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The role of mitochondria in the salvage and the injury of the ischemic myocardium

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Abstract

The relationships between mitochondrial derangements and cell necrosis are exemplified by the changes in the function and metabolism of mitochondria that occur in the ischemic heart. From a mitochondrial point of view, the evolution of ischemic damage can be divided into three phases. The first is associated with the onset of ischemia, and changes mitochondria from ATP producers into powerful ATP utilizers. During this phase, the inverse operation of F_0F_1 ATPase maintains the mitochondrial membrane potential by using the ATP made available by glycolysis. The second phase can be identified from the functional and structural alterations of mitochondria caused by prolongation of ischemia, such as decreased utilization of NAD-linked substrates, release of cytochrome *c* and involvement of mitochondrial channels. These events indicate that the relationship between ischemic damage and mitochondria is not limited to the failure in ATP production. Finally, the third phase links mitochondria to the destiny of the myocytes upon post-ischemic reperfusion. Indeed, depending on the duration and the severity of ischemia, not only is mitochondrial function necessary for cell recovery, but it can also exacerbate cell injury. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Mitochondrion; Heart; Ischemia; Cardiomyocyte; Calcium

1. Introduction

At first glance, a discussion about the participation of mitochondria in cell death might seem obvious. Indeed, the loss of mitochondrial function inevitably leads to cell necrosis, whereas an optimal energy metabolism is required to preserve cell viability. However, temporal, qualitative and quantitative aspects of the multi-faceted relationship between mitochondrial function and cell life have still to be elucidated. For instance, is mitochondrial failure an early step in the cascade of events leading to cell death? Is the alteration or the loss of a specific mitochondrial function necessary to commit cells to death? How much inhibition of mitochondrial function is required to jeopardize cell survival?

These and similar questions are the subjects of other papers in the present thematic issue of *Biochimica et Biophysica Acta*. Here we summarize the

Abbreviations: PFK, phosphofructokinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; $[Mg^{2+}]_i$, intracellular concentrations of Mg^{2+}; $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$, cytosolic and mitochondrial concentrations of Ca^{2+}; IF_1, ATPase inhibitor protein; ANT, adenine nucleotide translocase; $\Delta\psi_m$, mitochondrial membrane potential; LCACoA, long chain acyl-coenzyme A; ROS, reactive oxygen species; APD, action potential duration; PN, pyridine nucleotides; BDM, 2,3-butanedione monoxime

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changes in the function and metabolism of mitochondria in the ischemic heart to exemplify the complex relationships between mitochondrial derangements and cell necrosis. Apoptosis will not be considered thoroughly, since no information is available on the possible role of myocardial mitochondria in this type of cell death. On the other hand, the link between mitochondria and other myocardial pathologies is reviewed by Di Mauro in this same issue.

2. Functional and metabolic consequences of ischemia

In the heart, oxygen supply is generally reduced by coronary obstruction resulting in a critical reduction of flow (ischemia). In the affected region deprived of oxygen, drastic changes of metabolism and lack of adequate washout determine an abnormal accumulation of ions and metabolites.

2.1. Ischemia and energy metabolism

At a cellular level, the onset of ischemia is determined by an insufficient availability of oxygen for mitochondrial oxidations. As a consequence of the reduced or absent oxidative phosphorylation, intracellular creatine phosphate is rapidly depleted with a concomitant rise in P_i, both factors stimulating glycolysis and lactate production [1]. The accumulation of lactate and the hydrolysis of ATP decrease intracellular pH. During the early phase of ischemia, the failure of contraction and the rigor contracture, which are the most relevant changes in myocardial function, are caused by intracellular acidosis and severe reduction in ATP content, respectively [1–5]. Besides these relevant functional changes, several cellular activities are modified by the fall in pH and ATP content, including the inhibition of glycolysis which is observed under conditions of severe ischemia [6]. A low pH and the accumulation of lactate decrease the activity of both phosphofructokinase (PFK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [6]. It is also likely that PFK activity becomes depressed owing to the gradual lack of ATP, although hexokinase, inhibited in deenergized synaptosomes [7], is active in the ischemic myocardium as shown by the accumulation of glucose-6-phosphate [6]. In addition, we have recently shown that cytosolic proteins bind to myofibrillar proteins during the initial period of ischemia [8]. ATP depletion appears to be involved in the binding of GAPDH to myofibrils, which is associated with the loss of enzymatic activity. Similarly, the fall in ATP is associated with the binding to membranes and inactivation of PFK [9]. Thus, the failure in mitochondrial ATP production, which initially stimulates glycogen breakdown and glycolysis, is followed by the inhibition of the only alternative pathway for energy production in the cell.

Mitochondria not only cease to be the major ATP producers of the cell, but they can also become its major consumers owing to the hydrolytic activity of the F_0F_1 ATPase [10–12]. The inverse operation of F_0F_1 ATPase has also been recognized as a prominent pathway for ATP consumption in other cell types [13,14].

2.2. Mitochondrial information from intact cardiomyocytes

The temporal relationship between mitochondrial alterations and the evolution of ischemic damage cannot be elucidated entirely in the intact organ. Indeed, the function of mitochondria extracted from a damaged tissue and assayed in vitro does not allow a straight appraisal of the behavior of those same mitochondria in situ. The availability of both isolated cardiomyocytes and a variety of optical probes permitted the study of anoxia at the single cell level. Isolated cardiomyocytes show a rod shaped morphology and do not display spontaneous contractions. Rhythmic contractions can be evoked by electrical stimulation. Under deenergizing conditions, rod shaped myocytes can either change into square forms (rigor state or contracture) or hypercontract into rounded dysfunctional forms in which the typical sarcomeric striature is no longer distinguishable (hypercontracture) ([15,16], reviewed in [17]).

At the single cell level, hypercontracture is the paradigm of irreversible damage and is considered equivalent to the abrupt rise in diastolic pressure in the absence of contractile activity which is observed in the intact heart when reperfusion takes place after prolonged ischemia. However, in the intact heart irreversible damage is characterized by the loss of cell membrane integrity, easily demonstrated by the re-

lease of intracellular proteins in the coronary effluent. Conversely, hypercontracted myocytes often retain the integrity of sarcolemma, as demonstrated by the retention of small molecules such as intracellularly trapped fluorescent probes. The difference between single cells and intact hearts is likely explained by the lack of cell attachments. The same morphological distortion which determines the hypercontracture in isolated myocytes could probably cause the rupture of the sarcolemma in the intact heart by tearing off adjacent myocytes [18].

In single adult cardiomyocytes the relationship between cell function and mitochondrial membrane potential ($\Delta\psi_m$) was investigated by using JC-1, a carbocyanine derivative [12]. In addition, $\Delta\psi_m$ measurements were compared with the changes in the intracellular concentration of Mg^{2+} ($[Mg^{2+}]_i$) [12,19]. Since inside the cell most Mg^{2+} is bound to ATP, $[Mg^{2+}]_i$ increases mostly as a consequence of a fall in ATP content [19].

Anoxia does not induce an immediate drop in $\Delta\psi_m$. If glucose is not removed from the superfusing buffer, myocytes are not affected by a lack of oxygen. Their ability to twitch is maintained along with no changes in $\Delta\psi_m$ and $[Mg^{2+}]_i$. Anoxic changes can be observed by superfusing or incubating anoxic myocytes in glucose-free media. Under these conditions, the electrically-stimulated contraction is not modified during the initial anoxic phase. After a variable period of time (15–25 min), contraction fails before rigor contracture ensues. Only a limited reduction of $\Delta\psi_m$ occurs before asystole. Then, after the failure of contraction, ATP content falls, as shown by a gradual increase in $[Mg^{2+}]_i$, which reaches a plateau at the onset of rigor [19] when $\Delta\psi_m$ rapidly collapses [12]. Thus, the onset of rigor is concomitant with ATP depletion, as it was first suggested on the basis of results obtained in myocyte suspensions [21] and then directly confirmed in single myocytes injected with luciferase [21,22]. Both the contracture and the fall in $\Delta\psi_m$ occur in the absence of major changes in cytosolic and mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_c$ and $[Ca^{2+}]_m$) which rise progressively only after the onset of ATP-depletion contracture [23]. This sequence of events has also been reported in myocytes treated with cyanide and deoxyglucose [24].

These results support the concept that mitochondria maintain their $\Delta\psi_m$ by using glycolytically produced ATP. The fact that an active mitochondrial ATPase is necessary for the maintenance of $\Delta\psi_m$ is demonstrated by the rapid and complete collapse of $\Delta\psi_m$ occurring in oligomycin-pretreated myocytes [12,24]. This mechanism does not seem to pertain exclusively to cardiac myocytes. The acceleration of anoxia-induced mitochondrial depolarization after oligomycin administration has also been shown in carotid body type I cells [25].

2.3. F_0F_1 ATPase and ANT

Several factors are likely to limit the extent of ATP hydrolysis by mitochondria in situ. As it has recently been pointed out by Rouslin, in intact hearts, even in the presence of uncouplers, the rates of ATP depletion are far lower than those measurable in sonicated heart mitochondria [26]. ATPase activity is probably reduced as a consequence of intracellular acidosis. A pH decrease from 8.0 to 6.0 causes a two-fold reduction in ATPase activity [26]. In large mammals with slow heart rates a major role has been attributed to the ATPase inhibitor protein (IF_1) [27], which has been shown to reduce ATPase activity by 80% or more [29,30]. This possibility for ATP salvage is probably limited in small mammals by the scarce content of IF_1 [26,29].

The contribution of adenine nucleotide translocase (ANT) to ischemic damage is controversial. Under physiological conditions, the electrogenic exchange of ADP taken up by mitochondria with ATP released into the cytosol is driven by $\Delta\psi_m$. The inverse operation, which is necessary to move the glycolytically produced ATP into the matrix, could be reduced depending on the values of $\Delta\psi_m$ which result from the reverse proton pumping activity of F_0F_1 ATPase. Thus the build up of $\Delta\psi_m$ by means of ATP hydrolysis would serve as a brake to the further utilization of cytosolic ATP. However, the fact that ATP depletion induced by ischemia is slowed by oligomycin [11,30] rules out a primary role for ANT. On the other hand the inhibition of ANT in the ischemic heart has been suggested by several reports [30–32]. One major factor responsible for such inhibition should be the accumulation of long chain acyl-CoA (LCACoA) which occurs in ischemic hearts as a consequence of the inhibition of fatty acid oxidation [30,33,34]. The addition of these CoA esters to iso-

lated mitochondria inhibits ANT [35] in a process which is exacerbated by Ca^{2+} [36] and blunted by several cations such as Mg^{2+} and polyamines [37,38]. The abundance of these cations in the matrix could explain the lack of ANT inhibition, when LCACoA concentration is increased on the matrix side [39]. In addition, the mitochondrial content of total CoA (free+esterified) [33] is below the K_i of LCACoA for ANT [39] and the concentration of 'free' LCACoA is probably reduced in situ by the binding with various proteins [40], although it is possible that these esters do not accumulate homogeneously, reaching very high local concentrations within the hydrophobic core of the membranes.

2.4. Structural and functional alterations of mitochondria in the ischemic heart

The relationship between ischemic damage and mitochondria is not limited to the failure in ATP production. Indeed, if the oxygen restriction is maintained, mitochondria themselves become targets for ischemic damage, decreasing the chances of recovery for both metabolism and function. Mitochondria isolated from ischemic hearts show a pronounced decrease in respiration rates in spite of a normal or slightly reduced capacity for ATP production [41,42]. An increase in proton leak has been suggested to contribute to such alterations [41].

It has been known for more than 40 years that the structure of mitochondria is altered by a prolonged period (i.e., more than 30 min) of ischemia [43,44]. The morphologic changes include swelling, loss of matrical density and accumulation of dense granules. In a series of seminal studies Jennings first linked these morphological changes to the transition from reversible to irreversible injury [45] and then demonstrated that mitochondrial function is also compromised. In particular, mitochondria from ischemic tissues were unable to utilize pyruvate and α-ketoglutarate, whereas succinate oxidation was almost unaffected [46].

Supporting this initial observation, Complex I appears to be especially sensitive to ischemic injury [47–49]. In addition, we found that $\Delta\psi_m$ and oxidative phosphorylation are depressed even with succinate as the respiratory substrate when mitochondria from ischemic hearts are exposed in vitro to high Ca^{2+} [50]. The causes for impairment of the respiratory chain complexes are not known yet. One possible mechanism could be the generation of oxyradicals (ROS) which increases in mitochondria harvested from ischemic hearts [51]. Since ROS are able to elicit specific damage at the level of respiratory chain components [52], a vicious cycle of increasing damage could be generated. For instance, the oxidation of sulfhydryl groups consequent to ROS production is likely involved in the inhibition of other mitochondrial proteins, such as carnitine translocase [53].

Oxygen consumption could be reduced also by the loss of cytochrome *c*, which is released in the coronary effluent during anoxia in perfused guinea pig hearts [54]. Since in several cell types a relevant step of the apoptotic pathway appears to be the activation of caspase(s) by cytochrome *c* upon its release from mitochondria (see D. Jones' review in this same issue), it is tempting to speculate that cytochrome *c* might also trigger apoptosis in the heart. This is consistent with the notion that apoptosis occurs together with necrosis in post-ischemic reperfusion [55,56]. Preliminary results suggest that apoptosis in human failing hearts is associated with both a prevalent localization of cytochrome *c* around myofibrils and caspase activation [57].

Despite the alterations induced by ischemia in the respiratory chain, upon reperfusion oxidative phosphorylation is still active [58] and oxygen consumption is disproportionately high relative to the reduced contractility [59,60]. Indeed, as mentioned above, mitochondrial function is required both for myocardial recovery after a short period of ischemia as well as for its irreparable damage after a prolonged oxygen deprivation.

2.5. Metabolic derangements

2.5.1. CoA and carnitine

In addition to the above mentioned effects of LCACoA on ANT, these esters have been recently shown to inhibit the mitochondrial K_{ATP} channel by interacting with its cytosolic side [61]. This inhibition could be relevant in the evolution of the ischemic damage. In fact, agents which inhibit K_{ATP} channels abolish the protective effects exerted by K_{ATP} openers against the ischemic damage [62,63]. This beneficial effect was originally attributed to the opening of

similar channels located in the plasma membrane which should produce a cardioplegic effect resulting from the shortening of the action potential duration (APD) and the consequent reduction of Ca^{2+} entry. However, a cardioprotective effect by K_{ATP} openers was demonstrated in the absence of both APD changes and cardiodepression, ruling out the involvement of the sarcolemma K_{ATP} channel [64,65]. On the other hand, the mitochondrial K_{ATP} channel is likely responsible for the cardioprotection since the ischemic damage is also reduced by a selective mitochondrial K_{ATP} opener, diazoxide [66].

It has been suggested that K_{ATP} channels are also involved in preconditioning [67] which is the ability of a short period of ischemia to protect the heart from a further prolonged ischemic episode [68]. This phenomenon, which resembles the protection afforded by an initial exposure to sublethal concentrations of toxic agents in other cell types [69], implies that the physiological or pharmacological opening of the mitochondrial K_{ATP} channel is an early step in a self-defense mechanism. The maintenance of ATP contents in ischemic hearts treated with K_{ATP} openers [70] suggests that a reduced rate of ATP hydrolysis might underlie the cardioprotection. However, in the absence of an obvious link between opening of mitochondrial K_{ATP} channels and ATP salvage pathways, the protective mechanism is still far from being elucidated.

In isolated mitochondria as well as in the intact heart, LCACoA toxicity could be ascribed in part to a decreased CoASH availability. The role of CoASH/esterified-CoA and carnitine/esterified carnitine ratios in the evolution of ischemic damage has been investigated in isolated rat hearts perfused with glucose-containing buffer both in the presence and absence of propionate [50]. The ischemic damage was exacerbated by propionate owing to the reduction in both CoASH and carnitine contents. Indeed, the damaging effect of propionate was prevented by carnitine which restored the ratios between the free and esterified forms of CoA and carnitine.

The relevance of CoASH and carnitine availability for proper myocardial function is also supported by other reports. When the energy demand is increased, i.e., in the working heart perfusion system, propionate affects cardiac performance even under aerobic conditions and in the presence of glucose [71]. In addition, in working hearts perfused with acetoacetate, a fall in TCA cycle intermediates induced by inhibition of α-ketoglutarate dehydrogenase was associated with contractile failure [72,73].

Anoxia or ischemia also result in an increase of the esterified/free carnitine ratio [74]. This metabolic shift, which reproduces the analogous modification of CoA status, is caused by the inhibition of mitochondrial dehydrogenases consequent to the excess of reduced flavin and pyridine coenzymes. The reduced rates of both β-oxidation and the TCA cycle freeze CoA in the form of LCACoA or SCACoA, while available carnitine acts as a scavenger of acyl moieties to liberate CoA. This action of carnitine appears to be pertinent to pyruvate utilization. In fact, in hypoxic tissues pyruvate is mostly converted to lactate due to PDH inhibition. By decreasing the acetyl-CoA/CoA ratio, carnitine might stimulate PDH, thus diverting pyruvate from reduction to lactate and causing its oxidation to acetyl-CoA and then acetylcarnitine. Experimental and clinical evidence supports these concepts. Glucose oxidation is stimulated by carnitine in isolated hearts perfused under both normoxic and ischemic conditions [75,76]. In addition, carnitine administration was shown to reduce lactate formation in subjects suffering from coronary artery disease [77]. Similar results were also obtained in patients suffering from intermittent claudication [78].

2.5.2. *Pyridine nucleotides*

While several studies documented the expected increase in $NADH(H^+)/NAD^+$ ratio following a critical reduction in tissue oxygenation [79,80], the changes in the total content of pyridine nucleotides (PN) have been overlooked. Since the majority of PN is located within the mitochondrial matrix, it would be important to determine if the decrease in PN content induced by ischemia [81,82] occurs to the same extent in every cell compartment. In any case, a severe reduction in PN content implies that the mitochondrial pool is also affected. This appears to be the case upon post-ischemic reperfusion when tissue PN content is reduced to less than 30% of that measurable in normoxic controls (F. Di Lisa et al., unpublished data). Then the problem becomes that of the possible mechanisms causing the disappearance of mitochondrial PN. In isolated mitochondria a rapid

PN depletion is obtained upon incubation in the presence of high [Ca^{2+}] [83,84]. It has been proposed that NAD^+ is hydrolyzed by a Ca^{2+}-stimulated glycohydrolase located in the matrix [85]. Subsequently, ADP ribose formed by this hydrolysis would stimulate, by means of covalent modification, an intrinsic protein of the inner mitochondrial membrane which was suggested to be part of a specific pathway for mitochondrial Ca^{2+} efflux [86]. Such a mechanism was not supported by a careful investigation of the location of NAD^+ glycohydrolase, which rather ruled out its presence within the matrix space [87]. Accordingly, the PN pool of mitochondria can be hydrolyzed only upon its release into the intermembrane space. It is tempting to speculate that such an efflux might occur by means of MTP opening. This hypothesis is supported by the inhibition of mitochondrial PN degradation by CsA [88] which lacks any effect on NAD^+ glycohydrolase (F. Di Lisa et al., unpublished data). Thus, the measurement of mitochondrial PN content could represent a valuable tool for investigating MTP opening in intact cells or tissues.

3. Mitochondria in the transition from reversible to irreversible injury

Prolongation of ischemia (i.e., more than 20 min of no flow ischemia in perfused hearts) leads myocytes to a point of no return beyond which even readmission of oxygen fails to rescue cell viability. The concept of irreversible damage was originally defined by Jennings in 1957 as ''injury of sufficient severity and duration that the involved cells will continue to degenerate and become necrotic despite reoxygenation by reperfusion of arterial blood'' [44] (for a review of these early observations see [89]).

Myocardial tissue exhibits a peculiar transition from reversible to irreversible damage. In every cell type the lack of ATP production and a severe decrease of its content result in a slow degenerative process which eventuates in the loss of plasma membrane integrity with release of intracellular components. Thus, if the initial pathological stimulus (i.e., lack of oxygen) is not removed, the inhibition or the alteration of mitochondrial function result in cell death. In addition to this common mode of cell death, which occurs *gradually*, the myocardium exhibits a peculiar transition to irreversible injury, which occurs *suddenly* when oxygen supply is reestablished after a prolonged period of ischemia [89]. In this case the uncontrolled hypercontracture of myofilaments is associated with a rapid increase of cell membrane permeability followed by the release of intracellular constituents, such as enzymes [18,89]. The sudden changes produced by reperfusion on myocardial viability were concomitant with massive accumulation of calcium within the mitochondrial matrix [90,91].

This seminal finding highlighted the role of intracellular Ca^{2+} overload in myocardial pathology. However, the cautious comment of Jennings (''whether the calcium uptake is causally related to the development of irreversibility remains to be investigated'' [90,91]), was later overlooked by many investigators. In addition, the finding of mitochondrial calcium precipitates in situ as well as in isolated mitochondria [92] prompted a widespread interpretation of mitochondrial Ca^{2+} uptake as a pathological process. Only during the 1980's and early 1990's did mitochondrial Ca^{2+} homeostasis gain back its relevance in cell physiology owing to the recognition of the Ca^{2+} requirement for the activity of several dehydrogenases (reviewed in [93,94]), as shown for the first time with α-glycerophosphate dehydrogenase [95].

The mechanisms underlying the onset of irreversible damage are still a matter of debate and a detailed discussion about the various hypotheses is outside the scope of the present paper [96–99]. What is relevant for this review is that the rapid transition towards cell death requires a coupled mitochondrial respiration. This apparent paradox was first reported by Ganote, who demonstrated that cyanide blunted the enzyme release which occurs upon post-ischemic reperfusion in rat hearts [100]. Subsequently, the inhibition of the respiratory chain or the addition of uncouplers of oxidative phosphorylation were shown to limit the extent of enzyme release in different models of myocardial damage such as post-ischemic reperfusion [101], calcium paradox (reintroduction of Ca^{2+} after Ca^{2+}-free perfusion) and oxygen-paradox (readmission of oxygen after anoxia) [102,103]. These findings, obtained on perfused hearts and isolated myocytes, suggest that restoration of ATP produc-

tion by mitochondrial oxidative phosphorylation is essential for cell recovery, but can also contribute to the processes causing cell necrosis.

It could be hypothesized that upon reoxygenation mitochondria accumulate Ca^{2+} rather than producing ATP. The consequent failure of the mechanisms which extrude Ca^{2+} out of the sarcoplasm would result in a vicious cycle of a massive intracellular Ca^{2+} rise and mitochondrial derangement. In addition, the rise in $[Ca^{2+}]_i$ could promote opening of the cyclosporin-sensitive mitochondrial permeability transition pore, leading to a sudden $\Delta\psi_m$ dissipation (as reviewed in [104] and in this same issue by A. Halestrap). In general, any conditions associated with intracellular Ca^{2+} overload eventually result in hypercontracture. Under these circumstances, the increase in $[Ca^{2+}]_i$ precedes $\Delta\psi_m$ collapse [105]. For instance, in isolated cardiomyocytes exposed to conditions which determine Ca^{2+} overload, such as Ca^{2+} paradox (i.e., Ca^{2+} readmission to myocytes previously bathed in the absence of Ca^{2+}), a transient increase in $\Delta\psi_m$ is followed by its rapid and persistent collapse [105]. However, studies on isolated myocytes provide clear evidence that the transition towards the point of no return does not require Ca^{2+} overload and mitochondrial failure. In fact, upon reoxygenation, $\Delta\psi_m$ is partially restored independent of recovery of mechanical function, as demonstrated by the fact that FCCP addition is able to induce a further fall of $\Delta\psi_m$ in hypercontracted myocytes [12]. $\Delta\psi_m$ is likely utilized to produce ATP since, irrespective of the functional outcome, $[Mg^{2+}]_i$ decreases gradually to control levels [12,19]. Similarly, the removal of metabolic inhibition (cyanide plus deoxyglucose) was shown to induce hypercontracture along with a sudden recovery of ATP contents in cardiomyocytes microinjected with luciferase [22].

As far as Ca^{2+} homeostasis is concerned, reoxygenation is followed by a rapid fall of $[Ca^{2+}]_c$ towards preanoxic levels [23,106], whereas $[Ca^{2+}]_m$ shows a modest and transient decrease which is followed by a secondary rise. Interestingly, it appears that anoxic levels of $[Ca^{2+}]_m$ are inversely related to the probability of cell recovery upon reoxygenation, suggesting that even a slight increase in intramitochondrial Ca^{2+} levels could affect the anoxic damage [23].

Different effects of reoxygenation on intracellular $[Ca^{2+}]$ have been reported in cardiomyocytes from other animal species. For instance, in guinea pig cardiomyocytes a transient $[Ca^{2+}]_c$ rise [107,108] was related to the abundance of the sarcolemmal Na^+/Ca^{2+} exchanger in this species [109]. Nevertheless, since in isolated myocytes the irreversible damage occurs in the absence of large changes in intracellular Ca^{2+}, the large increase in tissue calcium which is observed in the intact heart upon reperfusion is probably the consequence rather than the cause of sarcolemma rupture. In addition, the recovered $\Delta\psi_m$ is utilized mostly to increase the cellular ATP content rather than to fill mitochondria with Ca^{2+}.

If $\Delta\psi_m$ is restored, ATP content increases and intracellular Ca^{2+} is not increased. Why, then, is mitochondrial function required to cause hypercontracture or tissue injury upon reoxygenation? This apparent paradox is probably related to the different ATP requirements of contraction and relaxation at the myofilament level, as shown by the elegant study of Altschuld et al. on permeabilized myocytes [110]. In this model, the absence of ATP produced contracture, whereas hypercontrature was obtained in the presence of submillimolar concentrations of ATP. Although these changes could be produced in the presence of EGTA, Ca^{2+} increased the ATP requirements for the maintenance or the recovery of the elongated morphology. Accordingly, a suboptimal mitochondrial function could produce low levels of ATP which, in the presence of even a modest rise in $[Ca^{2+}]_i$, might be sufficient for the formation of rigor bonds but not for the relaxation process, resulting in the hypercontracture. These concepts were validated by using 2,3-butanedione monoxime (BDM) which inhibits contraction by both interacting with myosin and reducing the sensitivity of myofibrils to Ca^{2+}. Indeed, when anoxic cardiomyocytes were supplemented with BDM just prior to reoxygenation, hypercontracture was prevented and the cells recovered their elongated morphology upon BDM withdrawal [111]. Since enzyme release from the reperfused heart is reduced by BDM as well [112], the hypercontracture appears to be a primary event in the generation of the irreversible damage. Thus, the severe intracellular calcium overload which follows the rupture of the plasma membrane would be in most cases the consequence rather than the cause of cell death. This concept should be taken into account when Ca^{2+}-dependent activities are suggested as causative

factors in the generation of the irreversible damage. For instance, based on the available evidence [113] the cause-effect relationship between MTP opening and reperfusion requires remains a matter of debate [104]. In a very fast sequence of events MTP opening could be the initiator of the process leading to sarcolemma rupture and enzyme release or it could be just one of the consequences of the intracellular flooding with extracellular Ca^{2+}. The powerful cooperation between cellular and molecular biology will undoubtedly help solve this question, and will be instrumental in clarifying the mechanisms by which mitochondria rule the complex balance between life and death.

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References

[1] D.G. Allen, C.H. Orchard, Circ. Res. 60 (1987) 153–168.
[2] W.G. Nayler, P.A. Poole Wilson, A. Williams, J. Mol. Cell. Cardiol. 11 (1979) 683–706.
[3] C. Hohl, A. Ansel, R. Altschuld, G.P. Brierley, Am. J. Physiol. 242 (1982) H1022–H1030.
[4] P.B. Kingsley, E.Y. Sako, M.Q. Yang, S.D. Zimmer, K. Ugurbil, J.E. Foker, A.H. From, Am. J. Physiol. 261 (1991) H469–H478.
[5] J.A. Lee, D.G. Allen, J. Clin. Invest. 88 (1991) 361–367.
[6] M.J. Rovetto, W.F. Lamberton, J.R. Neely, Circ. Res. 37 (1975) 742–751.
[7] M. Erecinska, D. Nelson, J. Deas, I.A. Silver, Brain Res. 726 (1996) 153–159.
[8] R. Barbato, R. Menabò, P. Dainese, E. Carafoli, S. Schiaffino, F. Di Lisa, Circ. Res. 78 (1996) 821–828.
[9] S.L. Hazen, M.J. Wolf, D.A. Ford, R.W. Gross, FEBS Lett. 339 (1994) 213–216.
[10] W. Rouslin, J.L. Erickson, R.J. Solaro, Am. J. Physiol. 250 (1986) H503–H508.
[11] R.B. Jennings, K.A. Reimer, C. Steenbergen, J. Mol. Cell. Cardiol. 23 (1991) 1383–1395.
[12] F. Di Lisa, P.S. Blank, R. Colonna, G. Gambassi, H.S. Silverman, M.D. Stern, R.G. Hansford, J. Physiol. (London) 486 (1995) 1–13.
[13] R. Imberti, A.L. Nieminen, B. Herman, J.J. Lemasters, J. Pharmacol. Exp. Ther. 265 (1993) 392–400.
[14] J.W. Snyder, J.G. Pastorino, A.P. Thomas, J.B. Hoek, J.L. Farber, Am. J. Physiol. 264 (1993) C709–C714.
[15] C. Hohl, A. Ansel, R. Altschuld, G.P. Brierley, Am. J. Physiol. 242 (1982) H1022–H1030.
[16] M.D. Stern, H.D. Silverman, S.R. Houser, R.A. Josephson, M.C. Capogrossi, C.G. Nichols, W.J. Lederer, E.G. Lakatta, Proc. Natl. Acad. Sci. USA 85 (1988) 6954–6958.
[17] H.S. Silverman, M.D. Stern, Cardiovasc. Res. 28 (1994) 581–597.
[18] C.E. Ganote, J.P. Kaltenbach, J. Mol. Cell. Cardiol. 11 (1979) 389–406.
[19] H.S. Silverman, F. Di Lisa, R.C. Hui, H. Miyata, S.J. Sollott, R.G. Hanford, E.G. Lakatta, M.D. Stern, Am. J. Physiol. 266 (1994) C222–C233.
[20] R.A. Haworth, D.G. Hunter, H.A. Berkoff, Circ. Res. 49 (1981) 1119–1128.
[21] K.C. Bowers, A. Allshire, P.H. Cobbold, J. Mol. Cell. Cardiol. 24 (1992) 213–218.
[22] I. Allue, O. Gandelman, E. Dementieva, N. Ugarova, P. Cobbold, Biochem. J. 319 (1996) 463–469.
[23] H. Miyata, E.G. Lakatta, M.D. Stern, H.S. Silverman, Circ. Res. 71 (1992) 605–613.
[24] A. Leyssens, A.V. Nowicky, L. Patterson, M. Crompton, M.R. Duchen, J. Physiol. (London) 496 (1996) 111–128.
[25] M.R. Duchen, T.J. Biscoe, J. Physiol. (London) 450 (1992) 13–31.
[26] W. Rouslin, R.B. Long, C.W. Broge, J. Mol. Cell. Cardiol. 29 (1997) 1505–1510.
[27] M.E. Pullman, G.C. Monroy, J. Biol. Chem. 238 (1963) 3762–3769.
[28] W. Rouslin, Am. J. Physiol. 252 (1987) H622–H627.
[29] W. Rouslin, C.W. Broge, I.L. Grupp, Am. J. Physiol. 259 (1990) H1759–H1766.
[30] A.L. Shug, J.H. Thomsen, J.D. Folts, Arch. Biochem. Biophys. 260 (1978) 14748–14755.
[31] V. Regitz, D.J. Paulson, R.J. Hodach, S.E. Little, W. Schaper, A.L. Shug, Basic Res. Cardiol. 79 (1984) 207–217.
[32] J.F. Hutter, C. Alves, S. Soboll, Biochim. Biophys. Acta 1016 (1990) 244–252.
[33] J.A. Idell Wenger, L.W. Grotyohann, J.R. Neely, J. Biol. Chem. 253 (1978) 4310.
[34] G. van der Vosse, J.F. Glatz, H.C. Stam, R.S. Reneman, Physiol. Rev. 72 (1992) 881–940.
[35] S.V. Pande, M.C. Blanchaer, J. Biol. Chem. 246 (1971) 402–411.
[36] F. Di Lisa, R. Menabò, G. Miotto, V. Bobyleva Guarriero, N. Siliprandi, Biochim. Biophys. Acta 973 (1989) 185–188.
[37] N. Siliprandi, F. Di Lisa, L. Sartorelli, in W. Gevers, L. Opie (Eds.), Membranes and Muscle, IRL Press, Oxford, 1985, pp. 105–119.
[38] A. Toninello, L. Dalla Via, S. Testa, D. Siliprandi, N. Siliprandi, Cardioscience 1 (1990) 287–294.
[39] K.F. LaNoue, J.A. Watts, C.D. Koch, Am. J. Physiol. 241 (1981) H663–H671.
[40] J.F. Glatz, G. van der Vosse, Mol. Cell. Biochem. 98 (1990) 237–251.

[41] V. Borutaite, V. Mildaziene, G.C. Brown, M.D. Brand, Biochim. Biophys. Acta 1272 (1995) 154–158.
[42] L. Kay, V.A. Saks, A. Rossi, J. Mol. Cell. Cardiol. 29 (1997) 3399–3411.
[43] R.E. Bryant, W.A. Thomas, R.M. O'Neal, Circ. Res. 6 (1958) 699–709.
[44] R.B. Jennings, W.B. Wartman, Z.E. Zudyk, Arch. Pathol. 63 (1957) 580–585.
[45] P.B. Herdson, H.M. Sommers, R.B. Jennings, Am. J. Pathol. 46 (1965) 367–386.
[46] R.B. Jennings, P.B. Herdson, H.M. Sommers, Lab. Invest. 20 (1969) 548–557.
[47] W. Rouslin, R.W. Millard, Am. J. Physiol. 240 (1981) H308–H313.
[48] W. Rouslin, S. Ranganathan, J. Mol. Cell. Cardiol. 15 (1983) 537–542.
[49] L. Hardy, J.B. Clark, V.M. Darley Usmar, D.R. Smith, D. Stone, Biochem. J. 274 (1991) 133–137.
[50] F. Di Lisa, R. Menabò, R. Barbato, N. Siliprandi, Am. J. Physiol. 267 (1994) H455–H461.
[51] D.K. Das, A. George, X.K. Liu, P.S. Rao, Biochem. Biophys. Res. Commun. 165 (1989) 1004–1009.
[52] Y. Zhang, O. Marcillat, C. Giulivi, L. Ernster, K.J. Davies, J. Biol. Chem. 265 (1990) 16330–16336.
[53] D. Pauly, S. Yoon, J. McMillin, Am. J. Physiol. 253 (1987) H1557–H1565.
[54] F. Naro, A. Fazzini, C. Grappone, G. Citro, G. Dini, A. Giotti, F. Malatesta, F. Franconi, M. Brunori, Cardioscience 4 (1993) 177–184.
[55] R.A. Gottlieb, K.O. Burleson, R.A. Kloner, B.M. Babior, R.L. Engler, J. Clin. Invest. 94 (1994) 1621–1628.
[56] J. Kajstura, W. Cheng, K. Reiss, W.A. Clark, E.H. Sonnenblick, S. Krajewski, J.C. Reed, G. Olivetti, P. Anversa, Lab. Invest. 74 (1996) 86–107.
[57] S. Kharbanda, E. Arbustini, F.D. Kolodgie, P. Morbini, B.A. Khaw, M.J. Semigran, G.W. Dec, D.W. Kufe, J. Narula, Circulation 96 (1997) I-115 (Abstract).
[58] E.Y. Sako, P.B. Kingsley Hickman, A.H. From, J.E. Foker, K. Ugurbil, J. Biol. Chem. 263 (1988) 10600–10607.
[59] S. Neubauer, B.L. Hamman, S.B. Perry, J.A. Bittl, J.S. Ingwall, Circ. Res. 63 (1988) 1–15.
[60] G. Gorge, P. Chatelain, J. Schaper, R. Lerch, Circ. Res. 68 (1991) 1681–1692.
[61] P. Paucek, Y.V. Yarov, X. Sun, K.D. Garlid, J. Biol. Chem. 271 (1996) 32084–32088.
[62] J.R. McCullough, D.E. Normandin, M.L. Conder, P.G. Sleph, S. Dzwonczyk, G.J. Grover, Circ. Res. 69 (1991) 949–958.
[63] G.J. Grover, J. Cardiovasc. Pharmacol. 24 (Suppl. 4) (1994) S18–S27.
[64] Z. Yao, G.J. Gross, Circulation 89 (1994) 1769–1775.
[65] G.J. Grover, A.J. D'Alonzo, C.S. Parham, R.B. Darbenzio, J. Cardiovasc. Pharmacol. 26 (1995) 145–152.
[66] K.D. Garlid, P. Paucek, V. Yarov-Yarovoy, H.N. Murray, R.B. Darbenzio, A.J. D'Alonzo, N.J. Lodge, M.A. Smith, G.J. Grover, Circ. Res. 81 (1997) 1072–1082.
[67] G.J. Gross, Basic Res. Cardiol. 90 (1995) 85–88.
[68] C.E. Murry, R.B. Jennings, K.A. Reimer, Circulation 74 (1986) 1124–1136.
[69] Y. Reiter, A. Ciobotariu, J. Jones, B.P. Morgan, Z. Fishelson, J. Immunol. 155 (1995) 2203–2210.
[70] C.D. McPherson, G.N. Pierce, W.C. Cole, Am. J. Physiol. 265 (1993) H1809–H1818.
[71] H. Bolukoglu, S.H. Nellis, A.J. Liedtke, Cardiovasc. Drugs Ther. 5 (Suppl. 1) (1991) 37–43.
[72] R.R. Russell, H. Taegtmeyer, J. Clin. Invest. 87 (1991) 384–390.
[73] R.R. Russell, H. Taegtmeyer, J. Clin. Invest. 89 (1992) 968–973.
[74] W.J. Idell, L.W. Grotyohann, J.R. Neely, J. Biol. Chem. 253 (1978) 4310–4318.
[75] T.L. Broderick, H.A. Quinney, G.D. Lopaschuk, J. Biol. Chem. 267 (1992) 3758–3763.
[76] T.L. Broderick, H.A. Quinney, C.C. Barker, G.D. Lopaschuk, Circulation 87 (1993) 972–981.
[77] R. Ferrari, F. Cucchini, O. Visioli, Int. J. Cardiol. 5 (1984) 213–216.
[78] G. Brevetti, F. Di Lisa, S. Perna, R. Menabò, R. Barbato, V.D. Martone, N. Siliprandi, Circulation 93 (1996) 1685–1689.
[79] C.H. Barlow, B. Chance, Science 193 (1976) 909–910.
[80] J. Eng, R.M. Lynch, R.S. Balaban, Biophys. J. 55 (1989) 621–630.
[81] R. Nunez, E. Calva, M. Marsch, E. Briones, S.F. Lopez, Am. J. Physiol. 231 (1976) 1173–1177.
[82] J. Schaper, W. Schaper, J. Am. Coll. Cardiol. 1 (1983) 1037–1046.
[83] A. Vinogradov, A. Scarpa, B. Chance, Arch. Biochem. Biophys. 152 (1972) 646–654.
[84] H.R. Lotscher, K.H. Winterhalter, E. Carafoli, C. Richter, J. Biol. Chem. 255 (1980) 9325–9330.
[85] B. Moser, K.H. Winterhalter, C. Richter, Arch. Biochem. Biophys. 224 (1983) 358–364.
[86] C. Richter, K.H. Winterhalter, S. Baumhuter, H.R. Lotscher, B. Moser, Proc. Natl. Acad. Sci. USA 80 (1983) 3188–3192.
[87] C.S. Boyer, G.A. Moore, P. Moldeus, J. Biol. Chem. 268 (1993) 4016–4020.
[88] C. Richter, M. Theus, J. Schlegel, Biochem. Pharmacol. 40 (1990) 779–782.
[89] R.B. Jennings, C.E. Ganote, K.A. Reimer, Am. J. Pathol. 81 (1975) 179–198.
[90] A.C. Shen, R.B. Jennings, Am. J. Pathol. 67 (1972) 417–440.
[91] A.C. Shen, R.B. Jennings, Am. J. Pathol. 67 (1972) 441–452.
[92] J.W. Greenawalt, C.S. Rossi, A.L. Lehninger, J. Cell Biol. 23 (1964) 21–38.
[93] R.G. Hansford, Rev. Physiol. Biochem. Pharmacol. 102 (1985) 1–72.
[94] J.G. McCormack, A.P. Halestrap, R.M. Denton, Physiol. Rev. 70 (1990) 391–425.
[95] R.G. Hansford, J.B. Chappell, Biochem. Biophys. Res. Commun. 27 (1967) 686–692.

[96] M. Tani, Annu. Rev. Physiol. 52 (1990) 543–559.
[97] W.H. Barry, Trends Cardiovasc. Med. 1 (1991) 162–166.
[98] K.A. Reimer, R.B. Jennings, in H. Fozzard, E. Haber, R.B. Jennings, A. Katz, H. Morgan (Eds.), The Heart and Cardiovascular System, 2nd Edn., Raven Press, New York, NY, 1992, pp. 1875–1973.
[99] C. Ganote, S. Armstrong, Cardiovasc. Res. 27 (1993) 1387–1403.
[100] C.E. Ganote, J. Worstell, J.P. Kaltenbach, Am. J. Pathol. 84 (1976) 327–350.
[101] J.S. Elz, W.G. Nayler, Am. J. Pathol. 131 (1988) 137–145.
[102] C.E. Ganote, J. McGarr, S.Y. Liu, J.P. Kaltenbach, J. Mol. Cell. Cardiol. 12 (1980) 387–408.
[103] J.P. Kehrer, Y. Park, H. Sies, J. Appl. Physiol. 65 (1988) 1855–1860.
[104] F. Di Lisa, P. Bernardi, Mol. Cell. Biochem. (1998), in press.
[105] K.K. Minezaki, M.S. Suleiman, R.A. Chapman, J. Physiol. (London) 476 (1994) 459–471.
[106] B. Siegmund, R. Zude, H.M. Piper, Am. J. Physiol. 263 (1992) H1262–H1269.
[107] S. Seki, K.T. MacLeod, Am. J. Physiol. 268 (1995) H1045–H1052.
[108] L. Ralenkotter, C. Dales, T.J. Delcamp, R.W. Hadley, Am. J. Physiol. 272 (1997) H2679–H2685.
[109] J.S. Sham, S.N. Hatem, M. Morad, J. Physiol. (London) 488 (1995) 623–631.
[110] R.A. Altschuld, W.C. Wenger, K.G. Lamka, O.R. Kindig, C.C. Capen, V. Mizushira, R.S. Vander Heide, G.P. Brierley, J. Biol. Chem. 260 (1985) 14325–14334.
[111] B. Siegmund, T. Klietz, P. Schwartz, H.M. Piper, Am. J. Physiol. 260 (1991) H426–H435.
[112] K.D. Schluter, P. Schwartz, B. Siegmund, H.M. Piper, Am. J. Physiol. 261 (1991) H416–H423.
[113] E.J. Griffiths, A.P. Halestrap, Biochem. J. 307 (1991) 93–98.