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·Review·

Hypoxic preconditioning in an autohypoxic animal model

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Abstract: Hypoxic preconditioning refers to the exposure of organisms, systems, organs, tissues or cells to moderate hypoxia/ischemia that results in increased resistance to a subsequent episode of severe hypoxia/ischemia. In this article, we review recent research based on a mouse model of repeated exposure to autohypoxia. Pre-exposure markedly increases the tolerance to or protection against hypoxic insult, and preserves the cellular structure of the brain. Furthermore, the hippocampal activity amplitude and frequency of electroencephalogram, latency of cortical somatosensory-evoked potential and spinal somatosensory-evoked potential progressively decrease, while spatial learning and memory improve. In the brain, detrimental neurochemicals such as free radicals are down-regulated, while beneficial ones such as adenosine are up-regulated. Also, antihypoxia factor(s) and gene(s) are activated. We propose that the tolerance and protective effects depend on energy conservation and plasticity triggered by exposure to hypoxia via oxygen-sensing transduction pathways and hypoxia-inducible factor-initiated cascades. A potential path for further research is the development of devices and pharmaceuticals acting on antihypoxia factor(s) and gene(s) for the prevention and treatment of hypoxia and related syndromes.

Keywords: hypoxia; hypoxic preconditioning; adaptive medicine

1 Introduction

Hypoxia is a common and important problem in both clinical settings and extreme environments. To date, adaptation to hypoxia has been investigated at the whole-body, systemic, organic and cellular levels. In 1963, we described this phenomenon as "a kind of induced tolerance of tissue-cells to hypoxia"[1]. This article reviews our recent findings on the relation of hypoxic preconditioning to brain plasticity, using the animal model of repeated autohypoxia.

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The terms preconditioning and tolerance were first described by Janoff in 1964[2]. The phenomenon of ischemic or hypoxic preconditioning (HPC) has been well documented in the heart and the brain[3-6].

2 An animal model of whole-body hypoxic preconditioning (WbHPC)

In general, two types of animal models, WbHPC and single-organ HPC, have been described[1,7-11]. Lu *et al.* created a WbHPC model of repetitive autohypoxia in adult BALB/C mice. The mouse is anesthetized with pentobarbital and placed into a container that is then sealed, so that the animal's own oxygen consumption induces autohypoxia. The animal is removed from the container and switched to another at

the onset of gasping. The procedure is performed once (H1) and repeated up to 5 times (H2, H3, H4 or H5). Animals exposed to hypoxia for 4 or 5 runs, groups H4 and H5, are judged to be preconditioned or tolerant. Those exposed once, H1, serve as experimental controls, and those having no exposure to hypoxia, H0, serve as normal blank controls[1,12,13].

The onset of gasping is judged to be the limit of the animal's tolerance to hypoxia in each exposure. The tolerance limit significantly increases run by run. The tolerance times in runs 2, 3, 4, and 5 are close to 2, 4, 6, and 8 times the first run respectively. In addition, when animals are matched for body weight and sex and randomly paired, normal control animals survive for only 17 min on average inside the container, whereas preconditioned animals survive for 146 min on average, 8.6 times the control time[1,12,13].

The *in vitro* methyl thiazolyl tetrazolium assay showed that PC12 cells co-cultured with aqueous brain extract from preconditioned animals are significantly more viable than those co-cultured with brain extract from control animals[14]. The leakage of lactic dehydrogenate (LDH) from cortical synaptosomes co-cultured with brain extract from preconditioned animals is markedly decreased[15]. The survival time inside a hypobaric chamber of normal mice injected with supernatant from brain homogenates of HPC mice (24.3 ± 6.2 min) is approximately double that of the saline control group (13.2 ± 4.8 min) and the H1 group (11.6 ± 3.5 min)[13]. These *in vitro* and *in vivo* results suggest the occurrence of an important modification or acclimatization in the brain during preconditioning.

3 Cerebral morphological and functional changes

Under the light microscope, no apparent difference is seen in the hippocampal neurons among the H0, H1, and H4 groups in the WbHPC model[16]. Histochemically, the acidic phosphatase activity remains unchanged in H1 and H4 animals. In comparison with normal controls, nitric oxide synthase (NOS)-positive neurons in the cortex and hippocampus stains more intensely and their processes are larger and longer in H1 animals. However, instead of further deterioration, these neurons stain lighter and their processes became smaller in H4 animals. The ratio of NOS-positive neurons in the cortex and hippocampus is 2.0 in the H0 group, 3.6 in H1, and 4.4 in H4. These findings indicate that no apparent damage occurs in the brain during HPC[16-18].

The activity of succinate dehydrogenase decreases and LDH increases in the hippocampus of preconditioned animals, leading to a transformation from aerobic oxidation of glucose to anaerobic glycolysis. No apparent difference occurs in the activity of glucose-6-phosphatase and ATPase, indicating no change in glycogenolytic activity and thus neuronal membrane pumps can be maintained under low energy conditions provided mainly by glycolysis[18].

Moreover, the hippocampal activity amplitude and frequency of electroencephalogram, cortical somatosensory-evoked potential and latency of spinal somatosensory-evoked potential are progressively reduced with increased hypoxic exposure time[19]. The mRNA and protein levels of neural cell adhesion molecule and the long-term potentiation induction rate decrease in the H1 group but recover to control levels in H4. The performance of preconditioned mice in maze testing improves over that in H1 and H0. These findings indicate that the spatial-cognitive ability of adult mice is not impaired but improves during HPC[20].

4 Neurochemical acclimatization

Antagonistic regulation of neurochemical elements is shown in the brain of the WbHPC model. Reactive oxygen species, lipid peroxides, calcium ions, excitatory amino acids, nitric oxide, phosphorylation level of extracellular signal-regulated kinase1/2, expression of alpha synuclein and other elements detrimental to brain function are down-regulated in the cortex of preconditioned animals. On the other hand, superoxide dismutase, glutathione peroxidase, inhibitory amino acids such as glycine and γ-aminobutyric acid, glycogen, cAMP response element-binding protein, c-Jun-N-terminal kinases, p38, membrane translocation of novel protein kinase ε and others beneficial to brain function are up-regulated during HPC[15,18,21-33].

Adenosine content and adenosine A1 receptor affinity

in the hippocampus in preconditioned mice are markedly higher than those in both the H0 and H1 groups[34]. Expression of Bcl-2 in hypoxia-tolerant animals is induced in the WbHPC model[35]. The newly-discovered neuroglobin (Ngb) protein, Ngb mRNA and Ngb-positive neurons in the cortex of preconditioned animals are up-regulated[36]. Hypoxia-inducible factor 1α (HIF-1α) mRNA increases rapidly and transitorily in the H1 group but decreases to the basal level in the H4 group. The HIF-1α protein level and HIF-1 DNA-binding activity increase in the H1 group and markedly increase in the H4 group. The expression of HIF-1α and one of its target molecules, vascular epithelial growth factor, is up-regulated in the hippocampus of preconditioned animals[37]. By patch clamp recording, we found that the outward potassium currents of cortical neurons treated with adenosine increase. Interestingly, a similar change is found when brain extract from preconditioned animals is added to the bath solution, indicating the generation of adenosine-like molecules in the brain of preconditioned animals, and ATP-dependent potassium channels can be activated by brain extract from preconditioned animals[38].

In addition to the down- and up-regulation of these known neurochemicals and molecules, an unknown component and gene are specifically found in the brain of preconditioned animals. The specific component or peak has been tentatively named antihypoxic factor. Similarly, a specific unknown antihypoxia-associated gene has been detected by mRNA differential display[39].

5 Mechanisms and implications of HPC

In WbHPC animals, the internal state seems to fluctuate to conform to the available oxygen challenge in the environment in terms of energy conservation (manuscript in preparation), brain plasticity, and antihypoxia factor/gene activation[39-43] (Fig. 1). The survival strategy under hypoxia is consistent with the adaptation of tissues and cells to hypoxia proposed in the early 1960s[2].

Repetitive exposure to hypoxia, as a preconditioning stimulus, triggers an oxygen sensing/signaling transduction cascade including the carotid and aortic bodies and tissue-specific receptors as well as corresponding pathways.

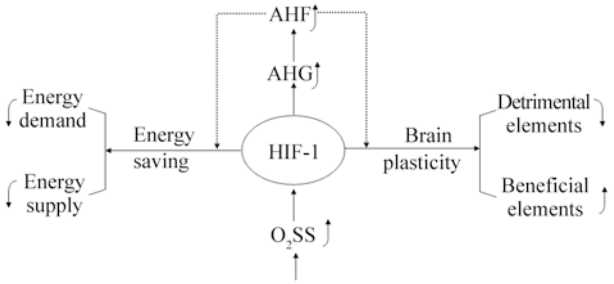

Fig. 1. Tentative framework of the mechanisms underlying hypoxic preconditioning. Under repetitive exposure to hypoxia, oxygen sensing/signaling/transduction pathways (O_2ss) including the carotid and aortic bodies and tissue-specific receptors as well as corresponding pathways and cascades are activated. Expression of hypoxia-inducible factor 1α (HIF-1α) is up-regulated by hypoxic signals and the target genes of HIF-1 are tissue-specifically activated initiating a cascade leading to energy conservation and cell protection in tissues and cells, especially in the brain. AHF: antihypoxic factor; AHG: antihypoxic gene.

Synthesis of HIF-1 is up-regulated by hypoxic signals and its target genes are tissue-specifically activated to initiate a cascade leading to energy conservation, brain plasticity and particularly the activation of antihypoxia factor(s)/gene(s)[42].

Interactions between HPC, synaptic transmission, synaptic plasticity, and newly generated/activated antihypoxia factor(s)/gene(s) in the brain are complex. An important requirement is to identify the newly-discovered antihypoxia factor(s)/gene(s) and the necessary preconditions that regulate the expression of brain plasticity at the neural network, synaptic and neuronal levels. Another requirement is to disclose the cellular mechanisms and conditions that transform the tissue-cell network into a low metabolic state without apparent loss of morphological and functional integrity[39,42].

With gains in understanding at these levels and the production/activation of antihypoxia factor(s)/gene(s), it may become possible to harness the brain plasticity and energy conservation as therapeutic tools and provide a basis for translational cytoprotection trials. HPC has emerged as a powerful experimental method of ameliorating ischemic/hypoxic injury in a variety of diseases. HPC, a strategy completely different from the traditional oxygen therapy,

is considered as an alternative strategy to combat hypoxia and other diseases, which can trigger the genetic potential of tissues and cells to cope with extreme environmental and other stresses[39,42].

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