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Review

Mitochondrial function and myocardial aging. A critical analysis of the role of permeability transition

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Abstract

Mitochondria have been suggested to be causally linked to age-related alterations through respiratory chain dysfunction and formation of reactive oxygen species, leading to damage of mitochondrial DNA. Impaired biosynthesis of respiratory chain and ATP synthase subunits encoded by mitochondrial genes would set up a vicious cycle contributing to the aging process. Mitochondria are also involved in the increased susceptibility to ischemic injury observed in aged hearts, a process where the mitochondrial permeability transition pore (PTP) may play a role. Here, we analyze (i) the possible mechanisms through which PTP opening might contribute to age-related myocardial alterations; (ii) the available evidence of an increased probability of PTP opening in mitochondria isolated from aged tissues; (iii) the current methodological limitations that complicate the elucidation of causal relationships between PTP opening, mitochondrial dysfunction, and myocardial aging.

Keywords: Aging; Mitochondria; Permeability transition; Reactive oxygen species; Ischemia

1. Introduction

Various aspects of the involvement of mitochondria in age-related myocardial impairment have been analyzed in previous reviews [1–5]. Here, besides summarizing current concepts, controversial matters and recent relevant contributions, we focus on the role that the mitochondrial transition pore (PTP) has in myocardial pathology and the possible links with mitochondrial derangements caused by senescence. Our analysis suggests that the causal relationships between mitochondrial dysfunction and age-related myocardial pathologies may still be ill-defined, in part because of methodological limitations that hamper the study of mitochondria and PTP in situ. Thus, the general and relevant question as to whether mitochondrial dysfunction (if any) is cause or consequence of myocardial aging does not have a definite answer yet.

2. Mitochondria in cardiac physiology and pathology

The relevance of mitochondria to the function and viability of any cell is not restricted to ATP synthesis. Indeed, mitochondria contribute to a variety of processes like cellular ionic homeostasis; synthesis of molecules such as heme, pyrimidines and urea, that largely occurs within mitochondrial matrix; generation of precursors for ex-novo synthesis of glucose, fatty acids and cholesterol. A relevant role of mitochondria is also recognized in cell death due to the release of proteins that promote apoptosis [6–8].

In the heart, the synthesis of ATP by means of oxidative phosphorylation matches the ATP demand dictated by contractile activity. However, when mitochondria become

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dysfunctional, FoF1 ATP synthase hydrolyzes ATP rather than synthesizing it. Therefore, glycolytically produced ATP is avidly consumed by mitochondria in a process that accelerates ATP depletion and jeopardizes cell viability. Besides the profound imbalance between ATP synthesis and utilization that occurs in ischemic cardiomyocytes [9,10], impairment of ionic homeostasis [11–15] and formation of reactive oxygen species (ROS) [16–18] represent two additional processes through which mitochondria accelerate, or even determine, the evolution of cell injury towards necrosis or apoptosis [6–8,19,20].

2.1. Mitochondria as sources of ROS

A large body of evidence supports the concept that ROS are formed within mitochondria under physiological and pathological conditions. This process has been reported to increase in heart during aging or post-ischemic reperfusion [16,17,21,22]. The common estimate that 2–4% of oxygen utilized by the respiratory chain undergoes univalent reduction becoming superoxide anion (O_2^-.) is probably too high, and it has been suggested that a correct estimate might be one order of magnitude lower [23,24]. O_2^-. formed at the level of Complex I and III is rapidly transformed into hydrogen peroxide (H_2O_2) by a family of metalloenzymes, superoxide dismutases (SOD) [25]. Particularly relevant in this process is the mitochondrial form of SOD (MnSOD or SOD-2). Widespread organ damage associated with severe mitochondrial dysfunction has been observed in mice lacking SOD-2 [26]. Recent work has demonstrated that CuZnSOD (SOD-1), commonly referred to as the cytosolic isoform, is also present in the intermembrane space of mitochondria [27]. SOD-1 mutations have been related to the pathogenesis of amyotrophic lateral sclerosis [28,29].

O_2^-. and H_2O_2 are not strong oxidants. However, H_2O_2 can be reduced to the hydroxyl radical (OH.), one of the strongest oxidants, in a reaction that is highly facilitated in the presence of reduced transition metals. Being exposed to this potential danger, it is not surprising that mitochondria have plenty of antioxidant defenses. Firstly, H_2O_2 can be safely reduced to water by catalase or glutathione peroxidase. In addition, ascorbate, tocopherols and quinones act as effective scavengers both in aqueous phase and within the hydrophobic core of mitochondrial membranes.

A dual function is played by coenzyme Q. Its partially reduced form, semiquinone, prompts the formation of O_2^-., as opposed to the antioxidant action displayed by coenzyme Q in its fully reduced form [30]. Since also cytochrome *c* and cytochrome oxidase might exert detoxifying roles against ROS [17,31–33], the inner mitochondrial membrane may represent not only a site for ROS production, but also a scavenging system.

The mitochondrial formation of ROS might be modulated by NO· [34,35] as a consequence of the inhibition of cytochrome oxidase [17,36–39]. This reversible process can be transformed into irreversible alterations of respiratory chain when NO. formation is sustained. Indeed, NO. reacting with O_2^- generates peroxynitrite, which can produce the irreversible nitration of proteins [40]. Interestingly, a proteomic study showed that one-third of the proteins nitrated during inflammatory challenge are of mitochondrial origin [41]. In brain mitochondria nitration has been shown to affect several subunits of both Complex I and FoF1 ATP synthase [42], suggesting a possible contribution to the vicious cycles described in Sections 2.2 and 3.

It must be pointed out that ROS are also produced within mitochondria at sites other than the inner mitochondrial membrane [21]. A relevant role in this respect is likely played by monoamine oxidases (MAO). These outer membrane flavoproteins catalyze electron transfer from various amine compounds (including catecholamines) to O_2, thus producing large amounts of H_2O_2 [18,43]. It has been calculated that in brain mitochondria MAO activity results in steady state concentrations of H_2O_2 48-fold higher than those originating from respiratory chain in the presence of antimycin A [18]. Therefore, under physiological conditions the inner mitochondrial membrane could scavenge ROS produced at other mitochondrial or cellular sites. Then, the increase in ROS formation detected under pathological conditions might result, at least in part, from the loss of scavenging properties of the inner mitochondrial membrane due to its dysfunction. The relationship among MAO, mitochondrial dysfunction and cell injury is supported by a growing body of evidence obtained in various cell types [44–46]. In addition, age-related increases in MAO-B levels have been described in human brains [47].

Recent results highlight the involvement of p66Shc in the complex relationships between mitochondria and ROS [48–50]. This protein, which localizes in part within mitochondria, acts downstream of p53 and is required for inducing the elevation of intracellular oxidants, cytochrome *c* release and apoptosis [49,50]. Although the molecular mechanism by which p66Shc catalyzes or participates in redox reactions has not yet been elucidated, great interest in this protein stems from the observation that the ablation of its gene results in lifespan prolongation along with increased resistance to oxidative stress [48]. Further studies have shown that p66Shc$^{-/-}$ mice display a decreased susceptibility to hindlimb ischemia [51], as well as a reduction in high-fat induced atherosclerosis [52].

The mitochondrial production of ROS might be part of transducing pathways whereby an initial oxidative stress originated at various cell sites is amplified by mitochondria. This mechanism has been demonstrated in isolated cardiac myocytes and termed ROS-induced ROS release (RIRR) [53]. Support for this mechanism has recently been provided by a study performed in astrocytes where increased ROS production and mitochondrial membrane potential were related to the activation of NADPH oxidase [54].

2.2. Mitochondria as targets of ROS

Besides being a major site for ROS production, mitochondria are compromised by severe and/or prolonged oxidative stress. Proteins, lipids and nucleic acids can be altered by ROS resulting in covalent changes that profoundly affect their structure and function. Although repair processes efficiently preserve mitochondrial structure and function, this line of defense is evaded by ROS attack occurring under conditions of severe or prolonged oxidative stress, such as post-ischemic reperfusion or aging, so that damage of mitochondrial components becomes detectable.

Results from different experimental models indicate that complex I is highly susceptible to ROS attack. Its activity is decreased by oxidative stress [55–57] in a process that is prevented in vitro by thiol antioxidants [58,59]. This type of protein oxidation involving cysteinyl residues is, at least theoretically, reversible. Other forms of protein oxidation, such as those thoroughly characterized by recent reports [42,60], result in irreversible covalent modifications of the affected proteins. In these cases the modified proteins have to be removed by proteolysis and replaced by de novo synthesis. As discussed in the following sections, errors in these repair processes result in the accumulation of oxidized products [61].

Since complex I is also considered to be the major site for ROS production within the respiratory chain [17,62,63], a vicious cycle is likely set up, eventually resulting in cell death. Interestingly, irreversible damage appears to correlate more with ROS formation than with respiratory chain dysfunction [64].

Functional and structural alterations are also a likely result of lipoperoxidation. A highly susceptible target is cardiolipin [2,3,65,66]. This phospholipid, which is especially abundant in the inner mitochondrial membrane, binds to cytochrome *c* and has been suggested to modulate the activity of crucial proteins, such as cytochrome oxidase and adenine nucleotide translocase [66,67]. Cardiolipin oxidation has been proposed to contribute to complex I impairment [65] and cytochrome *c* release [68]. The mitochondrial impairment that is acutely induced by oxidative derangements of lipids and proteins can be transformed into a long-term dysfunction by ROS-induced alterations of mitochondrial DNA (mtDNA) [5,69–72]. Among the DNA products generated by ROS attack, 8-oxo-deoxyguanosine is the most prevalent [73]. Its relationship with aging is discussed in Section 3.

2.3. Permeability transition

A major consequence, but also a cause, of mitochondrial dysfunction is represented by the permeability transition, a sudden increase of the inner membrane permeability to solutes with molecular weights up to 1500 Daltons (reviewed in [74–77]). This phenomenon is caused by the opening of a voltage-dependent, high-conductance channel located in the inner mitochondrial membrane that is defined as the permeability transition pore (PTP).

The open probability of this channel is controlled by several cellular factors and is decreased by cyclosporin A (CsA). Inducers of PTP opening, such as reactive oxygen species (ROS), matrix [Ca^{2+}] elevation and $\Delta\psi_m$ fall, are counteracted by physiological inhibitors, such as pH decrease and [Mg^{2+}] increase [76].

The delicate balance between agonists and antagonists appears to be lost in many pathological situations including post-ischemic reperfusion [7,78]. A prolonged opening of PTP determines conditions hardly compatible with cell survival. Indeed, in response to the collapse of mitochondrial membrane potential, the operation of FoF1 ATP synthase is inverted causing a rapid depletion of ATP. In addition, PTP-induced swelling of the mitochondrial matrix space may result in the rupture of the outer mitochondrial membrane causing the release of cytochrome *c* and other proteins that stimulate apoptosis.

The elucidation of the complex relationships linking PTP with cell death has been made possible by the development of specific methods for detecting PTP opening in isolated cells and intact issues [53,79–82]. In particular, a procedure developed in our laboratories that is based on the intracellular redistribution of calcein coupled with Co^{2+} quenching of cytosolic calcein fluorescence is currently the reference method for the direct assessment of PTP opening in intact cells [81]. Since this procedure can be used in intact cells only, two different methods were developed to detect PTP opening in intact hearts [80]. In the procedure devised in Halestrap's laboratory perfused hearts are first loaded with [^{3}H] 2-deoxyglucose, which accumulates within the cytosol as [^{3}H]-deoxyglucose-6-phosphate, but can only enter the mitochondria when the PTP opens. Using this approach it has been shown that PTP remains closed during ischaemia but opens upon reperfusion [80]. This conclusion has also been drawn by using a procedure developed in our laboratory that is based on the release of mitochondrial NAD^+ occurring upon PTP opening [82]. Besides representing a useful analytical tool, the loss of mitochondrial NAD^+ provides additional pathways linking PTP opening to cell death [83].

Results obtained with different techniques in different laboratories indicate that PTP opening occurs during post-ischemic reperfusion, and is causally related to the loss of myocyte viability [78,84,85]. Further supporting this concept, it has been reported that ischemic preconditioning (IPC) prevents PTP opening upon reperfusion [84,86]. The role of PTP in mediating or triggering IPC is less clear [87,88]. Such a role has been suggested based on the lack of IPC obtained when PTP inhibitors are administered during the IPC protocol [88]. However, since PTP inhibitors also abrogated uncoupler-induced IPC, it has been argued that effects other than PTP inhibition could contribute to the loss of IPC [87].

Recent studies suggest that the open probability of PTP may be modulated in response to the activation of upstream protein kinases [89,90]. Thus, signal cascades triggered by (de)phosphorylation might translate stress or injury stimuli to survival or death by determining PTP inhibition or opening, respectively. Further studies are necessary not only to identify the molecular components of PTP, but also to clarify how the activation of cytosolic kinases might affect structures and functions of the inner mitochondrial membrane.

3. Mitochondria and aging

A causal relationship between mitochondrial impairment and aging is likely considering the decline in physical activity associated with senescence. However, addressing such an apparently simple issue opens many questions, one of the most important being whether mitochondrial dysfunction is the cause or the consequence of aging. This question is far from having been answered. Even the occurrence of mitochondrial dysfunction in senescent muscles is questionable [91]. Nevertheless, a large body of evidence led to the formulation of the so-called mitochondrial theory of aging [69] elaborated from the original hypothesis relating oxidative stress to aging [92]. According to this theory ROS production, mtDNA damage and respiratory chain dysfunction are linked in a vicious cycle that generates a progressive decline of mitochondrial function that eventually impairs cell function and viability. Indeed, the oxidative damage of mtDNA is detected in many organs [73], and its extent is inversely correlated to maximal life span [93]. In addition, age-related mtDNA alterations such as deletions, rearrangements or point mutations, have been described in humans [94].

It has been hypothesized that mtDNA damage might preferentially affect the respiratory complexes containing a high proportion of mitochondrially-encoded subunits, while complexes composed only of nuclearly-encoded subunits (such as complex II) would be spared. This condition would generate a “disproportionate” respiratory chain with an enhanced rate of ROS formation (reviewed in [5]). This hypothesis appears questionable, since complex I assembly is abolished when mtDNA gene expression is suppressed [95]. Nevertheless, evidence of an imbalanced chain has been reported in failing mouse hearts. In fact, mtDNA damage was concomitant with a reduction in complex IV content, but complex II was unaffected [96]. On the other hand, factors other than subunit assembly could alter specific components of the respiratory chain. For instance, a 28% decrease in complex I activity was observed in failing human hearts despite the absence of mtDNA damage [97]. This result supports the high susceptibility of complex I to injury as discussed in Section 2.2.

The association between mtDNA damage and senescence could be interpreted as one of the many manifestations of aging, thus raising questions about its relevance. However, an elegant study has recently provided convincing proof of

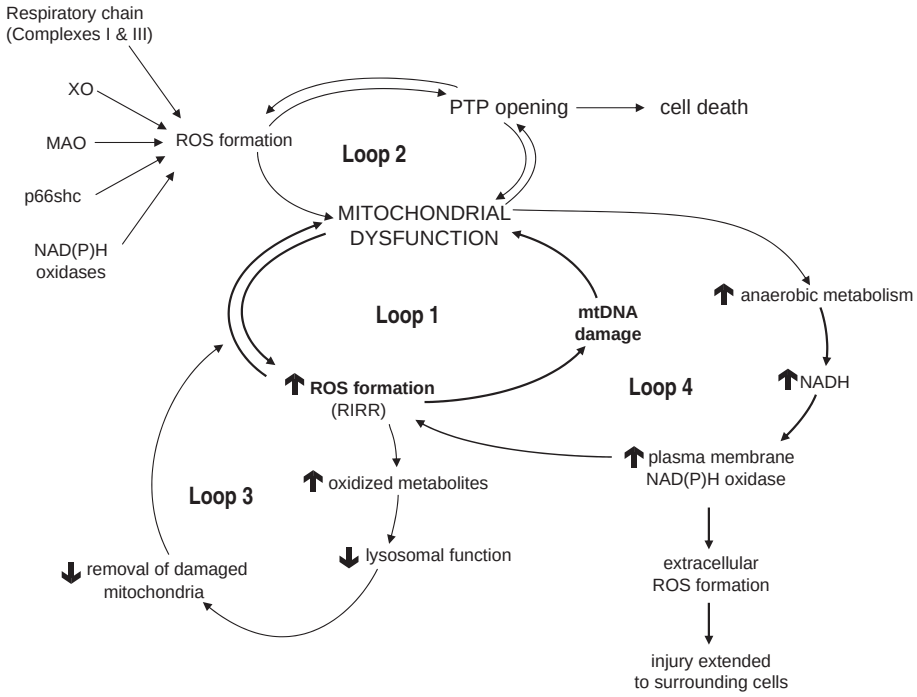

Fig. 1. Proposed mechanisms underlying cellular aging. The scheme highlights the central role of mitochondrial dysfunction in amplification (loop 1), accumulation (loop 3) and spreading (loop 4) of oxidative stress. PTP opening likely represents a critical element in the additional vicious cycle (loop 2) that might exacerbate mitochondrial dysfunction, thereby accelerating or determining the evolution towards cell death. MAO, monoamine oxidases; mtDNA, mitochondrial DNA; PTP, mitochondrial permeability transition pore, RIRR, ROS-induced ROS release; ROS, reactive oxygen species; XO, xantine oxidase.

the concept that mtDNA mutations are causally related to respiratory chain dysfunction and mammalian aging phenotypes [98]. A knock-in strategy was developed to hamper mtDNA repair in vivo by replacing a critical aspartate residue with alanine in DNA polymerase-γ, thus erasing the proof-reading activity of the enzyme without affecting its abilty to catalyze mtDNA replication. In 25-week-old mice harbouring the mutated polymerase, mtDNA alterations matched accelerated aging, that was accompanied by myocardial hypertrophy and dilatation [98].

The vicious cycle of ROS formation, mtDNA damage and mitochondrial dysfunction can spread to other organelles and eventually to surrounding cells (Fig. 1). It has been proposed that autophagocytosis, i.e. the process responsible for mitochondrial turnover, would be made less efficient by the gradual accumulation of oxidized products within lysosomes. Accordingly, a vicious cycle would be generated whereby dysfunctional mitochondria producing ROS would damage the rest of the cell while decreasing their own removal [61]. Besides impairing the mitochondrial-lysosomal axis, respiratory chain dysfunction produces the accumulation of NADH. Its reoxidation might also be catalyzed by plasma membrane redox systems that could regenerate NAD^+ along with ROS formation. According to the so-called reductive hostspot hypothesis, this process would cause secondary damage in the extracellular space and in neighbouring cells [99].

4. Mitochondrial dysfunction and myocardial aging

4.1. Respiratory chain

During the sixties and seventies most studies were performed on isolated mitochondria, where classical bioenergetic parameters, such as oxygen consumption and substrate utilization, were measured along with kinetics characterization of metabolic enzymes and respiratory chain components. The results of those initial studies were thoroughly reviewed by Hansford [1], who concluded that in senescent animals heart mitochondria are substantially unimpaired except for some substrate-specific changes, especially concerning fatty acid oxidation.

The occurrence of respiratory chain dysfunction and its causal relationship with age-related abnormalities are currently being called into question by many reports (reviewed in [100]). Despite the progressive lipid peroxidation occurring with aging [101], the activities of respiratory chain complexes [101] and of Complex V [102] were not found to be modified in elderly subjects. This lack of age-related dysfunction of the respiratory chain in humans is confirmed by the analysis performed on mitochondria from skeletal muscle biopsies [91].

Cytochrome measurements also provided conficting findings. A decline in cytochrome content has been reported in aged rat hearts [103]. However, studies on liver mitochondria from subjects aged 55 years and above detected an increase in cytochrome *c* along with unmodified cytochrome oxidase content [104]. This discrepancy highlights the difference between the respiratory chain in rat and human mitochondria as described in other reports [105], and prompts caution in drawing general conclusions from data obtained in single organs and/or animal species.

The discrepancies in experimental data concerning respiratory chain activities in aging could also originate from methodological issues. It has been proposed that the analyses usually performed on the entire population of heart mitochondria might have masked alterations occurring in subsets of mitochondria. Indeed, a procedure has been developed to separate two subpopulations of myocardial mitochondria. According to their locations they have been termed subsarcolemmal (SSM) and interfibrillar mitochondria (IFM), respectively [106]. The conventional procedures utilized for the isolation of heart mitochondria seem to result in a preferential extraction of SSM [3]. Using the Fischer 344 rat heart model of aging, SSM and IFM were isolated from 6-month adult and 24-month aging hearts. While no major changes were detected in SSM, aging resulted in decreased yield, oxidative phosphorylation capacity, complex IV activity and fatty acid oxidation rate of IFM [3]. More recently, the age-related increase in mitochondrial injury observed in hearts subjected to ischemia and reperfusion has been shown to affect IFM preferentially [107].

Concerns have also been raised on whether it is permissible to compare mitochondria extracted from animals of different ages. Indeed, different levels of fibrosis may interfere with the isolation procedures, and different degrees of mitochondrial purity may undermine conclusions based on measurements performed without proper normalization procedures [100,102,108].

4.2. Substrate utilization

Results from different laboratories indicate that fatty acid oxidation is depressed by about 30% in mitochondria from aged hearts [109–111]. This alteration has been attributed to decreased activities of enzymes and transporters rather than to changes in respiratory chain activity [1]. The decline in fatty acid utilization may also relate to the thyroid status [1], which is depressed in senescent animals [112]. On the other hand, age-related changes in oxygen consumption were not detected in perfused rat hearts utilizing fatty acids [113]. This finding casts some doubts on the large decreases in respiratory control observed in cardiac mitochondria isolated from aged animals [114,115].

Regarding carriers and transporters, initial studies described significant decreases in activity restricted to carnitine translocase and adenine nucleotide translocase (ANT) [1]. These declines were interpreted as resulting from a decrease in the countertransported substrate, affecting in turn the kinetic analysis performed in isolated mitochondria.

Indeed, age-linked decrements have been reported for both carnitine and adenine nucleotide contents [113,116,117]. However, besides possible changes in lipid-protein interactions due to modified or altered membrane lipids [2,117], direct protein changes are likely to play a relevant role since both carnitine translocase and ANT are highly susceptible to oxidative stress [118,119].

4.3. Aging, mitochondria and ischemic injury

The relationship between ROS and aging is likely to play a crucial role under pathological conditions, such as myocardial ischemia. A greater degree of injury is detected in aged hearts subjected to ischemia and reperfusion as compared to young adult hearts [2,3,120,121].

The increased damage likely results from a wide array of mechanisms that involve ROS formation and mitochondrial dysfunction. In particular, the vicious cycle linking complex I dysfunction to increased ROS formation (see Section 2.2) appears to play a relevant role [120]. The functional impairment of complex I could also result from oxidative modifications of upstream targets. For instance, α-ketoglutarate dehydrogenase appears to be highly susceptible to oxidative stress, and especially to 4-hydroxy-2-nonenal-induced inactivation [122].This alteration has been reported to be responsible for the age-dependent enhancement of reperfusion damage [122]. The possible contribution of α-ketoglutarate dehydrogenase is further highlighted by recent studies performed in isolated brain mitochondria demonstrating that this enzyme is a site for ROS formation [123,124]. Of note, this process should be stimulated under anoxic conditions because it requires a high $NADH(H^+)/NAD^+$ ratio. The ischemic injury is likely exacerbated by increases of cytosolic and mitochondrial $[Ca^{2+}]$. Modifications of cardiolipin content and composition are thought to impair mitochondrial Ca^{2+} homeostasis. In hearts from aged rats the cardiolipin content is lower and the ω6/ω3 ratio is higher than in organs from younger individuals [2]. Interestingly, the increased ω6/ω3 ratio associated with aging or obtained with a diet enriched with ω6 was accompanied by an increased mitochondrial Ca^{2+} content in isolated hearts perfused with norepinephrine or subjected to ischemia-reperfusion. Regarding IPC, conflicting results have been obtained in different species. Reduced IPC protection has been observed in aged hearts from the rat (reviewed in [125]) but not the rabbit [126], while discrepancies exist in studies of human atria [127,128]. These differences may depend on age-related modifications of the signaling pathways triggered by IPC, which might be differently affected by senescence in different species [129]. Although the mechanism(s) underlying IPC protection have not yet been completely elucidated, a pivotal role has already been attributed to ROS formed during the preconditioning phase. A slight formation of ROS induced by IPC has been suggested to protect the heart from the deleterious burst of ROS that characterizes post-ischemic reperfusion [130]. Since in aged hearts ROS formation could already be elevated under control conditions, it is tempting to speculate that a further increase might hardly elicit protection while facilitating detrimental effects. Further studies are necessary to test whether (i) IPC elicits an increase in ROS formation in aged hearts and (ii) antioxidants, which abrogate IPC in young adult hearts [131], protect aged hearts subjected to IPC protocols.

5. Permeability transition and aging

The derangements in mitochondrial function occurring in aging and related to ROS formation might suggest the involvement of PTP. This might also contribute to the increased susceptibility to ischemic injury and the reduced protection by IPC observed in aged hearts.

5.1. Available evidence

The relationship between PTP and age-related mitochondrial dysfunction critically reviewed in a recent article [132] has not been systematically investigated. An increased susceptibility to PTP opening was firstly documented in liver [133] and lymphocyte mitochondria [134] from old mice. In the study performed on lymphocyte mitochondria PTP was investigated by assessing the so-called calcium retention capacity (CRC). In this protocol isolated, energized mitochondria are exposed to a sequence of Ca^{2+} pulses that are accumulated until a threshold matrix load is reached that causes PTP opening, which can be assessed as an abrupt release of accumulated Ca^{2+} [135]. The impairment in CRC was subsequently demonstrated also in brain and liver mitochondria isolated from 20-month-old mice [136]. In all these studies the age-related dysfunctions were attributed to PTP based on the fact that in vitro CRC and swelling rate of mitochondria from old animals were restored to control values upon CsA addition. An increased susceptibility to PTP opening has also been documented in heart mitochondria as a reduced CRC in preparations from 24 month old Fischer 344 rats [137]. At present, these findings represent the only direct evidence of a link between aging and the PTP in the myocardium.

5.2. Possible roles and modulation

The above results suggest that age-related mitochondrial dysfunction and facilitated PTP opening are retained after mitochondrial isolation. Although these findings could be explained by covalent changes, such as proteolysis, oxidation, and (de)phosphorylation, the protein component(s) of the PTP still await identification.

PTP opening might be stimulated by the higher rates of mitochondrial ROS formation that have been documented in senescent animals as compared to younger littermates [1,120,138,139]. In the sequence of events linking aging

to functional and structural derangements of mitochondria, the causal relationship between PTP opening and ROS formation remains far from clear. Three major possibilities might be envisaged: (i) Ca^{2+}-induced PTP opening could be a primary event; (ii) PTP opening could be a later event triggered by an increased ROS formation; (iii) a vicious cycle could be generated whereby the initial opening of PTP, possibly involving only a fraction of mitochondria, might result in mitochondrial dysfunction causing an increase in ROS production. This latter event would cause the prolongation of PTP opening and/or the occurrence of PTP in the entire mitochondrial population of a given cell causing irreversible injury. Obviously, the derangements in Ca^{2+} homeostasis and ROS formation are likely to be exacerbated by the aforementioned alterations in NAD^+ content and metabolism.

Alternatively, or in addition, the PTP open probability might be increased by oxidative alterations of the lipid components of the inner mitochondrial membrane, especially of cardiolipin. The larger availability of arachidonic acid due to the higher ω6/ω3 ratio observed in aged hearts might prompt PTP opening and apoptosis [140]. These processes would be further stimulated by the increase in mitochondrial Ca^{2+} content associated with the age-related changes in cardiolipin composition [2].

Finally, an increased probability of PTP opening could be caused by changes in signaling pathways that characterize the aging process in many tissues [129,141]. Accordingly, PTP opening would transduce the decreased response of protective pathways, such as Akt activation [142], into bioenergetic impairments possibly evolving towards cell death.

5.3. Cautionary remarks

Although an involvement of the PTP in aging is appealing, we think that the available evidence is far from conclusive, and that unequivocal testing of this hypothesis will require additional work and methodological improvements. In particular:

(i) The aforementioned results were obtained by exposing isolated mitochondria to high Ca^{2+} concentrations. Although this approach may reveal an increased susceptibility to PTP opening in vitro, it is likely that such conditions are not experienced by mitochondria in situ.

(ii) PTP facilitation was investigated in isolated mitochondria only. It appears essential to investigate this issue in intact myocytes as well.

(iii) PTP was only investigated as an endpoint in very aged animals. No information is available as to when the change in PTP susceptibility might occur; and, more importantly, whether this process precedes or follows the appearance of other signs of mitochondrial dysfunction.

(iv) Although the above temporal relationships could, in principle, be assessed by isolating mitochondria at various ages, such an approach would not provide convincing evidence that an increased probability of PTP opening characterizes the aging cardiomyocyte in situ, nor that this process underlies the gradual dysfunction of heart mitochondria and cells. This causal relationship could only be elucidated by monitoring mitochondrial function and PTP opening in the intact organ, which is not feasible yet.

(v) The existence of causal links between PTP opening and aging are not easy to address with currently available drugs. Prolonged administration of CsA may cause adverse effects linked to calcineurin inhibition, and it would be very difficult, if at all possible, to dissociate the effects related to PTP desensitization through inhibition of mitochondrial cyclophylin D from those caused by inhibition of other cellular cyclophilins. This latter problem would also apply to CsA derivatives that inhibit the PTP but are devoid of calcineurin inhibiting activity.

6. Concluding remarks

The available information concerning mitochondrial function in aged hearts does not allow a conclusive elucidation of causal relationships with age-related alterations of myocardial function and structure. The decrease in mitochondrial function could represent an adaptive process to the reduced contractile performance that characterizes aged hearts [121]. This hypothesis, which might especially apply to healthy organs, is supported by the demonstration that in healthy subjects reduced physical activity is a major contributor to the decline in mitochondrial oxygen consumption during aging [143]. On the other hand, an increased ROS production by mitochondria might result in oxidative derangements of contractile proteins that in ischemic hearts appear to correlate with contractile impairment [144].

Although mtDNA damage can determine the appearance of aging phenotypes and this correlation holds true in the aging process of small organisms [145], the occurrence of significant mtDNA alterations and mitochondrial dysfunction is questionable in hearts of aged rodents and is not detectable in humans. At variance from results collected under physiological conditions in healthy hearts, mitochondria appear to be involved in the increased susceptibility to injury displayed by aged hearts. Under these conditions mitochondria damaged by an increased ROS formation become dysfunctional, further increasing ROS accumulation. This vicious cycle is likely to favour PTP opening. It must be pointed out that other mitochondrial channels might contribute to mitochondrial impairment. Unfortunately no information is available on the activity of the mitochondrial K_{ATP} channel and the anion

channel of the inner mitochondrial membrane (IMAC) in aged hearts. In particular, IMAC has been suggested to cause the propagation of ROS from a subset of damaged mitochondria to the rest of the cell generating oscillations of mitochondrial membrane potential [146]. It might be worth investigating the role of IMAC in ROS-induced aged-related alterations.

Despite these problems, elucidation of the mechanisms through which mitochondria contribute to the aging process appears to be an important task that will guide new therapeutic interventions aimed both at extending lifespan and at protecting aged hearts from oxidative stress and ischemic injury.

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