditioning against myocardial infarction is mediated by calcium-activated potassium channels: role of the mitochondrial permeability transition pore. Br J Anaesth 2012;108:594-601.

[177] Fukasawa M, Nishida H, Sato T, Miyazaki M, Nakaya H. 6-[4-(1-Cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydro-2-(1H)quinolinone (cilostazol), a phosphodiesterase type 3 inhibitor, reduces infarct size via activation of mitochondrial Ca2+-activated K+ channels in rabbit hearts. J Pharmacol Exp Ther 2008;326:100-104.

[178] Testai L, Martelli A, Marino A, D'Antongiovanni V, Ciregia F, Giusti L, Lucacchini A, Chericoni S, Breschi MC, Calderone V. The activation of mitochondrial BK potassium channels contributes to the protective effects of naringenin against myocardial ischemia/reperfusion injury. Biochem Pharmacol 2013;85:1634-1643.

[179] Gao Q, Yang B, Ye ZG, Wang J, Bruce IC, Xia Q. Opening the calcium-activated potassium channel participates in the cardioprotective effect of puerarin. Eur J Pharmacol 2007;574:179-184.

[180] Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, Ledgerwood EC, Smith RA, Murphy MP. Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. J Biol Chem 2001;276:4588-4596.

[181] Smith RA, Porteous CM, Coulter CV, Murphy MP. Selective targeting of an antioxidant to mitochondria. Eur J Biochem 1999;263:709-716.

[182] Porteous CM, Logan A, Evans C, Ledgerwood EC, Menon DK, Aigbirhio F, Smith RA, Murphy MP. Rapid uptake of lipophilic triphenylphosphonium cations by mitochondria in vivo following intravenous injection: implications for mitochondria-specific therapies and probes. Biochim Biophys Acta 2010;1800:1009-1017.

[183] Rodriguez-Cuenca S, Cochemé HM, Logan A, Abakumova I, Prime TA, Rose C, Vidal-Puig A, Smith AC, Rubinsztein DC, Fearnley IM, Jones BA, Pope S, Heales SJ, Lam BY, Neogi SG, McFarlane I, James AM, Smith RA, Murphy MP. Consequences of long-term oral administration of the mitochondria-targeted antioxidant MitoQ to wild-type mice. Free Radic Biol Med 2010;48:161-172.

[184] Biasutto L, Mattarei A, Marotta E, Bradaschia A, Sassi N, Garbisa S, Zoratti M, Paradisi C. Development of mitochondria-targeted derivatives of resveratrol. Bioorg Med Chem Lett. 2008;18:5594-7.

[185] Vyssokikh MY, Chernyak BV, Domnina LV, Esipov DS, Ivanova OY, Korshunova GA. SkBQ - prooxidant addressed to mitochondria. Biochemistry (Mosc) 2013;78:1366-70.

[186] Symonyan RA, Skulachev MV, Zinevich TV, Skulachev VP. [186] Adlam, VJ, Harrison JC, Porteous CM, James AM, Smith RA, Murphy MP, Sammut IA. Targeting an antioxidant to mitochondria decreases cardiac ischemia-reperfusion injury. FASEB J 2005;19:1088-1095.

[187] Smith RA, Hartley RC, Murphy MP. Mitochondria-targeted small molecule therapeutics and probes. Antioxid Redox Signal 2011;15:3021-3038.

[188] Smith RA, Murphy MP. Animal and human studies with the mitochondria-targeted antioxidant MitoQ. Ann N Y Acad Sci 2010;1201:96-103.

[189] Dickey JS, Gonzalez Y, Aryal B, Mog S, Nakamura AJ, Redon CE, Baxa U, Rosen E, Cheng G, Zielonka J, Parekh P, Mason KP, Joseph J, Kalyanaraman B, Bonner W, Herman E, Shacter E, Rao VA. Mito-Tempol and Dexrazoxane Exhibit Cardioprotective and Chemotherapeutic Effects through Specific Protein Oxidation and Autophagy in a Syngeneic Breast Tumor Preclinical Model. PLoS One 2013;8:e70575.

[190] Szeto HH, Schiller PW. Novel therapies targeting inner mitochondrial membrane-from discovery to clinical development. Pharm Res 2011;28:2669-2679.

[191] Zhao K, Zhao GM, Wu D, Soong Y, Birk AV, Schiller PW, Szeto HH. Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. J Biol Chem 2004;279:34682–34690.

[192] Szeto HH. Mitochondria-targeted cytoprotective peptides for ischemia-reperfusion injury. Antioxid Redox Signal 2008;10:601–619.

[193] Chernyak BV, Antonenko YN, Domnina LV, Ivanova OY, Lyamzaev KG, Pustovidko AV, Rokitskaya TI, Severina II, Simonyan RA, Trendeleva TA, Zvyagilskaya RA. Novel penetrating cations for targeting mitochondria. Curr Pharm Des 2013;19:2795-2806.

[194] Boengler K, Schulz R, Heusch G. Loss of cardioprotection with ageing. Cardiovascular Research 2009;83:247-261.

[195] Willems L, Zatta A, Holgren K, Ashton KJ, Headrick JP. Age-related changes in ischemic tolerance in male and female mouse hearts. J Mol Cell Cardiol 1996;38:245-256.

[196] Azhar G, Gao W, Liu L, Wei JK. Ischemia-reperfusion in the adult mouse heart influence of age. Exp Gerontol 1999; 34:699-714.

[197] Lesnefsky EJ, Gallo W, Ye J, Wittingham TS, Lust WD. Aging increases ischemia-reperfusion injury in the isolated, buffer-perfused heart. J Lab Clin Med 1994;124:843-851.

[198] O'Brien JD, Ferguson JH, Howlett SE. effects of ischemia and reperfusion on isolated ventricular myocytes from young adult and aged Fischer 344 rat hearts. Am J Physiol Heart Circ Physiol 2008; 294:H2174-H2183.

[199] Boengler K, Buechert A, Heinen Y, Roeskes C, Hilfiker-Kleiner D, Heusche G, Schulz R. Cardioprotection by ischemic postconditioning is lost in aged and STAT3-deficient mice. Circulation Research 2008;102:131-5.

[200] Boengler K, Konietzka I, Buechert A, Heinen Y, Garcia-Dorado D, Heusch G, Schulz R. Loss of ischemic preconditionig's cardioprotection in aged mouse hearts is associated with reduced gap junctional and mitochondrial levels of connexin 43. American Journal of Physiology heart Circulation Physiology 2007;292:H1764-9.

[201] Navarro A, Boveris A. The mitochondrial energy transduction system and the aging process. Am J Physiol Cell Physiol 2007;292:C670-C686.

[202] Liu L, Zhu J, Brink PR, Glass PS, Rebecchi MJ. Age-associated differences in the inhibition of mitochondrial permeability transition pore opening by cyclosporine A. Acta Anaesthesiol Scand 2011;55:622-630.

[203] Tricarico D, Camerino DC. ATP-sensitive K+ channels of skeletal muscle fibers from young adult and aged rats: possible involvement of thiol-dependent redox mechanisms in the

age-related modifications of their biophysical and pharmacological properties. Mol Pharmacol 1994;46:754-761.

[204] Tani M, Honma Y, Hasegawa H, Tamaki K. Direct activation of mitochondrial K_{ATP} channels mimics preconditioning but protein kinase C activation is less effective in middle-aged rat hearts. Cardiovasc Res 2001;49:56-68.

[205] Heinen A, Winning A, Schlack W, Hollmann MW, Preckel B, Frässdorf J, Weber NC. The regulation of mitochondrial respiration by opening of mKCa channels is age-dependent. Eur J Pharmacol 2008;578:108-113.

[206] Lee TM, Su SF, Chou TF, Lee YT, Tsai CH. Loss of preconditioning by attenuated activation of myocardial ATP-sensitive potassium channels in elderly patients undergoing coronary angioplasty. Circ 2002;105:334-340.

[207]Schulman D, Latchman DS, Yellon DM. Effect of aging on the ability of preconditioning to protect rat hearts from ischemia-reperfusion injury. Am J Physiol 2001;281:H1630-H1636.

[208] McCully JD, Toyoda Y, Wakiyama H, Rousou AJ, Parker RA, Levitsky S. Age- and gender- related differences in ischemia-reperfusion injury and cardioprotection: effects of diazoxide. Ann Thorac Surg 2006;82:117-123.

[209] Vasconsuelo A, Boland R. Actions of 17β-estradiol and testosterone in the mitochondria and their implications in aging. Ageing Res Rev 2013;12:907-17.

[210] Lancaster TS, Jefferson SJ, Hunter JC, Lopez V, Van Eyk JE, Lakatta EG, Korzick DH. Quantitative proteomic analysis reveals novel mitochondrial targets of estrogen deficiency in the aged female rat heart. Physiol Genomics 2012;44:957-969.

[211] Hausenloy DJ, Lim SY, Ong SG, Davidson SM, Yellon DM. Mitochondrial cyclophilin-D as a critical mediator of ischaemic preconditioning. Cardiovasc Res 2010;88:67-74.

[212] Yu TN, Hong H, Yang JQ, Gao Q, Xia Q. Role of mitochondrial calcium uniporter in cardioprotection induced by ischemic postconditioning in isolated rat heart. Zhejiang Da Xue Xue Bao Yi Xue Ban 2011; 40(3):304-8.

[213] Xu J, Wang NF, Zhang SZ, Gao Q, Xia Q. Mitochondrial calcium uniporter participates in remote preconditioning induced cardioprotection in rat hearts subjected to ischemia and reperfusion. Zhongguo Ying Yong Sheng Li Xue Za Zhi, 2009; 25(1):50-4.

[214] Zhang SZ, Gao Q, Cao CM, Bruce IC, Xia Q. Involvement of the mitochondrial calcium uniporter in cardioprotection by ischemic preconditioning. Life Sci. 2006;78(7):738-454.

[215] Hausenloy DJ, Ong SB, Yellon DM. The mitochondrial permeability transition pore as a target for preconditioning and postconditioning. Basic Res Cardiol. 2009;104(2):189-202.

[216] Szewczyk A, Kajma A, Malinska D, Wrzosek A, Bednarczyk P, Zabłocka B, Dołowy K. Pharmacology of mitochondrial potassium channels: dark side of the field. FEBS Lett. 2010;584:2063-9.

[217] Frezza C, Cipolat S, Scorrano L. Organelle isolation: functional mitochondria from mouse liver, muscle and cultured fibroblasts. Nature Protocols 2007;2:287-95.

[218] Saks V, Dos Santos P, Gellerich FN, Diolez P. Quantitative studies of enzyme-substrate compartmentation, functional coupling and metabolic channeling in muscle cells. Mol. Cell. Biochem. 1998;184:291-307.

[219] Sorgato MC, Keller BU, Stühmer W. Patch-clamping of the inner mitochondrial membrane reveals a voltage-dependent ion channel. Nature. 1987;330:498-500.

[220] Agafonov A, Gritsenko E, Belosludtsev K, Kovalev A, Gateau-Roesch O, Saris NE, Mironova GD. A permeability transition in liposomes induced by the formation of Ca2+/palmitic acid complexes. Biochim Biophys Acta 2003;1609:153-60

[221] García-Pérez C, Hajnóczky G, Csordás G. Physical coupling supports the local Ca2+ transfer between sarcoplasmic reticulum subdomains and the mitochondria in heart muscle. J Biol Chem 2008;283:32771-32780.

[222] Zick M, Rabl R, Reichert AS. Cristae formation-linking ultrastructure and function of mitochondria. Biochim Biophys Acta 2009;1793:5-19.

[223] Fieni F, Lee SB, Jan YN, Kirichok Y. Activity of the mitochondrial calcium uniporter varies greatly between tissues. Nat. Commun. 2012;3:1317

[224] Bednarczyk P, Wieckowski MR, Broszkiewicz M, Skowronek K, Siemen D, Szewczyk A. Putative Structural and Functional Coupling of the Mitochondrial BKCa Channel to the Respiratory Chain. PLoS One 2013;8:e68125.

[225] Garlid KD, Paucek P. Mitochondrial potassium transport: the K(+) cycle. Biochim Biophys Acta 2003;1606:23-41.

[226] Costa AD, Quinlan CL, Andrukhiv A, West IC, Jabůrek M, Garlid KD. The direct physiological effects of mitoK(ATP) opening on heart mitochondria. Am J Physiol Heart Circ Physiol 2006;290:H406-H415.

[227] Bernardi P. Mitochondrial transport of cations: channels, exchangers, and permeability transition. Physiol Rev 1999;79:1127-55.

[228] Kunz WS, Kunz W. Contribution of different enzymes to flavoprotein fluorescence of isolated rat liver mitochondria. Biochim Biophys Acta 1985;841:237-246.

[229] Halestrap AP, Clarke SJ, Khaliulin I. The role of mitochondria in protection of the heart by preconditioning. Biochim Biophys Acta 2007;1767:1007-1031.

[230] Liu Y, Sato T, Seharaseyon J, Szewczyk A, O'Rourke B, Marbán E. Mitochondrial ATP-dependent potassium channels. Viable candidate effectors of ischemic preconditioning. Ann N Y Acad Sci 1999;874:27-37.

[231] Holmuhamedov EL, Jovanović S, Dzeja PP, Jovanović A, Terzic A. Mitochondrial ATP-sensitive K+ channels modulate cardiac mitochondrial function. Am J Physiol 1998;275:H1567-H1576.

[232] Fornai M, Antonioli L, Colucci R, Pellegrini C, Giustarini G, Testai L, Martelli A, Matarangasi A, Natale G, Calderone V, Scarpignato C, Tuccori M, Blandizzi C. NSAID-induced enteropathy: are the currently available selective COX-2 inhibitors all the same? J Pharmacol Exp Ther 2013;in press.

[233] Costa AD, Garlid KD, West IC, Lincoln TM, Downey JM, Cohen MV, Critz SD. Protein kinase G transmits the cardioprotective signal from cytosol to mitochondria. Circ Res 2005;97:329-336.

[234] Jung DW, Davis MH, Brierley GP. Estimation of matrix pH in isolated heart mitochondria using a fluorescent probe Anal Biochem 1989;178:348-354.

[235] Holmuhamedov EL, Wang L, Terzic A. ATP-sensitive K+ channel openers prevent Ca2+ overload in rat cardiac mitochondria. J Physiol 1999;519:347-360.

[236] Gunter TE, Gunter KK, Sheu SS, Gavin CE. Mitochondrial calcium transport: physiological and pathological relevance. Am J Physiol 1994;267:C313-C339.

[237] Zoratti M, Szabò I. The mitochondrial permeability transition. Biochim Biophys Acta 1995;1241:139-176.

[238] Bernardi P, Petronilli V. The permeability transition pore as a mitochondrial calcium release channel: a critical appraisal. J Bioenerg Biomembr 1996;28:131-138.

9- LEGENDS

Figure 1

A) Schematic representation of events occurring during ischemic period.

The Na^+/K^+ ATPase is inhibited (1) because of the decline of ATP concentrations, leading to Na^+ accumulation and membrane depolarization, which triggers the opening of voltage gated Ca^{++} channels (2). Because of the high cytosol concentration of Na^+, the Na^+/Ca^{++} antiporter, that usually pumps Ca^{++} out of the cell and Na^+ into the cell, is inhibited or reversed, leading to further overload of Ca^{++} (3). The mitochondrial matrix massively accumulates Ca^{++} (4). During ischemia, the rate of glycolysis rises causing a metabolic production of lactic acid. The high intracellular concentration of Na^+ inhibits the Na^+/H^+ antiporter (5), with consequent lowering of the cytosol pH. Although the Ca^{++} accumulation in the matrix would be a triggering stimulus for MPTP opening, the acidic pH inhibits the activation of MPTP (6).

B) Schematic representation of events occurring duringreperfusion.

During reperfusion, the reactivation of aerobic metabolism restores the levels of ATP. The Na^+/K^+ ATPase activity is restored (1), leading to the normalization of the cytosol concentration of electrolytes (2) and H^+ (3). In conditions of physiological pH, the high levels of matrix Ca^{++} triggers the opening of MPTP (4), with consequent activation of pro-apoptotic pathways.

Figure 2

Schematic representation of I/R event, IPreC, IPostC and PhPreC protocols.

Figure 3

Schematic description of one of the different mechanisms which links the activation of mitoK channels to the control of mitochondrial calcium movements and modulation of the MPTP. The activation of mitoK channels causes inward flow of potassium ions, weak membrane

depolarization and reduced driving force for calcium accumulation in the matrix, limiting the formation of MPTP and its opening. This mechanism can, at least in part, reduce the release of mitochondrial pro-apoptotic factors during reperfusion and thus preserve mitochondrial membrane integrity.

Figure 4

Chemical structures of non-selective K_{ATP} channel openers (cromakalim, diazoxide and pinacidil), selective mito-K_{ATP} activators (BMS derivatives, F163, and KR-31466), BK_{Ca} openers (NS1619, CGS7184, NS11021) and SK activator (DCEBIO).

Figure 5

Chemical structures of "Dirty" drugs able to activate mitoK_{ATP} and mitoBK_{Ca} channels.

Figure 6

Chemical structures of mito-targeted drugs: (A) TPP^{+}-based conjugate drugs (B) peptide-based conjugate drugs (c)plastoquinone-based conjugate drugs.

Figure 7

Reliable synthetic strategy to obtain mitochondriotropic compounds, for the delivery of resveratrol (A) or SkBQ (B) into mitochondria.

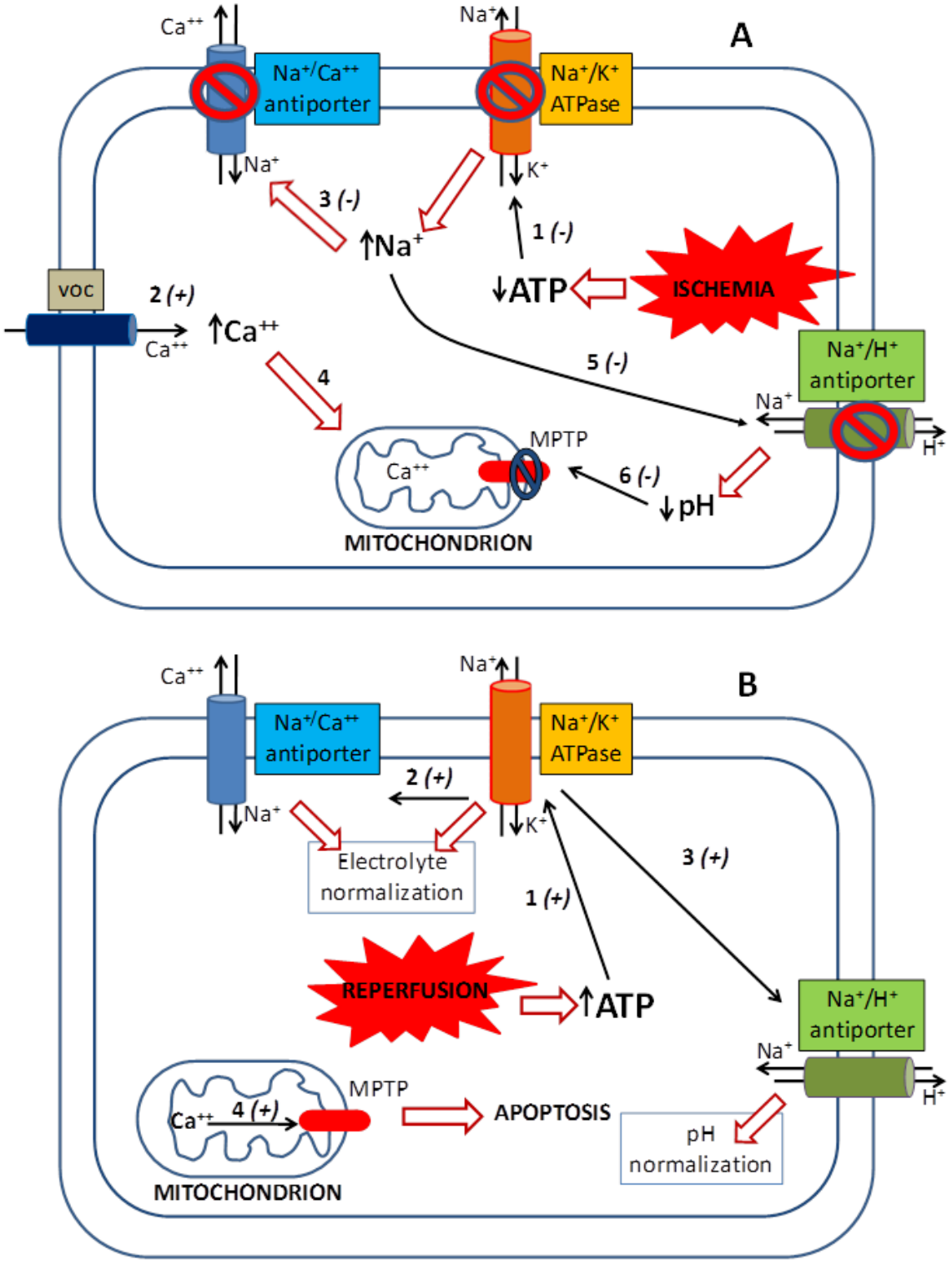
A
Ca++
Na+/Ca++ antiporter
Na+
Na+/K+ ATPase
K+
3 (-)
↑Na+
1 (-)
↓ATP
ISCHEMIA
voc
2 (+)
Ca++
↑Ca++
4
5 (-)
Na+/H+ antiporter
Na+
H+
MPTP
Ca++
6 (-)
↓pH
MITOCHONDRION
B
Ca++
Na+/Ca++ antiporter
Na+
Na+/K+ ATPase
K+
2 (+)
Electrolyte normalization
1 (+)
3 (+)
REPERFUSION
↑ATP
Na+/H+ antiporter
Na+
H+
MPTP
Ca++
4 (+)
APOPTOSIS
pH normalization
MITOCHONDRION

Fig. 1

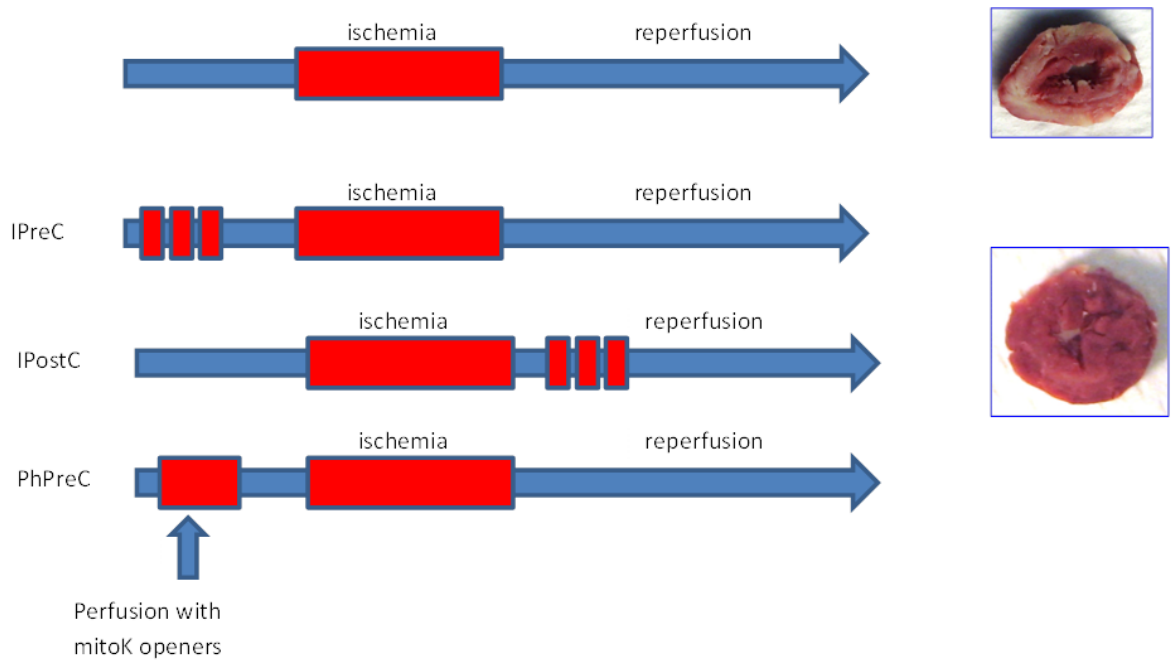
ischemia
reperfusion
IPreC
ischemia
reperfusion
IPostC
ischemia
reperfusion
PhPreC
ischemia
reperfusion
Perfusion with
mitoK openers

Fig. 2

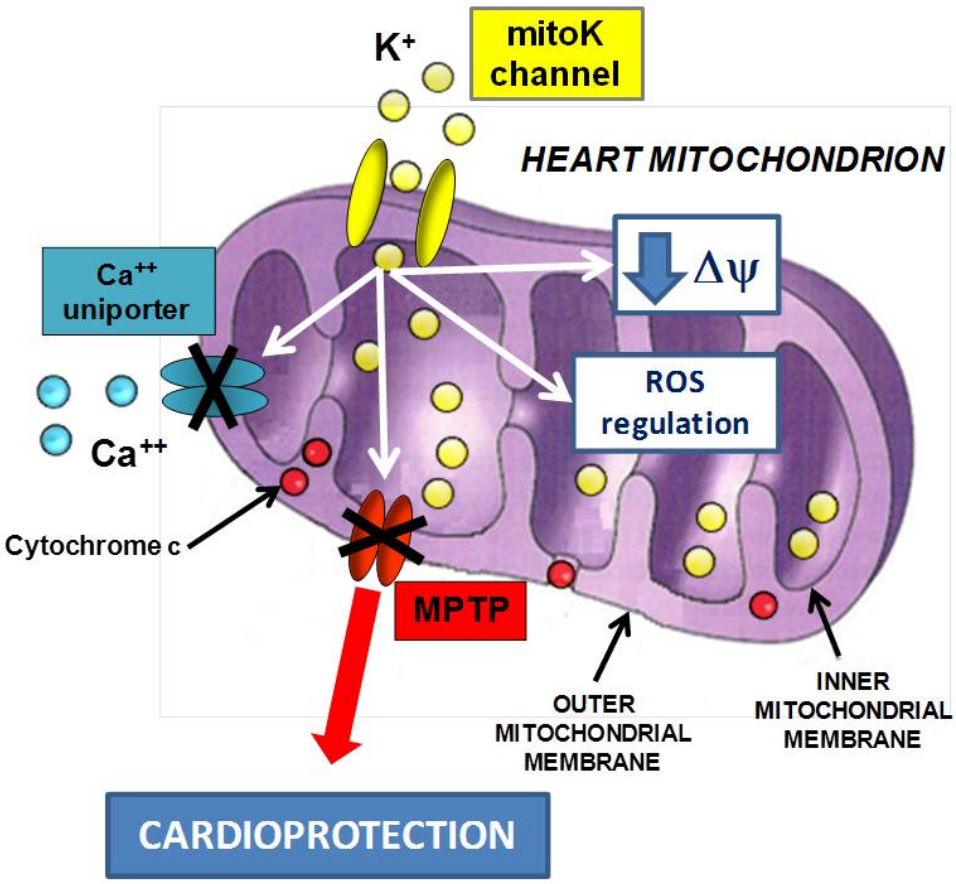
K+
mitoK channel
HEART MITOCHONDRION
Ca++ uniporter
Δψ
ROS regulation
Ca++
Cytochrome c
MPTP
OUTER MITOCHONDRIAL MEMBRANE
INNER MITOCHONDRIAL MEMBRANE
CARDIOPROTECTION

Fig. 3

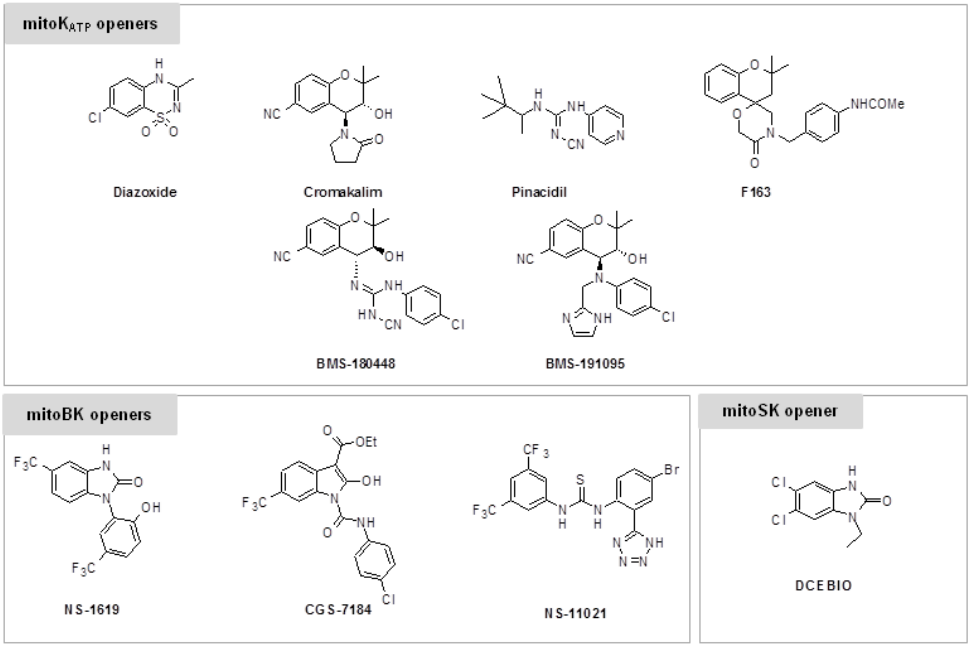
mitoK$_{ATP}$ openers
Diazoxide
Cromakalim
Pinacidil
F163
BMS-180448
BMS-191095
mitoBK openers
NS-1619
CGS-7184
NS-11021
mitoSK opener
DCEBIO

Fig. 4

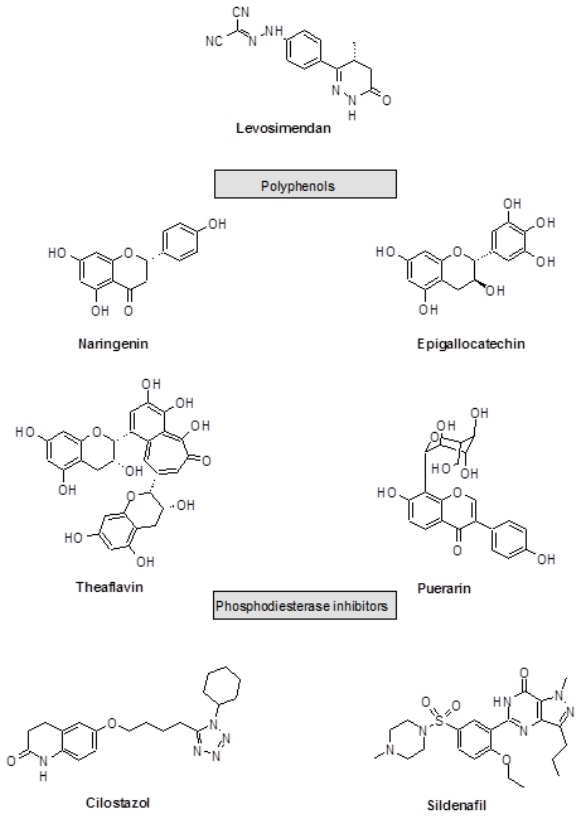
Levosimendan
Polyphenols
Naringenin
Epigallocatechin
Theaflavin
Puerarin
Phosphodiesterase inhibitors
Cilostazol
Sildenafil

Fig. 5

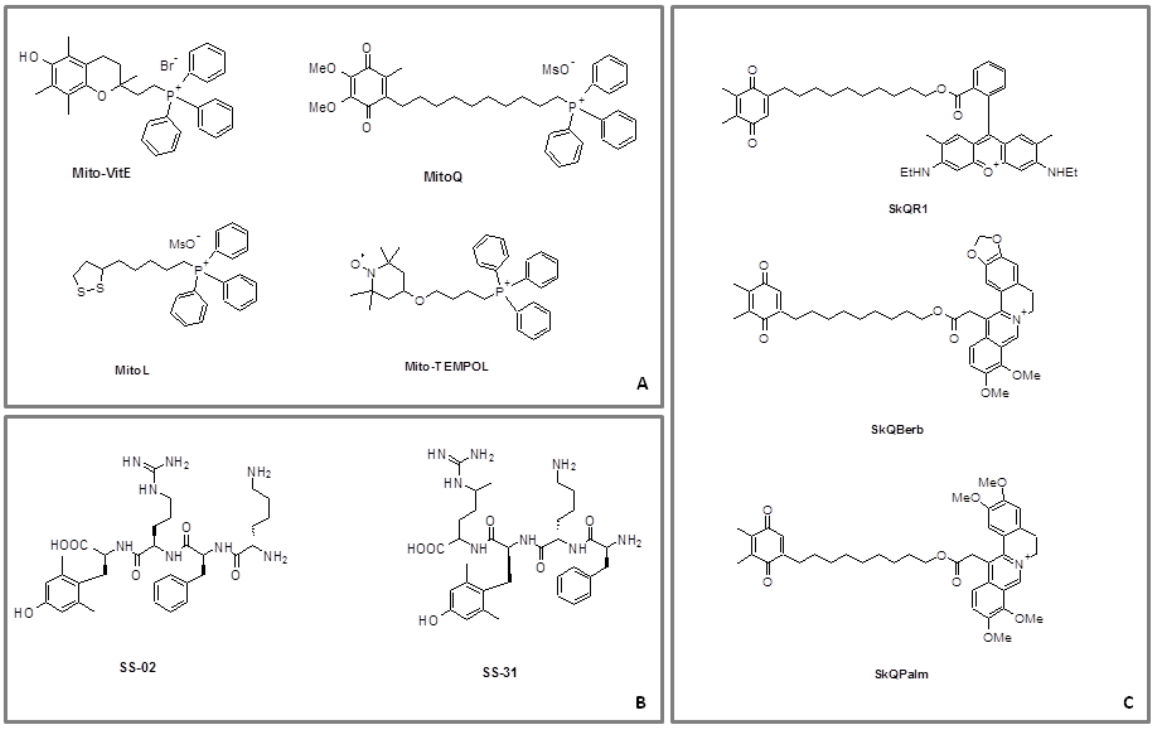
Mito-VitE
MitoQ
MitoL
Mito-TEMPOL
A
SS-02
SS-31
B
SkQR1
SkQBerb
SkQPalm
C

Fig. 6

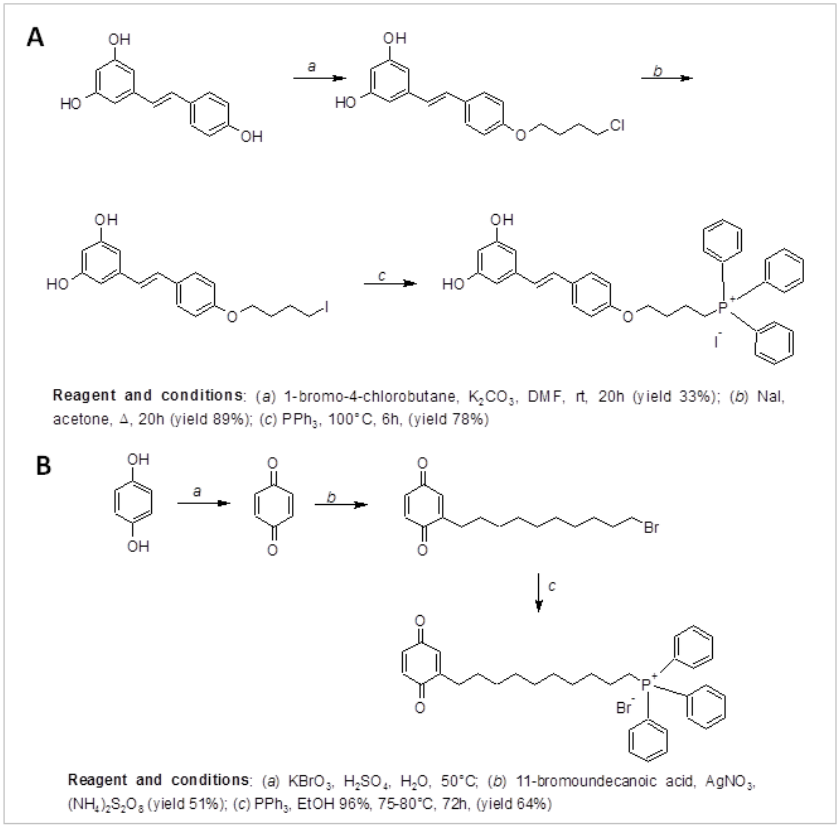
A
OH
HO
OH
a
OH
HO
O
Cl
b
OH
HO
O
I
c
OH
HO
O
P+
I-
Reagent and conditions: (a) 1-bromo-4-chlorobutane, K2CO3, DMF, rt, 20h (yield 33%); (b) NaI, acetone, Δ, 20h (yield 89%); (c) PPh3, 100°C, 6h, (yield 78%)
B
OH
OH
a
O
O
b
O
O
Br
c
O
O
P+
Br-
Reagent and conditions: (a) KBrO3, H2SO4, H2O, 50°C; (b) 11-bromoundecanoic acid, AgNO3, (NH4)2S2O8 (yield 51%); (c) PPh3, EtOH 96%, 75-80°C, 72h, (yield 64%)

Fig. 7