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Mitochondrial Ion Channels

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Abstract

In work spanning more than a century, mitochondria have been recognized for their multifunctional roles in metabolism, energy transduction, ion transport, inheritance, signaling, and cell death. Foremost among these tasks is the continuous production of ATP through oxidative phosphorylation, which requires a large electrochemical driving force for protons across the mitochondrial inner membrane. This process requires a membrane with relatively low permeability to ions to minimize energy dissipation. However, a wealth of evidence now indicates that both selective and nonselective ion channels are present in the mitochondrial inner membrane, along with several known channels on the outer membrane. Some of these channels are active under physiological conditions, and others may be activated under pathophysiological conditions to act as the major determinants of cell life and death. This review summarizes research on mitochondrial ion channels and efforts to identify their molecular correlates. Except in a few cases, our understanding of the structure of mitochondrial ion channels is limited, indicating the need for focused discovery in this area.

Keywords

bioenergetics; ischemia; preconditioning; mitochondria; oxidative phosphorylation; ion transport; energy metabolism; ATP synthesis

1. INTRODUCTION

1.1. Historical Perspectives

Interest in mitochondria has risen and fallen over the past century in the quest to describe the fundamental processes of the cell. In many cases, the significance of claims about the role of mitochondria is realized only after a period of intense criticism, gradual accumulation of evidence, and later recognition that part or all of the original conjecture was correct. This began in 1890 with Altmann's (1) description of mitochondria as the fundamental living elements of the protoplasm. He noted the similar morphology and staining characteristics of bacteria and the grains and filaments of cells (mitochondria), which he called bioblasts (bio, "life"; blasts, "germs"), and proposed that these bioblasts were the centers of biological function. Although he was roundly criticized at the time for his enthusiastic view of mitochondria, it seems that the next 100 years largely proved his point.

Novel functional roles for mitochondria often survive in modified form after the initial hypothesis has been overstated. The suggestion that mitochondria may be responsible for genetic inheritance came prematurely and was supplanted by recognition that the nucleus contained the bulk of the genetic material, only to be resurrected as our understanding of the mitochondrial genome and maternal inheritance emerged. Based on the observation that mitochondria can take up enormous amounts Ca^{2+}, researchers initially proposed that mitochondria could be the main intracellular storage organelles involved in Ca^{2+} cycling. This suggestion was superceded by the elucidation of the specialized role of the endo-(or sarco)

plasmic reticulum. However, recent experiments have rejuvenated interest in the role of mitochondria in fast Ca^{2+} responses (2).

Other roles for mitochondria have only grown in importance since they were originally suggested. Mitochondria's participation in oxidation-reduction reactions and cellular respiration was proposed early in the twentieth century, and the mechanisms underlying mitochondrial bioenergetics have been elucidated through a steady series of discoveries and conceptual realignments, including the description of the Krebs cycle, the concept of oxidative phosphorylation, and the chemiosmotic hypothesis (3,4).

The vectorial movement of ions is central to the chemiosmotic theory, and the four main postulates are as follows: (*a*) H^+ translocation down its electrochemical gradient across the mitochondrial inner membrane is reversibly coupled to ATP phosphorylation through the ATP synthase (F_1F_O ATPase). (*b*) The flow of electrons down the respiratory chain is coupled to H^+ pumping from the matrix to the intermembrane space to establish the protonmotive force. (*c*) Exchange-diffusion carrier proteins are present on the inner membrane to transport metabolites and selected inorganic ions into and out of the matrix. (*d*) The mitochondrial inner membrane is generally impermeable to ions other than H^+.

The last point applies only to mitochondria with tight coupling between oxygen consumption and the phosphorylation of ADP. The extent of coupling varies with membrane leakiness. An increase in the ion permeability of the mitochondrial inner membrane can be induced artificially with protonophoric chemical uncouplers, by a change in metabolic demand [through the stimulation of proton flux through the ATP synthase by ADP and phosphate (P_i)], or in response to increased flux through mitochondrial ion channels (e.g., by Ca^{2+} influx via the uniporter). The energy dissipated by ion flux has a depolarizing influence on mitochondrial membrane potential ($\Delta\Psi_m$), which stimulates NADH oxidation, proton pumping, and respiration. An increase in NADH production is required to compensate for higher rates of respiration and to avoid a mismatch in energy supply and demand.

To understand mitochondrial ion movements and their consequences, mitochondria must be considered as a subsystem of the cell's integrated processes, which include ion transport across the plasmalemma, intracellular Ca^{2+} cycling, and the mechanical or synthetic work of a given cell type. Only then can one appreciate the central role of mitochondria in maintaining the pseudohomeostasis known as normal cell physiology and understand how the failure of mitochondrial function can lead to cell death through a catastrophic necrotic event, autophagy, or the orchestrated process of apoptosis. Recognition of the role of mitochondria in cell life and death in the production of reactive oxygen species (ROS) and nitrogen species and in aging and disease has fueled a resurgence of mitochondrial interest in recent years. Mitochondrial ion channels, although still a nascent subject of investigation, appear to play a fundamental role in most of these functions.

1.2. Ion Transport Across the Inner Membrane: Background

Early observational studies of mitochondria in living cells suggested that mitochondria were sensitive to changes in osmolarity, suggestive of ion and water transport across the mitochondrial compartment. In 1915, Lewis & Lewis (5) reported that mitochondria in cultured embryonic chick cells could reversibly swell and contract in response to changes in the pH or osmotic pressure of the medium. These researchers also observed presciently that the morphology of mitochondria was extremely plastic (undergoing fission, fusion, elongation, etc.) and that mitochondrial structure degenerates in the presence of noxious agents, including hydrogen peroxide.

Other studies showed that a tissue could accumulate potassium against its concentration gradient in a metabolism-dependent manner, which supported the concept of active transport of ions. This in turn led investigators to study how isolated mitochondria actively accumulate ions. Lehninger (6) reviewed early work correlating mitochondrial electrolyte and water movement with oxidative phosphorylation and also discussed the behavior of mitochondria as "osmometers." The technique of measuring light-scattering changes as an indicator of mitochondrial volume has since been extensively employed to assay the activity of mitochondrial ion channels.

The binding (or uptake) of Ca^{2+} to mitochondria was first recognized in the 1950s. Work by Chance & Saris reported that energized mitochondria took up Ca^{2+} to uncouple mitochondria transiently (reviewed in Reference 7). Mitochondria eventually irreversibly uncoupled and discharged Ca^{2+} when a certain threshold level of Ca^{2+} was exceeded [now understood as the mitochondrial permeability transition (MPT)]. The ability of mitochondria to take up large quantities of Ca^{2+} in a manner that depended on the presence of the respiratory substrates ATP, P_i, and Mg^{2+} was reported soon after. An elegant study by Chance (8) in 1965 demonstrated that Ca^{2+} (*a*) induces a decrease in pH in the suspending medium, (*b*) stimulates respiration, (*c*) causes a cycle of swelling and contraction of the mitochondria, (*d*) oxidizes NADH, and (*e*) alters the redox state of respiratory chain carriers with millisecond response times (t1/2 ~ 70 msec). Additionally, Chance noted a crossover point in the redox chain between cytochromes *b* and *c*. This effect on the respiratory chain was relieved by the addition of 1.8 mM P_i, which also enhanced the maximal Ca^{2+}-stimulated rates of respiration and proton accumulation. Small additions of Ca^{2+} yielded cycles of oxidation and reduction. However, when the total amount of Ca^{2+} added exceeded 300 μM, irreversible, large-amplitude swelling occurred and was accompanied by sustained oxidation of the pyridine nucleotide pool and cytochrome *b* (8). These early studies have inspired abundant interest in the role of mitochondrial Ca^{2+} uptake in cell physiology.

Although the relative impermeability of the mitochondrial inner membrane to ions is a basic tenet of the chemiosmotic hypothesis, Mitchell recognized that several modes of ion transport are present, and he referred to them as symporters, antiporters, and uniporters (discussed in Reference 4). Symporters cotransport multiple ions (or an ion and a metabolite) in the same direction across the membrane (e.g., the mitochondrial P_i/H^+ carrier or the plasma membrane Na^+/glucose transporter), often utilizing the asymmetric electrochemical ion gradient to drive the transport in a thermodynamically favorable direction. Antiporters exchange ions on different sides of the membrane and can be either electroneutral (e.g., the Na^+/H^+ antiporter of the mitochondrial or plasma membrane) or electrogenic (e.g., the Na^+/Ca^{2+} exchanger of the plasma membrane). For electrogenic transporters, ion flux is driven by both the electrochemical gradients of the transported ions and the membrane potential. In a uniporter, ions flow electrophoretically down their electrochemical gradient, with transport rates in the range of 10^4–10^6 ions s^{-1} (e.g., the ion channels of the plasma membrane, the mitochondrial Ca^{2+} uniporter, etc.). With advancements in the ability to record ion channel activity in membranes and bilayers, the concept of the uniporter has evolved into the study of mitochondrial ion channels.

The large electrical driving force for ion movement (~180 mV) across the mitochondrial inner membrane strongly favors ion flow through any open ion channel, unless there is an equal and opposite chemical gradient for the ion (which is normally never the case for mitochondria). The theoretically enormous accumulation of cations in the mitochondrial matrix is limited by the restrictions imposed by the mitochondrial membrane–delimited compartment: Charge neutrality must be maintained, and changes in the osmolarity of the matrix have limits. For example, the entry of a cation through an open channel depolarizes $\Delta\Psi_m$ and stimulates respiration and proton pumping. This increases the pH gradient component of the protonmotive

force and also balances the positive charge entering with the ejection of a proton. If the cation accumulation in the matrix is accompanied by either the movement of a permeant weak acid (such as acetate) or the electroneutral uptake of P_i driven by the proton gradient, the pH change is prevented, and there is a net increase in matrix osmolarity and an increase in mitochondrial volume. Mitochondrial swelling can be counteracted by the concomitant stimulation of cation/H^+ antiporters. A futile cycle of K^+ influx [through a mitochondrial ATP–sensitive K^+ channel ($mitoK_{ATP}$)] and efflux (via the K^+/H^+ antiporter) may regulate physiological mitochondrial volume (9) (see Section 3.4.1). Conversely, the efflux of anions through an inner membrane anion channel [e.g., inner membrane anion channel (IMAC); see Section 3.5.1], coupled with cation efflux, may mediate mitochondrial contraction (10).

If the mitochondrial permeability transition pore (PTP) is not activated, mitochondria can take up large amounts of Ca^{2+}. This capacity as a Ca^{2+} sink is facilitated by the fact that P_i accumulates in parallel and can reversibly precipitate with Ca^{2+}, effectively lowering the free Ca^{2+} level to the μM range and minimizing osmotic effects. Ca^{2+} uptake is indirectly coupled to proton fluxes through direct H^+/Ca^{2+} exchange in some tissues, through the simultaneous uptake of P_i with H^+, and through Na^+/H^+ exchange secondary to the action of the Na^+/Ca^{2+} exchange Ca^{2+} efflux pathway. Mitochondrial Ca^{2+} overload can also result in rapid Ca^{2+} efflux from the matrix through PTP opening or PTP-independent pathways. In the case of PTP activation, matrix constituents with mass <~1.5 kDa exit the mitochondrion, and the ion permeability barrier of the inner membrane is lost, effectively short-circuiting proton-coupled energy transduction and dissipating $\Delta\Psi_m$.

The other principal rapid cation uptake pathway that has been studied extensively is the K^+ uniporter. K^+ conductance can be substantial in energized mitochondria, but recent work has indicated that energy depletion (e.g., ischemia) or treatment with K^+ channel openers activates fast K^+ uptake. K^+-selective ion channels may account for this response and are currently the subject of intense investigation because they confer protection against ischemia- or oxidative stress–mediated cell injury and apoptotic cell death (Section 3.4).

Researchers have also recognized for some time the transport of anions across the inner membrane in concert with cation movement. Mitchell & Moyle (11) reported that anions, including P_i, succinate, and malonate, accelerated the rate of decay of the pH gradient induced by a pulse of oxygen. This suggested the presence of anion transport systems coupled to proton movement, leading to the identification of the anion/metabolite-coupled cotransporter family. Inner membrane anion uniporters have been less well studied, but in the 1980s, an IMAC was postulated to account for anion-selective mitochondrial swelling responses (12). Subsequently, several anion channels were found in single-channel patch-clamp studies of mitoplasts (Section 3.5). Furthermore, investigators have identified certain mitochondrial membrane proteins that display anion channel activity under some conditions, including the mitochondrial uncoupling protein (UCP) (Section 3.1).

The best-studied mitochondrial ion channel in terms of structure and function is the voltage-dependent anion channel (VDAC), which is abundant in the mitochondrial outer membrane. VDAC is the primary route of entry and exit of metabolites and ions across the outer membrane and is a component of the mitochondrial contact site between the outer and inner membrane. Recent studies have focused on the regulation of outer membrane permeability as a physiological or pathophysiological control mechanism (see Section 2.1).

Table 1 summarizes known or postulated mitochondrial ion channels. The properties of mitochondrial ion channels are described below.

2. MITOCHONDRIAL ION CHANNELS OF THE OUTER MEMBRANE

2.1. The Voltage-Dependent Anion Channel

VDAC was originally purified and reconstituted in phospholipid bilayers in 1975 (13). A highly conserved protein with homology to bacterial porins, it forms an outer membrane pore with a diameter of 2.5–3 nm in the full conductance state (approximately 3 nS in 1 M NaCl). The channel is thought to be a polypeptide monomer that forms a beta barrel with 13 β-strands and one α-helix (14). It is partially anion selective in the open state, allowing the passage of metabolites such as ATP, ADP, and P_i, but it also permits the free diffusion of cations, including Ca^{2+}, K^+, and Na^+ (14). Nonelectrolytes (<3 nm diameter; <6 kDa) can also pass through the open channel. When the channel is in the closed state, the pore is still conductive but constricts to ~1.8 nm, at which point it favors cation permeability. The conductance to K^+ decreases by ~60%, and Ca^{2+} ions still permeate, but ATP flux is blocked, owing to a shift in the electrostatic profile and selectivity of the pore (15).

VDAC gating is highly voltage dependent, with peak currents near 0 mV and a dramatic reduction in channel open probability at positive or negative voltages in the range of 20–40 mV (15). This gating property may be physiologically important; recent evidence suggests that there is a significant membrane potential across the outer membrane (16). Although the large conductance of VDAC would tend to dissipate any membrane potential, the partial anion selectivity and the fact that fixed anionic charges on immobile macromolecules are asymmetrically disposed across the outer membrane would result in differential mobility of ions and the establishment of a Donnan potential. Assuming that protons rapidly equilibrate across the outer membrane, investigators hypothesized that if a pH gradient were detected, it could be justified only if a membrane potential existed. In fact, Porcelli et al. (17) detected a pH gradient by using pH-sensitive fluorescent proteins targeted to the intermembrane space. They reported a pH gradient of ~0.7 between the cytosol (pH 7.59) and the intermembrane space (pH 6.88) and calculated that the outer membrane potential necessary to support such a gradient would have to be 43 mV. If this is true, then VDAC would tend to be in the closed (low anion permeability) state in intact cells, raising the question of whether VDAC regulates mitochondrial metabolism by limiting metabolite flux in vivo.

Notably, several cytosolic factors decrease the open probability of VDAC channels reconstituted in bilayers. For example, the reduced pyridine nucleotides NADH and Mg^{2+}-NAD(P)H decrease ADP flux through VDAC, with K_is in the μM range (18). Moreover, endogenous inhibitors of VDAC, including an intermembrane space protein (19) and tbid (a cleaved form of Bid) (20), have been reported. Using a novel microfluidic and surface-plasmon resonance method, Roman and coworkers (21) recently surveyed a vast number of epitopes expressed from a liver cDNA library for these epitopes' ability to bind to reconstituted VDAC. Roman et al. were able to identify (*a*) positive interactions with ~40% of known VDAC binding partners and (*b*) 55 novel interactions, some of which inhibited VDAC in swelling assays.

VDAC is phosphorylated by protein kinase A (PKA) (22), protein kinase Cε (PKCε) (23), and tyrosine kinases (24). The phosphorylation of mitochondrial proteins by PKCε was correlated with the inhibition of PTP opening and may be related to protection against ischemic injury (23). VDAC is also a binding site for hexokinase II (HKII), and the dissociation of HKII from mitochondria has been correlated with induction of the MPT and apoptosis. The binding of HKII to VDAC appears to prevent VDAC closure (25), whereas hexokinase I may have the opposite effect (26).

Whether VDAC modulation of metabolite flux is a physiological control mechanism remains to be determined, and little is known about VDAC conductance in intact cells. However, recent reports indicate that a large outer membrane channel can be activated in intact neurons by a

train of synaptic action potentials (27) or by a proteolytically cleaved form of Bcl-xL and that NADH or hypoxia can inhibit these channels (28). Additionally, ethanol metabolism, by shifting the cytosolic NADH/NAD^+ redox potential to a more reduced state, may inhibit ADP flux across the outer membrane (29). Also, one study has reported that the molecular cut-off size and conductance of VDAC are Ca^{2+} dependent at submicromolar Ca^{2+} concentrations (30). Ca^{2+} pretreatment of partially permeabilized cells enhanced subsequent ATP uptake, implying VDAC-mediated regulation of metabolism. Notably, the large VDAC conductances seen in bilayers are not typically observed in patch-clamp experiments of intact mitochondria; rather, a variety of conductances in the range of 10–307 pS have been reported (31).

The implications of changes in outer membrane permeability on metabolism and cell death have been recognized in the context of various pathological states (29,32). Apoptosis is initiated by the release of proapoptotic factors from the mitochondria, including cytochrome *c*, Smac/Diablo, HtrA2/Omi, AIF and Endo G, and others (reviewed in Reference 33). For some noxious stimuli [e.g., ceramide (34)], mitochondrial outer membrane permeability increases prior to, or in the absence of, an immediate change in inner membrane permeability (35,36). At least three different mechanisms to explain the loss of intermembrane factors such as cytochrome *c* have been hypothesized: (*a*) physical rupture of the outer membrane as a result of mitochondrial swelling (usually linked to PTP opening), (*b*) a modification of VDAC structure, perhaps induced by proapoptotic proteins, such that the pore size increases enough to allow cytochrome *c* release (36), and (*c*) the formation of a new pore as a consequence of oligomerization and membrane insertion of proapoptotic proteins (37).

An oft-repeated model of the structure of the PTP (Section 3.6) includes, at a minimum, the adenine nucleotide translocase (ANT), VDAC, cyclophilin, and the F_1F_O ATPase (38). VDAC has been implicated both because it coimmunoprecipitates with the other proteins present at mitochondrial contact sites and because it is the main pathway for molecular transport across the outer membrane under normal conditions. Moreover, reconstitution of (at least) VDAC, ANT, and cyclophilin in phospholipid bilayers can form cyclosporin-sensitive channels (38). In addition, several common factors—for example, NADH and HKII binding—inhibit both the reconstituted VDAC channel and the PTP. The precise requirement for VDAC and the three-dimensional orientation of the components in the complex that form the pore are presently unknown, but possibly either a long pore spanning the two mitochondrial membranes forms from the association of VDAC with ANT or mitochondrial swelling simply ruptures the outer membrane (the role of VDAC is unclear in this case). In evaluating whether the current model of the PTP is correct (see Section 3.6), recent data demonstrating that the permeability transition can still be observed in ANT knockout mice (39) must be taken into account (40). Furthermore, large multiconductance channels in the inner membrane can still be observed in mitochondria devoid of VDAC (41), and Ca^{2+}-induced MPTs with properties indistinguishable from controls occurred in mitochondria from $VDAC1^{-/-}$ mice (42).

An ongoing debate is whether apoptosis is favored by VDAC opening or closure (14). Capano & Crompton (43) showed that the proapoptotic BH3-homology protein Bax coimmunoprecipitates with VDAC. In reconstitution studies in liposomes and phospholipid bilayers, Shimizu and coworkers (36) have reported that Bax can induce a novel high-conductance state of VDAC that permits cytochrome *c* to escape from the intermembrane space. Similarly, a recent report demonstrated that Bax and tBid increased the conductance of VDAC in planar bilayers and enhanced cytochrome *c* translocation from the *cis* to the *trans* chamber (44). Interestingly, VDAC phosphorylation by PKA inhibited the effects of Bax and tBid (45). Hexokinase II dissociation from VDAC and enhanced VDAC permeability as a trigger for cell death have been reviewed recently (32,46).

In contrast, others have observed no effect of monomeric or oligomeric Bax on VDAC gating or conductance (20), although the antiapoptotic protein Bcl-x_L promoted VDAC opening and helped to maintain the rate of ATP/ADP exchange across the outer membrane (47). In addition, the proapoptotic tBid was found to induce VDAC closure (20), and other studies have also linked the induction of apoptosis with a decrease in outer membrane permeability to metabolites (48).

2.2. The Mitochondrial Apoptosis–Induced Channel

Although debate is ongoing about whether VDAC constitutes a route for cytochrome *c* release, another candidate has emerged as a possible outer membrane permeation pathway. A recent study found that a novel high-conductance channel was frequently observed in proteoliposomes prepared from mitochondrial outer membranes that were isolated from cells undergoing apoptosis induced by growth factor withdrawal (49). This mitochondrial apoptosis–induced channel (MAC) had a fully open conductance of ~2.5 nS (~5 nm diameter) and multiple substates (37). Unlike VDAC, it was partially cation selective (P_{K+}:P_{Cl-} = 3) and was voltage independent. Because both the antiapoptotic Bcl-2 (50) and proapoptotic Bax (51) proteins can form channels when reconstituted in lipid bilayers, their role as mediators/regulators of MAC was investigated. MAC was not detected in proteoliposomes from cells overexpressing the antiapoptotic Bcl-2 protein. In contrast, the expression of human Bax in a VDAC-less yeast strain resulted in the appearance of channels identical to MAC (49). Moreover, the increased probability of observing MAC correlated with the translocation of Bax to the outer membrane and cytochrome *c* loss from the intermembrane space, and interventions that prevented Bax activation (e.g., Bcl-2 overexpression) reduced MAC formation. In agreement with MAC constituting a cytochrome *c* efflux pathway, cytochrome *c* may modify the conductance of reconstituted MAC. Bax and Bak appear to be functionally redundant with respect to the formation of MAC because MAC can still be observed in Bax knockout cell lines but not in Bax + Bak double knockouts.

The role of other outer membrane proteins in the formation of MAC and the precise time course of MAC activation in intact cells remain to be elucidated. With respect to the latter, Guihard et al. (52) detected MAC in mitochondria isolated from apoptotic rat liver only during the later stages of apoptosis, but Dejean et al. (53) argued that this was because of the particular apoptotic stimulus used (which activated the extrinsic pathway of apoptosis).

2.3. Translocase of the Outer Membrane

The insertion and translocation of mitochondrial preproteins encoded by the nucleus require specialized molecular machinery consisting of complexes of proteins on the outer and inner membranes. The constituent proteins of the translocase of the outer membrane of mitochondria (TOM) and its partner on the inner membrane, TIM, can form large conductance channels (54). Early electrophysiological studies of reconstituted mitochondrial membranes showed channels that were blocked by small basic peptides derived from mitochondrial presequences (e.g., the first 12 amino acids of cytochrome oxidase preprotein), and this peptide-sensitive channel (PSC) was proposed to be involved in mitochondrial protein import (55). Subsequent studies have shown that immunodepletion of the preparations with antibodies against TOM40p correlates with loss of channel activity, whereas control antibodies have no effect (56). Furthermore, antibodies against the carboxy terminus of TOM40p altered channel activity (56).

The properties of the channels formed by TIM or TOM proteins are almost identical (54). Both show a full conductance open state of 1 nS and a half-open state of 500 pS, and they have partial cation selectivity (P_{K+}:P_{Cl^-} = 5) (54). TOM channels show an asymmetric voltage dependence, with closure favored at positive voltages between +20 and +60 mV, but little

inactivation at negative voltages. Estimates of the pore diameter based on conductance are in the range of 2.4–2.7 nm, whereas polymer exclusion methods give a molecular weight cutoff of ~1000 and a dimension of ~2 nm (54). The slightly larger estimates of pore size based on conductance are complicated by the finding that the channel may consist of a double-barrel structure in which there may be cooperative gating of the two pores.

2.4. Peripheral (Mitochondrial) Benzodiazepine Receptor

Mitochondria contain binding sites for benzodiazepine receptor (BR) ligands that are pharmacologically distinguishable from the central-type receptor. Because these sites were also found in tissues outside the nervous system, they were labeled peripheral benzodiazepine receptors (PBR) (57). The PBR was preferentially enriched in tissues with high rates of oxidative phosphorylation, and membrane subfractionation studies demonstrated that the receptor was mitochondrial (58). The influence of the mitochondrial benzodiazepine receptor (mBzR) on mitochondrial physiology is incompletely understood, but we know that it plays a vital role in cholesterol transport and steroid biogenesis, accounting for the high density of this receptor in the adrenal gland and testes (59). The mBzR resides in the outer membrane as a complex of proteins; the primary component is the 18-kDa isoquinoline carboxamide–binding protein (IBP). As its name implies, IBP has a high affinity for isoquinoline carboxamide ligands like PK11195 (60), and it is found in close association with VDAC, ANT, and other proteins of the mitochondrial contact site. The binding of isoquinoline carboxamides and benzodiazepines is mutually competitive for the mBzR, and it was originally proposed that the binding site for benzodiazepines, such as the prototypical Ro 5–4864 (4′ Cl-diazepam), included the IBP, VDAC, and ANT (61). However, studies of yeast transformed with the IBP, but lacking either the VDAC or ANT protein, show high-affinity binding sites for isoquinoline carboxamides and benzodiazepines that are no different from binding sites of native receptors (62). More recently other proteins have been reported to associate with the IBP; these include the PBR-associated protein 1 [PRAX-1 (63)], the PBR-associated protein 7 [PAP7, or PKA with regulatory subunit 1α (64)], and a 10-kDa protein [pk10 (65)]. Several endogenous ligands (endozepines) interact directly with the mBzR, including an 86-amino-acid polypeptide known as the diazepam binding inhibitor (DBI; homologous to liver acyl CoA–binding proteins) (66,67) and its truncated derivative triakontatetraneuropeptide (TTN; DBI fragment 17–50) (68) as well as porphyrins (69), which bind to the receptor with nM affinities.

The first step of steroid synthesis and metabolism is the conversion of cholesterol to pregnenolone. This reaction is catalyzed by the cytochrome P-450 side-chain cleavage enzyme (P-450scc) located on the mitochondrial inner membrane. The overall reaction rate is limited by the transport of cholesterol across the outer membrane (70). PBR ligands facilitate this cholesterol transport (71) and enhance steroid production (72), whereas knocking out the IBP by homologous recombination in a cell line decreases steroid production (73). Molecular modeling indicated that the mBzR may form a barrel-like outer membrane cholesterol-permeable transport pathway composed of five membrane-inserted α-helices (74). Transmission electron and atomic force microscopic studies showed that anti-PBR antibodies are organized into complexes of 4–6 proteins (75).

If this structure forms a membrane-spanning pore in the outer membrane, it will need to accommodate the ~6.4-Å diameter of cholesterol, so ions (with Pauling radii <2 Å) should also be permeable. It is well known that benzodiazepine binding to $GABA_A$ receptors activates Cl^- conductance in the central nervous system. However, ion flux through the outer membrane via mBzR has not been demonstrated, although the close association between the IBP and VDAC begs the question. Several early studies showed that anions competitively inhibit ^{3}[H] Ro 5–4864 binding in kidney membranes with a profile correlating perfectly with their permeability through Cl^- channels, and inhibitors of anion transport similarly inhibited Ro 5–

4864, but not PK11195, binding (76). Other modulators of mBzR ligand binding include lipids (77), phospholipase A2 (78), König's polyanion, and cyclosporin A (CsA) (79), but their effects on mBzR-associated mitochondrial ion flux have not been investigated. The relationship between mBzR ligands, mitochondrial inner membrane ion channels, and function is discussed below (see Section 3.5.1).

3. MITOCHONDRIAL ION CHANNELS OF THE INNER MEMBRANE

3.1. Proton Leak and Uncoupling Protein

Proton transport across the mitochondrial inner membrane is at the heart of chemiosmotic theory. However, in addition to the passage of protons down the electrochemical gradient via the mitochondrial ATP synthase, other routes of "proton leak" are present. Proton leak can contribute significantly to the control of respiration in mitochondria in state 4 (limited by the availability of ADP) and, to a lesser extent, in mitochondria in state 3 (in the presence of substrate and ADP) (80). The proton conductance of the inner membrane displays an ohmic voltage dependence at low to intermediate $\Delta\Psi_m$ and a steep increase in conductance (nonohmic) at large $\Delta\Psi_m$ (>200 mV). The mechanisms responsible for the background conductances are incompletely understood, but several possible explanations include "slips" in the redox-driven proton pump stoichiometries and leaks of protons across the lipid bilayer.

In the context of this review, the contribution of proton-permeable channels must be considered. The pioneering work of Nicholls showed that in brown fat mitochondria, which have high rates of respiration dissipated as heat during nonshivering thermogenesis, GDP and albumin (which binds fatty acids) could regulate the proton leak (81). Later, this proton leak was attributed to a 32-kDa UCP (82). Three isoforms (UCP1, UCP2, and UCP3) are expressed in a variety of tissues (83). Besides the role of UCP1 in heat production, the function of UCP in physiology remains obscure; however, recent interest has focused on the modulation of ROS production, particularly in the context of diseases such as diabetes and obesity. Mild uncoupling of mitochondria, perhaps by altering the rate of production of free radicals, may be protective (84). Alternatively, partial uncoupling by UCP2 and/or UCP3 may optimize the efficiency of energy metabolism (85). Theoretical work suggests that the optimal ATP flow is achieved when the conductance of oxidative phosphorylation is matched to the conductance of the workload, most efficiently at coupling ratios slightly less than one (86).

It is not clear whether the proton and Cl^- conductance (87) mediated by UCP involves a carrier or ion channel–type mechanism. A 75pS anion channel was recorded in giant liposomes reconstituted with UCP1 (88), and reconstitution of the individual transmembrane domains of human UCP2 showed that all six transmembrane peptides form helical conformations in lipid model membranes. Only the second transmembrane peptide exhibited voltage-dependent anion channel behavior (89). Other evidence indicates that the anion and proton conductances are separable, depending on the presence or absence of coenzyme Q (90). Alternatively, UCP-mediated uncoupling may involve fatty acid cycling accompanied by protonation/deprotonation to cause net H^+ transport (85).

As a cautionary note, a leftward shift in the respiration-versus-$\Delta\Psi_m$ curve (i.e., higher oxygen consumption at any given $\Delta\Psi_m$) is often referred to as an increase in "H^+ leak." However, the ion selectivity of the leak pathway is usually not examined, so a sizable increase in the conductance of an ion channel with an equilibrium potential different from $\Delta\Psi_m$ (essentially any ion channel), be it selective or nonselective, would affect the curve similarly, by partially depolarizing $\Delta\Psi_m$. For example, mitochondria isolated from postischemic hearts have an increased proton (ion) leak rate that can be reversed by agents that block the PTP, a large nonselective channel (91). In mitochondria from hearts subjected to an ischemic preconditioning (IPC) protocol, GDP reversed the increase in proton leak, implicating UCP as

a leak pathway potentially responsible for the cardioprotective effect of preconditioning. Unfortunately, there are no selective pharmacological tools available to block UCP in intact cells or tissues, so GDP and/or albumin sensitivity of isolated mitochondria is the only way to test for the contribution of UCP to a given process.

3.2. The Mitochondrial Ca^{2+} Uniporter

As mentioned above, mitochondrial Ca^{2+} uptake has been recognized for more than 50 years, yet the protein mediating this essential physiological transport process is still unidentified. The mitochondrial Ca^{2+} uniporter (MCU) is present at low density (0.001 nmol mg^{-1} (92) on the mitochondrial inner membrane, but it has a high V_{max} of >1000 nmol min^{-1} mg^{-1}, corresponding to a flux of ~20,000 Ca^{2+} s^{-1} (93), in the range of a fast gated pore.

Other evidence also supports a model of the Ca^{2+} uniporter as an ion channel. For example, the dependence of Ca^{2+} uptake on $\Delta\Psi_m$ is consistent with electrodiffusion through a pore according to the Goldman constant field equation. Moreover, in a recent patch-clamp study of intact mitoplasts (94), an inwardly rectifying, highly Ca^{2+} selective (affinity 2 nM), voltage-dependent Ca^{2+} channel (MiCa) with properties that match the Ca^{2+} uniporter was recorded. Extramitochondrial concentrations of Ca^{2+} in the range of 20–100 μM yielded whole mitoplast currents of 20–30 pA, for a current density of ~55 pA pF^{-1} at −160 mV Remarkably, the Ca^{2+} flux did not saturate until $[Ca^{2+}]$ exceeded 100 mM, with a $K_{1/2}$ of 19 mM. Single-channel recordings in 105 mM Ca^{2+} showed a single-channel amplitude of 0.5–1 pA and a channel density of 10–40 channels μm^{-2} (channel conductance ~6 pS). MiCa had a high open probability at negative potentials and displayed rapid, but partial, Ca^{2+}-independent inactivation. It was argued that the much higher fluxes (5×10^6 Ca^{2+} s^{-1}) and $K_{1/2}$ recorded for MiCa in comparison to earlier studies of the Ca^{2+} uniporter were due to the inability to clamp the membrane potential in isolated mitochondria with such large currents flowing (94). Divalent ion selectivity for MiCa was also very similar to that reported for the MCU ($Ca^{2+} \approx Sr^{2+} \gg Mn^{2+} \approx Ba^{2+}$; blocked by Mg^{2+}), and the channel was sensitive to ruthenium red and Ru360 at low nM concentrations.

Despite a number of attempts to identify the MCU protein over the years, its molecular structure is still unknown. A soluble mitochondrial Ca^{2+}-binding glycoprotein, whose Ca^{2+} binding could be inhibited by La^{3+} or ruthenium red, was isolated in 1972 (95), and antibodies against it inhibited Ca^{2+} uptake (96). Later, Mironova and coworkers (97) also isolated a 40-kDa Ca^{2+}-binding glycoprotein from beef heart mitochondria and found that a 2-kDa peptide fragment in the preparation could reconstitute a ruthenium red–sensitive Ca^{2+} selective conductance in black-lipid membranes. Antibodies against this peptide inhibited Ca^{2+} uniporter activity in mitoplasts (98). The 2-kDa protein was further purified and formed 20-pS ruthenium red–sensitive channels upon reconstitution (99).

In other studies, antibodies were raised against a mitochondrial Ca^{2+} transporting extract; an antibody that recognized a 20-kDa protein was most effective in inhibiting Ca^{2+} transport (100). An 18-kDa protein fraction with high specificity for ^{103}Ru360 was further purified, and antibodies against it inhibited Ca^{2+} uptake in cytochrome oxidase–containing vesicles (101). None of the putative Ca^{2+} transporter proteins have been sequenced thus far.

Gunter and coworkers (102) have reported a second rapid mode of mitochondrial Ca^{2+} uptake (RaM). In liver and heart mitochondria, RaM transports Ca^{2+} very rapidly at the onset of a Ca^{2+} pulse and inactivates during a pulse. As compared with liver mitochondria, heart RaM requires a longer time to reset between pulses when a series of pulses is applied. The rapid uptake is less sensitive to activation by spermine, ATP, and GTP but is inhibited by AMP (103). It is presently unknown whether RaM is mediated by a separate transport protein or if

it is a second kinetic mode of the Ca^{2+} uniporter. The proteins involved in RaM-mediated Ca^{2+} uptake have not been identified.

3.3. Ryanodine Receptors

Mitochondrial Ca^{2+} uptake and endoplasmic reticular Ca^{2+} release are inhibited by common pharmacological inhibitors, including ruthenium red and ryanodine. Recent studies have reported that type I (skeletal type) ryanodine receptors (RyR1) are present on the cardiac mitochondrial inner membrane and may participate in either Ca^{2+} influx or efflux (104). Intriguingly, RyR1 immunoreactivity could not be detected in mitochondria from RyR1 knockout mice, providing strong support for the specificity of the antibodies used in these immunoprecipitation studies (104).

Any consideration of the ryanodine receptor as a viable candidate for the protein responsible for Ca^{2+} uniport activity in mitochondria must reconcile the discrepancy between the extremely high Ca^{2+} selectivity of the uniporter (94), which allows for mitochondrial Ca^{2+} uptake at submillimolar concentrations without permitting energy dissipation by monovalent ion flux, and the nonselectivity of ion permeation through ryanodine receptors. In addition, ion channels resembling RyR1 have not yet been reported in intact mitoplast patch-clamp recordings. Hence, further studies will be required to elucidate the role of RyR1 in mitochondrial Ca^{2+} handling.

Depending on the orientation of the Ca^{2+}-triggering site of the RyR1, i.e., toward the matrix or toward the intermembrane space, RyR1 may be a candidate either for rapid uptake of Ca^{2+} by mitochondria or for Ca^{2+}-triggered Ca^{2+} release from the mitochondrion through a non-PTP mechanism. Ruthenium red–sensitive Ca^{2+} efflux from Ca^{2+}-loaded mitochondria, attributed to reversal of the Ca^{2+} uniporter, has been reported (105).

3.4. K^+ Channels and Protection Against Ischemic Injury

More than 20 years ago, Lamping & Gross (106) demonstrated that pharmacological agents capable of opening K^+ channels protect hearts against ischemia-reperfusion injury. K^+ channel openers have come to be viewed as chemical preconditioners, i.e., compounds that can mimic the protective effects of brief cycles of ischemia and reperfusion. Moreover, the finding that K^+ channel inhibitors such as glybenclamide and 5-hydroxydecanoate could block the protective effects of either IPC or K^+ channel openers (107) suggested that K^+ channels were a native trigger and/or effector of preconditioning. Although earlier studies naturally presumed that the target of the K^+ channel openers was the sarcolemmal ATP–sensitive K^+ channel ($sarcK_{ATP}$), the focus has shifted recently to the mitochondria as the primary target of these compounds.

Plasma membrane K_{ATP} channels have been extensively studied at the molecular level, and their physiological roles in insulin release and in the modulation of vascular tone are indisputable. However, the presence of high densities of $sarcK_{ATP}$ in muscle cells has not been adequately explained. The recent availability of gene knockout mouse models in which components of the $sarcK_{ATP}$ channel have been ablated provides a new opportunity to answer this question. In the mouse, the primary physiological role of $sarcK_{ATP}$ apparently is to help the animal cope with metabolic stress. Mice that lack the pore-forming subunit of the cardiac $sarcK_{ATP}$ (Kir6.2) have a severely compromised ability to tolerate ischemia—even short periods of ischemia lead to rapid ischemic contracture of the heart (108,109). Similarly, when the K_{ATP} channel is pharmacologically inhibited in mice with HMR1098, a $sarcK_{ATP}$ blocker, ischemic dysfunction is accentuated. Neither ischemic nor chemical preconditioning can mitigate this effect. Function is also compromised with exercise, a more physiological form of metabolic stress (110). These studies indicate that the mouse is highly dependent on $sarcK_{ATP}$ channels for survival under conditions of high energy demand.

In contrast, in larger animal species (e.g., rabbits) (111) and in humans (112), sarcK_{ATP} appears to play a minor role in protecting the heart during ischemia. Selective pharmacological inhibition of sarcK_{ATP} has little or no effect on infarct size after ischemia and reperfusion or on the cardiac preconditioning response (113). Rather, sarcK_{ATP} contributes to postischemic electrical dysfunction by increasing the dispersion of repolarization and the heterogeneity of electrical excitability (114,115). Moreover, the action potential–shortening effects of sarcK_{ATP} activation during ischemia are not correlated with the extent of protection afforded by K^+ channel openers (107). Thus, other targets of these compounds, including the mitochondria, have been investigated in the context of protection against metabolic stress.

3.4.1. Mitochondrial K_{ATP}—In 1991, Inoue et al. (116) reported the presence of ATP-sensitive K^+ channels in the liver mitochondrial inner membrane, using the direct mitoplast patch-clamp method. The mitochondrial K_{ATP} channel (mitoK_{ATP}) had properties similar to the K_{ATP} channel observed in the sarcolemma of cardiac cells, albeit with a lower conductance. Thus, a link was established between the effects of K^+ channel openers on mitochondrial function and a specific ion channel target. The effects of K^+ channel openers on mitoK_{ATP} have been correlated with protection against ischemic injury in intact hearts (117) and in isolated myocytes (118). The general hypothesis that an increase in mitochondrial inner membrane permeability to K^+ improves cellular tolerance to ischemia-reperfusion injury has found widespread support in various tissues, including the liver, gut, brain, kidney, and heart.

Although studies of mitoK_{ATP} in mitoplasts by patch-clamp have been scarce (116,119), functional evidence supporting mitoK_{ATP} has been obtained by several methods, including (*a*) electrophysiological recordings of channels in proteoliposomes reconstituted with mitochondrial membrane proteins, (*b*) measurements of K^+ uptake into mitochondria or reconstituted liposomes, (*c*) mitochondrial swelling assays, (*d*) changes in mitochondrial redox potential, and (*e*) alterations in mitochondrial energetic parameters such as respiration and $\Delta\Psi_m$. Several groups have also reported the reconstitution of diazoxide-activated, 5-hydroxydecanoate-inhibited ion channels in planar lipid bilayers (for recent reviews of the evidence supporting a specific mitoK_{ATP} channel in the inner membrane, see References 120–122).

As for most of the mitochondrial channels, the lack of a specific molecular entity associated with mitoK_{ATP} has largely restricted putative identification to the use of pharmacological agents with known properties. A strong caveat is that some of these agents have substantial nonspecific effects on mitochondria that can alter the response to ischemia-reperfusion injury. A variety of K^+ channel openers activate mitoK_{ATP}; these include diazoxide, nicorandil, BMS191095, cromakalim, levcromakalim, EMD60480, EMD57970, pinacidil, RP66471, minoxidil sulfate, and KRN2391. Only the first three show significant selectivity toward the mitochondrial versus the sarcolemmal isoform of the K_{ATP} channel in cardiac myocytes. Another drawback is that there is only one widely available K^+ inhibitor that selectively inhibits mitoK_{ATP} without blocking sarcK_{ATP} (123), 5-hydroxydecanoate. The classical K_{ATP} channel inhibitor, glybenclamide, is a sulfonylurea that blocks both the sarcK_{ATP} and mitoK_{ATP} isoforms, whereas HMR1098 is usually selective for the sarcolemmal channel. In general, the pharmacological profile of the protective effect is consistent with mitoK_{ATP}, rather than sarcK_{ATP}, as the prime target (111).

Recent reports suggest that a number of other compounds—including sildenafil (124), levosimedan (125), YM934 (126), and MCC-134 (127)—modulate mitoK_{ATP}. MCC-134 could inhibit mitoK_{ATP} while activating sarcK_{ATP}. Importantly, MCC-134 prevented diazoxide-mediated protection against simulated ischemia, again supporting the argument that mitoK_{ATP} and not the sarcK_{ATP} channels mediated protection (127).

Another emerging area of interest is how signal transduction pathways either activate or are activated by the mitoK_{ATP} channel. Signaling pathways linked to phosphoinositide hydrolysis, protein kinase C (PKC) activation, or tyrosine kinases are mediators and/or effectors of cellular protection (128). In many cases, the downstream effects of receptor activation can be blocked not only by inhibitors of the kinases but by inhibition of the mitoK_{ATP} channel (129). This begs the question of whether the channel lies upstream or downstream of the posttranslational modifications mediated by either PKC or other kinases. One plausible link between the activation of mitoK_{ATP} and signaling would be a change in redox-sensitive pathways as a result of an increase in mitochondrially derived ROS (130). This could be due to an increase in respiratory rate (and consequent leak of electrons to superoxide) induced by the opening of the K^+ channel. A common effector, glycogen synthase kinase 3β (GSK-3β), has been proposed as the integrator of various preconditioning stimuli, including the actions of K^+ channel openers (131). Activation of GSK-3β blunts the effects of laser-induced oxidative stress on PTP activation in isolated cardiomyocytes. The mitoK_{ATP} channel is likely to be both a target and an effector in these pathways. For example, nitric oxide donors (132) and PKC activators (123) can enhance the activation of mitoK_{ATP} in isolated cardiac cells. In some studies, the mitoK_{ATP} inhibitor 5-hydroxydecanoate not only prevented IPC when applied during the preconditioning phase but also blocked the protection against infarction when given before the long index ischemia: this was the case for both early and delayed preconditioning protocols (133). More recently, the nitric oxide/cyclic GMP/G kinase signal cascade has been implicated in the activation of mitoK_{ATP} (134–136).

POSSIBLE STRUCTURE OF mitoK_{ATP}

Because the pharmacology of mitoK_{ATP} parallels certain combinations of known plasma membrane sulfonylurea receptor (SUR) and inward rectifier K^+ channel (Kir) subunits (191,192), these proteins have been obvious molecular candidates for the mitochondrial channel. Antibodies against either SUR or Kir6.x (193–200) have been reported to give a strong immunoreactive band in purified mitochondrial membranes. However, there are several criteria to meet when considering these results: (*a*) The mitochondrial inner membrane preparation must be shown to be completely free of other contaminating components, (*b*) the subunit of interest is co-enriched with mitochondrial markers, and (*c*) independent methods must confirm that the protein identified by the antibody is actually the original target (i.e., proteomic analysis should return an SUR or a Kir sequence in the preparation). Although some studies have satisfied the first two criteria, the last has not been achieved, leaving open the question of whether or not mitoK_{ATP} is a homolog of surface membrane K_{ATP} channels.

3.4.2. Mitochondrial K_{Ca}—The second selective K^+ channel to be identified in the mitochondrial inner membrane by direct patch-clamp of mitoplasts was the Ca^{2+}-activated K^+ channel (mitoK_{Ca}), detected in mitochondria from human glioma cells in 1999 (137) and in cardiac mitochondria by our laboratory in 2002 (138). In mitoplast patch-clamp experiments, this channel had a conductance of ~300 pS and was inhibited by the K^+ channel toxins charybdotoxin or iberiotoxin at nM concentrations, thus resembling the properties of Ca^{2+}-activated K^+ channels found in the plasma membranes of some cells. In partially permeabilized adult cardiomyocytes, these toxins also blunted K^+ uptake into mitochondria (138). A K_{Ca} opener, NS-1619, accelerated mitochondrial K^+ uptake and decreased infarct size in rabbit hearts subjected to 30 min of global ischemia and 2 h of reperfusion (138). We suggested that mitoK_{Ca} may be activated under pathophysiological conditions that increase mitochondrial Ca^{2+} uptake as a safeguard against excessive mitochondrial Ca^{2+} accumulation and may also play a physiological role to fine-tune mitochondrial volume and/or Ca^{2+} accumulation under conditions of increased cardiac workload. Ca^{2+} activation of this channel would be expected

to cause a partial depolarization of $\Delta\Psi_m$, which would decrease the driving force for Ca^{2+} entry under conditions of positive inotropic stimulation or ischemia.

Subsequent reports have confirmed that K_{Ca} channel openers protect hearts against ischemic injury (139,140). Moreover, similar to the effect of mitoK_{ATP} activation, mitoK_{Ca} opening has been implicated in early and delayed preconditioning (141) and may participate in the cardioprotection triggered by ischemia or receptor activation (142,143). NS-1619-mediated preconditioning can be prevented by blocking K_{Ca} channels with paxilline or by scavenging ROS during the exposure to the opener (140). The latter effect is reminiscent of the role of mitoK_{ATP} in preconditioning and implies that mitoK_{Ca} may be both a trigger and an effector of protection. A recent report (144) showed modulation of mitoK_{Ca} by PKA pathway but not by the PKC pathway, in contradistinction to the regulation of mitoK_{ATP} . Although both mitochondrial K^+ channels appear to have similar effects on mitochondrial function, each has a distinct and nonoverlapping pharmacology (138,144). This strongly supports the idea that enhanced mitochondrial K^+ uptake is the common factor associated with resistance to cell injury.

Antibodies against the BK type K_{Ca} channel cross-react with purified cardiac mitochondrial membranes (138), and a recent immuno-electron microscopy study revealed BK immunoreactivity in brain mitochondria (145). In addition, the β subunit of the BK channel is present in the mitochondria (146). However, it is still not clear whether the mitochondrial protein is identical, or just antigenically similar, to the surface membrane channel.

3.4.3. Kv1.3—Kv1.3, a Shaker-type K^+ channel, was reported to be present in the mitochondrial inner membrane of Jurkat T lymphocytes (147). In symmetric (134 mM) KCl, channels with a conductance of ~17 pS were detected in a mitoplast preparation and inhibited by margatoxin, a Kv1.3-selective toxin. The mitochondrial channels had properties that were identical to those of plasma membrane Kv1.3 channels, which are also present in this cell line. The channels were not present in mitoplasts from Kv1.3-null cells (CTLL-2) and were restored when Kv1.3 was overexpressed in a mouse cytotoxic T lymphocyte cell line (CTLL-2). Interpretation of the results is complicated by the concomitant expression of the Kv1.3 channel in the plasma membrane, possibly contributing surface membrane contamination to the preparation. However, the authors provide several lines of evidence supporting a mitochondrial inner membrane localization, including observations of the small conductance channel in patches that also contained the 107-pS channel (see Section 3.5.1) and the PTP (see Section 3.6) and the finding that margatoxin can hyperpolarize $\Delta\Psi_m$.

3.5. Anion Channels

3.5.1. IMAC—Mitochondrial swelling induced by cation and anion movements into the matrix compartment has been extensively employed to define ion permeabilities across the mitochondrial inner membrane (4). Following early studies of mitochondrial anion flux from the laboratories of Azzone, Selwyn, and Brierly (reviewed in Reference 12), a series of papers published in the 1980s provided experimental evidence that an inner membrane anion channel (IMAC) was active under certain conditions (e.g., Mg^{2+} depletion, alkalinization) (10,12, 148). IMAC is permeable to a variety of inorganic (e.g., $SCN^- > NO_3^- > Cl^- > P_i$) and organic (e.g., $oxaloacetate^{2-} > citrate^{3-} > malate^{2-} > ATP^{4-}$) anions (10). It is inhibited by protons and Mg^{2+} and by many different cationic amphiphiles, including amiodarone ($IC_{50} \sim 1$ μM), amitriptyline ($IC_{50} \sim 10$ μM), dibucaine ($IC_{50} \sim 20$ μM), propranolol ($IC_{50} \sim 25$ μM), and Ro 5–4864 (4′-chlorodiazepam; $IC_{50} \sim 34$ μM), among others (10). IMAC is modulated by thiol crosslinkers, including mersalyl and *N*-ethylmaleamide (10). IMAC activity is thought to be kept in check by endogenous inhibitors and by its strong voltage dependence under physiological conditions. However, the inhibition of IMAC by protons and Mg^{2+} is very

temperature sensitive, suggesting that IMAC is poised to respond to small changes in pH or Mg^{2+} under normal conditions (148), perhaps as a mechanism for modulating mitochondrial volume.

Direct single-channel patch-clamp methods revealed a number of partially anion-selective conductances in the inner membrane; the most prominent is the so-called centum pS (or mCtS) channel, a strongly voltage-dependent outwardly rectifying current (149–151). In the first patch-clamp study of the mitochondrial inner membrane, a 107-pS channel was recorded with a P_{Cl^-}:P_{K+} permeability ratio of 4.5; this channel comprises the main background conductance of the inner membrane (149). In this initial study, no pH dependence was observed, so the authors concluded that the 107 pS was not likely to be the single-channel equivalent of IMAC. However, subsequent investigations revealed properties of the ~100-pS channel in brown fat mitochondria that closely match the IMAC of swelling assays, including inhibition by propranolol, dihydropyridines, and the nucleotide analog cibacron Blue (10,150). More recently, Schonfeld et al. (152) showed that IMAC was activated by long-chain fatty acids in both intact rat liver mitochondria and in patch-clamp studies of the centum pS channel. Alternatively, a 15-pS anion-selective channel displaying many of the same properties as IMAC (i.e., both are activated by matrix alkalization and blocked by amiodarone, propranolol, and tributyltin) has been described (153).

Of particular interest is the inhibition of IMAC by benzodiazepines (10,154), considering that the mBzR (see Section 2.4) is present on mitochondrial membranes (albeit on the mitochondrial outer membrane). In studies comparing the effects of Ro 5–4894 and PK11195 on the mCtS and the large multiple-conductance channel (MCC) in mitoplasts, both channel types were blocked by mBzR ligands at concentrations of less than 1 μM (151), whereas central benzodiazepine ligands had no effect at concentrations up to 20 μM. The MCC was also sensitive to inhibition by CsA, but the mCtS channel was not. Another feature discriminating the two channel types was their sensitivity to protoporphyrin IX (PPIX), which is a known ligand of the mBzR (69). The MCC was inhibited by PPIX at low concentrations (IC_{50} ~ 24 nM) but was activated by PPIX at high concentrations (EC_{50} = 244 nM). In contrast, the mCtS was inhibited by PPIX at all concentrations (IC_{50} ~ 35 nM) (151). It is still unclear how the modulation of a presumed outer membrane receptor, mBzR, regulates inner membrane conductances such as the MCC and the mCtS. Researchers have reported other anion-selective channels, including ATP-sensitive anion channels found in yeast inner membranes (155). However, nothing is known about their physiological role and distribution in other species.

3.5.2. IMAC and mitochondrial criticality—IMAC and the mBzR play a role in postischemic electrical and contractile dysfunction in the heart (115). Inhibitors of IMAC and/or the mBzR (e.g., 4′-chlorodiazepam or PK11195) prevent or reverse oscillatory (PTP-independent) mitochondrial depolarizations induced by substrate deprivation (156) or oxidative stress (157) in adult cardiomyocytes. The underlying mechanism of the mitochondrial oscillator, explored in both experimental and computational studies (157–159), incorporates the concept of mitochondrial ROS–induced ROS release [originally coined to describe direct laser-induced PTP-mediated depolarization of $\Delta\Psi_m$ (160)], which triggers IMAC activation in a positive feedback loop (158). Remarkably, the local depolarization of just a few mitochondria in a cardiac cell can lead to cell-wide self-sustaining oscillations of $\Delta\Psi_m$ (157). Hence, the term mitochondrial criticality was forwarded to refer to the cellular conditions leading up to a breakpoint between stable and unstable $\Delta\Psi_m$ in the mitochondrial network (159). The approach to the critical state depends on whether mitochondrial ROS production by the respiratory chain exceeds a threshold level in a significant fraction of mitochondria (~60%) in the network. At the critical point, the weakly coupled fluctuations of individual mitochondria transition into an emergent spatiotemporal pattern of synchronized limit-cycle oscillations. This mitochondrial network phenomenon is tightly coupled to the

cardiomyocyte's electrophysiological response through energy-sensitive sarcK_{ATP} channels on the surface membrane, leading to scaling of the organelle-level dysfunction to the whole organ during ischemia and reperfusion (115).

3.5.3. CLC, mCLIC—Given the lack of information on the molecular structure of the anion-permeable mitochondrial channels described above, it is worthwhile to examine potential candidate channels known to be localized to mitochondrial membranes. Two classes of chloride channels, the ClC type and the CLIC type, have been reported to be present in mitochondria. The voltage-dependent chloride channel (ClC) family comprises a large class of structurally related membrane proteins with putative chloride channel activities. Although some members of the ClC family (ClC-3, ClC-4, ClC-5) may be targeted to intracellular locations, there is no evidence for mitochondrial localization. However, ClC-Nt, an anion channel isolated from tobacco plants, is enriched in mitochondrial membranes and has been proposed as a candidate for IMAC (161).

CLICs (chloride intracellular channels) represent a new class of intracellular anion channels that have been identified by their homology to the p64 protein. A mitochondrial homolog, mtCLIC, has been identified from differential display analysis of differentiating mouse keratinocytes from $p53^{+/+}$ and $p53^{-/-}$ mice. MtCLIC colocalized with cytochrome oxidase in keratinocyte mitochondria but also was detected in the cytoplasmic compartment. This p53-regulated putative channel has been associated with apoptosis (162) and may exist as either a soluble or transmembrane form that may translocate to the nucleus in response to cell stress (163).

3.6. Permeability Transition Pore

Permeabilization of the mitochondrial inner membrane, first detected as a large-amplitude swelling response in response to a variety of effectors (e.g., thyroxine, Ca^{2+}, P_i), has been known for more than 50 years (8,164). In the 1970s, the MPT was characterized in greater detail (165). It was proposed to involve the activation of a PTP (reviewed in References 40, 166, and 167). The finding that the immunosuppressant drug CsA could inhibit the MPT provided a crucial tool for subsequent investigation (168,169) and spurred the identification of its mitochondrial binding protein, cyclophilin D (170,171). Definitive evidence that the PTP was truly an ion-permeable channel was obtained upon the application of the technique of patch-clamping mitoplasts and the detection of MCC (153,172–175), or mitochondrial megachannels (176–179), which displayed conductances up to ~1.3 nS that were inhibited by CsA. The PTP allows the passage of ions and metabolites up to ~1500 molecular weight, for an apparent pore diameter of ~3 nm. In addition to the most commonly employed trigger, Ca^{2+} overload of the mitochondria, PTP opening is promoted by $\Delta\Psi_m$ depolarization, P_i, ROS, and thiol modification, and it can be inhibited by adenine nucleotides, Mg^{2+}, or matrix protons. Evidence that the ANT is a component of the PTP or even the pore itself was provided by the findings that bongkrekic acid, an inhibitor that stabilizes the ANT in the "m" conformation, inhibits the PTP and that atractylosides, which stabilize ANT in the "c" conformation, activate the PTP (180). Moreover, purified ANT preparations formed large Ca^{2+}-activated ion channels upon reconstitution in lipid bilayers (181). Similar channels of 600-pS conductance were obtained when *Neurospora crassa* ANT was expressed in *Escherichia coli* and reconstituted, a preparation that is devoid of possible contamination with other mitochondrial membrane components found in heart mitochondrial preparations (182). These channels were inhibited by ADP and bongkrekate, but not by carboxyatractylate, and were modulated by cyclophilin, whereas CsA abolished the cyclophilin effect.

Although the structure of the PTP is widely portrayed as consisting of a multiprotein complex prominently featuring the ANT, VDAC, cyclophilin, the F_1F_O ATPase, hexokinase, and other

modulatory proteins, recent observations have challenged this model. The two proteins central to prior models of PTP structure, ANT (39) and cyclophilin D (183–186), have been ablated in recent gene knockout mouse studies. The results support a modulatory (i.e., involving an increase in the threshold for Ca^{2+} activation) rather than an obligatory role for these proteins in the MPT. As discussed above (Section 2.1), data from mitoplast patch-clamp studies and gene-targeted mice also indicate that VDAC is not an obligatory member of the PTP protein complex.

Regardless of the structural details of the PTP, multiple lines of evidence support the idea that in tissues such as the heart, PTP opening occurs only during reperfusion after ischemia (115, 187) and is a major checkpoint on the route toward cell injury and death, although classic stimuli can readily induce apoptotic cell death in MPT-resistant mouse strains (186). However, mitochondrial depolarization in response to stress is not always the result of PTP opening (157). Thus, multiple assays must be used to determine whether a MPT has occurred; these include sensitivity to PTP inhibitors (e.g., CsA or sanglifehrin) and the direct demonstration that small molecules can permeate the mitochondrial inner membrane.

3.7. Connexin 43

Rodriguez-Sinovas et al. (188) recently demonstrated that connexin 43 (Cx43), the protein responsible for forming gap-junctional channels between cells, is present in mitochondrial membrane fractions and is increased in mitochondria from hearts subjected to an IPC protocol. Moreover, the protection afforded by IPC in wild-type mice is absent in heterozygous Cx43-deficient ($Cx43^{+/-}$) mice (189). Diazoxide-mediated protection is also lost in $Cx43^{+/-}$ mice. This effect was tentatively attributed to a decrease in cardiomyocyte ROS production during chemical preconditioning, as measured using a probe that loads (depending on $\Delta\Psi_m$) into the mitochondrial matrix, where it is then oxidized (190). Cx43 was specifically enriched in mitochondrial inner membrane fractions (188), and coimmunoprecipitation studies demonstrated an interaction of Cx43 with Tom20, part of the protein import machinery (see Section 2.5). However, it is presently unclear whether Cx43 forms a channel in the inner membrane. Several factors make it unlikely that it forms a pore: (*a*) Cx43 hemichannels generally do not conduct until the connexon is formed when the hemichannels meet at the junctional membrane, (*b*) there is no evidence that mitochondrial respiration or $\Delta\Psi_m$ is influenced by the mitochondrial Cx43 content, and (*c*) the Cx43 appeared to be in the phosphorylated form, which can induce channel closure. However, the activity of Cx43 may be altered by ischemia and/or reperfusion. Inhibiting the Hsp-90-dependent protein import pathway with geldanamycin prevents the IPC-mediated Cx43 translocation to the mitochondria. But the role of mitochondrial Cx43 in cardioprotection is unclear because geldamycin did not alter the protective effect (188). Furthermore, although geldanamycin abolished diazoxide-mediated protection, there was no correlation between this protection and Cx43 levels (188), nor was there a correlation between the extent of injury and the level of Cx43 in the absence of the chemical preconditioning stimulus.

4. CONCLUDING REMARKS

Ion channels can transport millions of ions per second, and the electrochemical driving forces for ion movement across the inner membrane are enormous. Thus, the knowledge gap regarding mitochondrial ion channel structure is perhaps understandable—these proteins must be present in extremely low abundance or have a very low open probability to maintain the low permeability to ions required to exploit the protonmotive force for ATP generation. Nevertheless, although mitochondrial ion channels may be highly controlled and open only briefly, the significance of their effects cannot be overstated. Mitochondrial ion channels are crucial to the mechanism of energy supply and demand matching and are the decisive factor in determining whether a cell lives or dies.

Mitochondrial ion channels for Ca^{2+}, K^+, or anions have been functionally and pharmacologically characterized at many levels, spanning from the single channel to the intact cell and to whole-organ function. The challenge ahead lies in defining the molecular structures responsible for forming the ion-selective mitochondrial pores. Achieving this goal will undoubtedly spur the development of novel and specific therapeutic agents targeted to the mitochondria. As the organelles responsible for integrating and responding to environmental challenges, mitochondria are the hub of all cellular functions and play a central role as determinants of cell life and death in a variety of pathologies, including acute coronary syndrome, neurodegeneration, cancer, and aging. Perhaps the key to managing these health problems will come from the next phase in the history of mitochondria to arise from mitochondrial ion channel discovery.

LITERATURE CITED

1. Altmann, R. Die Elementarorganismen und ihre Beziehungen zu den Zellen. Leipzig: Veit & Co; 1890. p. 145
2. Rizzuto R, Duchen MR, Pozzan T. Flirting in little space: the ER/mitochondria Ca^{2+} liaison. Sci. STKE. 20042004:re1
3. Mitchell P. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 1961;191:144–148. [PubMed: 13771349]
4. Nicholls, DG.; Ferguson, SJ. Bioenergetics3. London: Academic; 2002. p. 297
5. Lewis MR, Lewis WH. Mitochondria (and other cytoplasmic structures) in tissue cultures. Amer. J. Anat 1915;17:339–401.
6. Lehninger AL. Water uptake and extrusion by mitochondria in relation to oxidative phosphorylation. Physiol. Rev 1962;42:467–517. [PubMed: 14463788]
7. Saris NE, Carafoli E. A historical review of cellular calcium handling, with emphasis on mitochondria. Biochemistry 2005;70:187–194. [PubMed: 15807658]
8. Chance B. The energy-linked reaction of calcium with mitochondria. J. Biol. Chem 1965;240:2729–2748. [PubMed: 14304892]
9. Garlid KD. Cation transport in mitochondria—the potassium cycle. Biochim. Biophys. Acta 1996;1275:123–126. [PubMed: 8688444]
10. Beavis AD. Properties of the inner membrane anion channel in intact mitochondria. J. Bioenerg. Biomembr 1992;24:77–90. [PubMed: 1380509]
11. Mitchell P, Moyle J. Respiration-driven proton translocation in rat liver mitochondria. Biochem. J 1967;105:1147–1162. [PubMed: 16742541]
12. Garlid KD, Beavis AD. Evidence for the existence of an inner membrane anion channel in mitochondria. Biochim. Biophys. Acta 1986;853:187–204. [PubMed: 2441746]
13. Schein SJ, Colombini M, Finkelstein A. Reconstitution in planar lipid bilayers of a voltage-dependent anion-selective channel obtained from paramecium mitochondria. J. Membr. Biol 1976;30:99–120. [PubMed: 1011248]
14. Rostovtseva TK, Tan W, Colombini M. On the role of VDAC in apoptosis: fact and fiction. J. Bioenerg. Biomembr 2005;37:129–142. [PubMed: 16167170]
15. Levadny V, Colombini M, Li XX, Aguilella VM. Electrostatics explains the shift in VDAC gating with salt activity gradient. Biophys. J 2002;82:1773–1783. [PubMed: 11916837]
16. Colombini M. VDAC: the channel at the interface between mitochondria and the cytosol. Mol. Cell Biochem 2004;256–257:107–115.
17. Porcelli AM, Ghelli A, Zanna C, Pinton P, Rizzuto R, Rugolo M. pH difference across the outer mitochondrial membrane measured with a green fluorescent protein mutant. Biochem. Biophys. Res. Commun 2005;326:799–804. [PubMed: 15607740]
18. Zizi M, Forte M, Blachly-Dyson E, Colombini M. NADH regulates the gating of VDAC, the mitochondrial outer membrane channel. J. Biol. Chem 1994;269:1614–1616. [PubMed: 7507479]

19. Holden MJ, Colombini M. The outer mitochondrial membrane channel, VDAC, is modulated by a protein localized in the intermembrane space. Biochim. Biophys. Acta 1993;1144:396–402. [PubMed: 8399284]
20. Rostovtseva TK, Antonsson B, Suzuki M, Youle RJ, Colombini M, Bezrukov SM. Bid, but not Bax, regulates VDAC channels. J. Biol. Chem 2004;279:13575–13583. [PubMed: 14729675]
21. Roman I, Figys J, Steurs G, Zizi M. Hunting interactomes of a membrane protein: obtaining the largest set of voltage-dependent anion channel-interacting protein epitopes. Mol. Cell Proteom 2006;5:1667–1680.
22. Blachly-Dyson E, Peng S, Colombini M, Forte M. Selectivity changes in site-directed mutants of the VDAC ion channel: structural implications. Science 1990;247:1233–1236. [PubMed: 1690454]
23. Baines CP, Song CX, Zheng YT, Wang GW, Zhang J, et al. Protein kinase Cε interacts with and inhibits the permeability transition pore in cardiac mitochondria. Circ. Res 2003;92:873–880. [PubMed: 12663490]
24. Liberatori S, Canas B, Tani C, Bini L, Buonocore G, et al. Proteomic approach to the identification of voltage-dependent anion channel protein isoforms in guinea pig brain synaptosomes. Proteomics 2004;4:1335–1340. [PubMed: 15188400]
25. Vander Heiden MG, Chandel NS, Li XX, Schumacker PT, Colombini M, Thompson CB. Outer mitochondrial membrane permeability can regulate coupled respiration and cell survival. Proc. Natl. Acad. Sci. USA 2000;97:4666–4671. [PubMed: 10781072]
26. Azoulay-Zohar H, Israelson A, Abu-Hamad S, Shoshan-Barmatz V. In self-defense: Hexokinase promotes voltage-dependent anion channel closure and prevents mitochondria-mediated apoptotic cell death. Biochem. J 2004;377:347–355. [PubMed: 14561215]
27. Jonas EA, Buchanan J, Kaczmarek LK. Prolonged activation of mitochondrial conductances during synaptic transmission. Science 1999;286:1347–1350. [PubMed: 10558987]
28. Jonas EA, Hickman JA, Chachar M, Polster BM, Brandt TA, et al. Proapoptotic N-truncated BCL-xL protein activates endogenous mitochondrial channels in living synaptic terminals. Proc. Natl. Acad. Sci. USA 2004;101:13590–13595. [PubMed: 15342906]
29. Lemasters JJ, Holmuhamedov E. Voltage-dependent anion channel (VDAC) as mitochondrial governator—thinking outside the box. Biochim. Biophys. Acta 2006;1762:181–190. [PubMed: 16307870]
30. Bathori G, Csordas G, Garcia-Perez C, Davies E, Hajnoczky G. Ca^{2+}-dependent control of the permeability properties of the mitochondrial outer membrane and voltage-dependent anion-selective channel (VDAC). J. Biol. Chem 2006;281:17347–17358. [PubMed: 16597621]
31. Moran O, Sciancalepore M, Sandri G, Panfili E, Bassi R, et al. Ionic permeability of the mitochondrial outer membrane. Eur. Biophys. J 1992;20:311–319. [PubMed: 1373115]
32. Pastorino JG, Hoek JB. Hexokinase II: the integration of energy metabolism and control of apoptosis. Curr. Med. Chem 2003;10:1535–1551. [PubMed: 12871125]
33. Sharpe JC, Arnoult D, Youle RJ. Control of mitochondrial permeability by Bcl-2 family members. Biochim. Biophys. Acta 2004;1644:107–113. [PubMed: 14996495]
34. Siskind LJ, Kolesnick RN, Colombini M. Ceramide forms channels in mitochondrial outer membranes at physiologically relevant concentrations. Mitochondrion 2006;6:118–125. [PubMed: 16713754]
35. Madesh M, Hajnoczky G. VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome *c* release. J. Cell Biol 2001;155:1003–1015. [PubMed: 11739410]
36. Shimizu S, Tsujimoto Y. Proapoptotic BH3-only Bcl-2 family members induce cytochrome *c* release, but not mitochondrial membrane potential loss, and do not directly modulate voltage-dependent anion channel activity. Proc. Natl. Acad. Sci. USA 2000;97:577–582. [PubMed: 10639121]
37. Dejean LM, Martinez-Caballero S, Kinnally KW. Is MAC the knife that cuts cytochrome *c* from mitochondria during apoptosis? Cell Death Differ 2006;13:1387–1395. [PubMed: 16676005]
38. Crompton M, Barksby E, Johnson N, Capano M. Mitochondrial intermembrane junctional complexes and their involvement in cell death. Biochimie 2002;84:143–152. [PubMed: 12022945]
39. Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, et al. The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. Nature 2004;427:461–465. [PubMed: 14749836]

40. Di Lisa F, Bernardi P. Mitochondria and ischemia-reperfusion injury of the heart: Fixing a hole. Cardiovasc. Res 2006;70:191–199. [PubMed: 16497286]
41. Lohret TA, Kinnally KW. Multiple conductance channel activity of wild-type and voltage-dependent anion-selective channel (VDAC)-less yeast mitochondria. Biophys. J 1995;68:2299–2309. [PubMed: 7544166]
42. Krauskopf A, Eriksson O, Craigen WJ, Forte MA, Bernardi P. Properties of the permeability transition in VDAC1$^{-/-}$ mitochondria. Biochim. Biophys. Acta 2006;1757:590–595. [PubMed: 16626625]
43. Capano M, Crompton M. Biphasic translocation of Bax to mitochondria. Biochem. J 2002;367:169–178. [PubMed: 12097139]
44. Banerjee J, Ghosh S. Bax increases the pore size of rat brain mitochondrial voltage-dependent anion channel in the presence of tBid. Biochem. Biophys. Res. Commun 2004;323:310–314. [PubMed: 15351738]
45. Banerjee J, Ghosh S. Phosphorylation of rat brain mitochondrial voltage-dependent anion as a potential tool to control leakage of cytochrome *c*. J. Neurochem 2006;98:670–676. [PubMed: 16787424]
46. Shoshan-Barmatz V, Israelson A, Brdiczka D, Sheu SS. The voltage-dependent anion channel (VDAC): function in intracellular signaling, cell life and cell death. Curr. Pharm. Des 2006;12:2249–2270. [PubMed: 16787253]
47. Vander Heiden MG, Li XX, Gottleib E, Hill RB, Thompson CB, Colombini M. Bcl-xL promotes the open configuration of the voltage-dependent anion channel and metabolite passage through the outer mitochondrial membrane. J. Biol. Chem 2001;276:19414–19419. [PubMed: 11259441]
48. Duan S, Hajek P, Lin C, Shin SK, Attardi G, Chomyn A. Mitochondrial outer membrane permeability change and hypersensitivity to digitonin early in staurosporine-induced apoptosis. J. Biol. Chem 2003;278:1346–1353. [PubMed: 12403774]
49. Pavlov EV, Priault M, Pietkiewicz D, Cheng EH, Antonsson B, et al. A novel, high conductance channel of mitochondria linked to apoptosis in mammalian cells and Bax expression in yeast. J. Cell Biol 2001;155:725–731. [PubMed: 11724814]
50. Schendel SL, Xie Z, Montal MO, Matsuyama S, Montal M, Reed JC. Channel formation by antiapoptotic protein Bcl-2. Proc. Natl. Acad. Sci. USA 1997;94:5113–5118. [PubMed: 9144199]
51. Antonsson B, Conti F, Ciavatta A, Montessuit S, Lewis S, et al. Inhibition of Bax channel-forming activity by Bcl-2. Science 1997;277:370–372. [PubMed: 9219694]
52. Guihard G, Bellot G, Moreau C, Pradal G, Ferry N, et al. The mitochondrial apoptosis-induced channel (MAC) corresponds to a late apoptotic event. J. Biol. Chem 2004;279:46542–46550. [PubMed: 15328340]
53. Dejean LM, Martinez-Caballero S, Manon S, Kinnally KW. Regulation of the mitochondrial apoptosis-induced channel, MAC, by BCL-2 family proteins. Biochim. Biophys. Acta 2006;1762:191–201. [PubMed: 16055309]
54. Grigoriev SM, Muro C, Dejean LM, Campo ML, Martinez-Caballero S, Kinnally KW. Electrophysiological approaches to the study of protein translocation in mitochondria. Int. Rev. Cytol 2004;238:227–274. [PubMed: 15364200]
55. Henry JP, Chich JF, Goldschmidt D, Thieffry M. Ionic mitochondrial channels: characteristics and possible role in protein translocation. Biochimie 1989;71:963–968. [PubMed: 2478198]
56. Juin P, Thieffry M, Henry JP, Vallette FM. Relationship between the peptide-sensitive channel and the mitochondrial outer membrane protein translocation machinery. J. Biol. Chem 1997;272:6044–6050. [PubMed: 9038228]
57. Veenman L, Gavish M. The peripheral-type benzodiazepine receptor and the cardiovascular system. Implications for drug development. Pharmacol. Ther 2006;110:503–524. [PubMed: 16337685]
58. Anholt RR, Pedersen PL, De Souza EB, Snyder SH. The peripheral-type benzodiazepine receptor. Localization to the mitochondrial outer membrane. J. Biol. Chem 1986;261:576–583. [PubMed: 3001071]
59. Papadopoulos V. In search of the function of the peripheral-type benzodiazepine receptor. Endocr. Res 2004;30:677–684. [PubMed: 15666811]

60. Desjardins P, Bandeira P, Raghavendra Rao VL, Ledoux S, Butterworth RF. Increased expression of the peripheral-type benzodiazepine receptor-isoquinoline carboxamide binding protein mRNA in brain following portacaval anastomosis. Brain Res 1997;758:255–258. [PubMed: 9203558]
61. McEnery MW, Snowman AM, Trifiletti RR, Snyder SH. Isolation of the mitochondrial benzodiazepine receptor: association with the voltage-dependent anion channel and the adenine nucleotide carrier. Proc. Natl. Acad. Sci. USA 1992;89:3170–3174. [PubMed: 1373486]
62. Joseph-Liauzun E, Farges R, Delmas P, Ferrara P, Loison G. The M_r 18,000 subunit of the peripheral-type benzodiazepine receptor exhibits both benzodiazepine and isoquinoline carboxamide binding sites in the absence of the voltage-dependent anion channel or of the adenine nucleoti carrier. J. Biol. Chem 1997;272:28102–28106. [PubMed: 9346965]
63. Galieègue S, Jbilo O, Combes T, Bribes E, Carayon P, et al. Cloning and characterization of PRAX-1. A new protein that specifically interacts with the peripheral benzodiazepine receptor. J. Biol. Chem 1999;274:2938–2952. [PubMed: 9915832]
64. Steinberg RA, Symcox MM, Sollid S, Ogreid D. Arginine 210 is not a critical residue for the allosteric interactions mediated by binding of cyclic AMP to site A of regulatory (RIα) subunit of cyclic AMP-dependent protein kinase. J. Biol. Chem 1996;271:27630–27636. [PubMed: 8910352]
65. Blahos J, Whalin ME, Krueger KE. Identification and purification of a 10-kDa protein associated with mitochondrial benzodiazepine receptors. J. Biol. Chem 1995;270:20285–20291. [PubMed: 7657598]
66. Guidotti A, Forchetti CM, Corda MG, Konkel D, Bennett CD, Costa E. Isolation, characterization, and purification to homogeneity of an endogenous polypeptide with agonistic action on benzodiazepine receptors. Proc. Natl. Acad. Sci. USA 1983;80:3531–3535. [PubMed: 6304714]
67. Costa E, Guidotti A. Diazepam binding inhibitor (DBI): a peptide with multiple biological actions. Life Sci 1991;49:325–344. [PubMed: 1649940]
68. Duparc C, Lefebvre H, Tonon MC, Vaudry H, Kuhn JM. Characterization of endozepines in the human testicular tissue: effect of triakontatetraneuropeptide on testosterone secretion. J. Clin. Endocrinol. Metab 2003;88:5521–5528. [PubMed: 14602800]
69. Verma A, Nye JS, Snyder SH. Porphyrins are endogenous ligands for the mitochondrial (peripheral-type) benzodiazepine receptor. Proc. Natl. Acad. Sci. USA 1987;84:2256–2260. [PubMed: 3031675]
70. Simpson ER, Waterman MR. Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. Annu. Rev. Physiol 1988;50:427–440. [PubMed: 2837136]
71. Krueger KE. Peripheral-type benzodiazepine receptors: a second site of action for benzodiazepines. Neuropsychopharmacology 1991;4:237–244. [PubMed: 1678259]
72. Mukhin AG, Papadopoulos V, Costa E, Krueger KE. Mitochondrial benzodiazepine receptors regulate steroid biosynthesis. Proc. Natl. Acad. Sci. USA 1989;86:9813–9816. [PubMed: 2574864]
73. Papadopoulos V, Amri H, Li H, Boujrad N, Vidic B, Garnier M. Targeted disruption of the peripheral-type benzodiazepine receptor gene inhibits steroidogenesis in the R2C Leydig tumor cell line. J. Biol. Chem 1997;272:32129–32135. [PubMed: 9405411]
74. Bernassau JM, Reversat JL, Ferrara P, Caput D, Lefur G. A 3D model of the peripheral benzodiazepine receptor and its implication in intra mitochondrial cholesterol transport. J. Mol. Graph 1993;11:236–244. [PubMed: 8136326]
75. Papadopoulos V, Boujrad N, Ikonomovic MD, Ferrara P, Vidic B. Topography of the Leydig cell mitochondrial peripheral-type benzodiazepine receptor. Mol. Cell Endocrinol 1994;104:R5–R9. [PubMed: 7821699]
76. Lueddens HW, Skolnick P. 'Peripheral-type' benzodiazepine receptors in the kidney: regulation of radioligand binding by anions and DIDS. Eur. J. Pharmacol 1987;133:205–214. [PubMed: 3028841]
77. Beaumont K, Skowronski R, Vaughn DA, Fanestil DD. Interactions of lipids with peripheral-type benzodiazepine receptors. Biochem. Pharmacol 1988;37:1009–1014. [PubMed: 2833268]
78. Havoundjian H, Cohen RM, Paul SM, Skolnick P. Differential sensitivity of "central" and "peripheral" type benzodiazepine receptors to phospholipase A2. J. Neurochem 1986;46:804–811. [PubMed: 3005493]
79. Hirsch JD, Beyer CF, Malkowitz L, Loullis CC, Blume AJ. Characterization of ligand binding to mitochondrial benzodiazepine receptors. Mol. Pharmacol 1989;35:164–172. [PubMed: 2536464]

80. Brand MD, Chien LF, Ainscow EK, Rolfe DF, Porter RK. The causes and functions of mitochondrial proton leak. Biochim. Biophys. Acta 1994;1187:132–139. [PubMed: 8075107]
81. Nicholls DG, Grav HJ, Lindberg O. Mitochondrial from hamster brown-adipose tissue. Regulation of respiration in vitro by variations in volume of the matrix compartment. Eur. J. Biochem 1972;31:526–533. [PubMed: 4650156]
82. Heaton GM, Wagenvoord RJ, Kemp AJ, Nicholls DG. Brown-adipose-tissue mitochondria: photoaffinity labeling of the regulatory site of energy dissipation. Eur. J. Biochem 1978;82:515–521. [PubMed: 624284]
83. Ricquier D, Bouillaud F. Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. J. Physiol 2000;529(Pt 1):3–10. [PubMed: 11080246]
84. Starkov AA. "Mild" uncoupling of mitochondria. Biosci. Rep 1997;17:273–279. [PubMed: 9337482]
85. Jezek P. Fatty acid interaction with mitochondrial uncoupling proteins. J. Bioenerg. Biomembr 1999;31:457–466. [PubMed: 10653474]
86. Stucki JW. The optimal efficiency and the economic degrees of coupling of oxidative phosphorylation. Eur. J. Biochem 1980;109:269–283. [PubMed: 7408881]
87. Nicholls DG. Hamster brown-adipose-tissue mitochondria. The chloride permeability of the inner membrane under respiring conditions, the influence of purine nucleotides. Eur. J. Biochem 1974;49:585–593. [PubMed: 4442426]
88. Huang SG, Klingenberg M. Chloride channel properties of the uncoupling protein from brown adipose tissue mitochondria: a patch-clamp study. Biochemistry 1996;35:16806–16814. [PubMed: 8988019]
89. Yamaguchi H, Jelokhani-Niaraki M, Kodama H. Second transmembrane domain of human uncoupling protein 2 is essential for its anion channel formation. FEBS Lett 2004;577:299–304. [PubMed: 15527803]
90. Klingenberg M, Winkler E, Echtay K. Uncoupling protein, H^+ transport and regulation. Biochem. Soc. Trans 2001;29:806–811. [PubMed: 11709079]
91. Nadtochiy SM, Tompkins AJ, Brookes PS. Different mechanisms of mitochondrial proton leak in ischaemia/reperfusion injury and preconditioning: implications for pathology and cardioprotection. Biochem. J 2006;395:611–618. [PubMed: 16436046]
92. Reed KC, Bygrave FL. The inhibition of mitochondrial calcium transport by lanthanides and ruthenium red. Biochem. J 1974;140:143–155. [PubMed: 4375957]
93. Gunter TE, Buntinas L, Sparagna G, Eliseev R, Gunter K. Mitochondrial calcium transport: mechanisms and functions. Cell Calcium 2000;28:285–296. [PubMed: 11115368]
94. Kirichok Y, Krapivinsky G, Clapham DE. The mitochondrial calcium uniporter is a highly selective ion channel. Nature 2004;427:360–364. [PubMed: 14737170]
95. Sottocasa G, Sandri G, Panfili E, De Bernard B, Gazzotti P, et al. Isolation of a soluble Ca^{2+} binding glycoprotein from ox liver mitochondria. Biochem. Biophys. Res. Commun 1972;47:808–813. [PubMed: 4260315]
96. Panfili E, Sandri G, Sottocasa GL, Lunazzi G, Liut G, Graziosi G. Specific inhibition of mitochondrial Ca^{2+} transport by antibodies directed to the Ca^{2+}-binding glycoprotein. Nature 1976;264:185–186. [PubMed: 995204]
97. Mironova GD, Sirota TV, Pronevich LA, Trofimenko NV, Mironov GP, et al. Isolation and properties of Ca^{2+}-transporting glycoprotein and peptide from beef heart mitochondria. J. Bioenerg. Biomembr 1982;14:213–225. [PubMed: 7130161]
98. Saris NE, Sirota TV, Virtanen I, Niva K, Penttila T, et al. Inhibition of the mitochondrial calcium uniporter by antibodies against a 40-kDa glycoproteinT. J. Bioenerg. Biomembr 1993;25:307–312. [PubMed: 7688718]
99. Mironova GD, Baumann M, Kolomytkin O, Krasichkova Z, Berdimuratov A, et al. Purification of the channel component of the mitochondrial calcium uniporter and its reconstitution into planar lipid bilayers. J. Bioenerg. Biomembr 1994;26:231–238. [PubMed: 8056790]
100. Zazueta C, Masso F, Paez A, Bravo C, Vega A, et al. Identification of a 20-kDa protein with calcium uptake transport activity. Reconstitution in a membrane model. J. Bioenerg. Biomembr 1994;26:555–562. [PubMed: 7896770]

101. Zazueta C, Zafra G, Vera G, Sanchez C, Chavez E. Advances in the purification of the mitochondrial Ca^{2+} uniporter using the labeled inhibitor ^{103}Ru360. J. Bioenerg. Biomembr 1998;30:489–498. [PubMed: 9932651]
102. Sparagna GC, Gunter KK, Sheu SS, Gunter TE. Mitochondrial calcium uptake from physiological-type pulses of calcium. A description of the rapid uptake mode. J. Biol. Chem 1995;270:27510–27515. [PubMed: 7499209]
103. Buntinas L, Gunter KK, Sparagna GC, Gunter TE. The rapid mode of calcium uptake into heart mitochondria (RaM): comparison to RaM in liver mitochondria. Biochim. Biophys. Acta 2001;1504:248–261. [PubMed: 11245789]
104. Beutner G, Sharma VK, Lin L, Ryu SY, Dirksen RT, Sheu SS. Type 1 ryanodine receptor in cardiac mitochondria: transducer of excitation-metabolism coupling. Biochim. Biophys. Acta 2005;1717:1–10. [PubMed: 16246297]
105. Bernardi P, Paradisi V, Pozzan T, Azzone GF. Pathway for uncoupler-induced calcium efflux in rat liver mitochondria: inhibition by ruthenium red. Biochemistry 1984;23:1645–1651. [PubMed: 6202317]
106. Lamping KA, Gross GJ. Improved recovery of myocardial segment function following a short coronary occlusion in dogs by nicorandil, a potential new antianginal agent, and nifedipine. J. Cardiovasc. Pharmacol 1985;7:158–166. [PubMed: 2580137]
107. Grover GJ, Garlid KD. ATP-sensitive potassium channels: a review of their cardioprotective pharmacology. J. Mol. Cell Cardiol 2000;32:677–695. [PubMed: 10756123]
108. Suzuki M, Sasaki N, Miki T, Sakamoto N, Ohmoto-Sekine Y, et al. Role of sarcolemmal K_{ATP} channels in cardioprotection against ischemia/reperfusion injury in mice. J. Clin. Invest 2002;109:509–516. [PubMed: 11854323]
109. Suzuki M, Saito T, Sato T, Tamagawa M, Miki T, et al. Cardioprotective effect of diazoxide is mediated by activation of sarcolemmal but not mitochondrial ATP-sensitive potassium channels in mice. Circulation 2003;107:682–685. [PubMed: 12578868]
110. Zingman LV, Hodgson DM, Bast PH, Kane GC, Perez-Terzic C, et al. Kir6.2 is required for adaptation to stress. Proc. Natl. Acad. Sci. USA 2002;99:13278–13283. [PubMed: 12271142]
111. Sato T, Sasaki N, Seharaseyon J, O'Rourke B, Marban E. Selective pharmacological agents implicate mitochondrial but not sarcolemmal K_{ATP} channels in ischemic cardioprotection. Circulation 2000;101:2418–2423. [PubMed: 10821820]
112. Ghosh S, Standen NB, Galinanes M. Evidence for mitochondrial K_{ATP} channels as effectors of human myocardial preconditioning. Cardiovasc. Res 2000;45:934–940. [PubMed: 10728419]
113. Tanno M, Miura T, Tsuchida A, Miki T, Nishino Y, et al. Contribution of both the sarcolemmal K_{ATP} and mitochondrial K_{ATP} channels to infarct size limitation by K_{ATP} channel openers: differences from preconditioning in the role of sarcolemmal K_{ATP} channels. Naunyn-Schmiedebergs Arch. Pharmacol 2001;364:226–232. [PubMed: 11521165]
114. Billman GE, Englert HC, Scholkens BA. HMR 1883, a novel cardioselective inhibitor of the ATP-sensitive potassium channel. Part II: effects on susceptibility to ventricular fibrillation induced by myocardial ischemia in conscious dogs. J. Pharmacol. Exp. Ther 1998;286:1465–1473. [PubMed: 9732412]
115. Akar FG, Aon MA, Tomaselli GF, O'Rourke B. The mitochondrial origin of post-ischemic arrhythmias. J. Clin. Invest 2005;115:3527–3535. [PubMed: 16284648]
116. Inoue I, Nagase H, Kishi K, Higuti T. ATP-sensitive K^+ channel in the mitochondrial inner membrane. Nature 1991;352:244–247. [PubMed: 1857420]
117. Garlid KD, Paucek P, Yarov-Yarovoy V, Murray HN, Darbenzio RB, et al. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K^+ channels. Possible mechanism of cardioprotection. Circ. Res 1997;81:1072–1082. [PubMed: 9400389]
118. Liu Y, Sato T, O'Rourke B, Marban E. Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? Circulation 1998;97:2463–2469. [PubMed: 9641699]
119. Dahlem YA, Horn TF, Buntinas L, Gonoi T, Wolf G, Siemen D. The human mitochondrial K_{ATP} channel is modulated by calcium and nitric oxide: a patch-clamp approach. Biochim. Biophys. Acta 2004;1656:46–56. [PubMed: 15136158]

120. O'Rourke B. Evidence for mitochondrial K^+ channels and their role in cardioprotection. Circ. Res 2004;94:420–432. [PubMed: 15001541]

121. Garlid KD, Dos Santos P, Xie ZJ, Costa AD, Paucek P. Mitochondrial potassium transport: the role of the mitochondrial ATP-sensitive K^+ channel in cardiac function and cardioprotection. Biochim. Biophys. Acta 2003;1606:1–21. [PubMed: 14507424]

122. Facundo HT, Fornazari M, Kowaltowski AJ. Tissue protection mediated by mitochondrial K^+ channels. Biochim. Biophys. Acta 2006;1762:202–212. [PubMed: 16026967]

123. Sato T, O'Rourke B, Marban E. Modulation of mitochondrial ATP-dependent K^+ channels by protein kinase C. Circ. Res 1998;83:110–114. [PubMed: 9670924]

124. Ockaili R, Salloum F, Hawkins J, Kukreja RC. Sildenafil (Viagra) induces powerful cardioprotective effect via opening of mitochondrial K_{ATP} channels in rabbits. Am. J. Physiol. Heart Circ. Physiol 2002;283:H1263–H1269. [PubMed: 12181158]

125. Kopustinskiene DM, Pollesello P, Saris NE. Levosimendan is a mitochondrial K_{ATP} channel opener. Eur. J. Pharmacol 2001;428:311–314. [PubMed: 11689188]

126. Tanonaka K, Taguchi T, Koshimizu M, Ando T, Morinaka T, et al. Role of an ATP-sensitive potassium channel opener, YM934, in mitochondrial energy production in ischemic/reperfused heart. J. Pharmacol. Exp. Ther 1999;291:710–716. [PubMed: 10525091]

127. Sasaki N, Murata M, Guo Y, Jo SH, Ohler A, et al. MCC-134, a single pharmacophore, opens surface ATP-sensitive potassium channels, blocks mitochondrial ATP-sensitive potassium channels, and suppresses preconditioning. Circulation 2003;107:1183–1188. [PubMed: 12615799]

128. Cohen MV, Baines CP, Downey JM. Ischemic preconditioning: from adenosine receptor to K_{ATP} channel. Annu. Rev. Physiol 2000;62:79–109. [PubMed: 10845085]

129. Uchiyama Y, Otani H, Okada T, Uchiyama T, Ninomiya H, et al. Integrated pharmacological preconditioning in combination with adenosine, a mitochondrial K_{ATP} channel opener and a nitric oxide donor. J. Thorac. Cardiovasc. Surg 2003;126:148–159. [PubMed: 12878950]

130. Pain T, Yang XM, Critz SD, Yue Y, Nakano A, et al. Opening of mitochondrial K_{ATP} channels triggers the preconditioned state by generating free radicals. Circ. Res 2000;87:460–466. [PubMed: 10988237]

131. Juhaszova M, Zorov DB, Kim SH, Pepe S, Fu Q, et al. Glycogen synthase kinase-3β mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. J. Clin. Invest 2004;113:1535–1549. [PubMed: 15173880]

132. Sasaki N, Sato T, Ohler A, O'Rourke B, Marban E. Activation of mitochondrial ATP-dependent potassium channels by nitric oxide. Circulation 2000;101:439–445. [PubMed: 10653837]

133. Ockaili R, Emani VR, Okubo S, Brown M, Krottapalli K, Kukreja RC. Opening of mitochondrial K_{ATP} channel induces early and delayed cardioprotective effect: role of nitric oxide. Am. J. Physiol 1999;277:H2425–H2434. [PubMed: 10600865]

134. Xu Z, Ji X, Boysen PG. Exogenous nitric oxide generates ROS and induces cardioprotection: involvement of PKG, mitochondrial K_{ATP} channels, and ERK. Am. J. Physiol. Heart Circ. Physiol 2004;286:H1433–H1440. [PubMed: 14656708]

135. Costa AD, Garlid KD, West IC, Lincoln TM, Downey JM, et al. Protein kinase G transmits the cardioprotective signal from cytosol to mitochondria. Circ. Res 2005;97:329–336. [PubMed: 16037573]

136. Dang VC, Kim N, Youm JB, Joo H, Warda M, et al. Nitric oxide—cGMP—protein kinase G signaling pathway induces anoxic preconditioning through activation of ATP-sensitive K^+ channels in rat hearts. Am. J. Physiol. Heart Circ. Physiol 2006;290:H1808–H1817. [PubMed: 16339835]

137. Siemen D, Loupatatzis C, Borecky J, Gulbins E, Lang F. Ca^{2+}-activated K channel of the BK-type in the inner mitochondrial membrane of a human glioma cell line. Biochem. Biophys. Res. Commun 1999;257:549–554. [PubMed: 10198249]

138. Xu W, Liu Y, Wang S, McDonald T, Van Eyk JE, et al. Cytoprotective role of Ca^{2+}-activated K^+ channels in the cardiac inner mitochondrial membrane. Science 2002;298:1029–1033. [PubMed: 12411707]

139. Shintani Y, Node K, Asanuma H, Sanada S, Takashima S, et al. Opening of Ca^{2+}-activated K^+ channels is involved in ischemic preconditioning in canine hearts. J. Mol. Cell Cardiol 2004;37:1213–1218. [PubMed: 15572051]

140. Stowe DF, Aldakkak M, Camara AK, Riess ML, Heinen A, et al. Cardiac mitochondrial preconditioning by Big Ca^{2+}-sensitive K^+ channel opening requires superoxide radical generation. Am. J. Physiol. Heart Circ. Physiol 2006;290:H434–H440. [PubMed: 16126810]

141. Wang X, Yin C, Xi L, Kukreja RC. Opening of Ca^{2+}-activated K^+ channels triggers early and delayed preconditioning against I/R injury independent of NOS in mice. Am. J. Physiol. Heart Circ. Physiol 2004;287:H2070–H2077. [PubMed: 15217801]

142. Cao CM, Xia Q, Gao Q, Chen M, Wong TM. Calcium-activated potassium channel triggers cardioprotection of ischemic preconditioning. J. Pharmacol. Exp. Ther 2005;312:644–650. [PubMed: 15345753]

143. Gao Q, Zhang SZ, Cao CM, Bruce IC, Xia Q. The mitochondrial permeability transition pore and the Ca^{2+}-activated K^+ channel contribute to the cardioprotection conferred by tumor necrosis factor-α. Cytokine 2005;32:199–205. [PubMed: 16260145]

144. Sato T, Saito T, SaegUSA N, Nakaya H. Mitochondrial Ca^{2+}-activated K^+ channels in cardiac myocytes: a mechanism of the cardioprotective effect and modulation by protein kinase A. Circulation 2005;111:198–203. [PubMed: 15623543]

145. Douglas RM, Lai JC, Bian S, Cummins L, Moczydlowski E, Haddad GG. The calcium-sensitive large-conductance potassium channel (BK/MAXI K) is present in the inner mitochondrial membrane of rat brain. Neuroscience 2006;139:1249–1261. [PubMed: 16567053]

146. Ohya S, Kuwata Y, Sakamoto K, Muraki K, Imaizumi Y. Cardioprotective effects of estradiol include the activation of large-conductance Ca^{2+}-activated K^+ channels in cardiac mitochondria. Am. J. Physiol. Heart Circ. Physiol 2005;289:H1635–H1642. [PubMed: 16113069]

147. Szabo I, Bock J, Jekle A, Soddemann M, Adams C, et al. A novel potassium channel in lymphocyte mitochondria. J. Biol. Chem 2005;280:12790–12798. [PubMed: 15632141]

148. Beavis AD, Powers M. Temperature dependence of the mitochondrial inner membrane anion channel: the relationship between temperature and inhibition by magnesium. J. Biol. Chem 2004;279:4045–4050. [PubMed: 14615482]

149. Sorgato MC, Keller BU, Stuhmer W. Patch-clamping of the inner mitochondrial membrane reveals a voltage-dependent ion channel. Nature 1987;330:498–500. [PubMed: 2446143]

150. Borecky J, Jezek P, Siemen D. 108-pS channel in brown fat mitochondria might be identical to the inner membrane anion channel. J. Biol. Chem 1997;272:19282–19289. [PubMed: 9235923]

151. Kinnally KW, Zorov DB, Antonenko YN, Snyder SH, McEnery MW, Tedeschi H. Mitochondrial benzodiazepine receptor linked to inner membrane ion channels by nanomolar actions of ligands. Proc. Natl. Acad. Sci. USA 1993;90:1374–1378. [PubMed: 7679505]

152. Schonfeld P, Sayeed I, Bohnensack R, Siemen D. Fatty acids induce chloride permeation in rat liver mitochondria by activation of the inner membrane anion channel (IMAC). J. Bioenerg. Biomembr 2004;36:241–248. [PubMed: 15337854]

153. Antonenko YN, Kinnally KW, Tedeschi H. Identification of anion and cation pathways in the inner mitochondrial membrane by patch clamping of mouse liver mitoplasts. J. Membr. Biol 1991;124:151–158. [PubMed: 1722254]

154. Beavis AD. On the inhibition of the mitochondrial inner membrane anion uniporter by cationic amphiphiles and other drugs. J. Biol. Chem 1989;264:1508–1515. [PubMed: 2492277]

155. Ballarin C, Sorgato MC. An electrophysiological study of yeast mitochondria. Evidence for two inner membrane anion channels sensitive to ATP. J. Biol. Chem 1995;270:19262–19268. [PubMed: 7642599]

156. O'Rourke B. Pathophysiological and protective roles of mitochondrial ion channels. J. Physiol 2000;529(Pt 1):23–36. [PubMed: 11080248]

157. Aon MA, Cortassa S, Marban E, O'Rourke B. Synchronized whole cell oscillations in mitochondrial metabolism triggered by a local release of reactive oxygen species in cardiac myocytes. J. Biol. Chem 2003;278:44735–44744. [PubMed: 12930841]

158. Cortassa S, Aon MA, Winslow RL, O'Rourke B. A mitochondrial oscillator dependent on reactive oxygen species. Biophys. J 2004;87:2060–2073. [PubMed: 15345581]

159. Aon MA, Cortassa S, O'Rourke B. Percolation and criticality in a mitochondrial network. Proc. Natl. Acad. Sci. USA 2004;101:4447–4452. [PubMed: 15070738]

160. Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ. Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. J. Exp. Med 2000;192:1001–1014. [PubMed: 11015441]

161. Lurin C, Guclu J, Cheniclet C, Carde JP, Barbier-Brygoo H, Maurel C. CLC-Nt1, a putative chloride channel protein of tobacco, colocalizes with mitochondrial membrane markers. Biochem. J 2000;348(Pt 2):291–295. [PubMed: 10816421]

162. Fernandez-Salas E, Suh KS, Speransky VV, Bowers WL, Levy JM, et al. mtCLIC/CLIC4, an organellular chloride channel protein, is increased by DNA damage and participates in the apoptotic response to p53. Mol. Cell Biol 2002;22:3610–3620. [PubMed: 11997498]

163. Suh KS, Mutoh M, Nagashima K, Fernandez-Salas E, Edwards LE, et al. The organellular chloride channel protein CLIC4/mtCLIC translocates to the nucleus in response to cellular stress and accelerates apoptosis. J. Biol. Chem 2004;279:4632–4641. [PubMed: 14610078]

164. Cooper C, Tapley DF. The effect of thyroxine and related compounds on oxidative phosphorylation. J. Biol. Chem 1956;222:341–349. [PubMed: 13367007]

165. Hunter DR, Haworth RA, Southard JH. Relationship between configuration, function, and permeability in calcium-treated mitochondria. J. Biol. Chem 1976;251:5069–5077. [PubMed: 134035]

166. Bernardi P, Krauskopf A, Basso E, Petronilli V, Blachly-Dyson E, et al. The mitochondrial permeability transition from in vitro artifact to disease target. FEBS J 2006;273:2077–2099. [PubMed: 16649987]

167. Halestrap AP. Calcium, mitochondria and reperfusion injury: a pore way to die. Biochem. Soc. Trans 2006;34:232–237. [PubMed: 16545083]

168. Fournier N, Ducet G, Crevat A. Action of cyclosporine on mitochondrial calcium fluxes. J. Bioenerg. Biomembr 1987;19:297–303. [PubMed: 3114244]

169. Crompton M, Ellinger H, Costi A. Inhibition by cyclosporin A of a Ca^{2+}-dependent pore in heart mitochondria activated byinorganic phosphate and oxidative stress. Biochem. J 1988;255:357–360. [PubMed: 3196322]

170. Connern CP, Halestrap AP. Purification and N-terminal sequencing of peptidylprolyl *cis-trans*-isomerase from rat liver mitochondrial matrix reveals the existence of a distinct mitochondrial cyclophilin. Biochem. J 1992;284(Pt 2):381–385. [PubMed: 1599421]

171. Woodfield KY, Price NT, Halestrap AP. cDNA cloning of rat mitochondrial cyclophilin. Biochim. Biophys. Acta 1997;1351:27–30. [PubMed: 9116040]

172. Kinnally KW, Campo ML, Tedeschi H. Mitochondrial channel activity studied by patch-clamping mitoplasts. J. Bioenerg. Biomembr 1989;21:497–506. [PubMed: 2478535]

173. Kinnally KW, Antonenko YN, Zorov DB. Modulation of inner mitochondrial membrane channel activity. J. Bioenerg. Biomembr 1992;24:99–110. [PubMed: 1380510]

174. Zorov DB, Kinnally KW, Perini S, Tedeschi H. Multiple conductance levels in rat heart inner mitochondrial membranes studied by patch clamping. Biochim. Biophys. Acta 1992;1105:263–270. [PubMed: 1586662]

175. Zorov DB, Kinnally KW, Tedeschi H. Voltage activation of heart inner mitochondrial membrane channels. J. Bioenerg. Biomembr 1992;24:119–124. [PubMed: 1380499]

176. Petronilli V, Szabo I, Zoratti M. The inner mitochondrial membrane contains ion-conducting channels similar to those found in bacteria. FEBS Lett 1989;259:137–143. [PubMed: 2480918]

177. Szabo I, Zoratti M. The giant channel of the inner mitochondrial membrane is inhibited by cyclosporin A. J. Biol. Chem 1991;266:3376–3379. [PubMed: 1847371]

178. Szabo I, Zoratti M. The mitochondrial megachannel is the permeability transition pore. J. Bioenerg. Biomembr 1992;24:111–117. [PubMed: 1380498]

179. Zoratti M, Szabo I. Electrophysiology of the inner mitochondrial membrane. J. Bioenerg. Biomembr 1994;26:543–553. [PubMed: 7534761]

180. Schultheiss HP, Klingenberg M. Immunochemical characterization of the adenine nucleotide translocator. Organ specificity and conformation specificity. Eur. J. Biochem 1984;143:599–605. [PubMed: 6090134]

181. Brustovetsky N, Klingenberg M. Mitochondrial ADP/ATP carrier can be reversibly converted into a large channel by Ca^{2+} Biochemistry 1996;35:8483–8488. [PubMed: 8679608]

182. Brustovetsky N, Tropschug M, Heimpel S, Heidkamper D, Klingenberg M. A large Ca^{2+}-dependent channel formed by recombinant ADP/ATP carrier from Neurospora crassa resembles the mitochondrial permeability transition pore. Biochemistry 2002;41:11804–11811. [PubMed: 12269823]

183. Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, et al. Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. Nature 2005;434:652–658. [PubMed: 15800626]

184. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, et al. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. Nature 2005;434:658–662. [PubMed: 15800627]

185. Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P. Properties of the permeability transition pore in mitochondria devoid of cyclophilin D. J. Biol. Chem 2005;280:18558–18561. [PubMed: 15792954]

186. Schinzel AC, Takeuchi O, Huang Z, Fisher JK, Zhou Z, et al. Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. Proc. Natl. Acad. Sci. USA 2005;102:12005–12010. [PubMed: 16103352]

187. Halestrap AP, Clarke SJ, Javadov SA. Mitochondrial permeability transition pore opening during myocardial reperfusion—a target for cardioprotection. Cardiovasc. Res 2004;61:372–385. [PubMed: 14962470]

188. Rodriguez-Sinovas A, Boengler K, Cabestrero A, Gres P, Morente M, et al. Translocation of connexin 43 to the inner mitochondrial membrane of cardiomyocytes through the heat shock protein 90-dependent TOM pathway and its importance for cardioprotection. Circ. Res 2006;99:93–101. [PubMed: 16741159]

189. Schwanke U, Konietzka I, Duschin A, Li X, Schulz R, Heusch G. No ischemic preconditioning in heterozygous connexin43-deficient mice. Am. J. Physiol. Heart Circ. Physiol 2002;283:H1740–H1742. [PubMed: 12234831]

190. Heinzel FR, Luo Y, Li X, Boengler K, Buechert A, et al. Impairment of diazoxide-induced formation of reactive oxygen species and loss of cardioprotection in connexin 43 deficient mice. Circ. Res 2005;97:583–586. [PubMed: 16100048]

191. Liu Y, Ren G, O'Rourke B, Marban E, Seharaseyon J. Pharmacological comparison of native mitochondrial K_{ATP} channels with molecularly defined surface K_{ATP} channels. Mol. Pharmacol 2001;59:225–230. [PubMed: 11160857]

192. Mironova GD, Negoda AE, Marinov BS, Paucek P, Costa AD, et al. Functional distinctions between the mitochondrial ATP-dependent K channel (mitoKATP) and its inward rectifier subunit (mitoKIR). J. Biol. Chem 2004;279:32562–32568. [PubMed: 15138282]

193. Jiang MT, Ljubkovic M, Nakae Y, Shi Y, Kwok WM, et al. Characterization of human cardiac mitochondrial ATP-sensitive potassium channel and its regulation by phorbol ester in vitro. Am. J. Physiol. Heart Circ. Physiol 2006;290:H1770–H1776. [PubMed: 16361367]

194. Suzuki M, Kotake K, Fujikura K, Inagaki N, Suzuki T, et al. Kir6.1: a possible subunit of ATP-sensitive K channels in mitochondria. Biochem. Biophys. Res. Commun 1997;241:693–697. [PubMed: 9434770]

195. Zhou M, Tanaka O, Sekiguchi M, Sakabe K, Anzai M, et al. Localization of the ATP-sensitive potassium channel subunit (Kir6. 1uK_{ATP}-1) in rat brain. Brain Res. Mol. Brain Res 1999;74:15–25. [PubMed: 10640672]

196. Lacza Z, Snipes JA, Miller AW, Szabo C, Grover G, Busija DW. Heart mitochondria contain functional ATP-dependent K channels. J. Mol. Cell Cardiol 2003;35:1339–1347. [PubMed: 14596790]

197. Lacza Z, Snipes JA, Kis B, Szabo C, Grover G, Busija DW. Investigation of the subunit composition and the pharmacology of the mitochondrial ATP-dependent K channel in the brain. Brain Res 2003;994:27–36. [PubMed: 14642445]

198. Singh H, Hudman D, Lawrence CL, Rainbow RD, Lodwick D, Norman RI. Distribution of Kir6.0 and SUR2 ATP-sensitive potassium channel subunits in isolated ventricular myocytes. J. Mol. Cell Cardiol 2003;35:445–459. [PubMed: 12738227]

199. Cuong DV, Kim N, Joo H, Youm JB, Chung JY, et al. Subunit composition of ATP-sensitive potassium channels in mitochondria of rat hearts. Mitochondrion 2005;5:121–133. [PubMed: 16050978]

200. Zhou M, Tanaka O, Sekiguchi M, He HJ, Yasuoka Y, et al. ATP-sensitive K-channel subunits on the mitochondria and endoplasmic reticulum of rat cardiomyocytes. J. Histochem. Cytochem 2005;53:1491–1500. [PubMed: 15983113]

Table 1

Mitochondrial ion channels. A summary of mitochondrial ion channel types identified either in isolated mitochondria, proteolipid bilayers, or patch-clamp experiments. Detected single-channel conductances have been tentatively assigned to a given class of channel, but these assignments have not been unequivocally proven. See text for details and abbreviations

Location	Type	Conductance (~150 mM salt)	Modulators/inhibitors	Putative role(s)	Selected refs.
Outer membrane	VDAC (porin)	0.5–4 nS	Bax/Bak/Bcl-xL/Bcl-2, TOM20, Ca^{2+}, pH, ΔV, NADH, VDAC modulator	Metabolite transport, cytochrome *c* release/apoptosis, PTP complex	16
	TOM40 (PSC)	0.5–1 nS	Signal peptides	Protein transport	54
	MAC (BH proteins)	2.5 nS	Bax/Bak	Cytochrome *c* release/apoptosis	37
	Miscellaneous	10–307 pS	ΔV(for > 100 pS)	-	31
Inner membrane	Ca^{2+} uniporter	6 pS	Divalents, nucleotides, RuRed, ryanodine	Ca^{2+} uptake	94
	PTP MCC	0.03–1.5 nS	Ca^{2+}, ΔV, signal peptides, CsA	Protein transport	174
	MMC	0.3–1.3 nS	CsA, pH, Ca^{2+}, thiols, Bax, ANT inhibitors	Necrosis, apoptosis	178
	UCP	75 pS	Fatty acids	Thermogenesis	88
	K_{Ca}	295 pS	Ca^{2+}, ΔV, ChTx, IbTx	Volume regulation	137,138
	K_{ATP}	9.7 pS	ATP, GTP, palmitoyl CoA, Mg^{2+}, Ca^{2+}	Volume regulation, protection against apoptosis/ischemic injury	116,119
	$K_V1.3$	17 pS	Margatoxin	Cell death	147
	IMACs	45, 450 pS	ATP	Volume regulation (in yeast)	155
		15 pS(LCC)107 pS (centum pS)	Mg^{2+}, pH, P_i, thiols, DIDS,cationic amphiphiles	Volume regulation	149,150,153