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Review Article

Endothelial Derived Neuregulin-1 may be Important for Cardioprotection Induced by Ischemic Preconditioning

Gui-Zhen Yang, Fu-Shan Xue, Chao Sun, Xu Liao, and Jian-Hua Liu

ABSTRACT

Aim of review: The underlying mechanisms of ischemic preconditioning (IPC) have been studied for many years, but have not been elucidated completely. The available literatures indicate that endothelial derived neuregulin-1 (NRG-1) is involved in myocardial ischemia/reperfusion injury (IRI) and protects cardiomyocytes against H_2O_2-induced apoptosis by regulating endoplasmic reticulum stress (ERS). The aim of this review is to provide the evidence that endothelial derived NRG-1 maybe a crucial biomolecule mediating powerful cardioprotection by IPC.

Methods: According to the available literatures, this review will first discuss the factors attributable to cardioprotection of IPC and then provide an overview of the cellular and molecular mechanisms of endothelial derived NRG-1 maybe involved in IPC induced cardioprotection.

Recent findings: Multiple factors are attributable to cardioprotection induced by IPC. It has been shown that anoxia preconditioning *in vitro* can not provide cardioprotection as much as the IPC *in vivo*. During myocardial ischemic conditioning, endothelial cells may play several roles: a "receptor" for blood-borne conditioning moieties, a sensor for hypoxic stress and a paracrine organ. The NRG-1 produced by endothelial cells has been proved to protect against myocardial IRI through a PI3K/Akt pathway. Furthermore, unbalanced endoplasmic reticulum stress is one of the important mechanisms of IRI, and IPC has been demonstrated to protect myocardial IRI by regulating endoplasmic reticulum stress. In addition, NRG-1 may attenuate IRI by regulating cold inducible RNA-binding protein with its downstream endoplasmic reticulum stress related signaling pathways.

Summary: Endothelial derived NRG-1 and its downstream signaling pathways are involved in multiple aspects of cardiac physiology and function, and can provide a significant protection against myocardial injury. The available evidence indicates that selective deletion of endothelial derived NRG-1 *in vivo* decreases the tolerance to IRI, as demonstrated by impaired recovery of post-ischemic myocardial contraction function. Thus, endothelial derived NRG-1 maybe a crucial biomolecule mediating powerful cardioprotection by IPC. If this new view is proved by basic and clinical experiments, a crucial biomolecule mediated cardioprotection induced by IPC would be revealed.

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Ischemic preconditioning (IPC) is a known powerful strategy of cardioprotection and regarded as the gold standard for treatment of myocardial ischemia/reperfusion injury (IRI) (1). The underlying mechanisms of IPC have extensively been studied for many years, but have not been elucidated completely. The available evidence indicates that IPC can trigger the release of several autacoids including adenosine, bradykinin and opioids to activate cytoprotective pathways (2-4). Furthermore, IPC with limited ischemic insult can cause slight release of free radicals, which would confer protection on cells subsequently suffering from IRI (5). It has been shown that several pro-survival factors, including glucose transporter-1 and -4, heat shock protein 70 and vascular endothelial growth factors, are involved in the mechanisms of IPC (6). In addition, both the pro-survival mTOR pathway and the developmental Wnt pathway targeting glycogen synthase kinase-3β have also been attributable to the cardioprotection of IPC (7).

The cardioprotection of IPC has been proved *in vitro* and *in vivo* studies. Interestingly, however, we have noticed that anoxia preconditioning can not provide a powerful protection against cardiomyocyte anoxia/reoxygenation injury *in vitro* similar to that of the IPC against myocardial IRI *in vivo* (8, 9). Accordingly, we consider that some humoral cytokines or structures of intact heart may be necessary for powerful cardioprotection provided by the IPC *in vivo*. It has been shown that the endothelial cells can produce many active biomolecules that mediate cardioprotection. For example, growth factor, endothelin-1, bradykinin, prostaglandin E2, nitric oxide and hydrogen sulfide produced by vascular endothelial cells are involved in cardioprotection provided by IPC against myocardial IRI (10-14). Neuregulin-1 (NRG-1), a member of epidermal growth factor family, is produced by vascular endothelium (15). The available literatures indicate that endothelial derived NRG-1 is essential for multiple aspects of cardiac physiology and function during fetal development as well as after birth (16). Moreover, NRG-1 can provide protection against myocardial IRI through phosphoinositide 3 kinase (PI3K) signaling pathway (17). In view of important roles of endothelial derived NRG-1 in cardiac physiology and function, we consider that it maybe a crucial biomolecule mediating powerful cardioprotective features of IPC. This review will first discuss the factors attributable to cardioprotection of the IPC and then provide an overview of the cellular and molecular mechanisms that endothelial derived NRG-1 maybe involved in cardioprotection of the IPC.

Multiple Factors are Attributable to Cardioprotection of IPC

Available literatures indicate that IPC can trigger the release of several autacoids, including adenosine, bradykinin, opioids, etc (2-4). These autacoids in turn stimulate growth factor receptors and then activate related signaling pathways, relieving cell apoptosis via the mitogen activated protein kinases (MAPK)/extracellular signal regulated kinase 1/2 (ERK1/2) and reducing mitochondrial permeability transition pores (mPTP) opening by a PI3K signaling pathway (Figure 1). Furthermore, IPC has been associated with increased hypoxia-inducible factor α and subsequent expression of several prosurvival genes (6), slight release of free radicals (5), less opening of mPTP (18). A recent work also shows that ischemic conditioning can protect myocardium against IRI by attenuating endoplasmic reticulum stress (ERS), which is associated with p38 MAPK and JNK signaling pathways (19). Brooks et al. (20) demonstrated that the IPC can activate unfolded protein response, and ERS-dependent activation of activating transcription factor 3 mediates the late protection of IPC. It is noteworthy that even though some biomolecules and signaling pathways involved in the IPC have been revealed, the detailed mechanisms mediating powerful cardioprotection of IPC are still unclear.

Endothelial Derived NRG-1 may be Essential for IPC-Induced Cardioprotection

Bell and Yellon (21) had proposed that when ischemic conditioning was triggered, it was not an obligate phenomenon of cardiomyocytes alone, but rather a combination and interaction between many cells within the heart, including endothelial cells, fibroblasts, and extracellular matrix, etc. During myocardial ischemic conditioning, endo-

thelial cells may play several roles: a "receptor" for blood-borne conditioning moieties, a sensor for hypoxic stress and a paracrine organ. It has been shown that eluent from the isolated endothelial cells during IPC can increase resistance of isolated cardiomyocytes to hypoxic injury (22).

In the heart, NRG-1 is produced by endothelial cells and exerts its effect in a paracrine manner via the ErbB family of tyrosine kinase receptors (ErbB2, ErbB3, and ErbB4) (15). It has been shown that deficiency of NRG-1-ErbB signaling pathways can cause heart development failure or cardiac dysfunction (16). Furthermore, NRG-1-ErbB signaling pathway is involved in regulation of multiple processes, including cell growth (23), survival (17), glucose uptake (24) and mitochondrial function (25). In addition, administration of exogenous NRG-1 can decrease cardiomyocyte apoptosis by serum deprivation (26) and H_2O_2 stimulation (27).

In available literatures, a few studies have assessed the role of NRG-1 in the IRI process. It has been shown that IRI can activate NRG-1-ErbB signaling pathway, and selectively inhibiting expression of endothelial derived NRG-1 can obviously hinder the recovery of cardiac contraction function after ischemia (28). Fang et al. (17) had confirmed that NRG-1 can protect myocardium from IRI through a PI3K/Akt pathway. D′ Uva et al. (29) showed that selectively activating ErbB2 receptors on the myocardium can induce cardiomyocyte proliferation and improve the recovery of cardiac function, with less infarct size, more vascularization and less scarring.

Based on the above-mentioned comments, we consider that endothelial derived NRG-1 may be essential for cardioprotection of the IPC. Furthermore, deficiency of endothelial derived NRG-1 maybe an important reason why anoxia preconditioning can not provide a powerful protection against cardiomyocyte anoxia/reoxygenation injury *in vitro* similar to that of the IPC against myocardial IRI *in vivo* (8, 9).

How is Endothelial Derived NRG-1 Involved in IPC?

Endothelial Derived NRG-1 may Attenuate IRI by Regulating ERS Related Signaling Pathways

ERS is a common subcellular response to stress,

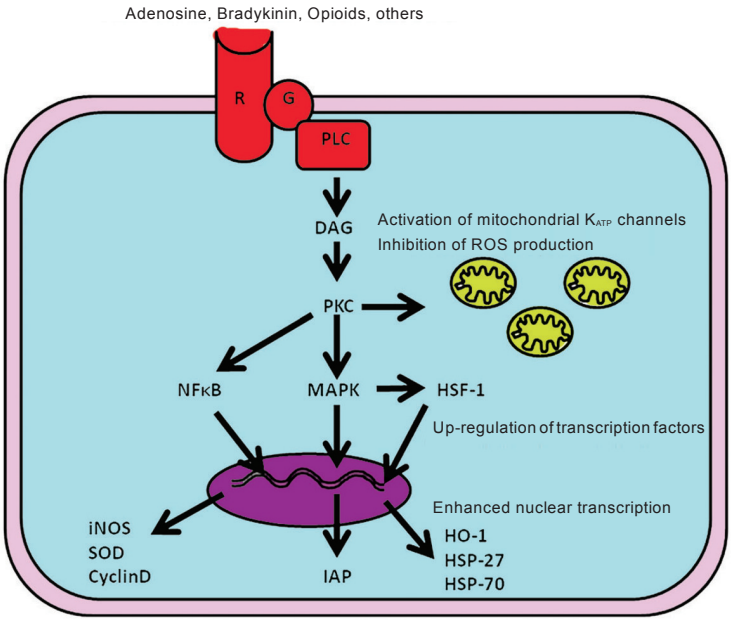

Figure 1. Mechanisms of Ischemic Preconditioning.
Autacoids including adenosine, bradykinin, opioids, or other mediators bind to the G-protein-coupled cell surface receptor. This induces a diacylglycerol-mediated activation of protein kinase C (PKC), mitogen activated protein kinases (MAPK), heat shock factor 1 (HSF-1), and nuclear factor κB (NFκB). This increases the nuclear transcription of protective mediators, such as heat shock proteins 27 and 70, iNOS, SOD, and inhibitors of apoptosis.

which can activate a set of signaling pathways, including three branches, inositol-requiring enzyme 1 (IRE1), PKR-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6), to regulate cell survival (30). In cells undergoing ERS, distinct unfolded protein response (UPR)-related responses are observed over time. Early UPR attenuates protein synthesis at the endoplasmic reticulum by inhibiting translation (which is dependent on the PERK-mediated phosphorylation of eukaryotic translation initiator factor 2α [eIF2α]), activating mRNA decay by regulated IRE1-dependent decay (RIDD), and activating autophagy through the IRE1α-JUN N-terminal kinase (JNK) pathway. In a second wave of events, the UPR transcription factors activating ATF6 fragment, spliced X box-binding protein 1 (XBP1s) and ATF4 promote many adaptive responses that work to restore endoplasmic reticulum function and maintain cell survival. Unmitigated ERS induces apoptosis to eliminate irreversibly damaged cells. The B cell lymphoma 2 (BCL-2) pro-

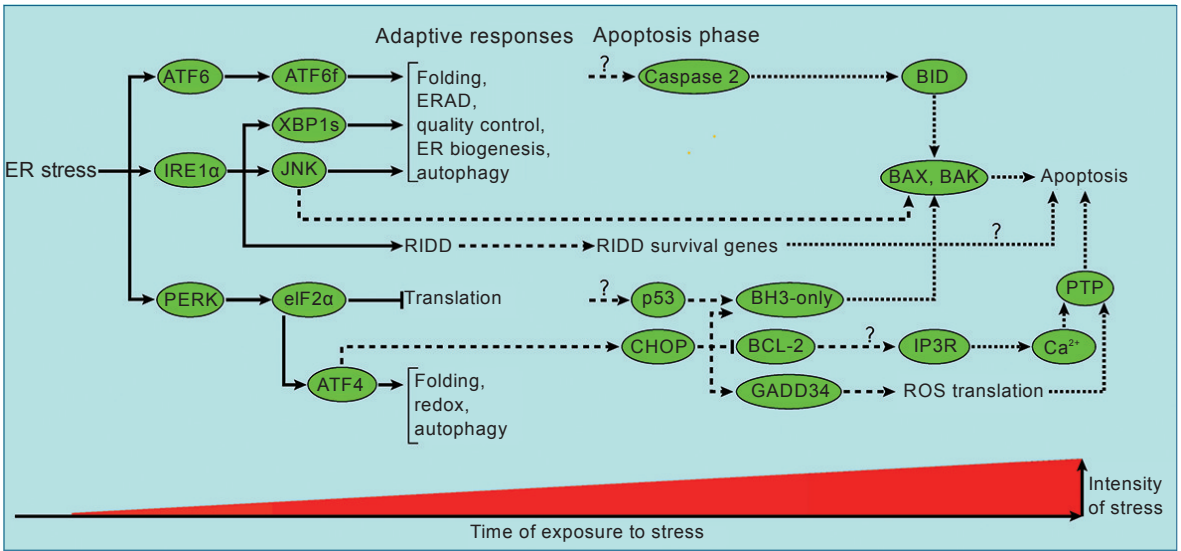

Figure 2. Cell Fate Decisions under Endoplasmic Reticulum Stress.
Dashed arrows exemplify transition steps from adaptive responses to apoptosis. Dotted arrows indicate events mediating apoptosis.

tein family is crucial for the control of ERS-induced apoptosis. When activated at the transcriptional or post-translational level, BCL-2 homology 3 (BH3)-only proteins regulate the activation of BAX and/or BH antagonist or killer (BAK) to trigger apoptosis. Sustained PERK signalling upregulates the pro-apoptotic transcription factor C/EBP-homologous protein (CHOP), which downregulates the anti-apoptotic protein BCL-2, induces the expression of some BH3-only proteins and upregulates growth arrest and DNA damage-inducible 34 (GADD34). The induction of GADD34 may induce the generation of reactive oxygen species (ROS) by enhancing protein synthesis through eIF2α dephosphorylation, and overloading cells with unfolded proteins. In addition to ROS, altered calcium homeostasis owing to inositol-1,4,5-trisphosphate receptor (IP3R) activation, may also contribute to the opening of the mPTP, which promotes apoptosis. CHOP, ATF4, and p53 can also control the expression of a subset of BH3-only proteins. Active IRE1α may sensitize cells to apoptosis through activation of JNK and RIDD of mRNA that encodes for chaperon escuchas BIP. Caspase 2 may also participate in ERS-mediated apoptosis by cleaving the BH3-interacting domain death agonist (BID), which activates BAK and BAX (31) (Figure 2).

It is generally believed that moderate ERS can improve cell survival, but excessive ERS can result in cell apoptosis (32). The different fates of cells regulated by ERS are determined by the expression of three downstream signaling pathways. If the pro-survival IRE1 signaling pathway is prominent, the cell survival will be improved. Conversely, eminence of the proapoptotic PERK and ATF6 signaling pathways will lead to cell fatality.

IRI can cause myocardial anoxia, acidosis, ATP exhaustion, calcium overload and excessive free radical production. All these can trigger ERS in the cardiomyocytes. Furthermore, apoptosis caused by severe ERS has been regarded as one of the important mechanisms of myocardial IRI (33). Martindale et al. (34) found that endoplasmic reticulum-targeted cytoprotective chaperone, glucose-- regulated proteins 78 and 94 (GRP78 and GRP94) increased dramatically during myocardial IRI process. Moreover, ischemia to cardiomyocytes can upregulate the expression of GRP78 and XBP1 in the early stage, and upregulate the expression of C/EBP homologous protein (CHOP) and caspase 12 in the late stage (32). Specially, Xu et al. (35) showed that endothelial derived NRG-1 protected cardiomyocytes against H_2O_2-induced apoptosis by regulating ERS. Accordingly, we speculate that endothelial derived NRG-1 maybe involved in IPC by regulating the ERS related signaling pathways.

Endothelial Derived NRG-1 may regulate ERS by Eliciting Cold-inducible RNA-Binding Protein during IPC

RNA-binding protein is one of the main regulators for posttranscriptional gene expression and can influence the physiological function of cells (36). Cold-inducible RNA-binding protein (CIRP) is a kind of RNA-binding protein (37). Other than cold stress (38), ultraviolet radiation (39) and hypoxia (40) can induce CIRP expression, exerting antiapoptotic and cell-protective effects under these stimuli. Recently, Li et al. (41) demonstrated that CIRP can regulate Ito channels in the heart. The rats with a CIRP gene knockout have a prolonged QT period and a shortened AP period, which is caused by increased Ito channels. Thus, it is deduced that CIRP may be a promising medicine for treatment of cardiovascular diseases. Furthermore, Zhu et al. (42) found that cold-inducible RNA-binding motif protein 3 can inhibit the expression of PERK by interacting with nuclear factor 90, thereby protecting cells from ERS-related injury. Knocking out cold-inducible RNA-binding motif protein 3 by SiRNA technique can upregulate PERK-eIF2a-CHOP signaling pathway and deteriorate HEK293 cell apoptosis, whereas overexpression of cold-inducible RNA-binding motif protein 3 can down-regulate PERK-eIF2a-CHOP signaling pathway during the ERS induced by thapsigargin or tunicamycin. Based on these findings, we consider that endothelial derived NRG-1 may regulate ERS by eliciting CIRP during IPC.

Prospects

IPC has always been a research hotspot in the field of IRI, but detailed mechanisms conferring its powerful cardioprotective properties have still not been clarified. In views of inconsistent cardioprotective efficiency of IPC from *in vitro* and *in vivo* studies, and important roles of endothelial derived NRG-1 in cardiac physiology and function, we suggest that endothelial derived NRG-1 maybe a crucial biomolecule mediating powerful cardioprotection of the IPC and function of endothelial derived NRG-1 in the IPC is achieved by regulating CIRP with its downstream ERS related signaling pathways. We believe that if this new view is proved by basic and clinical experiments, a crucial biomolecule mediating this IPC-induced powerful cardioprotection against myocardial IRI would be discovered. There is no doubt that this will increase new and interesting knowledge to the growing wealth of information regarding cardioprotective mechanisms of the IPC.

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