Title
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Journal
Circulation Research, 118(5)

ISSN
0009-7330

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Publication Date
2016-03-04

DOI
10.1161/circresaha.116.308291

Peer reviewed

New Roles for an Old Pore

James N. Weiss

Biological organisms depend on a variety of mechanisms to remove defective components. Such was originally thought to be the function of the mitochondrial permeability transition, in which high-conductance permeability transition pores (PTP) form in the inner mitochondrial membrane, causing immediate matrix depolarization, Ca release, reversal of ATP synthase, matrix swelling, and eventually rupture of the outer mitochondrial membrane, releasing proapoptotic signaling molecules, such as cytochrome c (Figure).[1] PTP formation is triggered primarily by reactive oxygen species (ROS) and matrix Ca overload. Originally interpreted as a suicide switch to remove defective mitochondria spewing excess ROS, PTP were later discovered to open transiently in a lower conductance mode hypothesized to serve a useful physiological role—namely as a mechanism to allow Ca-overloaded mitochondria to flush Ca from the matrix.[2]

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Physiologically, matrix-free [Ca] is similar to that in the cytoplasm, ≈100 nmol/L. With a matrix membrane potential around −180 mV, the equilibrium [Ca] in the matrix would be around 6 orders of magnitude higher, that is, 0.1 mol/L. Thus, the inner mitochondrial membrane normally has a low permeability to Ca, with influx primarily regulated by the mitochondrial Ca uniporter and efflux by Na–Ca exchange. When an imbalance occurs such that matrix-free [Ca] becomes elevated, the energetic cost of removing Ca against such an unfavorable electrochemical gradient is huge. Transient PTP openings allow the matrix to depolarize briefly, canceling out the electrochemical gradient and permitting excess matrix Ca to diffuse out of the matrix into the cytoplasm. Once matrix Ca has re-equilibrated with matrix buffers, the PTP closes and the respiration rapidly re-establishes membrane potential to resume ATP synthesis. Two key features are required for transient PTP openings to function effectively in this capacity: (1) a short duty cycle, such that a mitochondrion need only be depolarized <1% of the time, so that 99% time wise remains dedicated to ATP synthesis; (2) a lower conductance than that of long-lasting high-conductance PTP openings (permeable to molecules up to 1500 Da) to avoid depleting pyridine nucleotides and other essential respiratory cofactors required for the mitochondria to repolarize once the PTP closes. Both of these properties can be readily demonstrated in populations of isolated mitochondria studied in a cuvette.[3] If boluses of Ca are added to the cuvette, mitochondrial first take up Ca, but at a critical Ca load begin to release the stored Ca into the buffer. However, the average matrix membrane potential of the population remains unchanged. Moreover, if mitochondria have been preloaded with calcein (623 Da), they do not release calcein while releasing Ca.[3] These findings are consistent with a low-conductance mode and a short duty cycle such that <1% of mitochondria in the population are depolarized at any given time, thus having no detectable effect on the average membrane potential.

Despite previous studies visualizing PTP openings in single mitochondria, however, direct confirmation of the transient low-conductance mode of PTP openings in individual in situ mitochondrion in a cell has been challenging. In this issue, Lu et al[4] have tackled this issue using state-of-the-art imaging techniques and succeeded in identifying events that are convincing candidates for transient low-conductance PTP openings. They show that in intact and permeabilized cardiac myocytes exposed to progressive Ca loading by several methods, individual mitochondria occasionally demonstrate "MitoWinks," a sudden drop in matrix-free [Ca] lasting about 1 minute. They go on to show that these MitoWinks occur simultaneously with transient matrix membrane depolarizations but are not associated with loss of matrix calcein, implying a low-conductance state permeable to molecules <600 Da. MitoWinks are also suppressed by pharmacological or genetic PTP inhibition and mitochondrial Ca uniporter inhibition; conversely, they are promoted by progressive Ca loading, oxidative stress with H_2O_2, and heart failure. Together, these findings make a compelling case that the authors have succeeded in visualizing transient low-conductance PTP openings in single in situ mitochondria in isolated cardiac myocytes.

Despite this impressive achievement, however, several important questions remain. First, the frequency of the transient PTP openings was low, averaging once every 83 hours (0.02% duty cycle) under control conditions, which modestly increased to once every 3 hours (0.5%) under the most extreme conditions studied (including elevated extramitochondrial [Ca] up to 2000 nmol/L). Moreover, most of the experiments were performed with the mitochondrial Na–Ca exchange blocked to exacerbate matrix Ca accumulation. Thus, even under extreme Ca overload conditions, the transient PTP openings observed in this study were rare, raising a question of how robust this mechanism could be for regulating matrix Ca under rapidly changing conditions, such as ischemia/reperfusion. In populations of isolated mitochondria, matrix Ca release through transient PTP openings occurred much more rapidly during Ca loading,[3] bringing up the possibility that some regulatory

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(*Circ Res*. 2016;118:779-780.
DOI: 10.1161/CIRCRESAHA.116.308291.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/CIRCRESAHA.116.308291

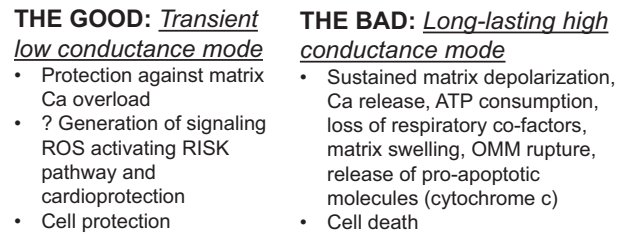

Figure. Protective and injurious roles of the mitochondrial permeability transition pore (PTP). OMM indicates outer mitochondrial membrane; RISK, reperfusion injury survival kinase; and ROS, reactive oxygen species.

component may have become disabled in the isolated myocyte preparations. Finally, a puzzling feature in this study is that after a transient PTP opening, the free [Ca] recorded in the matrix rapidly reaccumulated to a similar or even higher level without the pore reopening. The authors hypothesize accordingly that transient PTP opening may also deplete a cofactor (such as phosphate) that decreases the subsequent sensitivity of the pore to Ca. However, the details remain to be worked out.

What is the evidence that transient PTP openings are physiologically important to intact hearts? It has been previously demonstrated that when cyclophilin D, a key component promoting PTP formation, is knocked out in the mouse, their hearts are more resistant to acute ischemia/reperfusion injury, attributed to suppression of damaging long-lasting high-conductance PTP openings during reperfusion.[5] However, when cyclophilin D knockout mice are subjected to the chronic hemodynamic stress, their cardiac function deteriorates more rapidly than wild-type mice,[6] presumably because the loss of beneficial transient low-conductance PTP openings makes mitochondria more susceptible to matrix Ca overload and mitochondrial injury. Thus, suppressing PTP in the heart is a double-edged sword that can have either deleterious or beneficial effects depending on the setting. The double-edged sword effect also applies to acute ischemia/reperfusion injury. Although pharmacological or genetic PTP inhibition during reperfusion after prolonged ischemia reduces infarct size,[5,7] PTP inhibitors delivered during ischemic preconditioning (IPC) abrogate cardioprotection,[8] indicating that transient PTP openings during IPC play an important role in activating cardioprotective signaling through the RISK pathway. The mechanism may be related to the observation that transient low-conductance PTP openings in isolated mitochondrial populations are associated with significant ROS production,[3] consistent with the cyclosporin A-sensitive "superoxide flashes" imaged during PTP openings in in situ mitochondria.[9] During IPC, activation of the RISK pathway depends on signaling ROS generated during the IPC episodes because ROS scavengers administered during IPC block cardioprotection.[8,10] Thus, it is intriguing to speculate that the transient PTP openings, in conjunction with mitochondrial ATP-sensitive K channel openings, are important in generating the signaling ROS required to activate cardioprotective signaling during IPC. However, mechanism responsible for increased mitochondrial ROS production during PTP openings has yet to be fully defined.

These multifunctional aspects of the PTP make its further characterization an important subject, not only in heart but also in other organs as well. The Lu et al study[4] makes a key contribution by directly visualizing the PTP operating in its protective transient low-conductance mode, but also raises caution with respect to targeting PTP therapeutically. Like ROS, a little PTP activation (in the transient low-conductance mode) is protective, but a lot (in the long-lasted high-conductance mode) wreaks havoc.

Sources of Funding

This study was supported by National Institutes of Health/National Heart, Lung, and Blood Institute R01 HL117385 and the Laubisch and Kawata Endowments.

Disclosures

None.

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KEY WORDS: Editorials ■ heart failure ■ ion channels ■ mitochondria

Circulation Research
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Circ Res. 2016;118:779-780
doi: 10.1161/CIRCRESAHA.116.308291
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7330. Online ISSN: 1524-4571