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Mitochondrial ATP-sensitive K^+ channels, protectors of the heart

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Abbreviations K_{ATP} channels, ATP-sensitive K^+ channels; KCOs , K^+ channel opener compounds; mitoK_{ATP} channels, mitochondrial K_{ATP} channels; $\Delta\Psi m$, the potential of the inner mitochondrial membrane; 5-HD , 5-hydroxydecanoic acid; PTP, permeability transition pore; $[Ca^{2+}]_m$, the intra-mitochondrial Ca^{2+} concentration; SUR, sulfonylurea receptors; Kir6.x, inwardly rectifying K^+ channel subunits.

ATP-sensitive K^+ (K_{ATP}) channels were first identified in the sarcolemma of cardiac myocytes as inwardly rectifying K^+ channels that were inhibited by intracellular ATP (Noma, 1983). It was proposed that K_{ATP} channels would have a cardioprotective effect during ischemia by shortening action potential duration and thereby decreasing Ca^{2+} influx into myocytes. Then it was found that K^+ channel opener compounds (KCOs) that are known to activate sarcolemmal K_{ATP} channels exert cardioprotective effects under ischemia-reperfusion (Grover *et al.*, 1989; Grover & Garlid, 2000). However, several groups of investigators found that KCOs exerted cardioprotection at concentrations below those causing action potential shortening (Yao & Gross, 1994; Grover *et al.*, 1995a; Grover *et al.*, 1995b; Garlid *et al.*, 1997), indicating that KCOs may have targets other than sarcolemmal K_{ATP} channels. Alternative targets for KCOs include mitochondrial K_{ATP} (mitoK_{ATP}) channels (Grover & Garlid, 2000).

The mitochondrial inner membrane is polarized by ~180 mV with the matrix side negative ($\Delta\Psi m$) due to a H^+ gradient generated by respiratory enzyme complexes (Fig. 1) (Saraste, 1999). The energy stored in the form of $\Delta\Psi m$ is utilized to make ATP from ADP by ATP synthetase. The inner mitochondrial membrane possesses different ion channels (uniports) through which cations such as K^+, and Ca^{2+} flow into the matrix under the normal electrochemical gradient and diminish $\Delta\Psi m$ (Bernardi, 1999). Inoue et al. (1991) were the first to identify K^+-selective channels that were inhibited by ATP and glybenclamide in the inner membrane of liver mitochondria by using patch-clamp methods (Inoue *et al.*, 1991). Dahlem et al. also recently found similar channels in the inner mitochondrial membrane of Jurkat cells by using patch-clamp methods (Dahlem *et al.*, 2004). The existence of mitoK_{ATP} channels in the heart was confirmed by different techniques such as reconstitution of mitochondrial membranes into bilayer lipid membranes and purified mitochondrial proteins into proteoliposomes (Grover & Garlid, 2000; Ardehali & O'Rourke, 2005). In cardiac myocytes, diazoxide activated mitoK_{ATP} 1,000-2,000 times more potently than sarcolemmal K_{ATP} channels and it exerted cardioprotective effects during ischemia in this mitoK_{ATP}-selective concentration range (Garlid *et al.*, 1996; Garlid *et al.*, 1997). In addition, an inhibitor of mitoK_{ATP} channels, 5-hydroxydecanoic acid (5-HD) abolished the cardioprotective effect of diazoxide. Taken together, these results suggested that it was mitoK_{ATP} channels which play a pivotal role in cardioprotection evoked by KCOs. MitoK_{ATP} channels were also proposed to be the end-effecter of ischemic preconditioning (Cohen *et al.*, 2000), a mechanism by which brief periods of ischemia provide protection against subsequent longer ischemic periods (Murry *et al.*, 1986).

However, the mechanisms by which mitoK_{ATP} channels exert their cardioprotective effects were poorly understood. An article from Terzic's laboratory published in the Journal Physiology in 1999 shed light on this issue (Holmuhamedov *et al.*, 1999). By using mitochondria isolated from rat hearts, the authors showed that diazoxide (>1 μM) and another KCO, pinacidil (>10 μM) lead to reduction of Ca^{2+} influx through a ruthenium red-sensitive Ca^{2+} uniport and an increase in Ca^{2+} efflux through a cyclosporin A-sensitive mitochondrial permeability transition pore (PTP), a specific, voltage-dependent, nonselective high-conductance channel that is activated by an increase in the intra-mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_m$) and a decrease in $\Delta\Psi m$ (Bernardi, 1999; Halestrap *et al.*, 2004). Holmuhamedov et al. further showed that these effects of KCOs were inhibited by ATP, abolished by removal of extra-mitochondrial KCl and mimicked by the K^+ ionophore valinomycin. They ascribed these effects to a decrease in $\Delta\Psi m$ induced by the KCOs and thus a decrease in driving force for Ca^{2+} influx, a hypothesis initially proposed by Liu et al. (1998) (Liu *et al.*, 1998). They also showed that diazoxide exerted a similar effect in a 5-HD-sensitive manner in intact cardiac myocytes. Murata et al. (2001) extended this work and showed that diazoxide reduced $[Ca^{2+}]_m$ in isolated cardiac myocytes under simulated ischemia/reperfusion in a 5-HD-sensitive manner (Murata *et al.*, 2001). Wang et al. (2001) reported that this was also the case in isolated hearts during ischemia/reperfusion and that the reduction in $[Ca^{2+}]_m$ by diazoxide correlated with the recovery of the contractility after reperfusion (Wang *et al.*, 2001).

However, Holmuhamedov's view was challenged by Kowaltowski et al. (2001) (Garlid, 2000; Kowaltowski *et al.*, 2001). They argued that the bioenergetic effects observed by Holmuhamedov et al. with high concentrations of KCOs (i.e. >100 μM diazoxide or >50 μM pinacidil) resulted not from activation of mitoK_{ATP} channels but from drugs' protonophore activity

and inhibitory effect on respiration. Furthermore, they found that the decrease in ΔΨm induced in isolated mitochondria by diazoxide and pinacidil (<50 μM) was too small (1-2 mV) to account for their cardioprotective effect. Instead, they found that the KCOs significantly increased the mitochondrial volume by causing a K^+-influx and they suggested that this protected mitochondria during ischemia/reperfusion by preserving the architecture of the intermembrane space with consequent slowing of ATP hydrolysis and preservation of the ability to use creatine efficiently as substrate on reperfusion. On the other hand, Korge et al. (2002) found that although diazoxide hardly decreased ΔΨm in energized mitochondria, it did so clearly in de-energized mitochondria (Korge *et al.*, 2002). They showed that diazoxide thereby decreased Ca^{2+} influx and prevented Ca^{2+}-induced opening of PTP, consistent with Holmuhamedov's view. Because widespread irreversible opening of PTP inevitably results in the necrosis of cardiac myocytes (Halestrap *et al.*, 2004), they ascribed cardioprotection by KCOs to this effect. They also found that diazoxide prevented the release of cytochrome c from the intermembrane space perhaps by causing mitochondrial swelling. This would prevent cardiac myocytes from undergoing apoptosis (Akao *et al.*, 2001). It should be noted that KCO-induced opening of mitoK_{ATP} channels may also cause cardioprotection by regulating the synthesis of reactive oxygen species during ischemia/reperfusion (Ardehali & O'Rourke, 2005).

Thus, following Holmuhamedov's work (Holmuhamedov *et al.*, 1999), a number of investigators have proposed different mechanisms by which mitoK_{ATP} channels can cause cardioprotection. Probably, these mechanisms are not mutually exclusive but coordinately cause cardioprotection during ischemia/perfusion (Ardehali & O'Rourke, 2005). In spite of this remarkable progress, there still remain a number of questions regarding mitoK_{ATP} channels. For instance, diazoxide and 5-HD are reputed to specifically target mitoK_{ATP} channels but in fact both have other non-channel targets in mitochondria (Schafer *et al.*, 1969 ; Hanley, 2002 #23; Lim *et al.*, 2002; Ozcan *et al.*, 2002; Drose *et al.*, 2006) Furthermore, it has been shown that diazoxide can activate sarcolemmal K_{ATP} channels especially in the presence of intracellular ADP (D'Hahan *et al.*, 1999), and mouse atrial sarcolemmal K_{ATP} channels are highly sensitive to diazoxide (Zhang *et al.*, 2009). Thus, one must be cautious in interpreting the effects of these agents. In addition, the molecular identity of mitoK_{ATP} channels remains unclear (O'Rourke, 2000, 2004). The pharmacological similarities between mitoK_{ATP} and sarcolemmal K_{ATP} channels might suggest that mitoK_{ATP} channels are composed of sulfonylurea receptors (SUR1, SUR2A or SUR2B) (receptors for KCOs and sulfonylureas) and pore-forming subunits (Kir6.1 or Kir6.2) as sarcolemmal K_{ATP} channels (Seino, 1999). Indeed, Grover and Garlid (2000) tentatively identified a 63 kDa sulfonylurea-binding protein and a putative pore-forming subunit of 55 kDa from mitochondria (Grover & Garlid, 2000). Although some immunological analyses indicated the presence of these subunits in mitochondria (Suzuki *et al.*, 1997; Lacza *et al.*, 2003a; Lacza *et al.*, 2003b; Singh *et al.*, 2003; Cuong *et al.*, 2005; Jiang *et al.*, 2006), these observations were not confirmed by other investigators (Seharaseyon *et al.*, 2000; Kuniyasu *et al.*, 2003; O'Rourke, 2004; Foster *et al.*, 2008). Liu et al. indicated that SUR1/Kir6.1 channels closely resembled mitoK_{ATP} channels in their pharmacological properties (Liu *et al.*, 2001). However, diazoxide-induced protection of the brain from ischemia was observed equally in SUR1 knockout and wild-type mice in a 5-HD-sensitive manner, indicating that SUR1 is not a required component of mitoK_{ATP} channels (Munoz *et al.*, 2003). Sehararaseyon et al. (2000) showed that transfection of a dominant negative construct of Kir6.1 did not affect mitoK_{ATP} channel activity in isolated rabbit ventricular myocytes (Seharaseyon *et al.*, 2000), indicating that Kir6.1 is also not included in mitoK_{ATP} channels. Recently, Ardehali et al. proposed an alternative hypothesis that mitoK_{ATP} channels may be formed as a macromolecular complex containing mitochondrial ATP-binding cassette protein 1, phosphate carrier, adenine nucleotide translocator, ATP synthetase and succinate dehydrogenase (Ardehali *et al.*, 2004). Thus, mitoK_{ATP} and sarcolemmal K_{ATP} channels may be completely different molecules. Identification of the molecular structure of mitoK_{ATP} channels will lead to more precise delineation of the mechanism underlying regulation of the channels and the development of drugs selectively acting on the channels. Therefore, further investigations are clearly needed in order to deepen our understanding of this important field of cardiovascular pathophysiology and pharmacology.

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Author Contributions

Mitsuhiko Yamada: drafting the article or revising it critically for important content, and final approval of the version to be published.

Figure Legends

Figure 1: Ion transporters on the mitochondrial inner membrane described in the text. A horizontal gray bar indicates the mitochondrial inner membrane. This membrane is polarized by ~180 mV with matrix side negative ($\Delta\Psi m$) due to a H^+ gradient generated by respiratory enzyme complexes (REC) (Bernardi, 1999; Saraste, 1999). The energy stored in the form of $\Delta\Psi m$ is utilized to make ATP from ADP by ATP synthetase (AS). Mitochondrial ATP-sensitive channels ($mitoK_{ATP}$) mediate K^+ influx along $\Delta\Psi m$ and cause a decrease in $\Delta\Psi m$, matrix swelling and regulation of reactive oxygen species (ROS) generation. $MitoK_{ATP}$ channels are activated by K^+ channel opener compounds (KCOs) and inhibited by ATP, glybenclamide (Glb) and 5-hydroxydecanoic acid (5-HD). A decrease in $\Delta\Psi m$ inhibits Ca^{2+} influx through Ca^{2+} unipoters ($Ca^{2+}UP$). $Ca^{2+}UP$ is inhibited by ruthenium red (RR). Mitochondrial permeability transition pore (PTP) is a specific, voltage-dependent, nonselective high-conductance channel that is activated by an increase in the intra-mitochondrial Ca^{2+} concentration and a decrease in $\Delta\Psi m$. Ca^{2+} efflux through PTP is also facilitated by a decrease in $\Delta\Psi m$. PTP is inhibited by cyclosporin A (Cys A).

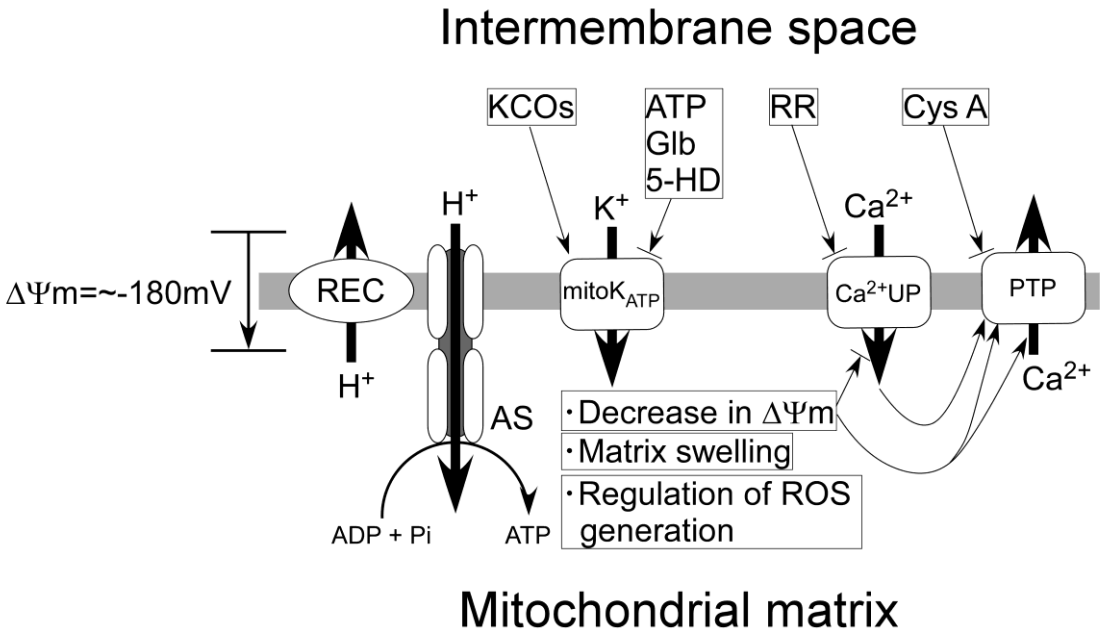
Intermembrane space
KCOs
ATP
Glb
5-HD
RR
Cys A
H+
K+
Ca2+
ΔΨm=~-180mV
REC
mitoKATP
Ca2+UP
PTP
H+
Ca2+
AS
·Decrease in ΔΨm
·Matrix swelling
·Regulation of ROS generation
ADP + Pi
ATP
Mitochondrial matrix

Figure 1