Influence of ischemia and myocardial reperfusion kinetics on the structure and functions of mitochondria in the pig

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Keywords: myocardial ischemia, reperfusion, kinetics, mitochondria, calcium overload, reactive oxygen species, oxidative phosphorylation

ABSTRACT

Prolonged cardiac ischemia induces severe lesions involving ROS production, structural and functional changes of mitochondria, reduced contractility and death by ventricular fibrillation. Reperfusion can paradoxically result in more severe lesions.

The objective of this study was to evaluate electrophysiological and hemodynamic changes together with structural and functional alterations of mitochondria in an ischemia/reperfusion pig model.

Five groups of 6 anesthetized pigs were used: GI. controls; GII. 45-min ischemia; GIII, GIV, GV. 45-min ischemia and 1-, 10- or 45-min reperfusion. Heart rate, duration of monophasic action potentials (dMAP), and left ventricle dP/dt max were measured after 1-, 5-, 10-, 20-, 30- and 45-min ischemia.

Compared to controls, ischemia and reperfusion induced tachycardia, reduced dMAP and LV dP/dt max, and produced ultrastructural and functional alterations of mitochondria. Electrophysiological and hemodynamic changes, and ultrastructural and functional alterations of mitochondria during ischemia were aggravated by reperfusion. The main mechanism seems to be the lack of O2 supply during ischemia and massive O2 supply during reperfusion with ROS overproduction leading to calcium overload and mPTP opening. Changes were more marked after 1-min than 10- or 45-min reperfusion. Improvement was correlated with conserved mitochondrial structure and function evidenced by moderately decreased O2 consumption and increased ROS production with augmented calcium retention.

These results support the use of pharmacological agents that prevent ischemia/reperfusion lesions by improving mitochondrial functions before reperfusion.
BACKGROUND

An interruption of the coronary blood flow results in myocardial ischemia whatever the causative event, either coronary spasm or thrombosis in the clinic, or experimental ligation of the anterior interventricular artery. Ensuing cardiac lesions are severe and irreversible when ischemia is prolonged up to 45 minutes [1]. These lesions involve various mechanisms such as the production of reactive oxygen species (ROS) [2] or disturbed activity of the Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\) exchangers [3]. Ischemia leads to depolarization of myocardial fibers resulting from reduced active ion transfers [4]: within seconds after coronary occlusion, K\(^+\) ions accumulate in interstitial spaces due to a lack of reintegration into the intracellular medium [5] where Na\(^+\) and Ca\(^{2+}\) ions tend to increase due to declining outflow [6].

Changes in the structure and functions of mitochondria have been evidenced during myocardial ischemia [7]. Mitochondrial structure anomalies are associated with calcium overload within cells [8] leading to electromechanical dysfunction [9] or cellular death [10]. Moreover, cardiac contractile energy is reduced by ischemia as evidenced by the decreased time derivative of left ventricular pressure (LV dP/dt max) [11]. This decrease can be explained by the inability of the ischemic myocardium to convert energy into contractile energy [12]. Myocardium attempts to survive to the lack of sufficient ATP concentrations during ischemia [13] by combining contraction inhibition (hibernation) and anaerobic glycolysis as most of ATP production in ischemic hearts seemingly derives from \(\beta\)-oxidation of fatty acids [14].

However, if ischemia is prolonged, death may occur, most often via ventricular fibrillation (VF) due to disturbances of myocardial electrical activity linked to both necrosis of the left ventricular mass and anomalies of metabolic energy presumably due to alterations in the structure and function of mitochondria. This latter aspect plays an important role in VF development because of the disturbed Ca\(^{2+}\) retention capabilities of mitochondria resulting in intracellular calcium overload during ischemia, and consequently disturbances of cellular electrogensis, hence arrhythmias and death.

The management of such a situation requires lifting obstruction in the thrombosed coronary artery [15], but paradoxically more severe lesions occur during the reperfusion phase – the so-called "oxygen paradox" whose consequences are severe and manifold [16]. One of these consequences, and probably the most important one, is the opening of mitochondrial permeability transition pores (mPTP) linked to ROS production and calcium overload [17]. The dysfunction of the respiratory chain, which is worsened during reperfusion, leads to a drop in ATP synthesis, whereas ROS production is maintained [18] with cytochrome c release in the cytosol and induction of apoptosis.

A large number of experimental data have shown that tissue lesions developing after reperfusion account for most of irreversible cellular damage in acute myocardial infarct (AMI). The negative role of mPTP opening is well recognized [19] and seems to be a critical event in cellular death after AMI [20]. However, there is no published study precisely describing the consequences of reperfusion over time. To the best of our knowledge, the kinetics of reperfusion consequences has not been thoroughly investigated. The aim of the present work was to concomitantly study changes in cardiac electrophysiological and hemodynamic parameters with structural and functional changes in mitochondria in a pig model of experimental myocardial ischemia followed by reperfusion.

METHODS

Animals and study design

The experiments were conducted on a total of 30 young domestic pigs of either sex weighing 20-25 kg. The experimental design was approved by the Animal Experimentation Ethics Committee of Claude Bernard University (n° BH 2009-16) prior to the initiation of the study. They conform with the Directive 2010/63/EU of the European Parliament.

The animals were randomly included into 5 groups. The first group (G1) consisted of sham animals: they were anesthetized and ventilated, but not submitted to experimental ischemia. Animals of the second group (G2) were submitted to 45 minutes of myocardial ischemia by total ligation of the distal branch of the anterior
interventricular artery. Animals of the 3 remaining groups (G3, G4, and G5) were submitted to 45 minutes of myocardial ischemia followed by 1, 10 or 45 minutes of reperfusion, respectively.

All animals were injected intramuscularly with 20 mg/kg ketamine (Imalgene 1000®, Merial, France) prior to intravenous injection of propofol (Diprivan®: Fresenius Kabi AB, Sweden) at the dose of 3 mg/kg, and then of chloralose at 100 mg/kg (Aldrich Chemie GmbH, Steinheim, Germany). In addition, chloralose was continuously perfused intravenously at 10 mg/kg/min throughout the experiment. Chloralose was used for its absence of effect on cardiovascular function. Thereafter, the animals were tracheotomized and ventilated via a Dräger SA2 respirator (Lubeck, Germany). The inspired oxygen fraction (FiO₂) and partial CO₂ pressure in expired gases at the end of expiration (PETCO₂) were monitored. The tidal volume was adjusted to maintain PETCO₂ within physiological values (35-40 mmHg). Adequacy of anaesthesia was monitored by checking every 5 minutes the absence of oculopalpebral reflex and of movements of the animal either spontaneous or in response to noise.

Ischemia was produced by total occlusion of the anterior interventricular coronary artery for 45 minutes. It was evidenced from the viola aspect of the non-perfused area, the shift of ST segment and the amplitude of T wave in the DII ECG lead.

At the end of the experiment, euthanasia was obtained by triggering ventricular fibrillation by means of an electrical shock applied through an electrode (placed in sub-epicardial position on the left ventricle) connected to a stimulator (Hugo Sachs Elektronik, Type 215, March-Hugstetten, Germany) delivering a current of 20 mA intensity for 100 ms duration.

Electrophysiological and hemodynamic recordings

HR was recorded via a standard DI or DII lead. The signal was amplified and digitized using the Lab-Pack system (Mp100; Biopac System, Santa Barbara, CA, USA). Myocardial electrical activity was analyzed locally through a Catronic ORX electrode 6F (Plastimed, Saint-Leu-La-Forêt, France) positioned in the sub-epicardial layer of the left ventricle wall close to the center of the hypoxic area and connected to the electrocardiograph. The duration of monophasic action potentials (dMAP) was measured at 90% repolarization.

An arterial pressure line was established through a catheter inserted into the left carotid artery and connected to a polygraph (M1166 A, model 66 S, Hewlett Packard Inc, USA) to monitor systolic (SBP), diastolic (DBP) and mean (MBP) arterial pressure (mmHg). Another catheter was introduced into the right carotid artery and then placed within the left ventricle to record left ventricular pressure. Data processing by the Lab-Pack system and Acqknowledge software (Biopac System, Santa Barbara, CA, USA) enabled to determine the time derivative of left ventricular pressure (LV dP/dt max).

All parameters were measured prior to ischemia and then 1, 5, 10, 20, 30 and 45 minutes after the start of ischemia, except in sham (group 1) animals. In addition, they were measured 1 minute after the start of reperfusion in group 3 animals; 1, 5 and 10 minutes after the start of reperfusion in group 4 animals; and 1, 5, 10, 20, 30 and 45 minutes after the start of reperfusion in group 5 animals.

Structural and functional parameters

Immediately after euthanasia, the heart of all animals was excised. One sample was taken from the ischemic zone of the left ventricle and then split into several small fragments immediately fixed by the addition of glutaraldehyde 4% and cacodylate 0.2N. The samples were transferred to the Molecular and Cellular Imaging Center (Lyon, France) for ultrastructural analysis using a JEOL 1200CX transmission electron microscope as previously described [21] to determine the number of nuclei with disrupted membranes and irregular chromatin agglomeration (20 examined nuclei), the number of disrupted or swollen sarcolemma (20 examined sarcolemma), the number of cell junction disruptions (20 examined cell junctions), the number of necrotic cardiomyocytes (20 examined cardiomyocytes), the number of displaced, swollen or degranulated mitochondria and disrupted mitochondrial crests (200 examined mitochondria) and the number of capillaries with thickening of the basal membrane and abnormal chromatin distribution in the nuclei (20 examined capillaries).
A second sample was taken from the ischemic zone of the left ventricle to study mitochondrial functions. The respiratory function was investigated with the method described by Gomez et al. [22]. Using a Gilson 5/6H oxygraph fitted to a Clark-type electrode, oxygen consumption from 500 µg of mitochondrial proteins was triggered by adding 5mM of glutamate 5 mM/malate 0.5 mM (G/M). Oxygen consumption was measured in oxygen nanoatoms/min/mg of proteins in the phosphorylation state (state 3) with ADP 200 µm and in the non-phosphorylation state (state 4) with or without oligomycin. The ratio of oxygen consumption in state 3 over state 4 is the respiratory control ratio (RCR).

ROS production by the mitochondrial respiratory chain was measured from hydrogen peroxide production using a SFM 25 spectrofluorometer (Kontron, France). Indeed, the measured fluorescence is directly proportional to \( H_2O_2 \) production [23]. The measurement was performed in a buffer (250 Mm sacharose, 1 Mm EDTA, 0.15 BSA, 1 Mm EGTA in Tris/HCL 20 mM, pH 7.4) containing the amplex Red probe (10 µM), peroxidase (0.6 Units) and 250 µg of proteins. The baseline level was determined with the buffer, and thereafter mitochondria and the glutamate/malate substrate (G/M) were added. All measurements were done at +25°C. Results are expressed in pmol of \( H_2O_2 \)/min/mg of mitochondrial proteins.

The calcium retention capacity of mitochondria (CRC) was determined using the method of article [24]. CRC allows determining the opening status of mPTP. CRC was measured after adding exogenous calcium (CaCl\(_2\)). The extramitochondrial calcium concentration was recorded from the fluorescence peak after pulses of 2.5 nmoles CaCl\(_2\). After each pulse, exogenous Ca\(^{2+}\) is captured by mitochondria, hence the return to baseline extra-mitochondrial calcium concentration as reflected by the reduced fluorescent signal. In case of marked mitochondrial overload, the extra-mitochondrial Ca\(^{2+}\) concentration (expressed in nmoles of exogenous Ca\(^{2+}\)/mg proteins) sharply increases, which indicates massive Ca\(^{2+}\) release by mitochondria, and thus mPTP opening.

**Statistical analysis**

Results are expressed as mean ± SEM. The statistical significance of differences between groups was estimated by one-way ANOVA, followed by Dunnett’s test or two-way ANOVA, followed by Bonferroni’s test. Differences were considered significant if \( p < 0.05 \).

**RESULTS**

No remarkable change in any of the studied parameters was observed in sham animals and their structural and functional aspects of mitochondria were considered normal. In contrast, ischemia alone as well as ischemia/reperfusion was found to significantly increase HR, shorten dMAP, and decrease LV dP/dt max as well as induce ultrastructural alterations and functional changes of mitochondria including reduced \( O_2 \) consumption, increased ROS production, and decreased CRC. Interestingly, the most severe ultrastructural and functional alterations of mitochondria were observed only after 1 minute of reperfusion and they were found to be progressively diminishing after 10 and 45 minutes of reperfusion.

**Ischemia and ischemia/reperfusion induce changes in electrophysiological and hemodynamic parameters**

As shown on figure 1, HR was increased during ischemia and ischemia/reperfusion as compared to sham animals (ischemia vs. sham, \( p < 0.05 \); 1-min reperfusion vs. sham, \( p < 0.001 \); 10-min reperfusion vs. sham, \( p < 0.05 \)). Importantly, HR was significantly decreased after 45-min reperfusion as compared to 1-min reperfusion. A significant shortening of dMAP was noted in animals submitted to ischemia and ischemia-reperfusion compared to sham animals (ischemia vs. sham, \( p < 0.05 \); 1-min reperfusion vs. sham, \( p < 0.001 \); 10-min reperfusion vs. sham, \( p < 0.05 \)). Importantly, dMAP was significantly shortened after 45-min reperfusion compared to 1-min reperfusion. LV dp/dt max was significantly during ischemia-reperfusion compared to sham animals (1-min reperfusion vs. sham, \( p < 0.05 \)). It is noteworthy that LV dp/dt max was significantly increased after 10- and 45-min reperfusion compared to 1 minute after reperfusion (\( p < 0.05 \)).
Figure 1: Changes in: A- the duration of monophasic action potentials (dMAP); and B- the time derivative of left ventricular pressure (LV dP/dt max) during ischemia and after different times of reperfusion.

![Graph showing changes in dMAP and LV dP/dt max during ischemia and after reperfusion.]

* p<0.05, ** p<0.01, *** p<0.001 = reperfusion vs. sham
$ p<0.05, $$ p<0.01, $$$ p<0.001 = reperfusion vs. ischemia
§ p<0.05, §§ p<0.01, §§§ p<0.001 = ischemia vs. sham
£ p<0.05,££ p<0.01, £££ p<0.001 = 10 or 45 min reperfusion vs. 1 min reperfusion.

Ischemia and ischemia/reperfusion alter the ultrastructure of cardiomyocytes, capillaries and mitochondria

Whereas there were no abnormal findings in sham animals, ischemia and ischemia-reperfusion induced ultrastructural changes in cardiomyocytes including picnotic nuclei, nuclear membranes with variable thickness, and irregular chromatin distribution; in capillaries with membrane thickening and disorganization; and in mitochondria with swelling and breaks of mitochondrial crests and sometimes mitochondrial membranes (Figure 2, Table I). Importantly, ultrastructural changes were the most severe after 1-minute reperfusion and they subsequently diminished.

Table 1: Structural alterations of nuclei, capillaries and mitochondria.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>45-min ischemia</th>
<th>1-min reperfusion</th>
<th>10-min reperfusion</th>
<th>45-min reperfusion</th>
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<tr>
<td><strong>Nuclei changes (%)</strong></td>
<td></td>
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<tr>
<td>11±2</td>
<td>65±1 §§</td>
<td>80±4***</td>
<td>46±5 **</td>
<td>40±4 **</td>
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<tr>
<td><strong>Capillary changes (%)</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>15±4</td>
<td>59±5§§</td>
<td>66+/6***</td>
<td>62±8**</td>
<td>55±2**</td>
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<tr>
<td><strong>Mitochondrial changes (%)</strong></td>
<td></td>
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<tr>
<td>13±2</td>
<td>66±3§§</td>
<td>85±3***</td>
<td>55±6***</td>
<td>48±3 **</td>
<td></td>
</tr>
</tbody>
</table>

Structural alterations seen after ischemia compared to sham animals (§) and after 1-, 10- or 45-min reperfusion compared to sham animals (*) and to 45-min ischemia ($).

* p<0.05, ** p<0.01, *** p<0.001; reperfusion vs. sham
§ p<0.05, §§ p<0.01, §§§ p<0.001; ischemia vs. sham
Figure 2: Ultrastructural examination of cardiomyocytes nuclei, capillaries and mitochondria (1200 CX, GEOL).

2A: Nuclei

2A1 (sham animals): nearly normal nuclei, regular membranes and chromatin. 2A2 (ischemia) & 2A3 (1-min reperfusion): picnotic nuclei, nuclear membranes of variable thickness, irregular chromatin.

2B: Capillaries


2C: Mitochondria

2C1 (sham animals): no remarkable changes in the structure of mitochondria. 2C2 (ischemia) & 2C3 (1-min reperfusion): swollen mitochondria with breaks of mitochondrial crests and sometimes membranes.

Ischemia and ischemia/reperfusion alter mitochondrial function

Oxygen consumption was significantly decreased during ischemia and after 1-, 10- or 45-min reperfusion (Figure 3, Table II). The most severe reduction in oxygen consumption was measured after 45 minutes of ischemia. Oxygen consumption progressively increased after 10 minutes of reperfusion, but remained significantly lower than in animals without ischemia. ROS production was already increased significantly after 1 minute of reperfusion, but significantly decreased 45 minutes of reperfusion. There was a significant decrease in calcium retention capacity during ischemia as well as after 1 minute of reperfusion. Calcium retention capacity was increased after 10 and 45 minutes of reperfusion, but this increase was not significantly different from results after 1 minute of reperfusion.
Figure 3: Determination of mitochondrial function.

Results are presented as mean ± SEM. A: production ROS (pmol of H$_2$O$_2$/min/mg mitochondrial proteins) using the G/M (glutamate/malate) substrate by mitochondria from pig hearts. B: Calcium retention capacity in mitochondria (nmoles of exogenous Ca$^{2+}$/mg of proteins).

* p<0.05, ** p<0.01, *** p<0.001; Reperfusion vs. sham
§ p<0.05, §§ p<0.01, §§§ p<0.001; Reperfusion vs. ischemia
§ p<0.05, §§ p<0.01, §§§ p<0.001; Ischemia vs. sham.

Table 2: Oxygen consumption in cardiac mitochondria.

<table>
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<th></th>
<th>Sham</th>
<th>45-min ischemia</th>
<th>1-min reperfusion</th>
<th>10-min reperfusion</th>
<th>45-min reperfusion</th>
</tr>
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<tbody>
<tr>
<td><strong>STATE 4</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>6.8±0.7</td>
<td>5.1±0.5</td>
<td>5.9±0.9</td>
<td>6.4±0.6</td>
<td>6.2±1.1</td>
</tr>
<tr>
<td><strong>STATE 3</strong></td>
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<tr>
<td></td>
<td>133±5.0</td>
<td>173±3.0 §§$§§$</td>
<td>33±9.0</td>
<td>60±9.0 **/§§</td>
<td>91±11 */§§§</td>
</tr>
<tr>
<td><strong>RCR</strong></td>
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<tr>
<td></td>
<td>19.5</td>
<td>3.33$§§§$</td>
<td>5.59**/§§</td>
<td>9.3*/§§</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Mitochondrial O$_2$ consumption under ischemia was compared to sham conditions (§), and that after 1-, 10- or 45-min reperfusion sham conditions (*) or ischemia ($)  
* p<0.05, ** p<0.01, *** p<0.001; reperfusion vs. sham  
§ p<0.05, §§ p<0.01, §§§ p<0.001; reperfusion vs. ischemia  
§ p<0.05, §§ p<0.01, §§§ p<0.001; ischemia vs. sham

DISCUSSION

The main objective of the present study was to follow the time course of events after 45-min ischemia and after 1-, 10- and 45-min reperfusion to determine when the most severe changes were seen. Myocardial ischemia induced significant changes in electrophysiological (tachycardia, shortened dMAP) and hemodynamic parameters (decreased LV dP/dt max), structural alterations in ventricular cardiomyocytes, and mitochondrial function alterations including a marked decrease in oxygen consumption, increased ROS production and reduced calcium retention capacity. Most of these alterations were present after 45 minutes of ischemia and were aggravated after only 1 minute of reperfusion. They progressively became less severe after 10 minutes and then 45 minutes of reperfusion.
The consequences of ischemia can be explained by structural and functional alterations of mitochondria [25] with ROS production [26] and calcium overload [27]. In turn, calcium overload can result in marked electrophysiological changes leading to ventricular fibrillation [28]. Indeed, during ischemia, muscle fibers lose K⁺, whereas Na⁺ ions accumulate in cardiac cells [29]. This global ion imbalance is responsible for membrane depolarization in cardiomyocytes resulting to decreased dMAP during ischemia as observed in the present study. This decrease reflects hyperexcitability leading to excessive automaticity of myocardial cells [30], hence to ventricular fibrillation. When coronary flow is near zero, Na⁺ and Ca²⁺ concentrations in cardiomyocytes can be increased 2-fold [31]. The increase in Na⁺ concentrations is due to decreased Na⁺ efflux via the Na⁺/K⁺ ATPase exchanger, which is inhibited during ischemia [32] and/or alternatively the entry of Na⁺ ions via the Na⁺/H⁺ exchanger whose activity is increased by intracellular acidosis. Moreover, Na⁺ overload stimulates the Na⁺/Ca²⁺ exchanger and thus increases intracellular calcium overload.

During ischemia, calcium overload can induce electrophysiological [33] and hemodynamic [34] disturbances. Electrophysiological changes seem to be mainly due to delayed afterdepolarizations as a consequence of calcium overload [35]. Globally, these changes are aggravated by reperfusion via excessive ROS generation and the resulting calcium overload [36]. Calcium overload is presumably the major contributing factor to the poor myocardial recovery after reperfusion [6].

The consequences of reperfusion mainly reflect the aggravation of lesions induced by ischemia. Long periods of ischemia/reperfusion have previously been shown to generate mitochondrial lesions [37]. During ischemia, the low oxygen supply leads to cardiac dysfunction, which accounts for most of the previously mentioned alterations. The survival of ischemic myocardial tissue can only be obtained via restoration of blood flow after reperfusion, which can be achieved in the clinic by thrombolytic drugs, angioplasty or coronary bypass. The restoration of blood flow, however, may cause severe lesions (the "oxygen paradox") including death of myocytes [38]. Ischemia/reperfusion lesions are the consequence of a multifactorial process involving ROS production and calcium overload, and to a lesser extent neutrophil activation and apoptosis [39]. Membrane lipid peroxidation associated with decreased activity of cardiac superoxide dismutase [40] and ROS production facilitates the entry of Ca²⁺ ions. As Ca²⁺ ions cannot enter the sarcoplasmic reticulum owing to alterations in the ATPase system [41] and Na⁺/Ca²⁺ exchanger [42], they tend to accumulate, hence calcium overload leading to decreased myocardial contractility [43].

ROS production and disturbed calcium homeostasis are considered to play a critical role during the first minutes of reperfusion [44]. Alterations in mitochondrial function are probably linked to opening mPTP as a consequence of ROS production [45]. Interestingly, mitochondria are the starting point of lesions associated with reperfusion via reduced oxidative phosphorylation leading to a drop in ATP synthesis. Within mitochondria, calcium load is increased and mPTP are opened, which allows the exit of cytochrome c and cardiolipin peroxidation. Cytochrome c in the cytosol triggers a cascade of events leading to apoptosis [46]. Structural lesions of mitochondria shown in the present study have been linked to intra-cellular calcium overload [8] resulting in electromechanical dysfunction [9] or cell death [10]. The conservation of mitochondrial membrane permeability, the inhibition of ROS production and the reduction of intra-cellular calcium overload can efficiently fight against ischemia-related lesions [47]. One example is controlled reperfusion to improve recovery of the cardiac function [39].

In the present study, severe alterations in the structure and function of mitochondria were observed after 45-min ischemia and they were aggravated after 1-min reperfusion. In contrast, electrophysiological and hemodynamic parameters were rather preserved after 10- and 45-min reperfusion, and changes in the structure and function of mitochondria were less pronounced. Structural and functional lesions of mitochondria are indeed early events in ischemia [48], and mitochondria play a key role in the reperfusion paradox [49]. Less severe alterations after 10- and 45-min reperfusion parallel the less pronounced decrease in oxidative phosphorylation and increase in ROS production and calcium...
retention capacity. All changes in electrophysiological and hemodynamic parameters as well as ultrastructural and functional alterations observed ischemia were aggravated very shortly after starting reperfusion in the present study. The primary causative mechanism is the shortage of oxygen supply during ischemia followed by an explosive oxygen supply during reperfusion, which results in excessive ROS production, and subsequently mitochondrial calcium overload and mPTP opening. The progressive improvement noted after 10- and 45-min reperfusion was correlated with conservation of the structure and functions of mitochondria. The fight against ischemia/reperfusion lesions should focus on those pharmacological agents, such as beta-blockers or anti-ischemic drugs that can improve mitochondrial function prior to reperfusion of the ischemic myocardium.

CONCLUSION

The alterations of cardiac electrophysiological and hemodynamic function and of ultrastructure and mitochondrial function due to ischemia are aggravated within 1 minute of reperfusion. The main mechanism of this aggravation seems related to massive reoxygenation upon reperfusion, following O2 deprivation during ischemia. This causes massive production of free radicals and subsequently mitochondrial calcium overload that triggers mPTP opening. These lesions are more severe after the first minute of reperfusion whereas they are partly relieved after 10 and 45 minutes of reperfusion. This improvement is correlated with an improvement of oxygen consumption, lower ROS production together with improved capacity of calcium retention by mitochondria.

REFERENCES

[7] Wang S, Radhakrishnan J, Ayoub IM, Kolarova JD, Taglieri DM, Gazmuri RJ. Limiting sarcolemmal Na+ A number of protective agents (e.g.: anti-ischemic drugs, beta blocking agents...) are used to reduce the deleterious consequences of reperfusion after ischemia by improving mitochondrial function. In order to avoid reperfusion lesions and the risk of sudden death, it is important that such protection is applied before reperfusion.

ABBREVIATIONS

ADP – Adenosine diphosphate
AMI – Acute myocardial infarct
ATP – Adenosine triphosphate
CRC – Calcium retention capacity of mitochondria
DBP – Diastolic blood pressure
dMAP – Duration of monophasic action potentials
G/M – Glutamate/malate
HR – Heart rate
LV dP/dt max – Maximum time derivative of left ventricular pressure
MBP – Mean blood pressure
mPTP – Mitochondrial permeability transition pores
RCR – Respiratory control ratio
ROS – Reactive oxygen species
SBP – Systolic blood pressure
VF – Ventricular fibrillation

CONFLICT OF INTEREST

There is no conflict of interest of any author with any public or private institution.


[32] van Emous JG, Lankamp CL, Ruigrok TJ, van Echteld CJ. Glycolytic ATP production is not


