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Review article

Hexokinases and cardioprotection

Guillaume Calmettes, Bernard Ribalet, Scott John, Paavo Korge, Peipei Ping, James N. Weiss *

UCLA Cardiovascular Research Laboratory, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
Department of Medicine (Cardiology), David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
Department of Physiology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

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ABSTRACT

As mediators of the first enzymatic step in glucose metabolism, hexokinases (HKs) orchestrate a variety of catabolic and anabolic uses of glucose, regulate antioxidant power by generating NADPH for glutathione reduction, and modulate cell death processes by directly interacting with the voltage-dependent anion channel (VDAC), a regulatory component of the mitochondrial permeability transition pore (mPTP). Here we summarize the current state-of-knowledge about HKs and their role in protecting the heart from ischemia/reperfusion (I/R) injury, reviewing: 1) the properties of different HK isoforms and how their function is regulated by their subcellular localization; 2) how HKs modulate glucose metabolism and energy production during I/R; 3) the molecular mechanisms by which HKs influence mPTP opening and cellular injury during I/R; and 4) how different metabolic and HK profiles correlate with susceptibility to I/R injury and cardioprotective efficacy in cancer cells, neonatal hearts, and normal, hypertrophied and failing adult hearts, and how these difference may guide novel therapeutic strategies to limit I/R injury in the heart. This article is part of a Special Issue entitled "Mitochondria: From Basic Mitochondrial Biology to Cardiovascular Disease".

Contents

1. Introduction

In the heart, ischemic preconditioning (IPC) is a process whereby repeated brief episodes of ischemia/reperfusion (I/R) protect the heart from injury during a subsequent prolonged I/R episode [1]. Although much research has been devoted to this phenomenon and its other variants, including pharmacologic preconditioning (PPC) and ischemic post-conditioning (IPoC), the underlying mechanisms of cardioprotection remain elusive. Common to all of these cardioprotective strategies is activation of a signaling cascade called the Reperfusion Injury Salvage Kinase (RISK) pathway involving phosphatidylinositol-3-kinase (PI3K), Akt, glycogen synthase kinase 3 beta (GSK3β) and other enzymes [2] (Fig. 1). In

* Corresponding author at: UCLA, 675 Charles E Young Dr. South, 3645 MRL Bldg, Los Angeles, CA 90095, USA. Tel.: +1 310 825 9029; fax: +1 310 206 5777.
E-mail address: jweiss@mednet.ucla.edu (J.N. Weiss).

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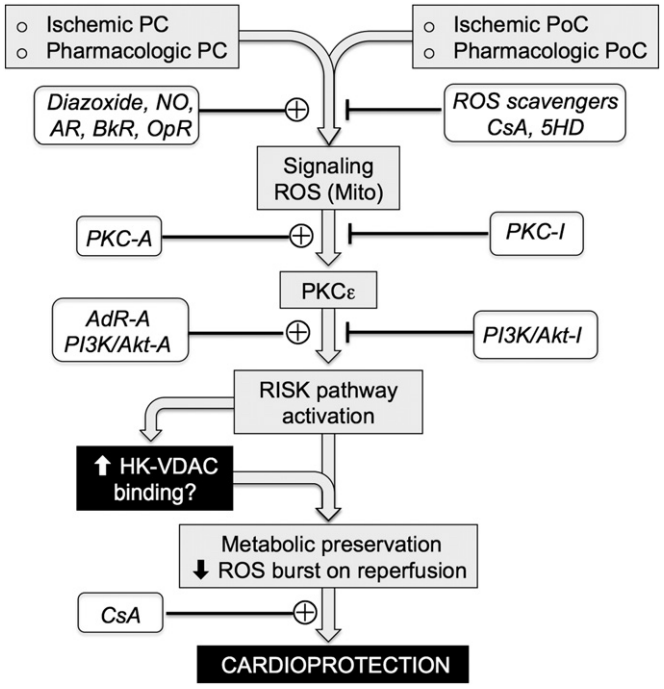

Fig. 1. Overview of cardioprotective signaling by ischemic and pharmacologic preconditioning (PC) and post-conditioning (PoC). ROS-dependent PKCε activation (often triggered by ROS originating from mitochondria due to mitoK$_{ATP}$ channel or transient low conductance mPTP opening) or other pathways such as adenosine receptor (AdR) stimulation lead to activation of the RISK pathway, initiating a signaling cascade that suppresses the massive ROS burst during reperfusion and prevents long-lasting mPTP opening. The Akt component of the RISK pathway enhances HK2 binding to mitochondria, contributing to an improved metabolic profile during prolonged ischemia and reduced ROS burst upon reperfusion characteristic of the cardioprotected state. Various activators (-A) and inhibitors (-I) of these steps are indicated. AR, adrenergic receptors; BkR, bradykinin receptors; OpR, opiate receptors; CsA, cyclosporine A; 5HD, 5-hydroxydecanoate; PI3K, phosphoinositol-3-kinase.

addition, cardioprotective signaling can also be transmitted via the Survivor Activating Factor Enhancement (SAFE) pathway involving the activation of tumor necrosis factor alpha (TNFα) and signal transducer and

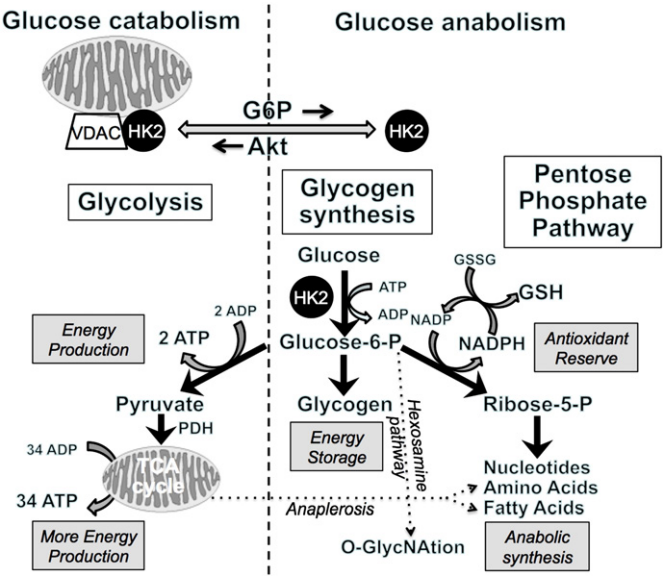

Fig. 2. Summary of catabolic, anabolic and antioxidant pathways in glucose metabolism. HK2 binding to mitochondria favors glycolysis, whereas cytoplasmic HK2 favors glycogen synthesis and the pentose phosphate shunt, which provides NADPH to reduce oxidized glutathione (GSSG) to reduced glutathione (GSH) and thereby bolsters antioxidant reserve.

activator of transcription-3 (STAT-3) [3–6], which may crosstalk with the RISK pathway. Although the details are still fuzzy, these signals appear to converge ultimately on the mitochondrion [7], and avert cell death by inhibiting mitochondrial permeability transition pore (mPTP) opening in the inner mitochondrial membrane. Of note, activation of the RISK pathway depends on a modest burst of reactive oxygen species (ROS) production during the preconditioning ischemia, because if ROS scavengers are present during this period, cardioprotection is abolished [8]. When the RISK pathway is activated by pre- or post-conditioning, the massive ROS burst that typically occurs after prolonged I/R in the absence of pre- or post-conditioning is markedly attenuated [9]. This may be a major factor in averting the mPTP opening and cell death, since oxidative stress is one of the major factors promoting mPTP opening [10].

The finding that post-conditioning confers almost equivalent cardioprotection as pre-conditioning suggests that RISK pathway activation at any time point up and during early reperfusion is the main requirement for effective cardioprotection [11]. Once activated, the cardioprotected state has two phases. The early phase, attributed to acute post-translational modification of target proteins by the RISK pathway, lasts about 1–2 h. Cardioprotection then dissipates, but returns again within 12–24 h, and lasts for another 48–72 h. This late phase is related to gene reprogramming and new protein synthesis triggered by RISK pathway activation [10].

How do hexokinases (HKs) fit into this picture? During low-flow ischemia or anoxia, glucose metabolism becomes the major source for ATP production via anaerobic glycolysis, and HKs mediate the first step in this process, namely the conversion of glucose to glucose-6-phosphate (G6P). G6P is a hub metabolite that can be directed to a number of catabolic or anabolic fates (Fig. 2). The main catabolic fate is glycolysis, which first generates ATP anaerobically via conversion to pyruvate and lactate, and then aerobically by mitochondrial oxidation of pyruvate and lactate. The main anabolic fates of G6P are two-fold: glycogen synthesis to store energy for deferred use, and the pentose phosphate shunt to generate ribose-5-phosphate (R5P). The conversion of G6P to R5P is a major source of cytoplasmic NADPH generation which is critical for maintaining antioxidant function by regenerating reduced glutathione (GSH) from oxidized glutathione (GSSG). R5P is also a precursor for synthesis of nucleotides, amino acids and fatty acids. Small amounts of G6P are also used by the hexosamine pathway for O-GlyNAtion of proteins, and some of the G6P directed to pyruvate is used in the TCA cycle for amino acid and fatty acid synthesis via anaplerosis.

In addition to acting as the gatekeeper for the metabolic and antioxidant roles of glucose, HKs have also been found to regulate mPTP opening directly. First described in the cancer field, the high-affinity HK isoforms HK1 and HK2 are strongly anti-apoptotic when bound to the outer mitochondrial membrane, putatively to the voltage-dependent anion channel (VDAC), an important regulator of the mPTP [12,13].

Thus, HKs are multifunctional proteins that orchestrate metabolic, antioxidant and direct anti-cell death effects. These functions are also strongly influenced by the subcellular distribution of HKs, with mitochondrially-bound HK promoting glucose catabolism and anti-cell death effects, while cytoplasmic HK promotes anabolic usages and antioxidant effects (Fig. 2). The predominant HK isoform in adult heart, HK2, dynamically shuttles between the mitochondria and cytoplasm in response to changes in intracellular G6P, pH and the cardioprotective signaling pathway Akt [14–22]. These factors set the stage for HKs to have a critical influence on the susceptibility of the heart to I/R injury.

Here we summarize the current state-of-knowledge about HKs and their role in protecting the heart from I/R injury. We begin by discussing the properties of different HK isoforms and how their function is regulated by their subcellular localization. Next, we discuss how HK modulates glucose metabolism and energy production during I/R, and then review the molecular mechanisms by which HKs influence mPTP opening and cellular injury during I/R. Finally, we describe the differences in susceptibility to oxidative or I/R injury in cancer cells, neonatal hearts,