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Review

Mitochondrial Ca^{2+} -activated K^+ channels and their role in cell life and death pathways Inge E. Krabbendam^a, Birgit Honrath^{a,b}, Carsten Culmsee^b, Amalia M. Dolga^{a,*}^a Faculty of Science and Engineering, Groningen Research Institute of Pharmacy, Department of Molecular Pharmacology, University of Groningen, 9713 AV Groningen, The Netherlands^b Institute of Pharmacology and Clinical Pharmacy, University of Marburg, 35043 Marburg, Germany

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ABSTRACT

Ca^{2+} -activated K^+ channels (K_{Ca}) are expressed at the plasma membrane and in cellular organelles. Expression of all K_{Ca} channel subtypes (BK, IK and SK) has been detected at the inner mitochondrial membrane of several cell types. Primary functions of these mitochondrial K_{Ca} channels include the regulation of mitochondrial ROS production, maintenance of the mitochondrial membrane potential and preservation of mitochondrial calcium homeostasis. These channels are therefore thought to contribute to cellular protection against oxidative stress through mitochondrial mechanisms of preconditioning. In this review, we summarize the current knowledge on mitochondrial K_{Ca} channels, and their role in mitochondrial function in relation to cell death and survival pathways. More specifically, we systematically discuss studies on the role of these mitochondrial K_{Ca} channels in pharmacological preconditioning, and according protective effects on ischemic insults to the brain and the heart.

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1. Mitochondrial dysfunction in disease

Mitochondria are vital for cell viability since they are responsible for energy production *via* oxidative phosphorylation (OXPHOS), regulation of calcium (Ca^{2+}) homeostasis, reactive oxygen species (ROS) generation, and apoptosis. Dysfunctional mitochondria have a major impact on neurodegenerative diseases. For instance, in Parkinson's Disease (PD), metabolic stress and mitochondrial damage can affect the viability of dopaminergic (DA) neurons in the substantia nigra [1–4], and α -synuclein aggregation, a hallmark of PD, has been strongly associated with mitochondrial dysfunction [5–8]. Specific α -synuclein forms bind to the mitochondrial TOM20 complex with high affinity, precluding the interaction of TOM20 with its co-receptor, TOM22 [9]. Translocation and binding of α -synuclein to TOM20 attenuates mitochondrial respiration, and depolarizes the mitochondrial membrane potential ($\Delta\Psi_m$), leading to increased mitochondrial superoxide production and impaired mitochondrial protein import. The loss of imported mitochondrial proteins was observed in DA neurons in the substantia nigra of post-mortem brain tissue from PD patients. These findings indicate a direct effect of α -synuclein on mitochondrial function and the involvement of mitochondrial integrity in the pathology of PD [5,7,10,11].

Increasing evidence suggests that accumulation of amyloid beta oligomers ($\text{A}\beta$ 1–42) in neuronal mitochondria *via* TOM proteins [12] may induce massive Ca^{2+} influx, leading to mitochondrial Ca^{2+} (mito[Ca^{2+}]) overload [13]. Subcellular fractionation studies and immune-electron microscopy demonstrated the import of $\text{A}\beta$ peptides into mitochondrial cristae in human cortical brain biopsies, which was independent of $\Delta\Psi_m$ [12,14]. Furthermore, $\text{A}\beta$ 1–40 and $\text{A}\beta$ 1–42 directly act on mitochondria by promoting mitochondrial dysfunction, oxidative damage and cell death [15]. Mitochondrial dysfunction can exert severe consequences to cell viability by releasing cytochrome c, triggering caspase activation and ultimately cell death [16–18].

Mitochondria actively regulate Ca^{2+} homeostasis within the cell. Mito[Ca^{2+}] uptake is one of the most efficient ways of lowering cytosolic Ca^{2+} in neurons under physiological conditions [19]. In these cells, cytosolic Ca^{2+} increase can arise from synaptic transmission, excitotoxicity or ischemic insults. The main function of mito[Ca^{2+}] is the stimulation of OXPHOS, by promoting both glycogen breakdown and glucose oxidation which leads to increased ATP supply [20–28]. On the other hand, pathological mito[Ca^{2+}] overload may lead to mitochondrial dysfunction and cell death. During ischemia, mito[Ca^{2+}] overload leads to increased ROS production which greatly increases with the rising oxygen tension at the onset of reperfusion [29]. Generation of mitochondrial ROS in association with mito[Ca^{2+}] overload triggers opening of the mitochondrial permeability transition pore (mPTP) at the inner mitochondrial membrane (IMM) [30]. mPTP formation causes fatal mitochondrial damage, attributed to $\Delta\Psi_m$ depolarization as a consequence to the dissipation of the electrochemical gradient. Loss of the electrochemical gradient causes ATP synthase to consume ATP, preceded by a transient but massive increase in ROS formation and release of Ca^{2+} to the cytosol. The consequent ROS release signals neighbouring mitochondria to do the same, eventually resulting in necrosis [31–38]. In conditions of oxidative stress, impaired Ca^{2+} homeostasis leads to mitochondrial dysfunction, which is characterized by changes in mitochondrial metabolism, such as alterations in ATP synthesis and NADP(H) oxidation, resulting in a vicious circle of further increased ROS production (Fig. 1) [39–41].

The superoxide anion is the main precursor of ROS, generating hydrogen peroxide that is further converted to oxygen and water by catalase enzymes or to the highly reactive hydroxyl radical through the Fenton reaction. The enzymes that generate superoxide anion include NADPH oxidases, cytochrome 450-dependent oxygenases

and xanthine oxidases. Within mitochondria, the superoxide anion is generated mainly by single electron transfer at redox centers of respiratory chain complexes I and III. In brain and muscle tissues, these sites of the mitochondrial respiratory chain complexes release the superoxide anion into the mitochondrial matrix by complex I and III, and into the intermembrane space by complex III [42]. Since generation of superoxides largely depends on the concentration of either oxygen or electron donors, processes that increase FMNH₂ concentration also increase superoxide production. Therefore, the process initiating superoxide formation by mitochondrial complex I activity is highly dependent on the mitochondrial NAD-redox state and on $\Delta\Psi_m$. Decreased $\Delta\Psi_m$ leads to increased respiratory chain electron flow and decreased complex I-mediated ROS formation [42–44].

In this context, diseased or damaged tissue often suffers from reduced mitochondrial activity, and is therefore susceptible to mitochondrial damage, examples being cardiac ischemia-reperfusion (I/R) injuries and pathological processes underlying neurodegenerative diseases [1,40,45–48]. Thus, mitochondria are major targets of cytoprotective approaches. Among these, one of the most promising approaches is mitochondrial preconditioning, in which harmful stimuli renders cells, tissues, and organs less susceptible to damage to subsequent harmful stimuli. The mechanism underlying this process seems to be partially related to the reduced driving force for mito[Ca^{2+}] influx or to superoxide radical synthesis. It has been proposed that mitochondrial potassium (K^+) channels regulate mitochondrial volume, acidification, Ca^{2+} homeostasis and mitochondrial integrity [49,50]. Thus, modifications of K^+ channel gating may have a major influence on the mitochondria-dependent cellular functions and, therefore on cell survival. In this review, we will discuss studies on preconditioning processes mediated through activation of mitochondrial K_{Ca} channels that results in cellular resilience (Graphical abstract). We will delineate the role of these different channels in mitochondrial function, and highlight their importance for the regulation of cell life and death pathways.

2. Mitochondrial Ca^{2+} -activated potassium (K_{Ca}) channels

The integrity of mitochondrial membranes, in particular the IMM, is essential for the generation and preservation of energy metabolism [51]. K^+ channels, such as the ATP-regulated K^+ (mito K_{ATP}) channel, the Ca^{2+} -activated K^+ (mito K_{Ca}) channel, the voltage-gated Kv1.3 K^+ (mitoKv1.3) channel and the two-pore domain TASK-3 K^+ (mitoTASK) channel are present at the IMM and regulate mitochondrial metabolism and $\Delta\Psi_m$ [50,52].

Since [Ca^{2+}] homeostasis is crucial for cell physiology, K_{Ca} channels in mitochondria are of particular importance. Activation of these channels modulates $\Delta\Psi_m$ and ROS production, and regulates mPTP opening. Maintenance of IMM integrity might therefore represent a novel target for neuroprotective strategies (Fig. 1) [53]. K_{Ca} channels include large conductance K_{Ca} channels (BK/ K_{Ca} 1/Slo1/Maxi-K/KCNMA1, 100–300 pS), intermediate conductance K_{Ca} channels (IK1/SK4/ K_{Ca} 3/KCNN4, 25–100 pS), and small conductance K_{Ca} (SK1-3/ K_{Ca} 2.1–2.3/KCNN1-3, 2–25 pS) channels. Recently a new terminology was introduced by Kaczmarek and colleagues [54]: the first group of K_{Ca} channels includes BK or K_{Ca} 1.1 channels and the sperm-specific K_{Ca} 5.1 (Slo3/KCNU1) channels. The second group includes two K^+ channels activated by Na^+ , such as K_{Na} 1.1 (Slack/Slo2.2/KCNT1; formerly K_{Ca} 4.1) and K_{Na} 1.2 (Slick/Slo2.1/KCNT2; formerly K_{Ca} 4.2). The third group includes the small conductance SK/ K_{Ca} 2.1–2.3 and the intermediate conductance IK/ K_{Ca} 3.1 channels [54]. K_{Ca} channels are expressed at the plasma membrane where they regulate after-hyperpolarization and the synaptic function of neuronal cells. On the other hand,

Fig. 1. Schematic overview of cell death and survival pathways. Ischemic stress insults on cell function and in particular on mitochondrial function are schematically shown, when mitochondrial K_{Ca} channels are closed (A) or opened/activated (B). Ischemic stress facilitates elevated levels of mito $[Ca^{2+}]$, increased ROS production, and higher probability of mitochondrial permeability transition pore (MPTP) opening and alterations, thereby leading to mitochondrial dysfunction and cell death. However, mito K_{Ca} activation preserves cell viability by lowering the generation of mitochondrial ROS and opening probability of MPTP.

K_{Ca} channels expressed at the IMM regulate mito $[Ca^{2+}]$, $\Delta\Psi_m$ and mitochondrial ROS formation [50]. K_{Ca} channel activation leads to flow of K^+ from the cytosol, where it is present at high concentrations, into the negatively charged mitochondrial matrix, resulting in mitochondrial membrane depolarization [55]. In consequence, the attenuated driving force for mito $[Ca^{2+}]$ uptake reduces mitochondrial Ca^{2+} overload. However, the function and regulation of these channels in different subcellular organelles is poorly understood.

2.1. Mitochondrial BK ($K_{Ca1.1}/BK_{Ca}$) channels

Among the K_{Ca} channels documented to be present in mitochondria, mitoBK channels were first described in LN229 human glioma cells in 1999 [54]. The presence of mitoBK channels at the IMM was demonstrated using patch-clamp recordings of the LN229 human glioma mitoplasts [56]. Afterwards, mitoBK channels were also identified in rat skeletal muscle, cardiac myocytes and human astrocytoma cells [57–59]. BK channel agonists such as NS1619 and NS004 decrease the $\Delta\Psi_m$. This depolarization was accompanied by inhibition of complex I of the mitochondrial respiratory chain in the human glioma cell line LN229, without reducing cell viability [60]. Hypoxia induces an increase in the opening probability of mitoBK channels, recorded in mitoplasts derived from LN229 and LN405 cells. The authors suggest that hypoxia causes a shift in the reversal potential of mitoBK channels into a positive direction, resulting in a greater mitoBK channel open probability and K^+ efflux from the mitochondrial matrix to the cytosol. It has also been indicated that the higher K^+ efflux might be a result of increased mito $[Ca^{2+}]$ concentrations. This rise in K^+ efflux is essential for maintaining cytosolic K^+ during hypoxia [61].

MitoBK channel topology indicates that toxin binding sites with affinity for charybdotoxin and iberiotoxin (mitoBK channel inhibitors) are exposed to the mitochondrial intermembrane space. Consequently, the mitochondrial matrix should contain the C-terminal tail domain with the Ca^{2+} binding site. Interestingly,

mitoBK channels in cardiac cells are encoded by the K^+ Ca^{2+} -activated channel subfamily M alpha 1 (*KCNMA1*) gene that also encodes the expression of BK channels at plasma membrane [62].

The α and $\beta 4$ subunit protein expression of BK channels were also detected in mitochondrial fractions isolated from H9c2 cells, a rat embryonic cardiomyoblast cell line [63]. MitoBK channels at the IMM of ventricular myocytes have been suggested to have similar properties as the plasma membrane BK channels [64]. Using single channel patch-clamp recordings of cardiac mitoplasts and mitochondrial K^+ flux measurements, mitoBK channels were identified as a component of the basal mitochondrial K^+ conductance.

The first study providing evidence for the existence of mitoBK channels in the brain was performed using fractionation of the rat CNS tissue, Western blot, immunocytochemistry and immunogold electron microscopy [65]. It was demonstrated that mitoBK channels are present at the IMM and co-reside with specific mitochondrial proteins, such as inner membrane marker cytochrome c oxidase (COX). Within the cell, expression of the $\beta 4$ subunit of mitoBK channels was restricted to a subpopulation of mitochondria. The highest expression level of the $\beta 4$ subunit was detected in the thalamus and in the brainstem [66]. These findings may support the perspectives for the neuroprotective role of mitoBK channels in specific brain structures and in brain pathologies related to these regions. In neurons, the BK channel at the IMM is sensitive to inhibition by iberiotoxin, but insensitive to charybdotoxin, suggesting different pharmacological properties than those classically described for BK channels located at the plasma membrane [67,68].

Dysregulated mitochondrial function with excessive ROS production and mito $[Ca^{2+}]$ overload are major factors underlying I/R injury [69,70]. K^+ channels that reside at the IMM regulate K^+ flux to the mitochondrial matrix. The K^+ entry through mitoBK channels depolarizes the $\Delta\Psi_m$, thereby reducing the driving force for Ca^{2+} influx into mitochondria [71]. Therefore, mitoBK channels have been recognized for their cytoprotective properties. Yet, there is not much agreement on how mitochondrial K^+ flux and mito $[Ca^{2+}]$

uptake influence the $\Delta\Psi_m$ in relation to mitoBK channel activation. Inconsistent findings might reside in the complexity of responses mediated by ROS generation sites, depending on either mitochondrial complex I or mitochondrial complex III in heart and brain mitochondria. Brain mitochondria are highly dependent on complex I-related superoxide generation sites (reverse electron flow) while heart mitochondria are largely dependent on complex III-related superoxide generation sites (forward electron flow). The “mild uncoupling” mediated by mitoBK channel activation in brain mitochondria induced a decrease in $\Delta\Psi_m$ and in the redox state of NAD. This uncoupling decreased mitochondrial superoxide production in a complex I-dependent manner [72–74]. Activation of pro-survival genes in the preconditioning phase is attributed to complex III-dependent ROS production [75,76]. The role of mitoBK channel openers in neuroprotection and cardioprotection will be further discussed in Sections 2.1.1 and 2.1.2.

2.1.1. Mitochondrial BK ($K_{Ca}1.1/BK_{Ca}$) channels in neuroprotection

While plasma membrane BK channels are major mediators of preconditioning, increasing evidence suggests mitoBK channels at the IMM of brain mitochondria contribute to protective effects against increased mito[Ca²⁺], thereby preserving cell viability in neurons [77,78]. For instance, the BK channel openers CGS7184 and NS1619 caused a flux of K⁺ from the cytosol into the mitochondrial matrix that lead to depolarisation of the $\Delta\Psi_m$ and a lower redox state of the NAD system, causing a reduction of ROS production by respiratory chain complex I of isolated rat brain mitochondria. The inhibition of ROS production was sensitive to the selective blockers of BK channel iberitoxin and charybdotoxin [72]. MitoBK channels were also shown to regulate mito[Ca²⁺], thereby decreasing the $\Delta\Psi_m$ [67].

In an *in vitro* model of oxidative stress, high glutamate concentrations led to mitochondrial dysfunction and cell death in immortalized hippocampal HT22 cells. Since HT22 cells do not express functional ionotropic glutamate receptors, the glutamate toxicity in these cells is mediated by competitive inhibition of cystine uptake by the glutamate/cystine antiporter, leading to the loss of glutathione-dependent antioxidant defenses. Subsequent generation of lipid peroxides and mitochondrial translocation of pro-apoptotic proteins leads to dissipation of $\Delta\Psi_m$ and potentiates ROS formation in the mitochondria. Together with excessive accumulation of mito[Ca²⁺] and inhibition of mitochondrial respiration, these events cause irreversible mitochondrial breakdown and cell death. [79,80,89,129]. The BK channel inhibitor paxilline protects HT22 cells against glutamate toxicity [81]. Paxilline is a mycotoxin produced by the fungus *Penicillium paxilli* and has been suggested to be an inhibitor of BK channels [82]. Patch-clamp experiments showed that paxilline was able to block plasma membrane BK channels [83]. Using flavoprotein fluorescence as an index to assess mitoBK channel activity, it was found that paxilline specifically inhibited the NS1619-induced oxidation of flavoproteins in intact cardiomyocytes. Paxilline is lipophilic, thus it is expected to cross plasma membranes and therefore it might affect mitoBK channels [82,84]. However, the protective effects of paxilline against glutamate toxicity in HT22 cells were independent of BK channel activation, since other mitoBK channel inhibitors such as charybdotoxin and iberitoxin, did not preserve cell viability in the presence of glutamate. Instead, paxilline could have induced the observed protection by modulation of Ca²⁺ homeostasis, since it blocked the activity of the inositol trisphosphate receptor (IP₃R) and the sarco/endoplasmic reticulum Ca²⁺ ATPase [85,86].

Substrates of the respiratory chain, such as NADH, succinate and glutamate/malate decreased mitoBK channel activity, and these effects were abolished by inhibitors of the respiratory chain such as rotenone, antimycin A and cyanide [59] indicating coupling of the

mitoBK channel to the mitochondrial respiratory chain. BK channel α and $\beta 4$ subunits are expressed at the IMM of neurons [87]. Modifications in mitoBK channel opening properties were found in membrane preparations from a rat model of A β neurotoxicity, an effect that could be linked to a change in mitoBK channel $\beta 4$ and - α subunit expression attributed to an A β -induced increased ROS production and to an enhanced accumulation of mitochondrial A β [88].

In hippocampal slice cultures exposed to glutamate, pre-incubation with NS1619 decreased neuronal cell death. Mitochondrial respiration measurements using high-resolution respirometry indicated an NS1619-induced increase in basal respiration, supporting a role for mitoBK channels in neuroprotection [89]. More specific effects of NS1619-induced neuronal preconditioning were studied in rat cortical neurons in models of oxygen-glucose deprivation, hydrogen peroxide (H₂O₂)-mediated oxidative damage, and glutamate excitotoxicity. To study the preconditioning effects, the immediate actions of NS1619 were studied on cultured neurons and isolated mitochondria where it depolarized mitochondria and induced a dose-dependent ROS generation. A slight increase in mitochondrial ROS generation enables specific adaptive cellular responses to promote cell resilience against subsequent toxic stimuli with an overall attenuating effect of ROS generation, underlying the molecular pathways of mitohormesis hypothesis. NS1619 hyperpolarized the neuronal cell membrane, and activated the phosphoinositide 3 kinase (PI₃K) signaling cascade. BK channel blockers only attenuated effects on cell membrane potential while other effects mediated by NS1619 such as the increased ROS production remained unchanged. Further studies revealed that NS1619 also inhibited the activation of caspase-3/7, a downstream target of caspase-9, the main enzyme responsible for apoptotic cell death [90]. These results indicated that NS1619 was a potent inducer of delayed neuronal preconditioning by regulating ROS generation, activating the PI₃K pathway, and inhibiting caspases. However, application of BK channel blockers did not counteract the observed neuroprotection [91].

To overcome the drawbacks of the nonselective agonist NS1619, the more selective and specific BK channel opener NS11021 has been used to investigate the potential neuroprotective effect of BK channel opening on glutamate-induced excitotoxicity in primary rat cortical neurons. Activation of BK channels via NS11021 induced neuroprotection in cortical neurons, suggesting a neuroprotective role of NS11021 in suppressing glutamate neurotoxicity, attenuating ER stress and preserving mitochondrial function [92,93]. Finally, the mitochondrial division inhibitor (mdivi-1), targeting dynamin-related protein1, exhibited protective effects in ischemic injury [94–96] by regulating the activation of BK channels as shown by increased BK channel expression levels, attenuated oxidative stress, mitochondrial dysfunction and neuronal apoptosis [97].

There is growing evidence that dysfunctional mitochondria are associated with hyperglycemia and insulin resistance and are therefore involved in the development and progression of diabetes [98,99]. Since mitochondrial K⁺ channels are involved in mitochondrial function, and since reports associated mitochondrial dysfunction with diabetes conditions, it was hypothesized that the function of mitoBK channel might be altered in diabetes. Indeed, the channel gating and permeation properties were affected in rat brain mitochondrial membranes prepared from a model of streptozotocin-induced diabetes. This effect was potentially linked to down-regulation of mitoBK α and $\beta 4$ subunits expression levels and an increase in ROS production in high glucose conditions [100].

Overall, studies described here indicate a protective role of mitoBK channels by attenuating overall ROS production, thereby preserving mitochondrial function and cell survival (Fig. 2).

Fig. 2. MitoK_{Ca} channel function. Increased mito[Ca²⁺] during ischemic insults would arise from activation of the *N*-methyl-D-aspartate receptors (NMDAR) in the plasma membrane or from efflux from endoplasmic reticulum (ER) via for example the sarco/endoplasmic reticulum Ca²⁺ ATPase (SERCA), inositol trisphosphate receptor (IP₃R) and ryanodine receptor (RyR) that coordinate release of Ca²⁺ from the ER (A). Opening of mitochondrial potassium (mitoK_{Ca}) channels residing at the IMM contribute to cell survival by regulation of ROS production and mitochondrial Ca²⁺ levels. MitoK_{Ca} channel activation restores the mitochondrial membrane potential, attenuates the formation of mitochondrial ROS as well as mito[Ca²⁺] overload (B). Moreover, it also prevents against the loss of ATP production at the site of the mitochondrial electron transport chain (C).

2.1.2. Mitochondrial BK ($K_{Ca}1.1/BK_{Ca}$) channels in cardioprotection

Mitochondria account for approximately one-third of the mass of the heart, play an essential role in maintaining cell function, and act as a buffer for increases in intracellular Ca^{2+} during ischemia [29].

Volatile anaesthetics, such as isoflurane, sevoflurane and desflurane, exhibit cardioprotective effects and attenuate myocardial infarct damage after I/R injury [101]. This process is termed anaesthetic-induced preconditioning when these volatile compounds are provided before ischemia is induced, and anaesthetic-induced postconditioning when they are administered in the early phase of reperfusion [102,103]. Increasing evidence suggests that activated mitoBK channels are involved in mechanisms of such preconditioning or postconditioning that reduce the infarct size following ischemic stress. The BK channel opener NS1619 halved the size of myocardial infarct of perfused guinea pig hearts subjected to global I/R, suggesting a protective function of mitoBK channels against necrotic and apoptotic cell injury after ischemia [64].

The NS1619-induced protection in the heart may be mediated through the process of preconditioning *via* inhibition of the mitochondrial respiratory chain [104]. Experiments in isolated ventricular cardiomyocytes from guinea pigs suggest that opening of mitoBK channels by NS1619 prevented mito[Ca^{2+}] overload upon reperfusion of the heart. The decrease in mito[Ca^{2+}] was accompanied by restored $\Delta\Psi_m$ and reduced ROS production. The effects of NS1619 were antagonized by the BK channel blocker paxilline. Furthermore, low concentrations of NS1619 induced mitochondrial ROS release, thereby initiating pharmacological-induced mitochondrial preconditioning. The NS1619-induced protective effects were dependent on the production of ROS [105–107]. The cAMP-dependent protein kinase A (PKA) pathway potentiated the opening of mitoBK channels and conferred cardioprotection [84]. It is already known that BK channels in smooth muscle cells can be activated by cAMP-dependent PKA [108]. However, to investigate protective mechanisms against cardiac injury and the role of kinase-mediated regulation of mitoBK channels in these processes, Sato and colleagues assessed mitoBK channel activity using flavoprotein oxidation measurements. It was found that the cell-permeable cAMP analogue 8Br-cAMP potentiated the NS1619-induced flavoprotein oxidation, suggesting that the mitoBK channel might be modulated by PKA [84]. Furthermore, Cilostazol, a phosphodiesterase type 3 inhibitor that activates cAMP-dependent PKA, activated mitoBK channels and reduced the infarct size in rabbit hearts [109] indicating a cytoprotective interplay between PKA signaling and mitoBK channels.

In addition to specific BK channel openers, a number of studies reported the involvement of mitoBK channels in the signaling pathway of agents leading to cardioprotection. This list includes among others adenosine [110], β -estradiol [111], and desflurane, which mediated anaesthetic preconditioning *via* activation of PKA [112]. The activity of cardiac mitoBK channels, containing an auxiliary $\beta 1$ -subunit was enhanced by 17 β -estradiol which increased the survival of myocytes under simulated ischemia [111]. Anti-ischemic effects of naringenin, a major flavonoid found in fruits of the genus *Citrus*, have been described in experimental I/R. Naringenin induced preconditioning in acute infarct in rat hearts, partly *via* activation of mitoBK channels [113]. Sildenafil, a phosphodiesterase-5-inhibitor, shares a common signaling pathway with mitoBK channels *via* regulation of protein kinase G (PKG), and sildenafil-mediated acute cardioprotection in rat hearts [114] was dependent on PKG mediated mitoBK channel activation [73]. The agents described above prevented I/R injuries in the heart when applied prior to ischemia, and this protection was reduced by co-administration of BK channel inhibitors such as paxilline, indi-

cating that pro-survival pathways are promoted by the BK channel activity. BK channels also mediate cardioprotection induced by tumor necrosis factor- α (TNF- α). The biological effects of TNF- α are mainly mediated through its two receptors TNF-R1 and TNF-R2. TNF-R1 is expressed in almost all cell types and exhibits apoptotic effects, while activation of TNF-R2 might promote cell survival, proliferation and differentiation [115–119]. TNF- α protects the myocardium against I/R injury by inhibiting mPTP opening as well as activating K_{Ca} channels, probably the mitoBK channel [120]. In addition, ischemic injury in cardiac myocytes was reduced by activating mitoBK channels with 12,14-dichlorodehydroabiatic acid [121] and rottlerin, likely *via* effects on restored $\Delta\Psi_m$ and reduced ROS generation [122]. Morphine induced preconditioning reduced the infarct size which was abolished by paxilline, confirming that this preconditioning effect of morphine also involved activation of BK channels [123].

MitoBK channels play a role in remote ischemic preconditioning (RPC) by protecting the heart from injury evoked by subsequent exposure to severe I/R. Treatment with NS1619 protected hearts against I/R injury. In addition, hypoxia was shown to contribute to increased mitoBK channel opening probability *via* improved mitochondrial function [124]. Using paxilline and NS1619, Borchert and colleagues found that mitoBK channels contribute to protection of cardiomyocytes isolated from chronically hypoxic rats [92]. In addition, the antioxidant tempol, a triphenylphosphonium (TPP)-based mitochondria targeting free radical scavenger [45,125], abolished neuroprotection mediated by mitoBK channel opening, indicating that the neuroprotective property of BK channel activators depends on ROS signaling. Indeed, chronic hypoxic rats that received brief re-oxygenation lost neuroprotective effects mediated by BK channel openers [126].

Extensive research describing the activity/effects of BK channel opening focused on the potency of the activator NS1619. It was indicated that the activator NS1619 partially inhibited mitochondrial respiration in isolated heart mitochondria, as evidenced by respiratory measurements of state 3 [127]. However, unspecific K^+ transport was observed after NS1619-induced activation of mitoBK channels [127] suggesting that NS1619 possibly exerted effects on mitochondrial function independent of mitoBK channels. In addition, the cardioprotective role of mitoBK channels was investigated using another BK channel activator, NS11021, a potent, selective, and chemically unrelated to NS1619. Electrophysiological recordings showed that NS11021 activated BK $\alpha + \beta 1$ channel subunits. Activation of BK channels is highly dependent on mitoBK α -subunits containing two N-glycosylation sites at their C-terminus being co-expressed with $\beta 1$ channel subunits [128]. According to the same study, in isolated perfused rat hearts subjected to I/R, NS11021 administered prior to ischemia or at the onset of reperfusion significantly attenuated the infarct size. Interestingly, ischemic – and anaesthetic preconditioning protocols efficiently protected BK-negative hearts from I/R injury [129] although neuroprotective effects of anaesthetic preconditioning were sensitive to paxilline in the BK-negative hearts. In contrast, Soltysinska and colleagues presented evidence for paxilline- and NS11021-sensitive BK channel-mediated currents in mitochondria from wild-type but not BK-negative cardiomyocytes [130].

BK channels have been shown to mediate ischaemic induced postconditioning [123,131]. The role of mitoBK channels in postconditioning induced by volatile anaesthetics remains unclear, however it was shown that administration of NS1619 decreased myocardial infarct size after I/R injury [132]. Overall, these data indicated that ischemic preconditioning requires mitoBK channels to enable a proper OXPHOS activity of mitochondria, allowing ADP stimulated respiration upon exposure to oxygen in the

myocardium, and limit the I/R injuries to the myocardium possibly via attenuating ROS generation.

2.2. Mitochondrial IK ($K_{Ca3.1}/SK4/IK_{Ca}/IK$) channels

Mitochondrial IK (mitoIK) channels were identified in mitochondria isolated from the human colon tumor cell line HCT116 [133]. Moderate increases in $\text{mito}[\text{Ca}^{2+}]$ caused mitoIK opening, thus linking K^+ permeability and transmembrane potential of the IMM to Ca^{2+} signaling. The mitochondrial presence of IK channels was demonstrated in HeLa cells of human cervix adenocarcinoma origin, and in mouse embryonic fibroblasts, but not in two colorectal adenocarcinoma cell lines: the human Caco-2, and the mouse C-26 cell line [134]. As yet, there are no studies reporting the presence and activation of mitoIK channels in relation to neuro- or cardioprotection.

2.2.1. Mitochondrial IK ($K_{Ca3.1}/SK4/IK_{Ca}/IK$) in disease pathology

Functional assays explored the hypothesis that mitoIK channels might regulate cell proliferation or play a role similar to that of the lymphocyte Shaker-type $\text{K}_v1.3$ channel which interacts with OMM-inserted apoptotic protein Bax, thereby initiating apoptosis [135]. However, the specific IK channel inhibitor TRAM-34 did not influence cell proliferation or cell death [134]. In contrast, mitoIK channels expressed in mitochondrial fractions of melanoma cells play a role in cell survival. The activation of mitoIK channels lead to hyperpolarization of $\Delta\Psi_m$ in response to TRAM-34 [136]. $\text{K}_v1.3$ inhibition induced a similar effect on $\Delta\Psi_m$ hyperpolarization and resulted in apoptosis [135].

Pancreatic ductal adenocarcinoma (PDAC) represents the most common form of pancreatic cancer, and frequent mutations in PDAC have severe impacts on the metabolism of tumor cells. To identify novel transporters or channels that regulate the OXPHOS in pancreatic tumor cells, a Seahorse XF Analyzer-based siRNA screen was established in the Mia PaCa-2 line, a cell model for PDAC. The siRNA that showed the greatest change in oxygen consumption rate (OCR) was targeting the *KCNN4* gene, which encodes for the IK channel. The presence of mitoIK channels in this cell line suggests a role of these channels in metabolic processes. Its importance in regulating oxygen consumption was suggested by metabolic analyses in permeabilized Mia PaCa-2 cells [137].

2.3. Mitochondrial SK ($K_{Ca2.1-3}/SK1-3$) channels

In addition to their expression at the plasma membrane, SK channels were identified at the IMM of both neurons and cardiomyocytes, where they play an important role in cell survival (Fig. 2) [138–140].

In murine hippocampal HT22 cells the expression of SK2 channels was identified at the IMM of isolated mitoplasts using patch-clamp recordings [138]. Pharmacological activation of these potassium channels promoted a slight $\Delta\Psi_m$ depolarization and a small increase in the generation of mitochondrial ROS [138]. However, transient overexpression of mitoSK2 channels did not increase mitochondrial superoxide production or depolarize $\Delta\Psi_m$ [141]. Mechanistic analysis revealed that mitoSK channel activation with CyPPA attenuated $\text{mito}[\text{Ca}^{2+}]$ uptake in response to ATP and in response to IP_3R -dependent ER-Ca^{2+} release induced by carbachol in HT22 cells. Furthermore, both pharmacological activation of SK channels with CyPPA and genetic overexpression of mitoSK channels substantially decreased mitochondrial respiration [141].

2.3.1. Mitochondrial SK ($K_{Ca2.1-3}/SK1-3$) channels in neuroprotection

The role of mitoSK channels has been established in a model of glutamate toxicity, which initiates oxidative stress and subse-

quent mitochondrial dysfunction in HT22 cells. In conditions of glutamate toxicity, SK channel activation by CyPPA and overexpression of mitoSK2 channels enhanced mitochondrial resilience and reduced cell death in HT22 cells [138,141]. Similarly, $\text{mito}[\text{Ca}^{2+}]$ uptake in primary cortical neurons (PCN) was reduced by CyPPA and NS309, another SK channel activator, in conditions of excitotoxicity and ATP-dependent $\text{mito}[\text{Ca}^{2+}]$ stimulation [141]. KB-R7943, a blocker of the reverse mode $\text{Na}^+/\text{Ca}^{2+}$ exchanger (mNCX) [142] prevented $\text{mito}[\text{Ca}^{2+}]$ uptake in HeLa cells [143] and in HT22 cells in response to ATP and carbachol [141]. In line with our studies where CyPPA or NS309 attenuated $\text{mito}[\text{Ca}^{2+}]$ uptake and promoted protection against oxidative stress in PCN, pre-treatment with KB-R7943 prevented glutamate-induced cell death in HT22 cells [141]. In the same study, inhibition of mitochondrial complex III with antimycin A attenuated $\text{mito}[\text{Ca}^{2+}]$ uptake and promoted neuroprotection against glutamate toxicity in HT22 cells. These studies indicate the tight association between $\text{mito}[\text{Ca}^{2+}]$ and mitochondrial respiration and their effect on cell survival and suggest that neuroprotection mediated by SK channel activation is regulated by attenuated $\text{mito}[\text{Ca}^{2+}]$ uptake and mitochondrial respiration.

Increased ROS and $\Delta\Psi_m$ depolarization under basal conditions might be linked to a preconditioning effect triggering mitohormesis, a process where initiation of endogenous mitochondrial oxidative stress responses leads to activation of adaptive mechanisms to prevent cell death in subsequent events of oxidative stress. Comparable results were found using cells challenged with exogenous H_2O_2 [144]. CyPPA induced ROS formation, slight ATP depletion and reduced mitochondrial respiration without inducing cell death under basal conditions. In contrast, during H_2O_2 toxicity, CyPPA pre-treatment preserved mitochondrial integrity and blocked cell death, further supporting a role for SK channel mediated preconditioning in neuroprotection.

As mitochondrial dysfunction has been associated with the pathogenesis of PD [145], it was also investigated whether mitoSK channels may exert protective effects in differentiated dopaminergic neurons. Indeed, in human dopaminergic neurons SK channel activation provided protection against rotenone-induced loss of the dendritic network and cell death [146].

2.3.2. Mitochondrial SK ($K_{Ca2.1-3}/SK1-3$) channels in cardioprotection

Functional expression of mitoSK channels was reported in guinea pig hearts [139]. It was postulated that activation of SK channels induced a preconditioning effect similar to that elicited by a BK channel opener, and that this effect was mediated via functional SK channels located at the IMM that modulate K^+ influx and are Ca^{2+} responsive. Indeed, it was shown that activation of SK channels by 1-EBIO prior to I/R injury reduced the infarct size [139], thus showing pharmacological preconditioning effects similar to the mitoBK channel opener NS1619 [147]. A prominent role for ROS in SK channel-mediated preconditioning was suggested as a superoxide dismutator targeting ROS in the mitochondrial matrix reversed the protective effects of 1-EBIO. During I/R injury, SK channel activation successfully restored redox homeostasis and attenuated $\text{mito}[\text{Ca}^{2+}]$ load similar to the effect of SK channel activation in models of neuronal cell death [139].

Recently, the expression of the SK3 channel splice variant in the IMM of guinea pig heart mitochondria was confirmed by Western blot [140]. In the same study, SK3 channel splice variants were identified in mitochondria of human ventricular tissue, as demonstrated by visualizing immunogold labeled particles in isolated mitochondria with high resolution immune-electron microscopy (IEM). By using a double-labeled immunofluorescence staining technique, confocal microscopy imaging and quantitative line scan analyses, SK3 channel protein was shown to co-localize with COX I (complex

IV) in mitochondria of rat ventricular myocytes. The SK channel agonist DCEB improved cardiac function and reduced infarct size after I/R injury in guinea pig isolated hearts and in rat hearts *in vivo*. These effects were reversed by the SK channel agonist NS5953. At the level of mitochondria, I/R influenced oxygen consumption. Respiratory control indexes were higher when treated with DCEB compared to I/R alone. The overexpression of SK3.1 channels in mouse atrial cardiomyocyte tumor cells (HL-1) enhanced Ca^{2+} -activated K^+ uptake, as directly measured by K^+ -selective fluorescent indicator PBFI-AM. Overexpression of SK3.1 channels in HL-1 cells was protective against hypoxia and reoxygenation injury. However, cell death was enhanced when knockdown of SK3 channels was induced by siRNA. In addition, in these SK3 channel knockdown HL-1 cells, the $\Delta\Psi_m$ was greatly lower. These results suggest that SK3 channel activation may protect against hypoxia and I/R induced cell damage in mitochondria of cardiomyocytes.

3. Concluding remarks

Mitochondrial dysfunction induced by excessive ROS production and $\text{mito}[\text{Ca}^{2+}]$ overload are major factors underlying pathological processes causing degeneration of neurons or heart cells. K^+ entry into the mitochondrial matrix through K_{Ca} channels located at the IMM depolarizes the mitochondrial membrane and reduces the driving force for Ca^{2+} influx into mitochondria, thereby influencing cell survival signaling. Large Ca^{2+} influx into mitochondria inhibits the mitochondrial respiratory chain, and this process can be prevented by pharmacological activation of mitoK_{Ca} channels. A large number of studies investigated the molecular mechanisms underlying preconditioning and identified a substantial role for mitoK_{Ca} channels in these processes in different paradigms of neurodegeneration and cardiac ischemia. Preconditioning and postconditioning mechanisms elicited by activated mitoBK channels reduce the infarct size due to ischemic stress via attenuation of mitochondrial ROS production, lowering $\text{mito}[\text{Ca}^{2+}]$ and inhibiting mPTP opening. Both neuronal mitoBK channels and mitoSK channels mediate protection by maintaining mitochondrial function, and mitoSK channels were recently shown to directly regulate mitochondrial respiration. However, the exact mechanism underlying the reduced mitochondrial respiration is still unclear and further studies are required as to identify the potential effects on individual mitochondrial complexes. mitoIK channels have not been studied at the IMM of neurons or cardiomyocytes, rendering them an interesting option for future studies. Although they are present at the IMM of several tumor cell lines where they could play a role in OXPHOS, their exact contribution to mitochondrial function in relation to cell function and survival still remains to be elucidated. Further investigations on the K_{Ca} channels and their influence on mitochondrial metabolism will help to understand how these intracellularly expressed channels influence cell viability, and how they can be distinguished from K_{Ca} channels at the plasma membrane.

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