The mitochondrial permeability transition pore as a target of cardioprotective signaling

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IT IS HAS BEEN OVER TWO DECADES SINCE Murry and colleagues (15) published their seminal study describing ischemic preconditioning, a phenomenon whereby brief antecedent periods of ischemia-reperfusion profoundly protect the heart against a subsequent lethal period of ischemia and reperfusion. This intrinsic cardioprotective mechanism has been demonstrated in every animal tested, including human cardiac tissue (20), and, obviously, exploitation of such a powerful protective mechanism would have considerable clinical applications. Consequently, considerable effort has been expended over the past twenty years to try to delineate the mediators and molecular mechanisms that underlie cardioprotection. We now know that various neurohormonal agents and peptides, such as adenosine, bradykinin, and nitric oxide, are released during the preconditioning bursts of ischemia and reperfusion (20). This trigger an increasingly complex series of signal transduction events within the myocyte that somehow confer a “memory” that enables the cell to survive a more pathological period of ischemia. These signal transduction elements primarily consist of kinases, especially protein kinase C (PKC), tyrosine kinases, and mitogen-activated protein kinases (14, 20), with Akt, glycogen synthase kinase 3B, and PKG recently added to the ever-expanding list (6, 14). Nevertheless, despite this extensive knowledge regarding the triggers and signal transduction networks of preconditioning, the critical targets of the protective machinery, the so-called “end effectors,” have remained elusive. However, recent evidence has implicated mitochondria and, in particular, the mitochondrial permeability transition pore, as important targets of cardioprotective signaling.

Ischemia-reperfusion induces rapid increases in mitochondrial permeability and volume, a phenomenon known as mitochondrial permeability transition (MPT). These large alterations in membrane permeability lead to a dissipation of the mitochondrial permeability transition (MPT). These large alterations in membrane permeability lead to a dissipation of the mitochondrial membrane potential (ΔΨm), inhibition of ATP synthesis, swelling, and ultimately mitochondrial rupture (8, 9). The MPT pore, a nonspecific channel that spans both mitochondrial membranes, mediates these increases in mitochondrial permeability (8, 9). The pore itself is permeable to solutes up to 1.5 kDa. This causes equilibration of H+ across the inner membrane, which dissipates ΔΨm. A simultaneous influx of water causes swelling of the mitochondrial matrix, which stretches the mitochondrial membranes to the point where the outer membrane fails. The MPT pore is redox, Ca2+, voltage, and pH sensitive. In particular, increases in matrix Ca2+ and oxidative stress induce MPT pore opening, whereas adenine nucleotides inhibit the pore (8, 9). This is especially germane since ischemia-reperfusion is associated with increases in pore activators (Ca2+ and oxidants) and reductions in pore inhibitors (ATP and ADP). Indeed, studies have shown that MPT occurs at reperfusion, and pharmacological and genetic inhibition of the MPT pore blunts ischemia-reperfusion injury (3, 5, 10, 16).

In the context of cardioprotection, several groups have reported that mitochondria isolated from preconditioned hearts are more resistant to Ca2+-induced MPT (2, 11, 12). Moreover, inhibition of key signaling enzymes such as PKC abrogates the inhibitory effect of preconditioning on the MPT pore (6, 13). Similarly, others have shown in cultured myocytes that cardioprotective agents prevent MPT and subsequent cell loss in response to hypoxia-reoxygenation (13, 19). Together, these data strongly indicate that the MPT pore, while not technically an end effector, certainly appears to be an end target of cardioprotection.

The excellent study by Townsend and colleagues (18), published in this issue of the American Journal of Physiology-Heart and Circulatory Physiology, offers further proof that inhibition of the MPT pore may ultimately be how cardioprotection actually works. Not content with restricting their studies to one particular model, the authors investigated the effects of a known cardioprotective agent, urocortin, on MPT in isolated mitochondria, both neonatal- and adult-cultured myocytes, as well as ex vivo perfused hearts. Using the “hot dog” technique whereby 2-[14C]deoxyglucose is entrapped in mitochondria that have undergone MPT, the authors showed that a 30-min pretreatment with urocortin significantly reduced the degree of ischemia-reperfusion-induced MPT and concomitant tissue damage in Langendorff-perfused rat hearts. Similarly, mitochondria isolated from the treated, ischemic hearts were less susceptible to Ca2+-induced MPT. In both neonatal and adult rat ventricular myocytes, acute and chronic exposure to urocortin profoundly reduced the loss of ΔΨm, an index of MPT, following simulated ischemia-reperfusion. Thus this state-of-the-art study provides comprehensive evidence that the inhibition of the MPT pore is involved in the cardioprotective effects of urocortin and serves as an excellent example as to how the MPT pore should be studied.

How does urocortin, and other cardioprotective agents, cause inhibition of MPT, i.e., how does the protective signal transduction network couple to the MPT pore? Many compounds, including urocortin, induce protection through the activation of the ε-isofrom of PKC (14, 20). Recent studies have suggested that PKC-ε may physically translocate to the mitochondria where it could potentially interact with and inhibit the MPT pore (4, 6, 13). However, in the present study, Townsend et al. (18) found very little PKC-ε in a highly purified mitochondrial fraction, with no additional translocation of PKC-ε to the mitochondria following either urocortin or ischemia (18). These data suggest that the coupling of PKC-ε to MPT inhibition may in fact be more indirect. One possibility, as proposed by the authors, is that activation of PKC-ε causes a reduction in the burst of reactive oxygen species upon reperfusion, thus greatly reducing one of the prime activators of MPT.
of the MPT pore. Indeed, using either a redox-sensitive dye or blotting for protein carbonylation, the authors demonstrated that urocortin reduced the increase in oxidative stress at reperfusion, an effect that could be blocked by the PKC inhibitor chelerythrine (18).

This of course raises the question of how PKC-ε prevents the production of reactive oxygen species, which at reperfusion are primarily mitochondrial in origin. PKCs have been shown to regulate NADPH oxidase activity, albeit in a positive manner (17), as well as levels of antioxidants, such as superoxide dismutase (7) and thioredoxin (1). Alternatively, although PKC-ε itself may not localize to mitochondria, other distal kinases, such as Akt and mitogen-activated protein kinases, may do so to inhibit mitochondrial oxidant production or even directly influence the MPT pore. Future studies addressing these suppositions are needed to continue to provide critical insight into the cardioprotective mechanism and, ultimately, into how it can be manipulated in the clinical setting.

REFERENCES


