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MitoK$_{ATP}$ activity in healthy and ischemic hearts

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Abstract In addition to their role in energy transduction, mitochondria play important non-canonical roles in cell pathophysiology, several of which utilize the mitochondrial ATP-sensitive K^+ channel (mitoK$_{ATP}$). In the normal heart, mitoK$_{ATP}$ regulates energy transfer through its regulation of intermembrane space volume and is accordingly essential for the inotropic response during periods of high workload. In the ischemic heart, mitoK$_{ATP}$ is the point of convergence of protective signaling pathways and mediates inhibition of the mitochondrial permeability transition, and thus necrosis. In this review, we outline the experimental evidence that support these roles for mitoK$_{ATP}$ in health and disease, as well as our hypothesis for the mechanism by which complex cardioprotective signals that originate at plasma membrane receptors traverse the cytosol to reach mitochondria and activate mitoK$_{ATP}$.

Keywords Mitochondrial K_{ATP} channel · Inotropy · Ischemia · Signaling pathways · Permeability transition

Introduction

Mitochondria are traditionally described as the power houses of the cell due to their role in ATP production. However, mitochondria carry out other essential physiological functions, such as regulation of intracellular calcium homeostasis (Kowaltowski 2000) and thermogenesis in hibernating mammals (Argyropoulos and Harper 2002). Mitochondria, through mitoK$_{ATP}$, are relay stations for signaling leading to gene transcription (Garlid et al. 2003). Mitochondria are the source and target for anti- as well as pro-apoptotic factors, such as cytochrome c and the Bcl-2 family of proteins (Fleury et al. 2002; Jeong and Seol 2008). Mitochondrial function is essential for tissue protection following ischemia-reperfusion via production of signaling ROS for activation of protective kinases (Hausenloy et al. 2005; Costa et al. 2008). Through activation of mitoK$_{ATP}$, mitochondria are able to maintain efficient energy exchange with the cytosol during inotropic stress (Garlid et al. 2006). Here, we will review briefly the effects of mitoK$_{ATP}$ opening on mitochondrial physiology in the healthy and ischemic heart.

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Effects of mitoK$_{ATP}$ activity in the healthy heart

During normoxia in the unstressed heart, respiration generates an electrical gradient across the mitochondrial inner membrane of 160–180 mV. This drives a steady diffusive K^+ influx into the matrix, which is balanced in the steady state by K^+ efflux via the K^+/H^+ antiporter (Garlid and Paucek 2003). If mitoK$_{ATP}$ is now opened, additional K^+ is driven into the matrix, accompanied by osmotically obligated water and electroneutrally transported anions, primarily phosphate. Within about 30 s, the mitochondria achieve a new steady state characterized by three changes: (i) a small increase in matrix volume, (ii) a modest alkalinization, and (iii) a very slight acceleration of respiration (Kowaltowski et al. 2001; Andrukhiv et al. 2006; Costa et al. 2006a). MitoK$_{ATP}$ opening during normoxia occurs in physiological response

to either plasma membrane receptor activation, whose signal migrates to mitochondria and phosphorylates the channel, or in response to a K_{ATP} channel opener. In both cases, the important effect is matrix alkalinization, which causes mild inhibition of Complex I and increased ROS production (Andrukhiv et al. 2006). In support of this interpretation, nice studies from Dos Santos' laboratory (Pasdois et al. 2008), in which fluorescence of redox-sensitive dyes were monitored noninvasively with an optic fiber probe placed near the left ventricular wall of Langendorff-perfused rat hearts, showed that diazoxide induced a moderate and transient increase in ROS production after diazoxide administration. Cardioprotection was reflected in a strong decrease in ROS generation during the subsequent ischemic and reperfusion phases. MitoK_{ATP}-dependent signaling during normoxia is an essential component of preconditioning and cardioprotection against ischemia-reperfusion injury. The ROS produced secondary to mitoK_{ATP} opening transmit the signal to specific mitochondrial PKCεs and to other protective kinases in the cell. The ROS is also an essential component of physiological signaling in the absence of subsequent ischemia, for example signaling the nucleus to trigger gene transcription (Garlid et al. 2003).

The situation is subtly different in the heart undergoing inotropic stress with associated high energy demand in response, for example, to adrenergic stimulation (MacGowan and Koretsky 2000; Saucerman et al. 2003). The great increase in ATP synthesis in response to this demand will decrease the membrane potential somewhat, thereby decreasing the driving force for K^+ influx. Uncompensated, this will result in matrix contraction and reciprocal expansion of the intermembrane space (IMS), causing dissociation of mitochondrial creatine kinase from VDAC and disruption of efficient energy transfer between mitochondria and contractile proteins via creatine/creatine phosphate (Saks et al. 1998). Energy transfer by ATP/ADP exchange across the outer membrane does not pose a problem when the heart is working in the lower 50% of its dynamic range, but it cannot sustain inotropy above this range. This was demonstrated in isolated hearts undergoing inotropic stress in response to elevated calcium, ouabain, or dobutamine (Garlid et al. 2006). In each case, the inotropic response was either prevented or reversed (depending on time of drug addition) by two different mitoK_{ATP} inhibitors, 5-hydroxydecanoic acid (5-HD) and tetraphenylphosphonium (Garlid et al. 2006). Our interpretation is that the inotropic signal goes to mitochondria to open mitoK_{ATP} by phosphorylation. Thus, inotropy requires mitoK_{ATP} to open, not to increase matrix volume, but to maintain constant IMS volume. The added K^+ permeability simply compensates for the lower driving force. Note that volume and K^+ content remain unchanged, but matrix alkalinization and consequent ROS production still occur, due to the lower membrane potential.

Effects of mitoK_{ATP} activity in the ischemic heart

Mitochondria appear to be the effectors responsible for both ischemia-reperfusion injury (IRI) and cardioprotection. The heart is strictly aerobic and therefore vulnerable to a decrease in oxygen supply (Jennings and Ganote 1976). Ischemia causes immediate disturbance of mitochondrial function, including failure of ATP synthesis, failure to respire, and a drop in $\Delta\Psi$. This is accompanied by changes in cytosolic composition, including increased Ca^{2+}, phosphate and fatty acids. This altered state is met during reperfusion by a large increase in reactive oxygen species (ROS) originating from the respiratory chain (Droge 2002; Turrens 2003). These factors promote opening of the mitochondrial permeability transition (MPT), a high-conductance pore in the inner mitochondrial membrane, which is the main cause of necrotic cell death in IRI (Crompton 1999; Di Lisa et al. 2001; Hausenloy et al. 2002; Weiss et al. 2003; Di Lisa and Bernardi 2006). It follows that any hope of protecting the heart from these consequences must ultimately involve the prevention of MPT opening (Weiss et al. 2003).

The heart posseses self-defense mechanisms that can reduce cell death and functional impairment after prolonged episodes of ischemia-reperfusion. Cardioprotective procedures include ischemic preconditioning (IPC), in which one or more periods of brief ischemia precede the index ischemia (Murry et al. 1986) and ischemic postconditioning (POC), in which staccato ischemia-reperfusions are administered immediately on reperfusion (Zhao and Vinten-Johansen 2006). A large variety of receptor agonists are also protective when administered prior to ischemia. Indeed, IPC and POC are receptor-mediated processes that are triggered by Gi-protein coupled receptor (GPCR) agonists released by the ischemic heart, primarily bradykinin, opioid peptides, and adenosine. Most other GPCR ligands are also cardioprotective (Downey et al. 2007). Cardioprotection is also afforded by several non-GPCR receptors, such as digitalis and calcium (Pasdois et al. 2007). A variety of non-receptor agents that act on intracellular targets are also protective and are reviewed in ref. (Garlid et al. 2008). Noteworthy among these are the K_{ATP} channel openers. All K^+ channel openers (KCO) are cardioprotective, and they have been shown to be cardioprotective in all species examined (Grover et al. 1989; Grover and Garlid 2000). It was initially assumed that these were acting on sarcolemmal K_{ATP}. About 12 years ago, we showed that it is in fact mitoK_{ATP} that is responsible for cardioprotection (Garlid et al. 1996; Garlid et al. 1997). Thus, diazoxide was shown to be a potent mitoK_{ATP} opener and was as effective as cromakalim in protecting the heart. But diazoxide protection, unlike that mediated by cromakalim, was not accompanied by action potential duration (APD) shortening, thus demonstrating that cardioprotection was not due to sarcK_{ATP} opening.

All of these protective mechanisms have been shown to require mitoK$_{ATP}$ opening, increased ROS production, and activation of one or more PKCεs (Downey et al. 2007; Costa et al. 2008; Garlid et al. 2008). Thus, cardioprotection involves both mitoK$_{ATP}$ opening and a decrease in MPT opening. We hypothesize that these two phenomena are part of the same signaling pathway (Costa et al. 2006b), as discussed in the next section.

Signaling from receptor to mitochondria by signalosome

We propose that cardioprotective signals are transmitted to mitochondria by signalosomes, which are vesicular, multimolecular signaling complexes that are assembled in caveolae and deliver signals to the mitochondrial outer membrane (MOM) (Garlid et al. 2008; Quinlan et al. 2008). A diagram of the signalosome hypothesis is contained in Fig. 1. Briefly, the activated receptor migrates to caveolae, where a signaling platform is assembled, then buds off as a signalosome, internalizes, and migrates to the mitochondria. There, the terminal kinase of the signalosome phosphorylates its receptor. GPCR-induced signalosomes use PKG as the terminal kinase, and ouabain- and calcium-induced signalosomes use Src and PKCε, which act in concert. Phosphorylation of receptors R1 or R2 causes the signal to be transmitted across the intermembrane space to PKCε1 on the mitochondrial inner membrane, leading to the intramitochondrial signaling pathway diagrammed in Fig. 2 and described previously (Kowaltowski et al. 2001; Andrukhiv et al. 2006; Costa et al. 2006a; Garlid et al. 2008).

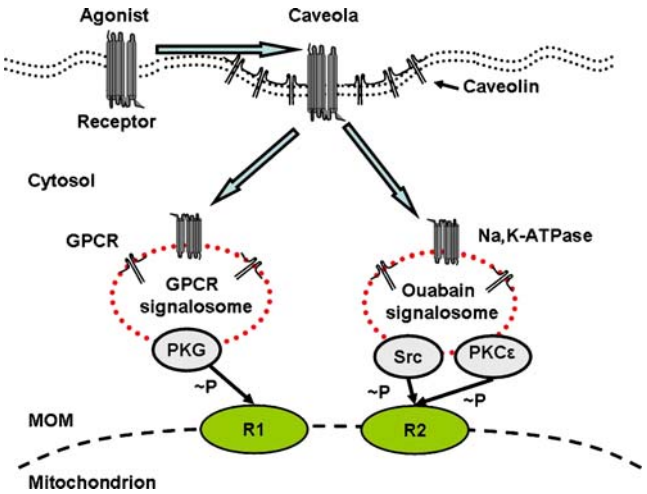

Fig. 1 The signalosome mechanism of cell signaling. Upon activation by its agonist, the receptor migrates to caveolae, where a signaling platform is assembled. This buds off as a signalosome and internalizes. The signalosome migrates via the cytoskeleton to mitochondria, where it binds to receptors on the MOM, designated as **R1** (for GPCR-induced signalosomes) and **R2** (for calcium or ouabain-induced signalosomes). The terminal kinase of the signalosome phosphorylates its specific MOM receptor. All GPCR signalosomes use PKG as the terminal kinase (Costa et al. 2005), whereas the ouabain and calcium signalosomes use Src and PKCε (Garlid et al. 2008). Phosphorylation of R1 or R2 causes the signal to be transmitted across the intermembrane space to PKCε1 on the mitochondrial inner membrane, leading to the intramitochondrial signaling pathway described in Fig. 2

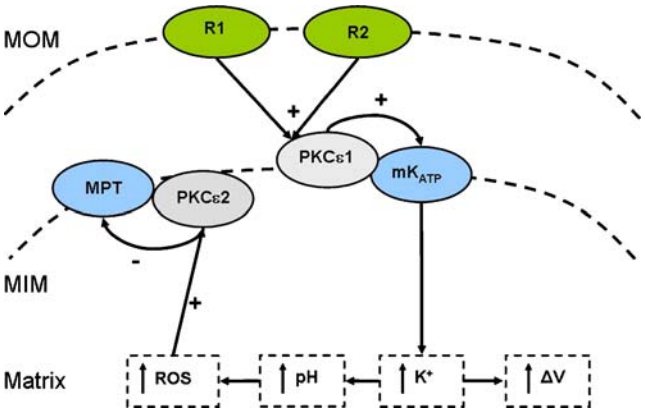

Fig. 2 Intramitochondrial signaling. PKCε1 opens mitoK$_{ATP}$ by phosphorylation (Costa et al. 2005; Jaburek et al. 2006). The ensuing K^+ influx leads to matrix alkalinization and increased ROS production from Complex I (Andrukhiv et al. 2006). The increased ROS activate a second inner membrane PKCε, PKCε2, which then inhibits the MPT (Costa et al. 2006b)

We purified signalosomes from hearts subjected to various conditioning protocols and then assayed their functional activity by adding them to mitochondria from untreated hearts. With no further additions, the signalosomes caused mitoK$_{ATP}$ opening and MPT inhibition (Quinlan et al. 2008). Functionally active signalosomes were obtained from hearts exposed to bradykinin, ouabain, calcium, ischemic preconditioning, ischemic postconditioning, acetylcholine and adenosine. The signalosomes were dissolved by the cholesterol binding agent methyl-β-cyclodextrin and were resistant to Triton X-100, properties reflecting their caveolar origin. Electron microscopy revealed that the signalosomes are about 140 nm in diameter and can be decorated with immunogold labeled caveolin 3 antibodies (Quinlan et al. 2008). The signalosome induced by bradykinin stimulation contains eNOS, guanylyl cyclase, and cGMP-dependent protein kinase (PKG), and we were able to demonstrate the participation of each of these enzymes in the mitoK$_{ATP}$ assay when proper substrates were supplied (unpublished).

Summary and conclusion

MitoK$_{ATP}$ play essential roles in the normal and ischemic heart. Inotropic stimuli open mitoK$_{ATP}$ to ensure efficient energy transfer from mitochondria to the cytosol during high workload situations. Protective stimuli against ische-

mia open mitoK_{ATP} to inhibit the onset of MPT, and thus prevent necrosis. Signals from both inotropy and protection are triggered at the level of the plasma membrane and must cross the cytosol to induce mitoK_{ATP} opening. The mechanism by which this occurs has been the subject of our recent work. The signalosome mechanism is a powerful new paradigm for cell signaling and cardioprotection. Although our findings do not yet establish the signalosome mechanism, they are sufficient to justify the suggestion as a working hypothesis, allowing exploration of the consequences for understanding ischemia-reperfusion injury, cardioprotection, and cell signaling in general.

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