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 (intmembrane 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 (Lesniesky et al. 2001).

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

![Fig. 1. Principle of generation of MMP in mitochondria.](image-url)
oxidative phosphorylation is modulated in relation to the
energy demand by the Ca\textsuperscript{2+} signal as the increased energy
demand is almost always accompanied by a rise in [Ca\textsuperscript{2+}],
e.g. for contraction. It has been suggested that
mitochondria may participate in the active propagation of
a [Ca\textsuperscript{2+}] signal across the cell through a process of Ca\textsuperscript{2+}-
induced Ca\textsuperscript{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\textsuperscript{2+} uptake into
mitochondria by the Ca\textsuperscript{2+}-uniporter and it is now
generally accepted that it is the Ca\textsuperscript{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\textsuperscript{2+} overload of mitochondrial
matrix and elevated mitochondrial matrix pH), and
possibly also physiological processes, can trigger a
mitochondrial permeability transition pore (PTP)
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 (ADP+P) results in a 10-fold decrease in the \( \text{H}_2\text{O}_2 \) 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 \( \text{Ca}^{2+} \), 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 cristal 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).

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 [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 Šťádal 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 Pi, 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 mitoK$_{ATP}$ channel openers exert cardioprotective effects in various animal models of ischemia-reperfusion situations (Garlid et al. 1997, Liu et al. 1998, Asemu et al. 1999). It has been indicated that the mitoK$_{ATP}$ 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 mitoK$_{ATP}$ 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

---

**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 [Ca$^{2+}$], (cytoplasmatic) and [Ca$^{2+}$]$_{m}$ (mitochondrial).
and structural integrity by diazoxide suggests a potential therapeutic application of mitoK<sub>ATP</sub> channel agonists in preventing ischemic injury.

Acknowledgement

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

References


---

**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