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A role for mitochondrial aquaporins in cellular life-and-death decisions?

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Lee, Wing-Kee, and Frank Thévenod. A role for mitochondrial aquaporins in cellular life-and-death decisions? Am J Physiol Cell Physiol 291: C195–C202, 2006; doi:10.1152/ajpcell.00641.2005.—Mitochondria dominate the process of life-and-death decisions of the cell. Continuous generation of ATP is essential for cell sustenance, but on the other hand, mitochondria play a central role in the orchestra of events that lead to apoptotic cell death. Changes of mitochondrial volume contribute to the modulation of physiological mitochondrial function, and several ion permeability pathways located in the inner mitochondrial membrane have been implicated in the mediation of physiological swelling-contraction reactions, such as the K⁺ cycle. However, the channels and transporters involved in these processes have not yet been identified. Osmotic swelling is also one of the fundamental characteristics exhibited by mitochondria in pathological situations, which activates downstream cascades, culminating in apoptosis. The permeability transition pore has long been postulated to be the primary mediator for water movement in mitochondrial swelling during cell death, but its molecular identity remains obscure. Inevitably, accumulating evidence shows that mitochondrial swelling induced by apoptotic stimuli can also occur independently of permeability transition pore activation. Recently, a novel mechanism for osmotic swelling of mitochondria has been described. Aquaporin-8 and -9 channels have been identified in the inner mitochondrial membrane of various tissues, including the kidney, liver, and brain, where they may mediate water transport associated with physiological volume changes, contribute to the transport of metabolic substrates, and/or participate in osmotic swelling induced by apoptotic stimuli. Hence, the recent discovery that aquaporins are expressed in mitochondria opens up new areas of investigation in health and disease.

Continuous generation of ATP by oxidative phosphorylation is the major task of mitochondria and is required to sustain general cellular functions, such as homeostasis of ion gradients, and to preserve specializations, for example, secretion, exocytosis, contraction, electrical activity, to name only a few. Mitochondria maintain cellular ATP levels to keep the intracellular milieu constant, while the cell feeds mitochondria with the necessary fuels and respiratory substrates. As long as the generation and consumption of ATP are at equilibrium or able to adapt to the varying requirements of cellular activity, the energetic and structural integrity of the cell upholds and can also easily match the damaging effects of metabolic by-products of the energetic conversion of fuels and O₂ to ATP and H₂O, such as reactive oxygen species (ROS).

The crucial role of mitochondria in normal cellular function accounts for the fact that mitochondrial dysfunction is frequently associated with cell death (24, 40, 62, 109, 110). Acute or chronic environmental stress can lead to a destabilization of mitochondrial function, and under certain conditions, become irreversible, triggering cell death. The mitochondrion itself is a source of cell death initiation since the respiratory transport chain is a prime location of O₂ radical formation. Although the respiratory transport chain quite efficiently uses O₂ in the oxidation of its components, the superoxide anion is generated as a by-product of oxidative phosphorylation, making the mitochondrion a major site of ROS production. It has been estimated that 1–5% of the electrons flowing through the electron transport chain leak into the production of ROS (106). These highly reactive and potentially harmful molecules are normally “mopped up” by mitochondrial glutathione, preventing cell death (51, 85). Hence mitochondria appear to play a pivotal role in determining life and death of the cell. It is therefore imperative to fully understand the processes and elements that control and determine mitochondrial function in health and disease.

For more than 50 years, mitochondrial research has focused on the bioenergetic aspects of mitochondrial function and dysfunction. The molecular determinants of these processes have been elucidated to a large extent, but the characterization of the membrane transport pathways not directly associated with energetic processes has lagged behind and remains largely phenomenological. Moreover, progress made in the field of ion and membrane transport, where cloning strategies have led to the discovery of a variety of ion channels and membrane transporters, has been neglected.

MITOCHONDRIA are the “powerhouses” of the cell; they represent the metabolic and bioenergetic centers for a variety of functions to preserve cell sustenance, including Ca²⁺ homeostasis, respiration, and ATP production. Under normal physiological conditions, mitochondria maintain an electrochemical gradient across the inner membrane, which is created through the electron transport chain as well as the extrusion of H⁺ from the matrix to the intermembrane space (IMS) during oxidation of respiratory substrates. A proton-motive force is generated consisting of an H⁺ concentration gradient and an electrical component reflected by the mitochondrial membrane potential (ΔΨₘ). As a result, the matrix is negatively charged and has a slightly higher pH than the cytosol. Subsequently, the proton-motive force is used by the F₀/F₁ ATP synthase in the inner membrane to drive ATP synthesis (80).

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ION CONDUCTANCES OF INNER MITOCHONDRIAL MEMBRANE: PHYSIOLOGICAL REGULATORS OF MITOCHONDRIAL VOLUME AND FUNCTION

Mitochondrial swelling and contraction are thought to control the rate of substrate oxidation under physiological conditions (44). When channels open, they initiate a flux of ions and water, thus contributing to the dissipation of ΔΨm, which enhances the flux of electrons through the respiratory chain and results in increased oxidation and ROS production. This causes alterations of the cellular redox state, which is known to function as an intracellular signaling mechanism that can alter the activity of transcription factors, enzymes, and other signaling events. The trans-mitochondrial membrane driving force for swelling is induced by the osmotic gradient provided by the mitochondrial matrix that consists of anionic proteins, monovalent cations, intermediates of the TCA cycle, and other small organic molecules (8).

$K^+$- and Anion-Selective Pores

Any cytosolic molecule entering the mitochondrial matrix or any matrix component released into the cytosol must first cross the outer and inner mitochondrial membranes. The outer mitochondrial membrane (OMM) is highly permeable to a variety of molecules and is nonselective in its permeability properties. The voltage-dependent anion channel (VDAC) is the most abundant protein present in the OMM. VDAC, or mitochondrial porin, is a large H2O-filled pore, which allows polar molecules up to 5 kDa to pass freely to reach the inner mitochondrial membrane (IMM) and to mediate the exchange of metabolites required for mitochondrial function (12). In contrast, permeability to solutes (ions, respiratory substrates, and metabolites) dramatically decreases at the IMM, which is freely permeable to just a few molecules, such as O2, CO2, and NH3. Other hydrophilic metabolites or ions can only cross the membrane via the many specific transport systems present within the membrane, such as exchangers, uniporters, and ion channels (10, 86). The regulation of the composition of the matrix is apparently crucial to the functioning of mitochondria. For example, matrix volume, ion concentrations (e.g., $K^+$, Ca2+, H+), cristae folding, and compartmentalization of proteins all have to be tightly regulated under normal physiological conditions to prevent mitochondrial dysfunction.

The physiological roles of many IMM ion permeability pathways are largely unknown, although $K^+$- and/or anion-selective pores are postulated to be important in the physiological regulation of mitochondrial volume (35). Electrogenic and electroneutral $K^+$ pathways of a “$K^+$ cycle” as well as anion permeability pathways are mainly involved, which have been characterized by indirect techniques such as swelling assays. Patch-clamp studies have demonstrated channel-like properties of these ion conductive pathways (21, 97), but their molecular identity has so far remained mostly elusive. Recently, however, it appears that the laborious search for the molecular identity of the mitochondrial ATP-sensitive $K^+$ (mitoKATP) channel of the IMM has reached a breakthrough: Ardehali and colleagues (5) have found that the mitoKATP channel consists of a macromolecular supercomplex of four mitochondrial proteins (mitoATP-binding cassette protein 1, phosphate carrier, adenine nucleotide translocator, ATP synthase), which associate with succinate dehydrogenase. For a more detailed characterization of the ion channels that participate in physiological mitochondrial swelling and contraction processes, see the reviews by Zoratti and Szabo (102) and Garlid (36).

Mitochondrial Ca2+ Uniporter

Mitochondrial Ca2+ entry into the matrix is mediated by the Ca2+ uniporter (MCU) located in the organelle’s inner membrane. The MCU opens once cytoplasmic Ca2+ concentrations rise. The main functions of the MCU are to transport Ca2+ down its electrochemical gradient and load the mitochondrial Ca2+ store (42, 98). In addition, the uptake of Ca2+ stimulates respiration by increasing the activities of Ca2+-sensitive dehydrogenases (pyruvate, isocitrate, and $\alpha$-ketoglutarate) in the TCA cycle, which results in increased production of the reducing agents NADH2 and FADH, and leads to augmentation of electron transport chain activity (18, 48, 79). The MCU has an important regulatory role in mitochondrial function because it does not transport K+ or Mg2+, preventing depolarization of the IMM by these abundant ions. From patch-clamp experiments conducted in mitoplasts, the MCU was recently found to be a highly selective ion channel with differing properties to conventional voltage gated Ca2+ channels (56). Its molecular identity, however, is still unresolved.

Mitochondrial Ca2+ uptake via the MCU has been implicated in many forms of cell death (26). Although mitochondria may be able to accumulate large amounts of Ca2+ without further affecting normal mitochondrial function, mitochondrial Ca2+ overload may induce cell injury and death by triggering a mitochondrial permeability transition (115).

NONSELECTIVE PERMEABILITY PATHWAYS IN INNER MITOCHONDRIAL MEMBRANE: WHEN VOLUME CHANGES TRIGGER CELL DEATH

In contrast to physiological swelling, which is reversible in nature and usually not associated with significant structural alterations of mitochondrial membranes and/or extensive matrix swelling, pathological conditions may be associated with morphological and functional changes of mitochondria that may even become irreversible. Apart from import of ions and H2O into the matrix, which is accompanied by dissipation of ΔΨm, progressive osmotic swelling of the matrix space and unfolding of the cristae of the IMM may ultimately lead to the disruption of the outer membrane, spilling the intermembrane contents into the cytosol (11, 46, 115). Proapoptotic factors, such as cytochrome c (72), apoptosis-inducing factor (101), endonuclease G (92), Smac/Diablo (25, 108), and pro-caspases (113) that are usually present on the inner membrane and/or in the IMS have been reported to be released in this way. Hence, increasing evidence displays the pivotal role of mitochondria in apoptosis, with the release of mitochondrial apoptotic factors exceeding a threshold as a “point of no return” (24, 63).

Proteins in the OMM as well as in the IMM have been implicated in generating large pores by forming protein oligomers that allow permeation of various molecules, including proapoptotic factors. For instance, recent patch-clamp and planar bilayer studies suggest that apoptogenic proteins of the Bcl-2 protein family, such as Bad, Bax, or t-Bid form channels or “megapores” in the OMM that allow passage of cytochrome c into the cytoplasm, or do so by modulating VDAC activity.
Mitochondrial Permeability Transition Pore

The mitochondrial permeability transition (MPT) is a widely studied phenomenon and has been implicated in cell death. During MPT, there is a sudden, dramatic increase in the permeability of the IMM, which is mediated by opening of a large conductance pore (~1 nS), the permeability transition pore (PTP), that permits the passage of molecules of mass <1.5 kDa in a nonselective manner, including cytosolic solutes and water. This leads to liberation of intermembrane contents via swelling and rupture of the OMM (11, 19, 46, 115).

The PTP is formed at contact points between the inner and outer mitochondrial membranes. However, its composition remains elusive. Until recently, it was thought to be a complex of VDAC, adenine nucleotide translocator (ANT), and a water-soluble matrix protein, cyclophilin D (19, 20), but recent studies indicate that ANT (57) and cyclophilin D (7, 81) are not essential for the formation and modulation of the PTP.

A wide variety of factors modulate the PTP. It is activated by physiological factors such as Ca2+ and Pi, by oxidative stress and H2O2 present in the matrix (58), by depolarization of the IMM, increase in pH of the matrix (77, 78) and also by decrease in adenine nucleotide concentrations (19, 47). The PTP is maintained in its closed state by other divalent cations (e.g., Mn2+, Sr2+) and increased ADP or ATP concentrations. Various pharmacological agents can also affect the PTP: while atractyloside opens the PTP, bongkrekic acid and cyclosporin A (CsA) block the pore. CsA is an immunosuppressive agent but also has an inhibitory action on PTP through interaction with the binding site of cyclophilin D to ANT (83).

During apoptosis, several proapoptotic proteins are liberated from the mitochondria into the surrounding cytosol as a result of swelling induced by PTP opening and/or the rupture of the OMM. Because the PTP opens as a consequence of significant cellular Ca2+ overload or severe energy depletion, and it mediates loss of matrix constituents, PTP activation probably presents an irreversible terminal event for the mitochondrion. Apoptotic factors, such as cytochrome c, apoptosis-inducing factor (AIF), and several pro-caspases, have been reported to be released via activation of the PTP (72, 101, 113), although the liberation of these factors can also be either independent of the PTP or even mitochondrial swelling (2, 3, 37).

PTP-independent Permeability Pathways

Sensitivity to CsA is one of the defining features of the “classic” PTP. However, more studies in intact cells and isolated mitochondria indicate that changes of mitochondrial membrane permeability and/or release of cytochrome c may also occur by processes that are independent of this canonical concept (2, 3, 37, 38, 45, 91). They may be Ca2+-dependent and activated by a plethora of unrelated stimuli (9, 16, 33, 52, 75, 88, 100) and thus contribute to the process of apoptosis. CsA-insensitive and Ca2+-independent mitochondrial swelling and cytochrome c release have also been reported in isolated mitochondria with other stimuli (28, 29, 37, 49, 61, 66, 71, 89).

The diversity of molecules inducing the increase of permeability of the IMM suggests that different mechanisms may underlie the effects of these agents. Certain compounds may modify the proteins present in the IMM or induce cluster formation, whereas others may affect membrane integrity or modulate the activity of ion permeability pathways.

The groups of Vercesi (33, 58, 59) and Lemasters (49) have proposed that high concentrations of PTP inducers or ROS generate CsA- and Ca2+-independent “unregulated” pores consisting of clusters of misfolded proteins that are also components of the PTP, such as ANT, in the IMM and would therefore exceed available chaperones, such as cyclophilin D, to regulate pore formation. The great abundance of ANT and VDAC in the IMM and OMM, respectively, would hence explain their involvement in PTP formation.

In addition, recent electrophysiological and molecular biological data suggest that the protein import complex of the IMM and OMM may participate in permeability transition that is CsA independent (64, 65, 73, 99, 116). Differences as well as analogies in the electrophysiological and pharmacological properties of the reconstituted channels to PTP were observed (65, 99). Evidence for the participation of this complex in cell death was provided by downregulating a component of the protein import complex, Tm 50, which resulted in increased cytochrome c release and an increased sensitivity to apoptotic cell death (43).

MITOCHONDRIAL AQUAPORINS: A NOVEL TRANSPORT PATHWAY INVOLVED IN VOLUME REGULATION OF THE MITOCHONDRIAL MATRIX

Aquaporins (AQP) are water-specific membrane channels that are widely distributed in different tissues, cells, and subcellular organelles. Of the known mammalian aquaporin analogs, a recent review by Agre and colleagues (55) listed AQP2, -6, and -8 as being expressed in intracellular vesicles. Additional evidence for expression of AQPs in secretory granules of parotid (76) and Brunner glands (87) has been recently published and the presence of AQP1 in pancreatic zymogen granules has also been postulated (1, 17).

However, only very recently have AQPs been found to be associated with mitochondria, where they may contribute to the regulation of mitochondrial matrix volume. The first immunohistochemical evidence for intracellular localization of AQP8 in rat liver and kidney proximal tubule (PT) was obtained by the group of Sören Nielsen in 2001 (30). Using immunoelectron microscopy, Ferri et al. (32) identified intracellular localizations of AQP8 in mouse liver, namely smooth endoplasmic reticulum, subapical vesicles and some mitochondria. The authors suggested that AQP8 might represent the molecular pathway underlying the osmotic movement of water across the IMM during changes in mitochondrial volume (32). Hence it was speculated that AQP8 may be important for mitochondrial function because this organelle must maintain its volume homeostasis to perform oxidative phosphorylation (44).

Subsequently, our laboratory, while attempting to elucidate the mechanisms underlying Cd2+-induced and PTP-independent osmotic swelling of isolated rat kidney cortex mitochondria, found it to be mediated by AQP8 expressed in the IMM (69). Thereafter, Calamita et al. confirmed the expression of AQP8 in the IMM of rat liver (13) and murine CNS stem cells.
ROLE OF AQPS EXPRESSION IN IMM: LESSONS FROM CADMIUM NEPHROTOXICITY

There is a general agreement that a hallmark of metal-induced toxicity is the formation of ROS (for reviews, see Refs. 103 and 107). By oxidizing membrane proteins and phospholipids, ROS may increase the permeability of plasma and intracellular membranes in a nonspecific manner to various ions, such as Ca²⁺, causing its liberation from intracellular stores and as its influx through the cell membrane, which ultimately results in mitochondrial damage. However, the feature of increased leakage of cell membranes induced by metals is a characteristic of cellular necrosis (74). Apoptosis, on the other hand, is morphologically distinct from necrosis and primarily does not involve leakage of cellular membranes (54, 74). The end point of the cell death process in apoptotic cells is the condensation and fragmentation of chromatin within the nucleus, while the plasma membrane remains intact, preventing leakage of the cytosolic contents into the extracellular space and the influx of extracellular components into the cytosol.

There are several possible signal transduction pathways proposed to be involved in apoptosis execution but it is becoming increasingly apparent that the exact apoptotic pathway activated is dependent on the cell death stimulus (for example, cytokines, death ligands, or formation of ROS), and the cell type (111). Apoptotic pathways can be divided into two main types: the death receptor, or extrinsic, pathway and the mitochondria-dependent, or intrinsic, pathway, which may cross-talk under certain conditions (53, 114). The death receptor pathway involves the binding of a death ligand, such as tumor necrosis factor-α or Fas, to its plasma membrane receptor. This leads to the recruitment of death domains and the direct activation of effector caspases via the initiator caspase-8. Caspases are cysteinyll proteases responsible for the cleavage of intracellular substrates leading to apoptosis (27). The mitochondria-dependent pathway may be induced by toxic compounds, including metals, and may also entail ROS formation and/or increase of cytosolic Ca²⁺ resulting in the opening of the PTP and the release of death-promoting factors from the mitochondrial IMS, which cause apoptosis in caspase-dependent and -independent manners. For example, cytochrome c release leads to the formation of the apoptosome complex resulting in caspase activation (72), but the liberation of AIF (101), a mitochondrial flavoprotein with oxidoreductase activity, from the IMS causes caspase independent DNA fragmentation (93). Despite the differing upstream pathways, it appears there is a convergence of the extrinsic and intrinsic pathways onto a common downstream mechanism, namely mitochondrial dysfunction (24, 63) and the activation of caspases.

Thus it becomes obvious that the signaling pathways for apoptosis and necrosis do not appear to be as specific for each type as originally thought; rather, there seems to be overlapping events which occur in both cell death types, e.g., increase of cytosolic Ca²⁺ and ROS formation. Whether the same stimulus induces apoptotic or necrotic cell death, may depend on its concentration and/or exposure time and hence on the magnitude of the damaging intracellular signal. Thus extreme care has to be taken to correctly decipher which steps are apoptotic and which are necrotic (112).

It is well established that Cd²⁺ has apoptosis inducing capacity in vitro (105) as well as in vivo (50) in kidney PT cells. Cd²⁺ induced apoptosis, however, appears to be a more complex process than expected which involves several time- and concentration-dependent signaling pathways. Low micromolar Cd²⁺ concentrations induce formation of ROS and apoptosis within 6–8 h (104). Interestingly, apoptosis at early time points is not associated with mitochondrial damage, release of cytochrome c, and AIF and caspase activation (68). Rather, apoptosis appears to be mediated by activation of calpains, another ubiquitously expressed family of cytosolic Ca²⁺-dependent cysteine proteases that are involved in the proteolytic degradation of cells undergoing apoptosis (14, 39). However, as the cytosolic Cd²⁺ concentration rises over time (31), other apoptotic pathways come into play: at 24 h, mitochondrial damage occurs concomitantly with the release of cytochrome c and AIF and caspase activation (68). At higher Cd²⁺ concentrations, toxicity also becomes associated with increased rates of necrotic cell death (68, 69).

To understand why ROS formation did not cause mitochondrial damage at early time points, we investigated the effect of micromolar Cd²⁺ concentrations on isolated kidney cortex mitochondria suspended in isotonic mannitol/sucrose/HEPES buffer. In the absence of PTP inducers, intact mitochondria did not exhibit net osmotic changes. Surprisingly, low micromolar Cd²⁺ concentrations blocked mitochondrial swelling and cytochrome c release elicited by activation of the PTP with 5 mM PO₄³⁻ or 2 mM H₂O₂ + 50 μM Ca²⁺ (69). These compounds are typical inducers of PTP (46) and mimic cellular toxicity caused by hypoxia or ROS formation. In contrast, micromolar Cd²⁺ concentrations alone triggered osmotic swelling and release of cytochrome c (69). Noticeably, the magnitude of mitochondrial swelling triggered by Cd²⁺ (10–15%) was smaller than that elicited by the PTP inducers (~40–50%) (70). Moreover, neither CsA nor bongkrekic acid affected Cd²⁺-induced swelling, which argues against the involvement of a classic PTP in this process. This excluded the PTP as the mechanism responsible for Cd²⁺-induced swelling of kidney cortex mitochondria. In a series of experiments, we demonstrated that Cd²⁺ enters the mitochondrial matrix and that the uptake pathway for Cd²⁺ uptake into mitochondria is the mitochondrial Ca²⁺ uniporter because ruthenium red or Ru₃60, two classic inhibitors of the MCU (10), blocked osmotic swelling and cytochrome c release induced by Cd²⁺. We concluded that for swelling to occur, Cd²⁺ must enter the matrix to affect a permeability pathway for H₂O and/or solutes.
Mannitol and/or sucrose permeability pathways in the IMM have not been described in the literature so far and the osmolality of Cd\(^{2+}\) entering the mitochondrial matrix through the MCU is too small to explain the observed magnitude of mitochondrial swelling (10–15% absorbance change). We reasoned that the only likely alternative mechanism was the activation of a hydraulic permeability pathway. Hence, to account for Cd\(^{2+}\)-induced osmotic swelling and H\(_2\)O flux into the mitochondrial matrix under these experimental conditions, the only reasonable guess was to postulate the presence of H\(_2\)O-permeable channels, such as AQPs, that would be activated by Cd\(^{2+}\). As a matter of fact, AQP8 was found to be expressed in the IMM. With the use of a rat AQP8 antibody, a weak band of \(~28\) kDa was detected in rat kidney cortex homogenate that was enriched in mitochondria and highly expressed in mitoplasts (69). With the use of AgNO\(_3\), a more potent inhibitor of aquaporins than Hg\(^{2+}\) (84), Cd\(^{2+}\)-induced swelling of mitoplasts was abolished (69). Interestingly, the kinetics of swelling induced by Cd\(^{2+}\) and inhibited by AgNO\(_3\) was slow and in the range of seconds to minutes, suggesting a low hydraulic permeability.

The slow kinetics of osmotic swelling induced by Cd\(^{2+}\) in kidney cortex mitochondria could reflect a differential sensitivity of various mitochondrial subpopulations to the effect of Cd\(^{2+}\) that may be caused by differences in expression levels of AQP8 in these mitochondrial subpopulations. The smaller magnitude of swelling elicited by Cd\(^{2+}\) compared with that observed with PTP inducers may also indicate that separate mitochondrial subpopulations are affected by PTP inducers respective by activation of AQP8 and that these subpopulations also differ in size. This is also suggested by the study of Calamita et al. (13), who found that larger mitochondria expressed more AQP8 than smaller ones and that subpopulations of mitochondria also exist, which do not express AQP8 at all.

Hence, we propose the following scenario to account for a role of mitochondrial AQPs in Cd\(^{2+}\)-induced apoptosis of kidney PT cells. Initially, Cd\(^{2+}\) uptake activates calpains, which causes apoptosis directly and/or indirectly. Simultaneously, ROS formation occurs that probably is largely buffered by the antioxidant defense mechanisms of the cells (such as glutathione or ROS-metabolizing enzymes). Moreover, Cd\(^{2+}\) prevents ROS-induced PTP formation, mitochondrial swelling, and release of proapoptotic factors. Once the concentration of Cd\(^{2+}\) exceeds a certain threshold, it directly triggers activation of AQP8 in a subpopulation of mitochondria that release cytochrome \(c\) and AIF and activate caspase-dependent apoptosis. Only at high cytosolic Cd\(^{2+}\) concentrations does the magnitude of ROS formation exceed the protective mechanisms of the cell and result in nonspecific damage of cellular membranes and necrotic cell death.

It is unclear whether the process of Cd\(^{2+}\) activation of AQP8 is irreversible. Our data can only indicate that Cd\(^{2+}\) must act from the matrix space (69). One can speculate that the mechanism of Cd\(^{2+}\)-induced activation of AQP8 occurs by binding to cysteine residues in the vicinity of the aqueous pore that are important for channel function. But because AQP8 is inhibited by Hg\(^{2+}\) at Cys\(^{210}\) (60, 90), this suggests that this residue does not mediate AQP8 activation by Cd\(^{2+}\). Furthermore, inhibition of AQP8 by Hg\(^{2+}\) means that it is unlikely that this AQP isoform is involved in Hg\(^{2+}\) toxicity.

PERSPECTIVES

Little is known about the mechanisms affecting AQP8 channel gating. Further investigations need to be carried out in this direction because mitochondrial AQP8/9 channel gating may have an important role in the regulation of the permeability of the IMM. In contrast, mechanisms affecting the gating behavior have been identified in other members of the AQP family, which may possibly involve phosphorylation, heteromerization, pH, Ca\(^{2+}\), pressure, solute gradients, and temperature (15, 55).

The study describing Cd\(^{2+}\)-induced osmotic swelling of kidney cortex mitochondria indicates that Cd\(^{2+}\) induces AQP8 gating by binding to a domain facing the matrix (69). Interestingly, Pb\(^{2+}\) also increases the H\(_2\)O permeability of astrocytes expressing AQP4 in their plasma membranes (41). Hence, divalent metals, such as Cd\(^{2+}\) or Pb\(^{2+}\), may interact with a Ca\(^{2+}\) binding site of AQPs. Ca\(^{2+}\) binding sites have been identified at the carboxy-terminus of AQP1 (34) and changes in cytosolic Ca\(^{2+}\) are known to modulate AQP0 water permeability (82). Hence it can be speculated that an increase of the Ca\(^{2+}\) concentration of the mitochondrial matrix, besides stimulating oxidative phosphorylation by direct interaction with dehydrogenases of the TCA cycle and sites in the electron transport chain (6, 18, 23), also induces swelling of the matrix that would be mediated by gating of water channels. Gating of AQPs would therefore participate in the physiological adaptation of mitochondrial function to the varying demands of the cell. In other words, AQP8 would be relevant in facilitating the increase in volume, which the mitochondrion undergoes when its ATP synthesis efficiency increases. Thus the rapidity of AQP8-mediated vesicle shrinkage observed in the study by Calamita and coworkers (13) suggests that mitochondria may well be able to adapt to rapid physiological demands of oxidative phosphorylation induced by local changes in cellular metabolism. Such considerations, however, are speculative and need to be tested in the future.

On the other hand, under pathological conditions, AQPs in mitochondrial subpopulations may cause swelling of these organelles independently of the PTP, resulting in rupture of the OMM and release of cytochrome \(c\) and other key proapoptotic factors into the cytoplasm leading to apoptosis. This possibility is corroborated by our recent study (69) demonstrating that opening of AQP8 expressed in the IMM of kidney cortex mitochondria may contribute to cytochrome \(c\) and AIF release from mitochondria and eventually lead to apoptosis of kidney PT cells. Additional evidence for a role of AQP8 in apoptotic processes is provided by Calamita et al. (13), who showed the absence of AQP8 in mitochondria of immortalized hepatocytes. Consequently, in the light of a likely role of mitochondrial AQPs in proapoptotic processes induced by Cd\(^{2+}\), it may be worthwhile to reinvestigate experimental conditions, in which CsA-insensitive and PTP-independent mitochondrial swelling and/or apoptosis occur.

In summary, the recent discovery that AQP8/9 are expressed in the IMM (4, 13, 69) opens up exciting avenues in the investigation of new mechanisms involving mitochondrial volume changes in health and disease. Moreover, mitochondrial AQPs should be considered as specific therapeutic targets for various pathophysiological conditions, in which cell death is associated with mitochondrial swelling and damage.
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REFERENCES


4. Amiry-Moghaddam M, Lindland H, Zelenin S, Roberg BA, Gun-
dersen BB, Petersen P, Rinvik E, Torgner IA, and Ottersen OP. Brain mitochondria contain aquaporin water channels: evidence for the expression of a short AQP9 isoform in the inner mitochondrial mem-


9. Belosludtsev KN, Belosludtseva NV, and Mironova GD. Possible mechanism for formation and regulation of the palmitate-induced cyclo-


12. Belosludtsev KN, Belosludtseva NV, and Mironova GD. Possible mechanism for formation and regulation of the palmitate-induced cyclo-

13. Balaban RS, Bose S, French SA, andTerrito PR. Role of calcium in metabolic signaling between cardiac sarcoplasmic reticulum and mito-


19. Chaumont F, Moshelion M, and Daniels MJ. Multiprotein complex containing succinate dehydrogenase confers mito-


22. Crompton M. Aqp9 is highly expressed in the inner mitochondrial mem-


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