

## Critical Review

# The Mitochondrial Potassium Cycle

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### Summary

The mitochondrial  $K^+$  cycle consists of influx and efflux pathways for  $K^+$  and anions. Net movement of  $K^+$  salts across the inner membrane causes changes of matrix volume, so regulation of the cycle is vital for maintaining the structural integrity of the organelle. The mitochondrial  $K^+$  cycle also appears to play important roles in cellular pathophysiology in vivo. Opening the mitochondrial ATP-sensitive  $K^+$  channel (mito $K_{ATP}$ ) prior to ischemia protects the heart from ischemia-reperfusion injury. Mito $K_{ATP}$  is an important player in the cell signaling pathways for ischemic protection and also for gene transcription, roles that appear to depend on the ability of mito $K_{ATP}$  opening to trigger increased mitochondrial production of reactive oxygen species. Mito $K_{ATP}$  opening during both ischemia and reperfusion and during the high work state is found to preserve the structure of the intermembrane space and thereby maintains the normally low outer membrane permeability to adenine nucleotides. This review discusses the properties of the mitochondrial  $K^+$  cycle that help to understand the basis of these effects.

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### COMPONENTS OF THE MITOCHONDRIAL $K^+$ CYCLE

The mitochondrial  $K^+$  cycle consists of five components. 1) The electron transport system (ETS) ejects protons, leading to generation of a protonmotive force,  $\Delta p = \Delta \Psi + Z \Delta pH$  (1); where  $Z = (RT \ln 10)/F$  (59.2 mV at 25°C), and  $\Delta \Psi$  is the membrane potential. In isolated mitochondria respiring in state 2,  $\Delta \Psi$  is about 190 mV, and  $\Delta pH$  is about 0.3. 2)  $\Delta \Psi$  drives  $K^+$  influx by diffusion (“ $K^+$  leak”) and 3) through the mito $K_{ATP}$ . 4)  $K^+$  influx will alkalize the matrix, causing phosphate to enter

via the electroneutral  $P_i/OH$  exchanger. 5) Excess  $K^+$  is ejected by the  $K^+/H^+$  antiporter (2, 3).

### Potassium Leak Across the Inner Membrane

As postulated by Mitchell (1), the inner membrane must have a low diffusive permeability to protons and ions generally; otherwise, ion leaks would short-circuit the protonmotive batteries and ATP would not be synthesized. Nevertheless, cation leaks occur at significant rates in respiring mitochondria, and they are physiologically important. Inward potassium leak causes matrix swelling (3), and inward proton leak dissipates energy and contributes to the basal metabolic rate (4).

Garlid et al. (5) used Eyring rate theory to develop the proper model for describing ion leak across the mitochondrial inner membrane. At high potentials ( $>100$  mV), the expression for diffusive flux of cations,  $J$ , reduces to a simple exponential function of  $\Delta \Psi$ :

$$J = f P C_{10} e^{u/2} \quad [1]$$

where  $u \equiv -zF\Delta \Psi/RT$ , and  $P$  is the permeability constant.  $C_{10}$  is the bulk ion concentration, and  $f$  describes partitioning into the energy wells at the surface of the membrane. Measurements of cation and proton leak in mitochondria are in excellent agreement with the predictions of Eqn. 1 (6). This relationship is also relevant to considerations of volume homeostasis in vivo.  $\Delta \Psi$  will decrease as ATP production increases. A 10% decrease in  $\Delta \Psi$ , from 190 to 170 mV, will result in a 32% decrease in the rate of diffusive  $K^+$  uptake.

### The $K^+/H^+$ Antiporter

Early studies indicated that mitochondria possess a very active  $Na^+/H^+$  antiporter, whereas  $K^+/H^+$  antiport activity was either very low or absent altogether (7). However, Brierley's laboratory (8) recognized that  $^{42}K^+/K^+$  exchange may reflect uniport-antiport cycling of  $K^+$ , as originally proposed by Mitchell (1). The first evidence establishing the existence of

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the  $K^+/H^+$  antiporter came from experiments showing that simple matrix swelling unmasked an electroneutral  $K^+$  efflux from mitochondria (9).

*Regulation of the  $K^+/H^+$  Antiporter by Matrix  $Mg^{2+}$ .* The simplest explanation for activation of the  $K^+/H^+$  antiporter by matrix swelling was that a matrix solute acts as a reversible, regulatory inhibitor of the antiporter and that swelling increases activity by simple dilution of the inhibitor. This mechanism was supported by the finding that activation of  $K^+/H^+$  antiport was independent of the cause of matrix swelling (2, 9, 10). We subsequently showed that the physiological inhibitor is  $Mg^{2+}$  ion, acting from the matrix side of the antiporter (11–17). Inhibition of  $K^+/H^+$  exchange by divalent cations follows the sequence  $Mn^{2+} < Ca^{2+} < Mg^{2+} < Sr^{2+}$  in both rat heart and rat liver mitochondria. The  $K_i$  for inhibition is 50–90  $\mu M$  for  $Ca^{2+}$  and 200–400  $\mu M$  for  $Mg^{2+}$  in both heart and liver (3). This result was confirmed with the reconstituted  $K^+/H^+$  antiporter, where we found a  $K_i$  for  $Mg^{2+}$  of 300–350  $\mu M$  (16).

The  $Mg^{2+}$  Carrier-Brake Hypothesis states that the  $K^+/H^+$  antiporter is under continuous partial inhibition by  $Mg^{2+}$  ions and that variations in matrix  $Mg^{2+}$  activity are one means of regulating matrix volume in vivo (2). The most important quality of this mechanism is its sensitivity to changes in matrix volume. Swelling in vivo is caused by net uptake of salts, and the anions of these salts will include citrate, phosphate, and other anions that form complexes with  $Mg^{2+}$ . Small fluctuations in free anion content must necessarily result in changes in free matrix  $[Mg^{2+}]$  because  $Mg^{2+}$  content is relatively stable. In the steady state, the system will be poised so that the rate of  $K^+/H^+$  antiport precisely equals the rate of electrophoretic  $K^+$  uniport. By attuning  $K^+$  efflux to influx in this manner, “the  $Mg^{2+}$  carrier brake prevents excessive futile cycling of  $K^+$  and minimizes the energetic cost of doing business in a high potassium environment” (2).

*Allosteric Regulation of the  $K^+/H^+$  Antiporter by Matrix Protons.* The  $K^+/H^+$  antiporter is stimulated by alkaline pH up to pH 8.3 (13, 18), and we have shown that the antiporter is allosterically inhibited by matrix protons (17). It is likely that this regulation is important in regulation of matrix volume in vivo, where the challenge to be met is excess uptake or loss of  $K^+$  salts. As described next, these movements will be associated with changes in matrix pH in the direction favoring an appropriate response of the  $K^+/H^+$  antiporter.

*Regulation of the  $K^+/H^+$  Antiporter by Matrix Volume.* An increase in matrix volume is found to activate  $K^+/H^+$  antiport even in the absence of  $Mg^{2+}$  (18–20). Bernardi and Azzone (19) attributed the effect to conformational changes induced by membrane stretching, and we have no alternative mechanism at present.

*Reversible Inhibition of the  $K^+/H^+$  Antiporter by Amphiphilic Amines.* Inhibition of  $K^+/H^+$  antiport by quinine was first reported in 1982 (13). We have observed inhibition by a wide variety of amphiphilic amines, including phenothiazines, antidepressants, antihistamines, antiarrhythmics, and local anesthetics (20). Most agents inhibited in the 50- $\mu M$  range. D- and

L-isoforms of propranolol have identical effects, arguing against receptor specificity of the inhibitory site. Quinine and quinacrine inhibit the  $K^+/H^+$  antiporter of rat liver (14) and beef heart mitochondria (21) with  $K_i$  values at pH 7.8 of 27  $\mu M$  and 6  $\mu M$ , respectively.

*Irreversible Inhibition of the  $K^+/H^+$  Antiporter by Dicyclohexylcarbodiimide (DCCD).* DCCD irreversibly inhibits the  $K^+/H^+$  antiporter, but only when it is in the active conformation.  $K^+/H^+$  antiport is protected from DCCD by its reversible ligands,  $Mg^{2+}$ ,  $H^+$ , and quinine (14, 22, 23). These selective conditions permitted identification of a unique 82-kD band that was radiolabeled by [ $^{14}C$ ]DCCD (22). The kinetics of binding and inhibition were in good agreement and allowed us to estimate that rat liver mitochondria contain 7 to 8 pmol of  $K^+/H^+$  antiporter per mg of protein (23). Assuming a maximal activity at 25°C of 300 nmol/mg.min, the turnover number of the  $K^+/H^+$  antiporter is about 700 per s (14). We also found that mitochondria from beef heart, rat heart, and brown adipose tissue contain the DCCD-labeled 82-kD protein.

*Isolation and Reconstitution of the  $K^+/H^+$  Antiporter.* The  $K^+/H^+$  antiporter was extracted from beef heart and rat liver mitochondrial membranes and reconstituted into lipid vesicles (16, 24, 25). The reconstituted protein exhibited electroneutral  $^{85}Rb$  transport, which was reversibly inhibited by  $Mg^{2+}$  and quinine. Subsequently, we introduced a  $K^+$ -sensitive fluorescent probe, PBFI, for measurement of intraliposomal  $[K^+]$  and established that DCCD completely and irreversibly inhibits  $K^+/H^+$  antiport, in accord with findings in intact mitochondria. In a multistep purification protocol, the 82-kD  $K^+/H^+$  antiporter was purified from rat liver mitochondria to apparent homogeneity on SDS-PAGE. The purified protein catalyzed electroneutral  $K^+/H^+$  antiport and was inhibited by DCCD,  $Mg^{2+}$  or timolol. We subsequently learned that the 82-kD band also contained a subunit of ubiquinone reductase.

*$Na^+/H^+$  and  $K^+/H^+$  Antiporters of Mitochondria.* It was initially believed that  $K^+/H^+$  exchange was mediated by a single  $Na^+/H^+$  antiporter that had a much lower affinity for  $K^+$  than for  $Na^+$ . Nakashima and Garlid (13) showed, however, that mitochondria possess two distinct  $Na^+/H^+$  antiporters. The  $Na^+$ -selective  $Na^+/H^+$  antiporter (26) is cation-selective for  $Na^+$  and does not transport  $K^+$ ,  $Rb^+$ , or  $Cs^+$  (27). The  $K^+/H^+$  antiporter is non selective for alkali cations:  $Na^+$ ,  $K^+$ ,  $Li^+$ ,  $Rb^+$ , and  $Cs^+$  are transported at similar rates with  $K_m$  values that vary between 30 and 120 mM.

*Molecular Identity of Mitochondrial  $K^+/H^+$  and  $Na^+/H^+$  antiporters.* The  $Na^+$ -specific  $Na^+/H^+$  antiporter has been identified in yeast as NHA2 (28). Although its functional characterization is limited, its properties are consistent with this identification. Our own studies indicating that yeast mitochondria lack the  $Na^+/H^+$  antiporter (29) are not in conflict with this identification, because our assays would not have detected the very low activity of this porter. Human NHE6, which is the only mammalian NHE so far identified that is localized to mitochondria, was obtained based on its strong sequence similarities to

Yeast NHA2 (28). NHE6 migrates on SDS-PAGE with a molecular weight of about 65 kD, consistent with our identification of the mitochondrial  $\text{Na}^+/\text{H}^+$  antiporter as a 59-kD protein (30). Thus, the data are consistent with identification of NHE6 as the mitochondrial  $\text{Na}^+$ -specific  $\text{Na}^+/\text{H}^+$  antiporter.

NHE7 (31) is localized to the Golgi and has no mitochondrial targeting signal. It is of interest, with respect to its partial characterization as a nonspecific ( $\text{Na}^+$ ,  $\text{K}^+$ )/ $\text{H}^+$  antiporter, that NHE7 transports  $\text{Na}^+$ ,  $\text{Li}^+$ ,  $\text{K}^+$ , and  $\text{Rb}^+$  and is inhibited by quinine. Moreover, the molecular weight of NHE7 is in the expected range for the 82-kD mitochondrial  $\text{K}^+/\text{H}^+$  antiporter. Thus, this protein appears to be similar to the  $\text{K}^+/\text{H}^+$  antiporters of mitochondria (3) and plasma membrane (32, 33), whose molecular identities remain unknown. There is about 70% amino acid identity between NHE6 and NHE7.

### The ATP-Sensitive $\text{K}^+$ Channel

$\text{K}^+$  uniport following reconstitution was first observed by Mironova et al. (34) and subsequently by Diwan et al. (35). Both laboratories identified  $\text{K}^+$  uniport activity with an inner membrane protein in the 53 to 57-kD range. The channel was  $\text{K}^+$ -selective; however, characterization of reconstituted  $\text{K}^+$  transport was very limited, and no attempts were made to determine its regulation. In 1991, Inoue et al. (36) showed that giant mitochondria exhibited  $\text{K}^+$  channel activity that was inhibited by ATP and glibenclamide. We then published our reconstitution studies establishing that the inner membrane contains an ATP-sensitive  $\text{K}^+$  channel (37). We showed that the partially purified fraction catalyzed electrophoretic  $\text{K}^+$  flux and was highly selective for  $\text{K}^+$ . The conductance was in agreement with values observed by Mironova et al. (34). Importantly,  $\text{K}^+$  flux was inhibited with high affinity by ATP and glibenclamide.

**Subunit Structure of  $\text{mitoK}_{\text{ATP}}$ .** Plasma membrane  $\text{K}_{\text{ATP}}$  channels ( $\text{cellK}_{\text{ATP}}$ ) consist of an inward-rectifying 51-kD  $\text{K}^+$  channel (KIR6.1 or KIR6.2) and a 140-kD sulfonylurea receptor (SUR1, 2A, or 2B). These subunits co-assemble in a 4:4 complex to form an octameric channel (38–40). The SURs are members of the ATP-binding cassette (ABC) family, which also includes p-glycoprotein and CFTR.

$\text{MitoK}_{\text{ATP}}$  also consists of two subunits—a 55-kD mitoKIR and a 63-kD mitoSUR, and we predict that mitoSUR will turn out to be a half-molecule ABC protein. MitoSUR was identified through its labeling with high affinity by azido- $^{125}\text{I}$ -glyburide and fluorescent BODIPY-FL-glyburide (FL-GLY) (41). Labeling was prevented by inclusion of 1  $\mu\text{M}$  glyburide, and purification by preparative SDS-PAGE showed that only the 63-kD protein was labeled. The  $K_d$  for FL-GLY is about 13 nM (42), de facto evidence that the 63-kD band contains the sulfonylurea receptor; yet these subunits remain associated during Triton extraction and purification. MitoKIR can also be purified absent the mitoSUR by ethanol extraction as originally described by Mironova et al. (34).

Experiments designed to determine the topological location of the  $\text{mitoK}_{\text{ATP}}$  regulatory sites for  $\text{Mg}^{2+}$ , nucleotides, and

long-chain CoA esters revealed that these ligands all react on the same side of  $\text{mitoK}_{\text{ATP}}$  and face the intermembrane space (43).

**Regulation of  $\text{mitoK}_{\text{ATP}}$  by Nucleotides, CoA esters, and  $\text{Mg}^{2+}$ .** Reconstituted  $\text{mitoK}_{\text{ATP}}$  is inhibited by ATP (39  $\mu\text{M}$ ), ADP (280  $\mu\text{M}$ ), palmitoyl CoA (260 nM), and oleoyl CoA (40 nM), with  $K_{1/2}$  values given in parentheses. Inhibition exhibits an absolute requirement for  $\text{Mg}^{2+}$  (50  $\mu\text{M}$ ) or  $\text{Ca}^{2+}$  (50  $\mu\text{M}$ ). Because CoA esters are not  $\text{Mg}^{2+}$  chelators, these results imply that  $\text{Mg}^{2+}$  ion interacts independently with the protein. Note that the effects of ADP and oleoyl CoA (inhibition) are opposite to what is normally observed with  $\text{cellK}_{\text{ATP}}$  (opening). Inhibition by ATP is reversed by GTP (7  $\mu\text{M}$ ), GDP (230  $\mu\text{M}$ ), and UDP (13  $\mu\text{M}$ ) (37, 44).

MitoKIR is also inhibited by ATP but with lower affinity (500  $\mu\text{M}$ ) and lacking the requirement for  $\text{Mg}^{2+}$ , suggesting that the  $\text{Mg}^{2+}$  binding site is located on mitoSUR. Low affinity inhibition of mitoKIR by ATP is remarkably similar to observations with truncated KIR6.2 (45). In the first instance of nucleotide opening of a KIR, we found that UDP reversed ATP inhibition of mitoKIR noncompetitively with  $K_{1/2}$  of 70 to 75  $\mu\text{M}$ .

It is not at all clear how or whether these complex interactions regulate  $\text{mitoK}_{\text{ATP}}$  in vivo. We favor the view that  $\text{mitoK}_{\text{ATP}}$  is closed during low work states in vivo, and that it is opened by phosphorylation; however, there is no direct evidence for this at present.

**Pharmacological Blockers of  $\text{mitoK}_{\text{ATP}}$ .** In isolated, respiring rat heart or liver mitochondria,  $\text{mitoK}_{\text{ATP}}$  is inhibited by glyburide (1–6  $\mu\text{M}$ ) or 5-OH-decanoate (5-HD) (45–75  $\mu\text{M}$ ), but only when ATP,  $\text{Mg}^{2+}$ , and an opener are present. These are the conditions that would obtain in vivo. We found that although different open states of  $\text{mitoK}_{\text{ATP}}$  catalyze identical  $\text{K}^+$  fluxes, they exhibit very different susceptibilities to channel inhibitors (46).

MitoKIR is NOT sensitive to glyburide or 5-HD, due to the absence of the mitoSUR. However, we have recently identified a number of other drugs that block  $\text{K}^+$  flux through mitoKIR. Unlike 5-HD, these drugs inhibit all open states of the channel, and they are equally effective on holo- $\text{mitoK}_{\text{ATP}}$  in both liposomes and intact mitochondria (unpublished data).

**Pharmacological Openers of  $\text{mitoK}_{\text{ATP}}$ .** In the first report showing that  $\text{mitoK}_{\text{ATP}}$  is a receptor for  $\text{K}^+$  channel openers (KCOs), we reported the effects of cromakalim (1  $\mu\text{M}$ ), diazoxide (0.4 nM), and two cromakalim analogues (6 nM) (47). We have now extended these studies to additional KCOs (48, 49).

No pharmacological agents have been identified that open plasma membrane KIR, and mitoKIR is completely insensitive to the classical KCOs. However, Mironova and coworkers (50) have observed that a derivative of the local anesthetic benzocaine (*p*-diethylaminoethylbenzoate, DEB) activates mitoKIR channel activity in bilayer membranes. We have confirmed that this agent opens both mitoKIR and holo- $\text{mitoK}_{\text{ATP}}$  in the reconstituted system and in mitochondria. DEB has a  $K_{1/2}$  of about 10  $\mu\text{M}$ .

### **Electroneutral Anion Exchange Porters in Mitochondria**

When  $K^+$  influx occurs in exchange for  $H^+$  efflux driven by electron transport, the resulting alkalization will drive electroneutral  $P_i$  uptake via the  $P_i/OH^-$  exchanger. In liver mitochondria, tricarboxylate and dicarboxylate exchange porters are linked to each other and to the  $H_2PO_4^-/H^+$  symporter through which, in turn, substrate anions are coupled to the pH gradient [reviewed in (51–55)]. In heart and brain, which lack several of the anion exchangers, the  $P_i/OH^-$  exchanger will make the most significant contribution.

### **Consequences of Net $K^+$ Uptake in Mitochondria**

Our hypothesis that  $mitoK_{ATP}$  plays a key role in cardioprotection against ischemia-reperfusion injury (47, 56) is now generally accepted (42, 57–60). However, there is no consensus on the mechanisms of protection and considerable controversy over what happens when  $mitoK_{ATP}$  is opened in vivo (61). To understand these events, it is necessary to understand what happens to mitochondria when  $mitoK_{ATP}$  is opened.

*MitoK<sub>ATP</sub> Opening Does Not Cause Significant Uncoupling.* Increased  $K^+$  cycling will dissipate energy and reduce  $\Delta\Psi$ , but it is important to note that the degree of uncoupling depends on the magnitude of the added  $K^+$  flux through  $mitoK_{ATP}$ . Although several laboratories have reported results that seem to indicate uncoupling by  $mitoK_{ATP}$  opening in isolated mitochondria (62–64), we have shown directly that this is not the case (65). Similarly, the suggestion that diazoxide uncouples in vivo (57) seems to be refuted by measurements of uncoupling in vivo (66–68).

The reason for lack of significant uncoupling is that the magnitude of  $K^+$  flux through  $mitoK_{ATP}$  is very low: 24 to 30 nmol  $K^+ \cdot \text{min}^{-1} \text{mg}^{-1}$  at 25°C in rat heart mitochondria, enough to depolarize  $\Delta p$  by only 1–2 mV (65). Brain  $mitoK_{ATP}$  has higher activity, but not enough to cause significant uncoupling (41).

*MitoK<sub>ATP</sub> Opening Increases Matrix Volume.* The inner membrane is highly permeable to water (69), so uptake or loss of  $K^+$  salts will be accompanied by osmotically obligated water and swelling or shrinking of the matrix. We find that  $mitoK_{ATP}$  opening causes a 15 to 20% increase in steady state matrix volume in mitochondria from rat heart, liver, and brain, an effect that was blocked by 5-HD (41, 65).  $MitoK_{ATP}$  opening was also shown to regulate matrix volume during simulated ischemia, in which  $K^+$  uptake is supported by ATP hydrolysis. Addition of antimycin A caused depolarization, a decrease in  $K^+$  leak, and matrix contraction. The matrix contraction was reversed by diazoxide (65).

*MitoK<sub>ATP</sub> Opening Decreases IMS Volume.* Electron micrographs of mitochondria indicate that the matrix is tightly packed within the outer membrane, leading to a narrow intermembrane space (IMS). Addition of diazoxide causes the matrix to expand by about 0.35  $\mu\text{l}/\text{mg}$ . Conversely, inhibition of state 3 respiration, which decreases  $\Delta\Psi$ , causes the matrix to contract by about the same amount (65). Most of this change will occur at the expense of IMS volume. We estimate that

the intermembrane distance varies between 90 and 160 Å between these extremes of matrix volume (unpublished) and suggest that these changes may have important effects on IMS structure-function. For example, the octameric mitochondrial isoform of creatine kinase (Mi-CK), which is proposed to be the dominant form in vivo, is cubic with a side length of 93 Å (70, 71).

*MitoK<sub>ATP</sub> Opening Regulates VDAC Permeability to Nucleotides.* VDAC controls outer membrane permeability to ADP and ATP (72). In heart, VDAC is normally in a low-conductance state that is poorly permeable to these nucleotides, and energy transfers between mitochondria and cytosol are mediated instead by creatine and creatine phosphate (73). Two measurements that reflect outer membrane permeability to nucleotides are the  $K_{1/2}$  (ADP) for respiration (74) and the rate of ATP hydrolysis in nonrespiring mitochondria. We have found that diazoxide-induced changes in matrix volume have profound effects on both of these parameters and that an intact outer membrane is required for these effects (75). We hypothesize that binding of octameric Mi-CK to VDAC requires a narrow intermembrane distance and confers a low conductance to nucleotides. When IMS expands due to matrix contraction, Mi-CK dissociates from VDAC, leading to a high conductance state. Preventing this effect causes reduced ATP hydrolysis, which may contribute to the cardioprotective effects of  $mitoK_{ATP}$  opening (41).

*MitoK<sub>ATP</sub> Opening Increases Matrix pH.* The pH change that results from increased  $K^+$  uptake is mitigated by uptake of  $P_i$  with protons. pH will nevertheless increase, because cytosolic  $[P_i]$  is much lower than  $[K^+]$ . This pH increase may be significant for heart, in which resting  $P_i$  levels are on the order of 1 mM.

*MitoK<sub>ATP</sub> Opening Increases Mitochondrial ROS Production.* Adding a  $mitoK_{ATP}$  opener to cardiomyocytes or perfused hearts causes a moderate rise in mitochondrial ROS production, which is blocked by 5-HD (61, 76, 77). The ROS production is caused by increased  $K^+$  influx into the matrix, because it can be mimicked by low concentrations of valinomycin. As expected valinomycin-induced ROS is insensitive to 5-HD (unpublished). We observe in isolated heart mitochondria that mild matrix alkalization causes increased ROS production and suggest that this may be the mechanism by which KCOs induce increased ROS production.

## **CONCLUSIONS**

The considerations of the preceding section may be extrapolated to the intact cell:  $MitoK_{ATP}$  is opened in vivo by administration of a  $K_{ATP}$  channel opener or by endogenous signaling, perhaps involving direct phosphorylation of the channel. The immediate consequence of  $mitoK_{ATP}$  opening is a moderate  $K^+$  influx into the matrix. What happens next will depend on the underlying bioenergetic state of the cell. In the low-work state (high  $\Delta\Psi$ ), influx of  $K^+$  will cause matrix swelling, matrix alkalization, and increased ROS production. In the high-work state,

or during ischemia or hypoxia,  $K^+$  influx through  $\text{mitoK}_{\text{ATP}}$  will compensate for the decrease in  $K^+$  diffusion at the lower  $\Delta\Psi$ , so that matrix and IMS volumes are maintained. These features lead to several hypotheses for multiple roles of  $\text{mitoK}_{\text{ATP}}$  in cardiac physiology that will be detailed in future publications.

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