Review Article

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The Pathophysiology of Brain Ischemia and Ischemic Preconditioning

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Abstract

Ischemic tolerance can protect the brain against cerebral ischemia and neurodegenerative diseases. However, several studies demonstrate ischemic tolerance by various methods, the exact mechanisms of ischemic tolerance has not been clearly understood.

In this study, we first studied brain ischemia-related mechanisms and then evaluated the outcomes of mitochondrial pathophysiology of ischemic tolerance in focal and full stroke animal models.

In this study, mitochondrial and peroxisomal reactions are considered critical in ischemic tolerance. In rats and Syrian mice, the middle cerebral artery occlusion (MCAO) model was employed to represent cerebrovascular stroke. Ischemic tolerance exhibits different types of adaptation responses associated with a number of sub-cellular changes.

Changes in the cellular non-genomic pathways are usually short and reversible; while, the consequences of genes expression are a long-term process and can lead to permanent alternations in the genes expression pattern. The ischemic tolerance can be clinically significant. Therefore, it is important to address the risks and advantages of ischemic tolerance in non-infarcted tissues such as transplanted ones.

Introduction

Stroke is the third most common cause of death and disability in the United States. About 80% of strokes occur due to ischemia caused by thrombosis, embolism, and reduction in the systematic perfusion pressure [1]. Brain ischemia causes excessive release of excitatory amino acids and activation of their receptors, and consequently leads to calcium influx into the cell, electrophysiological and metabolic disorders, lipid peroxidation, and other oxidative processes [2]. Ischemia/reperfusion launches a process, called oxidative stress, which can exacerbate ischemic injury [3, 4]. Oxidative stress can cause the formation of nitric acid and superoxide. Any disturbance in the production or metabolism of each of these can have pathological effects.

Ischemic preconditioning (IPC): Preconditioning is an endogenous phenomenon caused by sub-lethal ischemia in the cells. It leads to adaptive and protective responses against future severe ischemic injuries. This resistance to sever ischemic injury caused by IPC is known as ischemic tolerance [5]. Ischemic tolerance can be described in different organs such as heart, kidney, liver, skeletal muscle, small intestine, lung, and brain [6, 7]. IPC triggers in two acute and delayed phases. The acute phase develops within a few minutes and wanes after 2-3 hours. Delayed phase appears within a few hours and lasts for several days. The signals that develop these two phases of IPC may have similar compositions [8, 9]. They differ in that acute phase is protein synthesis-independent mediated by post-translational protein modifications, and so are short-lived. Delayed phase, on the other hand, requires new protein synthesis and are long-lived. Pharmacologic pre conditioning could lead us to stroke treatment [10].

Laboratory methods for inducing tolerance to ischemia include short periods of transient ischemia [11], anoxia [12], hyperthermia and hypothermia [13], chemical inhibition of oxidative phosphorylation [14], hypoxia [15], oxidative stress [16], spreading depression [17], and dietary restriction [18].

Several protein are involved with IT development including potassium channels [19], caspase-3 and HSP70 [20, 21], NMDA receptors [22], Protein Kinase C [23], superoxide dismutase, glutathione peroxidase [24], increased glutamate transporters, TNF-α [25], NF- κB [26], HIF-1 [27], and erythropoietin [28]. The objective of this review article is to describe these proteins.

Potassium transportation across the mitochondrial inner membrane: Efflux of potassium from matrix is done by sodium-proton (K^+/H^+) exchanger. Influx of potassium into the matrix is done by simple diffusion through potassium leak channels or ATP-sensitive channels. These influx and efflux safeguard the matrix size. Potassium influx into the matrix, which is accompanied by H+ efflux, causes alkalinization of it. Then, these protons return to the matrix along with Pi [29]. Electron transport system in mitochondrial inner membrane, which causes membrane potential ($\Delta\Psi$), which occurs in two states [30]: high state occurs during

resting or preconditioning, and low state happens during ischemia or reperfusion.

The role of mitK$_{ATP}$ opening in different stages: Preconditioning leads to alkalinization and increase in production of reactive oxygen species (ROS), which act as a secondary messenger on signal pathways. In ischemia, potassium influx causes water uptake. For that reason, intra-membrane space (IMS) expands, so creatine kinase allows ADP to exit. Therefore, during ischemia, ATP is not produced, and is only consumed. On the other hand, the produced ADP exits from mitochondrial permeability transition (MPT) pores (Fig. 1). During reperfusion, creatine kinase existing in intra-membrane space translates creatine (Cr) into phosphate creatine (PCr). As we know, creatine phosphate groups supply 67% of energy needed by the heart cells (Fig. 2) (pay attention to ATP and ADP influx and eflux signs).

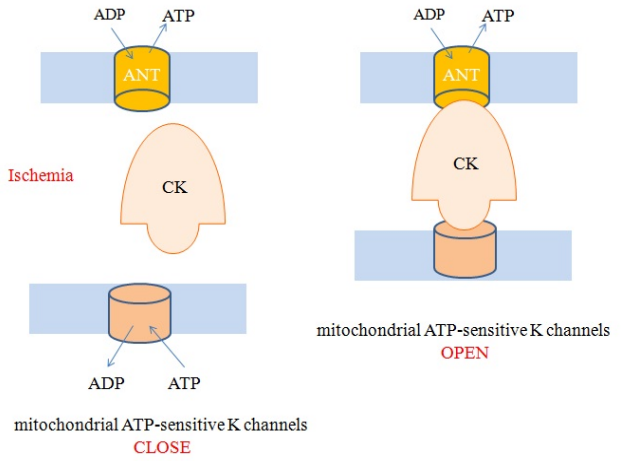

Figure 1. The role of ATP-sensitive channels opening in ischemia

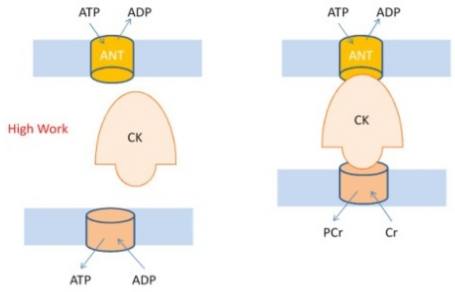

Figure 2. The role of ATP-sensitive channels opening in reperfusion

Mitochondrial permeability transition (MPT) pores: Increase of calcium in the matrix and reduction of ATP lead to MPT. Ischemic preconditioning (IPC) restrains these factors, in that it inhibits proton from leaving the matrix. In the following process, F1 inhibitor binds to F0F1-ATPase enzyme and inhibits it. Consequently, ATP is stored, membrane energy decreases, and calcium influx into the matrix through voltage-related channels is constrained. Thereby, MPT and cell death (necrosis and apoptosis) would happen (Fig. 3).

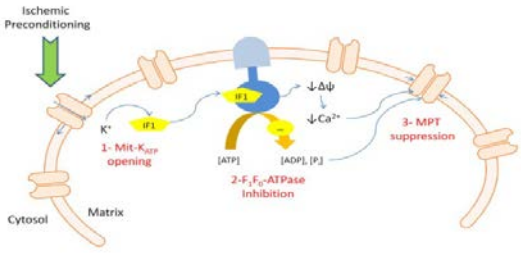

Figure 3. IPC inhibits MPT

The proteins that leave mitochondria during ischemia and the influence of IPC on them: 1. Cytochrome C: ischemia and reperfusion causes ATP reduction, due to ROS production, and mitochondrial ATP-sensitive channels opening. This leads to loss of mitochondrial K^+ and H^+ gradients and release of cytochrome C occurs [15, 16]. Cytochrome C binds to Apaf-1 (apoptotic protease activating factor-1) and forms apoptosome, transfers apoptosome caspase 9 to caspase 3, and activate caspase DNase 3. In addition, this reaction leads to DNA damage, and so apoptosis. In IPC, HSC70 (heat shock chaperone70) catches caspase 3. This process causes HSC70 reduction, which results in HSP70 increases. HSP70 inhibits the release of cytochrome C (Fig. 4).

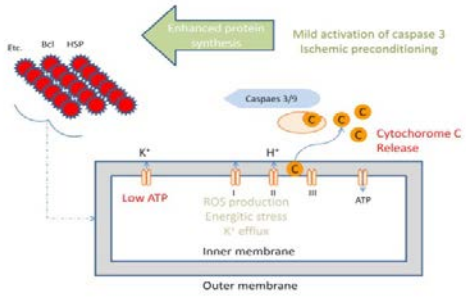

Figure 4. IPC inhibits the release of cytochrome C

2. HSP70 apoptosis inducing factors (AIF) cause release of these factors [15].

3. Smac (Second mitochondrial-derived activator of caspase/DIABLO (Direct Inhibitor of Apoptosis protein-Binding Domain of Smac protein) binds to IAP (Inhibitors of Apoptosis), and inhibits anti-apoptosis function by cleaving it from caspase. IPC inhibits Smac/DIABLO release (not IAP and HSP70) [15].

During ischemia, cytoplasmic calcium concentration increases. Thereby, protein phosphatase calcineurin is activated. It results in BAD (bcl-2-associated death promoter) dephosphorylation, and release of it from its dormant position in the cytoplasm and transportation to mitochondrion, where forms heterodimer with Bcl-2 and

causes MPT. IPC activates PI_3K and Akt/PKB signals. These signals lead to phosphorylation of BAD, resulting in apoptosis inhibition. In addition, IPC causes increase in Bcl-2 family members, which are anti-apoptosis.

Caspase 3 enzyme

Caspase 3 enzyme causes proteolysis of the target proteins including inhibitor of caspase-3-activated DNase. Thereby, this DNase is activated and launches apoptosis. IPC acts downstream of signal transduction pathway of caspase 3 activation and upstream of signal transduction pathway of its target, i.e. caspase-activated DNase, to prevent cell death [15].

Potassium channels that protect against increased intracellular calcium

1. Calcium- sensitive potassium channel (K_{Ca2+}) that opens due to large conductance of calcium.
2. ATP-sensitive potassium channel opens due to intracellular ATP reduction, and exist in cell membrane surface and mitochondrial inner membrane.
3. Acid-sensitive and mechanosensitive potassium channels (open due to strain, as the cells swell during ischemia, and because of PUFA (polyunsaturated fatty acid), such as arachidonic acid, stimulation and cell acidizing due to arachidonic acid desorption from membrane into cytoplasm during ischemia).

When these channels open, due to hyper-polarization of the cell, potassium leaves it, and calcium influx through voltage-dependent calcium channels is inhibited. This is so because membrane potential rests away from the threshold activity of these channels. In addition, NMDA (N-Methyl-D-Aspartate) receptors are blocked by magnesium ion, so inhibits Ca^{2+} influx. In the presynaptic cells, hyper-polarization causes reduction in the amount of glutamate release, and so decrease in apoptosis [14].

Hypoxia induce factor (HIF-α)

The ROS produced during IPC binds to iron group in prolyl hydroxylase enzyme and inhibits it. Therefore, HIF-α would no longer be degraded by above enzyme, its concentration increases, and enters into the nucleus as a transcription factor and results in erythropoietin expression increase, activation of VEGF (Vascular Endothelial Growth Factor) and GLUT1 (Glucose Transporter 1), and cell proliferation. Finally, all of these factors lead to maintenance of cells viability potential and inhibition of apoptosis.

Erythropoietin

Erythropoietin concentration increases during IPC and binds to its receptors on cell surface, and causes induction of following protective responses through phosphorylation of Jack 2 [23]: (Fig. 5)

1) Phosphorylation STAT-5, its movement towards the nucleus, binding to DNA, and Bcl-2 and Bcl-XL expressions. The two latter cases cause inhibition of caspase 3 activities.
2) Separation of of IκB (Inhibitor of NF-kB) form NF-κB, activation of (Nuclear Factor κB) NF-κB, movement towards the nucleus, neuroprotection genes expression such AIP and SOD.
3) Phosphorylation of PI3K and Akt/PKB, and thereby, inhibition of caspase 3 formation, inhibition the activity of the BAD and Bax, and inhibition of apoptosis

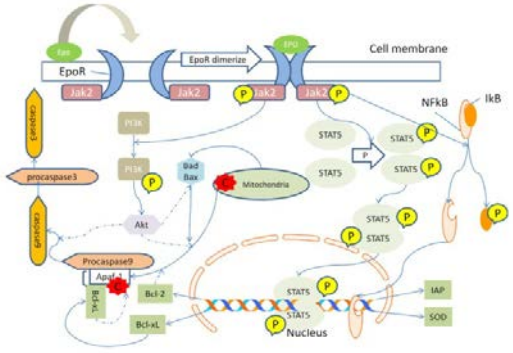

Figure 5. The role of erythropoietin in ischemia preconditioning

Protein Kinase C and NMDA receptor: Adding NMDA (N-Methyl-D-aspartate) causes activation of its receptor, resulting in temporary increase of intracellular calcium. Then, phosphatidylinositol 4, 5-bisphosphate degrades into inositol triphosphate (IP_3) and diacylglyserol (DAG). IP_3 causes the release of calcium from endoplasmic network, and DAG with calcium activate protein kinase (PKC) and launches Ras-MAPK pathway. This pathway phosphorylates protein kinase and neuroprotection transcription factors [17, 18] (Fig. 6). The use of NMDA antagonist inhibits neuroprotection.

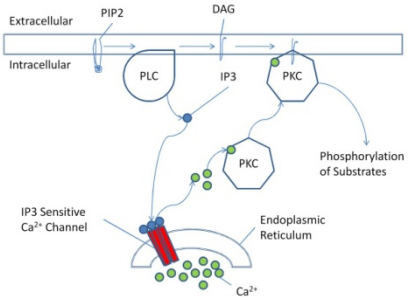

Figure 6. Activation of protein kinase C

Nuclear factor kappa B (NF-κB): This protein is usually disabled in cytoplasm, as is bound with inhibitory proteins such as IκB. As a result, when is phosphorylated by its activating agents, it would become isolated from IκB, enter into the nucleus, and act as transcription factor. NF-κB is activated via different signals. For example, cytokines such as erythropoietin and TNF-α, neurotrophic factors, neurotransmitters, oxidative stress, increased intracellular calcium, glutamate receptors excitement, and depolarization of membrane [31]. The activation of NF-κB causes induction of anti-apoptosis genes, such as SOD and Bcl-2, which provide neuroprotection, induction of genes engaged in controlling inflammatory response, cell

growth regulation, and transcription IκB manufacturing gene. Consequently, it leads to the movement towards the nucleus, isolation of NF-κB from DNA, and finally termination of its activity. Then, NF-κB either returns to cytoplasm or is wiped out in the nucleus [32].

Glutamate transporters (EAAT: Excitatory Amino Acid Transporter)

Ischemia causes increased glutamate release in synaptic space. Glutamate is an amino acid with resultant toxicity properties, caused by excitement that ultimately results in apoptosis [33]. IPC activates TACE enzyme that converts TNF-α into a solution [34]. Thereby, TNF-α can activate NF-κB. Therefore, due to NF-κB activation, the level of glutamate transporters increases. These transporters then collect glutamate from synaptic space and decrease their destructive consequences [20].

Superoxide dismutase (SOD) and glutathione peroxidase (GSHPx)

The occurrence of MPT during ischemia causes the release of superoxide from mitochondrial matrix. The superoxide dismutase enzyme [35], in combination with two hydrogen ions, can convert superoxide to hydrogen peroxide. Then, hydrogen peroxide is split either into two water molecules via glutathione peroxide [36] or into water and oxygen by catalase enzyme [19].

Adenosine receptors

During hypoxia, hypoglycemia and convulsion, ATP decreases and is converted into ADP and AMP. The 5'-nucleodize enzyme produces adenosine by dephosphorylating AMP. Intra-cellular AMP-derived adenosine is released from the cell by nucleozid transporter [28, 37]. OF the adenosine receptors (including A1, A2A, and A3), A1 and A3 have more salient role in IPC [38]. They exercise their protective influence by activating various paths and signals: phospholipase C and D, mitochondrial ATP-sensitive potassium channels, PI3-K and Akt pathways, nitric oxide synthase, and MAPK/Erk pathway. Activation of these receptors causes reduction in formation of mitochondrial radicals, increase of cell's antioxidant capacity, and inhibition of MPT pores opening. The expression of adenosine sub-receptors controls ATP reduction. Thereby, it inhibits influx of sodium, calcium, and proton into the cell, during ischemia [29].

Nitric oxide synthase

Heart ischemia activates nitric oxide synthase (eNOS: endothelial Nitric Oxide Synthase). The produced nitric oxide triggers PKC. PKC then launches Scr (SH3 homology domain), Lck (Lymphocyte-specific protein), tyrosine kinase, ERK, and NF-κB. These signals cause the induction of transcription of inflammatory or immunologic oxide synthase. The produced nitric oxide causes mitochondrial ATP-sensitive potassium channels opening [5].

However, brain ischemia activates NMDA receptors. These receptors are paired with neural nitric acid, so nNOS is triggered and produces nitric oxide. After that, p21, Ras, Raf, Mek, and Erk proteins are activated, thereby, ELK-1, CREB (cAMP response element binding protein) expression factors are triggered and cause synthesis of such proteins as Bcl-2 and different types of HSC. In addition, they result in reduction of apoptosis carrier proteins

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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