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Opening mitochondrial K_{ATP} in the heart – what happens, and what does not happen

Abstract There is considerable evidence that opening the mitochondrial ATP-sensitive potassium channel (mitoK_{ATP}) is cardioprotective in ischemia-reperfusion. Two prominent questions surround the role of mitoK_{ATP} in the cardiomyocyte: How does opening mitoK_{ATP} protect? What is the normal physiological role of mitoK_{ATP} in the heart? Before these questions can be addressed, it is necessary to agree on the bioenergetic consequences of opening mitoK_{ATP}, and this distills down to a single question – does opening mitoK_{ATP} cause significant uncoupling or not? The evidence strongly indicates that it does not and that reports of uncoupling and inhibition of Ca^{2+} uptake are the result of using toxic concentrations of K_{ATP} channel openers. Thus, opening mitoK_{ATP} results in increased K^+ flux that is sufficient to change mitochondrial volume but is insufficient to cause significant depolarization of membrane potential. The volume changes, however, have significant bioenergetic consequences for energy coupling in the cell.

Key words Mitochondria – ATP-sensitive potassium channel – cardioprotection – ischemia – heart

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Introduction

The discovery that the mitochondrial K_{ATP} channel (mitoK_{ATP}) may be the receptor for the cardioprotective effects of K_{ATP} channel openers (7, 11) is merely the latest in a series of surprising events in the quest to understand ischemic protection – a series whose beginnings may be traced to the discovery of ischemic preconditioning (21). Many laboratories are now focusing on mitoK_{ATP}, primarily with pharmacological approaches, and this may be a good time to reflect briefly on some of the issues surrounding the role of mitoK_{ATP} in ischemic protection. I will focus primarily on the consequences of opening mitoK_{ATP} – what does opening mitoK_{ATP} do? And, perhaps equally important, what does opening mitoK_{ATP} not do?

Does opening mitoK_{ATP} depolarize mitochondria?

K^+ cycling in mitochondria will dissipate energy – the energetic cost of volume homeostasis (3). K^+ influx will be driven by an equal rate of H^+ ejection by the electron transport system (ETS), and the increased proton current will reduce the electrical potential ($\Delta\psi$) by an amount determined by the internal resistance of the ETS battery. Therefore, an increase in K^+ flux will result in lower $\Delta\psi$, but it must be emphasized that the extent of depolarization will depend entirely on the magnitude of the increase in K^+ flux.

Plant mitochondria are completely uncoupled in K^+ medium, whereas they remain coupled in Na^+ or Li^+ media (24). This observation is complemented by earlier findings that plant mitochondria possess unusually high K^+/H^+ antiport activity, perhaps 10-fold greater than mammalian mitochondria. Two important principles are illustrated by these findings: (i) K^+ cycling can uncouple if fluxes are sufficiently high, and

(ii) if the K^+ influx capacity is large, then mitochondria will express a correspondingly large capacity for K^+ efflux in order to prevent swelling and lysis.

These principles can be applied to mammalian mitochondria and to the in vivo state. From estimates of the V_{max} of the K^+/H^+ antiporter (19), we infer that total K^+ influx cannot exceed about 10 % of the maximal proton pumping rate. K^+ leak accounts for at least half of this value, so flux through $mitoK_{ATP}$ should amount to no more than 5 % of maximum respiratory capacity, sufficient to depolarize the membrane by 2–4 mV. Direct measurements on isolated rat heart mitochondria confirm this expectation. K^+ flux through $mitoK_{ATP}$ is 24 to 30 nmol $K^+ \cdot min^{-1}\ mg^{-1}$ at 25 °C, enough to depolarize by 1–2 mV. Consistent with the lack of significant depolarization, opening $mitoK_{ATP}$ had no effect on Ca^{2+} uptake (Kowaltowski and Garlid, unpublished results). Thus, not only consideration of K^+/H^+ exchange capacity but also direct measurements lead us to the firm conclusion that uncoupling and depolarization secondary to opening $mitoK_{ATP}$ in vivo are too small to cause significant direct effects on mitochondrial energetics (5).

This conclusion appears to be contradicted by reports from several laboratories who reported massive depolarizations in mitochondrial suspensions and in cardiomyocytes treated with a variety of $mitoK_{ATP}$ openers (1, 14, 15, 18, 32). The apparent uncoupling led Liu et al. (18) to hypothesize that mitochondrial depolarization secondary to opening $mitoK_{ATP}$ protects the heart by reducing mitochondrial Ca^{2+} uptake. It is necessary to resolve these mutually exclusive hypotheses surrounding the primary bioenergetic consequences of opening $mitoK_{ATP}$ before we can begin to understand how $mitoK_{ATP}$ plays a role in cardioprotection.

Studies on isolated mitochondria

Two laboratories have reported that K_{ATP} channel openers caused a profound depolarization of isolated mitochondria (1, 14, 15, 32). Holmuhamedov et al. (14, 15) also demonstrated inhibition of Ca^{2+} uptake in the presence of $mitoK_{ATP}$ openers, in apparent support of the Ca^{2+} hypothesis of Liu et al. (18). A common feature of these studies is that the $mitoK_{ATP}$ were already open under the conditions of the experiments, because ATP and Mg^{2+} were omitted from the assays. Under these conditions, K_{ATP} openers have no effect on $mitoK_{ATP}$ – they cannot open a channel that is already open (6). Therefore, these results are independent of $mitoK_{ATP}$ activity.

A second common feature is the use of drug concentrations far in excess of those required to open $mitoK_{ATP}$ – in the range of 100–800 M. It should be noted that concentration-dependent inhibition of electron transport is observed with nearly all hydrophobic drugs (20), and is also observed with K_{ATP} channel openers (8). Thus, when given in excess doses, diazoxide and pinacidil do indeed reduce and Ca^{2+} uptake, but these effects are unrelated to $mitoK_{ATP}$ – the same effects are observed in Li^+ medium, and Li^+ is not transported by $mitoK_{ATP}$. Rather, the effects originate from drug toxicity. When used at pharmacological concentrations necessary to open $mitoK_{ATP}$, neither pinacidil nor diazoxide promoted detectable changes in mitochondrial membrane potential, nor did they affect Ca^{2+} flux (Kowaltowski and Garlid, unpublished results).

These results emphasize the necessity of recognizing the difference between toxic and pharmacological concentrations of K_{ATP} channel openers. They show that K^+ flux through $mitoK_{ATP}$ is sufficient to change mitochondrial volume, as discussed below, but is insufficient to cause significant changes in $\Delta\psi$ or Ca^{2+} uptake.

Studies on cardiomyocytes

Flavoprotein fluorescence in cardiomyocytes, arising from oxidized FAD, was shown to increase after administration of K_{ATP} openers by Liu et al. (18). Their interpretation is that opening $mitoK_{ATP}$ uncouples electron transport, thereby accelerating respiration and leading to oxidation of proximal electron transport carriers, including FAD-linked enzymes such as succinate dehydrogenase (SDH). This interpretation is open to serious question.

- The doses required are far greater than the $K_{1/2}$ values that have been observed in intact hearts. 100 µM diazoxide was used by Liu et al. (18), whereas 30 µM is probably sufficient to give maximal protection (7). In a study with nicorandil, fluorescence was still increasing at 1000 µM (28), whereas the $K_{1/2}$ value for this drug in heart mitochondria is about 5 µM (Garlid, unpublished data). The requirement for high doses suggests toxic effects, which have in fact been demonstrated previously with diazoxide in heart mitochondria using the same fluorescence technique: 150 µM diazoxide caused 60 % inhibition of SDH and also inhibited pyruvate oxidation in the intact cell (30).
- The signal appears not to be a direct response to $mitoK_{ATP}$ opening. There is an extremely long delay of 10–12 min between administration of diazoxide and the onset of the signal, whereas the drug almost certainly opens $mitoK_{ATP}$ within a few seconds. This discrepancy supports the contention (below) that the signal is not reporting uncoupling secondary to $mitoK_{ATP}$ opening. If the signal arises from opening $mitoK_{ATP}$, which remains to be established, it must be a secondary event.
- The signal does not fully accord with what is known about K_{ATP} – mediated protection. Phorbol ester (PMA), a protein kinase C activator, augmented the effect of 100 µM diazoxide

on flavoprotein fluorescence to a robust 68 % of the uncoupled level, which was interpreted as up-regulation of mitoK_{ATP}. PMA alone had no effect on flavoprotein fluorescence, despite the fact that activation of PKC is thought to involve K_{ATP} opening (31).

- The interpretation that the FAD signal reflects uncoupling secondary to opening mitoK_{ATP} (18, 28, 29) appears to be incorrect. Compared to the uncoupled response, diazoxide caused 48 % uncoupling. If the heart were 48 % uncoupled, it could scarcely survive, to say nothing of contract. Moreover, such a massive rate of K^+ influx would greatly exceed the capacity of the efflux pathway, and mitochondria would swell and burst. Indeed, direct measurement of cardiac efficiency of oxygen utilization (work/oxygen consumption) show that K_{ATP} openers have no effect on efficiency (9, 10). Therefore, it seems clear that K_{ATP} channel openers do not cause significant uncoupling in vivo.

These protocols are very attractive, because they permit assay of mitoK_{ATP} activity in situ. Favoring involvement of mitoK_{ATP} is the prevention of FAD oxidation by 5-HD, but it is troubling that glyburide was without effect. Given that the signal does not arise from uncoupling, its origin remains obscure, especially in view of the high concentrations of K_{ATP} channel openers required.

The physiological role of mitoK_{ATP} in heart

We continue to believe that opening mitoK_{ATP} has negligible direct effects on respiration, $\Delta\psi$ and ΔpH, and that the primary effect is on matrix volume and the volume of the intermembrane space (IMS). Work in progress in our laboratory indicates that these volume changes have a profound secondary effect on cellular bioenergetics.

Consider the working heart as it undergoes the transition to a high-work state, in which ATP production and oxygen consumption may increase as much as 8-fold. Increased current through the ETS will cause $\Delta\psi$ to drop, and K^+ diffusion into the matrix will drop as an exponential function of $\Delta\psi$ (4). Indeed, if $\Delta\psi$ drops by 35 mV, diffusive K^+ influx will drop by 50 %. If mitoK_{ATP} does not open, matrix volume will contract until the K^+/H^+ antiporter senses the drop in volume, resulting in a lower steady-state volume in the high work state. We have estimated the extent of the volume contraction caused by high phosphorylation rates to be about 20 % in isolated rat heart mitochondria, and this contraction is largely reversed by diazoxide (Kowaltowski and Garlid, unpublished results). Thus, mitoK_{ATP} is well suited to maintain constant matrix volume when $\Delta\psi$ falls, because the additional conductance pathway compensates for the reduced driving force, thereby minimizing the matrix contraction that would otherwise occur during high rates of ATP synthesis.

Why is it important to prevent such a small contraction of the mitochondrial matrix? The answer is provided by the work of Saks and coworkers (26, 27), who have elucidated the role of metabolic channeling of ~P by creatine kinase (CK). In brief, an intact mitoCK assembly involves functional association of mitoCK with the ATP/ADP translocase (ANT) at the inner membrane and also interaction with porin at the outer membrane. These associations are essential for the high work state, and they are strongly dependent on the volume of the intermembrane space (IMS). When the matrix contracts by 20 %, the IMS will expand reciprocally to a much greater extent. If IMS expansion is not prevented, mitoCK will dissociate during the high-work state, precisely when metabolic channeling through this complex is most needed. Thus, we hypothesize that matrix contraction, which would normally accompany high phosphorylation rates, must be prevented by opening mitoK_{ATP} in order for metabolic channeling to proceed. The hypothesis predicts that increased work states in heart cannot proceed if mitoK_{ATP} is blocked, and preliminary evidence supports this prediction (25). The physiological signal to open mitoK_{ATP} is not known, but it is assumed to derive from the signal leading to increased contraction rates and most likely involves phosphorylation of mitoK_{ATP}.

Maintaining matrix volume has a second important consequence. It has been known since 1948 that substrate oxidation is tightly controlled by matrix volume (17), independently of the means used to change volume (22). Volume activation of electron transport has been demonstrated in liver, heart and brown adipose tissue mitochondria (12, 13, 22). Activation of ETS will also contribute to the support of high energy throughput in the high work state, further emphasizing the need to maintain volume in the face of the reduced $\Delta\psi$ associated with high rates of ATP synthesis.

What happens when mitoK_{ATP} is opened in the resting state, when oxygen consumption is low and $\Delta\psi$ is high? There are several such situations, for example, when K_{ATP} channel openers are added to the normal heart, or during preconditioning. To our surprise, these conditions cause a moderate increase in mitochondrial production of reactive oxygen species (ROS) (Xie and Garlid, unpublished results). This suggests that ROS production is also regulated by volume, possibly deriving from activation of the ETS, described above. Elevated ROS have been shown to trigger gene transcription (2, 34) and to be required for protection afforded by ischemic preconditioning (33). Preliminary data suggest that the ROS signal requires opening of mitoK_{ATP}.

The cardioprotective role of mitoK_{ATP}

The mechanism of cardioprotection by mitoK_{ATP} is poorly understood. We have been focusing on the role of mitoK_{ATP} in

preserving the architecture of the IMS and consequent preservation of energy transfer processes between mitochondria and cytosol. The basis for this is that the earliest ischemic-induced alteration in mitochondrial function is the loss of functional coupling between adenine nucleotide translocase (ANT) and mitochondrial creatine kinase (mi-CK) (16). Studies were carried out on Langendorff-perfused rat hearts and included an assessment of energetics using permeabilized skinned fibers. (The experiments are being carried out in the laboratory of Pierre Dos Santos. M. N. Laclau, et al., abstracts submitted to AHA 2000).

We found that ischemic preconditioning protects mitochondrial function in situ, as evidenced by the maintenance of the high $K_{1/2}$ for ADP, the high V_{max} of respiration, the preservation of functional coupling between mi-CK and ANT, and the absence of stimulation of respiration by cytochrome c. These effects were reproduced by diazoxide and abolished by 5-HD. Hearts treated with diazoxide prior to ischemia maintained the same $K_{1/2}$ for ADP as controls, as well as the same coupling between ANT and CK. These data suggest that ischemic preconditioning and K_{ATP} channel openers preserve the low permeability of the outer membrane for ADP by maintaining the architecture of the inter-membrane space. The consequences are preservation of cellular ATP levels during ischemia and better functional recovery of hearts upon reperfusion.

These findings indicate that mitoK_{ATP} is open during ischemia; however, Pain et al. (23) have reported that open K_{ATP} channels during ischemia is not a requirement for protection. Clearly, the timing requirements for mitoK_{ATP} opening and closing need to be resolved.

Summary and conclusions

To understand the role of mitoK_{ATP} in the normal and ischemic heart, it is necessary to establish the bioenergetic consequences of opening this channel. Ongoing work in our laboratory leads us to conclude that the only primary effect of K^+ flux through mitoK_{ATP} is to regulate mitochondrial volume and that reported changes in $\Delta\psi$ and Ca^{2+} uptake are epiphenomena of suprapharmacological drug doses. The volume changes are very important. They regulate energy flow through the electron transport system, and they preserve the architecture of the intermembrane space, thereby permitting efficient energy transfers between mitochondria and cellular ATPases. A new and poorly understood development in the field is the finding that opening mitoK_{ATP} is associated with up-regulation of ROS production by mitochondria, which, in turn, appear to signal gene transcription.

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