Mapping Preconditioning's Signaling Pathways: An Engineering Approach

JAMES M. DOWNEY[1], THOMAS KRIEG[2] AND MICHAEL V. COHEN[1]

1Dept of Physiology and Medicine, University of South Alabama, Mobile, AL USA and
[2]Deptt of Cardiology, Ernst-Moritz-Arndt University Greifswald, Greifswald, Germany

ABSTRACT: Preconditioning the heart by exposure to brief cycles of ischemia-reperfusion causes it to become very resistant to ischemia-induced infarction. This protection has been shown to depend on a large number of signal transduction components whose arrangements within the cardiomyocyte are unknown. To aid the translation of this phenomenon to the clinical setting we have attempted to map the signal transduction pathways responsible for this protection. To resolve the signaling order we have injected a signal at an intermediate point in the system transduction pathway and monitored it at a downstream site. System analysis reveals both parallel and series signaling arrangements. Separate trigger and mediator phases could be identified. *The trigger phase* is now well mapped. During the preconditioning ischemia autacoids, including adenosine, opioids, and bradykinin, are released from the heart. These substances occupy their respective G_i-coupled receptors. Opioid and bradykinin receptors activate PI3-kinase which, through the Phosphoinositide Dependent Protein Kinase (PDKs), causes activation of Akt. Opioid couples through transactivation of the Epidermal Growth Factor (EGF) receptor while bradykinin's coupling to PI3-kinase is unknown. PI3-kinase causes Extracellular Signal Regulated Kinase (ERK)-dependent activation of endothelial Nitric Oxide Synthase (eNOS). The resulting nitric oxide activates soluble guanylyl cyclase resulting in Guanosine dependent Protein Kinase (PKG) activation through production of cGMP. PKG initiates opening of ATP-sensitive potassium channels on the inner membrane of the mitochondria. Potassium entry into mitochondria causes the generation of free radicals during reperfusion when oxygen is reintroduced. Through redox signaling these radicals activate Protein Kinase C (PKC) and put the heart into the protected phenotype that persists for one to two hours. Although adenosine receptors activate PI3-kinase, they also have a second direct coupling to PKC and thus bypass the mitochondrial pathway. *The mediator phase* occurs during the first minutes of reperfusion following the lethal ischemic insult and is still poorly defined. Briefly, PKC somehow potentiates adenosine's ability to activate signaling from low-affinity A_{2b} adenosine receptors. These receptors couple to the survival kinases, Akt and ERK, believed to inhibit the formation of deadly mitochondrial permeability transition pores through the phosphorylation of Glycogen Synthase Kinase (GSK-3∃). The proposed signaling maps reveal many points at which drugs can trigger the protected phenotype.

KEYWORDS: free radicals, ischemia, mitochondria, nitric oxide, permeability transition pores, ATP-sensitive potassium channels, redox signaling, protein kinase, EGF receptor, adenosine receptor

Address for correspondence: James Downey, PhD, Room 3074, Medical Science Bldg., University of South Alabama, Mobile, AL 36688, USA. Tel: 251-460-6818, Fax: 251 460 6464. Email: jdowney@usouthal.edu