Since its original description nearly 25 years ago, the phenomenon of ischemic preconditioning (IPC) continues to captivate a great amount of research interest. The ability to render the myocardium resistant to lethal ischemia/reperfusion injury by preconditioning it with a brief episode of ischemia and reperfusion has remained largely a laboratory phenomenon, with only a handful of proof-of-concept clinical studies realizing its true potential. Of course, the need to apply the IPC protocol before the index myocardial ischemic event has restricted its clinical application to planned cardiac surgery or percutaneous coronary intervention, settings in which the index myocardial ischemic event can be reliably predicted. The ability to noninvasively reproduce IPC cardioprotection by applying the IPC stimulus to the upper arm or leg with a blood pressure cuff to induce ischemia and reperfusion has also facilitated its clinical translation. In contrast, interrupting myocardial ischemia/reperfusion has also facilitated its clinical translation. The sheer number and diversity of these signal pathways before the index myocardial ischemia has been attributed to the binding of autacoids to their respective receptor on the cardiomyocyte membrane. However, the transfer of the cardioprotective signal from the receptor to the signaling pathway may involve other mechanisms.

Although an enormous research effort has been invested in elucidating the underlying mechanism of IPC, the actual pathway that mediates cardioprotection remains unclear. The current paradigm suggests that ≥1 brief episodes of ischemia and reperfusion that make up the IPC stimulus generate autacoids such as adenosine, bradykinin, and opioids, which then recruit a number of signaling pathways through the activation of their respective receptors (see the Figure). Many of these signal transduction pathways converge on the mitochondria, resulting in the generation of oxidative stress, which activates downstream kinase mediators of cardioprotection. The sheer number and diversity of these signal transduction pathways linked to IPC, and the complexity with which they interact, can at times be bewildering. Presumably, this complicated array of potential signal pathways allows a degree of redundancy within the system so that even if one particular signaling pathway is inhibited, IPC may still be possible if a strong enough stimulus is applied. Therefore, one cannot conclude that something is an obligatory signaling mediator of IPC until it has been comprehensively demonstrated that increasing the intensity of the IPC stimulus has no effect.

The signaling pathways underlying IPC have conventionally been categorized into those recruited in direct response to the IPC stimulus before myocardial ischemia and those that protect the ischemic heart at the time of myocardial reperfusion. In this framework, the phosphatidylinositol-3-kinase (PI3K-Akt) pathway and mitogen activated protein kinase-extracellular signal regulated kinase (MEK1/2-Erk1/2) protein kinase signaling cascades have been implicated as mediators of IPC both before myocardial ischemia and at the onset of reperfusion. Their specific recruitment at the time of myocardial reperfusion has resulted in their being named the reperfusion injury salvage kinase (RISK) pathway. The activation by IPC of these prosurvival kinase pathways before the index myocardial ischemia has been attributed to the binding of autacoids to their respective receptor on the cardiomyocyte membrane. However, the transfer of the cardioprotective signal from the receptor to the signaling pathway may involve other mechanisms.

For example, recent data have suggested that caveolae may contribute to IPC signaling through the PI3K-Akt pathway. Caveolae are lipid-rich microdomains of the cell membrane that have been reported to localize multiple cardioprotective signaling components such as the PI3K-Akt pathway. They are formed by the scaffolding proteins caveolins, of which caveolin-3 is the predominant isoform present in the heart and striated muscle. Tsutsumi and coworkers have demonstrated that mice lacking caveolin-3 limited myocardial infarct size through the activation of PI3K-Akt and that mice lacking caveolin-3 were resistant to the cardioprotection elicited by a standard IPC stimulus. A previous study has demonstrated that mice lacking caveolin-3 are also resistant to pharmacological preconditioning with the volatile anesthetic isoflurane. In an interesting article by Cao and coworkers in this week’s issue of Circulation, the potential involvement of cell membrane physiology in IPC cardioprotection has been further extended. In a prior study published only last year, the same authors discovered that caveolin-3 forms a functional complex with a novel protein called mitsugumin 53 (MG53) and contributes to intracellular vesicle trafficking and skeletal myocyte differentiation. MG53, which is expressed only in the heart and skeletal muscle, is a member of the tripartite motif/RING B-box coiled coil family of proteins (TRIM72) that contribute to vesicle trafficking under normal physiological conditions. In response to damage to the muscle cell membrane and

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From The Hatter Cardiovascular Institute and University College London Hospital and Medical School (D.J.H., D.M.Y.), London, UK.
Correspondence to Derek M. Yellon, The Hatter Cardiovascular Institute, University College London, 67 Chenes Mews, London, WC1E 6HX, UK. E-mail d.yellon@ucl.ac.uk
(Circulation. 2010;121:2547-2549.)

© 2010 American Heart Association, Inc.
Circulation is available at http://circ.ahajournals.org
DOI: 10.1161/CIRCULATIONAHA.110.958462
oxidative stress, MG53 attaches to phosphatidylserine at the inner surface of the cell membrane and forms a functional complex with caveolin-3, which facilitates vesicle translocation to the sites of membrane injury, where the vesicles are then used to patch the membrane through a calcium-dependent fusion process. Cao and colleagues have demonstrated that MG53, which is present in the heart, acts as a mediator of IPC protection in a manner that is dependent on both caveolin-3 and the downstream recruitment of the PI3K-Akt and MEK1/2-Erk1/2 signaling cascades but is independent of the janus kinase–signal transducer and activator of transcription-3 pathway. Using a rather unusual IPC protocol comprising four 10-minute cycles of ischemia and reperfusion, these authors first demonstrated that in vivo IPC prevented the reduction in MG53 induced by ischemia/reperfusion injury. They then found that hearts excised from mice lacking MG53 sustained larger myocardial infarct sizes in response to ischemia/reperfusion injury and were resistant to a standard IPC stimulus comprising two 5-minute cycles of ischemia and reperfusion. However, whether MG53 is actually an obligatory mediator of IPC as stated in the article can be concluded only if the murine hearts were found to be resistant to a number of different IPC protocols. In terms of signaling, the authors have provided convincing evidence that IPC requires the presence of both MG53 and caveolin-3 to activate PI3K-Akt and MEK1/2-Erk1/2. Interestingly, they demonstrate that the p85 subunit of PI3K and caveolin-3 interact in preconditioned hearts, thereby providing the link between MG53, caveolin-3, and PI3K. From this study, although the role of cell membrane repair as a phenomenon in IPC cardioprotection has not been explored, downstream signaling through known prosurvival kinase pathways have clearly been demonstrated to be of vital importance.

At this point, it is important to distinguish between the IPC-induced activation of these prosurvival kinases before the index myocardial ischemia, which these authors have clearly demonstrated, and the recruitment of the RISK pathway at the onset of myocardial reperfusion, which their data have not shown, even though the authors refer to the RISK pathway throughout their article. In other words, even though both MG53 and caveolin-43 have been linked to the activation of PI3K-Akt and MEK1/2-Erk1/2 before the index ischemic event, it remains to be proven that this relationship operates during the first few minutes of myocardial reperfusion. The same applies to the survivor activating factor enhancement pathway, which the authors state they had excluded as mediators of MG53-induced cardioprotection; in fact, they had only shown that the janus kinase–signal transducer and activator of transcription-3 pathway was not required before the index myocardial ischemia, but whether it played a role at the onset of reperfusion as part of the survivor

**Figure.** This hypothetical scheme depicts the potential interaction between MG53, caveolin-3, and the prosurvival kinases (PI3K-Akt and MEK1/2-Erk1/2) in the setting of IPC. IPC generates autacoids such as adenosine, bradykinin, and opioids, which recruit the prosurvival kinase pathways via the activation of their respective receptors on the cell membrane, which converge on the mitochondria and result in the release of reactive oxygen species (ROS) through the activation of the mitochondrial ATP-dependent potassium channel (MitoKATP channel), which in turn activates the downstream mediator of IPC cardioprotection. Damage to the cell membrane (perhaps in the setting of ischemia/reperfusion injury [IRI]) activates MG53 via oxidant signaling, which attaches to the cell membrane via phosphatidylserine, where it forms a functional complex with caveolin-3 and traffics vesicles to the cell membrane, which then fuse with and repair the cell membrane in a calcium-dependent manner. Whether this cell membrane repair system operates in preconditioned hearts is an intriguing possibility that remains to be proven. Caveolin-3, which is also required for IPC, spatially localizes cardioprotective signaling components such as the PI3K-Akt pathway into endocytic vesicles. Evidence suggests that caveolin-3 binds to the p85 subunit of PI3K. NO indicates nitric oxide; eNOS, endothelial NO synthase; PKC, protein kinase C; and PKG, protein kinase G.
activating factor enhancement pathway remains to be investigated.

The findings from this study by Cao and coworkers raise some interesting questions that merit further investigation. For example, is the physiological role of MG53 in the heart the same as that in skeletal muscle? That is, are these proteins also responsible for cell membrane repair in the heart and, if so, under which circumstances? Can IPC protect against cell membrane damage mediated by ischemia/reperfusion injury (see the Figure)? What is the interrelationship between the MG53–caveolin-3 system and the autacoid stimulation of their respective cell membrane surface receptor during IPC? Does the former system provide the spatial localization required for the latter, thereby amplifying the cardioprotective response? Finally, does MG53 actually activate the RISK pathway? In other words, does MG53 confer cardioprotection at the onset of reperfusion via PI3K-Akt and MEK1/2-pathway? If so, does this novel cardioprotective response provide a novel cardioprotective therapeutic strategy for the treatment of acute myocardial infarction.

Acknowledgment

The authors thank the British Heart Foundation for continued support.

Disclosures

None.

References

Cell Membrane Repair as a Mechanism for Ischemic Preconditioning?
Derek J. Hausenloy and Derek M. Yellon

_Circulation_. 2010;121:2547-2549; originally published online June 1, 2010;
doi: 10.1161/CIRCULATIONAHA.110.958462

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/121/23/2547

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ 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_ is online at:
http://circ.ahajournals.org//subscriptions/