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Review article

The mitochondrial permeability transition: A current perspective on its identity and role in ischaemia/reperfusion injury

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ABSTRACT

The mitochondrial permeability transition pore (MPTP) is a non-specific pore that opens in the inner mitochondrial membrane (IMM) when matrix [Ca^{2+}] is high, especially when accompanied by oxidative stress, high [Pi] and adenine nucleotide depletion. Such conditions occur during ischaemia and subsequent reperfusion, when MPTP opening is known to occur and cause irreversible damage to the heart. Matrix cyclophilin D facilitates MPTP opening and is the target of its inhibition by cyclosporin A that is cardioprotective. Less certainty exists over the composition of the pore itself, with structural and/or regulatory roles proposed for the adenine nucleotide translocase, the phosphate carrier and the FoF1 ATP synthase. Here we critically review the supporting data for the role of each and suggest that they may interact with each other through their bound cardiolipin to form the ATP synthasome. We propose that under conditions favouring MPTP opening, calcium-triggered conformational changes in these proteins may perturb the interface between them generating the pore. Proteins associated with the outer mitochondrial membrane (OMM), such as members of the Bcl-2 family and hexokinase (HK), whilst not directly involved in pore formation, may regulate MPTP opening through interactions between OMM and IMM proteins at "contact sites". Recent evidence suggests that cardioprotective protocols such as preconditioning inhibit MPTP opening at reperfusion by preventing the loss of mitochondrial bound HK2 that stabilises these contact sites. Contact site breakage both sensitises the MPTP to [Ca^{2+}] and facilitates cytochrome *c* loss from the intermembrane space leading to greater ROS production and further MPTP opening. This article is part of a Special Issue entitled 'Mitochondria'.

Contents

Abbreviations: Akt, also known as protein kinase B; ANT, adenine nucleotide translocase; Bad, Bcl-2-associated death promoter; Bak, Bcl-2 homologous antagonist/killer; Bax, Bcl-2-like protein 4; Bcl2, B cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; Bid, BH3 interacting-domain death agonist; CAT, carboxyatractyloside; CK, creatine kinase; CsA, cyclosporin A; CyP-D, cyclophilin D; G-6-P, glucose-6-phosphate; GSK3β, glycogen synthase kinase 3β; HK, hexokinase; IF1, ATP synthase inhibitor factor 1; IMM, inner mitochondrial membrane; IP, ischaemic preconditioning; I/R, ischaemia reperfusion; MCF, mitochondrial carrier family; MPTP, mitochondrial permeability transition pore; NHE, sodium/proton exchanger; OMM, outer mitochondrial membrane; OPA-1, optic atrophy 1; PCr, phosphocreatine; PiC, phosphate carrier; PKA, protein kinase A; PKCε, protein kinase Cε; pmf, proton motive force; PPIase, peptidylprolyl isomerase; ROS, reactive oxygen species; SR, sarcoplasmic reticulum; T_0, time of ischaemic rigour start; TAT-HK2, cell permeable peptide of HK2 binding domain; tBid, truncated BID; TP, temperature preconditioning; TSPO, translocator protein of the outer membrane; VDAC, voltage-dependent anion channel; ρ^0, mitochondrial DNA-depleted cells.

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1. Introduction

The major role of mitochondria in the heart is the provision of ATP by oxidative phosphorylation to drive the contractile cycle and maintain ionic homeostasis. Oxidative phosphorylation requires the permeability barrier of the inner mitochondrial membrane (IMM) to be maintained. However, mammalian mitochondria contain a latent non-specific pore within their inner membrane, known as the mitochondrial permeability transition pore (MPTP). Opening of the MPTP not only prevents mitochondria from synthesising ATP by oxidative phosphorylation, but also allows reversal of the FoF1 ATP synthase causing hydrolysis of the ATP produced by glycolysis or any remaining "healthy" mitochondria [1]. If this occurs for any length of time, cells become depleted of ATP and will eventually die by necrosis. In essence, MPTP opening converts mitochondria from ATP providers that energise the cell to agents of cell death, akin to the caring Dr Jekyll turning into the murderous Mr Hyde [2]. It is now widely accepted that the MPTP plays a major role in determining the extent of injury the heart suffers during reperfusion after a prolonged period of ischaemia; such ischaemia/reperfusion injury (I/R) is reflected in the size of the necrotic area or infarct (see [2–4]). In this article we will first review what is currently known about the mechanism and molecular identity of the MPTP, paying particular attention to significant new developments since our previous review in this journal [1]. We will then briefly summarise the evidence that MPTP opening is a key event in I/R injury and finally review how inhibiting MPTP opening during reperfusion is cardioprotective.

2. The properties of the MPTP

2.1. Historical perspective

It has been known for more than sixty years that mitochondria become leaky, uncoupled and massively swollen if they are exposed to high calcium concentrations, especially in the presence of phosphate and when accompanied by oxidative stress (see [2,5]). This phenomenon became known as the permeability transition and was originally thought to reflect activation of endogenous phospholipase A_2 leading to phospholipid breakdown within the IMM [6]. However, seminal studies in the late seventies by Haworth and Hunter [7,8] revealed that the permeability transition involved the opening of a non-specific channel permeable to any molecule <1.5 kDa. This was subsequently confirmed by Crompton [9] who demonstrated that the pore could be specifically blocked by sub-micromolar concentrations of cyclosporin A (CsA) [10]. This critical observation was rapidly confirmed by others [11,12] and provided the first clue as to the identity of one component of the MPTP, cyclophilin-D. Since then several laboratories in addition to ours, most notably those of Crompton, Bernardi and Molkentin, have been involved in characterising the properties of the MPTP and its molecular identity (see [1,5,13,14]).

2.2. The open pore is a non-selective channel — evidence and consequences

Measurement of the permeability properties of mitochondria that have undergone the permeability transition demonstrated that the MPTP is a non-specific pore with a diameter of about 2.3 nm [7,15]. Further evidence for this came from patch-clamp studies that identified the presence of a megachannel within the IMM whose opening and electrophysiological properties matched those predicted for the MPTP [16,17]. However, it is important to note that such megachannel behaviour may also reflect other molecular entities [18,19]. This becomes important when the role of specific proteins in the formation of the MPTP is investigated using electrophysiological techniques as discussed in Section 3.

Opening of the MPTP makes the IMM freely permeable to protons and hence uncouples mitochondria as noted above; but it also allows all small molecular weight metabolites, cofactors and ions to equilibrate between the mitochondrial matrix and the cytosol. This includes $[Ca^{2+}]$ and there is evidence that this provides a mechanism for releasing excessive accumulated calcium from the mitochondrial matrix in some (patho)physiological situations [20,21]. This is discussed further in Section 2.4. MPTP opening also induces swelling of mitochondria. This occurs because small molecular weight metabolites and cofactors equilibrate across the IMM leaving proteins within the matrix to exert a colloidal osmotic pressure [1,2]. In vitro, in the absence of extramitochondrial proteins to provide an osmotic support, this causes extensive swelling of the matrix accompanied by cristae unfolding and rupture of the outer mitochondrial membrane (OMM). Such swelling is accompanied by a decrease in light scattering which is often used to monitor the progress of MPTP opening [22,23]. In vivo, swelling is still observed but it is of lesser magnitude because of the colloidal osmotic support provided by proteins in the cytosol. However, it may still be sufficient to cause rupture of the outer membrane and release of intermembrane proteins such as cytochrome *c* that can induce apoptosis [24,25].

2.3. MPTP opening is triggered by elevated matrix $[Ca^{2+}]$

A matrix-facing Ca^{2+} binding site is essential to trigger MPTP opening, and any factor that influences calcium loading, such as modulators of Ca^{2+} import and efflux pathways, will affect MPTP opening, whilst chelation of matrix calcium causes rapid closure [7,15,26–29]. Interestingly, unlike most mitochondrial Ca^{2+}-sensitive processes such as the Ca^{2+}-activated dehydrogenases, Sr^{2+} cannot substitute for Ca^{2+} as a trigger for MPTP opening. Indeed, the Ca^{2+} trigger site can be inhibited by Sr^{2+} and other divalent cations such as Mg^{2+} [7,30,31], and also by H^+ which accounts for the potent inhibition of MPTP opening by low pH [8,32,33]. There is an additional divalent cation regulatory site on the MPTP that faces the cytosolic side of the inner membrane and inhibits MPTP opening. This has a broader specificity than the Ca^{2+}-trigger site, and inhibition is observed with many divalent cations including Ca^{2+} and Mg^{2+} [34].

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