

Mitochondrial K⁺ transport and cardiac protection during ischemia/reperfusion

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Abstract

Mitochondrial ion transport, oxidative phosphorylation, redox balance, and physical integrity are key factors in tissue survival following potentially damaging conditions such as ischemia/reperfusion. Recent research has demonstrated that pharmacologically activated inner mitochondrial membrane ATP-sensitive K⁺ channels (mitoK_{ATP}) are strongly cardioprotective under these conditions. Furthermore, mitoK_{ATP} are physiologically activated during ischemic preconditioning, a procedure which protects against ischemic damage. In this review, we discuss mechanisms by which mitoK_{ATP} may be activated during preconditioning and the mitochondrial and cellular consequences of this activation, focusing on end-effects which may promote ischemic protection. These effects include decreased loss of tissue ATP through reverse activity of ATP synthase due to increased mitochondrial matrix volumes and lower transport of adenine nucleotides into the matrix. MitoK_{ATP} also decreases the release of mitochondrial reactive oxygen species by promoting mild uncoupling in concert with K⁺/H⁺ exchange. Finally, mitoK_{ATP} activity may inhibit mitochondrial Ca²⁺ uptake during ischemia, which, together with decreased reactive oxygen release, can prevent mitochondrial permeability transition, loss of organelle function, and loss of physical integrity. We discuss how mitochondrial redox status, K⁺ transport, Ca²⁺ transport, and permeability transitions are interrelated during ischemia/reperfusion and are determinant factors regarding the extent of tissue damage.

Key words

- Mitochondria
- Potassium
- Calcium
- Free radicals
- Heart
- Ischemia

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Ischemia, reperfusion and ischemic preconditioning

The interruption of blood supply to a tissue (ischemia) followed by re-establishment of oxygenation and nutrition (reperfusion) causes extensive damage to cardiac tissue. Indeed, heart infarction is a leading cause of morbidity and mortality worldwide (1).

A major consequence of ischemia is the inhibition of aerobic energy metabolism due to a quick reduction in tissue oxygen levels. Mitochondrial oxidative phosphorylation stops and tissue ATP levels decline, stimulating glycolysis, which quickly depletes fermentable substrates. The decline in ATP levels is increased by the inverse function of mitochondrial F₀F₁ ATP synthase which, in

the absence of oxygen and a membrane potential, hydrolyzes ATP in a manner only partially decreased by its inhibitory subunit (2).

Given the central role of mitochondrial energy metabolism in the maintenance of intracellular levels of high-energy phosphates, it is not surprising that mitochondrially active drugs are effective regulators of tissue damage following ischemia/reperfusion. In the early 1990's, cyclosporin A, which prevents mitochondrial permeability transition (MPT), was shown to be a potent inhibitor of cardiac cell death promoted by ischemia/reperfusion (3). MPT consists of a loss of inner membrane impermeability to ions, water and even small proteins caused by excessive mitochondrial Ca^{2+} accumulation and oxidative stress (4,5). Thus, the finding that this process participates in ischemic cell death indicated that not only a lack of oxidative phosphorylation was involved in this damage, but also functional and structural alterations of mitochondria themselves. Indeed, MPT occurs predominantly during reperfusion, when most cardiac damage occurs, despite the possibility of re-establishing oxidative phosphorylation (6).

In 1997, another mitochondrially active drug, diazoxide (DZX), a selective agonist of ATP-sensitive K^+ channels (K_{ATP}) in inner mitochondrial membranes, was found to be strongly cardioprotective (7). Since this finding, the consequences of mitochondrial K_{ATP} ($\text{mitoK}_{\text{ATP}}$) opening and possible mechanisms of cardioprotection promoted by this channel have attracted much attention in the literature (for a review, see Ref. 8). In addition, the role of $\text{mitoK}_{\text{ATP}}$ in the protection of other tissues has also been studied (9).

In the heart, $\text{mitoK}_{\text{ATP}}$ opening is a key step in ischemic preconditioning (IP) (7,10), an endogenous mechanism which leads to the prevention of damage promoted by ischemia/reperfusion. IP consists of one or

more brief sublethal episodes of ischemia, each separated by brief periods of reperfusion (11). This procedure paradoxically protects the heart against necrosis, arrhythmia and functional damage promoted by subsequent ischemia (11,12). IP promotes both an early phase of protection that lasts a few hours and a second window of protection (13,14) that appears 1-2 days after the preconditioning event and may persist for a few days. Evidence that $\text{mitoK}_{\text{ATP}}$ is involved in IP includes measurements of increased ATP-sensitive mitochondrial K^+ transport after IP (15) and the finding that IP is reversed by $\text{mitoK}_{\text{ATP}}$ antagonists (16,17).

Mitochondrial K^+ transport

While the outer mitochondrial membrane is permeable to ions due to the presence of the voltage-dependent anion channel (18), the inner membrane must be highly impermeable to cations in order to maintain the inner membrane potential ($\Delta\psi$) necessary for oxidative phosphorylation (19). Proton pumping from the matrix into the intermembrane space by the electron transport chain generates $\Delta\psi$ and also creates a strong tendency toward the uptake of cations into the matrix due to its negative charge. Because of the relative impermeability of the inner mitochondrial membrane, cation leakage is limited. However, K^+ , the main intracellular cation, continuously leaks into the matrix at a slow rate. In order to compensate for this leakage and to prevent excessive matrix swelling with accumulated K^+ , it has long been known that mitochondria present an inner membrane K^+/H^+ antiporter, which removes K^+ using the proton gradient (8) (see Figure 1).

In 1991, Inoue et al. (10) showed that rat liver mitochondria present ATP-sensitive K^+ transport in their inner membrane, the first indication of the presence of a K^+ uniporter in mitochondria. An K_{ATP} ($\text{mitoK}_{\text{ATP}}$) was later isolated and reconstituted by Garlid's

group (20). This group found ATP-sensitive K⁺ transport in reconstituted protein samples extracted from mitochondria containing two bands: a 55-kDa protein (mitoKIR) which functions as the K⁺ channel and a 63-kDa protein (mitoSUR) which functions as a regulator (21). On the other hand, Marban's group (22) has found mitoK_{ATP} activity in reconstituted fractions containing a different set of proteins including succinate dehydrogenase. These investigators suggest that succinate dehydrogenase inhibition is a necessary step for pharmacological activation of mitoK_{ATP}, while Garlid's group believes succinate dehydrogenase inhibition is a consequence of toxic concentrations of some mitoK_{ATP} agonists such as DZX (23). As a result, the molecular identity and regulatory mechanisms of this channel remain controversial.

Although the mitoK_{ATP} model proposed by Garlid's group presents structural similarities to plasma membrane K_{ATP}, its regulatory properties are different. Unlike other K_{ATP}, mitoK_{ATP} is inhibited by both ATP and ADP, and activated by both GTP and GDP. Inhibition by ATP or ADP requires the presence of Mg²⁺ ions (20). MitoK_{ATP} is also physiologically inhibited by long-chain acyl-CoA esters (8,24). Since mitoK_{ATP} affinity for ATP and ADP is much higher than intracellular concentrations, cytosolic levels of GDP control the open state while long-chain acyl-CoA esters determine the closed state of this channel *in vivo* (24). In addition, mitoK_{ATP} is regulated pharmacologically by a variety of drugs, including nicorandil, DZX, BMS-191095, and pinacidil (agonists), and 5-hydroxydecanoate and glibenclamide (an antagonist). DZX and 5-hydroxydecanoate are useful as experimental tools since they do not affect plasma membrane K_{ATP} when used at micromolar concentrations sufficient to affect mitoK_{ATP} (7,17,25).

The presence of a K⁺ uniporter allows the entry of positively charged ions into the matrix, an effect that could potentially disrupt $\Delta\psi$ and oxidative phosphorylation. How-

ever, mitoK_{ATP} does not have the ability to hamper oxidative phosphorylation because it presents very low K⁺ transport rates capable of decreasing $\Delta\psi$ only marginally (23). This small increase in K⁺ influx causes a significant increase in matrix volume (23), leading to the hypothesis that prevention of excessive mitochondrial contraction is a central function of mitoK_{ATP}.

Another physiological function of mitoK_{ATP} was proposed initially by Holmuhamedov et al. (26), who suggested that channel opening could regulate mitochondrial Ca²⁺ uptake. Although later experiments (23) demonstrated that the changes in $\Delta\psi$ promoted by mitoK_{ATP} under physiological conditions are insufficient to alter Ca²⁺ uptake, recent evidence has suggested that mitoK_{ATP} may have a significant effect on Ca²⁺ transport under conditions in which respiration is hampered (27,28, see discussion below).

Finally, an important function of mitoK_{ATP} may be to regulate the release of mitochondrial reactive oxygen species (ROS). Mitochondria are a significant intracellular source of ROS, generated mostly from superoxide radicals formed by monoelectronic reduction of oxygen at early steps of the electron transport chain. Electron "leakage" at these points is strongly dependent on electron transport rates, with faster rates decreasing elec-

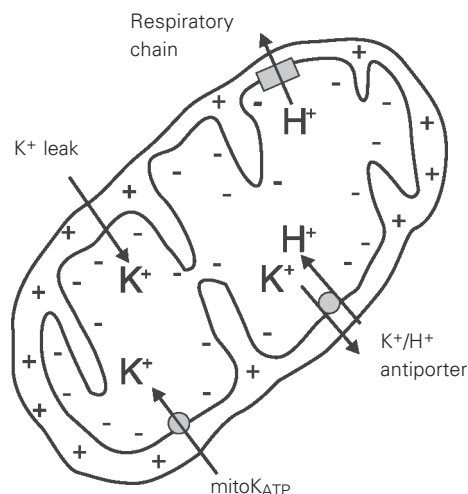


Figure 1. K⁺ transport across mitochondrial membranes. Mitochondrial inner membranes present a K⁺ leak dependent on $\Delta\psi$, generated by H⁺ pumping by the respiratory chain. mitoK_{ATP} opening allows K⁺ entry into the matrix causing an increase in volume. Excess K⁺ can be removed by the K⁺/H⁺ antiporter. The outer mitochondrial membrane is freely permeable to ions. mitoK_{ATP} = mitochondrial membrane ATP-sensitive K⁺ channels.

tron leakage and ROS generation. Thus, decreases in $\Delta\psi$ associated with enhanced respiration prevent mitochondrial ROS release. Enhanced respiration is remarkably effective in decreasing ROS, and very small changes in $\Delta\psi$ ("mild uncoupling") lead to significant alterations in ROS release (29). Indeed, we have found that the marginal effects of mitoK_{ATP} opening on $\Delta\psi$ are sufficient to decrease mitochondrial ROS release by up to 30% (30). Other groups have found conflicting results using intact cells and indirect ROS measurements (31), but our results showing that mitoK_{ATP} prevents mitochondrial oxidative stress are fully compatible with those reported by Schumacker's group (32) who found that mitochondrial ROS release decreased within cardiomyocytes treated with mitoK_{ATP} agonists (see discussion below).

MitoK_{ATP} channels in ischemia/reperfusion and ischemic preconditioning

As stated previously, the use of mitoK_{ATP} agonists has been shown to protect against damage observed in a variety of heart ischemia/reperfusion protocols, including experiments using *in vivo* hearts, *ex vivo* hearts (33,34) and isolated cardiomyocytes (28,32). In addition, mitoK_{ATP} agonists have been shown to mediate ischemic protection in other tissues (9). Halestrap's group (35) has recently questioned the ability of these agonists to increase mitochondrial ATP-inhibited K⁺ uptake, and has suggested that the effects of these drugs are related to other cellular functions (35). However, the method adopted by this group to measure matrix volume changes promoted by mitoK_{ATP} agonists involves the use of radioactive markers and mitochondrial centrifugation, which definitely changes organellar conformation and may not be sensitive enough to detect changes promoted by low K⁺ fluxes. In addition, the idea that mitochondrial K⁺ uptake is

cardioprotective is supported by the fact that activation of a different type of mitochondrial K⁺ channel, the recently described mitochondrial Ca²⁺-activated K⁺ channel, is also beneficial (36). Furthermore, the wide variety of drugs which activate mitoK_{ATP} and also promote cardioprotection practically rules out the possibility of non-mitoK_{ATP} effects being responsible for the protective results obtained (7,25,32). Finally, the finding that a variety of mitoK_{ATP} antagonists prevent the beneficial effects of IP (16,17,32) further supports a direct role of this channel.

The exact mechanism by which mitoK_{ATP} activity is enhanced during IP is still not fully understood. IP involves the activation of protein kinases such as protein kinase C, tyrosine kinase, and downstream kinases, which can phosphorylate mitoK_{ATP} (25,37). However, although K_{ATP} are activated by phosphorylation, there is no direct evidence to date that mitoK_{ATP} activity can be enhanced in this manner. On the other hand, mitoK_{ATP} activity is enhanced by oxidation (38), and ROS levels are moderately augmented during IP (15,39). Thus, moderate increases in mitochondrial ROS occurring during IP may lead to mitoK_{ATP} oxidation and activation (15). This mechanism of activation of mitochondrial K⁺ channels has been best demonstrated in plant mitochondria, in which superoxide anion formation stimulates K⁺ transport (40).

There is also some controversy concerning whether mitoK_{ATP} plays a major role as a trigger or mediator of IP. Some investigators have found that protection conferred by DZX can be decreased by protein kinase C (41,42) and tyrosine kinase inhibitors (43), suggesting that mitoK_{ATP} activates protein kinases, triggering the protective effects of IP. However, other studies support the idea that mitoK_{ATP} acts downstream of kinases (44). It is most probable that this channel plays a role both as a trigger and a mediator, activating a kinase cascade that feeds back in a positive manner to keep the channel open

during ischemia.

Several mechanisms by which mitoK_{ATP} may mediate ischemic protection have been proposed (see Figure 2). The finding that mitoK_{ATP} regulates mitochondrial volume under physiological conditions suggests that this may be an important function during ischemia/reperfusion. Indeed, mitoK_{ATP} activity improves oxidative phosphorylation during reperfusion and prevents ATP hydrolysis by mitochondrial F₀F₁ ATP synthase during ischemia (33,34). These effects can also be observed when mitochondria are osmotically swollen, indicating they are a consequence of increases in matrix volume promoted by K⁺ uptake. This increase leads to physical alterations in the relationship between the inner and outer membrane and changes transport properties, decreasing influx of ATP into the matrix, where it can be hydrolyzed by ATP synthase (33). As a consequence, ATP levels in the heart decrease less dramatically during ischemia (34), ensuring cell survival.

ATP hydrolysis by ATP synthase is accompanied by proton pumping from the mitochondrial matrix to the intermembrane space, partially re-establishing $\Delta\psi$ in the absence of oxygen (2). This generation of $\Delta\psi$ is futile, since it cannot lead to net ATP generation, but may be sufficient to provide the driving force for Ca²⁺ uptake. Thus, it is possible that mitoK_{ATP} opening, in addition to inhibiting ATP loss due to the reverse activity of ATP synthase, may prevent excessive mitochondrial Ca²⁺ uptake during ischemia. This hypothesis is supported by the finding that Ca²⁺ uptake is slightly smaller in non-respiring mitochondria treated with DZX (34) and that mitochondrial Ca²⁺ levels are decreased by mitoK_{ATP} agonists in non-respiring cells (28).

Finally, a controversial role for mitoK_{ATP} during ischemia/reperfusion is the regulation of mitochondrial ROS release. Schumacker's group (32) originally demonstrated that the mitoK_{ATP} agonist pinacidil efficiently

reduces ROS production and limits cell death when added only during reperfusion, in a manner sensitive to 5-hydroxydecanoate. In addition, these investigators found that 5-hydroxydecanoate does not inhibit moderate increases in ROS formation measured during IP. Taken together, these results indicate that mitoK_{ATP} opening directly prevents ROS release, and that increases in ROS found during preconditioning are independent of mitoK_{ATP}. Despite these earlier findings, some later studies suggested that mitoK_{ATP} opening increased, rather than decreased, mitochondrial ROS release (31,45). We find these results questionable since MitoTracker Red, the probe used to detect ROS in the latter study, does not respond to classical mitochondrial ROS regulators such as uncouplers or respiratory inhibitors (45). Furthermore, we have found (Facundo HTF and

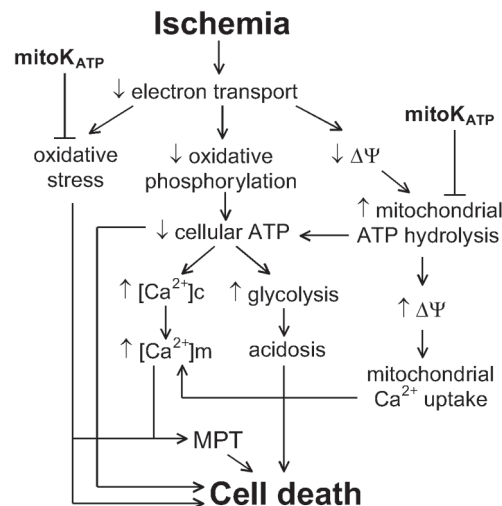


Figure 2. Proposed cardioprotective effects of mitoK_{ATP} opening. Electron transport is diminished during ischemia, leading to decreased oxidative phosphorylation, decreased $\Delta\psi$ and oxidative stress. Cellular ATP is depleted as a consequence of diminished oxidative phosphorylation and ATP hydrolysis by ATP synthase. These phenomena cause an increase in intracellular and mitochondrial Ca²⁺ concentrations, which, when accompanied by oxidative stress, cause MPT, ultimately leading to cell death. mitoK_{ATP} opening blocks the occurrence of oxidative stress and ATP hydrolysis, protecting the ischemic tissue against cell death. mitoK_{ATP} = mitochondrial membrane ATP-sensitive K⁺ channels; $\Delta\psi$ = inner membrane potential; MPT = mitochondrial permeability transition.

Kowaltowski AJ, unpublished observations) that dichlorofluorescein, used to measure ROS in our previous study (30), presents increased fluorescence when in the presence of DZX, in a manner completely independent of the presence of mitochondria. Indeed, we have found a clear decrease in heart, brain and liver mitochondrial ROS release upon physiological and pharmacological opening of mitoK_{ATP} (30). Our finding agrees with the well-established relationship between mitochondrial uncoupling and ROS release (29). mitoK_{ATP} opening promotes mild uncoupling by allowing cations to enter the matrix and stimulating K⁺/H⁺ exchange (8). The idea that mild uncoupling promoted by DZX is important in cardioprotection is supported by the finding that both artificial uncouplers and endogenous uncoupling proteins can prevent ischemic damage (46,47).

A common consequence of mitochondrial oxidative stress is MPT, a non-selective inner membrane permeabilization caused by the oxidation of membrane proteins (4,48). Since MPT is a known cause of post-ischemic heart damage (6), it seems logical to suggest that mitoK_{ATP} opening prevents ischemic damage by inhibiting MPT. Indeed, in addition to decreasing ROS generation in mitochondria (30), mitoK_{ATP} also prevents Ca²⁺ accumulation, the depletion of ATP and accumulation of phosphate, conditions that trigger MPT (4,5; see Figure 2). Recent studies have supported this idea, demonstrating that IP prevents MPT (49).

Thus, evidence obtained to date indicates that mitoK_{ATP} prevents post-ischemic heart

damage by exerting a variety of actions which participate both as triggers and as mediators of cardioprotection. The mediator activities of mitoK_{ATP} which most probably determine tissue survival include improving tissue levels of high energy phosphates during ischemia and reperfusion, preventing mitochondrial Ca²⁺ uptake, decreasing mitochondrial ROS release and inhibiting MPT (see Figure 2).

Final remarks

The highly efficient cardioprotection conferred by mitoK_{ATP} agonists against damage promoted by infarction and the perspective of immediate applicability of these drugs have generated a lot of interest among basic researchers intent on understanding and improving the effects of these drugs. In clinical trials, perhaps the most convincing evidence supporting the applicability of these drugs *in vivo* is the efficacy of nicorandil. *In vitro*, nicorandil activates cardiac mitoK_{ATP} (50,51) and exerts a direct cardioprotective effect on heart muscle cells, mediated by the selective activation of these channels (51). In addition, the treatment of patients with stable angina with nicorandil decreases the incidence of cardiovascular events and reduces infarct size (52-54), strongly supporting the immediate applicability of this class of drugs. We hope that a better understanding of the mechanisms of action of these chemicals, along with the development of more effective and selective mitoK_{ATP} agonists, will contribute to the development of even better cardioprotective strategies.

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