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Review

The mitochondrial permeability transition pore and cyclophilin D in cardioprotection ☆

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ABSTRACT

Mitochondria play a central role in heart energy metabolism and Ca^{2+} homeostasis and are involved in the pathogenesis of many forms of heart disease. The body of knowledge on mitochondrial pathophysiology in living cells and organs is increasing, and so is the interest in mitochondria as potential targets for cardioprotection. This critical review will focus on the permeability transition pore (PTP) and its regulation by cyclophilin (CyP) D as effectors of endogenous protective mechanisms and as potential drug targets. The complexity of the regulatory interactions underlying control of mitochondrial function in vivo is beginning to emerge, and although apparently contradictory findings still exist we believe that the network of regulatory protein interactions involving the PTP and CyPs in physiology and pathology will increase our repertoire for therapeutic interventions in heart disease. This article is part of a Special Issue entitled: Mitochondria and Cardioprotection.

1. Introduction

Our current understanding of ischemia/reperfusion (I/R) injury is greatly contributed by the notion that mitochondria are both a pivotal site for determining the loss of cell viability and a central target of processes triggered by ischemia, such as elevation in intracellular Ca^{2+} and reactive oxygen species (ROS). Therefore, it is not surprising that strategies aimed at protecting against I/R damage have focused on mitochondria. During the Seventies and Eighties major attention was given to metabolic pathways, Ca^{2+} homeostasis and oxidative stress, yet mitochondrial dysfunction as such was less studied. During the Nineties the number of mitochondrial studies rose exponentially in almost every biomedical field mostly due to the recognized involvement of mitochondria in apoptosis, which from the very beginning was linked to the mitochondrial permeability transition pore (PTP) [1,2]. Concomitantly, the inhibition of PTP opening by cyclosporine A (CsA) was shown to afford significant cardioprotection against I/R injury [3,4]. At present, a general consensus exists that the PTP is a major factor in determining cell death, and that PTP inhibition affords significant cardioprotection (reviewed in [5–7]), a concept that has been successfully translated to clinical settings [8].

The PTP can be described as a voltage- and Ca^{2+}-dependent, CsA-sensitive, high-conductance channel located in the inner mitochondrial membrane (IMM) and regulated by the OMM through specific protein–protein interactions [9,10]. Its opening causes a sudden increase in IMM permeability (i.e., a permeability transition) to solutes with molecular masses up to 1500 Da [11]. Surprisingly, the molecular identity of the proteins responsible for this relevant mitochondrial process has not been elucidated yet. Adenine nucleotide translocase (ANT) and the voltage-dependent anion channel (VDAC) have been proposed to represent PTP components, but genetic studies have demonstrated that PTP opening can still be observed in mitochondria devoid of these proteins, which therefore might rather modulate PTP function [12].

2. PTP modulation and cyclophilin D

Factors involved in modulating the open probability of the PTP have been analyzed in several reviews [5,11,13,14]. The long list of PTP modulators includes ions and metabolites, lipids and components of mitochondrial membranes, as well as soluble and membrane proteins. Briefly, PTP opening is favored by elevation in intramitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_m$), ROS, inorganic phosphate (Pi) and mitochondrial depolarization. These factors are counteracted by physiological PTP antagonists, such as elevated values of mitochondrial membrane potential ($\Delta\psi_m$), pH values above or below the

Abbreviations: ANT, adenine nucleotide translocase; CsA, cyclosporine A; CyP, cyclophilin; Drp1, dynamin-related protein 1; $\Delta\psi_m$, mitochondrial membrane potential; ERK, extracellular signal regulated kinase; GSK, glycogen synthase kinase; IMM, inner mitochondrial membrane; IPC, ischemic preconditioning; IPoC, ischemic post-conditioning; mitoK_{ATP}, mitochondrial K_{ATP} channel; OMM, outer mitochondrial membrane; PKA, cyclic AMP-dependent protein kinase; PKG, cyclic GMP-dependent protein kinase; PPIase, peptidylprolyl *cis-trans* isomerase; PTP, permeability transition pore; ROS, reactive oxygen species; VDAC, voltage-dependent anion channel

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optimum 7.3 for pore opening [15], Mg^{2+} and adenine nucleotides, especially ADP.

The PTP is modulated also by the IMM surface potential independently of changes in $\Delta\psi_m$, consistent with the observations that PTP opening is favored by polyanions and counteracted by polycations [16]. Besides these modulators acting at the level of the interface between phospholipid polar heads and the surrounding aqueous milieu, also the hydrophobic core of the IMM appears to be involved in PTP regulation. Indeed, heart mitochondria isolated from rats fed with docosahexaenoic acid (DHA) display an increased DHA content in mitochondrial phospholipids associated with a decreased susceptibility to Ca^{2+}-induced permeability transition [17].

Many proteins have been described to be involved in PTP regulation, yet major attention has been focused on the matrix protein cyclophilin (CyP) D due to its importance in pharmacological control of PTP opening and to the pivotal information provided by its genetic deletion (reviewed in [18]). PTP opening is facilitated by binding of CyPD to the inner mitochondrial membrane (IMM) in a process modulated by Ca^{2+}, Pi and ROS [19]. Notably, CyPD binding to the IMM is prevented by CsA and by other molecules interacting with CyPD that are usually described as PTP inhibitors. However, since increasing $[Ca^{2+}]$ results in PTP opening even in the presence of these molecules, "PTP desensitization" describes better than "PTP inhibition" the effect of this family of compounds, a concept that also applies to mitochondria devoid of CyPD [20]. It must be mentioned that PTP desensitization by CsA or by CyPD ablation requires Pi, which under these conditions behaves like a PTP inhibitor [19,21]. The evolutionary implications of this finding have been discussed in a recent review [22]. Recent work from the Halestrap laboratory has shown that the polyclonal antibody used in previous studies to identify a CyPD-binding protein of 32 kDa as ANT1 is rather labeling the Pi carrier, which has now been incorporated in the model of the PTP proposed by Halestrap's laboratory [23].

CyPD, the product of the *Ppif* gene, is a member of a family of highly conserved peptidylprolyl *cis-trans* isomerases (PPIases or rotamases, see below). Although this enzymatic activity relates potentially all the CyPs to protein folding, the specific functions played by the various members of this family are likely to depend more on individual, specific structural determinants than on the common denominator of rotamase activity [24]. Due to its unique N-terminus CyPD is targeted to the mitochondrial matrix. Increased intramitochondrial levels of Ca^{2+} and/or ROS promote a tight binding of CyPD to the IMM in a process that is associated with PTP opening and is antagonized by CsA. Although CsA inhibits the PPIase activity of all CyPs, the enzymatic activity is not required for membrane binding and stimulation of PTP opening [25]. The relationship between CyPD and PTP has initially been demonstrated by pharmacology through the use of CsA, and then corroborated by other CyPD ligands such as sanglifehrin A, NIM-811, Debio-025 and antamanide [26–29]. However, conclusive evidence for a regulatory role of CyPD was provided by genetic studies demonstrating that the propensity of the PTP to open is reduced in mitochondria devoid of CyPD [20,30–32], while susceptibility to PTP opening and to cell death is increased when CyPD is overexpressed [33,34], but see [35].

Recent observations indicate that CyPD can undergo covalent modifications. In tumor cell models CyPD undergoes phosphorylation on serine/threonine residues in a process that appears to be counteracted by extracellular signal regulated kinase (ERK) and promoted by glycogen synthase kinase (GSK) 3 [36]. Consistent with this hypothesis, a recombinant GSK3 could phosphorylate CyPD in vitro, and an in silico analysis identified possible GSK3 target residues on CyPD [36]. On the other hand, GSK3 inhibition results from its phophorylation catalyzed by ERK. Notably, CyPD phosphorylation was associated with an increased susceptibility to PTP opening. This link between GSK3 and CyPD fits the model proposed by Sollott and coworkers concerning the role of the PTP in ischemic preconditioning (IPC) ([14], discussed below). However, besides the fact that at present CyPD phosphorylation has not been documented in cardiac myocytes, further studies are necessary to elucidate the mechanism (s) whereby the (de)phosphorylation status of CyPD might control PTP opening and its modulation by the other factors described above. In addition, other CyPD sites could be phosphorylated by potentially competing kinases resulting in a decreased probability of PTP opening, or phosphorylation of PTP components could modulate the interaction with CyPD. Thus, at this early stage PTP regulation by CyPD phosphorylation should be regarded as an interesting possibility rather than as an established fact.

Other covalent modifications of CyPD could be induced by ROS, and redox changes have in fact been described in CyPs from different species [37–39]. Also human CyPD has been reported to display redox regulation by means of thiol modification [40]. Among its four cysteinyl residues Cys^{203} oxidation has been shown to be especially relevant in hampering isomerase activity. In addition, the formation of a disulfide bond between Cys^{203} and Cys^{157} was shown to result in a significant conformational change [40]. However, no information is available on the relationship between CyPD oxidation and PTP opening. Early studies showed that treatment with oxidants promoted CyPD binding to the inner mitochondrial membrane, and that the bound fraction was enzymatically inactive [41]. Future studies on the link between oxidative stress, CyPD and the PTP should also address the paradoxical finding that a mild increase in ROS formation might be protective by reducing PTP opening [42] as opposed to the well-established damaging effects induced by severe oxidative stress [41]. In this respect, and depending on the amount of ROS, cysteinyl residues in CyPD might be available for either inter- or intra-molecular disulfide cross-bridge formation. The formation of intermolecular disulfide bridges might promote CyPD aggregation or its covalent interaction with surrounding proteins, which would in turn be prevented by generation of intramolecular disulfide bonds.

3. Possible role of cyclophilin A

CyPs are ubiquitous proteins endowed with PPIase activity that have been conserved during evolution [43,44]. They have been found in the genomes of mammals, plants, insects, fungi and bacteria, and share a common domain of approximately 109 amino acids, the CyP-like domain [45]. In humans, cytosolic CyPA is the prototype of the family and 16 unique CyPs have been found [43–45]. After binding CsA, the PPIase activity is inhibited [46] and the CsA/CyPA complex binds to and inhibits the cytosolic phosphatase calcineurin [47] resulting in immunosuppression [48,49]. The PPIase activity of the protein (or its inhibition) is not relevant for calcineurin inhibition [50], suggesting that CyPs have specific cellular functions that may be of importance for a variety of processes relevant to human disease [45]. Specific functions are also suggested by the existence of tissue- and organelle-specific isoforms characterized by the combination of the signature CyP domain with the proper targeting and/or retention sequence(s) [45]. Since all CyPs are inhibited by CsA and its analogs, a role for isoforms other than CyPD in in vivo paradigms should always be considered even when ligands that do not cause calcineurin inhibition (like NIM811 and Debio 025) are used.

Cytosolic CyPA can be relevant to the cardioprotective effects of CsA, which would add to those afforded by inhibition of CyPD. Indeed, recent studies have revealed that translocation of the pro-fission dynamin-related protein 1 (Drp1) to mitochondria (which favors mitochondrial fission and thus fragmentation of the network) requires its calcineurin-dependent dephosphorylation at Ser637 [51]. Since CsA in complex with cytosolic CyPA inhibits calcineurin [47], CsA also affects mitochondrial shape and function independent of its interactions with matrix CyPD, a fact that should be taken into account when interpreting the effects of CsA on mitochondrial function and cell survival [52]. The relevance of this problem is

highlighted by recent reports showing that genetic ablation or pharmacological inhibition of Drp1 affords a significant protection against cardiomyocyte injury caused by ceramide, hyperglycemia and post-ischemic reperfusion [53–56]. In particular, upon treatment with high glucose Drp1 translocation was shown to be responsible for an increase in ROS formation that was followed by PTP opening [55]. Future studies will be necessary to clarify whether this sequence of events can be translated from isolated cardiomyocytes exposed to an extremely high glucose level to intact hearts in various pathological conditions.

4. Additional mitochondrial effects of cyclophilin D

Recent work has identified interactions of CyPD with other mitochondrial proteins that may be related to the PTP but could also affect it indirectly through the modulation of PTP-regulatory factors. One of the best characterized interactions is with the F_1F_O-ATP synthase, which was recently documented in bovine heart mitochondria [57]. Cross-linking studies demonstrated that CyPD forms a complex with the OSCP, b, and d subunits, i.e., that it interacts with the extrinsic part of the lateral stalk [57]. CyPD binding was favored by Pi and decreased the enzyme-specific activity, while CsA increased the enzymatic activity and displaced CyPD from the ATP synthase. Whether this striking analogy with Pi-dependent PTP regulation by CyPD indicates a link between the two processes, or rather modulation of the ATP synthase regulates the PTP through the matrix levels of ADP, Pi and Mg^{2+} remains to be established.

CyPD interacts with a complex chaperone network involving Hsp90 and its related molecule TRAP-1 [58]. CyPD bound to the complex would no longer be available for PTP opening, and CyPD displacement by selective Hsp90 antagonists like Shepherdin may disrupt this protective network and activate the PTP with onset of cell death. Given the prominence of the Hsp90–TRAP-1–CyPD interactions in tumor cells, this strategy has been successfully used for their selective killing [58]. A recent study also demonstrated a CsA-sensitive interaction of CyPD with Bcl2. CsA increased tBid-dependent release of cytochrome *c* without PTP opening [59]. Consistent with previous results [35], CyPD overexpression made cells more resistant to apoptotic stimuli, a finding that is difficult to reconcile with a predominant effect of overexpression on the PTP [35,59]. This set of studies suggests that CyPD overexpression may be a mechanism through which tumor cells become resistant to death, and therefore that CyPD inhibition might be an effective anti-cancer therapy.

5. PTP, cyclophilin D and cardioprotection

Although PTP structure is still elusive and its modulation is far from having been conclusively elucidated, the relationship between PTP opening and cell death is supported by a large body of evidence, especially in the field of acute myocardial ischemia and reperfusion. PTP opening appears to be causally linked to the loss of cell viability as demonstrated by the reduction in infarct size obtained when PTP opening is counteracted by pharmacological inhibitors or by genetic ablation of CyPD.

As detailed in several reviews [5,11,13,14], PTP opening favors ATP depletion, deregulation of cellular Ca^{2+} homeostasis and oxidative stress, all of which synergize in jeopardizing cell survival. The sequence of events can be summarized as follows. PTP opening determines an immediate collapse of $\Delta\psi_m$ that is followed by ATP depletion. When opening is prolonged, the initial uncoupling-like effect is rapidly followed by respiratory inhibition caused by loss of pyridine nucleotides and of cytochrome *c*. This latter event can be contributed by the rupture of the outer mitochondrial membrane (OMM) that follows PTP-dependent matrix swelling, or by making more cytochrome *c* available for release through cristae junction widening followed by release through Bax/Bak channels through an otherwise intact OMM [60]. The resulting inhibition of electron flow might explain the increased ROS formation induced by PTP opening. Since the latter event is favored by ROS [6,61], a vicious cycle of injury amplification is likely to be established, especially at the onset of reperfusion.

Regarding ischemia/reperfusion injury, during ischemia the build-up of factors favoring PTP opening (e.g., depolarization and increased Ca^{2+} and Pi matrix levels) is balanced by the presence of PTP antagonists (e.g., intracellular acidosis, high levels of Mg^{2+} and ADP), which may prevail and prevent PTP opening. Conversely, upon reperfusion the recovery of pH together with a burst in ROS formation in the presence of high matrix concentrations of Ca^{2+} and Pi creates optimal conditions for PTP opening in spite of the antagonizing effect of $\Delta\psi_m$ recovery. The concept that PTP is more likely to open upon reperfusion is supported by findings obtained in intact hearts using direct methods for PTP assessment [4,62].

Decreased susceptibility to PTP opening appears also to be involved in endogenous defense mechanisms that are triggered during ischemia and reperfusion to maintain cardiomyocyte viability. Repeated short episodes of ischemia and reperfusion protect from a subsequent prolonged ischemia are a process termed “ischemic preconditioning” (IPC) [63]. The deleterious effects of post-ischemic reperfusion have been shown to be limited also by performing a series of very short periods of ischemia and reperfusion at the time of reperfusion. This maneuver is termed “ischemic post-conditioning” (IPoC), and has received great attention as a protective strategy in the clinical setting [64,65]. Both IPC and IPoC have been related to PTP [66–70] based upon the following major lines of evidence: (i) IPC results in a decreased occurrence of PTP opening during post-ischemic reperfusion [69,71]; (ii) IPC and IPoC reduce PTP susceptibility in mitochondria isolated during reperfusion [66]; and (iii) cardioprotection by IPC and IPoC is similar to that obtained using compounds (or interventions) that decrease PTP opening [26,67], although from a theoretical standpoint similar degrees of protection might result from completely different mechanisms.

It has to be pointed out that the features of PTP opening in isolated mitochondria are not necessarily reflecting the status of the pore in situ, since conditions applied in the test tube are quite different from those occurring in cardiomyocytes subjected to ischemia and reperfusion. In addition, mitochondria extracted from protected hearts are expected to perform better even if the PTP is *not* involved in the protective mechanism in vivo.

The notion that the PTP contributes to mediate IPC has been corroborated by observations suggesting that CyPD inhibition or deletion abolishes the cardioprotective effects of IPC itself [72,73]. This apparent paradox reminds of, and fits well with, the concept that a slight increase in ROS formation during the preconditioning phase prevents the large accumulation in ROS induced by reperfusion, and thus results in maintenance of myocardial viability [74,75]. Indeed, cardiomyocytes devoid of CyPD were shown to (i) lack the increase in ROS formation that is otherwise induced by hypoxic preconditioning and (ii) fail at activating Akt and Erk 1/2. In addition, hearts lacking CyPD could not be further protected by IPC [72]. This interpretation was put forward by Hausenloy et al., who proposed that IPC induces transient, non-lethal opening of the PTP that protects from reperfusion-induced prolonged opening. Although this hypothesis fits our demonstration that the PTP actually opens in intact cells under “resting” conditions, as assessed with the calcein loading/cobalt quenching technique [76], it might prove quite difficult to assess in intact hearts. For instance, under ischemic conditions intracellular pH is likely to differ between isolated cardiomyocytes and intact hearts and the same applies to pH recovery upon reperfusion. In addition, the degree of protection elicited by CyPD deletion might already be maximal, thus limiting the possibility of additional benefit. Alternatively, CyPD might be required for IPC-induced protection independently of its role in PTP modulation (see above).

The mechanisms by which IPC and/or IPoC affect mitochondrial function and structure, as well as PTP opening, have not yet been elucidated. A large body of reports provided evidence of multiple relationships between IPC/IPoC-induced changes in signaling pathways and the increased resistance of mitochondria to I/R injury [14,77–81]. A comprehensive scheme has been proposed where IPC-induced inactivation of GSK-3β is transduced to mitochondria resulting in PTP inhibition [71]. The constitutively active GSK-3β would be inactivated by phosphorylation at Ser^9 catalyzed by upstream protein kinases. These have been grouped under the acronyms of RISK (Reperfusion Injury Salvage Kinase), including phosphatidylinositol-3-OH kinase (PI3K)-Akt and Erk 1/2 [80], and SAFE (Survivor Activating Factor Enhancement) describing the activation of the cytokine tumor necrosis factor alpha (TNFα) and signal transducer and activator of transcription-3 (STAT-3) [81]. In addition, activation of cyclic GMP-dependent protein kinase (PKG) [82], cyclic AMP-dependent protein kinase (PKA) [83] and PKCε [84] has been reported to contribute to the maintenance of mitochondrial function in hearts undergoing I/R, whereas PKCδ (together with GSK-3β) appears to facilitate mitochondrial dysfunction [85].

Despite the large amount of data linking protein kinases with mitochondrial (dys)function, how signaling pathways activated in the cytosol might affect energy-linked process occurring within the IMM is still far from being clear. Even assuming that signals are relayed or proteins are translocated across the OMM to reach the intermembrane space and/or inner mitochondrial compartments, the (de)phosphorylated proteins need to be defined, and their causative role in PTP modulation assessed. Candidate (de)phosphorylation reactions may involve the OMM VDAC, thus affecting adenine nucleotide traffic between mitochondria and cytosol and/or binding of the antiapoptotic protein Bcl-2 [86]. A PTP-regulatory role has been proposed also for IPC-induced opening of the mitochondrial K_{ATP} channel (mitoK_{ATP}) by PKCε, which may cause a slight increase in H_2O_2 formation eventually causing PTP inhibition [42]. The ROS-mediated link between PKCε and mitoK_{ATP} involves additional factors. For instance, the increase in ROS formation induced by diazoxide is blunted in cardiomyocytes and hearts of heterozygous connexin 43 deficient ($Cx43^{+/-}$) mice [87]. Therefore, it is tempting to speculate that Cx43 favors PKCε interaction with mitoK_{ATP} or modulates directly mitoK_{ATP} activity [88].

The possibility that PTP opening might be prevented in response to signaling pathways triggered by IPC or IPoC has been criticized because susceptibility to PTP opening is not decreased in mitochondria isolated at the end of the preconditioning phase, at variance from what is observed at the onset of reperfusion [70]. Therefore, changes occurring during ischemia rather than those produced during preconditioning would be responsible for the IPC-induced resistance to PTP opening. This criticism also matches the observation that in some experimental models activation of the RISK pathway appears to be independent of any protective effects [89,90]. It has been suggested that rather than causing direct and immediate modifications in protein (de)phosphorylation, IPC reduces the extent of oxidative stress during ischemia and reperfusion [6,74]. Although the burst in ROS formation occurring during reperfusion is clearly reduced in IPC-treated cardiomyocytes [74], the mechanism by which IPC or IPoC might affect oxidative stress is yet ill-defined. Since ROS formation and PTP opening are linked in a cycle, at present it cannot be established whether reduced oxidative stress is upstream or downstream of decreased PTP opening. In addition, evidence that ischemia-induced protein carbonylation is reduced in IPC-treated hearts [6] does not help to clarify the underlying protective mechanisms. Indeed, the carbonylated proteins in mitochondria were not identified [6], and a major contribution might have come from contaminating actin. Finally, oxidation of mitochondrial proteins is not necessarily linked to adverse effects. For instance, oxidative modification (i.e., nitrosylation) of Complex I has been suggested to be involved in IPC-induced cardioprotection [91].

6. The PTP as a Ca^{2+} release channel

Early work from our laboratories has indicated that in cultured cells transient PTP openings take place under "resting" conditions [92], and that PTP agonists induce cytochrome *c* release and cell death only for prolonged PTP open times [76]. A physiological role was proposed for these transient PTP openings, and we have argued that the PTP could act as a reversible fast Ca^{2+} release channel [93]. The advantage of an unselective channel of high conductance would be to provide charge compensation within the channel itself, thus allowing mitochondrial Ca^{2+} release at zero potential, i.e., even for vanishingly small Ca^{2+} gradients [94]. This hypothesis is consistent with some early as well as very recent observations related to heart mitochondria.

In 1992 Altschuld et al. showed that in isolated adult rat ventricular cardiomyocytes the mitochondrial uptake of $^{45}Ca^{2+}$ was doubled upon treatment with CsA [95]. This rather overlooked finding has been recently confirmed and extended by a report showing that in cardiomyocytes isolated from $Ppif^{-/-}$ mice the mitochondrial matrix displays a 2.6-fold elevation in total Ca^{2+} levels as compared to wild type littermates [96]. The matrix Ca^{2+} rise was shown to be linked to activation of intramitochondrial Ca^{2+}-dependent dehydrogenases resulting in a stimulation of glucose oxidation at the expense of a reduced fatty acid oxidation—a condition that resembles one of the hallmarks of decompensated cardiac hypertrophy [97]. Perhaps even more importantly, the absence of CyPD resulted in an increased propensity for heart failure in three different models, i.e., transaortic constriction, overexpression of Ca^{2+}/calmodulin-dependent protein kinase IIδc and swimming exercise [96]. The link with metabolic changes might be not crucial, since the increased glucose oxidation obtained by inhibiting pyruvate dehydrogenase kinase prevents contractile derangements in two models of right ventricular hypertrophy [98]. Nevertheless, the study of Elrod et al. attributes a relevant role to CyPD as a necessary factor for the physiological response of the heart to mechanical stresses [96]. Apparently, this concept is disputed by findings also reported by Molkentin's laboratory showing that CyPD deletion prevented contractile impairment induced by overexpression of sarcolemmal L-type Ca^{2+} channel [99]. However, in this latter model CyPD protection might be ascribed to a reduced occurrence of cell death caused by PTP opening following mitochondrial Ca^{2+} overload, a condition that might not apply to other forms of heart failure. Thus, the protection against cardiomyocyte necrosis afforded by CsA (or CyPD ablation in mice) in experimental and clinical myocardial infarction may come at the price of reduced myocardial resistance to chronic conditions of mechanical overload, that are possibly associated with an increased susceptibility to apoptosis.

Recent work in mouse primary adult neurons also indicates that PTP is only activated in response to the combined action of more than one physiological stimulus affecting cytosolic Ca^{2+}, and that under these conditions PTP opening does not induce neuronal death but rather takes part in cellular Ca^{2+} dynamics [100].

7. Conclusions

The role of the PTP and CyPs in cardioprotection is a rapidly evolving area whose complexity is being increasingly appreciated. In Fig. 1 we have summarized the potential benefits and drawbacks of PTP inhibition, which today is possible only through inhibition of CyPD. We believe that the emerging network of regulatory protein interactions involving the PTP and CyPs in physiology and pathology will increase the repertoire of rationale therapeutic interventions in heart disease.

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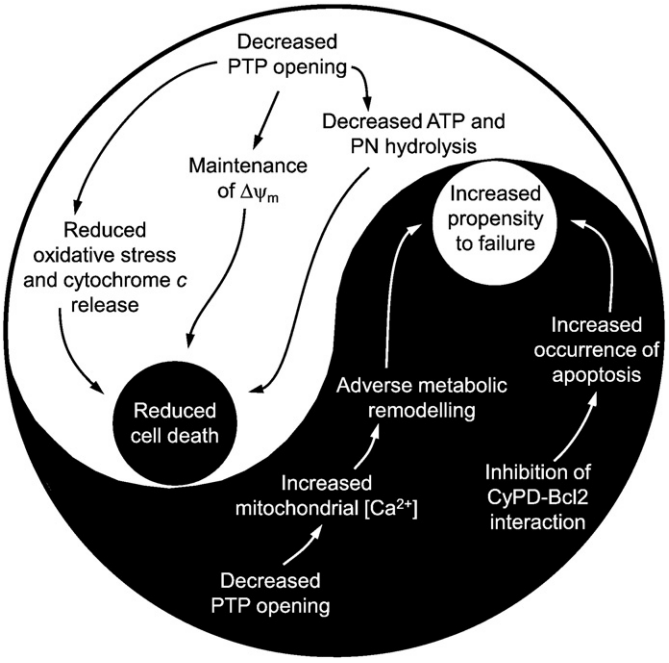

Fig. 1. Beneficial and adverse effects induced by genetic deletion or pharmacological inhibition of CyPD. Additional protection by CsA may derive from CyPA-dependent inhibition of calcineurin, which prevents Drp1 dephosphorylation and mitochondrial fragmentation. PN, pyridine nucleotides. For further explanation see text.

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