Ischemic Preconditioning Slows Energy Metabolism and Delays Ultrastructural Damage During a Sustained Ischemic Episode

Murry et al

A classic article by Murry et al published in 1990 in *Circulation Research* showed that preconditioning slowed the rate of ATP breakdown during a subsequent sustained period of ischemia. The mechanism responsible for reduced ATP breakdown is still unknown, but perhaps it is related to the inhibition of F1-F0 ATPase. This is an attractive hypothesis, given several recent studies suggesting that the F1-F0 ATPase can form the mitochondrial permeability transition pore.

Ischemic preconditioning (PC) is one of the more reproducible and robust forms of cardioprotection. A classic article by Murry et al1 was one of the original descriptions of PC. This article, along with others, demonstrated that the heart could initiate an adaptive signaling program that could lead to a significant reduction in infarct size. This article and an earlier article by this group2 reinvigorated the field of cardioprotection. This article showed that subjecting the heart to several brief periods of intermittent ischemia and reperfusion could protect the heart from a subsequent longer period of sustained ischemia. It was somewhat surprising that although the ischemically preconditioned hearts had an additional 20 minutes of ischemia, they had significantly less necrosis. This protection was initiated by as little as 5 minutes of reperfusion between short PC protocol and the extended period of ischemia and was lost if the last period of reperfusion was extended beyond 30 to 60 minutes. These data suggested that a rapid signaling pathway was involved in initiating cardioprotection. This classic article led to a revolution in the field of cardioprotection. The discovery of ischemic PC was followed by a large number of studies showing that this protection could also be initiated by pharmacological agents (pharmacological PC). A large number of signaling pathways were shown to be involved in cardioprotection.

In the years since 1990, >10,000 articles have been published in the field of PC. The concept of ischemic PC also led to the development of remote PC and ischemic postconditioning.

Also of note, this article made several interesting observations that are relevant and largely unexplained >2 decades after publication. In the 23 years since publication of this classic article, PC has been extensively studied, and we have learned much about the signaling pathways involved. However, we still do not fully understand the details of the downstream mechanism by which PC delays cell death.

This article showed that PC slowed the rate of decrease in ATP and reduced the rate of lactate production and concluded that preservation of ATP resulted from reduced ATP utilization and not from increased ATP production. This study also showed that the PC hearts had less glucose-1-phosphate and glucose-6-phosphate, which could have a role in regulating the hexokinase association with mitochondria.

The data in this article suggest that PC protection involves reduced energy demand; however, despite enormous effort, the mechanism by which PC reduces energy demand is still unclear. Previous studies have shown that 50% of ATP consumption during ischemia is a result of the reverse mode of the F1-F0 ATPase.22,23 Thus, either inhibition of the reverse mode of the F1-F0 ATPase (as occurs with binding of inhibitor factor) or inhibition (direct or indirect) of F1-F0 ATPase during ischemia would reduce the decline in ATP and reduce energy demand. In fact, several studies in the mid-1990s tested whether PC resulted in inhibition of the F1-F0 ATPase.22–24 Although the findings were mixed, it was generally concluded that the F1-F0 ATPase activity was not inhibited in submitochondrial particles isolated from PC hearts compared with non-PC hearts. These studies focused largely on inhibition by the inhibitor factor and were performed under conditions in which the inhibitor factor would stay bound during the isolation of the submitochondrial particles. However, any labile posttranslational modification or other protein-protein interactions would likely be lost during the preparation of submitochondrial particles.

The observations of Murry et al1 are of interest in light of recent studies suggesting that F1-F0 ATPase may be the long-sought mitochondrial permeability transition pore (mPTP). The mPTP is a large conductance channel in the mitochondria, which in its open conformation is permeable to solutes <1.5 kDa. Opening of this channel leads to loss of mitochondrial membrane potential and loss of mitochondrial cofactors, leading to dysregulated electron transport and increased production of reactive oxygen species.25 The resultant loss of membrane potential, decrease in ATP, and increase in reactive oxygen species are thought to lead to cell death.17 An increase in mitochondrial matrix Ca and reactive oxygen species, which occurs at the start of reperfusion, is a well-known activator of the mPTP. However, despite the...
important proposed role of the mPTP in cardioprotection, its molecular identity has eluded investigators. A complex between the voltage-dependent anion channel and the adenine nucleotide translocator has been proposed to form the mPTP. However, mice lacking various voltage-dependent anion channel or adenine nucleotide translocator isoforms were still capable of undergoing mPTP opening, suggesting that these components are not essential components of

**Figure.** The hypothesis that ischemic preconditioning (PC)-mediated inhibition of the F1-F0 ATPase reduces ATP breakdown and thereby reduces anaerobic glycolysis, leading to a reduction in cytosolic acidification. It is further speculated that the modifications that lead to inhibition of F1-F0 ATPase might also block ATPase dimer formation, which has been proposed to be the open form of the mitochondrial permeability transition pore (mPTP). Thus, modification of F1-F0 ATPase is proposed both to inhibit the reverse mode of the ATPase and to inhibit mPTP. ANT indicates adenine nucleotide translocator; and VDAC, voltage-dependent anion channel.
the mPTP. Cyclosporin A was shown in the 1990s to inhibit mPTP opening by binding to cyclophilin D, a mitochondrial peptidyl-prolyl cis-trans isomerase. Consistent with a role for cyclophilin D as a regulator of mPTP, mice lacking cyclophilin D have reduced susceptibility to Ca-activated mPTP opening.26 Inhibition of mPTP opening is proposed to be a primary target of PC and other types of cardioprotection. Loss or inhibition of cyclophilin D reduces Ca activation of mPTP and reduces infarct size after ischemia and reperfusion. In fact, a small clinical trial has reported that administration of cyclosporine to patients at reperfusion is cardioprotective.27 Although the mPTP is considered a primary target of PC, the lack of information on the identity of the mPTP has hampered detailed mechanistic studies. It now seems that there was an important clue about the identity of the mPTP in the original articles on PC.

Recent data have suggested that mitochondrial F1-F0 ATPase can form the mPTP.28–30 Cyclophilin D has recently been reported to bind to subunits b and d and ATPO of the lateral stalk of the F1-F0 ATPase.31 Decreasing ATPO levels using RNAi reduced the Ca sensitivity of the mPTP. Additional data have been presented showing that dimers of the ATP synthase comprise the mPTP.28 Another recent article has suggested that the c subunits of F1 ATPase comprise the mPTP and that downregulation of the c subunit reduces the mPTP.29 These recent data suggesting a role for F1-F0 ATPase in regulating cell death are consistent with reports from more than a decade ago. Taken together, these data suggest the novel but speculative hypothesis that inhibition of F1-F0 ATPase would reduce the breakdown of ATP, which would thereby reduce the rate of glycolysis. This would account for the ability of PC to reduce the ischemic cytosolic acidification, and the inhibition of the F1-F0 ATPase might also explain the reduced mPTP opening and infarct size. It is proposed that PC activates cardioprotective signaling cascades that lead (by still-to-be-determined mechanisms likely involving posttranslational modifications) to altered conformation and transient inhibition of the F1-F0 ATPase. This transient inhibition of the F1-F0 ATPase reduces ischemic acidification and leads to a reduced breakdown of ATP. The conformational change associated with this transient inhibition of the F1-F0 ATPase is further proposed to place the ATPase in a conformation that opposes mPTP opening. Consistent with this hypothesis, recent studies have reported that posttranslational modifications such as S-nitrosylation or acetylation of components of F1-F0 ATPase lead to its inhibition.32,33 We have also found that inhibition of glycogen synthase kinase 3 leads to phosphorylation of ATP and that mutation of this site blocked cardioprotection initiated by glycogen synthase kinase 3 inhibition (Figure).34 It has taken >25 years, and we still do not have the complete answer, but perhaps we are on the way to understanding the mechanism responsible for the reduced ATP breakdown and reduced ischemic acidification that occurs in preconditioned hearts.

Disclosures

None.

References


Did a Classic Preconditioning Study Provide a Clue to the Identity of the Mitochondrial Permeability Transition Pore?

Elizabeth Murphy and Charles Steenbergen

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