Acute myocardial ischaemia induces specific alterations of ventricular mitochondrial function in experimental pigs

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Abstract

Aims: As cardiac metabolic flexibility is crucial, this study examined whether acute ischaemia can induce specific qualitative alterations of the mitochondrial metabolic pathways as well as energy transfer systems.

Methods: Left descending coronary artery ligation was performed after sternotomy in eight pigs and the heart was excised after 45 min of ischaemia. Maximal O_2 uptake (V_{max}, μmol O_2 min^{-1} g^{-1} dry weight) of saponin-skinned myofibres were measured from ischaemic and non-ischaemic area of ventricular myocardium.

Results: V_{max} decreased by ~20% in ischaemic myocardium with both glutamate-malate (18.1 ± 1.3 vs. 22.1 ± 1.7 in control, $P < 0.05$) and pyruvate substrates (19.3 ± 1.0 vs. 23.3 ± 2.0 in control, $P < 0.05$) whereas no difference was observed with palmitoyl carnitine (15.6 ± 1.8 vs. 16.6 ± 0.9 in control). The K_m of mitochondrial respiration for ADP decreased in ischaemic heart by 24% (679 ± 79 vs. 899 ± 84 μM of ADP in control, $P < 0.05$). Moreover, the mitochondrial creatine kinase efficacy (K_m without creatine/K_m with creatine), representative of the coupling of oxidative phosphorylation process with the mitochondrial creatine kinase, was reduced in ischaemic heart (11.6 ± 2.5 in ischaemic vs. 18.0 ± 2.2 in control, $P < 0.05$).

Conclusions: These findings argue for specific mitochondrial impairments at the level of pyruvate oxidation and creatine kinase channelling system after an acute period of *in vivo* ischaemia, whereas the lipid mitochondrial oxidation pathway seems to be preserved. Such a loss of metabolic flexibility following acute ischaemia could become an early feature of metabolic dysregulation of the heart.

Keywords creatine kinase, fatty acid, ischaemia, mitochondria, myocardial infarction, substrate oxidation.

Cardiac contractile function depends on a constant flow of cellular energy production through mitochondrial aerobic ADP and creatine rephosphorylation. The heart adapts to acute and chronic workload changes by oxidizing the most efficient fuel for respiration, a phenomenon recently defined as metabolic flexibility (Taegtmeyer *et al.* 2004). Loss of this flexibility is a hallmark of maladaptation, which could lead to myocardial contractile dysfunction.

During and following ischaemia (Isch), the heart becomes less efficient at converting energy into contractile force (for review see Dyck & Lopaschuk 2002).

However, the relationship between ischaemic damage and mitochondrial function is not yet fully understood and may not only result in adenosine triphosphate (ATP) production failure because of oxygen deprivation.

Indeed, if oxygen restriction is maintained, mitochondria themselves become targets for ischaemic damage, decreasing the chances of myocytes recovery for both metabolism and function (Di Lisa *et al.* 1998). In response to Isch, a majority of ATP production seems to be derived from the β-oxidation of fatty acids in the heart (Liedtke *et al.* 1988, Lopaschuk *et al.* 1990). Particularly, in perfused myocardium, a poor recovery of mechanical function has been shown to be associated with low rates of glucose utilization, which might be inhibited by fatty acids (Liedtke *et al.* 1988, Lopaschuk *et al.* 1990, 1993, Opie & Sack 2002). Ischaemia-induced reduction in cardiac efficiency could also result from alterations in energy substrates oxidation (Liedtke *et al.* 1988, Renstrom *et al.* 1990, Zuurbier & Ince 2002).

Although, experimental results on Isch-induced mitochondrial alterations, obtained by evaluating *in situ* mitochondrial function using the permeabilized fibres technique (Veksler *et al.* 1987), are scarce and somewhat conflicting. Saks *et al.* detected a decrease in creatine-stimulated oxygen uptake as well as a reduction of the maximal respiratory rates with pyruvate substrate in the arrested rat heart subjected to a 35 min Isch (Saks *et al.* 1989, Kay *et al.* 1997). On the other hand, Veksler *et al.* claimed that there was no significant change in mitochondrial function after 45 min of Isch in the arrested guinea-pig hearts with glutamate-malate substrates (Veksler *et al.* 1990). In fact, the effects of acute Isch on the principal mitochondrial metabolic pathways have never been studied together, despite the importance of the metabolic flexibility of the heart (Taegtmeyer 2004).

We recently showed that besides the known differences among muscles in maximal oxidative rates (Saks *et al.* 1998, Ventura-Clapier *et al.* 1998), left ventricular mitochondria can use both fatty acid and pyruvate substrates (Ponsot *et al.* 2005). We concluded from this study that the permeabilized fibres technique represent a good way to measure the balance between the different mitochondrial metabolic pathways contributing to ATP production (Ponsot *et al.* 2005), and then allows to characterize the possible loss of the metabolic flexibility of the heart after acute Isch.

Impairments in energy transfer from mitochondria to myofibrils could also reduce the cardiac efficiency after Isch (Liu *et al.* 1996a,b). Indeed, mitochondrial creatine kinase damage has been demonstrated in perfused heart, suggesting an alteration of the ATP production adjustment to the ATP demand (Kay *et al.* 1997, Rossi *et al.* 1998, Dos Santos *et al.* 2000).

To date, most experiments studying mitochondrial impairments following Isch were conducted on isolated heart (*ex vivo*) and not in models mimicking acute myocardial Isch secondary to coronary occlusion that occurs in patients. Unlike in isolated heart, this research protocol allows to study the mitochondrial function of myocytes not only suffering oxygen and nutriments deprivation but also submitted to an increased mechanical load and to the systemic environment characterized by an intense neuro-hormonal stimulation (Rouleau *et al.* 1994).

We hypothesized that acute Isch could induce specific qualitative impairments of the mitochondrial metabolic pathways as well as energy transfer systems in the heart, leading to a decrease of the metabolic flexibility.

The goal of this study was therefore to investigate the functional capacities of mitochondria to oxidize different substrates (i.e. glutamate-malate, pyruvate and palmitoyl carnitine) and the mitochondrial respiration control by ADP and creatine in ischaemic myocardium after coronary artery ligation *in vivo* in experimental pigs.

Materials and methods

Production of the model

This investigation was carried out in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publications no. 85-23, revised 1996) and has been approved by our Institute Animal Ethics Committee.

Anaesthesia and ventilation. Eight pigs (26–30 kg) were medicated with IM administration of ketamine (20 mg kg^{-1}) and azaperone (2 mg kg^{-1}). An IV catheter (22 Ga) was inserted into an auricular vein. IV induction was obtained with IV thiopenthal (10 mg kg^{-1}). Anaesthesia was maintained with isoflurane up to a maximum of 1.5 vol% end-tidal fraction.

Endotracheal intubation with a Portex 6 mm tube (SIMS Portex, Keene, NH, USA) was facilitated with pancuronium (0.1 mg kg^{-1}). Ventilation was controlled with a Dräger Cicero Ventilator (Dräger Medical, Antony, France) with an inspiratory fraction of 0.66 N_2O in oxygen at a fresh gas flow of 2.0 L min^{-1}. Minute ventilation was adjusted to keep the $PETCO_2$ between 35 and 45 mmHg.

Coronary artery ligation. A sternotomy was performed. Then, the left descending coronary artery was ligated at its distal third. After the ligation, the animals were observed in the open chest state for 45 min. If ventricular fibrillation occurred, electrical defibrillation was performed immediately. Nevertheless, two pigs die from non-retractable ventricular fibrillation. Then, all the results are given with the six remaining pigs.

Tissue processing of left ventricular muscle

After 45 min Isch, two biopsies were realized in the left ventricular beating myocardium, one in the ischaemic area and one in the non-ischaemic area of the myocardium, far from the area at risk. At the end, all the pigs were killed.

Functional properties of mitochondria

The respiratory parameters of the total mitochondrial population were studied *in situ* in saponin-skinned fibres using a method described earlier (Veksler *et al.* 1987, De Sousa *et al.* 2000).

Briefly, thin fibre bundles (100–250 μm in diameter) were excised from ventricular muscles. Fibres were then incubated for 30 min at 4 °C in skinning solution S containing 50 μg mL^{-1} saponin. This procedure was followed by washing fibres for 10 min at +4 °C in solution S without saponin. Respiratory rates were determined using a Clark electrode (Strathkelvin Instruments, Glasgow, UK) in an oxygraphic cell containing 3 mL solution R (see below) at 22 °C with continuous stirring. Solutions S and R used were based on the information of the composition of the intracellular milieu. They contained: CaK_2 EGTA 2.77 mM, K_2 EGTA 7.23 mM (free Ca^{2+} concentration 100 nM), $MgCl_2$ 6.56 mM, imidazole 20 mM, taurine 20 mM, dithiothreitol 0.5 mM. Ionic force was adjusted to 160 mM by addition of potassium methanesulfonate. The pH of the solution was 7.1. Solution S also contained 5 mM MgATP and 15 mM PCr, and solution R contained 3 mM phosphate and 2 mg mL^{-1} fatty acid free bovine albumin serum.

All biopsies underwent both protocols.

Protocol 1: After addition of malate (2 mM) and glutamate (5 mM), the ADP-stimulated respiration (V_{ADP}) above basal oxygen consumption (V_0) was measured by stepwise addition of ADP from 10 to 2000 μM with or without creatine (20 mM), as previously described (Zoll *et al.* 2002, 2003). The apparent K_m's for ADP, inversely proportional to ADP sensitivity, and V_{ADP} were calculated using a non-linear mono exponential fitting of the Michaelis–Menten equation. Maximal respiration rate (V_{max}) was (V_{ADP} + V_0) and the Acceptor Control Ratio (ACR) was V_{max}/V_0. The ACR represents the degree of coupling between oxidation and phosphorylation. Addition of creatine produced a decrease in K_m values because of mitochondrial creatine kinase (CK) activity that ensured an efficient ADP regeneration near the inner mitochondrial membrane. The CK efficacy was evaluated by the ratio between the apparent K_m for ADP in the absence of creatine and in the presence of creatine. This ratio is representative of the coupling of oxidative phosphorylation process with the mitochondrial creatine kinase (De Sousa *et al.* 1999).

Protocol 2: In presence of ADP (2 mM), we determined the maximal muscular oxidative capacities with each mitochondrial substrate added to the preparation (N'Guessan *et al.* 2004). The substrates used were: (1) glutamate (5 mM) + malate (2 mM) which activate the Krebs cycle enzyme malate dehydrogenase providing NADH to the respiratory chain; (2) pyruvate 500 μM + malate 2 mM which activate the pyruvate dehydrogenase complex (PDH) localized in the mitochondrial matrix; and (3) palmitoyl carnitine (PC, 135 μM) + malate (2 mM) which is transferred into the matrix by the inner membrane-localized carnitine translocase (CT) and carnitine palmitoyl transferase II (CPTII) and then activate the β-oxidation. Under these conditions, ADP, inorganic phosphate and O_2 are present at saturating concentration in the oxygraphic chamber. After measurements, fibre bundles were carefully removed and dried. Respiration rates were expressed as μmol O_2 min^{-1} g^{-1} dry weight. As an example, mitochondrial oxygen consumption in function of ADP concentration and the fitting procedure for a representative curves from ischaemic and control myofibres are shown in Figure 1.

Saponin-skinned fibres technique characteristics

This technique allows the study of intracellular organelles, such as mitochondria. The morphological integrity and the good preservation of mitochondria has been demonstrated after permeabilization (Saks *et al.* 1995, 1998). The technique of saponin-skinned fibres allows to study all the population of mitochondria, including mitochondria of infarcted myocytes, which was not the case when mitochondria were isolated, inducing a loss of a fraction of them which could include mitochondria altered by Isch (Saks *et al.* 1998).

Statistical analysis

Values are expressed as mean values ± standard error of the mean (SEM). Differences between ischaemic and control myocardial fibres were analysed using an unpaired Student *t*-test. The level of significance was taken at $P < 0.05$.

Results

Functional properties of mitochondria in the left ventricular muscle (LV)

To assess the alterations of the main mitochondrial metabolic pathways in the LV, the respiratory rates of *in situ* mitochondria at saturating ADP concentration

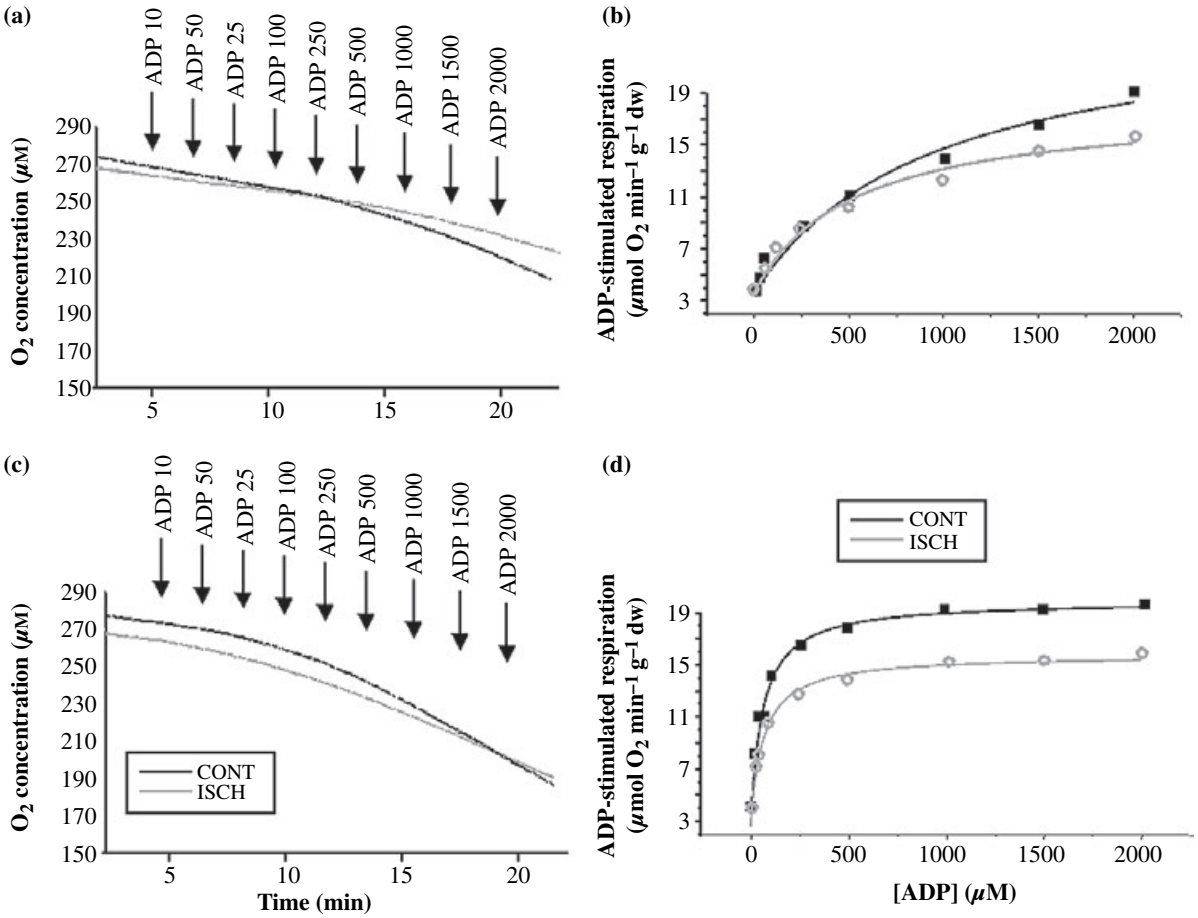

Figure 1 Examples of the Michaelis–Menten kinetics of the oxygen consumption of permeabilized myocardial fibres to increasing levels of the phosphate acceptor adenosine diphosphate (ADP). a and c represent Raw data obtained by myofibres respiration within the oxygraphic chamber from a representative control myocardial (black lines) and ischaemic (grey lines) fibres. The tissue O_2 consumption (V, mmol O_2 min^{-1} g^{-1} dry weight) increases with increasing doses of the phosphate acceptor ADP. Panel b and d show the ADP-related respiration characterizing the Michaelis and Menten kinetics of ADP phosphorylation for control myocardial fibres (black lines) and ischaemic myocardial fibres (grey lines) groups. These experiments were performed in the absence (a and b) or in the presence (c and d) of 20 mM creatine. The striking difference in maximal tissue oxygen uptake between the ischaemic and control myocardial fibres is reflected by the differing asymptotic values of O_2 uptake.

(V_{max}) in the presence of different mitochondrial substrates are reported in Figure 2.

A 45 min of Isch decreased the LV V_{max} by 18 and 17% with glutamate-malate (18.1 ± 1.3 in Isch vs. 22.1 ± 1.7 μmol O_2 min^{-1} g^{-1} dry weight in control, $P < 0.05$) and pyruvate as substrates (19.3 ± 1.0 in Isch vs. 23.3 ± 2.0 μmol O_2 min^{-1} g^{-1} dry weight in control, $P < 0.05$). In opposition, no significant difference was observed between the ischaemic and the control parts of the LV when using the substrate palmitoyl carnitine (15.6 ± 1.8 in Isch vs. 16.6 ± 0.9 μmol O_2 min^{-1} g^{-1} dry weigh in control).

In order to reveal the relative substrate alterations by Isch in the LV, values for glutamate-malate and palmitoyl carnitine were normalized to their respective pyruvate V_{max} and results are shown in Figure 2b.

The relative palmitoyl carnitine respiration rate was higher in Isch than in control LV (+24%, $P < 0.05$), whereas the relative glutamate-malate respiration rate was not altered. This demonstrates the specificity of the mitochondrial function alterations at the level of the pyruvate and glutamate-malate oxidation pathways.

Mitochondrial respiration control

Respiratory rates of *in situ* mitochondria in the absence of nucleotides (V_0) were not significantly altered in LV after 45 min of Isch (2.5 ± 0.2 in Isch vs. 2.8 ± 0.3 μmol O_2 min^{-1} g^{-1} dry weight in control).

The ACR, defined as the ratio V_{max}/V_0 was not significantly different between Isch and control LV (7.6 ± 0.6 in Isch vs. 8.2 ± 0.7 in control).

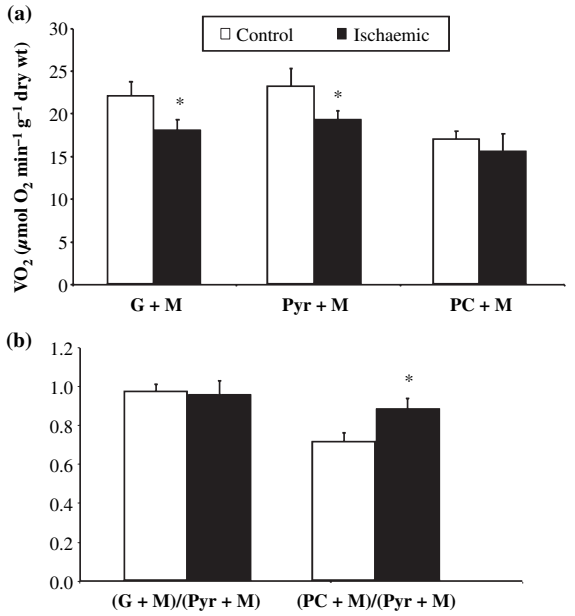

Figure 2 (a) Maximal ADP-stimulated respiration rates (V_{max}) of *in situ* mitochondria from ischaemic and non-ischaemic myocardial fibres in presence of different substrates. Oxygen consumption was measured in saponin permeabilized muscle fibres in an oxygraph cell and with a Clark electrode. Values are expressed as μmol O_2 min^{-1} g^{-1} fibre dry wt. (b) The ratio of (PC + M V_{max})/(Pyr + M V_{max}) as well as the ratio (G + M V_{max})/(Pyr + M V_{max}) show the specific alterations of the mitochondrial oxidation pathways because of acute *in vivo* ischaemia. The substrates used were: G + M: glutamate-malate; Pyr + M: pyruvate- malate; PC + M: palmitoyl carnitine – malate. Values are means ± SEM. Significantly different from control, $^*P < 0.05$.

To establish control of the respiratory activity by the principal regulator, ADP, we studied the oxygen consumption rates of saponin-permeabilized fibres at various ADP concentrations. Representative data for control and ischaemic myocardial fibres in the absence (a and b panel) or presence (c and d panel) of creatine are presented in Figure 1. The significant difference in maximal ADP-stimulated respiration between control and ischaemic fibres is reflected by the differing asymptotic values of O_2 uptake. As can be seen in Figure 3a, in the absence of creatine, the K_m for ADP was high in control LV and was significantly diminished by 24.5% in Isch LV (679 ± 79 in Isch vs. 899 ± 84 μM of ADP in control, $P < 0.05$).

Creatine addition induced a significant decrease in K_m in both Isch and control LV (66 ± 7 in Isch and 52 ± 6 μM of ADP in control) but the CK efficacy as evaluated by the ratio between the apparent K_m for ADP in the absence of creatine and in the presence of creatine, was significantly decreased by 35.7% in Isch compared with control LV (11.6 ± 2.5 in Isch vs. 18.0 ± 2.2 in control, $P < 0.05$, Fig. 3b), showing an impairment of the coupling between the oxidative phosphorylation process and the mitochondrial creatine kinase. There was no difference in the K_m values with creatine between Isch and control LV (Fig. 3a).

Discussion

This study investigated the metabolic profile of ischaemic heart by measuring the functional capacities of mitochondria to oxidize different substrates after coronary artery ligation *in vivo* in experimental pigs, model close to acute human myocardial Isch.

The main results demonstrate that acute *in vivo* Isch impaired qualitatively the cardiac mitochondrial function measured *in situ*. Theses alterations were characterized by (1) a specific reduction of myocardial maximal oxidative capacities to use pyruvate and glutamate-malate as substrates whereas mitochondrial capacity to oxidize palmitoyl carnitine was maintained; (2) a decrease of functional efficacy of the mitochondrial creatine kinase, altering the cellular energy transfer system.

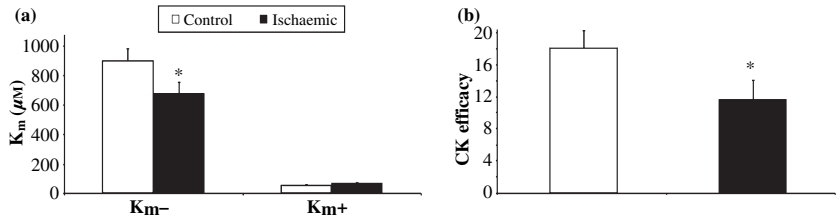

Figure 3 (a) K_m for ADP of *in situ* mitochondria from control and ischaemic myocardial fibres. The ADP-stimulated respiration was measured as a function of ADP with (K_m+) or without (K_m–) creatine (20 mM) in skinned fibres. The apparent K_m for ADP was calculated using a non-linear fitting of the Michaelis–Menten equation. Significantly different from control, $^*P < 0.05$. (b) Creatine Kinase efficacy of *in situ* mitochondria from control and ischaemic myocardial fibres. It represents the coupling between oxidative phosphorylation and the mitochondrial creatine kinase, and was calculated as the ratio of the apparent K_m for ADP in the absence to that in the presence of creatine. Data are means ± SEM. Significantly different from control, $^*P < 0.05$.

Substrates-specific alterations of mitochondrial function

Specific mitochondrial function impairments with glutamate-malate and pyruvate. Myocardial maximal oxidative capacities with pyruvate and glutamate-malate decreased by about 20% after 45 min of *in vivo* Isch in the experimental pigs. Similarly, in the isolated rat heart model, 30 min of Isch reduced the maximal mitochondrial respiration by approximately 30% when using pyruvate-malate as substrates (Kay *et al.* 1997). Impairments of maximal oxidative capacities with pyruvate and glutamate-malate are in line with the mitochondrial ultrastructural and functional injuries occurring early in the course of Isch (Rouslin 1983, Asimakis & Conti 1984, Huang & Liedtke 1989, Lesnefsky *et al.* 1997, Suleiman *et al.* 2001, Xiao & Allen 2003). The glutamate-malate oxidation, which bypassed PDH complex, was altered in the same proportion than pyruvate oxidation, suggesting that a specific decrease of PDH activity is unlikely to explain the decrease of carbohydrate oxidation.

Interestingly, this specific decrease of V_{max} when pyruvate substrate was used, support a relative increase in respiration rate with palmitoyl carnitine in the ischaemic LV, demonstrating that the mitochondrial metabolic pathways were differently affected in ischaemic myocardium.

Preservation of mitochondrial V_{max} with palmitoylcarnitine. The fatty acid oxidation with palmitoylcarnitine implies the carnitine-acylcarnitine translocase and CPTII located on the matrix side of the inner mitochondrial membrane. These enzymes transfer acyl-Coa within the mitochondrial matrix to sustain β-oxidation and to produce acetyl-CoA taken up by the citric acid cycle. The reducing equivalents produce fuel to both complex I and II of the phosphorylation chain (Ponsot *et al.* 2005).

A major observation of the present study is the preservation of the mitochondrial capacity to oxidize carnitine palmitoyl after 45 min Isch. This finding demonstrates that fatty acids oxidation pathway, downstream from the carnitine palmitoyl transferase I (CPT-I), was not functionally impaired after acute Isch.

It is well known that fat oxidation is less efficient in energy production than oxidation of carbohydrates (Hinkle *et al.* 1991, Taegtmeyer 2002). In our experimental conditions, this is further supported by the fact that lipid oxidation does not stimulate the mitochondrial respiratory chain at the maximal power (only at 72% of the maximum, compared with pyruvate V_{max}). This might explain why in our ischaemic conditions, a 20% decrease of the maximal power of the mitochondrial respiratory chain (i.e. observed with pyruvate V_{max}) could have no functional consequences on lipid V_{max}, affording for the specific preservation of lipid metabolism after Isch.

Consequences of the substrates-specific mitochondrial alterations. A key to better understand energy metabolism in the heart may be found in the comprehension that the heart, a metabolic omnivore, is readily able to switch from one substrate to another. Switches in substrate utilization occur in response to multiple stimuli, including substrate availability, coronary blood flow, oxygen supply, circulating hormones, and workload of the heart (Taegtmeyer 2002). This property allows the heart to choose the most efficient substrate for a given physiologic environment, a phenomenon defined by Taegtmeyer *et al.* (2004) as *metabolic flexibility*. Theses authors proposed that the preservation of metabolic flexibility is critical in the normal adaptation of the heart to an external stimulus (Taegtmeyer *et al.* 2004). The specific impairments of the pyruvate pathway after acute Isch represent a loss of metabolic flexibility in the heart, which could become an early feature of metabolic dysregulation in the failing heart. Accordingly, an acute increased heart work could be associated with a deficit in ATP production pathway in ischaemic myocardium since, in this condition, the preferentially mitochondrial substrate oxidized will be pyruvate (Goodwin *et al.* 1998).

Alteration of the mitochondrial respiration control

Besides the disclosure of a specific functional alteration of glucose oxidation pathway, we looked for Isch-induced alterations in respiration control by ADP and modifications of mitochondrial creatine kinase (CK) coupling with oxidative phosphorylation. Indeed, such abnormalities could participate to the mismatch between the sites of energy production and the sites of energy utilization.

Firstly, the ACR, proportional to the functional coupling between oxidations and phosphorylations, remained unchanged after Isch suggesting that the electron transport in the respiratory chain was correctly coupled to the synthesis of ATP (Saks *et al.* 1998). The ACR of our pig cardiac fibres are in agreement with data obtained in rat's heart suggesting the appropriate quality of the preparation (Saks *et al.* 1998).

Secondly, we observed that the sensitivity of mitochondrial respiration to external ADP, which is low in control heart as it has been described in rodents (Saks *et al.* 1995, Ventura-Clapier *et al.* 1998), was increased after 45 min of *in vivo* Isch, decreasing the coupling between mitochondrial CK and mitochondrial oxidative phosphorylation process.

This is in agreement with data obtained in a model of isolated heart demonstrating early alterations related to

the control of energy production after 30 min of Isch (Kay *et al.* 1997), and with previous works showing that activities of total, mitochondrial and MM isoenzymes of CK were decreased in post-ischaemic myocardium (Greenfield & Swain 1987, Neubauer *et al.* 1988). Our findings extend these previously results to a model closer to human myocardial Isch and infarction, and demonstrated that in contrast to isolated heart model (Kay *et al.* 1997), the ACR was not altered, and the sensitivity of mitochondria for ADP was also more increased in isolated heart than in our *in vivo* model (respectively +55% vs. +24%). Therefore, *in vivo* myocardial Isch may be less harmful for intracellular energy transfer systems than isolated heart Isch.

Nevertheless, this change in CK channelling efficacy at the level of mitochondria represents an important impairment of energy distribution and signal channelling between sites of cellular energy production and utilization (Ventura-Clapier *et al.* 1998, Kaasik *et al.* 2001). Several mechanisms can contribute to produce these alterations: an increase in Pi concentration and a decrease in pH, resulting from cardiac Isch (Veksler & Ventura-Clapier 1994), as well as the Isch-induced alterations at the level of the outer mitochondrial membrane and intermembrane space, may participate in the decrease of functional mitochondrial CK activity (Kay *et al.* 1997).

Interestingly, theses disturbances of mitochondrial CK coupling have been demonstrated in a model of chronic heart failure, making the myocyte enter a vicious cycle of energy mismatch and calcium dysregulation, especially in period of increased workload (De Sousa *et al.* 1999). The phosphocreatine shuttle is not necessary to maintain a low, stable levels of intracellular energetic flux, but is definitely required to fuel the ATPases when the contractile reserve of the heart is recruited, in particular in condition of increased heart rate. This adaptation process has also been shown in the *vastus lateralis* muscle of athletic subjects, confirming the importance of this system in condition of high energetic demand (Zoll *et al.* 2002, 2003). Thus, alteration of the phosphocreatine shuttle decreases the adjustments of ATP production to energetic demand, especially in conditions of increased workload and adrenergic stimulation that prevail *in vivo* during the first phases of acute myocardial infarction.

Conclusions

This work demonstrates the existence of specific mitochondrial impairments at the level of pyruvate but not lipid oxidations after an acute period of *in vivo* Isch, suggesting a decrease of the metabolic flexibility of the heart.

Finally, together with alterations of the creatine kinase channelling system, not only quantitative but also qualitative mitochondrial alterations come out following acute Isch. Such impairments could have important consequences for the adjustment of ATP production to ATP demand, especially in conditions of increased workload.

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