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Review

Mitochondrial potassium transport: the K^+ cycle

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Abstract

Potassium transport plays three distinct roles in mitochondria. *Volume homeostasis to prevent excess matrix swelling* is a housekeeping function that is essential for maintaining the structural integrity of the organelle. This function is mediated by the K^+/H^+ antiporter and was first proposed by Peter Mitchell. *Volume homeostasis to prevent excess matrix contraction* is a recently discovered function that maintains a fully expanded matrix when diffusive K^+ influx declines due to membrane depolarization caused by high rates of electron transport. Maintaining matrix volume under these conditions is important because matrix contraction inhibits electron transport and also perturbs the structure–function of the intermembrane space (IMS). This volume regulation is mediated by the mitochondrial ATP-sensitive K^+ channel (mitoK_{ATP}). *Cell signaling* functions to protect the cell from ischemia-reperfusion injury and also to trigger transcription of genes required for cell growth. This function depends on the ability of mitoK_{ATP} opening to trigger increased mitochondrial production of reactive oxygen species (ROS). This review discusses the properties of the mitochondrial K^+ cycle that help to understand the basis of these diverse effects.

Keywords: Adenosine triphosphate; Mitochondria; Heart; ATP-sensitive potassium channel; Ion channel gating; Membrane transport; Volume regulation

1. Introduction

The inner membrane of mitochondria contains the essential components of the electron transport proteins, the F1F0-ATPase, and all of the exchange carriers that transfer substrates between the mitochondrial matrix and the cytosol. As uniquely recognized by Peter Mitchell, this picture is incomplete without considering the physiology of mitochondria. These organelles function in a cytosolic milieu containing Na^+, K^+, and Ca^{2+}, which are transported across the inner membrane. The enzymes of oxidative phosphorylation must be in communication with the cytosol, in order to receive signals calling for increased ATP production. Mitochondria also deliver signals to the cytosol, including changes in reactive oxygen species (ROS), which activate kinases, or cytochrome *c* from the intermembrane space (IMS), leading to apoptosis. These components of mitochondrial physiology will be discussed in this review.

Mitochondria are structurally complex. The internal compartment, containing the enzymes of the tricarboxylic acid cycle, is called the matrix, which is bounded by the inner membrane. External to the inner membrane is a narrow compartment called the IMS, which, in turn, is bounded by the outer mitochondrial membrane (OM). The inner membrane is highly invaginated, creating internal structures called the cristae. The region adjacent to the OM is the peripheral inner membrane. The cristal and peripheral inner membranes are continuous, and, although they may have subtly different functions, they will be treated in this review as a single inner membrane. The inner membrane surface: volume (S/V) ratio is very high, about 500 $cm^2/\mu l$ of matrix volume, roughly two orders of magnitude greater than the S/V of cells. This means that ion traffic across the inner membrane will be very high. Moreover, the gradients driving salt uptake are very high, and the inner membrane is highly permeable to water. These factors increase considerably the hazard of excessive osmotic swelling and lysis, disrupting the vesicular structure essential for mitochondrial function.

This review and its companion [1] will discuss three distinct roles played by the mitochondrial K^+ cycle in mitochondrial and cell physiology: (i) to provide volume homeostasis to prevent excessive matrix swelling; (ii) to provide volume regulation to prevent excessive matrix

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contraction, and (iii) to regulate mitochondrial ROS production for the purpose of cell signaling. This paper will review what is known about the components of the K^+ cycle and the consequences of changes in their activities.

2. The potassium cycle and chemiosmotic theory

Mitchell [2] proposed that the mitochondrial inner membrane contains vectorially oriented electron transport enzymes that transform the energy of substrate oxidation into an electrogenic proton efflux. The energy is thereby stored as a proton electrochemical potential gradient across the inner membrane, called the protonmotive force, Δp:

$$\Delta p = \Delta\psi + Z\Delta\mathrm{pH} \quad (1)$$

where $Z \equiv (RT\ln 10)/F$ (59.2 mV at 25 °C), and $\Delta\psi$ is the membrane potential. In isolated mitochondria respiring in the absence of ADP (state 2), $\Delta\psi$ is about 190 mV and ΔpH is about 0.3 units.

Chemiosmotic theory [3] is the basis for understanding the mitochondrial K^+ cycle. The very high value of $\Delta\psi$ required for oxidative phosphorylation is not only a powerful driving force for K^+ uptake, but also assures that K^+ diffusion will be highly sensitive to physiological fluctuations in $\Delta\psi$, as discussed in Section 4. Thus, dynamic regulation of mitochondrial K^+ flux in vivo is essential for maintaining the structural and functional integrity necessary for oxidative phosphorylation. This was clearly recognized by Mitchell [4], who wrote:

> While the introduction of the foregoing sophistication [the chemiosmotic theory of energy coupling] solved one problem it created another; for, the membrane potential that would now be required to reverse the ATPase reaction would cause the ions of opposite sign of charge to the internal aqueous phase to leak in through the coupling membrane. To prevent swelling and lysis, the ion leakage would have to be balanced by extrusion of ions against the electrical gradient. It was therefore necessary to postulate that the coupling membrane contains exchange diffusion systems… that strictly couple the exchange of anions against OH^- ions and of cations against H^+ ions.

This hypothesis was stated long before electroneutral cation and anion exchangers were known to exist in nature and reveals an extraordinary depth of physiological insight.

3. The mitochondrial potassium cycle—an overview

The mitochondrial K^+ cycle consists of influx and efflux pathways for K^+, H^+, and anions and is diagrammed in Fig. 1. Note that these ions are exchanged between the matrix and the IMS; however, the OM does not present a barrier to further exchange of small ions with the cytosol. Electrogenic proton ejection by the electron transport system generates an electrical membrane potential (1), which drives K^+ influx by diffusion ("K^+ leak") and via the mitochondrial ATP-sensitive K^+ channel (mitoK_{ATP}). This K^+ for H^+ exchange will alkalinize the matrix, causing phosphate to enter via the electroneutral Pi-H^+ symporter. Net uptake of K^+ salts will be accompanied by osmotically obligated water, resulting in matrix swelling. Excess matrix K^+ is then ejected by the K^+/H^+ antiporter.

Early work on the K^+ cycle focused on diffusive K^+ influx and K^+/H^+ antiport (for reviews, see Refs. [5–7]). Because mitochondria operate at a very high $\Delta\psi$, there is an inexorable influx of K^+ salts and water that threatens the integrity of the organelle. Diffusive K^+ influx is too small to have a significant effect on energy conservation, but if it were allowed to continue unchecked, it would be sufficient to cause matrix water content to increase by about 15% per minute, with eventual lysis. This catastrophe is avoided by means of the K^+/H^+ antiporter, which is regulated to sense volume changes and eject excess K^+ from the matrix [7,8].

Considering the large electrical driving force for K^+ uptake, it seemed unlikely that Nature would insert a K^+ channel in the inner membrane, but work from Mironova's and Diwan's laboratories indicated that was indeed the case [9,10]. The K^+ uniporter turned out to be a

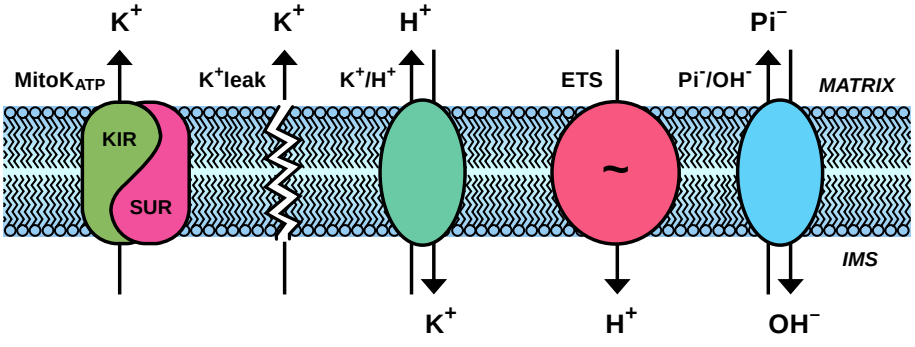

Fig. 1. The mitochondrial potassium cycle. See text for description.

mitoK$_{ATP}$ [11]. MitoK$_{ATP}$ meets a different need in volume regulation. When mitochondria are synthesizing ATP at very high rates, $\Delta\psi$ decreases. K^+ diffusion is very sensitive to changes in $\Delta\psi$ and the transient imbalance between influx and efflux causes matrix volume to contract to a lower steady state volume. Since matrix contraction interferes with efficient energy transfers from mitochondria to cytosol [1], these events occur at precisely the wrong time in relation to the cell's energetic needs. We propose that endogenous signals open mitoK$_{ATP}$ so that K^+ influx and matrix volume are maintained in the face of a lower driving force.

It turns out that mitoK$_{ATP}$ plays a third role in cell physiology. When mitoK$_{ATP}$ is opened in the resting state of high $\Delta\psi$, the resulting K^+ influx induces a moderate increase in mitochondrial production of ROS [12,13]. The increased ROS levels, in turn, activate a variety of kinases involved in the signaling pathways of cardioprotection against ischemia-reperfusion injury and in pathways leading to gene transcription and cell growth.

A major reason for renewed interest in the mitochondrial K^+ cycle arose when it was found that pharmacological agents that open mitoK$_{ATP}$ protect the heart against ischemia-reperfusion injury [14]. Moreover, we have found that mitoK$_{ATP}$ plays important roles in normal cardiac physiology. These aspects are reviewed in the companion paper [1].

4. Potassium leak across the inner membrane

One of the four postulates of the chemiosmotic theory is that 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 [2]. Notwithstanding the low permeability, cation leaks occur at significant rates, and they are physiologically important: inward K^+ leak causes matrix swelling [7], and inward proton leak contributes to the basal metabolic rate [15].

Diffusive transport of ions obeys the same laws that govern transport of nonelectrolytes across thin membranes. The rate of transport is proportional to the concentration difference, and the proportionality constant (the permeability coefficient) is a function of the energy barrier that must be crossed during transport. The ionic charge adds a new feature that derives from long-range effects of the electric field on the local free energy of the diffusing particles. An ion diffusing across the inner membrane of mitochondria must traverse a single, sharp energy barrier located at the center of the membrane, and only those ions having sufficient energy to reach this peak will cross to the energy well on the opposite side. The process of overcoming this extremely unfavorable Gibbs energy of transfer is clearly the rate-limiting step of ion diffusion, and net flux will be proportional to the differential probability of getting to this peak from either side. This probability is given by the Boltzmann function, $\exp(-\Delta\tilde{\mu}_p/RT)$, where $\Delta\tilde{\mu}_p \equiv \tilde{\mu}_p - \tilde{\mu}_{aq}$ is the Gibbs energy of the ion at the peak (p) relative to its value in the aqueous energy well at the surface of the membrane (aq). $\Delta\tilde{\mu}_p$ is the activation energy of ion diffusion and defines the permeability coefficient. As described in detail by Garlid et al. [16], these considerations lead to the following expression for diffusive flux of cations and hydronium ions across thin biomembranes:

$$J = fP(C_{1o}e^{u/2} - C_{2o}e^{-u/2}) \quad (2)$$

where u is the reduced voltage ($zF\Delta\psi/RT$), C_{1o} and C_{2o} are bulk aqueous concentrations, f is the surface partition coefficient (energy well/bulk), and P is the permeability constant, given by

$$P \equiv ke^{-\Delta\mu_p^o/RT} \quad (3)$$

The factor 1/2 in the exponents of Eq. (2) arises from the fact that the maximum energy barrier is found at the midpoint of the membrane. This barrier splits $\Delta\psi$ in half when we make the customary constant field assumption.

The second term in Eq. (2) represents back-flux of cations from the matrix and becomes negligible at the high values of $\Delta\psi$ maintained by respiring mitochondria. Under these conditions, Eq. (2) reduces to a simple exponential function of $\Delta\psi$:

$$J = fPC_{1o}e^{u/2} \quad (4)$$

Eq. (4) emphasizes the point that ion flux at high potentials is not affected by the concentration gradient across the membrane and also makes the important prediction that all diffusing cations will experience the same rate-limiting step. This prediction is affirmed by the flux-voltage plots in Fig. 2, which show that TEA^+ (tetraethylammonium ion) and H^+ (hydronium ion) diffusion across the mitochondrial inner membrane are identical with respect to their voltage dependence.

The finding that protons and cations experience an identical rate-limiting step is at variance with the suggestion that proton leak is "anomalous" and that protons are transported by a unique mechanism [17]. In fact, the difference between proton and cation leak is only quantitative and largely reflects events at the membrane surface, whose influence has been ignored in much of the work on proton leaks. It must be emphasized that the concentration terms in all flux equations for membrane transport, including Fick's law, apply to the energy wells at the surface of the barrier and not to the bulk aqueous solution [16].

Eq. (4) is relevant to conditions facing mitochondria in vivo. When ATP production increases in response to

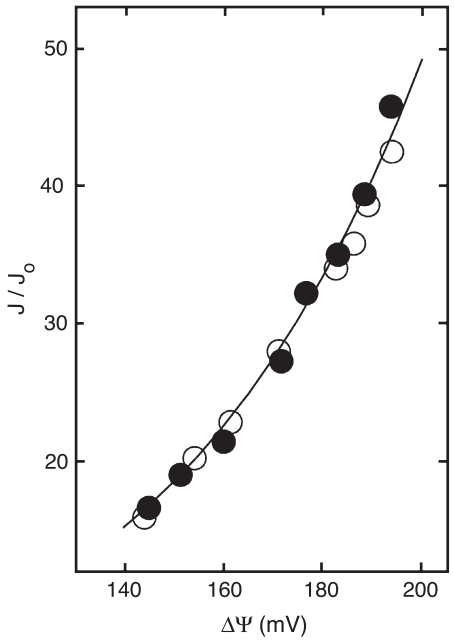

Fig. 2. The exponential dependence of mitochondrial cation and proton leak on $\Delta\psi$. Normalized fluxes of TEA^+ (●) and H^+ (○) in mitochondria, J/J_o, are plotted versus $\Delta\psi$ as in Eq. (4). $J_o \equiv f\,P[C]_o$, where $[C]_o$ is the aqueous concentration of TEA^+ or H^+, respectively, and f and P are defined in the text. J_o was obtained from the extrapolated intercept of a semilogarithmic plot, ln(J) vs. $F\Delta\psi/RT$. The solid line is the function $e^{F\Delta\psi/2RT}$, showing that the data obey Eq. (4).

cellular demands, $\Delta\psi$ will decrease, because more current is being drawn from the protonmotive batteries. Eq. (4) tells us that a 10% decrease in $\Delta\psi$, from 190 to 170 mV, will result in a 32% decrease in the rate of diffusive K^+ uptake.

5. The mitochondrial K^+/H^+ and Na^+/H^+ antiporters

5.1. Existence of the K^+/H^+ antiporter

The first evidence for Na^+/H^+ and K^+/H^+ antiport in mitochondria was obtained by Mitchell and Moyle [18], who measured swelling in NaOAc and KOAc media. These findings were confirmed by several groups, and the view emerged that mitochondria possess a very active Na^+/H^+ antiporter while K^+/H^+ antiport activity is either very low or absent altogether [19–21].

In studies predating the chemiosmotic hypothesis, Gamble [22] showed that $^{42}K^+/K^+$ exchange was respiration-dependent, suggesting K^+ cycling in mitochondria. Using similar protocols, Brierley et al. [23–25] suggested that $^{42}K^+/K^+$ exchange may reflect uniport–antiport cycling of K^+ and proposed a regulated interplay between K^+ uniport and K^+/H^+ antiport [26]. The problem remained that K^+/H^+ antiport was inferred and not demonstrated directly. It was necessary to dissect K^+/H^+ antiport from K^+ uniport experimentally in order to establish its existence.

This was accomplished in our laboratory with the demonstration that mitochondria, which retain matrix K^+ when suspended in isotonic sucrose, lose K^+ in hypotonic sucrose [27]. Swelling-induced K^+ efflux was reversed by valinomycin in respiring mitochondria but not in the absence of respiration. These and other results [8,28] established the existence of a latent, electroneutral K^+/H^+ antiporter. The finding that latency of K^+/H^+ antiport was released by matrix swelling provided an essential clue to the mechanism of regulation of the carrier, as described below. Swelling-induced K^+ efflux also permitted, for the first time, an experimental separation of K^+/H^+ antiport from K^+ uniport.

5.2. Mitochondria possess two cation/H^+ antiporters

Prior to 1982, it was generally 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 [29] showed, however, that mitochondria possess two distinct cation/H^+ antiporters. The Na^+/H^+ antiporter [18] is selective for Na^+ and does not transport K^+, Rb^+, or Cs^+. The K^+/H^+ antiporter transports all alkali cations (Na^+, K^+, Li^+, Rb^+ and Cs^+) at similar rates, with K_m values varying between 30 and 120 mM. The Na^+/H^+ antiporter exhibits a characteristic pH profile with maximal activity at about pH 7.3 and lacks the complex regulation exhibited by the K^+/H^+ antiporter. The K^+/H^+ antiporter, but not the Na^+/H^+ antiporter, is inhibited by quinine [29].

A word about nomenclature. Both antiporters transport Na^+ under in vitro conditions. However, a consideration of cytosolic Na^+ and K^+ concentrations and the respective K_m values indicates that Na^+ will not undergo significant exchange on the K^+/H^+ antiporter under in vivo conditions. Therefore, its designation as the K^+/H^+ antiporter seems appropriate on physiological grounds.

Garlid [30] has suggested that the plasmalemma may contain modified versions of both mitochondrial Na^+/H^+ antiporters. The mitochondrial Na^+/H^+ antiporter resembles the renal tubular Na^+/H^+ antiporter [31] in its discrimination against K^+ and its competitive inhibition by Li^+ [32]. The mitochondrial K^+/H^+ antiporter resembles a cardiac sarcolemmal $Na^+–K^+/H^+$ antiporter that transports all alkali cations, including Na^+ and K^+, and is inhibited by dicyclohexylcarbodiimide (DCCD) and amphiphilic amines [33,34]. The latter class of antiporter would tend to catalyze Na^+/K^+ exchange in cells and dissipate the effects of the Na^+, K^+-ATPase. A sound design principle would be followed if the cell, like mitochondria, were to regulate volume by governing a passive backflow process rather than an active transport process.

5.3. Regulation of the mitochondrial K^+/H^+ antiporter

We showed in a series of papers that the mitochondrial K^+/H^+ antiporter is reversibly inhibited by Mg^{2+},

protons, and amphiphilic amines; and is irreversibly inhibited by DCCD.

5.3.1. Regulation of the K^+/H^+ antiporter by matrix Mg^{2+}—the carrier brake mechanism

To explain activation of the K^+/H^+ antiporter by matrix swelling, we proposed that a matrix solute acts as a reversible inhibitor of the antiporter and that swelling reduces inhibitor concentration by dilution. This mechanism was consistent with the finding that activation of K^+/H^+ antiport was independent of the cause of matrix swelling [8,27,28].

A strong clue to the identity of this inhibitor came from the dependence of swelling-induced K^+ efflux on matrix anion composition. Anions that form complexes with divalent cations (citrate, phosphate) stimulated electroneutral K^+ efflux, while anions with poor chelating ability (acetate, succinate, malate) retarded it [8]. We concluded that the K^+/H^+ antiporter was regulated by matrix divalent cations, and we subsequently showed that Mg^{2+} ion is the physiological inhibitor, acting from the matrix side of the antiporter [29,35–40]. Inhibition of K^+/H^+ antiport 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 Mg^{2+} inhibition is 200–400 μM in both heart and liver [7], a result that was confirmed with the reconstituted K^+/H^+ antiporter [39].

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. 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+}], since 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 K^+ uptake 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'' [8]. This hypothesis also explains the apparent absence of K^+/H^+ antiport from freshly isolated mitochondria: as K^+ and anions are lost to the K^+-free medium used during the isolation procedure, matrix [Mg^{2+}] rises to levels at which the antiporter is fully inhibited. Indeed, it is this process which determines the matrix volume of isolated mitochondria; they would lose all their K^+ salts without Mg^{2+} inhibition of the K^+/H^+ antiporter.

5.3.2. Allosteric regulation of the K^+/H^+ antiporter by matrix protons

The K^+/H^+ antiporter is also inhibited by matrix protons [40]. This inhibition is allosteric and additive with the competition that occurs between H^+ and K^+ for the transport site. As a consequence, K^+/H^+ antiport increases with alkaline pH and reaches a maximum at pH 8.3 [29,41]. Proton regulation of the K^+/H^+ antiporter may be important in heart mitochondria, which lack significant citrate transport activity.

5.3.3. Regulation of the K^+/H^+ antiporter by matrix volume

Increased matrix volume itself activates K^+/H^+ antiport, even when matrix Mg^{2+} is depleted [41–43]. The mechanism of this effect is unknown. It may be due to conformational changes induced by membrane stretching [42], or the allosteric site may interact with other matrix solutes in the nonphysiological case of very low [Mg^{2+}] and [H^+].

5.3.4. Reversible inhibition of the K^+/H^+ antiporter by amphiphilic amines

A wide variety of amphiphilic amines were found to inhibit the K^+/H^+ antiporter, including phenothiazines, antidepressants, antihistamines, antiarrhythmics, and local anesthetics [43,44]. Most agents inhibited in the 50 μM range. This inhibition is not due to interaction with a β-receptor-like site, because D and L isomers of propranolol were found to have identical effects. Quinine and quinacrine inhibit the K^+/H^+ antiporter of liver mitochondria with Hill coefficients of 1.0 and K_i values at pH 7.8 of 27 and 6 μM, respectively [37]. The discovery of these pharmacological inhibitors of K^+/H^+ antiport [29] proved to be very useful for the study of cation/proton antiport in mitochondria, because it enabled the demonstration for the first time that mitochondria contain two different Na^+/H^+ antiporters [29].

5.3.5. Irreversible inhibition of the K^+/H^+ antiporter by DCCD

DCCD is a non-selective probe of ion transport proteins [45] which reacts irreversibly with carboxylic groups that are buried within the hydrophobic core of the membrane. We showed that DCCD inhibits the K^+/H^+ antiporter irreversibly and completely [46]. We subsequently made the interesting observation that, although Rb^+/H^+ exchange was completely inhibited, Rb^+/Rb^+ exchange was only partially inhibited by DCCD [39].

DCCD was found to possess the useful property that it only inhibits the active conformation of the K^+/H^+ antiporter. Thus, K^+/H^+ antiport is protected from DCCD inhibition by quinine, matrix Mg^{2+}, and matrix acidity [37,46,47]. This property was ideally suited to protocols designed to identify the protein by DCCD radiolabeling, and the K^+/H^+ antiporter was identified as an 82 kDa protein in rat heart, beef heart, and hamster brown adipose tissue mitochondria [38,46]. DCCD labeled submitochondrial particles, confirming that the protein resides in the inner membrane [47]. The kinetics of [^{14}C] DCCD binding to the 82 kDa protein are in good agreement with inhibitory kinetics [47], and the amount of radiolabeled 82 kDa band yields an estimate of 7–8 pmol of

K^+/H^+ antiporter per milligram of rat liver mitochondrial protein. Assuming a maximal activity at 25 °C of 300 nmol/mg min, the turnover number of the K^+/H^+ antiporter is about 700 per second [37].

5.4. Purification and reconstitution of the K^+/H^+ antiporter

The K^+/H^+ antiporter was extracted from mitochondrial membranes using Triton X-100 and reconstituted into liposomes [39]. This preparation exhibited electroneutral $^{86}Rb^+$ transport that was reversibly inhibited by Mg^{2+} and quinine. We then introduced a fluorescence assay for liposomal K^+ transport, using the K^+-sensitive probe PBFI, which was far superior to the radioisotope method [48,49]. The 82 kDa K^+/H^+ antiporter was purified to apparent homogeneity on SDS-PAGE, using a multistep protocol. The purified protein catalyzed electroneutral K^+/H^+ antiport that was inhibited by DCCD, Mg^{2+}, and timolol [50]. We subsequently learned, however, that the 82 kDa band contains a subunit of ubiquinone reductase in addition to the K^+/H^+ antiporter (unpublished results).

5.5. The molecular identity of the mitochondrial Na^+/H^+ and K^+/H^+ antiporters

The yeast mitochondrial Na^+/H^+ antiporter was identified as NHA2 [51]. Isolated yeast mitochondria were assayed for $^{22}Na^+$ uptake from acetate medium. Flux was inhibited by benzamil, an inhibitor of the mitochondrial Na^+/H^+ antiporter [52], and no flux was observed in mitochondria from yeast lacking the NHA2 gene. Although this functional characterization is very limited, it is consistent with identification of NHA2 as a mitochondrial Na^+/H^+ antiporter. Our laboratory had shown that yeast mitochondria lack the Na^+-specific Na^+/H^+ antiporter [53]. However, there is no real conflict between these results, because our light scattering assay would not have been able to detect the very low rates of $^{22}Na^+$ uptake by NHA2 (1 nmol/mg/min) reported by Numata et al. [51].

Human NHE6 was obtained based on its strong sequence similarities to yeast NHA2 [51]. Seven mammalian Na^+/H^+ antiporter isoforms (NHE1 to NHE7) have so far been identified [54,55]. Of these, only NHE6 appears to be localized to mitochondria. NHE6 contains a putative mitochondrial inner membrane targeting signal; dual labeling experiments indicate mitochondrial localization; and it was found to distribute in all tissues examined. NHE6 migrates on SDS-PAGE with a molecular weight of about 65 kDa, which is also consistent with our identification of the mitochondrial Na^+/H^+ antiporter as a 59 kDa protein [56]. Thus, the data are consistent with identification of NHE6 as the mitochondrial Na^+-specific Na^+/H^+ antiporter.

NHE7 [57] is not a mitochondrial protein, but its properties may be relevant to future molecular identification of the K^+/H^+ antiporter. NHE7 is localized to the Golgi and has no mitochondrial targeting signal. Partial characterization indicates that it is a non-specific (Na^+, K^+)/H^+ antiporter that transports Na^+, Li^+, K^+, and Rb^+ and is inhibited by quinine. Moreover, its molecular weight is in the expected range for the 82 kDa mitochondrial K^+/H^+ antiporter. NHE7 appears to be similar to the K^+/H^+ antiporters of mitochondria [7] and plasma membrane [33,34], whose molecular identities remain unknown.

6. The mitochondrial ATP-sensitive K^+ channel

The existence of a specific K^+ uniport pathway was difficult to establish in isolated mitochondria, due to the coexistence of a considerable parallel K^+ leak pathway. Accordingly, two laboratories undertook to reconstitute a specific K^+ uniporter, and this was achieved by Mironova et al. [9] and Diwan et al. [10]. The channel was K^+-selective; however, its regulation was not characterized. Although activity was identified with an inner membrane protein in the 53–57 kDa range, attempts to obtain amino acid sequence were unsuccessful.

We began purification–reconstitution of mitoK_{ATP} in 1990 and presented the first results in 1991 [58,59]. In the same year, Inoue et al. [60] obtained channel conductances by patch clamping fused giant mitoplasts from rat liver mitochondria and showed that channel activity was inhibited by ATP and glyburide, consistent with identification as a K_{ATP} channel. In 1992, we published the results of our reconstitution studies, which confirmed that the inner membrane contains an ATP-dependent K^+ channel [11]. We observed in that paper that mitoK_{ATP} exhibits many properties similar to those of the plasma membrane K_{ATP} channel (cellK_{ATP}), which is discussed in Section 6.6.1.

6.1. Detection of mitoK_{ATP} activity in isolated mitochondria

Respiring mitochondria take up K^+ salts and osmotically obligated water when suspended in K^+ medium. Therefore, matrix swelling measured using the light scattering technique [61,62] is a reasonably direct measure of K^+ influx. We have also estimated the magnitude of mitoK_{ATP}-mediated K^+ flux by measuring the attendant respiratory stimulation in rat heart [63], and rat brain [64] mitochondria. As is always the case in our laboratory, both protocols employ negative controls, which consist of making the same measurements in tetraethylammonium (TEA^+) or Li^+ medium (in contrast to the sarcolemmal K_{ATP}, TEA^+ has no effect on K^+ flux via mitoK_{ATP} [11]).

Other techniques have not proved useful for studying mitoK_{ATP} activity in isolated mitochondria. Thus, direct measurement of K^+ flux is not possible, because K^+ concentrations are approximately the same on both sides of the inner membrane. Changes in $\Delta\psi$ are too small to detect, because K^+ flux through mitoK_{ATP} is very small in magnitude [63]. In principle, it should be possible to detect salt and water uptake by isotopic measurements

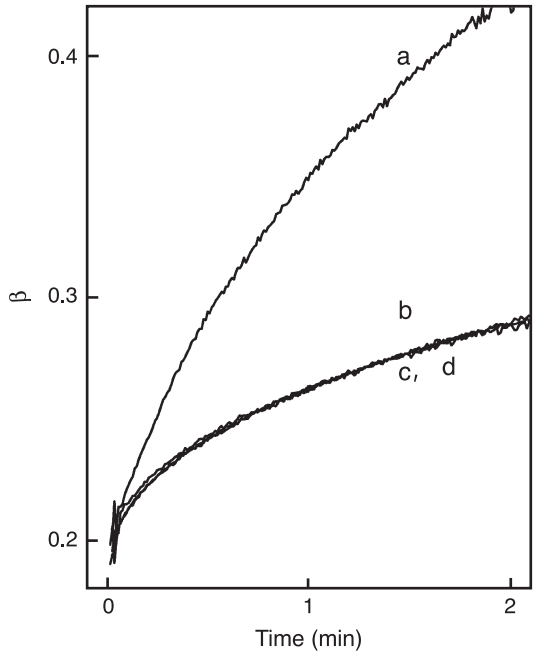

Fig. 3. ATP-dependent K^+ uptake by rat heart mitochondria, with TEA^+ control. Light-scattering traces from rat heart mitochondria respiring on ascorbate/TMPD in K^+ or TEA^+ medium. Trace a, K^+ influx in the absence of ATP. Trace b, K^+ influx in the presence of 0.2 mM ATP. Trace c, TEA^+ influx in the absence of ATP. Trace d, TEA^+ influx in the presence of 0.2 mM ATP. These data show that mitoK_{ATP} is K^+-selective and that ATP does not cause volume-independent changes in the light scattering signal. From Jaburek et al. [66].

of matrix water content. However, the small increases in matrix volume (10–15%) are similar in magnitude to the error of measurement of matrix volume, which is the difference between the waters of distribution of ^3H_2O and ^{14}C-sucrose [65]. This error is magnified at 25 °C or higher temperatures.

Using the light scattering technique, we demonstrated ATP-sensitive K^+ flux in rat heart [63,66,67], rat liver [68], and rat brain [64] mitochondria, using substrates of sites I, II, and IV. The basic observation, contained in traces a and b of Fig. 3, is that matrix swelling in K^+ medium is inhibited by ATP in the presence of Mg^{2+}. Control experiments in K^+-free medium are absolutely essential to exclude other effects of ATP. In this experiment, TEA^+ was substituted for K^+ in traces c and d of Fig. 3. Note two important aspects of matrix swelling in TEA^+ medium, which is due to diffusive ion leak [16]: (a) it is completely unaffected by ATP, and (b) it occurs at the same rate as K^+-induced swelling in the presence of ATP, i.e., when mitoK_{ATP} is inhibited. These simple control experiments, reported for both rat liver [68] and rat heart [66] mitochondria, give us confidence that the light scattering technique is accurately reporting mitoK_{ATP}-dependent volume changes.

Additional experiments are also necessary to convince us that we are looking at mitoK_{ATP}-dependent processes. The ATP-inhibited channel must be opened by K_{ATP} channel openers and GTP, and the open channel must be re-inhibited by a variety of channel blockers, including 5-HD and glyburide [63,64,66,67,69–71]. An example of these measurements is given in Fig. 4, in which the effects of ATP, diazoxide, and glibenclamide are shown.

Das et al. [72] have repeated some of these experiments, and Fig. 2 of this reference shows good evidence for inhibition of K^+ flux in heart and liver mitochondria by ADP and ATP, both of which inhibit mitoK_{ATP} [11]. When they add ATP or ADP after 4 min, they observe a slow contraction, which we also observe. This contraction is due to the to the temporary imbalance between K^+ influx, reduced by inhibition of mitoK_{ATP}, and K^+ efflux on the K^+/H^+ antiporter.

Das et al. [72] interpretation of this data, however, is that the effects of ADP and ATP are not due to changes in matrix volume: "The use of light scattering to measure changes in matrix volume and thus K^+ transport is questionable since it is well established that ATP and ADP can induce conformational changes in the mitochondria that can lead to an increase in light scattering without any change in matrix volume." This claim cannot be supported by any theoretical arguments. The light scattering measurements use about one billion mitochondria per milliliter. The 520 nm wavelength used is of the same order as mitochondrial dimensions and is ideal for detecting average volume changes [61]. It is simply impossible to detect

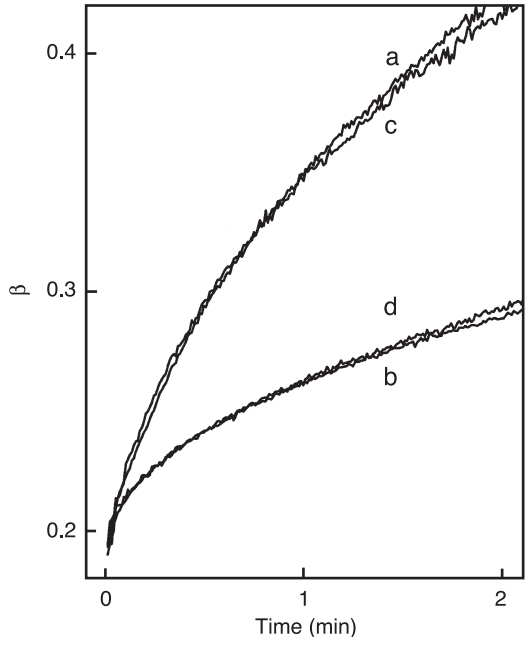

Fig. 4. Effects of glyburide and diazoxide on mitoK_{ATP}. Light-scattering traces from rat heart mitochondria respiring on ascorbate/TMPD in K^+ medium. Trace a, K^+ influx in the absence of ATP. Trace b, K^+ influx in the presence of 0.2 mM ATP. Trace c, reversal of ATP inhibition by 10 μM diazoxide. Trace d, re-inhibition by 10 μM glyburide in the presence of 10 μM diazoxide and 0.2 mM ATP. From Jaburek et al. [66].

volume-independent "conformational changes" with such a technique.

Indeed, we have shown directly that ATP does not cause "conformational changes" in the light scattering signal. Our first paper on mitoK$_{ATP}$ activity in isolated rat liver mitochondria, published 10 years ago, showed that swelling was unaffected by ATP in TEA$^+$ medium [68]. The data in Fig. 3, published 5 years ago, shows a similar result in rat heart mitochondria [66]. We have also examined mitochondrial swelling due to passive diffusion of erythritol and malonamide [62] and found ATP to have no effect. We routinely carry out such controls, and in hundreds of experiments, we have never observed effects of ATP in non-K$^+$ media. If Das et al. [72] had carried out these simple control experiments, they would have avoided spurious claims about conformational change.

6.2. Purification and reconstitution of mitoK$_{ATP}$

Mitochondrial membrane proteins were extracted with Triton X-100, and the extract was fractionated on a variety of columns. We identified the fraction containing mitoK$_{ATP}$ by reconstitutive activity [11,49]. The partially purified proteins were reconstituted into liposomes containing the trapped K$^+$-sensitive fluorescent probe, PBFI (Fig. 5A), permitting quantitative measurements of K$^+$ flux. As shown in Fig. 5B, this preparation catalyzed electrophoretic K$^+$ flux that was inhibited with high affinity by ATP and glyburide. MitoK$_{ATP}$ was highly selective for K$^+$ [11]. We also measured electrical activity after reconstitution of mitoK$_{ATP}$ in lipid bilayer membranes [71]. A second technique, pioneered by Mironova et al. [9], uses ethanol extraction of mitochondrial membranes [49]. This procedure extracts mitoKIR but not mitoSUR, presumably because mitoKIR is a more hydrophilic protein. MitoKIR has been reconstituted into liposomes and inserted into lipid bilayer membranes for study of electrical activity [73,74].

6.3. Subunit structure of mitoK$_{ATP}$

The subunit structure of mitoK$_{ATP}$ appears to be qualitatively similar to that of cellK$_{ATP}$, which are heteromultimers consisting of a 49–51 kDa inward-rectifying potassium channel (KIR6.1 or KIR6.2) and a 140–180 kDa sulfonylurea receptor (SUR1, 2A, or 2B), as described in Section 6.6. MitoK$_{ATP}$ also consists of two subunits—a 55 kDa mitoKIR and a 63 kDa mitoSUR (Fig. 6). MitoSUR was identified by specific, high-affinity labeling with fluorescent BODIPY-FL-glyburide (FL-GLY) [64], with a K_d about 13 nM [75]. Preparative SDS-PAGE showed that only the 63 kDa protein was labeled by FL-GLY. On the basis of its molecular size and its biochemical similarity to plasma membrane SUR, we predict that mitoSUR will turn out to be a half-molecule ABC protein.

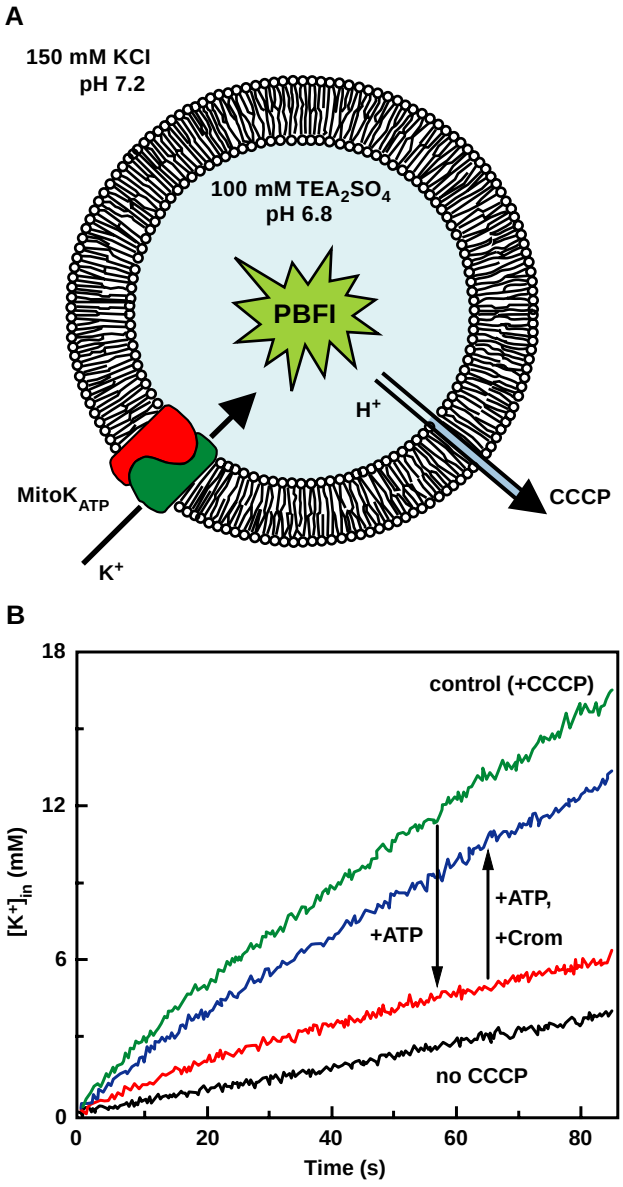

Fig. 5. Assays of K$^+$ flux via mitoK$_{ATP}$ following purification and reconstitution in liposomes. (Panel A) Protocol for assay of K$^+$ flux in liposomes. MitoK$_{ATP}$ is reconstituted in buffer containing the K$^+$-sensitive fluorescent probe, PBFI. External probe is then removed, and intraliposomal K$^+$ is followed by fluorescent assay [11,49]. (Panel B) K$^+$ flux assay. Little K$^+$ influx is observed in the absence of CCCP (bottom curve); however, a rapid K$^+$ uptake is observed in the presence of CCCP (top curve), reflecting electrophoretic K$^+$ flux via mitoK$_{ATP}$. This flux is inhibited by ATP (in the presence of Mg^{2+}), and the ATP-inhibited flux is restored by the K$_{ATP}$ channel opener, cromakalim.

Several lines of evidence show that the mitoKIR preparation contains the K$^+$ channel moiety of holomitoK$_{ATP}$: (a) both active fractions contain a 55 kDa inner membrane protein; (b) both channels are K$^+$-selective [9,11,73]; (c) the unitary conductance of both preparations is 10 pS in 100/100 mM KCl [71,73,74]; (d) pharmacological agents that open or close mitoKIR exhibit the same effects and $K_{1/2}$ values when applied

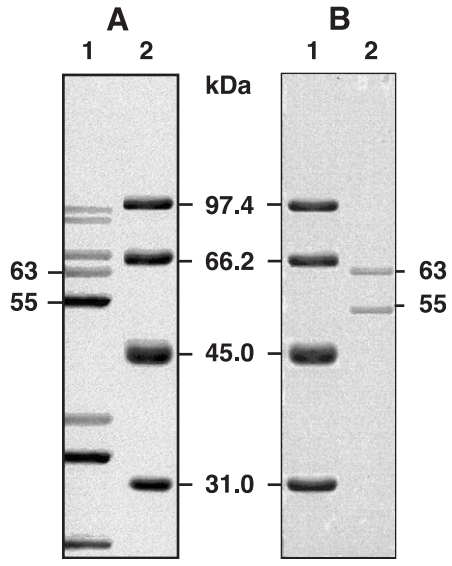

Fig. 6. Purification of the mitochondrial ATP-sensitive K^+ channel. MitoK_{ATP} was first purified from brain mitochondria [64] on a DEAE-cellulose column and eluted with 250 mM KCl (panel A, lane 1). This fraction was further purified on an ATP-affinity column (panel B, lane 2). The reconstitutively active fraction separates on 10% SDS-PAGE as two protein bands of 63 kDa (mitoSUR) and 55 kDa mitoKIR).

to holo-mitoK_{ATP} (unpublished); (e) the 55 kDa protein is an inward-rectifying K^+ channel [9,73].

6.4. Orientation of nucleotide regulatory sites in mitochondria

We found that the presence of Mg^{2+} in the reconstitution buffer causes 90% of the regulatory sites to face inward in liposomes, while the absence of Mg^{2+} causes the reverse orientation. This finding enabled us show that the binding sites for Mg^{2+}, nucleotides, and long-chain CoA esters all reside on the same side of the mitoK_{ATP} complex. Further experiments showed that these regulatory sites face the IMS [71].

6.5. Regulation of mitoK_{ATP}

MitoK_{ATP} is subject to a rich variety of regulation by biochemical and pharmacological agents, some of which interact with mitoSUR, whereas others interact with mitoKIR.

6.5.1. Regulation of mitoK_{ATP} by nucleotides, CoA esters, and Mg^{2+}

Reconstituted mitoK_{ATP} is inhibited by ATP, ADP, and long-chain CoA esters, with $K_{1/2}$ values shown in Table 1. Note that the effects of ADP and oleoyl CoA (inhibition) are opposite to what is normally observed with cellK_{ATP} (opening). Inhibition exhibits an absolute requirement for Mg^{2+} (50 μM) or Ca^{2+} (50 μM) [11,70]. Because CoA esters do not chelate Mg^{2+}, this requirement implies that Mg^{2+} interacts independently with the protein. The ATP-inhibited protein is opened by GTP, GDP, or UDP ([64,70] and unpublished data).

6.5.2. Pharmacological blockers of mitoK_{ATP}

When mitochondria are incubated under standard conditions (without addition of ATP or Mg^{2+}), mitoK_{ATP} is open and sensitive to ATP + Mg^{2+}, but it is completely insensitive to glyburide and 5-OH-decanoate (5-HD). We recognized, however, that standard in vitro conditions are very far removed from those that are obtained in vivo when inhibitors are added. When mitochondria are incubated in the presence of ATP, Mg^{2+}, and GTP (or a pharmacological K_{ATP} channel opener), mitoK_{ATP} is again open, but it is now highly sensitive to glyburide and 5-HD, with $K_{1/2}$ values of 1–6 and 45–75 μM, respectively. These results show that although different open states of mitoK_{ATP} catalyze identical K^+ fluxes, they may exhibit very different susceptibilities to channel inhibitors [66].

6.5.3. Pharmacological openers of mitoK_{ATP}

We showed that mitoK_{ATP} is a high-affinity receptor for K^+ channel openers (KCO), including cromakalim, diazoxide, and two cromakalim analogues [69]. Cromakalim opened mitoK_{ATP} with micromolar potency, and we proposed that mitoK_{ATP} may be the site of action for the cardioprotective effects of KCOs. Ongoing studies of the pharmacology of mitoK_{ATP} reveal that all KCOs known to act on plasma membrane K_{ATP} channels also act to open mitoK_{ATP} (Paucek and Garlid, unpublished data).

6.5.4. Regulation via mitoKIR

A great deal has been learned about cellK_{ATP} from studies of KIR in the absence of SUR. These studies were made possible by the finding that removal of the C-terminus of KIR6.2 enabled functional expression of this channel [76]. Native mitoKIR is reconstitutively active without truncation, and its properties have been studied extensively by Mironova et al. [73,74,77]. Reminiscent of findings with KIR6.2 [78], mitoKIR is inhibited by ATP with low affinity ($K_{1/2}$ = 500 μM) and without the requirement for Mg^{2+}, suggesting that the Mg^{2+} binding site is

Table 1
Nucleotide modulation of mitoK_{ATP}

Ligand	Action	$K_{1/2}$ value
ATP (no Mg^{2+})	no effect	–
ATP (+Mg^{2+})	inhibit	39 μM
ADP (+Mg^{2+})	inhibit	280 μM
Palmitoyl CoA (+Mg^{2+})	inhibit	260 nM
Oleoyl CoA (+Mg^{2+})	inhibit	40 nM
GTP (+ATP+Mg^{2+})	open	7 μM
GTP (+PCoA+Mg^{2+})	open	232 μM
GDP (+ATP+Mg^{2+})	open	140 μM
UDP (+ATP+Mg^{2+})	open	13 μM

The values reported are primarily from Ref. [11] and Ref. [70].

located on mitoSUR. Furthermore, UDP reverses ATP inhibition of mitoKIR noncompetitively (Mironova, Paucek and Garlid, unpublished).

MitoKIR is insensitive to glyburide, 5-HD, and the classical K_{ATP} channel openers, because these drugs act via mitoSUR. However, we have recently identified a number of other agents that block or open K^+ flux through mitoKIR. These drugs are equally effective on holo-mito-K_{ATP} in both liposomes and intact mitochondria. One such agent is the benzocaine derivative DEB (*p*-diethlyaminoethylbenzoate), which activates mitoKIR channels in bilayer membranes [79]. We have confirmed that DEB opens both mitoKIR and holo-mitoK_{ATP} in the reconstituted system and in mitochondria, with $K_{1/2}$ about 10 μM (Mironova, Paucek and Garlid, unpublished). The membrane potential probe, tetraphenylphosphonium (TPP^+), is a potent inhibitor and inhibits both mitoKIR and holo-mitoK_{ATP} in the reconstituted system and in mitochondria, with $K_{1/2}$ about 50 nM (Paucek and Garlid, unpublished data).

6.5.5. Regulation of mitoK$_{ATP}$ in vivo

As we have seen, both mitoSUR and mitoKIR are nucleotide-binding proteins. It must be noted that ATP inhibits K^+ flux through mitoK_{ATP} at nonphysiologically low concentrations of ATP, and there is no evidence that mitoK_{ATP} activity in vivo is affected by changes in ATP concentration. For these reasons, we do not consider ATP to be a regulator of mitoK_{ATP} in the true sense of the word. There is strong pharmacological evidence that mitoK_{ATP} is normally closed in vivo and can be opened by a variety of endogenous signaling pathways that may be triggered by Ca^{2+}, ouabain, plasma membrane receptor ligands, and by brief ischemia followed by reperfusion [1]. Thus, in vivo evidence indicates that mitoK_{ATP} is opened by endogenous signaling pathways. Given that numerous kinases are activated by these pathways, it is reasonable to speculate that mitoK_{ATP} is regulated in vivo by phosphorylation.

6.6. A comparison of cardiac mitoK$_{ATP}$ and cellK$_{ATP}$

From our first experiments, we were struck by the fact that mitoK_{ATP} reacts with the same biochemical and pharmacological ligands as those that regulate cellK_{ATP}. On this basis, we predicted that molecular identification of mitoK_{ATP} would reveal it to be a member of the K_{ATP} channel family [80]. At the same time, mitoK_{ATP}, like most individuals in a family, has its own unique features, and these identify it as a distinct subtype of the family. A brief review of cellK_{ATP} properties is included to facilitate these comparisons.

6.6.1. Plasma membrane K$_{ATP}$ channels (cellK$_{ATP}$)

CellK_{ATP} were first identified as 80 pS channels by Noma in 1983 by patch clamp in cardiac myocytes [81], and have subsequently been found in many cell types. In pancreatic β-cells, cellK_{ATP} regulate glucose-stimulated insulin secretion and are the targets for sulfonylureas used to treat type 2 diabetes [82]. In vascular smooth muscle, they regulate vascular tone and are targets for diazoxide and pinacidil in the treatment of hypertensive emergencies [83,84]. In skeletal muscle, cellK_{ATP} may be activated in fatigue [85]. In cardiac muscle, they were originally thought to be involved in ischemic preconditioning and ischemic protection by KCO [86,87].

CellK_{ATP} are heteromultimers of two subunits, an inward-rectifying potassium channel (KIR), and a regulatory sulfonylurea receptor (SUR). KIR6.x co-assemble with SUR in a 4:4 complex to form an octameric channel [88–90]. An RKR domain in the C-terminus of KIR6.x and in the sixth intracellular loop of SUR prevents membrane expression of either of these proteins in the absence of its partner [91].

The SURs are members of the ATP-binding cassette (ABC) family, which also includes P-glycoprotein and CFTR. SUR is thought to contain 17 transmembrane domains (TMD) arranged in three groups [92–94]. The genes encoding SUR1 and SUR2 are 70% homologous, while SUR2A (cardiac) and 2B (smooth muscle) are splice variants that differ only in 42 amino acids encoded by the final exon [95]. SUR1 regulates the β-cell channel, SUR2A the cardiac channel, and SUR2B the smooth muscle channel [94–99].

SUR contains two separate nucleotide binding domains (NBDs), each composed of a Walker A and B domain separated by a linker region. Mutating the lysine in Walker A or the aspartate in Walker B in either of the NBDs of SUR1 causes loss of MgADP activation of the channel [90,100]. SUR is the receptor for sulfonylureas and KCOs [95,96,101]. By exchanging domains between SUR1 and SUR2B, two TMDs were identified that are critical for KCO binding. The receptor sites for KCOs and sulfonylures are closely associated within a specific regulatory domain (Thr^{1059}–Asn^{1320}) in TMDIII of SUR1 [102,103]. KCO binding to SUR1, SUR2A and SUR2B (measured from the effect on labeled glyburide displacement) requires ATP, Mg^{2+} or Mn^{2+}, and non-hydrolyzable ATP-analogues do not support binding. These results suggest that KCO binding requires a conformational change induced by ATP hydrolysis [104–106]. Glyburide blocks both KIR6.2-SUR1 and KIR6.2-SUR2A with high affinity, but 5-HD does not inhibit cellK_{ATP}.

All KIR channels are tetramers, usually of identical subunits. They have two TMDs linked by a pore loop containing either GYG or GFG [107]. C-terminal truncations of KIR6.2 (KIR6.2ΔC26) permit expression and channel measurements [76]. K_{ATP} channel openers and sulfonylureas do *not* block KIR; however some drugs, such as phentolamine [108], cibenzoline [109], and DIDS and SITS [110,111] block KIR6.2ΔC26 in the absence of SUR. Quaternary ions such as TPP^+ block cellK_{ATP} [112], most likely by acting directly on KIR.

It is thought that ATP inhibits cellK_{ATP} by interacting with KIR6.2 and that SUR confers high affinity to this binding [76,113]. ATP inhibition kinetics suggest that four

ATP bind, but one is sufficient to inhibit, and models consistent with this interpretation have been presented [114,115].

An ATP-insensitive, but MgGDP-activated channel is observed when KIR6.1 and SUR2B are coexpressed, and this is thought to correspond to the nucleotide-activated channels found in smooth muscle [99,116,117] and the K_{ATP} channels in hypothalamic glucose-receptive neurons [118]. Suzuki et al. [119] have shown that mitochondria are immunostained with antibodies to KIR6.1. We have examined these antibodies with the following results: (a) they have no effect on K^+ flux via reconstituted mito-K_{ATP}; (b) they react with at least three mitochondrial proteins; however, (c) they do not react with any protein in the reconstitutively active purified fraction of mito-KATP. The antibodies were raised to a 12 amino acid fragment of KIR6.1, and it seems likely that they recognize non-KATP proteins with homologies in a limited domain. It is also important to note that KIR6.1 migrates in our hands as a 47–49 kDa protein on SDS-PAGE, whereas mitoKIR migrates as a 54–55 kDa protein.

6.6.2. MitoK_{ATP} and cellK_{ATP}—similarities and differences

After more than 10 years of study, it is abundantly clear that mitoK_{ATP} and cellK_{ATP} are generally regulated by the same ligands and drugs. However, mitoK_{ATP} possesses many unique properties that identify it as a distinctive subtype of the K_{ATP} family.

We compared mitoK_{ATP} and cellK_{ATP} purified from the same tissue (beef heart) using identical flux/reconstitution protocols [14,69,120]. We found that they may be extracted and fractionated using the same protocols, but that activities elute at widely different salt concentrations [69]. On SDS-PAGE, mitoKIR migrates at a higher MW than KIR6.1 or 6.2, and mitoSUR migrates at a far lower MW than any of the plasma membrane SURs [64].

There are numerous biochemical and biophysical differences between mitoK_{ATP} and cellK_{ATP}. The conductances of cardiac mitoK_{ATP} and cellK_{ATP} are very different—10 and 80 pS, respectively. MitoK_{ATP} is unique in its absolute requirement for Mg^{2+} for ATP inhibition. MgADP and long-chain acyl CoA esters inhibit mitoK_{ATP} [11,70], but they open cellK_{ATP} [121].

MitoK_{ATP} is a distinct pharmacological receptor. When cardiac mitoK_{ATP} and cellK_{ATP} were studied under identical conditions, diazoxide was found to be 1000 times more potent in opening mitoK_{ATP} than cellK_{ATP} and 5-HD was found to inhibit mitoK_{ATP} but not cellK_{ATP} [14,69]. Moreover, TEA^+ inhibits most KIRs of the plasma membrane, but has no effect on mitoK_{ATP}.

Finally, we raised polyclonal antibodies to the mixture of 55 kDa proteins in the reconstitutively active mitoK_{ATP} fraction. These antibodies do not react with proteins in the reconstitutively active cellK_{ATP} fraction from beef heart. Moreover, the antibodies inhibit K^+ flux through reconstituted mitoK_{ATP}, but have no effect on K^+ flux through reconstituted cellK_{ATP} (Paucek and Garlid, unpublished data).

7. The K^+ cycle in yeast mitochondria

Yeast mitochondria presumably have the same need for volume homeostasis as mammalian mitochondria, and indeed an active K^+/H^+ antiporter was described by Villalobo et al. [122]. Welihinda et al. [53] investigated the regulation of this process in an effort to determine whether yeast mitochondria contain a separate Na^+-specific Na^+/H^+ antiporter. Na^+ and K^+ transport were both completely inhibited by propranolol and quinine, and they were inhibited to a similar extent by Mg^{2+}, Ca^{2+}, and DCCD. Moreover, the pH profile of this antiport was similar to that of the mammalian K^+/H^+ antiporter, with a pK_i of 7.7. These results are consistent with a single, non-specific K^+/H^+ antiporter; however, the existence of a specific Na^+/H^+ antiporter with very low activity would not have been detected in these studies. Increasing the K^+ cycle by increasing $[K^+]$ was found to stimulate oxidative phosphorylation in yeast mitochondria without uncoupling [123].

Yeast also appear to increase mitochondrial ion permeability to avoid imbalances due to excess energy conservation. This was first described by Prieto et al. [124] as a proton-conducting pathway and subsequently as an anion uniporter that transports Br^- and Cl^- [125], although it is not clear how the latter pathway can uncouple in vivo. It seems more likely to be a non-selective cation uniport pathway, as described by Guerin et al. [126] and Manon [127]. It has been suggested that this pathway is the yeast counterpart of the permeability transition [128]. This pathway is stimulated by ATP or GTP and inhibited by Pi or ADP. Lu and Beavis [129] found that leader sequence peptides activate a non-selective cation uniport in yeast mitochondria. Because transport was not affected by ATP or cyclosporin, they concluded that it does not reflect mitoK_{ATP} or the permeability transition.

8. The K^+ cycle in plant mitochondria

An indication that plant mitochondria possess a very active K^+ cycle was the demonstration of very high K^+/H^+ antiport activity by Diolez and Moreau [130]. This was later complemented by the demonstration, in a variety of plant mitochondria, that a K^+ uniport pathway with characteristics of mitoK_{ATP} catalyzes sufficient flux to collapse $\Delta\psi$ [131]. K^+ influx was inhibited by ATP, and this inhibition was reversed by diazoxide or GTP. The authors proposed a K^+ cycle similar to that in Fig. 1, but differing quantitatively in that K^+ flux in plant mitochondria is sufficient to uncouple respiration completely. Petrussa et

al. [132] also obtained evidence for a plant mitoK$_{ATP}$, and found that it was opened by cyclosporin A. In recent studies, these authors confirmed that ATP inhibition of K^+ flux was reversed by GTP and diazoxide and, in contrast to Pastore et al. [131], found that glyburide and 5-HD inhibited the GTP-stimulated K^+ flux [133].

Plant mitoK$_{ATP}$ appears to play a major role in the in vitro regulation of succinate dehydrogenase (SDH). It had been known for some years that plant SDH is activated by ATP and ADP, but the mechanism was unknown. Affourtit et al. [134] showed that SDH is inactivated by K^+ and reactivated by nucleotides acting from the IMS, and they hypothesize that these effects are mediated via plant mitoK$_{ATP}$.

9. The effect of net K^+ influx on anion influx and matrix pH

As shown in Fig. 1, each mode of K^+ flux is associated with an equal counter-flux of protons. Electrophoretic K^+ influx is electrically coupled to electrogenic H^+ efflux, and electroneutral K^+ efflux is intrinsically coupled to electroneutral H^+ influx. Consider the consequence of net K^+ influx when mitoK$_{ATP}$ is opened. The resulting increase in matrix pH, δpH_i, is related to net loss of protons from the matrix, $-\delta H_i^+$, as follows:

$$\delta pH_i = -\delta H^+ / B_i \quad (5)$$

where B_i (nmol/mg pH) is the buffering capacity of the matrix, about 25 nmol/mg [135]. If mitoK$_{ATP}$ opening causes a total net K^+ uptake of 70 nmol/mg, then matrix pH would increase by 2.8 units! However, the increasing pH gradient drives uptake of phosphoric acid on the P_i transporter, which largely mitigates the pH change. In liver mitochondria, tricarboxylate and dicarboxylate exchange porters are linked to each other and to the P_i transporter, and these anions will also enter with protons. The most rapid response will be given by the P_i transporter itself, because of its greater capacity. This is especially true in heart and brain, in which several of the anion exchangers are lacking or possess low activity.

Palmieri et al. [136] showed that substrate anions that participate in this cascade distribute across the inner membrane so that the fully protonated acid has equal activity in both phases, just as if they penetrated by non-ionic diffusion. This result can be expressed as follows:

$$[A^{z-}]_i / [A^{z-}]_o = 10^{z\Delta pH} \quad (6)$$

where z is the valency of the unprotonated anion (excellent reviews of mitochondrial anion transport are found in Refs. [137–141]).

Eq. (5) may be expressed in terms of the amounts of K^+ and anions taken up into the matrix:

$$\delta pH_i = (\delta K^+ - z\delta A^-)/B_i \quad (7)$$

Eq. (7) shows that matrix pH can only remain constant if uptake of anionic equivalents equals uptake of K^+. In vivo, cytosolic anion concentrations are low, and matrix alkalinization will always accompany net K^+ uptake (the extent of alkalinization can be estimated from simultaneous solution of Eqs. (6), (7), and (8)). The increase in matrix pH secondary to mitoK$_{ATP}$ opening may be significant in heart, in which resting P_i levels are on the order of 1 mM.

10. The effect of changes in K^+ influx on matrix and IMS volumes

10.1. Osmotic equilibrium in mitochondria

The inner membrane is highly permeable to water [61], which keeps mitochondria in a state of osmotic equilibrium with its environment. As shown in Fig. 7, osmotic equilib-

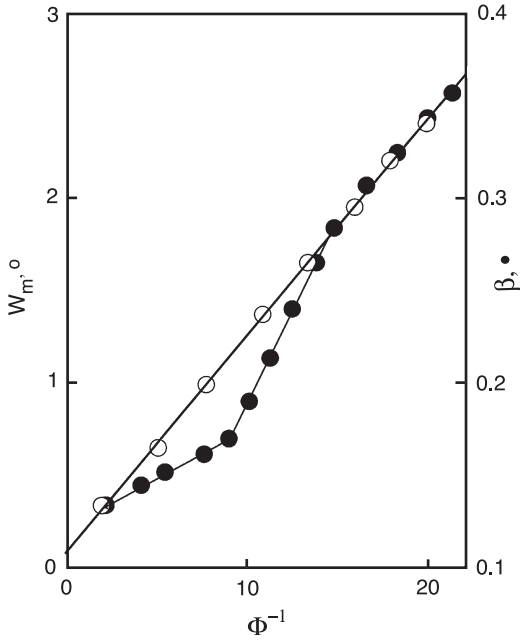

Fig. 7. Osmotic behavior of rat liver mitochondria. W_m is linear and reversible over the range shown, provided that there is no solute loss during the measurement. β is linear and reversible over the initial range, and then undergoes an irreversible transition when the outer membrane ruptures. Rat liver mitochondria (0.1 mg/ml) were incubated in media containing 5 mM Hepes, pH 7.2, 0.1 mM EGTA, 2 μM rotenone and concentrations of KCl to obtain the osmolalities indicated. Total mitochondrial volume from light scattering (β) and matrix water content (W_m) were measured as described in Ref. [61].

rium imposes a relationship between matrix water content, W_m (μl H_2O/mg), and matrix solute content, S_m (nmol/mg) [65]:

$$W_m = W_a + g_m S_m/\phi \quad (8)$$

where g_m is the osmotic coefficient of the internal phase (estimated to be about 0.94) and ϕ is the osmolality, about 300 mosM in vivo. W_a is the osmotic intercept and corresponds to water that is occupied by protein hydration and is therefore osmotically inactive [65]. It can be seen that uptake or loss of K^+ salts, causing a change in S_m, will be accompanied by osmotically obligated water and swelling or shrinking of the matrix.

Tedeschi and Harris [142,143] showed that changes in matrix volume can be followed by light scattering, which is a very important tool for studies of mitochondrial ion transport. Most studies follow absorbance (A) of the suspension, but this approach is only useful for gross irreversible changes, called high-amplitude swelling. The proper variable is the quantity, $P[1/A - 1/A_\infty]$, which we call β, and which is proportional to the volume of the scattering particle. P is the mitochondrial protein concentration in the assay, and $1/A_\infty$ is the intercept of a double reciprocal plot relating A to protein concentration in the assay [61].

Fig. 7 contains a plot of β versus inverse osmolality, $1/\phi$, for rat liver mitochondria. Entirely similar results were obtained for rat heart mitochondria [67]. We have shown that β is fully reversible in the isosmotic range [61]. Stoner and Sirak [144] showed that matrix swelling–contraction in the isosmotic range (the first linear segment of the light scattering osmotic curve) occurs at the expense of the IMS. Thus, IMS volume varies reciprocally with matrix volume under physiological conditions. At a certain point (occurring in Fig. 7 at $\phi = 115$ mosM), β is no longer reversible. Thus, addition of hypertonic solution to the assay causes the matrix to contract, but β does not return to its original value [61]. This is caused by outer membrane rupture, which leads to the presentation of a larger scattering surface and which, of course, is irreversible under these conditions. We have called this the "Humpty Dumpty" effect [7].

The data in Fig. 7 illustrate a number of important aspects of the light scattering technique: (a) β can be quantitatively related to matrix water content, and the kinetics of light scattering changes, $d\beta/dt$, can be transformed to solute transport rates [62]; (b) light scattering kinetics can be measured reliably in the range after the outer membrane has ruptured, because this part of the light scattering curve is also linear with matrix volume [38,62]; (c) light scattering is a very useful method for determining the osmolality and matrix volume at which the outer membrane ruptures; (d) given that the IMS is very small in vivo, the measurement of matrix volume at which outer membrane ruptures provides an estimate of the matrix volume that obtains in vivo; (e) it must be emphasized that the transition point depends very strongly on conditions of mitochondrial isolation. Prolonged incubation in K^+ free medium causes loss of matrix K^+ salts, and the transition will occur at low osmolalities. Isolation performed rapidly or in the presence of quinine to inhibit the K^+/H^+ antiporter, will cause retention of matrix K^+ salts, and the transition will occur at higher osmolalities.

10.2. The isolation artifact in matrix and IMS volumes

Perhaps the most important and least appreciated isolation artifact of mitochondria is the excess matrix contraction due to loss of K^+ (via K^+/H^+ exchange) and anions during isolation in K^+-free medium [8]. Studies described in the companion paper [1] indicate that this artifactual matrix contraction has profound effects on electron transport and on the outer membrane permeability to nucleotides. As shown diagrammatically in Fig. 8, the changes can readily be reversed in vitro by two independent means that are entirely equivalent. With reference to Eq. (8), it is evident that matrix volume can be restored either through respiration-driven uptake of K^+ salts (Eq. (9)) or by changing the osmotic strength of the medium (Eq. (10)):

$$\Delta W_m = \Delta S_m/\phi \quad (9)$$

$$\Delta W_m = S_m \Delta(1/\phi) \quad (10)$$

10.3. MitoK_{ATP} opening increases matrix volume

Matrix volume can be restored to near-normal (in vivo) values by means of respiration in K^+ salts, as illustrated in Fig. 8. From this steady state, mitoK_{ATP} opening causes a

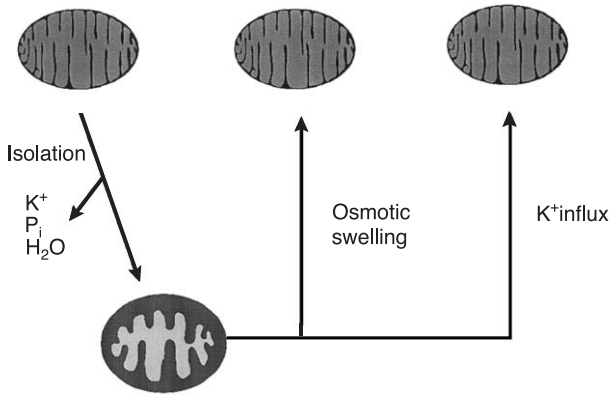

Fig. 8. Correcting the isolation artifact in mitochondria volume. During isolation, mitochondria lose K^+ salts and water via the K^+/H^+ antiporter and anion porters. This causes the matrix to become highly contracted relative to its normal state in vivo. Matrix and IMS volumes can be restored to their original values by mild osmotic swelling in hypotonic media or by respiring in K^+ salts.

15–20% increase in steady state matrix volume in mitochondria from rat heart, liver and brain, and this effect is blocked by 5-HD [63,64]. MitoK_{ATP} opening was also shown to regulate matrix volume during simulated ischemia and state 3 respiration. Addition of antimycin A to simulate ischemia caused a 10–15% contraction in matrix volume, and a contraction of similar magnitude was observed following addition of ADP to trigger state 3 respiration [63]. In both cases, the contraction is due to depolarization and decrease in diffusive K^+ influx. In both simulated ischemia and state 3 respiration, diazoxide reversed the contraction and caused steady state volume to return to near its original steady state levels. This effect of diazoxide was blocked by 5-HD. It is important to emphasize that in these conditions, opening mitoK_{ATP} provides a parallel K^+ conductance that compensates for the lower driving force for K^+ influx. It may be physiologically significant that the magnitude of K^+ flux through mitoK_{ATP} is so well-matched to the changes associated with both ischemia (in the presence of ATP) and maximum ATP production.

10.4. The kinetics of matrix volume changes

We have shown that under specific conditions, matrix water content, W_m, depends on matrix K^+ content as follows [65]:

$$W_m = W_a + 1.52K^+/\phi \tag{11}$$

Let $A \equiv \phi/1.52$, and $v_{KNET} \equiv dK^+/dt$ (net K^+ uptake). Then

$$dW_m/dt = v_{KNET}/A \tag{12}$$

where v_{KNET} is net K^+ influx into the matrix, and A is 160–200 mM. v_{KNET} is the sum of individual K^+ flux pathways:

$$v_{KNET} = v_{KATP} + v_{KLEAK} - v_{K/H} \tag{13}$$

We will make the approximation that the sensitivity ($\alpha_{K/H}$) of the K^+/H^+ antiporter to changes in matrix volume is constant over the limited range of volumes that occur between steady states,

$$dv_{K/H}/dt = \alpha_{K/H} dW_m/dt = \alpha_{K/H} v_{KNET}/A \tag{14}$$

Of the pathways in Eq. (13), only $v_{K/H}$ is capable of responding to changes in matrix volume; therefore,

$$dv_{K/H}/dt = -dv_{KNET}/dt, \text{ and} \tag{15}$$

$$dv_{KNET}/dt = -\alpha_{K/H} v_{KNET}/A \tag{16}$$

Eqs. (12) and (16) are integrated in succession to yield

$$\Delta W_m(t) = (\Delta v_{KNET}/\alpha_{K/H})(1 - e^{-(\alpha/A)\Delta t}) \tag{17}$$

Eq. (17) describes the approach of matrix volume to the new steady state in response to a sudden change in electrophoretic K^+ uptake, such as mitoK_{ATP} opening or a drop in $\Delta\psi$. The data published in Kowaltowski et al. [63] enable us to determine how well this equation fits the experimental results. We found in heart mitochondria at 25 °C that Δv_{KNET} for mitoK_{ATP} opening is about 30 nmol/mg min, based on the difference in respiration observed when mitoK_{ATP} was open or closed. A similar comparison showed that the difference in steady state matrix water contents in these two states (ΔW_m at $t = \infty$) is about 0.36 μl/mg. This allows us to estimate $\alpha_{K/H}$ at 83 mM/min, using Eq. (17) at $t = \infty$. If we assume that $A \approx 180$ mM, we may calculate the half-time to achieve the new steady state from Eq. (17). This value is about 1.5 min, which is in good agreement with the experiments on isolated mitochondria [63].

10.5. MitoK_{ATP} opening decreases IMS volume

Electron micrographs of mitochondria in vivo indicate that the matrix is tightly packed within the outer membrane, leading to a narrow average intermembrane distance between inner and outer membranes, r_{IMS}. Most of the changes in matrix volume caused by mitoK_{ATP} opening, ischemia, or the high-work state, will be reflected in a reciprocal change in IMS volume. We can estimate the corresponding change in r_{IMS} from membrane surface areas (SA). Inner membrane SA for isolated rat liver mitochondria are in excellent agreement [15,145], and Cieciura et al. [146] have provided comparative measurements of SA per unit mitochondrial volume for heart and liver mitochondria in situ. The values for isolated heart mitochondrial SA were derived by assuming that mg protein per unit volume is about the same for heart and liver mitochondria. Liver inner and outer membrane SA are 521 and 155 cm^2/mg [145], and the corresponding derived values for heart mitochondria are 1094 and 194 cm^2/mg. From these values, we estimate that a change in IMS volume of 0.35 μl/mg corresponds to a change in r_{IMS} of 32 Å. If r_{IMS} is normally 130 Å, diazoxide would reduce this to 88 Å if all of the volume changes were transmitted to the IMS. Since the outer membrane is capable of some expansion, as judged by light scattering [61], the actual r_{IMS} would be somewhat greater. Conversely, ischemia or the high-work state would decrease matrix volume and increase r_{IMS} to about 162 Å. Although these are rough approximations, they suggest that physiological changes in matrix volume 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 [147,148]. The importance of these changes in IMS volume in relation to Mi-CK is discussed in the companion paper [1].

11. Bioenergetic consequences of opening mitoK$_{ATP}$

It is now generally agreed that mitoK$_{ATP}$ plays a key role in cardioprotection against ischemia-reperfusion injury [1,14,69]; however, there is considerable controversy over the bioenergetic consequences of opening mitoK$_{ATP}$ in vivo [12]. We have discussed in previous sections that the matrix will become more alkaline and matrix volume will expand. Now we turn to the effects of mitoK$_{ATP}$ opening on respiration, $\Delta\psi$, and mitochondrial production of ROS.

11.1. The effect of mitoK$_{ATP}$ opening on respiration and $\Delta\psi$

We have measured the effects of ATP, ATP + diazoxide, and ATP + diazoxide + 5-HD on respiration in both rat heart [63] and rat brain [64] mitochondria. Based on these studies, the magnitude of K^+ flux through mitoK$_{ATP}$ is 24–30 nmol K^+/min^{-1} mg^{-1} at 25 °C in rat heart mitochondria. Similar measurements in mitochondria utilizing site I substrates by Kopustinskiene [149] yields an implied rate of 50 nmol K^+/mg/min at 37 °C. Considering the difference in temperature, these values are in good agreement. A respiratory stimulation of 5 ngatomO/min^{-1} mg^{-1} is a very low rate indeed, considering that rat heart mitochondria can respire in state 3 at over 1000 ngatomO/min^{-1} mg^{-1}. This comprises important evidence against the hypothesis that mitoK$_{ATP}$ opening uncouples respiration. This issue is discussed in greater detail in the companion paper [1].

As expected, the increased respiration due to mitoK$_{ATP}$ opening is too small to perturb $\Delta\psi$ very much. Indeed, the change in $\Delta\psi$ is too small to measure, and we estimate that it is 1–2 mV [63].

11.2. MitoK$_{ATP}$ 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 [12,13,150–152]. 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 [152]. We observe in isolated mitochondria that mild matrix alkalinization causes increased ROS production. As pointed out in Section 9, net uptake of K+ salts always leads to matrix alkalinization, and we suggest that this may be the mechanism by which KCOs induce increased ROS production [64].

12. Summary

The mitochondrial potassium cycle is highly regulated to respond to changing conditions originating from both the mitochondrion and the cytosol. Under physiological conditions, continual diffusive K^+ uptake, accompanied by anions and water, threatens the integrity of the organelle. This threat is countered by the K^+/H^+ antiporter, whose activity increases in response to increases in matrix volume. K^+ leak into the matrix is exponentially dependent on membrane potential, and physiological and pathological decreases in $\Delta\psi$ cause matrix contraction. Decreased matrix volume causes decreased K^+/H^+ antiport, and a new steady state balance is achieved at a lower matrix volume. Although this response prevents a total collapse of matrix volume, this is apparently not sufficient, because the inner membrane also contains a mitoK$_{ATP}$ channel that can restore matrix volume by adding a parallel conductance pathway to compensate for the reduced driving force for K^+ influx. We consider this volume regulation to be important for cellular bioenergetics and cardioprotection [1]. The mitoK$_{ATP}$ channel also plays a role in cell signaling by causing a moderate increase in mitochondrial production of ROS, which in turn, triggers physiological and cardioprotective signaling pathways [1].

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References

[1] K.D. Garlid, P. Dos Santos, Z. Xie, P. Paucek, Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K^+ channel in cardiac function and cardioprotection, Biochim. Biophys. Acta 1606 (2003) (preceding paper: doi:10.1016/S0005-2728(03)00109-9).
[2] P. Mitchell, Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism, Nature 191 (1961) 144–148.
[3] D.G. Nicholls, S.J. Ferguson, Bioenergetics 3, Academic Press, London, 2002.
[4] P. Mitchell, Chemiosmotic coupling in oxidative and photosynthetic phosphorylation, Biol. Rev. Camb. Philos. Soc. 41 (1966) 445–502.
[5] G.P. Brierley, The uptake and extrusion of monovalent cations by isolated heart mitochondria, Mol. Cell. Biochem. 10 (1976) 41–63.
[6] G.P. Brierley, in: B. Trump, A.U. Arstila (Eds.), Pathobiology

of Cell Membranes, vol. III, Academic Press, New York, 1983, pp. 23–61.
[7] K.D. Garlid, in: J.J. Lemasters, C.R. Hackenbrock, R.G. Thurman, H.V. Westerhoff (Eds.), Integration of Mitochondrial Function, Plenum, New York, 1988, pp. 257–276.
[8] K.D. Garlid, On the mechanism of regulation of the mitochondrial K^+/H^+ exchanger, J. Biol. Chem. 255 (1980) 11273–11279.
[9] G.D. Mironova, N.I. Fedotcheva, P.R. Makarov, L.A. Pronevich, G.P. Mironov, Protein from beef heart mitochondria inducing the potassium channel conductivity of bilayer lipid membrane, Biophysik (USSR) 26 (1981) 458–465.
[10] J.J. Diwan, T. Haley, D.R. Sanadi, Reconstitution of transmembrane K^+ transport with a 53 kilodalton mitochondrial protein, Biochem. Biophys. Res. Commun. 153 (1988) 224–230.
[11] P. Paucek, G. Mironova, F. Mahdi, A.D. Beavis, G. Woldegiorgis, K.D. Garlid, Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K^+ channel from rat liver and beef heart mitochondria, J. Biol. Chem. 267 (1992) 26062–26069.
[12] K.D. Garlid, Opening mitochondrial K(ATP) in the heart—what happens, and what does not happen, Basic Res. Cardiol. 95 (2000) 275–279.
[13] J. Tian, J. Liu, K.D. Garlid, J.I. Shapiro, Z. Xie, Involvement of mitogen-activated protein kinases and reactive oxygen species in the inotropic action of ouabain on cardiac myocytes. A potential role for mitochondrial KATP channels, Mol. Cell. Biochem. 242 (2003) 181–187.
[14] K.D. Garlid, P. Paucek, V. Yarov-Yarovoy, H.N. Murray, R.B. Darbenzio, A.J. D'Alonzo, N.J. Lodge, M.A. Smith, G.J. Grover, Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K^+ channels. Possible mechanism of cardioprotection, Circ. Res. 81 (1997) 1072–1082.
[15] R.K. Porter, A.J. Hulbert, M.D. Brand, Allometry of mitochondrial proton leak: influence of membrane surface area and fatty acid composition, Am. J. Physiol. 271 (1996) R1550–R1560.
[16] K.D. Garlid, A.D. Beavis, S.K. Ratkje, On the nature of ion leaks in energy-transducing membranes, Biochim. Biophys. Acta 976 (1989) 109–120.
[17] D.W. Deamer, J.W. Nichols, Proton flux mechanisms in model and biological membranes, J. Membr. Biol. 107 (1989) 91–103.
[18] P. Mitchell, J. Moyle, Translocation of some anions cations and acids in rat liver mitochondria, Eur. J. Biochem. 9 (1969) 149–155.
[19] G.P. Brierley, Passive permeability and energy-linked ion movements in isolated heart mitochondria, Ann. N.Y. Acad. Sci. 227 (1974) 398–411.
[20] M.G. Douglas, R.S. Cockrell, Mitochondrial cation–hydrogen ion exchange. Sodium selective transport by mitochondria and submitochondrial particles, J. Biol. Chem. 249 (1974) 5464–5471.
[21] B.P. Rosen, M. Futai, Sodium/proton antiporter of rat liver mitochondria, FEBS Lett. 117 (1980) 39–43.
[22] J.L.J. Gamble, Potassium binding and oxidative phosphorylation in mitochondria and mirochondrial fragments, J. Biol. Chem. 228 (1957) 955–971.
[23] D.W. Jung, E. Chavez, G.P. Brierley, Energy-dependent exchange of K^+ in heart mitochondria, K^+ influx, Arch. Biochem. Biophys. 183 (1977) 452–459.
[24] G.P. Brierley, M. Jurkowitz, E. Chavez, D.W. Jung, Energy-dependent contraction of swollen heart mitochondria, J. Biol. Chem. 252 (1977) 7932–7939.
[25] D.W. Jung, G.P. Brierley, Energy-dependent contraction of swollen heart mitochondria—activation by butacaine, Arch. Biochem. Biophys. 193 (1979) 76–87.
[26] G.P. Brierley, in: S. Fleischer, Y. Hatefi, D.H. MacLennan, A. Tzagoloff (Eds.), The Molecular Biology of Membranes, Plenum, New York, 1978, pp. 295–308.
[27] K.D. Garlid, Unmasking the mitochondrial K/H exchanger: swelling-induced K^+-loss, Biochem. Biophys. Res. Commun. 83 (1978) 1450–1455.
[28] K.D. Garlid, Unmasking the mitochondrial K/H exchanger: tetraethylammonium-induced K^+-loss, Biochem. Biophys. Res. Commun. 87 (1979) 842–847.
[29] R.A. Nakashima, K.D. Garlid, Quinine inhibition of Na^+ and K^+ transport provides evidence for two cation/H^+ exchangers in rat liver mitochondria, J. Biol. Chem. 257 (1982) 9252–9254.
[30] K.D. Garlid, Sodium/proton antiporters in the mitochondrial inner membrane, Adv. Exp. Med. Biol. 232 (1988) 37–46.
[31] P.S. Aronson, Kinetic properties of the plasma membrane $Na^+–H^+$ exchanger, Annu. Rev. Physiol. 47 (1985) 545–560.
[32] S. Nath, K.D. Garlid, in: J.J. Lemasters, C.R. Hackenbrock, R.G. Thurman, H.V. Westerhoff (Eds.), Integration of Mitochondrial Function, Plenum, New York, 1988, pp. 355–362.
[33] S.M. Periyasamy, P. Paucek, K.D. Garlid, A. Askari, Reconstitution and partial purification of cardiac sarcolemmal Na^+/H^+ (K^+/H^+) antiporter, Arch. Biochem. Biophys. 313 (1994) 184–188.
[34] S.M. Periyasamy, S.S. Kakar, K.D. Garlid, A. Askari, Ion specificity of cardiac sarcolemmal Na^+/H^+ antiporter, J. Biol. Chem. 265 (1990) 6035–6041.
[35] R.S. Dordick, G.P. Brierley, K.D. Garlid, On the mechanism of A23187-induced potassium efflux in rat liver mitochondria, J. Biol. Chem. 255 (1980) 10299–10305.
[36] R.A. Nakashima, R.S. Dordick, K.D. Garlid, On the relative roles of Ca^{2+} and Mg^{2+} in regulating the endogenous K^+/H^+ exchanger of rat liver mitochondria, J. Biol. Chem. 257 (1982) 12540–12545.
[37] K.D. Garlid, D.J. DiResta, A.D. Beavis, W.H. Martin, On the mechanism by which dicyclohexylcarbodiimide and quinine inhibit K^+ transport in rat liver mitochondria, J. Biol. Chem. 261 (1986) 1529–1535.
[38] D.J. DiResta, K.P. Kutschke, M.D. Hottois, K.D. Garlid, $K^+–H^+$ exchange and volume homeostasis in brown adipose tissue mitochondria, Am. J. Physiol. 251 (1986) R787–R793.
[39] S.S. Kakar, F. Mahdi, X.Q. Li, K.D. Garlid, Reconstitution of the mitochondrial non-selective Na^+/H^+ (K^+/H^+) antiporter into proteoliposomes, J. Biol. Chem. 264 (1989) 5846–5851.
[40] A.D. Beavis, K.D. Garlid, Evidence for the allosteric regulation of the mitochondrial K^+/H^+ antiporter by matrix protons, J. Biol. Chem. 265 (1990) 2538–2545.
[41] G.P. Brierley, M.S. Jurkowitz, T. Farooqui, D.W. Jung, K^+/H^+ antiport in heart mitochondria, J. Biol. Chem. 259 (1984) 14672–14678.
[42] P. Bernardi, G.F. Azzone, Electroneutral $H^+–K^+$ exchange in liver mitochondria. Regulation by membrane potential, Biochim. Biophys. Acta 724 (1983) 212–223.
[43] W.H. Martin, Pharmacology, Medical College of Ohio, Toledo, OH, 1984, p. 174.
[44] D.W. Jung, T. Farooqui, E. Utz, G.P. Brierley, Effects of quinine on K^+ transport in heart mitochondria, J. Bioenerg. Biomembranes 16 (1984) 379–390.
[45] A. Azzi, R.P. Casey, M.J. Nalecz, The effect of *N*,*N*′-dicyclohexylcarbodiimide on enzymes of bioenergetic relevance, Biochim. Biophys. Acta 768 (1984) 209–226.
[46] W.H. Martin, A.D. Beavis, K.D. Garlid, Identification of an 82,000-dalton protein responsible for K^+/H^+ antiport in rat liver mitochondria, J. Biol. Chem. 259 (1984) 2062–2065.
[47] W.H. Martin, D.J. DiResta, K.D. Garlid, Kinetics of inhibition and binding of dicyclohexylcarbodiimide to the 82,000-dalton mitochondrial K^+/H^+ antiporter, J. Biol. Chem. 261 (1986) 12300–12305.
[48] P. Jezek, F. Mahdi, K.D. Garlid, Reconstitution of the beef heart and rat liver mitochondrial K^+/H^+ (Na^+/H^+) antiporter. Quantitation of K^+ transport with the novel fluorescent probe, PBFI, J. Biol. Chem. 265 (1990) 10522–10526.
[49] K.D. Garlid, X. Sun, P. Paucek, G. Woldegiorgis, Mitochondrial cation transport systems, Methods Enzymol. 260 (1995) 331–348.
[50] X.Q. Li, M.G. Hegazy, F. Mahdi, P. Jezek, R.D. Lane, K.D. Garlid, Purification of a reconstitutively active K^+/H^+ antiporter from rat liver mitochondria, J. Biol. Chem. 265 (1990) 15316–15322.
[51] M. Numata, K. Petrecca, N. Lake, J. Orlowski, Identification of a

mitochondrial Na^+/H^+ exchanger, J. Biol. Chem. 273 (1998) 6951–6959.
[52] A. Kapus, G.L. Lukacs, E.J. Cragoe Jr., E. Ligeti, A. Fonyo, Characterization of the mitochondrial $Na^+–H^+$ exchange. The effect of amiloride analogues, Biochim. Biophys. Acta 944 (1988) 383–390.
[53] A.A. Welihinda, R.J. Trumbly, K.D. Garlid, A.D. Beavis, On the regulation of Na^+/H^+ and K^+/H^+ antiport in yeast mitochondria: evidence for the absence of an Na(+)-selective Na^+/H^+ antiporter, Biochim. Biophys. Acta 1144 (1993) 367–373.
[54] J. Orlowski, Na^+/H^+ exchangers. Molecular diversity and relevance to heart, Ann. N.Y. Acad. Sci. 874 (1999) 346–353.
[55] J. Orlowski, S. Grinstein, Na^+/H^+ exchangers of mammalian cells, J. Biol. Chem. 272 (1997) 22373–22376.
[56] K.D. Garlid, Z. Shariat-Madar, S. Nath, P. Jezek, Reconstitution and partial purification of the Na(+)-selective Na^+/H^+ antiporter of beef heart mitochondria, J. Biol. Chem. 266 (1991) 6518–6523.
[57] M. Numata, J. Orlowski, Molecular cloning and characterization of a novel $(Na^+, K^+)/H^+$ exchanger localized to the trans-Golgi network, J. Biol. Chem. 276 (2001) 17387–17394.
[58] M.G. Hegazy, F. Mahdi, X. Li, G. Gui, G. Mironova, A.D. Beavis, K.D. Garlid, Purification and reconstitution of the rat liver mitochondrial K^+ uniporter, Biophys. J. 59 (1991) A136.
[59] G. Gui, M.G. Hegazy, G. Mironova, F. Mahdi, A. Beavis, K.D. Garlid, Purification and reconstitution of the mitochondrial K^+ channel, J. Mol. Cell. Cardiol. 23 (1991) S78.
[60] I. Inoue, H. Nagase, K. Kishi, T. Higuti, ATP-sensitive K^+ channel in the mitochondrial inner membrane, Nature 352 (1991) 244–247.
[61] A.D. Beavis, R.D. Brannan, K.D. Garlid, Swelling and contraction of the mitochondrial matrix: I. A structural interpretation of the relationship between light scattering and matrix volume, J. Biol. Chem. 260 (1985) 13424–13433.
[62] K.D. Garlid, A.D. Beavis, Swelling and contraction of the mitochondrial matrix: II. Quantitative application of the light scattering technique to solute transport across the inner membrane, J. Biol. Chem. 260 (1985) 13434–13441.
[63] A.J. Kowaltowski, S. Seetharaman, P. Paucek, K.D. Garlid, Bioenergetic consequences of opening the ATP-sensitive K(+) channel of heart mitochondria, Am. J. Physiol. 280 (2001) H649–H657.
[64] R. Bajgar, S. Seetharaman, A.J. Kowaltowski, K.D. Garlid, P. Paucek, Identification and properties of a novel intracellular (mitochondrial) ATP-sensitive potassium channel in brain, J. Biol. Chem. 276 (2001) 33369–33374.
[65] K.D. Garlid, The state of water in biological systems, Int. Rev. Cyt. 192 (2000) 281–302.
[66] M. Jaburek, V. Yarov-Yarovoy, P. Paucek, K.D. Garlid, State-dependent inhibition of the mitochondrial KATP channel by glyburide and 5-hydroxydecanoate, J. Biol. Chem. 273 (1998) 13578–13582.
[67] P. Dos Santos, A.J. Kowaltowski, M.N. Laclau, S. Seetharaman, P. Paucek, S. Boudina, J.B. Thambo, L. Tariosse, K.D. Garlid, Mechanisms by which opening the mitochondrial ATP-sensitive K(+) channel protects the ischemic heart, Am. J. Physiol. 283 (2002) H284–H295.
[68] A.D. Beavis, Y. Lu, K.D. Garlid, On the regulation of K^+ uniport in intact mitochondria by adenine nucleotides and nucleotide analogs, J. Biol. Chem. 268 (1993) 997–1004.
[69] K.D. Garlid, P. Paucek, V. Yarov-Yarovoy, X. Sun, P.A. Schindler, The mitochondrial KATP channel as a receptor for potassium channel openers, J. Biol. Chem. 271 (1996) 8796–8799.
[70] P. Paucek, V. Yarov-Yarovoy, X. Sun, K.D. Garlid, Inhibition of the mitochondrial KATP channel by long-chain acyl-CoA esters and activation by guanine nucleotides, J. Biol. Chem. 271 (1996) 32084–32088.
[71] V. Yarov Yarovoy, P. Paucek, M. Jaburek, K.D. Garlid, The nucleotide regulatory sites on the mitochondrial KATP channel face the cytosol, Biochim. Biophys. Acta 1321 (1997) 128–136.
[72] M. Das, J.E. Parker, A.P. Halestrap, Matrix volume measurements challenge the existence of diazoxide/glibencamide-sensitive KATP channels in rat mitochondria, J. Physiol. 547 (2003) 893–902.
[73] G.D. Mironova, Y.Y. Skarga, S.M. Grigoriev, A.E. Negoda, O.V. Kolomytkin, B.S. Marinov, Reconstitution of the mitochondrial ATP-dependent potassium channel into bilayer lipid membrane, J. Bioenerg. Biomembranes 31 (1999) 159–163.
[74] G.D. Mironova, Y.Y. Skarga, S.M. Grigoriev, V.M. Yarov-Yarovoy, A.V. Alexandrov, O.V. Kolomytkin, The ATP-dependent potassium channel from rat liver mitochondria: 1. Isolation, purification, and reconstitution in a bilayer lipid membrane, Membr. Cell Biol. 10 (1996) 429–437.
[75] G.J. Grover, K.D. Garlid, ATP-sensitive potassium channels: a review of their cardioprotective pharmacology, J. Mol. Cell. Cardiol. 32 (2000) 677–695.
[76] S.J. Tucker, F.M. Gribble, C. Zhao, S. Trapp, F.M. Ashcroft, Truncation of Kir6.2 produces ATP-sensitive K^+ channels in the absence of the sulphonylurea receptor, Nature 387 (1997) 179–183.
[77] G.D. Mironova, S.M. Grigoriev, Y. Skarga, A.E. Negoda, O.V. Kolomytkin, ATP-dependent potassium channel from rat liver mitochondria: inhibitory analysis, channel clusterization, Membr. Cell Biol. 10 (1997) 583–591.
[78] F.M. Ashcroft, F.M. Gribble, Correlating structure and function in ATP-sensitive K^+ channels, Trends Neurosci. 21 (1998) 288–294.
[79] S.M. Grigoriev, Y.Y. Skarga, G.D. Mironova, B.S. Marinov, Regulation of mitochondrial KATP channel by redox agents, Biochim. Biophys. Acta 1410 (1999) 91–96.
[80] K.D. Garlid, Cation transport in mitochondria—the potassium cycle, Biochim. Biophys. Acta 1275 (1996) 123–126.
[81] A. Noma, ATP-regulated K^+ channels in cardiac muscle, Nature 305 (1983) 147–148.
[82] F.M. Ashcroft, F.M. Gribble, ATP-sensitive K^+ channels and insulin secretion: their role in health and disease, Diabetologia 42 (1999) 903–919.
[83] J.M. Quayle, M.T. Nelson, N.B. Standen, ATP-sensitive and inwardly rectifying potassium channels in smooth muscle, Physiol. Rev. 77 (1997) 1165–1232.
[84] R. Paulissian, Diazoxide, Int. Anesthesiol. Clin. 16 (1978) 201–237.
[85] N.W. Davis, N.B. Standen, P.R. Stanfield, ATP-dependent potassium channels of muscle cells: their properties, regulation, and possible functions, J. Bioenerg. Biomembranes 23 (1991) 509–535.
[86] G.J. Grover, J.R. McCullough, D.E. Henry, M.L. Conder, P.G. Sleph, Anti-ischemic effects of the potassium channel activators pinacidil and cromakalim and the reversal of these effects with the potassium channel blocker glyburide, J. Pharmacol. Exp. Ther. 251 (1989) 98–104.
[87] C.G. Nichols, W.J. Lederer, Adenosine triphosphate-sensitive potassium channels in the cardiovascular system, Am. J. Physiol. 261 (1991) H1675–H1686.
[88] J.P.T. Clement, K. Kunjilwar, G. Gonzalez, M. Schwanstecher, U. Panten, L. Aguilar-Bryan, J. Bryan, Association and stoichiometry of K(ATP) channel subunits, Neuron 18 (1997) 827–838.
[89] N. Inagaki, T. Gonoi, S. Seino, Subunit stoichiometry of the pancreatic beta-cell ATP-sensitive K^+ channel, FEBS Lett. 409 (1997) 232–236.
[90] S. Shyng, C.G. Nichols, Octameric stoichiometry of the KATP channel complex, J. Gen. Physiol. 110 (1997) 655–664.
[91] N. Zerangue, B. Schwappach, Y.N. Jan, L.Y. Jan, A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels, Neuron 22 (1999) 537–548.
[92] C.F. Higgins, The ABC of channel regulation, Cell 82 (1995) 693–696.
[93] G.E. Tusnady, E. Bakos, A. Varadi, B. Sarkadi, Membrane topology distinguishes a subfamily of the ATP-binding cassette (ABC) transporters, FEBS Lett. 402 (1997) 1–3.
[94] J. Bryan, L. Aguilar-Bryan, Sulfonylurea receptors: ABC transporters that regulate ATP-sensitive K(+) channels, Biochim. Biophys. Acta 1461 (1999) 285–303.

[95] N. Inagaki, T. Gonoi, J.P. Clement, C.Z. Wang, L. Aguilar-Bryan, J. Bryan, S. Seino, A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels, Neuron 16 (1996) 1011–1017.
[96] L. Aguilar-Bryan, C.G. Nichols, S.W. Wechsler, J.P.T. Clement, A.E. Boyd III, G. Gonzalez, H. Herrera-Sosa, K. Nguy, J. Bryan, D.A. Nelson, Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion, Science 268 (1995) 423–426.
[97] W.A. Chutkow, M.C. Simon, M.M. Le Beau, C.F. Burant, Cloning, tissue expression, and chromosomal localization of SUR2, the putative drug-binding subunit of cardiac, skeletal muscle, and vascular KATP channels, Diabetes 45 (1996) 1439–1445.
[98] S. Isomoto, C. Kondo, M. Yamada, S. Matsumoto, O. Higashiguchi, Y. Horio, Y. Matsuzawa, Y. Kurachi, A novel sulfonylurea receptor forms with BIR (Kir6.2) a smooth muscle type ATP-sensitive K^+ channel, J. Biol. Chem. 271 (1996) 24321–24324.
[99] M. Yamada, S. Isomoto, S. Matsumoto, C. Kondo, T. Shindo, Y. Horio, Y. Kurachi, Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K^+ channel, J. Physiol. 499 (1997) 715–720.
[100] C.G. Nichols, S.L. Shyng, A. Nestorowicz, B. Glaser, J.P. Clement, G. Gonzalez, L. Aguilar-Bryan, M.A. Permutt, J. Bryan, Adenosine diphosphate as an intracellular regulator of insulin secretion, Science 272 (1996) 1785–1787.
[101] C. Ammala, A. Moorhouse, F.M. Ashcroft, The sulphonylurea receptor confers diazoxide sensitivity on the inwardly rectifying K^+ channel Kir6.1 expressed in human embryonic kidney cells, J. Physiol. 494 (1996) 709–714.
[102] I. Uhde, A. Toman, I. Gross, C. Schwanstecher, M. Schwanstecher, Identification of the potassium channel opener site on sulfonylurea receptors, J. Biol. Chem. 274 (1999) 28079–28082.
[103] N. D'Hahan, H. Jacquet, C. Moreau, P. Catty, M. Vivaudou, A transmembrane domain of the sulfonylurea receptor mediates activation of ATP-sensitive K(+) channels by K(+) channel openers, Mol. Pharmacol. 56 (1999) 308–315.
[104] M. Schwanstecher, C. Sieverding, H. Dorschner, I. Gross, L. Aguilar-Bryan, C. Schwanstecher, J. Bryan, Potassium channel openers require ATP to bind to and act through sulfonylurea receptors, EMBO J. 17 (1998) 5529–5535.
[105] M.J. Dunne, Protein phosphorylation is required for diazoxide to open ATP-sensitive potassium channels in insulin (RINm5F) secreting cells, FEBS Lett. 250 (1989) 262–266.
[106] R.Z. Kozlowski, C.N. Hales, M.L. Ashford, Dual effects of diazoxide on ATP-K^+ currents recorded from an insulin-secreting cell line, Br. J. Pharmacol. 97 (1989) 1039–1050.
[107] C.G. Nichols, A.N. Lopatin, Inward rectifier potassium channels, Annu. Rev. Physiol. 59 (1997) 171–191.
[108] P. Proks, F.M. Ashcroft, Phentolamine block of KATP channels is mediated by Kir6.2, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 11716–11720.
[109] M. Horie, M. Watanuki, K. Tsuji, H. Ishida, A. Ishida-Takahashi, Y. Yuzuki, Y. Seino, S. Sasayama, Blockade of cardiac ATP-sensitive K^+ channel by cibenzoline targets its pore-forming subunit, J. Cardiovasc. Pharmacol. 35 (2000) 434–442.
[110] T. Furukawa, L. Virag, T. Sawanobori, M. Hiraoka, Stilbene disulfonates block ATP-sensitive K^+ channels in guinea pig ventricular myocytes, J. Membr. Biol. 136 (1993) 289–302.
[111] P. Proks, P. Jones, F.M. Ashcroft, Interaction of stilbene disulphonates with cloned K(ATP) channels, Br. J. Pharmacol. 132 (2001) 973–982.
[112] G.A. McPherson, A.E. Piekarska, Antagonism by lipophilic quaternary ions of the K^+ channel opener, levcromakalim, in vascular smooth muscle, Br. J. Pharmacol. 112 (1994) 1223–1229.
[113] A.P. Babenko, L. Aguilar-Bryan, J. Bryan, A view of sur/KIR6.X, KATP channels, Annu. Rev. Physiol. 60 (1998) 667–687.
[114] C.G. Nichols, W.J. Lederer, M.B. Cannell, ATP dependence of KATP channel kinetics in isolated membrane patches from rat ventricle, Biophys. J. 60 (1991) 1164–1177.
[115] S. Trapp, P. Proks, S.J. Tucker, F.M. Ashcroft, Molecular analysis of ATP-sensitive K channel gating and implications for channel inhibition by ATP, J. Gen. Physiol. 112 (1998) 333–349.
[116] A. Fujita, Y. Kurachi, Molecular aspects of ATP-sensitive K^+ channels in the cardiovascular system and K^+ channel openers, Pharmacol. Ther. 85 (2000) 39–53.
[117] U. Russ, A. Hambrock, F. Artunc, C. Loffler-Walz, Y. Horio, Y. Kurachi, U. Quast, Coexpression with the inward rectifier K(+) channel Kir6.1 increases the affinity of the vascular sulfonylurea receptor SUR2B for glibenclamide, Mol. Pharmacol. 56 (1999) 955–961.
[118] K. Lee, A.K. Dixon, P.J. Richardson, R.D. Pinnock, Glucose-receptive neurones in the rat ventromedial hypothalamus express KATP channels composed of Kir6.1 and SUR1 subunits, J. Physiol. 515 (1999) 439–452.
[119] M. Suzuki, K. Kotake, K. Fujikura, N. Inagaki, T. Suzuki, T. Gonoi, S. Seino, K. Takata, Kir6.1: a possible subunit of ATP-sensitive K^+ channels in mitochondria, Biochem. Biophys. Res. Commun. 241 (1997) 693–697.
[120] P. Paucek, K.D. Garlid, Reconstituted K^+ flux via the partially purified KATP channel from cardiac sarcolemma, Biophys. J. 64 (1993) A311.
[121] O. Larsson, J.T. Deeney, R. Branstrom, P.O. Berggren, B.E. Corkey, Activation of the ATP-sensitive K^+ channel by long chain acyl-CoA. A role in modulation of pancreatic beta-cell glucose sensitivity, J. Biol. Chem. 271 (1996) 10623–10626.
[122] A. Villalobo, M. Briquet, A. Goffeau, Electrogenic proton ejection coupled to electron transport through the energy-conserving site 2 and K^+/H^+ exchange in yeast mitochondria, Biochim. Biophys. Acta 637 (1981) 124–129.
[123] S. Manon, X. Roucou, M. Rigoulet, M. Guerin, Stimulation of oxidative phosphorylation by electrophoretic K^+ entry associated to electroneutral K^+/H^+ exchange in yeast mitochondria, Biochim. Biophys. Acta 1231 (1995) 282–288.
[124] S. Prieto, F. Bouillaud, D. Ricquier, E. Rial, Activation by ATP of a proton-conducting pathway in yeast mitochondria, Eur. J. Biochem. 208 (1992) 487–491.
[125] S. Prieto, F. Bouillaud, E. Rial, The nature and regulation of the ATP-induced anion permeability in *Saccharomyces cerevisiae* mitochondria, Arch. Biochem. Biophys. 334 (1996) 43–49.
[126] B. Guerin, O. Bunoust, V. Rouqueys, M. Rigoulet, ATP-induced unspecific channel in yeast mitochondria, J. Biol. Chem. 269 (1994) 25406–25410.
[127] S. Manon, Dependence of yeast mitochondrial unselective channel activity on the respiratory chain, Biochim. Biophys. Acta 1410 (1999) 85–90.
[128] S. Manon, X. Roucou, M. Guerin, M. Rigoulet, B. Guerin, Characterization of the yeast mitochondria unselective channel: a counterpart to the mammalian permeability transition pore? J. Bioenerg. Biomembranes 30 (1998) 419–429.
[129] Y. Lu, A.D. Beavis, Effect of leader peptides on the permeability of mitochondria, J. Biol. Chem. 272 (1997) 13555–13561.
[130] P. Diolez, F. Moreau, Correlation between ATP synthesis, membrane potential, and oxidation rate in potato *Solanum tuberosum* mitochondria, Biochim. Biophys. Acta 806 (1985) 56–63.
[131] D. Pastore, M.C. Stoppelli, N. Di Fonzo, S. Passarella, The existence of the K(+) channel in plant mitochondria, J. Biol. Chem. 274 (1999) 26683–26690.
[132] E. Petrussa, V. Casolo, E. Braidot, E. Chiandussi, F. Macri, A. Vianello, Cyclosporin A induces the opening of a potassium-selective channel in higher plant mitochondria, J. Bioenerg. Biomembranes 33 (2001) 107–117.
[133] E. Chiandussi, E. Petrussa, F. Macri, A. Vianello, Modulation of a plant mitochondrial K^+ATP channel and its involvement in cytochrome *c* release, J. Bioenerg. Biomembranes 34 (2002) 177–184.

[134] C. Affourtit, K. Krab, G.R. Leach, D.G. Whitehouse, A.L. Moore, New insights into the regulation of plant succinate dehydrogenase. On the role of the protonmotive force, J. Biol. Chem. 276 (2001) 32567–32574.
[135] A.D. Beavis, On the inhibition of the mitochondrial inner membrane anion uniporter by cationic amphiphiles and other drugs, J. Biol. Chem. 264 (1989) 1508–1515.
[136] F. Palmieri, E. Quagliariello, M. Klingenberg, Distribution of anions across the mitochondrial membrane, Biochem. J. 116 (1970) 36P.
[137] J.B. Chappell, Systems used for the transport of substrates into mitochondria, Br. Med. Bull. 24 (1968) 150–157.
[138] M. Klingenberg, Metabolite transport in mitochondria: an example for intracellular membrane function, Essays Biochem. 6 (1970) 119–159.
[139] K.F. LaNoue, A.C. Schoolwerth, Metabolite transport in mitochondria, Ann. Rev. Biochem. 48 (1979) 871–922.
[140] R.S. Kaplan, Structure and function of mitochondrial anion transport proteins, J. Membr. Biol. 179 (2001) 165–183.
[141] F. Palmieri, F. Bisaccia, L. Capobianco, V. Dolce, G. Fiermonte, V. Iacobazzi, C. Indiveri, L. Palmieri, Mitochondrial metabolite transporters, Biochim. Biophys. Acta 1275 (1996) 127–132.
[142] H. Tedeschi, D.L. Harris, Some observations on the photometric estimation of mitochondrial volume, Biochim. Biophys. Acta 28 (1958) 392–402.
[143] H. Tedeschi, D.L. Harris, The osmotic behavior and permeability to non-electrolytes of mitochondria, Arch. Biochem. Biophys. 58 (1955) 52–67.
[144] C.D. Stoner, H.D. Sirak, Osmotically-induced alterations in volume and ultrastructure of mitochondria isolated from rat liver and bovine heart, J. Cell Biol. 43 (1969) 521–538.
[145] K. Schwerzmann, L.M. Cruz-Orive, R. Eggman, A. Sanger, E.R. Weibel, Molecular architecture of the inner membrane of mitochondria from rat liver: a combined biochemical and stereological study, J. Cell Biol. 102 (1986) 97–103.
[146] L. Cieciura, K. Rydzynski, W. Klitonczyk, Stereologic studies on mitochondrial configuration in different organs of the rat, Cell Tissue Res. 196 (1979) 347–360.
[147] K. Fritz-Wolf, T. Schnyder, T. Wallimann, W. Kabsch, Structure of mitochondrial creatine kinase, Nature 381 (1996) 341–345.
[148] U. Schlattner, T. Wallimann, Octamers of mitochondrial creatine kinase isoenzymes differ in stability and membrane binding, J. Biol. Chem. 275 (2000) 17314–17320.
[149] D.M. Kopustinskiene, Effect of Adenosine-Triphosphate-Sensitive Potassium Channel Openers on Mitochondrial Functions, Library of Kaunas Univ., School of Medicine, Kaunas, Lithuania, 2002.
[150] R.A. Forbes, C. Steenbergen, E. Murphy, Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism, Circ. Res. 88 (2001) 802–809.
[151] T. Obata, Y. Yamanaka, Block of cardiac ATP-sensitive K(+) channels reduces hydroxyl radicals in the rat myocardium, Arch. Biochem. Biophys. 378 (2000) 195–200.
[152] M. Krenz, O. Oldenburg, H. Wimpee, M.V. Cohen, K.D. Garlid, S.D. Critz, J.M. Downey, J.N. Benoit, Opening of ATP-sensitive potassium channels causes generation of free radicals in vascular smooth muscle cells, Basic Res. Cardiol. 97 (2002) 365–373.