Signaling Mechanisms in Ischemic Preconditioning
Interaction of PKCε and MitoKATP in the Inner Membrane of Mitochondria
Hossein Ardehali

The cardiac “warm up” phenomenon, described more than 50 years ago in patients with coronary artery disease, refers to improvement in cardiac symptoms and physical performance following exposure to short periods of ischemia. Several mechanisms, such as adaptive reduction in oxygen consumption by the ischemic myocardial region, improved oxygen supply via collateral recruitment or dilation of the stenotic vessel, and activation of an intrinsic phenomenon called ischemic preconditioning (IPC) have been proposed to account for this phenomenon. IPC refers to a process in which brief periods of ischemia improves the ability of the heart to tolerate subsequent prolonged ischemic periods. It was first identified in the heart in 1986 by Murry et al. and has since been demonstrated in various experimental and animal models.

Several triggers have been proposed for IPC, including adenosine, bradykinin, protaglandins, opioid receptors, nitric oxide, and Ca2+. These triggers lead to the activation of several intracellular pathways that ultimately protect myocardial cells against injury. Although the details of these pathways have not been totally characterized, mitochondria have been shown to be key mediators of IPC. Specifically, the opening of a mitochondrial channel, called the mitochondrial ATP-sensitive potassium channel or mitoKATP, is believed to be critical for the induction of IPC; drugs that activate this channel protect against ischemia and inhibitors of mitoKATP reverse these protective effects.

The signaling pathways that lead to the activation of mitoKATP are still under investigation. In a recent article, Oldenburg et al. demonstrated that in isolated rabbit adult cardiomyocytes, bradykinin increased the levels of reactive oxygen species (ROS) and this effect was reversed by inhibitors of both mitoKATP and protein kinase G (PKG). Subsequent studies demonstrated that mitoKATP can be activated by the addition of exogenous cGMP and PKG, and that this effect is reversed by inhibitors of protein kinase C (PKC). These results suggest that PKG transmits the cardioprotective signal to mitoKATP through a PKC-dependent pathway. It is unclear how this signal is transmitted and which isoforms of PKC are involved in this process.

In this issue of Circulation Research, Jabúrek et al. demonstrate in a series of elegant studies that mitoKATP and PKCε directly interact in the inner mitochondrial membrane, and that PKCε is required for the opening of mitoKATP. They first demonstrate the presence of PKCε in highly enriched mitochondrial fractions. Subsequently, they show that PKCε activators induce the opening of mitoKATP, while its inhibitors and a protein phosphatase reverse these effects.

The PKC family of enzymes play a role in several cellular signal transduction pathways and is implicated in numerous physiological and pathological processes. Thus far, 11 members of the PKC family have been characterized. These proteins are divided into 3 groups based on their responsiveness to diacylglycerol (DAG) or Ca2+ for enzyme activity. The α, β, βII, and γ isoforms are both DAG- and Ca2+-dependent, while the ε, δ, θ, and η are only DAG-dependent and do not need Ca2+ for activity. The atypical PKCs (ζ and λ) are neither DAG- nor Ca2+-dependent and require lipid-derived molecules for activity.

The potential role of PKC enzymes in cardioprotection has been the subject of many investigations. To evaluate the role of the individual isoforms of PKCs in cardioprotection, recent studies have used isozyme-specific modulators as well as transgenic and knockout mice of specific PKC isozymes. PKC isoforms translocate to distinct cellular locations after activation by binding to their specific anchoring proteins, called receptor for activated C-kinase or RACKs. Peptides against the RACK binding site of each PKC isozyme can inhibit the translocation and activity of the corresponding enzyme and have been used as isozyme-specific inhibitors. Peptide activators promote PKC isozymes to translocate to a specific subcellular location by mimicking the function of isozyme-specific RACKs.

Ping et al demonstrated that all 11 isoforms of PKC are present in rabbit myocardium and that IPC activates the ε and η isoforms. Subsequent studies have supported a major role of PKCε in IPC. Mochly-Rosen’s group has demonstrated that PKCε is activated in IPC, and that treatment with a PKCε selective inhibitor during preconditioning reverses the protective effects of IPC. Furthermore, overexpression of PKCε in the heart of transgenic mice resulted in a lesser degree of ischemic damage, and PKCε knockout mice did not retain the protective effects of preconditioning. These results suggest that PKCε is required and sufficient for the protective effects of IPC in the heart. Another PKC isozyme, PKCδ, also plays a role in myocardial cell death. However, unlike PKCε, this isozyme is believed to promote damage from an ischemic insult. Activation of PKCδ causes a higher degree of cell death in response to ischemia and its inhibition...
leads to protection in isolated cardiomyocytes and intact hearts.17

Although the signaling mechanisms that link PKC to ischemic preconditioning are not completely characterized, several pathways have been proposed (Figure). These include generation of free radicals, changes in the levels of the pro- and antiapoptotic proteins of Bcl-2 family, and activation of the mitoK<sub>ATP</sub>.18 ROS that are produced during both ischemia and antiapoptotic proteins of the Bcl-2 family, and activation of the mitoK<sub>ATP</sub>. ROS that are produced during both ischemia and reperfusion have deleterious effects on cardiomyocytes. However, these molecules are also believed to activate multiple signaling pathways, including the activation of PKC enzymes.19,20 ROS have been shown to lead to PKCe activation and translocation.19,20 However, there is currently no evidence linking ROS formation to PKCe mediated cardioprotection. PKCe is also shown to enhance the phosphorylation of a proapoptotic Bcl-2 related protein (Bcl-2 associated death domain or BAD), inactivating it and blocking its ability to induce apoptosis.21 The expression of Bcl-2 protein may also be regulated by PKCe.22 However, a recent study showed that although knockdown of PKCe induces apoptosis in glioma cells, it does not affect the expression of Bcl-2 or Bax.23

A number of previous studies provided evidence for a possible link between PKC and mitoK<sub>ATP</sub> in IPC. Hassouna et al demonstrated that PKCe inhibitors block protection from IPC, and that diazoxide (a mitoK<sub>ATP</sub> activator) did not affect the phosphorylation of PKCe, suggesting that PKCe may act upstream of mitoK<sub>ATP</sub>.24 Korge et al has shown that a non-specific PKC activator can induce cardioprotection and this effect was reversed by mitoK<sub>ATP</sub> inhibitors.25 Finally, PKCe is shown to interact with several mitochondrial proteins, suggesting that it may translocate into the mito-

dria.26 The current manuscript for the first time shows that PKCe interacts and activates mitoK<sub>ATP</sub> in the inner membrane of the mitochondria.

Although the findings by Jaburek et al are interesting and have addressed an important question, they have also raised many new questions. What is the mechanism for PKCe translocation into the mitochondria? Does PKCe phosphorylate any protein components of mitoK<sub>ATP</sub>? Is there a RACK linking PKCe to mitoK<sub>ATP</sub> in the mitochondria? Besides PKG, what other pathways lead to PKC-mediated activation of mitoK<sub>ATP</sub>? The answer to these questions may better delineate the protective roles of PKCe and mitoK<sub>ATP</sub>.

In summary, the current manuscript by Jaburek et al demonstrates that mitoK<sub>ATP</sub> and PKCe functionally interact with each other in the inner membrane of the mitochondria. These results improve our understanding of the signals that leads to the opening of mitoK<sub>ATP</sub> and the protective effects of IPC (Figure). Although the mechanism of PKCe activation of mitoK<sub>ATP</sub> is not totally clear, it is tempting to speculate that it directly phosphorylates the protein components of the channel, resulting in the opening of the channel and entrance of K<sup>+</sup> into the mitochondria. This potential mechanism for the regulation of the mitoK<sub>ATP</sub> channel remains to be elucidated.

Acknowledgments

I thank Drs Mike Burke and Kannan Mutharasan for critical reading of the manuscript.

Sources of Funding

H.A. is supported by NIH grant K08 HL079387 and grants from the Schweppe foundation and the Northwestern Memorial Foundation.

Disclosures

None.

References


Signaling Mechanisms in Ischemic Preconditioning: Interaction of PKCε and MitoKATP in the Inner Membrane of Mitochondria
Hossein Ardehali

Circ Res. 2006;99:798-800
doi: 10.1161/01.RES.0000247029.31997.a4
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/99/8/798

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/