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EDITORIAL

Inhibition of mitochondrial permeability transition pore opening: the holy grail of cardioprotection

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Cardioprotection is a fairly vague term which refers to the reduction of damage from myocardial ischemia/reperfusion by several endogenous phenomena, such as hibernation [23, 37], ischemic preconditioning [32, 41], ischemic postconditioning [20, 43], and remote conditioning [19, 22] as well as by pharmacological interventions. With the recognition of the postconditioning phenomenon [43], myocardial reperfusion injury has been appreciated as a reality [42] from which protection is clinically feasible [36, 38, 39]. The signal transduction of cardioprotection is under intense investigation, with the ultimate aim to identify targets for pharmacological recruitment of cardioprotection [21]; three major pathways have been characterized—the cGMP/PKG-pathway [11], the reperfusion injury salvage kinase (RISK)-pathway [18] and the survivor activating factor enhancement (SAFE)-pathway [31]. These three major pathways are not mutually exclusive, but potentially cooperative, and they are recruited by the above cardioprotective phenomena to a different extent.

The cardioprotective signaling pathways are thought to converge on the mitochondria [21], and various mitochondrial proteins without or with channel structure have been identified as central elements in cardioprotection. Several signaling pathways of cardioprotection converge to inhibit glycogen synthase kinase-3β which in its phosphorylated state increases the threshold for mitochondrial permeability transition pore (MPTP) opening [25, 26].

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The MPTP is a large-conductance megachannel which is traditionally thought to be formed by an arrangement of the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane, the adenine nucleotide transporter (ANT) in the inner membrane and cyclophilin D in the matrix [30]. The MPTP is either not present or mostly closed under physiological conditions, but opens in response to high concentrations of calcium. The calcium-induced opening of MPTP is favoured by high concentrations of inorganic phosphate, reactive oxygen species, and nitric oxide and a reduction of the inner membrane potential—all conditions which occur during myocardial ischemia and reperfusion; acidosis and magnesium ions inhibit/delay MPTP opening [8, 13, 29, 30, 40, 44]. In addition, pro- and anti-apoptotic members of the Bcl-family interact with the MPTP [3]. Formation and opening of the MPTP results in depolarization of the inner membrane potential and matrix swelling and ultimately in rupture of the outer membrane. Proteins, among them cytochrome C, are then released from the intermembrane space into the cytosol and activate caspase cascades to initiate cellular fragmentation and ultimately cell death.

In 1993, both the groups of Crompton [12] and Halestrap [16] demonstrated that MPTP opened upon reperfusion following myocardial ischemia and that cyclosporine A protected from reperfusion injury. Cyclosporine A inhibits binding of cyclophilin D to ANT, thereby MPTP opening and ultimately cell death; ablation of the Ppif gene which encodes for cyclophilin D [4, 33] and cyclosporine A [4] therefore reduce infarct size resulting from myocardial ischemia and reperfusion.

Yellon et al. first proposed that inhibition of MPTP opening could be the effector of ischemic preconditioning [17], and Ovize et al. shortly thereafter proposed MPTP inhibition as the effector of ischemic postconditioning [2];

their experimental evidence was largely based on the use of MPTP inhibition by cyclosporine A or its non-immunosuppressive derivative and analysis of respective changes in the calcium retention capacity of isolated mitochondria.

Mechanistic analyses of MPTP are derived from biochemistry of purified and reconstituted putative constituent proteins, from electrophysiology of isolated mitoplasts and/or mitochondria and from confocal microscopy using marker molecules and dyes in isolated cells. The involvement of the MPTP is obviously more difficult to identify in ischemic/reperfused myocardium in vivo, and evidence for the involvement of the MPTP in vivo is therefore largely indirect, e.g. using genetic ablation of putative constituent proteins or pharmacological inhibitors such as cyclosporine A.

Apart from quantitative analysis of mitochondrial depolarization using the fluorescent probe tetramethylrhodamine ethyl ester (TMRE) which may, however, not be specific for MPTP opening [34], the determination of calcium retention capacity in isolated cardiac mitochondria has recently emerged as a popular tool to address the mitochondrial resistance to MPTP opening, and changes in calcium retention capacity may occur independently from changes in membrane potential [35]. Originally this method was developed in liver mitochondria where exposure to pulses of extramitochondrial calcium was buffered by calcium uptake into the mitochondrial matrix such that the extramitochondrial calcium concentration returned back to baseline upon each pulse until finally the extramitochondrial calcium concentration abruptly and markedly increased, presumably secondary to massive calcium release from open MPTP [24]. Now, for proper interpretation of calcium retention capacity in the context of MPTP opening, the functional viability of the mitochondria after isolation must be ascertained; this is most convincingly done by the determination of their respiratory capacity. Respiration should be measured using complex I substrates, because electron flux through complex I is essential to maintain the sensitivity of MPTP for opening [14]. Respiration should not only be measured at baseline, but also with stimulation by ADP and finally with uncoupling using substances such as dinitrophenol or FCCP (carbonylcyanide-4-(trifluoromethoxy)-phenylhydrazone) [9]. The comparison of calcium retention capacity between two different mitochondrial preparations, e.g. protected versus non-protected, can only be interpreted in relation to their respective respiratory capacity. Given proper determination of respiration, it is then essential to observe a true return of the extramitochondrial calcium concentration back to baseline to make sure that all mitochondria in the given population under study still have their MPTP closed, before after several calcium pulses with return to baseline an abrupt and massive calcium release is detected [6]. The comparison of mitochondrial preparations undergoing different interventions by the amount of calcium needed to induce MPTP opening assumes that baseline calcium load is comparable; in fact, reperfusion per se increases mitochondrial calcium, and preconditioning but not postconditioning attenuates this increase [1]. The administration of exogenous cyclosporine A and the consequent delay of MPTP opening should be part of each protocol to determine mitochondrial calcium retention capacity. With use of cyclosporine A, however, only the role of cyclophilin D in MPTP opening is assessed [6]. With measurement of mitochondrial calcium retention capacity in the absence and in the presence of cyclosporine A, therefore, the cyclophilin D-dependent and -independent alterations in MPTP opening can be determined; the cyclophilin D-independent changes in MPTP opening might reflect the regulator, e.g. inorganic phosphate [13, 15].

Apart from these important technical considerations, conceptually data on calcium retention capacity from isolated mitochondria ex vivo can only be retrospectively associated to the observed infarction and protection from it, but can never be put into a causal context: is better calcium retention capacity ex vivo cause or consequence of reduced infarct size in vivo? Finally, not all mitochondria are created equal, and there may be differences between subsarcolemmmal and interfibrillar mitochondria which are relevant for cardioprotection [10].

The traditional view of the MPTP is far from firmly established; each single putative constituent of the MPTP appears dispensable under certain conditions [8, 44]: VDAC [5, 28], ANT [27], and cyclophilin D [6, 7]. From a pragmatic point of view, however, cyclosporine A when given at reperfusion clearly reduced infarct size by about 40% in a clinical proof-of-concept study [36]; whether or not such protection translates into improved prognosis remains to be seen in larger-scale prospective trials.

In the end, inhibition of the MPTP is indeed like a holy grail in cardioprotection: it is an extremely attractive target, but the true identity and precise function of the MPTP in myocardial ischemia/reperfusion remain enigmatic and elusive—you just have to believe in it, or not.

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