
MINIREVIEW

Mitochondrial Membrane Potential in Cardiac Myocytes

L. ŠKÁRKA, B. OŠŤÁDAL

*Institute of Physiology, Academy of Sciences, Centre for Experimental Cardiovascular Research,
Prague, Czech Republic*

Received January 9, 2002

Accepted March 25, 2002

Summary

Mitochondria are involved in cellular functions that transcend the traditional role of these organelles as the energy factory of the cell. Their relative inaccessibility and the difficulties involved in attempts to study them in their natural environment – the cytosol – has delayed much of this understanding and they still have many secrets to yield. One of the relatively new fields in this respect is undoubtedly the analysis of mitochondrial membrane potential. The realization that its alteration may have important pathophysiological consequences has led to an increased interest in measuring this variable in a variety of biological settings, including cardiovascular diseases. Measurements of mitochondrial membrane potential tell us much about the role of mitochondria in normal cell function and in processes leading to cell death. However, we must be aware of the limitations of using isolated mitochondria, single cells and different fluorescent indicators.

Key words

Heart mitochondria • Membrane potential • Hypoxia

Hypoxic states of the cardiovascular system are undoubtedly associated with the most frequent diseases of modern times. They result from disproportion between the amount of oxygen supplied to the cardiac cell and the amount actually required by the cells. The degree of hypoxic (ischemic) injury depends not only on the intensity and duration of the hypoxic (ischemic) stimulus, but also on the level of cardiac tolerance to oxygen deprivation. This variable is determined by myocardial blood flow and oxygen transporting capacity of the blood on the one hand, and the functional (level of contractile function, systolic wall tension, heart rate, external work) and metabolic state of the cardiac muscle on the other.

The myocardial tissue is typically aerobic and its metabolism is closely dependent upon oxygen availability, as confirmed by the abundance of mitochondria (30 % of total cell volume) and myoglobin. The high-energy requirements of contraction are met almost exclusively by mitochondrial oxidative phosphorylation. This, in turn, leads to the high sensitivity of myocardial cells to oxygen deficiency. Unfortunately, since the award of the Nobel Prize for chemistry to Peter Mitchell for his formulation of the chemiosmotic theory of oxidative phosphorylation (Mitchell and Moyle 1967), most physiologists have regarded mitochondria as "solved". They have been

considered as divorced from those aspects of physiology that are deemed exciting and significant, and dismissed as irrelevant structures, whose sole function is to manufacture ATP unobtrusively and subservient to the energetic demands of the cell when necessary (Duchen 1999). Over the last few years, however, mitochondria re-emerged into the spotlight of experimental cardiologists: their interest in the potential role of mitochondria in the pathogenesis of cardiac diseases, particularly of ischemia and reperfusion, has markedly increased (for review see Lesnefsky *et al.* 2001). Moreover, we can see an enormous resurgence of interest in the role of mitochondria within cells. Recognition that, besides their traditional role as "powerhouses" of the cell in generating ATP, mitochondria play an important role in other aspects of normal cell functioning, e.g. in calcium signaling in the cardiac cell. But possibly the greatest interest has been in the emerging role of mitochondria as regulators of cell life - cell death transition, in both necrotic and apoptotic forms of cell death (Griffiths 2000). The normal performance and survival of cardiac cells that have high-energy requirements depend on the maintenance of the mitochondrial membrane potential (MMP). Measurement of MMP is therefore essential for extending the understanding of molecular mechanisms controlling cardiomyocyte function (Mathur *et al.* 2000). The present review briefly summarizes our knowledge of the structure and function of cardiac mitochondria and draws attention to the recent developments in the study of the role of MMP in cardiac mitochondria under normal and pathological conditions.

Cardiac mitochondria

Structure

Mitochondrial structure provides compartmentation of mitochondrial metabolism (for review see Lesnefsky *et al.* 2001). The outer membrane encapsulates the organelle, while the inner membrane surrounds the central matrix space of the mitochondrion. The outer membrane represents a permeability barrier to cytosolic molecules larger than 1500 Da and separates the intermembrane space from the cytosol. The intermembrane space has an ionic composition similar to that of cytosol, and contains a distinct group of proteins, including cytochrome *c*. The inner mitochondrial membrane consists of regions of inner boundary membrane that are parallel to the outer membrane, and regions invaginating into the matrix as cristae. These

infoldings of the inner membrane are of various shapes: in some mitochondria, the cristae are tubular and in some cases, the infoldings are longitudinal rather than lateral. The cristae include the electron transport chain, the phosphorylation apparatus, and transporter proteins. The inner boundary membrane participates in transport reactions, including the formation of contact sites. Contact sites are dynamic structures that involve fusion of the inner and outer mitochondrial membranes and are key participants in protein import, energy coupling with the cytosol *via* formation of creatine phosphate, and uptake of fatty acids of oxidative metabolism. Their number varies according to the intensity of oxidative metabolism with an increased number present in actively respiring mitochondria. The mitochondrial matrix space contains metabolic enzymes, mitochondrial DNA (mtDNA), and RNAs (ribosomal and transfer RNA). This genome has only a restricted set of genes coding for some proteins involved in oxidative phosphorylation.

Cardiac mitochondria exist in two functionally distinct populations: subsarcolemmal mitochondria (SSM) residing beneath the plasma membrane and interfibrillar mitochondria (IFM) located between the myofibrils (Palmer *et al.* 1977). The ADP-stimulated respiratory rates (state 3) are greater in IFM than in SSM, whereas the coupling of respiration is similar in both populations. IFM have an increased content of respiratory cytochromes, and the activity of ETC complexes is greater in IFM than in SSM (Palmer *et al.* 1985). Differences in respiratory rates and enzyme contents persist following exposure of each population to the methods used to isolate the other population. A distinct structural marker for each population has not been identified (Škárka *et al.* 2000). However, the two populations are affected differently in pathological states, including calcium overload (Palmer *et al.* 1986), cardiomyopathy (Hoppel *et al.* 1982), aging (Fannin *et al.* 1999), and ischemia (Piper *et al.* 1985). Thus, consideration of regional differences in the mitochondrial response to disease is required in order to identify novel mitochondrial defects present in various pathophysiological states. Ultrastructural studies (Lawrie *et al.* 1996) suggest that the cellular organization of mitochondria may be arranged in relation to cell function; while mitochondria may be juxtaposed to endoplasmic reticulum in nonexcitable cells, they are more likely to lie close to the plasmalemma in excitable cells. However, how this organization might be controlled is still not clear.

Function

Mitochondria are the primary consumers of oxygen and it could be argued that mitochondrial requirement for oxygen delivery has driven the evolution of the respiratory and cardiovascular system. Mitochondria are primarily ATP generators. This is far from trivial: ATP is the major currency in the energy economy in all living things, from bacteria to plants and humans. It is necessary for the phosphorylation reactions that modulate so many fundamental cellular processes. It may be stored and used as a transmitter and it controls the activity of several classes of ion channel (such as the ATP-sensitive K channel (mitoK_{ATP}), the calcium-release channel of the sarcoplasmic reticulum and voltage-gated calcium channels). Mitochondrial energy production may also be diverted from ATP synthesis to the generation of heat through the expression of catecholamine-regulated uncoupling proteins (UCP) in brown fat in neonates and in hibernating mammals (Palou *et al.* 1998, Baumruk *et al.* 1999). It has been suggested that the presence of UCP on the inner mitochondrial membrane changes its proton conductivity and may thus influence MMP; UCP-induced decrease of MMP may lower ATP synthesis and generate heat. These proteins may be involved not only in the control of energetic and lipid metabolism but also in the control of production of free radical species by the respiratory chain (Boss *et al.* 1998). The heart and skeletal muscle express genes of at least three different UCPs (UCP2, 3, 5). UCP2 and 3 probably function as protonophores (Echtay *et al.* 2001). UCP3 and 5 were shown to lower the mitochondrial membrane potential when overexpressed in cultured human muscle (Garcia-Martinez *et al.* 2001) or kidney (Yu *et al.* 2000) cells (for review see Ricquier and Bouillaud 2000). However, the UCP function is not yet clear.

Carbohydrates and fats are the principal substrates for mitochondrial oxidation. Carbohydrate metabolism generates pyruvate for mitochondrial uptake. Triglyceride hydrolysis or myocyte uptake of fatty acids provides acyl groups for mitochondrial activation and uptake. Mitochondrial substrate selection is tightly controlled in response to exogenous substrate availability and the physiological state of myocytes. Pyruvate metabolism requires uptake by the pyruvate transporter followed by oxidation by pyruvate dehydrogenase to acetyl-CoA. The enzymes involved in fatty acid activation and the transporter are located in the outer membrane and contact sites. Fatty acid oxidation requires activation of fatty acids by long-chain-fatty-acid-CoA ligase, followed by formation of acylcarnitines by

carnitine palmitoyltransferase-I (CPT-I) (Brdiczka *et al.* 1990).

The matrix space contains the enzymes of the tricarboxylic acid (TCA) cycle, biosynthetic enzymes, and antioxidant defense enzymes. TCA cycle oxidation of acetyl-CoA generates NADH and FADH₂ for oxidation by the ETC. The ETC consists of four multi-subunit enzyme complexes and the mobile electron carriers, coenzyme Q (inner membrane) and cytochrome c (intermembrane space), that pass electrons sequentially from high NADH or FADH₂ to low (molecular oxygen) redox potential. The multi-subunit complexes of electron transport chain (ETC) diffuse individually in the semi-fluid inner membrane cristae. The subunit composition, 3-dimensional structure, and structure-function relationships of the ETC complexes I, II, III, and IV (cytochrome oxidase) have been extensively studied and reviewed (Lesnefsky *et al.* 2001).

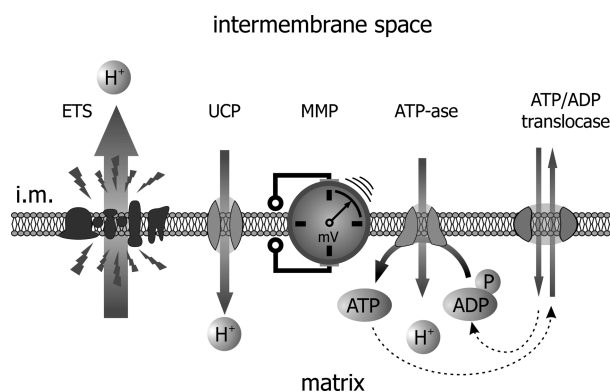


Fig. 1. Principle of generation of MMP in mitochondria. Electron transport system (ETS) pumps protons across the inner mitochondrial membrane (i.m.) and thus generates membrane potential (MMP); this proton gradient is necessary for conversion of ADP to ATP (ATP synthase). ATP is transported into the matrix in exchange with ADP by ADP/ATP translocase. The presence of uncoupling protein (UCP) on the inner membrane may decrease MMP in expense of heat instead of ATP generation.

Mitochondria also take up calcium and are functionally tightly integrated into the mechanism of cellular calcium signaling (for review see Duchen 1999). Isolated mitochondria will accumulate huge amounts of Ca²⁺ in the presence of inorganic phosphate. It now seems likely that the major consequence of the Ca²⁺ uptake in terms of mitochondrial function is the up-regulation of energy metabolism. This makes good sense since

oxidative phosphorylation is modulated in relation to the energy demand by the Ca^{2+} signal as the increased energy demand is almost always accompanied by a rise in $[\text{Ca}^{2+}]$, e.g. for contraction. It has been suggested that mitochondria may participate in the active propagation of a $[\text{Ca}^{2+}]$ signal across the cell through a process of Ca^{2+} -induced Ca^{2+} release (Ichas and Mazat 1998).

Mitochondrial membrane potential

Definition

The supply of energy by the mitochondrion depends on the maintenance of the chemiosmotic gradient across its inner membrane (Mitchell 1979). This gradient, also known as the proton motive force, is generated by three respiratory enzyme complexes which use the free energy released during electron transport to translocate protons from the mitochondrial matrix into the intermembrane space (Fig. 1). Proton motive force has two components: the membrane potential (MMP), which arises from the net movement of positive charge across the inner membrane and the pH gradient. Of these two components, MMP contributes most of the energy stored in the gradient, typically 150 mV. Hence, for practical purposes, MMP may be used on its own as an indicator of the energization state of mitochondria (Mathur *et al.* 2000).

Changes in MMP are integral to cell life - death transition although the answer whether as a primary cause or a secondary event is not yet known. In normal cell function, the maintenance of MMP is essential for ATP synthesis. MMP is highly negative, approximately -180 mV, due to the chemiosmotic gradient of protons across the inner mitochondrial membrane, the energy of which is used for ATP synthesis by the respiratory chain. MMP also provides the driving force for Ca^{2+} uptake into mitochondria by the Ca^{2+} -uniporter and it is now generally accepted that it is the Ca^{2+} signal in the mitochondria that stimulates ATP production in response to an increased energy demand by the cell (Hansford and Zorov 1998).

Measurement of mitochondrial membrane potential

Major advances in this field came with the development of fluorescent indicators, which could be localized into the mitochondrial compartment. The most commonly used fluorescent indicators for measuring MMP either in single cells or in isolated mitochondria are rhodamine 123, its derivatives tetramethylrhodamine

methyl and ethyl esters (TMRM and TMRE), and carbocyanine JC-1. These compounds accumulate in the mitochondrial matrix because of their charge and solubility in both the inner mitochondrial membrane and matrix space. In many cases, the distribution of the free dye across the inner membrane has been shown to follow the Nernst equation (Scaduto and Grotyohann 1999). However, binding of the fluorescent probes to both inner and outer mitochondrial membranes may cause deviations from the predicted theoretical behavior (Scaduto and Grotyohann 1999), and result in enhanced apparent uptake which makes calculations of exact MMP unreliable. Hence, the results are usually compared with those obtained in the presence of a mitochondrial uncoupler, such as FCCP, to attain maximum depolarization. The rhodamines are single-excitation, single-emission dyes, whereas JC-1 emits light at red and green wavelengths according to its concentration: at high concentrations, J-aggregates form and emit red light, whereas at low concentrations the monomer form emits green light (Griffiths 2000). Several criteria must be met for these probes to be useful in the estimation of MMP: they should not cause loss of mitochondrial function and/or depletion of MMP and the compound must be easily detected to estimate the distribution across the inner membrane. Mathur *et al.* (2000) have compared and re-evaluated the use of the dyes in neonatal cardiomyocytes using confocal microscopy of individual cells, and, for the first time, flow cytometry of cell cultures. The study of MMP by flow cytometry has several advantages over other techniques. In particular, it allows the analysis of heterogeneous cell populations and is amenable to multiparametric measurements. Mathur *et al.* (2000) concluded that JC-1 is the best dye for detecting changes in MMP; however, they questioned the use of JC-1 as a ratiometric indicator, and found that much information could be gained by studying changes in the fluorescence of the individual wavelengths.

Changes of mitochondrial membrane potential under physiological and pathological conditions

Mitochondrial permeability transition pore

Certain pathological conditions (e.g. oxidative stress, ATP depletion, Ca^{2+} overload of mitochondrial matrix and elevated mitochondrial matrix pH), and possibly also physiological processes, can trigger a mitochondrial permeability transition pore (PTP)

(Heinrich and Holz 1998), where a nonspecific pore opens in the mitochondrial membrane (Griffiths 2000). This is a pore with a large maximal conductance (12 000 pS) but with multiple subconductance states, and an estimated pore diameter of 2 nm (Crompton and Costi 1990), apparently concentrated at the contact sites between the mitochondrial inner and outer membranes. First discovered by the observation of Ca^{2+} -induced mitochondrial swelling (Haworth and Hunter 1979), this phenomenon was intensively studied for many years in a small number of laboratories before emerging into prominence recently through the proposal that it may play a role in apoptosis, although whether as an initial or a late event is under debate. According to Griffiths (2000), collapse of MMP itself does not necessarily indicate that the PTP has occurred and this, together with the use of nonspecific inhibitors of the PTP (such as cyclosporin A), may partly explain the conflicting data in this area.

Maintenance of MMP is fundamental for the normal performance and survival of cells that have a high-energy requirement, such as beating cardiomyocytes. Furthermore, the collapse of MMP due to the opening of a high-conductance pore in the inner mitochondrial membrane has been implicated in the molecular mechanism associated with ischemic injury to the heart. Therefore, the development of reliable methods to evaluate MMP in cardiomyocytes is of high relevance for cardiovascular research (Mathur *et al.* 2000).

MMP and mitochondrial antioxidant defense

Mitochondria represent the most important intracellular source of reactive oxygen species (ROS), although alternative sources in other organelles and the cytoplasm are also present in cells. Based upon a large body of experimental evidence, reactive oxygen species appear to participate in aging and aging-associated pathology. In the mitochondria and the cytosol, both enzymatic and non-enzymatic systems are available, which serve to decrease the reactive oxygen species steady-state concentration. It has been shown that a very important factor involved in the regulation of ROS production is MMP. During normal respiratory conditions, 2-4 % of the electron flow through the ETC only partially reduces O_2 , thus generating superoxide (Turrens and Boveris 1980, Turrens 1997). At higher oxygen concentrations, the diminished availability of reduced cofactors of the respiratory chain and a high MMP tend to increase this mitochondrial radical formation, which is substantially enhanced in the presence of defects within the respiratory chain (Turrens

1997). Mitochondria respond to this radical-induced oxidative stress with a defined antioxidant defense system: enzymatic antioxidant systems (mitochondrial superoxide dismutase, the glutathione redox system and mitochondrial catalase) (Radi *et al.* 1991). If the enzymatic scavengers are exhausted, another more potent mechanism takes place: mild uncoupling mediated by uncoupling proteins (Skulachev 1998). In this process, membrane conductance rises slightly, resulting in some dissipation of MMP without influencing ATP synthesis, but with a marked decrease of radical formation. Korshunov *et al.* (1998) showed that a 15 % decrease in MMP under resting conditions caused by an uncoupler, a respiratory inhibitor or oxidative phosphorylation substrates ($\text{ADP}+\text{P}_i$) results in a 10-fold decrease in the H_2O_2 production by heart mitochondria. During prolonged oxidative stress in a situation when a certain MMP decline is reached due to mild uncoupling, a reversible opening of the permeability transition pore (PTP) can occur. This process increases the permeability of the inner mitochondrial membrane for solutes up to 1 500 Da and results in a much greater decrease of MMP than during the previous steps. Further prolongation of oxidative stress usually results in an irreversible PTP opening when MMP is completely dissipated and oxidation is uncoupled from phosphorylation (ATP synthesis stops). The next step is the starting point of an apoptotic process. If a sufficient number of mitochondria follow this path and release enough cytochrome c, the interplay between cytochrome c, ATP, intracellular Ca^{2+} , apoptotic protease-activating factor-1 (APAF-1) and other factors can finally activate the caspase cascade, which eventually leads to the death of radical-producing cells – this can be interpreted as the final step in the antioxidant defense system (Fig. 2).

MMP in ischemia/reperfusion

Shorter periods of ischemia are characterized by reversible ultrastructural changes, such as slight mitochondrial swelling. Depending on the duration and intensity, ischemia will initiate more severe ultrastructural alterations, causing an irreversible phase of myocardial injury. This period is characterized by swollen mitochondria with rounded configuration, clear matrix, and fragmentation of cristae. External membranes appear to be occasionally damaged and two types of amorphous densities in mitochondrial matrix can be observed: large rounded and finger-shaped amorphous densities of different length. Several cristae in afflicted mitochondria make a close position of their membranes

resulting in an increased electron density (Feuvray 1981, Hegstad *et al.* 1999). The onset of reperfusion is characterized by the development of crystal adhesions, which are recognized as electron-dense, rod-shaped,

pentalaminar condensed profiles located in the intracristal compartment. Both the frequency and distribution of crystal adhesions increase during reperfusion (Hegstad *et al.* 1999).

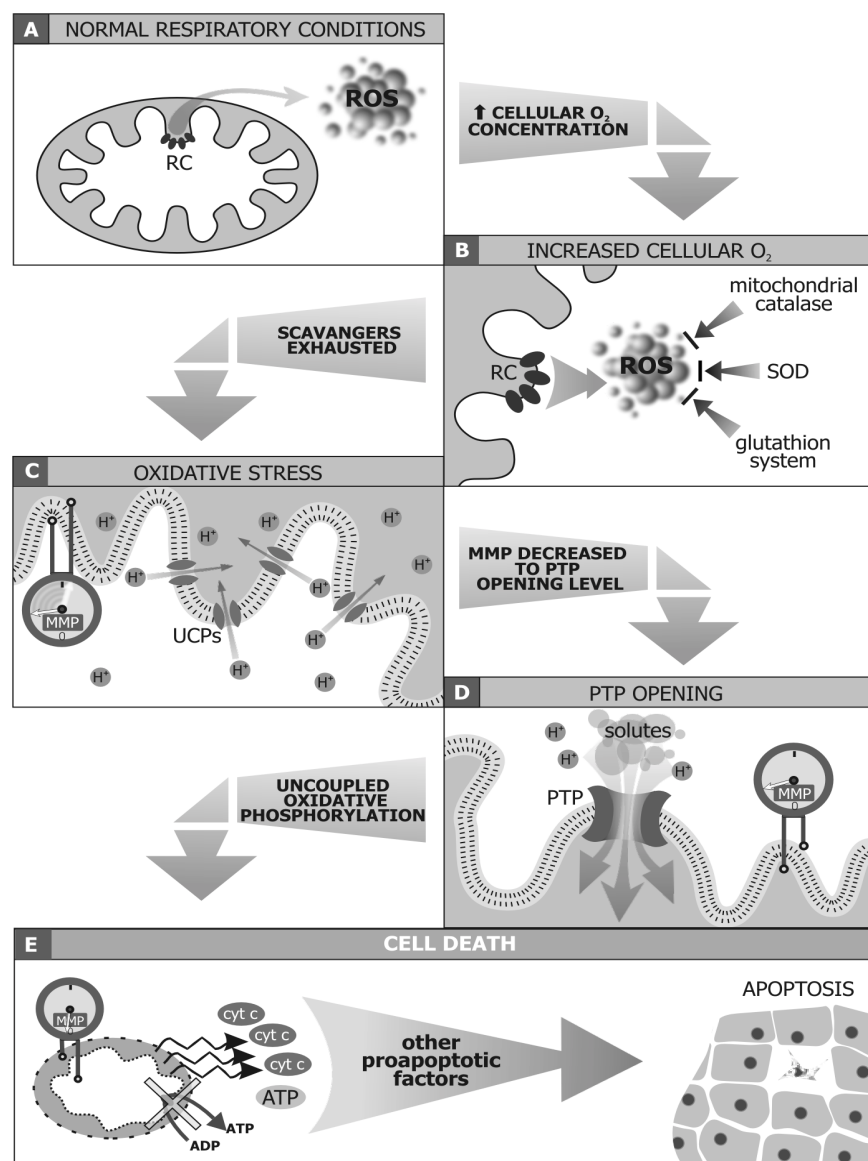


Fig. 2. Mitochondrial defense cascade against radical-induced oxidative stress. **A:** Under normal conditions, respiratory chain (RC) produces 2-4 % of reactive oxygen species (ROS) from an electron flux. **B:** Higher cellular oxygen concentration and a high membrane potential cause increase of ROS formation. The first steps of the mitochondrial defense cascade are enzymatic radical scavengers. **C:** If the scavengers are exhausted, the increased oxidative stress is suggested to result in a slight increase of mitochondrial inner membrane proton conductance in a process called mild uncoupling. The result is a small dissipation of mitochondrial membrane potential (MMP) not affecting the ATP synthesis. **D:** If a certain MMP decline is reached under oxidative stress due to mild uncoupling, a reversible opening of permeability transition pore (PTP) follows. **E:** Ongoing oxidative stress is assumed to result in an irreversible permeability transition causing a complete breakdown of MMP, uncoupling of ADP phosphorylation

from oxidation of substrates and hydrolysis of all available ATP. Factors involved in the induction of programmed cell death, such as cytochrome *c* (cyt *c*) are released from mitochondria and together with other proapoptotic factors (ATP, APAF-1) activates procaspase 9. The final step in the mitochondrial anti-ROS defense cascade is ROS producing cell removal by way of apoptosis.

Ischemia/reperfusion-induced structural changes of cardiac mitochondria are accompanied by an alteration of mitochondrial function. It has been repeatedly confirmed that even a partial decrease in the membrane potential value caused by a relatively small decrease of membrane resistance, dramatically changes all mitochondrial functions. In such a case, uncoupling of respiration and phosphorylation seems to be the most

frequent consequence because e.g. a twofold decrease in the proton potential is sufficient to stop the highly endergonic reaction of ADP phosphorylation by inorganic phosphate (Skulachev 1999).

It has been proposed that inappropriate PTP opening during ischemia/reperfusion injury might represent a key event in the ensuing tissue damage. In fact, dissipation of the MMP caused by PTP opening

could well result in a massive and abrupt release of accumulated Ca^{2+} into the cytosol, leading to cell death. Immediately at the onset of anoxia, only a limited reduction of MMP occurs before the asystole. Then, after the failure of contraction, ATP content falls as showed by a gradual increase in $[\text{Mg}^{2+}]$ which reaches a plateau at the onset of rigor. Consequently, the complete collapse of MMP would follow the glycolytic failure and ATP depletion after glycogen exhaustion. It is obvious that an active mitochondrial ATPase is necessary for the maintenance of MMP. MMP is not modified by the prolongation of anoxia following rigor (Di Lisa and Bernardi 1998) (Fig. 3). Most damage to the heart under the condition of severe ischemia/reperfusion occurs by necrosis but there is evidence for cell death by apoptosis (Haunstetter and Izumo 1998, for review see Ošťádal and Kolář 1999). Recently, it has been observed that hypoxia-induced PTP opening and loss of MMP is associated with cytochrome c release and apoptosis of ventricular myocytes (Gurevich *et al.* 2001). Similarly, reoxygenation after anoxia produced a variable but often profound loss of MMP, which mediated the release of cytochrome c from the mitochondria, which again leads to apoptosis (Adams *et al.* 2000, Xu *et al.* 2001, Gurevich *et al.* 2001).

The feasibility of PTP opening cannot be predicted easily by considering the changes which characterize the ischemic myocardium. In fact, a complete picture of cytosolic and mitochondrial components is complicated by the coexistence of factors that can either promote or reduce the probability of pore opening. According to Di Lisa and Bernardi (1998), ischemia-induced calcium overload, mitochondrial depolarization, increase in cytosolic P_i , increased long-chain-acyl-CoA content, and oxygen radicals belong to this group of inducing factors. On the other hand, the opening of PTP is reduced by a decrease in intracellular pH, an increase of free cytoplasmic ADP, and an increase of intracellular Mg^{2+} during ischemia. These authors concluded that although occurrence of permeability transition in ischemic damage is supported by a variety of data, the evidence in favor of its causative role in ischemia-reperfusion is still not sufficient.

MMP in cardiac protection

In this connection, it is necessary to mention the possible role of MMP in cardiac protection. It has been reported that $\text{mitoK}_{\text{ATP}}$ channel openers exert cardioprotective effects in various animal models of ischemia-reperfusion situations (Garlid *et al.* 1997, Liu

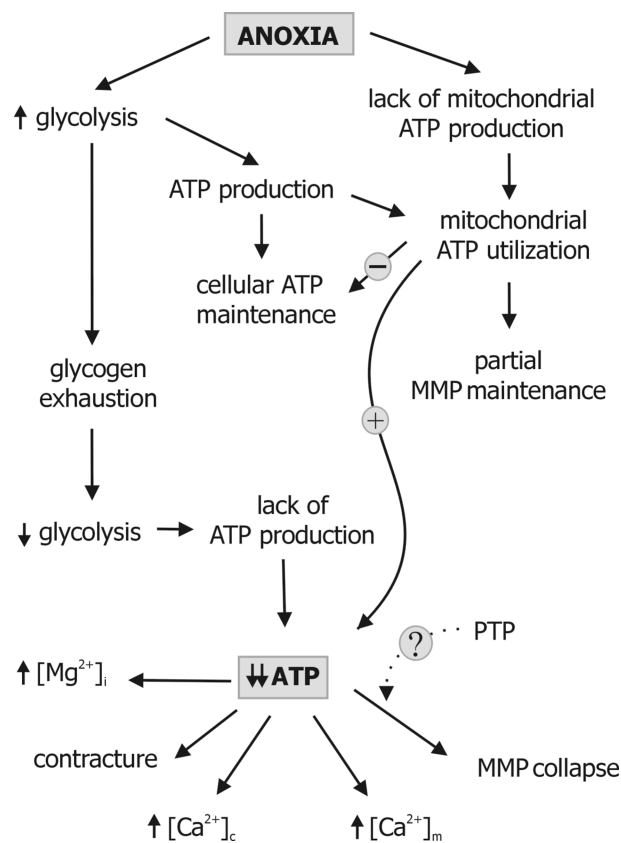


Fig. 3. Sequence of events following anoxia in isolated cardiomyocytes. In the first phase of anoxia no major changes apparently occur, due to the maintenance of cell morphology, function and metabolism by anaerobic glycolysis. However, mitochondria switch from ATP producers into ATP consumers during anoxia; when glycolytic substrates are no longer available, they contribute to a rapid fall of the ATP content, resulting in myocyte contracture and MMP collapse. The prolongation of anoxia after contracture is mostly characterized by a parallel rise in both $[\text{Ca}^{2+}]_c$ (cytoplasmic) and $[\text{Ca}^{2+}]_m$ (mitochondrial).

et al. 1998, Asemu *et al.* 1999). It has been indicated that the $\text{mitoK}_{\text{ATP}}$ channel opener, diazoxide, attenuated ischemia/reperfusion injury due to the preservation of mitochondrial function (Iwai *et al.* 2000). Xu *et al.* (2001) demonstrated that diazoxide stabilized MMP by attenuating the loss of MMP and high polarization observed during ischemia/reperfusion. Stabilization of MMP by activation of $\text{mitoK}_{\text{ATP}}$ channel was accompanied by remarkable recovery of ATP and absence of calcium accumulation in mitochondria. The precise mechanism of the effect of diazoxide on MMP remains, however, unknown. Nevertheless, it may be assumed that the maintenance of mitochondrial function

and structural integrity by diazoxide suggests a potential therapeutic application of mitoK_{ATP} channel agonists in preventing ischemic injury.

Acknowledgement

Supported by grant GAUK 56/2000 and GACR 305/00/1659.

References

- ADAMS JW, PAGEL AL, MEANS CK, OKSENBERG D, ARMSTRONG RC, BROWN JH: Cardiomyocyte apoptosis induced by Gαq signaling is mediated by permeability transition pore formation and activation of the mitochondrial death pathway. *Circ Res* **87**: 1180-1187, 2000.
- ASEMU G, PAPOUSEK F, OSTADAL B, KOLAR F: Adaptation to high altitude hypoxia protects the rat heart against ischemia-induced arrhythmias. Involvement of mitochondrial K_{ATP} channel. *J Mol Cell Cardiol* **31**: 1821-1831, 1999.
- BAUMRUK F, FLACHS P, HORAKOVA M, FLORYK D, KOPECKY J: Transgenic UCP1 in white adipocytes modulates mitochondrial membrane potential. *FEBS Lett* **444**: 206-210, 1999.
- BOSS O, MUZZIN P, GIACOBINO JP: The uncoupling proteins, a review. *Eur J Endocrinol* **139**: 1-9, 1998.
- BRDICZKA D, BUCHELER K, KOTTKE M, ADAMS V, NALAM VK: Characterization and metabolic function of mitochondrial contact sites. *Biochim Biophys Acta* **1018**: 234-238, 1990.
- CROMPTON M, COSTI A: A heart mitochondrial Ca²⁺-dependent pore of possible relevance to re-perfusion-induced injury. Evidence that ADP facilitates pore interconversion between the closed and open states *Biochem J* **266**: 33-39, 1990.
- DI LISA F, BERNARDI P: Mitochondrial function as a determinant of recovery or death in cell response to injury. *Mol Cell Biochem* **184**: 379-391, 1998.
- DUCHEN MR: Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death. *J Physiol Lond* **516**: 1-17, 1999.
- ECHTAY KS, WINKLER E, FRISCHMUTH K, KLINGENBERG M: Uncoupling proteins 2 and 3 are highly active H⁺ transporters and highly nucleotide sensitive when activated by coenzyme Q (ubiquinone). *Proc Natl Acad Sci USA* **98**: 1416-1421, 2001.
- FANNIN SW, LESNEFSKY EJ, SLABE TJ, HASSAN MO, HOPPEL CL: Aging selectively decreases oxidative capacity in rat heart interfibrillar mitochondria. *Arch Biochem Biophys* **372**: 399-407, 1999.
- FEUVRAY D: Structural, functional, and metabolic correlates in ischemic hearts: effects of substrates. *Am J Physiol* **240**: H391-H398, 1981.
- GARCIA-MARTINEZ C, SIBILLE B, SOLANES G, DARIMONT C, MACE K, VILLARROYA F, GOMEZ-FOIX AM: Overexpression of UCP3 in cultured human muscle lowers mitochondrial membrane potential, raises ATP/ADP ratio, and favors fatty acid vs. glucose oxidation. *FASEB J* **15**: 2033-2035, 2001.
- GARLID KD, PAUCEK P, YAROV-YAROVY V, MURRAY HN, DARBENZIO RB, D'ALONZO AJ, LODGE NJ, SMITH MA, GROVER GJ: Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K⁺ channels. Possible mechanism of cardioprotection. *Circ Res* **81**: 1072-1082, 1997.
- GRIFFITHS EJ: Mitochondria - potential role in cell life and death. *Cardiovasc Res* **46**: 24-27, 2000.
- GUREVICH RM, REGULA KM, KIRSHENBAUM LA: Serpin protein CrmA suppresses hypoxia-mediated apoptosis of ventricular myocytes. *Circulation* **103**: 1984-1991, 2001.
- HANSFORD RG, ZOROV D: Role of mitochondrial calcium transport in the control of substrate oxidation. *Mol Cell Biochem* **184**: 359-369, 1998.
- HAUNSTETTER A, IZUMO S: Apoptosis: basic mechanisms and implications for cardiovascular disease. *Circ Res* **82**: 1111-1129, 1998.
- HAWORTH RA, HUNTER DR: The Ca²⁺-induced membrane transition in mitochondria. II. Nature of the Ca²⁺ trigger site. *Arch Biochem Biophys* **195**: 460-467, 1979.
- HEGSTAD AC, YTREHUS K, LINDA S, MYKLEBUST R, MYKLEBUST R, JORGENSEN L: The initial phase of myocardial reperfusion is not associated with aggravation of ischemic-induced ultrastructural alterations in isolated rat hearts exposed to prolonged global ischemia. *Ultrastruct Pathol* **23**: 93-105, 1999.

- HEINRICH H, HOLZ J: Myocardial apoptosis in the overloaded and the aging heart: a critical role of mitochondria? *Eur Cytokine Netw* **9**: 693-695, 1998.
- HOPPEL CL, TANDLER B, PARLAND W, TURKALY JS, ALBERS LD: Hamster cardiomyopathy. A defect in oxidative phosphorylation in the cardiac interfibrillar mitochondria. *J Biol Chem* **257**: 1540-1548, 1982.
- ICHAS F, MAZAT JP: From calcium signaling to cell death: two conformations for the mitochondrial permeability transition pore. Switching from low- to high-conductance state. *Biochim Biophys Acta* **1366**: 33-50, 1998.
- IWAI T, TANONAKA K, KOSHIMIZU M, TAKEO S: Preservation of mitochondrial function by diazoxide during sustained ischaemia in the rat heart. *Br J Pharmacol* **129**: 1219-1227, 2000.
- KORSHUNOV SS, KORKINA OV, RUUGE EK, SKULACHEV VP, STARKOV A: Fatty acids as natural uncouplers preventing generation of O₂⁻ and H₂O₂ by mitochondria in the resting state. *FEBS Lett* **435**: 215-218, 1998.
- LAWRIE AM, RIZZUTO R, POZZAN T, SIMPSON AW: A role for calcium influx in the regulation of mitochondrial calcium in endothelial cells. *J Biol Chem* **271**: 10753-10759, 1996.
- LESNEFSKY EJ, MOGHADDAS S, TANDLER B, KERNER J, HOPPEL CL: Mitochondrial dysfunction in cardiac disease: ischemia--reperfusion, aging, and heart failure. *J Mol Cell Cardiol* **33**: 1065-1089, 2001.
- LIU Y, SATO T, O'ROURKE B, MARBAN E: Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? *Circulation* **97**: 2463-2469, 1998.
- MATHUR A, HONG Y, KEMP BK, BARRIENTOS AA, ERUSALIMSKY JD: Evaluation of fluorescent dyes for the detection of mitochondrial membrane potential changes in cultured cardiomyocytes. *Cardiovasc Res* **46**: 126-138, 2000.
- MITCHELL P: Keilin's respiratory chain concept and its chemiosmotic consequences. *Science* **206**: 1148-1159, 1979.
- MITCHELL P, MOYLE J: Chemiosmotic hypothesis of oxidative phosphorylation. *Nature* **213**: 137-139, 1967.
- OŠŤÁDAL B, KOLÁŘ F: *Cardiac Ischemia: From Injury to Protection*. Kluwer Academic Publishers, Dordrecht /Boston/London, 1999.
- PALMER JW, TANDLER B, HOPPEL CL: Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle. *J Biol Chem* **252**: 8731-8739, 1977.
- PALMER JW, TANDLER B, HOPPEL CL: Biochemical differences between subsarcolemmal and interfibrillar mitochondria from rat cardiac muscle: effects of procedural manipulations. *Arch Biochem Biophys* **236**: 691-702, 1985.
- PALMER JW, TANDLER B, HOPPEL CL: Heterogeneous response of subsarcolemmal heart mitochondria to calcium. *Am J Physiol* **250**: H741-H748, 1986.
- PALOU A, PICO C, BONET ML, OLIVER P: The uncoupling protein, thermogenin. *Int J Biochem Cell Biol* **30**: 7-11, 1998.
- PIPER HM, SEZER O, SCHLEYER M, SCHWARTZ P, HUTTER JF, SPIECKERMANN PG: Development of ischemia-induced damage in defined mitochondrial subpopulations. *J Mol Cell Cardiol* **17**: 885-896, 1985.
- RADI R, TURRENS JF, CHANG LY, BUSH KM, CRAPO JD, FREEMAN BA: Detection of catalase in rat heart mitochondria. *J Biol Chem* **266**: 22028-22034, 1991.
- RICQUIER D AND BOUILLAUD F: The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *Biochem J* **345**: 161-179, 2000.
- SCADUTO RC JR, GROTYOHANN LW: Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. *Biophys J* **76**: 469-477, 1999.
- SKULACHEV VP: Uncoupling: new approaches to an old problem of bioenergetics. *Biochim Biophys Acta* **1363**: 100-124, 1998.
- SKULACHEV VP: Mitochondrial physiology and pathology; concepts of programmed death of organelles, cells and organisms. *Mol Aspects Med* **20**: 139-184, 1999.
- ŠKÁRKA L, BAUMRUK F, KOPECKÝ J, OŠŤÁDAL B: Mitochondrial membrane potential of cardiomyocytes during early ontogenetic development of the rat. *Physiol Res* **49**: P15, 2000.
- TURRENS JF: Superoxide production by the mitochondrial respiratory chain. *Biosci Rep* **17**: 3-8, 1997.
- TURRENS JF, BOVERIS A: Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J* **191**: 421-427, 1980.

-
- XU M, WANG Y, AYUB A, ASHRAF M: Mitochondrial K(ATP) channel activation reduces anoxic injury by restoring mitochondrial membrane potential. *Am J Physiol* **281**: H1295-H1303, 2001.
- YU XX, MAO W, ZHONG A, SCHOW P, BRUSH J, SHERWOOD SW, ADAMS SH, PAN G: Characterization of novel UCP5/BMCP1 isoforms and differential regulation of UCP4 and UCP5 expression through dietary or temperature manipulation. *FASEB J* **14**: 1611-1618, 2000.
-

Reprint requests

Dr. Libor Škárka, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic, Fax: +420 2 4106 2125, e-mail: skarka@biomed.cas.cz