Journal of Molecular and Cellular Cardiology 46 (2009) 858–866

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Review article

Cardioprotective signaling to mitochondria

Keith D. Garlid [a,*], Alexandre D.T. Costa [b], Casey L. Quinlan [a], Sandrine V. Pierre [c], Pierre Dos Santos [d]

[a] Department of Biology, Portland State University, Portland, OR 97201-0751, USA
[b] Instituto Carlos Chagas (Fiocruz), Rua Prof. Algacyr Munhoz Mader 3775, Curitiba, PR 81350-010, Brazil
[c] Department of Physiology and Pharmacology, University of Toledo College of Medicine, Toledo, Ohio, USA
[d] INSERM Unit 828, University Victor Segalen-Bordeaux 2, University Hospital of Bordeaux, Bordeaux, France

ARTICLE INFO

Article history:
Received 14 August 2008
Received in revised form 7 November 2008
Accepted 26 November 2008
Available online 11 December 2008

Keywords:
Mitochondrial K_{ATP} channel
Protein kinase C
Reactive oxygen species
Permeability transition
Signaling pathways

ABSTRACT

Mitochondria are central players in the pathophysiology of ischemia–reperfusion. Activation of plasma membrane G-coupled receptors or the Na,K-ATPase triggers cytosolic signaling pathways that result in cardioprotection. Our working hypothesis is that the occupied receptors migrate to caveolae, where signaling enzymes are scaffolded into signalosomes that bud off the plasma membrane and migrate to mitochondria. The signalosome–mitochondria interaction then initiates intramitochondrial signaling by opening the mitochondrial ATP-sensitive K^+ channel (mitoK$_{ATP}$). MitoK$_{ATP}$ opening causes an increase in ROS production, which activates mitochondrial protein kinase C epsilon (PKCε), which inhibits the mitochondrial permeability transition (MPT), thus decreasing cell death. We review the experimental findings that bear on these hypotheses and other modes of protection involving mitochondria.

Published by Elsevier Inc.

Contents

* Corresponding author. Fax: +1 503 725 3888.
E-mail address: garlid@pdx.edu (K.D. Garlid).

0022-2828/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.yjmcc.2008.11.019

1. Mitochondria: the target for ischemia–reperfusion injury and cardioprotection

Mitochondria are effectors of both ischemia–reperfusion injury (IRI) and cardioprotection. As pointed out 30 years ago by Jennings and Ganote [1], the heart is strictly aerobic and consequently extremely vulnerable to a decrease in oxygen supply. Thus, ischemia causes profound and immediate mitochondrial derangements. These include cessation of ATP synthesis, inhibition of respiration, and a drop in $\Delta\Psi$. This is accompanied during ischemia by cellular changes, especially an increase in Ca^{2+} and phosphate, and, during reperfusion, by large increases in reactive oxygen species (ROS) originating from the respiratory chain [2,3]. Together, these factors promote opening of the mitochondrial permeability transition (MPT), a high-conductance pore in the inner mitochondrial membrane, which is the main cause of necrotic cell death in IRI [4–8]. Consequently, as pointed out by Weiss et al. [4], cardioprotection by preconditioning or postconditioning must ultimately involve the prevention of MPT.

In addition to their role as mediators of cell death, mitochondria have been shown to be major effectors of diverse self-defense mechanisms, including ischemic pre- and post-conditioning [7,9–11]. These and other conditioning protocols have been shown to require opening of the mitochondrial ATP-sensitive K^+ channel (mitoK$_{ATP}$) and inhibition of MPT opening. Since cardioprotection involves both mitoK$_{ATP}$ opening and a decrease in MPT opening, it is reasonable to hypothesize that these two phenomena are part of the same signaling pathway. Indeed, this connection has been demonstrated [12], and will be discussed in this review.

This review describes our current understanding of the signaling mechanisms that originate at plasma membrane receptors, go to mitochondria, and terminate with MPT inhibition. For space reasons, we have not discussed mechanisms for the prevention of apoptosis. For different perspectives, readers are referred to excellent reviews by other authors [13–18].

2. Receptor-mediated signaling to open mitoK$_{ATP}$

2.1. Gi-protein coupled receptor (GPCR) pathways

Ischemic preconditioning (IPC) and ischemic postconditioning are receptor-mediated processes that are triggered by GPCR agonists released by the ischemic heart, primarily bradykinin, opioid peptides, and adenosine [19]. Other GPCR ligands, including acetylcholine, catecholemines, endothelin, and angiotensin II, are also cardioprotective [20–24], but they were found not to be physiological triggers of IPC [14]. A composite diagram of the GPCR signaling pathways is given in Fig. 1. GPCR signaling has been extensively studied by Downey and Cohen and their coworkers, and is the subject of an excellent review by these authors [14]. It should be emphasized that each G_i-coupled receptor ligand triggers its own unique signaling cascade. Opioids and acetylcholine instigate transactivation of the epidermal growth factor receptor (EGFR), leading to downstream activation of phosphatidylinostitol 3-kinase (PI3-K) and Akt. Bradykinin also induces activation of PI3-K and Akt, but without transactivation of EGFR. These two pathways then converge and ultimately lead to mitoK$_{ATP}$ opening and production of ROS. The adenosine signaling pathway has not yet been fully characterized. MitoK$_{ATP}$ opening is not involved during the *trigger* phase of adenosine preconditioning (i.e., when 5-HD administration brackets adenosine perfusion) [25], but mitoK$_{ATP}$ opening is required during the *mediator* phase (i.e., when 5-HD administration precedes ischemia) [26–29].

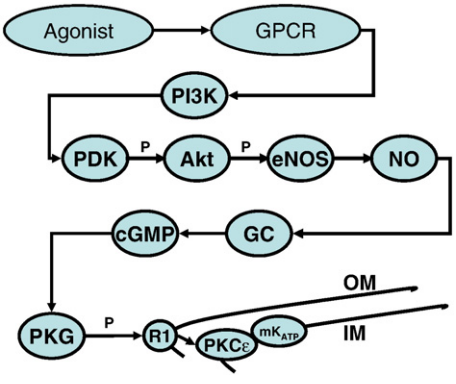

Fig. 1. GPCR-mediated signaling to mitochondria. Occupation of the GPCR leads to activation of PI3-kinase, phosphorylation of phosphatidylinositol bisphosphate, and activation of the phosphatidylinositol-dependent kinases (PDKs) [128]. PDKs then phosphorylate Akt, which initiates the remainder of the cytosolic signaling pathway: endothelial nitric oxide synthase (eNOS) is phosphorylated, leading to production of NO. NO stimulates guanylyl cyclase, and the cGMP produced activates protein kinase G (PKG) [129], which causes mitoK$_{ATP}$ opening [62,130–132].

Protection afforded by all of the trigger substances is blocked by PKC inhibitors, and PKC, probably PKCε, is thought to be a common target of cardioprotective signaling [14]. It has been difficult to localize the critical PKCε, because multiple PKCε isoforms participate in cardioprotection [30]. In ouabain signaling, PKCε acts proximally in conjunction with EGFR transactivation [31]. The adenosine A1 receptor is believed to directly stimulate PLC and PLD to activate PKC [21]. These PKCs are cytosolic. As discussed below, two PKCε isoforms regulate mitoK$_{ATP}$ and MPT at the level of the inner mitochondrial membrane [32]. Thus, the physiological effect of PKCε activation depends entirely on its location and not on its biochemistry, which appears to be invariant.

2.2. Non-GPCR pathways of protection — digitalis

Cardiac glycosides are classic inhibitors of the plasma membrane Na^+/K^+-ATPase, but this enzyme also has important non-canonical functions that are triggered by digitalis. Thus, ouabain interaction with the Na,K-ATPase activates src kinase, causing formation of a "binary receptor" that phosphorylates and assembles other proteins into signaling modules that transmit signals to intracellular compartments [33,34]. Ouabain signaling has been shown to depend on mitoK$_{ATP}$ opening and mitochondrial ROS production [35]. Ouabain is cardioprotective in rat heart [31,36,37], and this cardioprotection is blocked by the mitoK$_{ATP}$ blocker 5-hydroxydecanoate (5-HD), the ROS scavenger N-2-mercaptopropionylglycine (MPG), and the src kinase inhibitor PP2 [36]. It is interesting to note that, whereas inhibition of the pump and consequent increase in intracellular Na^+ and Ca^{2+} is required for positive inotropy, ouabain cardioprotection occurs at doses (about 10 μM in rat) that do not produce significant enzyme inhibition [37] or increased contractility [31,36,37]. These distinctions further emphasize the dissociation of the pumping and signaling functions of Na,K-ATPase. Ouabain cardioprotection does not depend on guanylyl cyclase or PKG activities, showing that this signaling pathway differs from that triggered by GPCR agonists [36]. Ouabain-induced inotropy also requires mitoK$_{ATP}$ opening and ROS production [36,38].

The rat heart Na,K-ATPase exhibits a low sensitivity to cardiac glycosides; however, we have observed qualitatively similar phenomena in the ouabain-sensitive rabbit heart. Thus, cardioprotection occurs at lower ouabain doses than those required for inotropy, and both cardioprotection and inotropy require mitoK$_{ATP}$ opening (S. Pierre, unpublished data).

3. From receptor to mitochondria by signalosomes

We propose that cardioprotective signals are transmitted to mitochondria by signalosomes, which are vesicular, multimolecular signaling complexes that are assembled in caveolae and deliver signals