Chapter

Mitochondrial Channels and Their Role in Cardioprotection

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Abstract

Mitochondria play a pivotal role in cardioprotection. The major cardioprotective mechanism is ischemic preconditioning (IpreC), through which short periods of ischemia protect a subsequent prolonged acute ischemic episode. Mitochondria channels, particularly the potassium channels (mitoK) such as ATP-dependent and calcium-activated potassium channels, have been suggested as trigger or end effectors in IpreC. Activators of mitoK are promising therapeutic agents for the treatment of the myocardial injury due to ischemic episodes. In this chapter, we are summarizing our current knowledge on the physiology function of different mitochondrial channels with a focus on the potassium channels and their mechanism in cardioprotection. Furthermore, the currently under development therapy by targeting the mitochondrial channels for the treatment of heart failure are also discussed.

Keywords: cardioprotection, ischemic preconditioning (IpreC), ischemic postconditioning (IpreC), oxidative phosphorylation, reactive oxygen species (ROS), cell death, mitochondrial permeability transition pore, mitochondrial potassium channels, ischemia, reperfusion, heart failure

1. Introduction

Heart failure is a major public health issue that is still having a poor prognosis despite all the advancements in scientific research and technologies [1]. The approaches for the drug development of heart disease are majorly relying on the pathophysiology of the cellular mechanisms and inter and intracellular channels in the failing heart. Heart being an organ of extensively high energy demand and mitochondria being the powerhouse of the eukaryotic organisms, they are meant to be closely connected. Any change in mitochondrial function inevitably affects the health of the heart irrespective of the etiology. Recent advances in the field indicate that besides having a compromised powerhouse, mitochondrial malfunctioning accompanies certain pathogenic mechanisms leading to heart failure [2, 3]. Current therapies like ischemic pre- and postconditioning provide symptomatic benefit but do not address the abnormalities at a molecular level. Since the mitochondria play an important role in the pathophysiology of a failing heart, understanding its mechanism can potentially improve the approaches for the therapies for direct improvement of cardiac functions. Among the abnormalities shown by the mitochondria, ruptured

electron transport chain, excessive formation of reactive oxygen species (ROS), perturbed ion homeostasis are the basic concerns [4]. An important and potential substrate for therapeutics in heart failure is mitochondrial channels [5]. In this chapter, we intend to discuss the available information about the mitochondrial channel with regards to its pathophysiological effects on heart health and their responses to the ischemic conditioning alongside the available agonist for the mitochondrial channel.

2. Mitochondria and its functions specific to heart cells

Due to high energy demand, the number of mitochondria in the heart cells is excessively high, with a daily production of approximately 65 kg ATP through oxidative phosphorylation [6]. In the neonatal cardiac myocytes, the mitochondria are highly motile in the cytosol generating energy through glycolysis and glucose metabolism. Whereas, in an adult myocyte the mitochondria have reduced motility, and energy generation occurs from the metabolism of fatty acid [7].

Mitochondria are known to arise billions of years ago through the engulfment of alpha proteobacteria by the precursors of modern eukaryotic cells and it evolved to become an essential multifunctional organelle [8]. Mitochondria are made up of an outer comparatively permeable and inner highly folded relatively impermeable lipid bilayer. The folded inner membrane with a high surface area contains the complexes for the generation and transportation of adenosine triphosphate (ATP) through oxidative phosphorylation. In the myocardial cells, the substrates are oxidized to produce acetyl coenzyme A, which in turn drives the Krebs cycle to produce nicotinamide adenine dinucleotide hydrogen (NADH) and flavin adenine dinucleotide ($FADH_2$) in the mitochondrial matrix. The oxidation of NADH and $FADH_2$ leads to the establishment of proton motive force later fetched by F1F0 ATP synthase to convert adenosine diphosphate (ADP) and inorganic phosphate to ATP [9].

In the process of energy production, approximately 2% of the electrons flowing in the electron transport cycle are reduced to form a superoxide anion which is reduced to H_2O_2 followed by H_2O generation by antioxidant enzymes. Excessive production of these ROS is toxic to the cell, yet these natural byproducts of oxygen metabolism trigger a variety of oxygen sensing machinery including gene expression, however, the overload of ROS impairs the redox potential of the cell leading to various oxidative damages [10].

The dynamics of Ca^{2+}, an important element to trigger various enzymatic processes and a second messenger for contractile functions, is also organized by mitochondria by either transmembrane Ca^{2+} transport or ROS-mediated signaling pathways [11]. In case of increased workload rapid mitochondrial Ca^{2+} uptake is facilitated by Ca^{2+} uptake channel for elevated ATP production. The elimination of Ca^{2+} from the mitochondrial matrix however is slower and mediated either directly by Na^+/Ca^{2+} exchanger or indirectly by multiple mitochondrial K^+ channels with unknown mechanisms [12].

Alongside the role as a life-supporting system, mitochondria can also trigger programmed cell death in the required conditions. The mitochondrial permeability transition pore (MPTP) opens in response to stress and leads to loss of membrane potential, which stops ATP production and release of cytochrome C and other mitochondrial protein causing necrosis and cell apoptosis [13]. In cases of heart failure mitochondria-induced cell death is an important mechanism. Here we intend to discuss important parameters of mitochondrial dysfunction which lead to heart failure, and therapeutic approaches to circumvent the situation.

3. Mitochondrial dysfunction and heart failure

In cardiac cells the energy consumption should meet the energy production rate on a beat-by-beat basis, failing which the stored energy cannot last more than a few seconds. In a pathological remodeling the oxidative metabolism switch from fatty acid metabolism to glycolysis, which only contributes less than 5% of the total ATP demand of an adult heart [14]. On the other hand, during pathological remodeling the required energy increases due to disturbed cardiac geometry, and impaired ATP homeostasis. Studies have shown that the mitochondrial mechanisms involved in pathological remodeling in efforts to restore the energy homeostasis eventually led to a vicious cycle that drives pathological remodeling towards heart failure. The most puzzling scenario suggests that in the failing heart the ATP content is largely maintained after an initial glitch, thus whether the heart failure occurs due to energy starvation or in efforts to fight that starvation is a question yet to be addressed. Further in-depth analysis of the mitochondrial mechanisms can clarify if the efforts of maintaining the energy hemostasis are either helpful or potentially worsen the failing heart.

The catalysis of degradative oxidation of the nutrients through anaerobic dehydrogenases is facilitated by the reduction of oxidized pyridine and flavin nucleotide like NAD(P^+) and FAD. These coenzymes should be again reoxidized since they are non-replenishable and cannot permeate the cell membrane with the degradation rate. During an ischemic episode since the respiratory chain is impaired the oxidation of the above-stated substrates is also hampered, moreover, the NADH(H^+) oxidation is carried out by lactate dehydrogenase. Therefore, the anaerobic glycolysis takes over as the only pathway for ATP synthesis provided the phosphocreatinine is depleted with the onset of ischemia. Therefore, in a failing heart the oxidative metabolism switch for alternative carbon sources such as glucose which can be beneficial due to increased ATP production and oxygen uptake but when it takes over the usual fatty acid metabolism the energy production is not sufficient for an adult heart [14]. Increased glycolysis causes anaplerosis, increased lactate production, triggers the heart to go into pathological remodeling, and also inhibits branched-chain amino acid (BCAA) catabolism, and causes the accumulation of BCAA. A hyperacetylation of mitochondrial protein has also been seen as a failing heart the cause of which is not clearly understood [15].

The decrease in ATP concentration causes an immense ionic imbalance across the cell cytoplasm leading to the lowering of the pH of the cell. The inhibition of Na^+/K^+ ATPase, Na^+/H^+, Na^+/Ca^{2+} antiporters leads to an overload of Ca^{2+} inside the cells causing hypercontracture and triggering the irreversible opening of mitochondrial permeability transition pore (MPTP) [16]. The frequently converting ATP into ADP and phosphate seeps out of the cell which further contributes to reduced performance of the heart. Opening of only one pore causes frequent depolarization and triggers the opening of other pores, following which the rapid influx of small molecular weight solutes enters the mitochondrial matrix to compensate for the depolarization and causes the mitochondrial matrix to swell. The expansion of the inner mitochondrial membrane leads to the rupture of the outer membrane which releases proapoptotic proteins leading eventually to cell death. Therefore, it is believed that altering the MPTP pore opening can be helpful in the prevention of cardiac reperfusion and cell death [17].

During an ischemic episode, the release of ROS is formed under the physiological and pathological conditions within the mitochondria. In a regular respiratory chain

reaction, 2–4% oxygen undergoes an univalent reaction and produces superoxide [4]. The superoxides that are formed at complex I and complex III level are rapidly transformed by metalloenzymes like superoxide dismutase into hydrogen peroxide. In the first minute, it is small but in a later stage, it increases dramatically, leading to the disruption of mitochondrial membrane potential. Therefore, the consequence of ROS formation has been linked to the opening of the MPTP channel leading to apoptosis. These episodes put together in series lead to a gradual and irreversible decline of the cell integrity.

The opening of MPTP can occur through all the factors mentioned here, such as an increase in Ca^{2+} ion, depolarization, increase in the ROS, and phosphate concentration [18]. Certain factors like a high concentration of H^{+}, Mg^{2+}, and ADP can counteract the MPTP opening and work as antagonists [19, 20]. On the contrary, in the condition of reperfusion, the change in the pH is recovered by the burst formation of ROS in the presence of Ca^{2+}, which creates the most favorable condition for MPTP opening even though the antagonizing effect of membrane potential recovery occurred. In isolated mitochondria, the MPTP opens at a very high Ca^{2+} concentration which is practically not possible in vivo therefore the increased Ca^{2+} alone is not responsible for MPTP opening rather can be triggered by several processes like ROS generating Ca^{2+} dependent enzyme.

4. Cardioprotection

It is believed that to reduce the damage occurring in the heart cells in a prolonged ischemic episode, the heart cells can be trained beforehand through small and regulated episodes of either cardiac ischemia or reperfusion that resulted from ATP deprivation or concentration increase of ROS and Ca^{2+} (**Figure 1**). This method has been tested in dogs [21] and higher mammals including humans [22, 23]. This process is known as ischemic preconditioning (IpreC). Similarly, ischemic postcondition

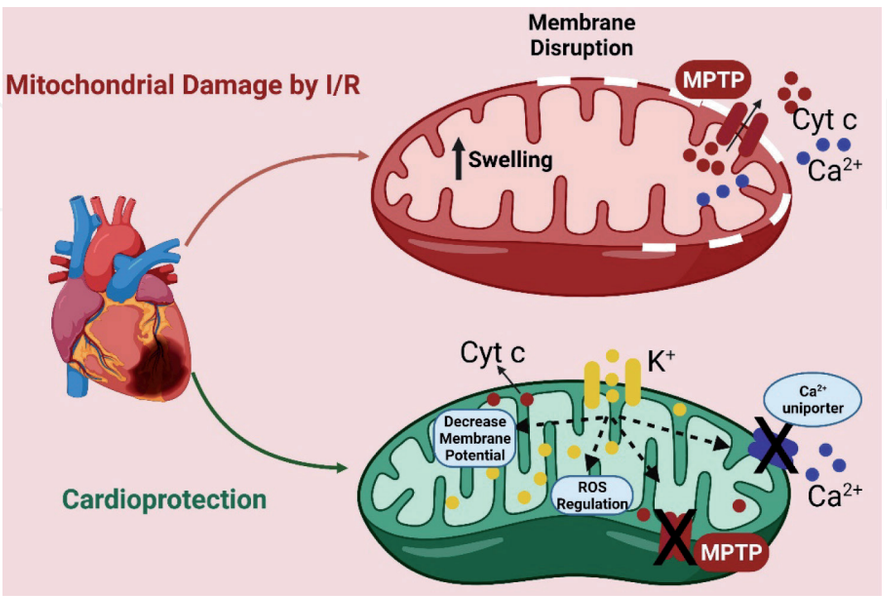

Figure 1.
The ischemic/reperfused heart mitochondria in comparison to the cardioprotected mitochondria. In cardioprotected mitochondria MPTP and MCU channels are closed and mitoK channel is opened.

(IpostC) can also be done in a brief intermittent cycle after a severe event [24]. These are performed by natural or artificial biomolecules which will be discussed later in this chapter.

The process of IpreC and IpostC usually activates protein kinase C isozymes [25] and other kinases [26] whose roles in cardioprotection are very dicey, because as ε isozyme protects the mitochondrial function by activating $ALDH_2$ aldehyde dehydrogenase which removes the lipid peroxidation products, Baines et al. showed that the translocation of ε isozyme prevents the opening of MPTP pore [27]. Whereas δ isozyme of protein kinase C increases the tissue injury by flawed perfusion of myocytes and inhibits ATP and pyruvate dehydrogenase regeneration [27]. Several mitochondrial pathways are activated in the conditioning process contributing significantly to the process of cardioprotection and therefore they are considered attractive pharmacological targets.

5. Mitochondrial channels with an integral role in cardioprotection

The multifaceted relationship of mitochondria with cell death makes it an ideal target for aiming to preserve cardiomyocytes viability. In the lack of oxygen during ischemia although the ATP synthesis cannot be restored yet can protect through decreasing ATP hydrolysis. Several self-defense mechanisms are triggered by ischemic preconditioning like the depolarization of mitochondrial matrix promotes F1F0 ATPase binding to its natural inhibiter Factor (IF) [28]. A similar effect has been shown by overexpressed the BCL-2 gene in mice hearts, to conclude that ATP hydrolysis is modulated by BCL-2 as well since the oligomycin addition did not possess any additional effect. BCL-2 is upregulated in the preconditioned heart and downregulated by ischemia and reperfusion [29]. However, the cardioprotective effect caused by preconditioning can be abolished by antisense nucleotide in a perfused rat heart [30]. Another way to prevent ATP hydrolysis is by MPTP inhibition, which presents a wide range of protective actions like maintaining Ca^{2+} homeostasis, NAD^+ depletion prevention, and preventing the release of pro-apoptotic protein [20, 31, 32]. The preconditioned heart prevents the opening of MPTP pores conferring stress-tolerant condition of the cardiomyocytes [33, 34].

In addition to protective effects posed by MPTP inhibition, numerous studies have vouched for the supporting effect of the mitochondrial potassium channel, specially $mitoK_{ATP}$ and calcium-dependent $mitoK_{Ca.}$ The influx of K^+ into the inner mitochondrial matrix causes depolarization, with pH increase and matrix swelling [35–37]. It is suggested that matrix swelling due to K^+ uptake compensates for the contraction of the matrix caused by increased potential difference due to lack of oxygen. The K^+ uptake and matrix swelling are suggested to increase the recovery of ATP concentration, by preventing the loss of substrate channeling which happened due to increased potential difference at the onset of reperfusion [38].

5.1 Mitochondrial permeability transition pore

A sudden increase in the permeability of the solute in the inner mitochondrial membrane (IMM) is known as the permeability transition [16]. The MPTP was first described by Haworth and Hunter in 1979, who showed that the addition of high levels of calcium to bovine myocardial mitochondria induced a nonspecific increase in permeability of the inner mitochondrial membrane [39]. Although the occurrence

of permeability transition and its inhibitor as adenine dinucleotide has been known since 1950 [40]. Our understanding of mitochondrial physiology and the acceptance of the pore theory of permeability transition is greatly attributed to the study of a mitochondrial channel.

The opening of the MPTP channel causes depolarization, blocks ATP synthesis, releases Ca^{2+}, depletes pyridine nucleotide, inhibits respiration, causes matrix swelling, which subsequently leads to cytochrome C mobilization and outer mitochondrial membrane rupture which ultimately releases endonuclease G and apoptosis-inducing factor (AIF) and other proapoptotic protein to kill the cell (**Figure 1**) [41, 42]. It should be noted though, that this detrimental effect of MPTP opening occurs only when the pore opening is long-lasting [43]. Whereas the short-term opening, both in vivo and in vitro [44], is suggested to be involved in the physiological regulation of Ca^{2+} and the homeostasis of ROS [4], subsequently providing mitochondria a fast mechanism for Ca^{2+} release. According to a study performed on a mitochondria calcium uniporter (MCU), null mice had an equal I/R injury as the wildtype littermates overruling cyclophilin-D (CyPD) protection (**Figure 1**). It leads to challenging the established concept and awaits the molecular details of the myocardial reperfusion mechanism and the precise roles of the channels for answers to these contradicting observations. The potential role of MPTP opening in heart failure was recognized way before the discovery of the role of mitochondria in apoptosis.

Although the molecular nature and precise composition of MPTP remain unknown it is believed that some proteins regulate the function of MPTP like CyPD (**Figure 1**). After the observation that cyclosporin (CsA) is a potent inhibitor of MPTP opening [45, 46], Halestrap et al. demonstrated that it occurred due to an inhibition of a peptidyl-prolyl cis-trans isomerase PPIase in the matrix [47]. They further purified and demonstrated the protein to be CyPD, which is an 18 kDa matrix protein. A range of other CsA analogs and sanglifehrin A (SfA) that showed their potency in preventing MPTP opening also acted as inhibitors of PPIase of CyPD. On the other hand, the MPTP opening is also inhibited by ATP and ADP but their complexes with Mg^{2+} and other nucleotides like AMP, GTP, or GDP fail to show a similar effect, it is worth noting that none of them are transported by the adenine nucleotide translocase (ANT) [48]. Furthermore, the increased sensitivity of MPTP opening towards Ca^{2+} is attributed to the inhibition in the binding of the ATP and ADP with ANT either by depleting the matrix of adenine nucleotides or by modifying ANT by thiol [49]. Helstrap group developed a model for MPTP, where CyPD binds to ANT and they undergo conformational changes to induce pore formation under Ca^{2+} trigger, and they showed that matrix Ca^{2+} favored 'C' conformation for ANT. Several matrices facing glutamate and aspartate residues on ANT are present whose carboxyl groups might play the role of Ca^{2+} binding as there is no Ca^{2+} binding motif established on ANT [49]. Another data consistent with the model showed the coprecipitation of CyPD specifically with ANT and the bonding increases with rising oxidative stress and decreases with the introduction of CsA but not with inactive CsH analog [50, 51]. The crystal structure of bovine ANT1 [52] showed a constriction provided by 3 helices, block the channel and if these are rearranged by the change facilitated by CyPD, then an extensive conformational change might account for MPTP formation. Phosphate ion has been known as an MPTP activator and carboxyatractyloside (CAT) prevents ANT from binding the phenyl arsine oxide (PAO) column but still does not prevent MPTP activation, which suggests that PAO can have an additional MPTP activation site apart from the ANT. When CAT treated beef heart mitochondria was passed through the PAO column phosphate carrier protein (PiC) was bound to

the column [53]. Pretreatment of the column with MPTP inhibitors like ubiquinone (UQo) prevents the PiC binding to the column which suggests a key role in MPTP formation. Other proteins have also been suggested to have the structural and regulatory role in MPTP formation like peripheral benzodiazepine receptor and voltage-dependent anion channel, hexokinase, creatinine kinase, BCL2 proteins, and Bcl 2 associated X (BAX) proteins may also be associated with MPTP, but which proteins eventually constitute the formation of the pore is still unknown [54, 55].

Recently, another theory of multiple pores in MPTP has been proposed. Studies have been supporting the potential roles of ANT, PiP, F1F0 ATP synthase, and CyPD to be inner membrane component but all of them has shown CsA sensitive permeability despite the genetic deletion of the responsible gene, which raises a question on the hypothesis and further investigation led to propose the multiple pore-forming mechanisms. Deletion of the C subunit of F1F0 ATP synthase showed that CsA induced MPTP synthesis showed much lower conductance as compared to wild-type MPTP [56]. This C-subunit lacking channel could be inhibited by an ANT inhibiter bongkrekic, therefore it was suggested that a classic MPTP was not formed in the knockout mitochondria. It was concluded that the MPTP formation could be enhanced through other proteins e.g. ANT in the lack of c-subunit. Another study proposed that dimer of F1F0 ATP synthase, ANT and PiC can assemble into synthasome complex, and it requires CyPD for disassembly into its components. They further suggest that ATP synthasome assembles and disassembles in high work conditions and MPTP formations respectively. Low ADP, high calcium enhancement leading to increase the membrane potential, and ROS formation trigger the disassembly of ATP synthasome leading to MPTP formation. Additional studies will be required to completely understand all the components of synthasome in generating MPTP [57].

As a result of its central role in myocardial infarction, MPTP poses itself as an obvious target for cardioprotection. A wide variety of cardioprotective protocols have been demonstrated to prevent MPTP opening during reperfusion. Certain drugs directly inhibit MPTP like CsA and SfA and their non-immunosuppressant derivatives like 4-methyl-val-CsA and D-3-MeAla-4-EtVal-CsA etc. and certain protocols that decrease oxidative stress and pH for inhibiting MPTP pore opening such as ischemic preconditioning [34] and ischemic postcondition [58], temperature preconditioning [59], Na^+/H^+ exchanger inhibiter like cariporide [60], mitochondrial ubiquinone antioxidants [61], the anesthetic propofol [62], urocortin [63], antioxidants including pyruvate [64].

The drugs that directly inhibit MPTP pose great value in protecting the heart during cardiac surgery, it has been shown that CsA improved cardiac performance following angioplasty treatment [65]. However, CsA and Sfa administration pose unwanted side effects because they interact with other cyclophilins like CypA moreover, their MPTP opening inhibition is overruled by the intensity of the pore opening stimulus [66]. This situation requires the development of new MPTP inhibitor drugs which can overcome these constraints. The development of new drugs requires structural insight into the MPTP pores.

5.2 Inner mitochondrial anion channel (IMAC)

The inner mitochondrial anion channel (IMAC) was the first mitochondrial channel to be identified using the patch-clamp method [67]. The pharmacological drug testing on the cardiomyocytes, for analysis of the mitochondrial matrix swelling, led to the discovery of its role in membrane potential perturbation. Its activity

is promoted under stressed oxidizing conditions [68]. O'Rourke and co-workers proposed that the arrhythmias and electrophysiological alteration in cardiomyocytes are the results of disturbed membrane potential due to failed cellular mitochondrial network under oxidative stress [69]. The inhibition of IMAC mediated mitochondrial membrane potential oscillation with 4-chlorodiazepam showed a significant reduction and stabilization of the sarcolemmal action potential [70]. High-resolution optical action potential mapping showed that the introduction of 4-chlorodiazepam facilitates the restoration of action potential duration and prevents ventricular fibrillation. The thiol oxidants trigger the oscillation of membrane potential, glutathione, and NADH, which in turn increases the ROS concentration [71]. The inhibition of IMAC activity is triggered by the binding of 4-chlorodiazepam with benzodiazepine receptors. The inhibited IMAC preserves the membrane potential; however, the prohibited efflux of superoxide from IMAC further increases the ROS concentration [72]. The increasing ROS and decreasing glutathione concentration in the mitochondrial matrix trigger the opening of the MPTP pore, and therefore, IMAC can be considered as an instigator of MPTP opening [71].

5.3 Mitochondrial Ca^{2+} uniporter

The macromolecular structural assembly responsible for mitochondrial Ca^{2+} uptake machinery is known as the mitochondrial calcium uniporter (MCU) complex. It was initially assumed that an active uptake and passive release are required for the transport of Ca^{2+} across the inner mitochondrial membrane [73], but multiple groups showed that the uptake is energetically favored whereas efflux requires electrogenic ion-exchange [74].

Ca^{2+} uptake in mitochondria results from a single transport mechanism by a Ca^{2+} sensitive channel of mitochondria known as MCU (**Figure 1**). The molecular identification of the MCU protein complex which was closely connected to a comprehensive protein compendium MitoCarta was done in 2008 [75]. Following the establishment of a compendium Ca^{2+} sensing regulator, mitochondrial Ca^{2+} uptake 1(MICU1) was discovered in 2010 [76]. MICU1 was predicted to contain no transmembrane domain and was therefore not considered forming a pore. Later 40 kDa two transmembrane domains were identified termed MCU in 2011 [77, 78] followed by the identification of other regulatory subunits.

The concentration Ca^{2+} increases in the mitochondrial matrix during ischemia and reperfusion and this increase is proposed to activate MPTP opening [16]. Therefore, the inhibition of mitochondrial calcium uniporter is studied to reduce cell damage in I/R. Studies from MCU knockout mice in the germline [79] and MCU mutated gene [80], in both the cases the Ca^{2+} uptake was hindered leading to no MPTP opening, but neither of the situations reduced the size of cardiac infarct at the onset of I/R. In contrast, where the MCU was deleted after birth in adult hearts showed cardioprotection in an in vivo model [81]. The reason for this kind of difference is not very clear but apparently, the MCU knockout before birth could generate a more robust MPTP pore not regulated by CsA as well. Alongside MCU, the other two core structural components are mitochondrial calcium uniporter b MCUb and an ion transport component termed "essential MCU regulator" or EMRE. MCUb is closely related to MCU with 50% amino acid homology, containing two similar transmembrane domains linked with coiled-coil domain. On the other hand, EMRE is a 10 kDa protein span in the inner mitochondrial membrane that contains an aspartate-rich, highly conserved, C-terminal region, whose topology however is still unclear [82]. It was proposed by

Mootha et al. that EMRE is required for Ca^{2+} channeling activity and also helps in keeping the MICU1/MICU2 intact to the MCU complex [83].

Altering the levels of regulators of MCU complex the calcium uptake can also be regulated in mitochondria, subsequently altering the susceptibility to MPTP-induced cell death. A mitochondria Ca^{2+} uptake protein1 (MICU1) mutation causing a loss of function in a human patient is associated with ataxia, attributed to mitochondrial Ca^{2+} overload [84, 85]. In a failing heart, an increase of MICU1 and $Na^{+}/Li^{+}/Ca^{2+}$ exchanger (NCXL) has been observed to compensate for the Ca^{2+} overload [86]. MICU2 on the other hand has been observed to increase, with cardiovascular disease in both humans and mice, at the transcriptional level [87]. Mice with deletion of MICU2 showed a certain degree of diastolic dysfunction. The low ratio of MICU/MCU maintains a low threshold of calcium entry in mitochondria and the overexpression of MICU1 causes contractile dysfunction to the heart. Therefore, the rise in MICU1 and MICU2 with age and disease alters the susceptibility of calcium overload and MPTP inhibition.

In a cardiac muscle, constant rhythmic cycles of contraction are dependent on permanent uptake and release of Ca^{2+} in the cytoplasm and buffering organelles [88]. After a myocardial contraction, the removal of the Ca^{2+} from the cytoplasm is provided by Na^{+}/Ca^{2+} exchanger (NCX) in the endoplasmic reticulum, on the other hand in non-muscle cells the cytosolic Ca^{2+} signals and Ca^{2+} buffering depends on mitochondrial Ca^{2+} uptake [89]. Although this mitochondrial Ca^{2+} uptake in cardiomyocytes possesses a very low MCU current and constitutes less than 1% of total Ca^{2+} uptake [88, 90], it plays a key role in coordinating between excitation and metabolism coupling [91]. In a healthy heart, two models of mitochondrial Ca^{2+} dynamics have been suggested by Cao et al., the first model suggests that Ca^{2+} concentration oscillates in a beat-to-beat manner in cardiomyocytes whereas, the second model emphasizes gradual Ca^{2+} uptake by cardiac mitochondria. On the contrary, in the damaged heart the Ca^{2+} mishandling within the mitochondria is well documented [92]. The MICU1 protein content is significantly low following I/R due to inhibition of translocase expression of the outer membrane. Furthermore, treatment attempts using siRNA on myocardial MICU1 aggravated the ischemic episode increasing tissue damage and depressing cardiac function due to apparent Ca^{2+} overload [86].

As it is quite evident that the uncontrolled influx of Ca^{2+} is disastrous for cardiomyocytes, and MCU is the major route for Ca^{2+} entry. Therefore, alteration in the expression of MCU can be a promising target for cardioprotection. For the inhibition of MCU ruthenium red and its derivatives are generally used, however, ruthenium red has nonspecific activity towards other ion channels [93] as well which does not make it a suitable inhibitor and prevents it from usage as a therapeutic agent. Recently, two new highly selective MCU inhibitors were developed one is DS16570511 prevents Ca^{2+} overload and raises cardiac contractility without affecting heart rate [94]. The second one is Ru265 is negligibly toxic and prevents hypoxia in the cell model [95]. Mitoxanthrone, an anticancer drug that showed its efficiency in inhibiting MCU [96], similarly kaempferol known as an anticancer [97] and cardioprotective drug [98] could prevent Ca^{2+} created arrhythmias [99]. These can be promising drugs in preventing Ca^{2+} related risks to cardiomyocytes but they require more animal study, and careful modeling and validation before adapting as therapeutics.

5.4 Mitochondrial potassium channel

On one side where the opening of mitochondrial mega channels like MPTP and Ca^{2+} uniporter represents a hallmark of cell death, on the other hand, the transport of

K^+ through ion channels is known to play a central role in neural and cardioprotection [100–102]. The membrane potential and permeability of the inner mitochondrial membrane are strictly controlled for efficient ATP production. The presence of an electrophoretic pathway for entry and antiporter mechanism for the exit of K^+ has been well established and they critically regulate the mitochondrial volume and function. The transport of K^+ ions from the cytosol to the mitochondrial matrix is carefully conducted through ion channels by utilizing electrogenic transport, where the proton ejection by the electron transport system generates enough membrane potential for the influx of K^+. There are four kinds of mitochondrial K^+ channels (**Figure 2**) present in the inner membrane the ATP regulated [103], Ca^{2+} regulated [104], Twin pore TASK channel [105], and voltage-gated Kv1.3 potassium channel [106]. These channels resemble the plasma membrane potassium channels in their basic biophysical properties and are regulated to avoid the membrane potential collapse.

5.4.1 ATP sensitive potassium channel

Several mitochondrial K^+ channels (**Figure 2**) have been discovered so far but ATP sensitizing uptake of K^+ has gazed maximum attention. The cardiac ischemic conditioning was first believed to be working on K_{ATP} of the plasma membrane counterpart but based on pharmacological analysis with channel openers and inhibitors shown to affect mitochondrial K_{ATP} channel. The mitoK_{ATP} channel was first identified in rat liver mitochondria using the patch-clamp method [90] and was later found in the inner mitochondrial membrane [107, 108]. They are situated at the crossroad of metabolism and membrane sensitivity. The molecular identities of a mitoK_{ATP} were recently determined by Angela Paggio et al. [109], which is similar to its plasma membrane counterparts that consisting of pore-forming potassium channel CCDC51 (MITOK) and ATP-binding cassette (ABC) transporter ABCB8 (MITOSUR); however, the detailed assembly and function mechanism is still unknown due to the missing of structural information. The plasma membrane K_{ATP} is heterooctameric, containing four inward rectifying potassium channel subunits of Kir6.1 and four

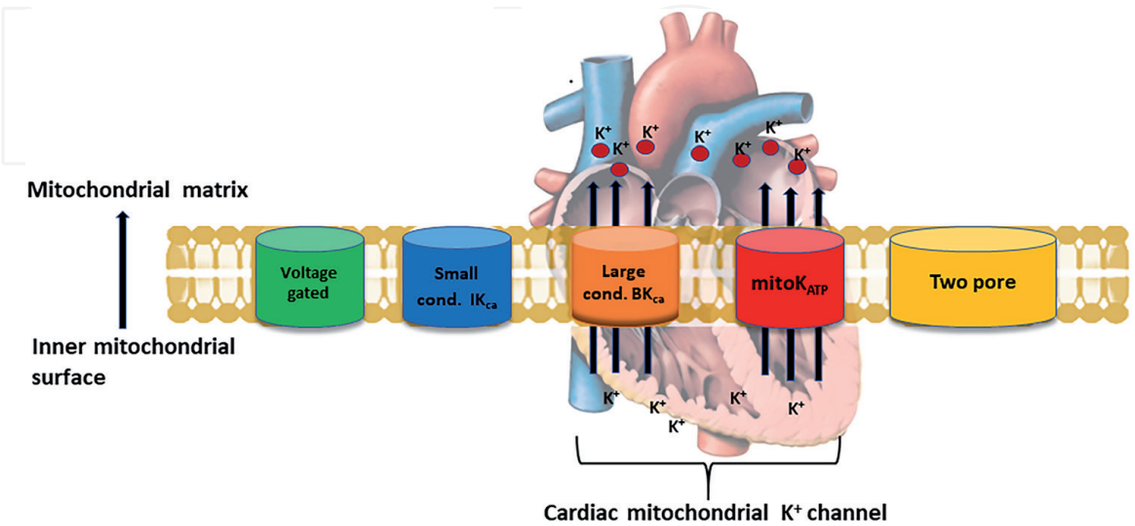

Figure 2.
Mitochondrial potassium channels (a) voltage-gated potassium channels (Kv 1.3, Kv 1.1, Kv 1.5), (b) small and large conductance mitoK_{ATP} channel (IK_{CA}, BK_{Ca}), (c) ATP sensitive K^+ channel, (d) twin pore K^+ channel. mitoK_{ATP} and mitoBK_{Ca} having extensively studied for cardioprotection.

sulfonylurea receptor subunits, which belong to the ABC transporter family [110]. Whether this newly identified mitoK_{ATP} is occupying a similar octameric assembly as the plasma membrane K_{ATP} is still unknown and moreover, it may not present the only version of mitoK_{ATP} as channels such as Kir6.1 has also been suggested in the formation of mitoK_{ATP} [111]. Nevertheless, they are believed to play a central role in cardioprotection, since the bizarre method of cardioprotection called ischemic conditioning was introduced. Ischemic preconditioning was first observed with plasma membrane using K_{ATP} channels as effectors, but later mitochondrial potassium channel became an interesting target for the same. The pathway involves activating the protein kinase and generating ROS, but the precise role of mitoK_{ATP} is not very well established. Therefore, evidence of molecular structure for the mitoK_{ATP} will subside the pharmacology-based arguments of its existence and role in the process of preconditioning. According to a study by Peng Duan et al., mitoK_{ATP} channel opening is helpful in the optimal expression of protein kinase B (p-AKT) and forkhead box protein O1 (pFoxo1) in an insulin-resistant cell. The increased p-Foxo1 is phosphorylated by p-AKT, reducing its transcriptional efficiency, and transferred out of the nucleus, and prevents the expression of pro-apoptotic protein, thereby preventing apoptosis [112].

A study done by Garlid et al. in 2006, showed that the K^+ ion uptake in the mitochondria leads to increased ROS production as they explain that the opening of the mitoK_{ATP} channel will lead to a small amount of K^+ uptake but this lowered potential will increase the matrix volume and pH by a persistent steady state. Valinomycin was used to induce the mitoK_{ATP} opener and an increased pH caused an increased ROS production, when the acetic acid influx increased for compensation of the alkaline matrix the ROS production also reduced proving that the alkalinity is a cause for ROS production. It was also proved by them that the ROS was generated from the complex I of the electron transport system [113].

5.4.2 Ca^{2+} activated potassium channel

These channels were first discovered in the glioma cell line LN-229 and have been extensively studied in the brain and cardiac cells since then [104]. The calcium-activated potassium channel is of two types small and intermediate conductance K^+ channel and large or big conductance K^+ channel. The small and intermediate channels are only calcium-dependent and not voltage-dependent they possess calmodulin for the Ca^{2+} binding at the C-terminal region. Their major function is promoting proliferation and migration of dendritic cells and smooth muscle [114].

The first evidence of its presence in the inner mitochondrial membrane was found in the late 90s by Siemen and coworkers although showing that it possesses a conductance of 300 pS [104]. Its role in protecting the heart from ischemic insult was first discovered by Xu et al. and their structural characterization in the plasma membrane indicates that it originates from potassium calcium-activated channel subfamily M alpha 1 (Kcnma1) gene containing extracellular N terminus and intracellular C-terminus [115]. It has been proven in 2013 that the BK_{Ca} pore-forming alpha subunits are encoded by the same genes (Kcnma1) as the basis of why they possess the same physiological properties [116].

The big conductance Ca^{2+} sensitive K^+ channel also known as MitoBK_{Ca} on the other hand is intuitive to voltage and mechanical stress alongside Ca^{2+} sensitivity. The knockout experiments have proven for the MitoBK_{Ca} channels to have a cardioprotective effect by reversing the ROS production and opening MPTP. It has also

been shown that these channels form a multiprotein complex with several proteins involved in apoptotic machinery [117].

mitoBK$_{Ca}$ channel [118] represent themselves as a key pathophysiological target due to their sensitivity towards calcium, voltage, and a range of cellular components. Several small molecular openers for BK$_{Ca}$ and pharmacological agents have provided very insightful information to decipher the role of BK$_{Ca}$ channel. Pharmacological agents like NS1619 and NS11021 have been used to activate BK$_{Ca}$ can potentially play a vital role in cardioprotection. However, they fail to reach the clinical applications due to their non-specificity [119–122]. Although it is posing a great deal of difficulty in developing the BK$_{Ca}$ activators, it becomes essential considering that expression of BK$_{Ca}$ is vital for cardioprotection [116, 123].

The first representation BK$_{Ca}$ playing an essential role in cardioprotection from I/R injury was performed by using NS1619, whose effect was blocked by praxilline [115]. A 3 mM NS1619 preconditioning showed an improved reduction of infarct size, possibly by modulated Ca^{2+} and ROS concentration [117]. BK$_{Ca}$ mediated cardioprotection involves ROS, $Ca^{2+,}$ and MPTP and their interplay. It is anticipated that reduction of deleterious ROS through BK$_{Ca}$ activation prevents the excess release of Ca^{2+} from the endoplasmic reticulum subsequently reducing the influx and overload in mitochondria preventing the cell from injury.

5.4.3 Voltage-gated potassium channel

These are the most diverse family of K^+ channels. They are grouped into 12 families comprising 40 of the 90 genes present in human cells [124]. These channels mostly consist of six transmembrane helices (S1-S6) where two of them (S5-S6) form the loop and four of them are proceedings to the loop (S1-S4). The fourth positively charged loop senses the change in the membrane potential. These channels have a wide variety and therefore, they represent a fine regulation of K^+ flux in the homeostasis and pathological processes. The mitochondrial counterpart mitoKv1.3 is found in lymphocytes [125] and many carcinogenic cells [126, 127], they present similar physiological functions as the plasma membrane counterpart and, they are translated from the same gene. mitoKv1.3 is a target for pro-apoptotic protein Bax [128]. Their complex prevents the opening of mitoKv1.3 channel for K^+ influx and therefore causes the disturbance in membrane potential and eventually leads to apoptotic cell death. Therefore, mitoKv1.3 has represented itself as a new tool for targeted cell death for many tumor cells by triggering mitochondria-induced apoptosis. Their presence in cardiac cells has not been reported. Similar to Kv1.3 other potassium channels like Kv1.1 and Kv1.5 have also shown dual origin in both mitochondrial and plasma membrane causing cell apoptosis by targeting macrophages [129, 130].

5.4.4 Twin pore potassium channel

The mitochondrial TASK-3 was discovered in human keratinocyte HeCaT cells using the patch-clamp method [131]. It shows similarity with its plasma membrane counterpart and its activity is inhibited at acidic pH [132]. Lidocaine and low pH completely block the task channel activity in mitochondria. TASK-3 is essential for the survival of WM35 melanoma cells [133] but its activity in the mitochondrial dysfunction in cardiac reperfusion is not known.

6. Mitochondria channels as a therapeutic target of heart failure

The above discussions have made it clear that since the inner mitochondrial channels regulate the onset of apoptosis and cell death, they present an important target for cardioprotective therapeutics. Not only mitochondrial potassium channel but also MPTP, MCU, connexin-43, and protein uncoupling have shown their potential roles in reducing myocardial infarct size and preventing heart failure.

A sudden opening of MPTP can be triggered through a high concentration of Ca^{2+}, high amount of ROS production, and decrease in mitochondrial membrane potential, which results in the loss of proton gradient appearing as an uncoupling effect, which prevents ATP formation and promotes its hydrolysis [40]. Subsequently, the proton gradient utilizes the Ca^{2+} uptake and causes the matrix swelling as an approach of the MPTP to prevent the detrimental rise in Ca^{2+} in the mitochondrial matrix [134]. It is known that opening of MPTP for a short duration can proceed without affecting cell viability and can also contribute to cardioprotection through participating in pre ischemic conditioning and it later prevents the opening of MPTP pore during ischemic reperfusion preventing cell damage and the onset of heart failure [17]. Although the evidence to support its cardioprotective functions is majorly based on the pharmacology and genetic observation that avoided MPTP opening. It has been shown that the administration of cyclosporin A shows a cardioprotective effect by preventing MPTP opening in the mice model; however, the results are mixed for the large mammalian model [135].

As mentioned earlier an increase in the Ca^{2+} concentration contributes to the opening of the MPTP channel, it is also necessary to mention that the Ca^{2+} is essential for the key enzyme activation in the oxidation of the substrates that fuel the respiratory chain, followed by ATP formation. It is very unfortunate that despite the advancement in technologies we are still unable to determine the physiological and pathological concentration of Ca^{2+} in the mitochondrial matrix [136]. Nevertheless, the Ca^{2+} homeostasis is maintained within the mitochondrial matrix by the uptake of Ca^{2+} through uniporters and the release is catalyzed by Na^{+}/Ca^{2+} exchanger. The understanding of the molecular nature of Ca^{2+} uniporter has advanced our knowledge about Ca^{2+} homeostasis. The deletion of mitochondrial calcium uniporter gene from the embryonic and adult mice has shown completely contradicting results and the reasons of which have not yet been fully understood. However, the results that appeared in adult mice fully support the role of calcium overload leading to MPTP opening and eventually cell death. This is further supported by the $Na^{+}/Ca^{2+}/Li^{+}$ exchanger knockout mice which showed the overload of Ca^{2+} leading to MPTP opening on the onset of ischemic reperfusion leading to cell death. Therefore, the drugs that intend to target Ca^{2+} uniporter for therapeutics need to validate the contrast effects before large animal and clinical testing [137].

Connexin43(Cx43) is a well-known channel for the intercellular connections by forming the gap junctions, but apart from the plasma membrane occurrence, they are also known to be present in cellular organelle like subsarcolemmal mitochondria [138], nucleus [139], and exosomes [140]. Cx43 plays an important role in ischemic-reperfusion injury and its prevention. According to pharmacological evidence the concentration of Cx43 increases with the introduction of diazoxide DZX or fibroblast growth factor 2 to prevent myocardial injury, but this increase is not observed after the ischemic preconditioning protocol [141]. It also interacts with the mitochondrial potassium channel [141] and regulates nitric oxide

formation. The role of Cx43 is certain in cardioprotection but the exact mechanism and function remain to be elucidated.

At last, the presence of several mitochondrial K^+ channels and their activity in the failing heart presents them as a crucial target in the therapeutics of myocardial dysfunction. The K^+ uptake and release play a central role in the maintenance of mitochondrial matrix volume. The electrophoretic influx of K^+ is balanced by the K^+/H^+ antiporter [142]. Valinomycin triggers the uncontrolled K^+ influx disturbing the mitochondrial polarization and causing the swelling of the matrix. The function of potassium channels is basically to maintain the matrix volume. Initially, surface K^+ channel was suggested to play an essential role in ischemic pre and postconditioning but later when diazoxide (DXZ), which was involved in cardioprotection of non-contractable heart, did not show any effect on surface K^+ channel, whereas drugs that were only targeting surface K^+ did not show cardioprotective effect. On the contrary, the isolated mitochondria showed restored activity of ATP inhibited flux and showed inhibition caused by 5-hydroxydecanoate (5HD) [143]. This shifts the attention to the mitochondrial K channel for cardioprotection but ever since the DXZ and 5HD also affect mitochondrial physiology in general it requires the molecular structure information and in vivo attempts for concrete statements. The structural information will provide tools for determining its exact function in myocardial ischemia/reperfusion in a failing heart, Ca^{2+} transport and MPTP opening, and protein involved in ROS formation, followed by improving therapeutic approaches [144]. Similar to the ATP activated K^+ channel, Ca^{2+} and voltage-activated channels are also pharmacologically proven to play a similar role as mitoK_{ATP}, the ischemic conditioning protocol triggers the formation of protein kinase C which is helpful in an increased opening mitoK_{Ca} channel.

7. Current progress in the field

The strategies used to protect the heart from opening MPTP and mitochondrial calcium uniporter pores and in the case of ischemia conditioning, the opening of mitoK_{ATP} and BK_{Ca} channel plays a vital role in the cardioprotection. CsA is a well-known desensitizer for MPTP, but it did not prove to be the best option in clinical trials [65]. CsA exerts its activity by binding to CyPD, but in cases of intense stimuli, the pore opening becomes independent of CyPD. Therefore, there is a need of developing more pharmacological agents that can directly inhibit the MPTP openings, but they require further information about the structural insight of the pore. Although CyPD activates pore opening the complete mechanism is still unclear. Similarly, although it is evident that ROS and Ca^{2+} influence the MPTP opening but without knowing the structural details of MPTP we fail to conclude how they do so.

Mitochondrial calcium regulates a range of myocyte functions alongside energy production like cell division and trophism. With the development of MCU structures over the years, they have emerged as a very important target for cardioprotection, but the development of a reliable drug is still in process. Potassium channels are also widely accepted as an important target and are closely linked to modulating the apoptotic process. This information is present due to the pharmacology of the channel openers and inhibitors. Very limited knowledge is present to show concrete evidence. The molecular structure can be helpful in understanding and curing several mitochondria-associated diseases.

The inhibition of MPTP channel opening and the mitoK channel both elicit the cardioprotection and are likely to be related. Uptake of K^+ through mitoK decreased the mitochondrial membrane potential which reduces the mitochondrial Ca^{2+}, which in turn decreases the possibility of MPTP opening. Along with ATP production, and ROS regulation, mitochondrial channels like MPTP, Ca^{2+} channel, and mitoK channels are established to play a crucial role in cardioprotection. The mechanism, however, for their connection and coordination with each other in the process of cardioprotection is far from conclusive.

Recent attempts to translate cardioprotective strategies that target some of these mitochondrial ion channels have been hugely disappointing, and the translational of these strategies in clinical settings have not been successful. Several drugs have been tested on various animal models that have shown certain cardioprotective mechanisms. However, the lack of knowledge about the underlying mechanism of protective actions needs a lot of following studies to design modulators specific for mitochondrial channels with regards to cardioprotection in human trials. In the light of studies available, we still have a long way to go in the depth of the cardioprotective mechanism.

8. Conclusions

Conclusively, heart failure is an outcome of cardiac injury that originated due to a variety of etiologies and denotes a complex clinical syndrome. Several mitochondrial channels associated mechanisms have been recognized that drive the depletion of cardiomyocytes before cell death. These observations not only provide a link of overall heart health with mitochondrial channel opening and closing but also inspires therapeutic approaches. The core molecular identity of some mitochondrial channels like MCU and mitoK$_{ATP}$ are discovered recently, whereas most mitochondrial potassium channels are in their intermediate state. These channels act as switches to control the development of ischemic injury either towards recovery or the loss of viability. The progress towards understanding the molecular identity and mechanism of channel opening and inhibition will help to translate the experimental approaches into promising therapeutic development to combat a deadly health concern.

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Conflict of interest

The authors declare no conflict of interest.

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