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Review article

The mitochondrial permeability transition pore: Molecular nature and role as a target in cardioprotection

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ABSTRACT

The mitochondrial permeability transition (PT) – an abrupt increase permeability of the inner membrane to solutes – is a causative event in ischemia–reperfusion injury of the heart, and the focus of intense research in cardioprotection. The PT is due to opening of the PT pore (PTP), a high conductance channel that is critically regulated by a variety of pathophysiological effectors. Very recent work indicates that the PTP forms from the F-ATP synthase, which would switch from an energy-conserving to an energy-dissipating device. This review provides an update on the current debate on how this transition is achieved, and on the PTP as a target for therapeutic intervention. This article is part of a Special Issue entitled 'Mitochondria: from basic mitochondrial biology to cardiovascular disease'.

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1. Introduction

The permeability transition (PT) is an abrupt increase of the inner mitochondrial membrane (IMM) permeability to solutes, which in mammalian mitochondria has a cutoff of about 1500 Da. Occurrence of the PT and its inhibition by adenine nucleotides is known since the 1950s [1,2], and the phenomenon has been investigated in a number of laboratories (e.g. [3–13]). The term "permeability transition" was introduced in 1979 by Haworth and Hunter, who carried out a thorough characterization of its basic features in heart mitochondria, and provided the important insight – which is today generally accepted – that the PT could be due to opening of an IMM channel, the PTP [14–17]. This hypothesis was confirmed by patch-clamp studies on mammalian mitoplasts, which revealed the presence of a high-conductance (≈1 nS) channel,

Abbreviations: ANT, adenine nucleotide translocase; CsA, cyclosporin 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; I/R, ischemia–reperfusion; MMC, mitochondrial megachannel; OMM, outer mitochondrial membrane; PKA, cyclic AMP-dependent protein kinase; PKG, cyclic GMP-dependent protein kinase; PPIase, peptidylprolyl *cis-trans* isomerase; PT, permeability transition; PTP, permeability transition pore; ROS, reactive oxygen species; TSPO, transport protein of 18 kDa; VDAC, voltage-dependent anion channel.

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the mitochondrial megachannel (MMC) [18,19]. The MMC possesses all the basic features of the PTP [20,21] including sensitivity to cyclosporin A (CsA) [22], and represents the electrophysiological equivalent of the pore [23]. The study of mitochondrial channels has greatly contributed to our understanding of mitochondrial physiology, and to the acceptance of the pore theory of the PT (see [24] for a recent review).

PTP opening is traditionally linked to mitochondrial dysfunction because its occurrence leads to mitochondrial depolarization, cessation of ATP synthesis, Ca^{2+} release, pyridine nucleotide depletion, inhibition of respiration and, in vitro at least, matrix swelling; in turn, swelling causes mobilization of cytochrome c, outer mitochondrial membrane (OMM) rupture and eventually release of proapoptotic proteins such as cytochrome c itself, endonuclease G and AIF [25,26]. It should be mentioned that these detrimental effects on energy conservation and cell viability are only seen for long-lasting openings of the PTP [27], while short-term openings – which have been documented both in isolated mitochondria and in situ [27–30] – may be involved in physiological regulation of Ca^{2+} and reactive oxygen species (ROS) homeostasis [31], and provide mitochondria with a fast mechanism for Ca^{2+} release [32–35]. The potential role of the PTP in heart injury has been recognized very early [36,37], well before the role of mitochondria in apoptosis was discovered [38–40]. PTP desensitization with CsA proved beneficial in heart ischemia–reperfusion injury, as well as in pre- and post-conditioning through mechanisms that await clarification [41–49].

Matrix Ca^{2+} is an essential permissive factor for PTP opening, but the role of mitochondrial "Ca^{2+} overload" as a causative event in I/R injury of the heart has recently been challenged. In MCU null mitochondria – where Ca^{2+} overload does not occur during reperfusion – the extent of necrosis was the same as that observed in the hearts from wild type littermates, and the cardioprotective effect of CyPD ablation was abrogated [50]. These surprising observations raise many issues that still await an answer, such as the cause of cell death, the mechanism of activation of mitochondrial metabolism and the mechanism of PTP opening in MCU null mice. Yet these experiments do show that cardiomyocyte cell death can occur without mitochondrial "Ca^{2+} overload"; and suggest that there is enough Ca^{2+} in the matrix of MCU null mitochondria to allow pore PTP opening, possibly a consequence of the burst of ROS that follows reperfusion [31].

2. Molecular nature of the permeability transition pore: the early days

The molecular nature of the PTP has been the matter of debate for the last 30 years. In the early 1990s Snyder and coworkers found that the peripheral benzodiazepine receptor, an OMM protein today called TSPO [51], copurified with the adenine nucleotide translocator (ANT) and the voltage-dependent anion channel (VDAC) in protocols based on detergent extraction followed by hydroxylapatite chromatography; radiolabeled high-affinity ligands of TSPO were recovered in fractions where TSPO could be detected together with VDAC and ANT [52]. This finding was of great interest because nanomolar concentrations of the same TSPO ligands affected the channel properties of MMC in electrophysiological experiments, suggesting that all of these proteins could be involved in formation of the PTP [53].

This suggestion was strengthened a few years later by work from the Brdiczka laboratory during the characterization of OMM and IMM "contact sites", i.e. specialized structures where the two membranes form close contacts mediated by protein–protein interactions [54]. These sites would include hexokinase on the cytosolic surface of, and VDAC within, the OMM, creatine kinase and nucleoside diphosphate kinase in the intermembrane space, and ANT in the IMM; they were proposed to mediate channeling of adenine nucleotides to and from mitochondria [54–56]. The link with the PTP was made when the same laboratory showed that hexokinase-enriched fractions from low detergent extracts of mitochondria formed channels with the conductance expected of the PTP, and conferred permeability properties to liposomes that could be inhibited by N-methylVal-4-cyclosporin [57]. It must be stressed that the preparation contained a very large number of proteins, which makes assignment of the channel activity to a specific species quite problematic. Furthermore – and unlike the case of PTP – currents were inhibited rather than induced by atractylate, and the active fractions were not enriched in VDAC and/or ANT [57]. The same preparations were shown to also contain proteins of the Bcl-2 family [58], and this set of observations led to a model where the PTP would be a multiprotein complex spanning both mitochondrial membranes and comprising ANT, VDAC, TSPO, cyclophilin (CyP) D as well as hexokinase and Bcl-2 proteins [59]. This model did not stand the test of genetics, as a CsA-sensitive PT could be easily detected in the absence of ANT [60], VDAC [61,62] as well as of TSPO [63]. An alternative model is the formation of the PTP by the Pi carrier following its interaction with CyP-D and ANT [64]. However, results obtained by patch-clamp analysis of the reconstituted Pi carrier do not match the electrophysiological PTP features [65] and genetic deletion of the Pi carrier does not support the idea that this protein is essential for PTP formation [66].

Studies on *Ppif*$^{-/-}$ mice (*Ppif* is the unique gene encoding CyPD in the mouse) have demonstrated that this protein is an important modulator which sensitizes the PTP to Ca^{2+} and confers sensitivity to CsA, but not an essential pore component [67–70]. By following the interactions of the matrix CyPD with other mitochondrial proteins it has recently been possible to identify a novel structure for the PTP, which will be described in the following paragraph.

3. The permeability transition pore forms from F-ATP synthase

By monitoring the presence of CyPD in blue native gels of mitochondrial proteins Giorgio et al. discovered that CyPD interacts with the F-ATP synthase, and that it can be crosslinked to the stalk proteins b, d and OSCP [71]. Binding of CyPD to the F-ATP synthase required Pi, and caused a decrease of the enzyme's catalytic activity; while it was counteracted by CsA, which displaced CyPD and increased the catalytic activity [71]. It was then found that CyPD interacts with the OSCP subunit of F-ATP synthase [72]. Gel-purified dimers of F-ATP synthase incorporated into lipid bilayers displayed currents activated by Ca^{2+}, Bz-243 and phenylarsine oxide (but not atractylate) with a unit conductance of about 500 pS, which is identical to that of the bona fide mammalian MMC-PTP [72]. The channel-forming property is shared by purified F-ATP synthase dimers of yeast mitochondria, which also displayed Ca^{2+}-dependent currents of slightly lower conductance (about 300 pS) [73]. Furthermore, yeast strains lacking the e and/or g subunits, which are necessary for dimer formation, showed a remarkable resistance to PTP opening [73]. Although strains lacking subunits e [74] or g [75] display abnormal morphology, with balloon-shaped cristae and F-ATP synthase monomers distributed randomly in the membrane, they did develop a normal membrane potential [73], suggesting that the increased resistance to PTP opening may not depend on these structural differences. Based on these findings, it has been proposed that the PTP forms from F-ATP synthase dimers, possibly in the lipid region between two adjacent stalks [76].

The idea that the pore forms from the F-ATP synthase is also supported by two independent studies. Bonora et al. used targeted inactivation of the c subunit of F-ATP synthase – which forms the H^+-transporting c ring of F-ATP synthases – to show that HeLa cells become resistant to PTP opening and cell death [77]; while Alavian et al. reconstituted the c subunit or the purified F-ATP synthase in liposomes, and measured Ca^{2+}-activated channels [78] with properties similar to those described by Giorgio et al. with purified dimers [72]. It is not possible to derive mechanistic insights about the nature of the PTP-forming channel from the study of Bonora et al. because the consequences of knockdown of the c subunit on other components of the F-ATP synthase and on other mitochondrial proteins were not addressed, and it is unclear whether and how many functional F-ATP synthases were left after the knockdown of the c subunit [77]. Alavian et al., on the other

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