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Hypoxic Preconditioning

A Novel Intrinsic Cytoprotective Strategy

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Abstract

A concept of tissue-cell adaptation to hypoxia (hypoxic preconditioning) is raised and its corresponding animal model is introduced. A significantly strengthened tolerance to hypoxia and a protective effect of the brain extracts from the preconditioned animals are presented. Changes in animals' behavior, neuromorphology, neurophysiology, neurochemistry and molecular neurobiology during preconditioning are described. Energy saving, hypometabolism, and cerebral protection in particular are thought to be involved in the development of hypoxic tolerance and tissue-cell protection. The essence and significance of the hypoxic tissue-cell adaptation or preconditioning are discussed in terms of biological evolution and practical implication.

Index Entries: Hypoxic tolerance; energy saving; hypometabolism; brain protection; mouse.

Introduction

Hypoxia is a common and important problem in conditions of both clinic and extreme environments *(1)*. A large number of data has been accumulated over a century. However, the action of hypoxia and hypoxia adaptation remains elusive. Medical approaches in counteracting the devastating effects of hypoxia have been limited to supplementation of oxygen via traditional oxygen inspiration and a modern high-pressure oxygen chamber.

Ischemic/hypoxic preconditioning of the heart and brain is a well-documented phenomenon since the late 1980s *(2–9)*. The brain requires a continuous supply of oxygen and glucose to maintain its viability and function. The mechanisms allowing the brain to survive hypoxia are the adaptation to hypoxia. The mammalian brain is the most vulnerable to oxygen deprivation and has thus been one of the major subjects of intensive studies for a long period of time.

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Attention has been paid to the activity of isolated mammalian brain and the response of in vitro brain slice preparation to hypoxia *(5,6).* Little is known, however, regarding the acute adaptation of a brain to hypoxia in vivo and its cerebral mechanisms. A concept of tissue-cell adaptation to hypoxia or hypoxic preconditioning was raised in 1963 and an intrinsic ability of tissues and cells to protect themselves from severe hypoxic injury was proposed to be triggered or motivated by repetitive exposures of the organism or its tissue cells to the condition of hypoxia/ischemia *(10).* A unique animal model of hypoxic preconditioning was developed and the effects and mechanisms of the preconditioning have been studied in our laboratory since the early 1960s.

A Model of Repetitive Autohypoxia *(11–14)*

Experiments were conducted at room temperature (18 ± 1°C) on adult BALB/C mice of both sexes, weighing 16.0 to 22.0 g. The animals were anesthetized with 1% sodium pentobarbital (5.5 mL/kg interperitneally) and randomly divided into three basic groups: blank control group with no exposure to hypoxia (H_0), hypoxia control group with only one time of hypoxic exposure (H_1), and hypoxic preconditioning group subjected to four or five times the hypoxic exposures (H_4 or H_5). The four or five times hypoxia-exposed mice were regarded as hypoxia-preconditioned or hypoxia-tolerant/resistant animals. For dynamic observation, groups exposed to hypoxia two or three times (H_2 or H_3) were also added.

The animal was placed into a 125-mL jar with fresh air and the jar was sealed with a rubber plug. Immediately after gasping breath appeared, the animal was removed from the jar and switched to another jar containing fresh air of similar volume within 30 s. The jar was immediately hermetically sealed again. This procedure was performed once (H_1) and repeated two or three, and four or five times (H_2 or H_3, and H_4 or H_5, respectively).

At least three factors (lower oxygen, high carbon dioxide, and low atmospheric pressure) were thought to be involved in the airless condition in the present procedure. It is generally recognized that the main consequence is hypoxia, and the procedure is simply described as "autohypoxia" *(15).* To confirm the postulation, the carbon dioxide was absorbed by calcium hydroxide inside the jar, and the atmospheric pressure was kept constant with a capsule, leaving only hypoxia in the jar (Fig. 1). The postulation was shown to be true because hypoxia itself could also produce hyoxic tolerance or hyoxic adaptation that is comparable to the condition of low oxygen, high carbon dioxide, and low atmospheric pressure.

The appearance of gasping breath was regarded as the tolerance limit in each trial. The time period between the beginning of air tightness (t_0) and the appearance of the first gasping (t_1) was termed "original duration of tolerance" (T_o). The standard tolerance duration (T) in a standard jar with an effective fresh air volume of 100 mL (V_e) was calculated as follows:

$$\begin{aligned} T &= (T_o/\mathrm{V_e}) \times 100 \\ &= [(t_1 - t_0)/(V_0 - V_a)] \times 100 \\ &= [\,(t_1 - t_0)/(V_0 - W_a/\mathrm{D_a})] \times 100 \\ &= [\,(t_1 - t_0)/(V_0 - W_a/0.94)] \times 100 \end{aligned}$$

where T is the standard tolerance time (min), t_0 is the starting time of sealing, V_e is the effective jar volume (mL), V_0 is the original jar volume (mL), V_a is the animal's volume (mL), W_a is the animal's body weight (g), and D_a is the animal's density determined from displacement V_a and measured body weight. The average D_a was 0.94, ranging from 0.92 to 1.00.

Strengthened Tolerance to Hypoxia *(11–14,16–63)*

In our model, the tolerance of the animals to hypoxia was significantly enhanced. The hypoxic tolerant time was increased by several times after repetitive hypoxic exposures. The

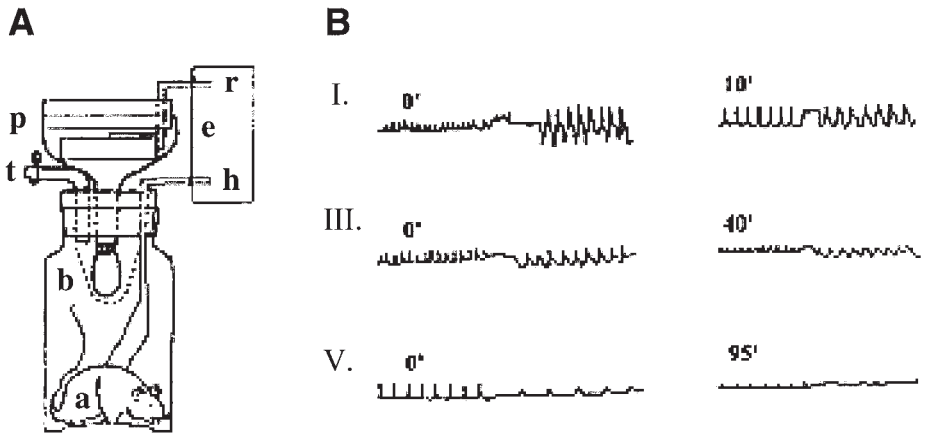

Fig. 1. Diagram showing apparatus for recording electrocardiogram (ECG) and respiration movement **(A)** and examples of these recordings **(B). (A)** a, animal; b, balloon; e, ECG; h, ECG recording; p, piezoelectrical crystal microphone; r, respiration movement recording; t, tube for taking air from the jar. The dashed line represents the metal net. **(B)** Left traces are ECG, and right traces are respiration movement in runs 1 (I), 3 (III), and 5 (V). Numbers are time-points (in minutes) at which the records were taken in runs 1, 3, and 5.

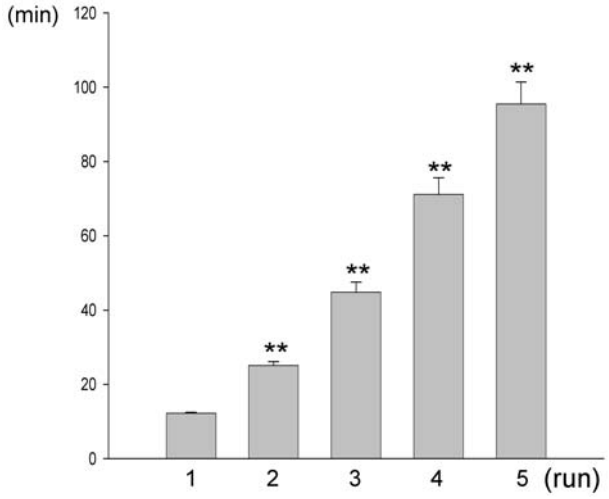

Fig. 2. Tolerance times in different runs of hypoxic exposure. $^{**}p < 0.05$ and 0.01, respectively, compared with the preceding run.

average *standard tolerance duration* in runs 1, 2, 3, 4, and 5 was 12.2, 25.1, 44.8, 71.1, and 95.5 min, respectively (Fig. 2). The tolerance limit in the successive second, third, fourth, and fifth run of exposure was as two, four, six, and eight times longer, respectively, compared to that of the first run.

Normal animals in the control group (H_0, n = 15) survived for only 1.60 ± 0.36 min in the hypobaric chamber with pO_2 at 2.7 kPa. However, the mean *survival time* of the preconditioned animals (H_5, n = 15) under the hypobaric condition survived as long as 15.3 ± 2.9 min, almost 10 times than that of the control animals ($p < 0.001$). A few animals in the H_5 group survived for 50 min.

When animals were randomly paired based on their body weight and sex, normal animals (H_0, n = 5) survived only for 1.7 min on average in the chamber, whereas the hypoxia-preconditioned ones (H_5, n = 5) lived for 146 min on average, 86 times longer than the survival time of their normal partners ($p < 0.001$). One of the preconditioned animals survived as long as 185 min and behaved well the next day.

The other groups, similarly matched, were intraperitoneally injected with lethal doses of potassium cyanide (50 mg/kg) immediately after completion of the fourth run of hypoxic exposure (H_4, n = 8) and survived for 9.4 ± 2.4 min, 4.1 times longer than the normal controls (H_0, 2.3 ± 0.3 min, n = 8) ($p < 0.01$).

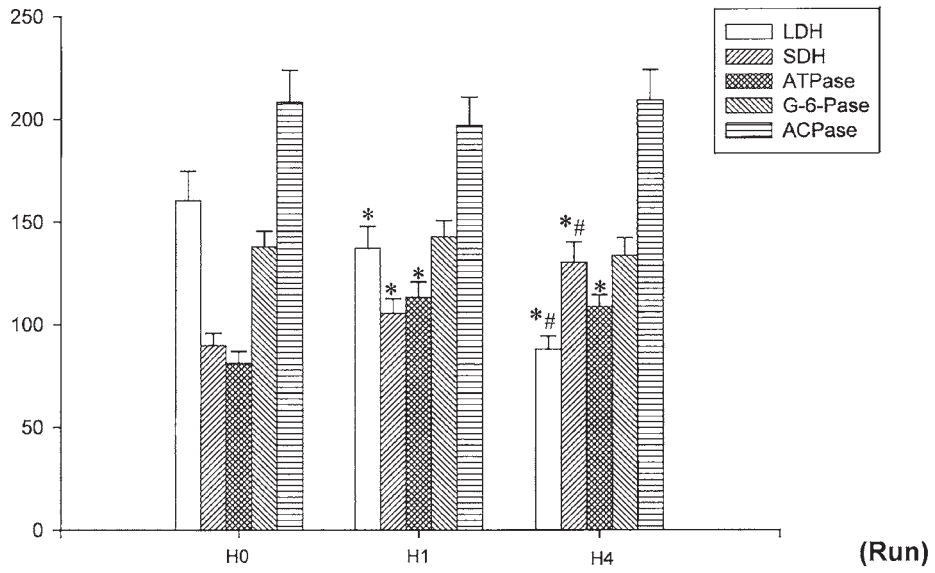

Fig. 3. Gray scales of enzyme stain by image analysis in groups H_0, H_1, and H_4. *$p < 0.05$ compared with H_0; #$p < 0.05$ compared with H_1.

The *Residual activity* of the isolated medulla and the spinal cord after decapitation of the hypoxia-tolerant mice (H_5, $n = 8$) lasted for 124 and 66 s on average, respectively, five and three times longer than that of the control mice with no exposure to hypoxia (H_0, 25 and 22 s, $n = 8$).

Maintained Structure of Brain Cells *(16–18)*

Under a light microscope, no apparent differences were seen in the *hippocampal neurons* among the H_0, H_1, and H_4 groups. Nissl bodies existed in the cytoplasm of these three groups. A few necrotic neurons with cytoplasmic eosinophilia and nuclear pyknosis were detected in the hippocampal CA1 in group H_0 but no further increase was seen in groups H_1 and H_4. At the level of electron microscopy, the structures of mitochondria and endoplasmic reticula in the *cerebral cortex neurons* were almost normal in most hypoxia-preconditioned animals. However, they were slightly destroyed in some control animals exposed to hypoxia once (H_1) (this is not acceptable because the destroyed structures cannot be recovered so quickly as no any changes can be found in H_4).

Histochemically, the acidic phosphophatase *(ACPase)* activity remained unchanged in H_1 and H_4 animals, indicating that no necrosis occurred in the hippocampal neurons by hypoxic exposures (Fig. 3). In comparison with normal controls, nitric oxide synthase (NOS)-positive neurons in the cortex and hippocampus stained more intensively and their processes looked larger and longer in H_1 animals. However, instead of further deterioration, these neurons stained lighter and their processes became smaller in H_4 animals. The ratio in the number of these neurons was 2.0, 3.6, and 4.4, respectively, in H_0, H_1, and H_4 animals.

Reduced Energy Demand *(19–21)*

Overall Behavior

During the first run of hypoxic exposure, the animals' respiration gradually quickened, cyanosis gradually increased, and, finally, spasmlike

activity and gasping breath appeared. Similar behavior was evident during the second run. Starting from the third run, the animals remained quiet most of the time and their respiration became slow and deep but regular in pattern (Fig. 1). Cyanosis became more apparent and the eyeballs showed a black-violet color.

Spontaneous Movement and Righting Reflex

Spontaneous movement and righting reflex of the mice disappeared in 10.4 and 12.0 min on average, respectively. The disappearance lasted for 3.1 min on average in the first run. During the second run, the animals' spontaneous movement recovered in 0.2 min following airtightness and then apparently decreased and disappeared in 13.0 min. Their righting reflex disappeared in 25.0 min. No recovery in spontaneous movement was seen from the third run to the fifth run and the animals kept quiet all the time, which lasted as long as 256.0 min on average.

Spontaneous EEG

Spontaneous EEG in the cerebral cortex gradually decreased in both amplitude and frequency and became a straight line. Epilepticlike waves were frequently shown in the hippocampal CA1 region, whereas a lower frequency of delta rhythm mostly occurred in the CA3 region since run 4.

Evoked Cerebral Potentials

Evoked cerebral potentials by stimulation of the peroneal nerve gradually reduced and became a straight line in run 4. The amplitude of population spikes induced by stimulation of the septal area gradually decreased and disappeared first in the CA1 region and then in the CA3 region.

Respiratory Movement and ECG

The rates of respiration and heart beat decreased as the exposure runs increased. The respiration rate was 315 cycles (C)/min at the beginning of the first run and decreased to 78 C/min at the end of the fifth run, decreasing by 75%. The heart rate was 744 C/min at the beginning of the first run and deceased to 157 C/min at the end of the fifth run, decreasing by 80% (Figs. 1 and 4). In addition to the reduction in the rates of respiration and heart beat, the amplitude of respiratory movement and ECG wave were decreased; however, no abnormal change was seen in the respiration rhythm and ECG (Figs. 1 and 4).

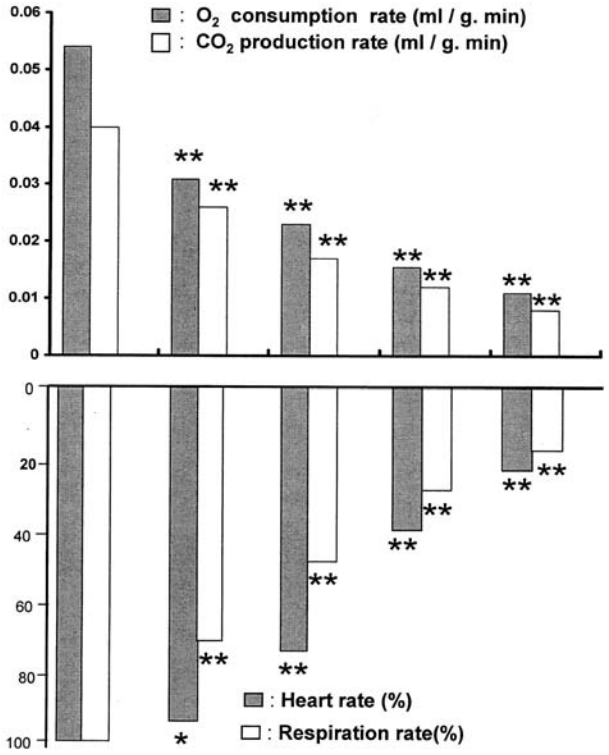

Fig. 4. Heart and respiration rates **(lower)** and rates of oxygen consumption and carbon dioxide production **(upper)** in runs 1 to 5 (from left to right).

Reduced Energy Supply *(17,22–24)*

Gas Metabolism

Oxygen consumption and carbon dioxide production were exponentially decreased as

the exposure run increased. The oxygen consumption rate was 0.05 mL/g min at the tolerance limit in run 1 and decreased to 0.01 mL/g min at the tolerance limit in run 5, decreasing 80% (Fig. 4).

Energy Metabolism

The average survival times (min) in the sealed environment after administration of normal saline, iodoacetic acid, malonic acid, potassium cyanide, and potassium cyanide plus iodoacetic acid for the animals exposed repeatedly to hypoxia for three runs (H_3) were, 3.1, 3.9, 1.4, 2.6, and 2.8 times, respectively, those of the control animals that had been treated with the corresponding chemicals but without exposure to hypoxia (H_0). The efficacy of energy via the pathway glycolysis, CREBS, and respiratory chain in preconditioned mice was thus 3.9, 1.4, and 2.6 times that of the unexposed animals.

Enzyme Histochemistry

The activity of lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) was found to be increased and decreased, respectively, in the hippocampus of the hypoxia-preconditioned animals, leading to an increase in anaerobic glycolysis and a decrease in the aerobic oxidation of glucose. The activity of adenosine triphosphatase (ATPase) was decreased in H_1 animals but remained at the same level without continuing decrease in H_4 animals. Glucose-6-phosphatase (G-6-Pase) activity remained unchanged and the inferring hippocampal neurons maintained the production of glucose via glycogeneolysis (Fig. 3).

Body Temperature

The body temperature of the preconditioned animals was also exponentially decreased. It was 35°C on average before the first exposure and decreased to 20°C close to room temperature immediately following the conclusion of the fifth run.

Protective Effects of Brain Homogenate Extract *(11,13,25,26)*

The survival time in vivo in the hypobaric chamber of the animals injected with brain homogenate supernatant from hypoxic-resistant mice (24.3 ± 6.2 min, n = 15) was about double that of the saline control group (13.2 ± 4.8 min, n = 15) and the non-hypoxia-exposed controls (11.6 ± 3.5 min, n = 15).

When acutely dissociated synaptosomes of the rat cortex were coincubated under condition of hypoxia with cerebral homogenate extract taken from animals exposed to hypoxia for different runs, the LDH leakage rate was found to be significantly decreased in groups H_2, H_3, and H_4 in particular in comparison with that in group H_0 (Fig. 5).

When cultured PC12 cells were cocultured under condition of anoxia with homogenate extract taken from the brains of hypoxic resistant mice for 48 h, the cells were alive and their methyl thiazolyl tetrazolium (MTT) activity and LDH leakage were 13% and 47%, respectively, whereas almost all those cocultured with brain extract taken from the brain of normal animals died (95%) and the cell's measurement of MTT and LDH was 0.02% and 96%, respectively.

These results indicate that a kind of adaptation to hypoxia was quickly developed by acute and repetitive exposures of mice to progressive autohypoxia and some adaptive elements, including water-soluble antihypoxic substances, occur in the brain of the hypoxia-preconditioned mice.

Down-Regulation of Sensitive/Destructive Elements in the Brain *(27–46)*

The level of lipid peroxide (LPO) was 150.5, 189.5 and 178.2 nmol/g respectively in groups H_0, H_1, and H_4. The level was first significantly increased in group H_1 and then significantly decreased and tended to return to control (H_0).

Compared with control groups, the positive cell percentage of reactive oxygen species

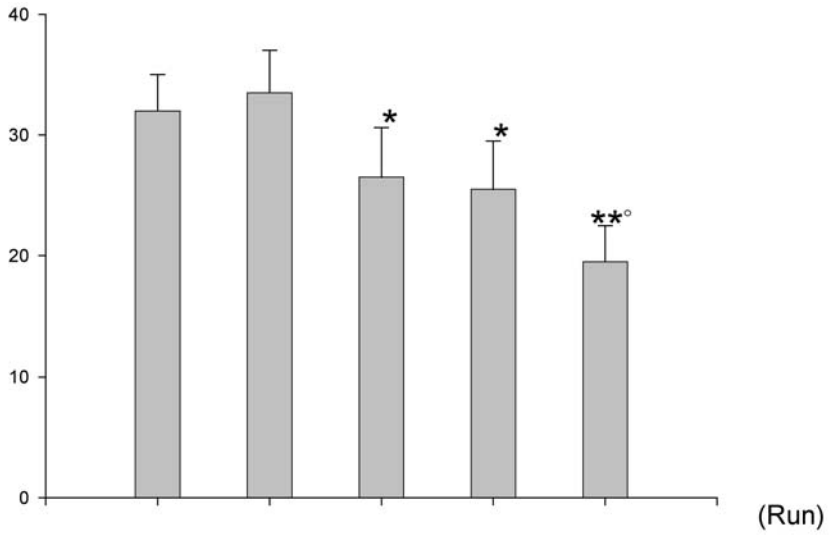

Fig. 5. Efflux rates of LDH in groups H_0, H_1, H_2, H_3, and H_4. *,**$p < 0.05$ and 0.01, respectively, compared with H_0; $\Delta p < 0.05$, compared with H_1.

(ROS) in the brain in group H_1 was found to be increased. However, the positive percentage of ROS decreased with the increase of exposure runs, and in H_4, it was significantly lower than that in H_1.

The increase in tolerance of animals to hypoxia was accompanied by a significant elevation of $[Ca^{2+}]_i$ and calcium ion content in the whole-brain homogenate of the animals exposed to hypoxia once and no apparent elevation was seen during the successive exposure to hypoxia.

Similar changes were also seen in the absorption values of phospholipids, contents of free fatty acids, concentrations of aspartate and glutamate in the whole brain and different brain regions such as the telencephalon, diencephalons, hippocampus, brainstem, and cerebellum (Fig. 6). Phospholipid A_2 (PLA_2) activity was 9.531, 13.420, and 10.528 mol/h g. respectively in groups H_0, H_1, and H_4. No significant difference was found in lactate content in the brain of groups H_4 and H_1 in comparison with group H_0.

L-Arginine concentration, NOS activity, and NO content in the the whole brain and the subregions such as telencephalon, diencephalons, and brainstem were significantly increased during the first exposure. Instead of continuing to increase, the concentration, activity, and content were significantly decreased in run 4 after the second and third exposures. The content of noradrenaline was 0.191, 0.189, and 0.101 nmol/g respectively in groups H_0, H_1, and H_4 neuropeptide (PY)-like immunoreactivity in the mice brain was 10.61, 15.44, and 9.94 pg/mg in groups H_0, H_1, and H_4, respectively. A similar tendency to change was also shown in the contents of calcitonian gene related peptide (CGRP) and angiotensin II (ANGII). The phosphorylation level of extracellular signal-related kinase $^1/_2$ (ERK1/2) was found to be progressively decreased as the exposure runs increased (Fig. 7).

α-Synuclein (α-Syn), a key molecule implicated in the pathogenesis of Parkinson's disease, was also regulated by hypoxic preconditioning. As for most of downregulated molecules shown earlier, α-Syn was increased after the first hypoxic exposure (H_0). However, after repeated successive hypoxic exposures, the levels of this protein in both the cortex and hippocampus gradually declined to control levels.

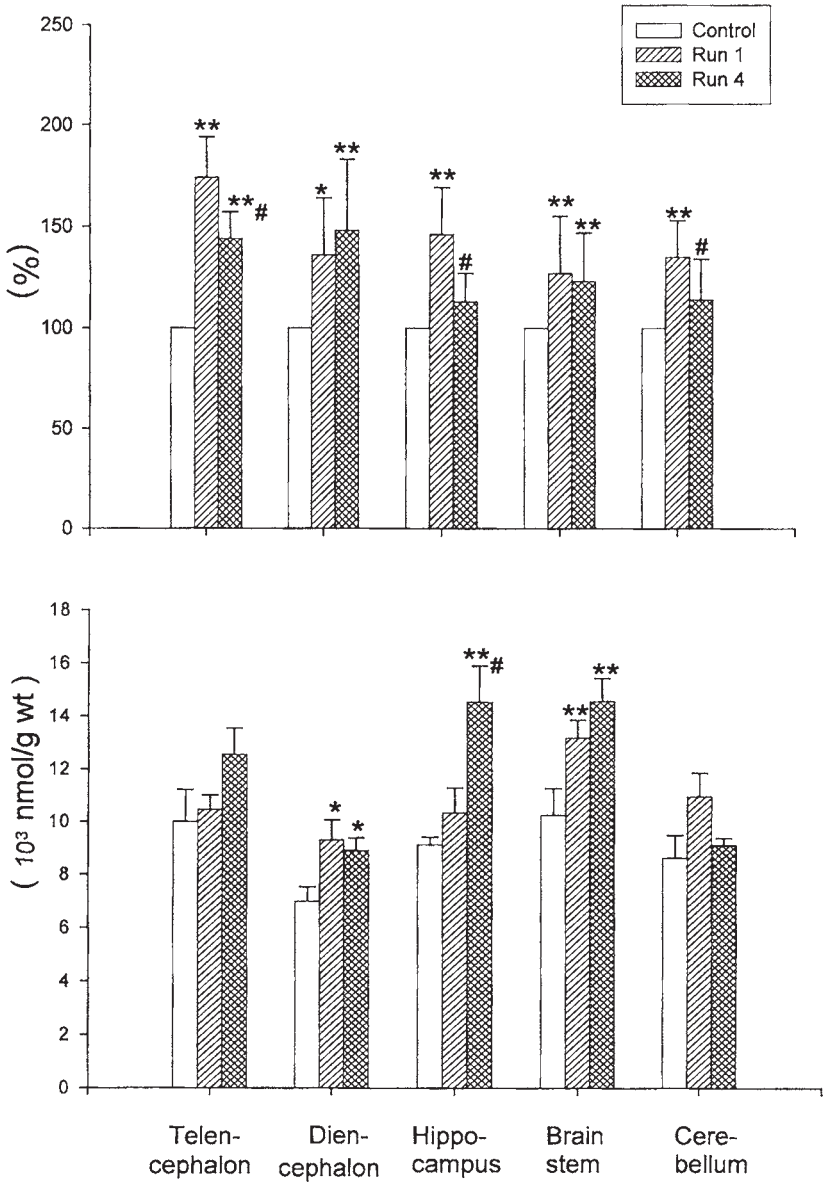

Fig. 6. Levels of glutamate **(upper)** and GABA **(lower)** in different subregions of the brain. *,**$p < 0.05$ and 0.01 compared with control; #$p < 0.05$ compared with run 1.

Up-regulation of Defensive/Protective Elements in the Brain *(47–63)*

The activity (μmol/L min protein) of glutathione peroxide (GSH-Px) in group H_1 (8.42 ± 0.93) was significantly lower than that in group H_0 (12.33 ± 1.54), and in group H_4, it was significantly higher (9.06 ± 1.67) than that in group H_1. Similar change was seen in activity of superoxide dismutase (SOD).

Adenosine content and adenosine A1 receptor affinity in the hippocampus in group H_4 were

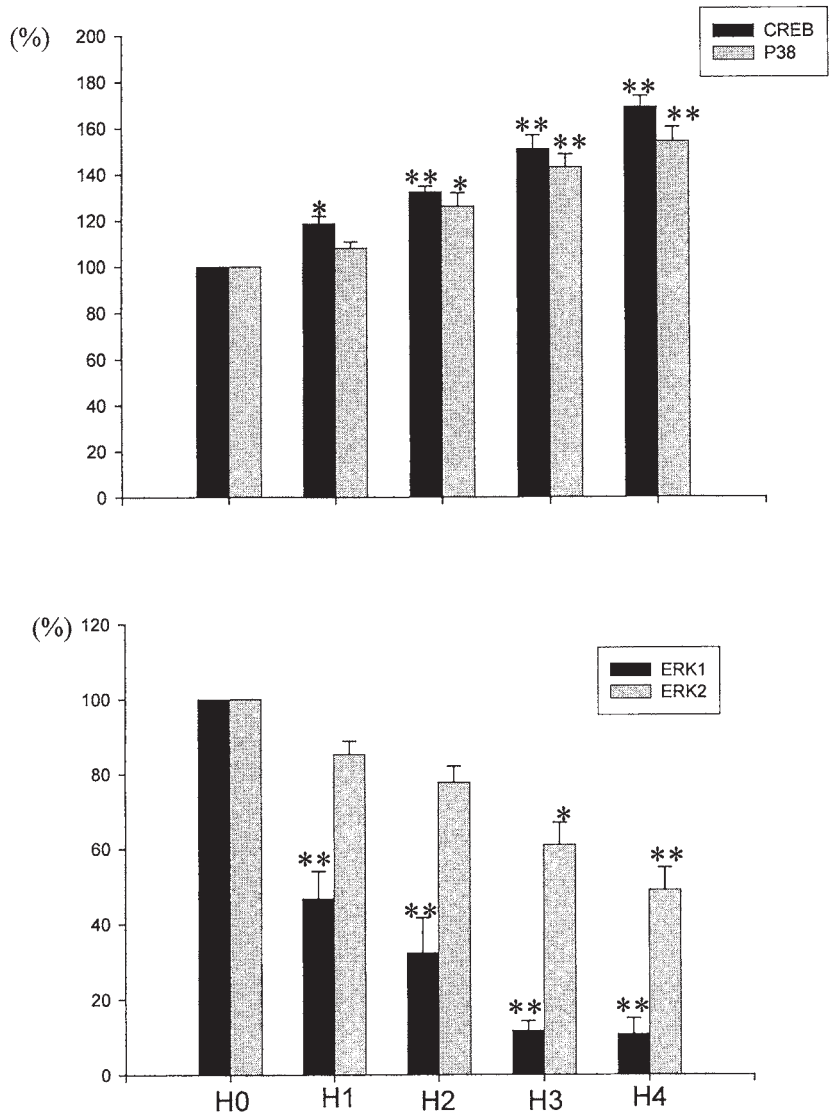

Fig. 7. Levels of ERK1/2 **(lower)** and CREB/p38 in the control, H_1, H_2, H_3, and H_4 groups **(upper).** *,**$p < 0.05$ and 0.01, respectively, compared with H_0.

markedly higher than that in both groups H_1 and H_0. The contents of glycine and GABA in the whole brain, diencephalon, hippocampus, and brainstem were significantly increased as the animals' tolerance to hypoxia increased (Fig. 6). The content of 5-hydroxy-tryptamine (5-HT)/dopamine was 0.2226/0.645, 0.198/0.712, and 0.244/0.865 nmol/g respectively in groups H_0, H_1, and H_4. Glycogen content of whole brain in group H_4 was markedly higher than that in the corresponding areas of both groups H_1 and H_0. cAMP response element binding protein (CREB) and p38 phosphorylation levels were found to be progressively increased as the exposure run increased (Fig. 7).

The level of hypoxic-inducible factor (HIF)-1α was found to be significantly increased in group H_1 (vs H_0) and decreased in group H_4 (vs

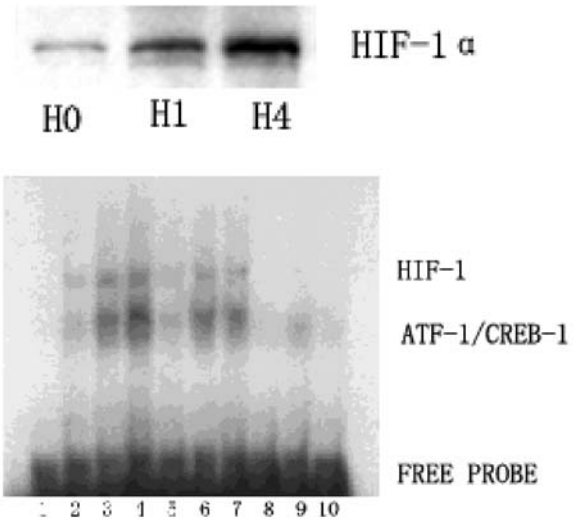

Fig. 8. Western blot analysis of HIF-1α in the hippocampus in groups H_0, H_1, and H_4 (n = 6) and DNA-binding activity on erythropoietin hypoxia response element in different groups (n = 3). Protein free (lane 1); electrophoretic mobility shift assays (EMSA) using nuclear extract was prepared from group H_0 (lanes 2, 5, and 8); group H_1 (lanes 3, 6, and 9), and group H_4 (lanes 4, 7, and 10). For supershift assays (lanes 5–7), binding reactions contained 2 μL antiserum raised against HIF-α. For competition assays (lanes 8–10), binding reactions included 100-fold excess of unlabled oligonucleotides (n = 3).

H_1), whereas the level of HIF-α protein was significantly increased in group H_1 (1.3–3.4 times H_0) and even markedly increased in group H_4 (3.7–13.1 times H_0). The HIF-1α DNA-binding activity was significantly increased in group H_1 (20 times vs H_0) and H_4 in particular (1.2 times vs H_1) (Fig. 8).

Outward potassium current (Ik) was significantly increased and inhibited (1298→2413→ 1713 pA) respectively when brain extract of preconditioned mice and glibenclamide were applied to the bath solution of acutely dissociated hippocampal neurons, suggesting that the ATP-dependent potassium channel (K_{ATP}) of hippocampal neurons is activated by the brain extract of preconditioned animals (Fig. 9).

Expression of neuroglobin (Ngb) RNA tended to be gradually increased by *in situ* hybridization of Ngb RNA in the cerebral cortex of hypoxic-preconditioned mice. When the newly discovered Ngb was cloned into pEGFP-N1 vector, pEGFP-N1-Ngb was transformed into Sy5y cells, and the cells' endurance to hypoxia was observed under the condition of lack of oxygen, the cells' ability to survive was found to be improved in the Ngb expressed SY5Y cells under the condition of hypoxia (unpublished data, not shown here).

Interestingly, an unknown mRNA band and component were specifically found in the brain of the hypoxia-preconditioned mice by differential display reverse transcription–polymeras chain reaction and high-performance liquid chromatography, respectively (unpublished data, not shown here). The specific band and component were thought to be specifically related to the tolerance increase and were named temporarily as hypoxia-related gene and hypoxia-related factor, respectively. Further work is in process in our lab to clarify their molecular structure and biological activity.

Theoretical Consideration *(1,7,9,10,64–95)*

Mammals are at the end of a gradual metabolic evolution. The increased metabolic rate and the resulting endogenous heat production are the preconditions for enhanced long-term performance and homothermy. The drop in metabolic rate during hypoxia is a well-known phenomenon and a frequent occurrence in both newborn and adult mammals.

The physiological features such as apnea, bradycardia, and peripheral vasoconstriction with hypometabolism of hypoperfused tissues are maintained mainly by stabilizing selection during evolution and is referred to as the diving response serving to conserve oxygen for the heart and brain in pinnipeds. Brain metabolic organization in humans is recognized as an example of a highly conserved system, and genes specifying structure and function of the central nervous system involved in regulated

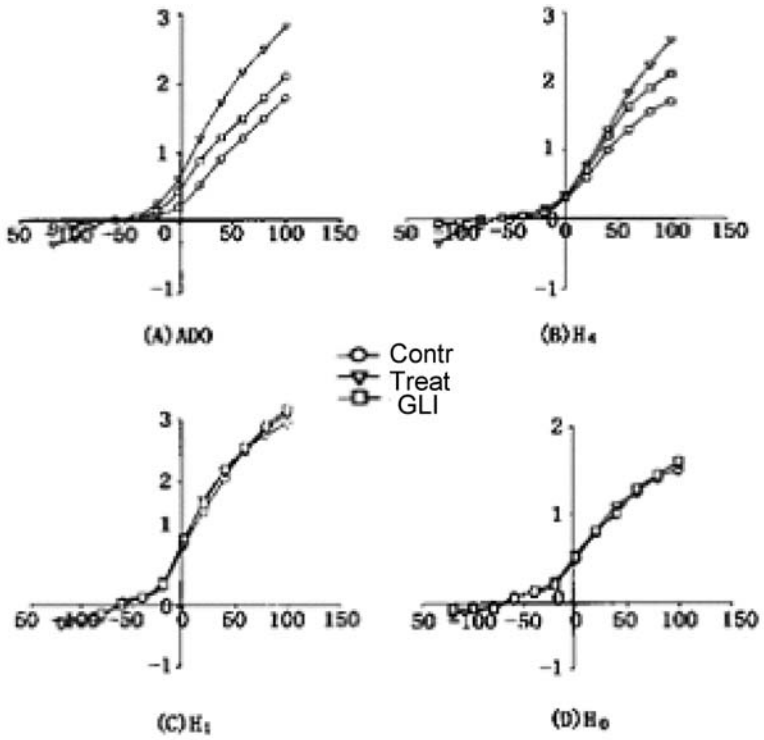

Fig. 9. Effects of cerebral homogenate extracts on *I–V* curve of Ik of rat hippocampal neurons. The brain extract of H_4 and adenosine (ADO, as reference) increases Ik, which is blocked by Gli **(A,B).** No effects are shown by extracts of H_1 and H_0 **(C,D).**

ATP demand and ATP supply pathways are also maintained by stabilizing selection through phylogeny.

Options available to living organisms against changes in environmental conditions range within two extremes: protect and maintain homeostasis against environmental challenges by active work of internal regulatory mechanisms at one end and, at the opposite end, internal state fluctuates to conform to external situation. The former option is the product of long-term evolution; it can be considered in practice but might cause energy exhaustion and organ-system damage. The later is conserved through long-term evolution for life surviving under condition of severe hypoxia or anoxia. The hypoxic survival strategy is all the way down to the cellular level in hypoxia-tolerant vertebrates.

The above-presented data suggest that hypoxic preconditioning seems to be in the range of the later option and a biological strategy to motivate/initiate potential energy largely conserved at the tissue-cell level through evolution. Its theoretical mechanisms might be framed in Fig. 10 based on the above-mentioned facts and related theory. The precondition is the repetitive exposure to hypoxia and the activation of carotid body, glomus cells, and other tissue-specific oxygen-sensing/signal transdution pathways, which, in turn, regulate the synthesis of HIF-1. HIF-1 targets several different genes in a tissue-specific manner, initiating cascades shown in Fig. 10. HIF-1 is the critical factor based on the recent finding that HIF-1 plays a central role in both hypoxic signal-sensing/transduction and modulation/control of its target genes.

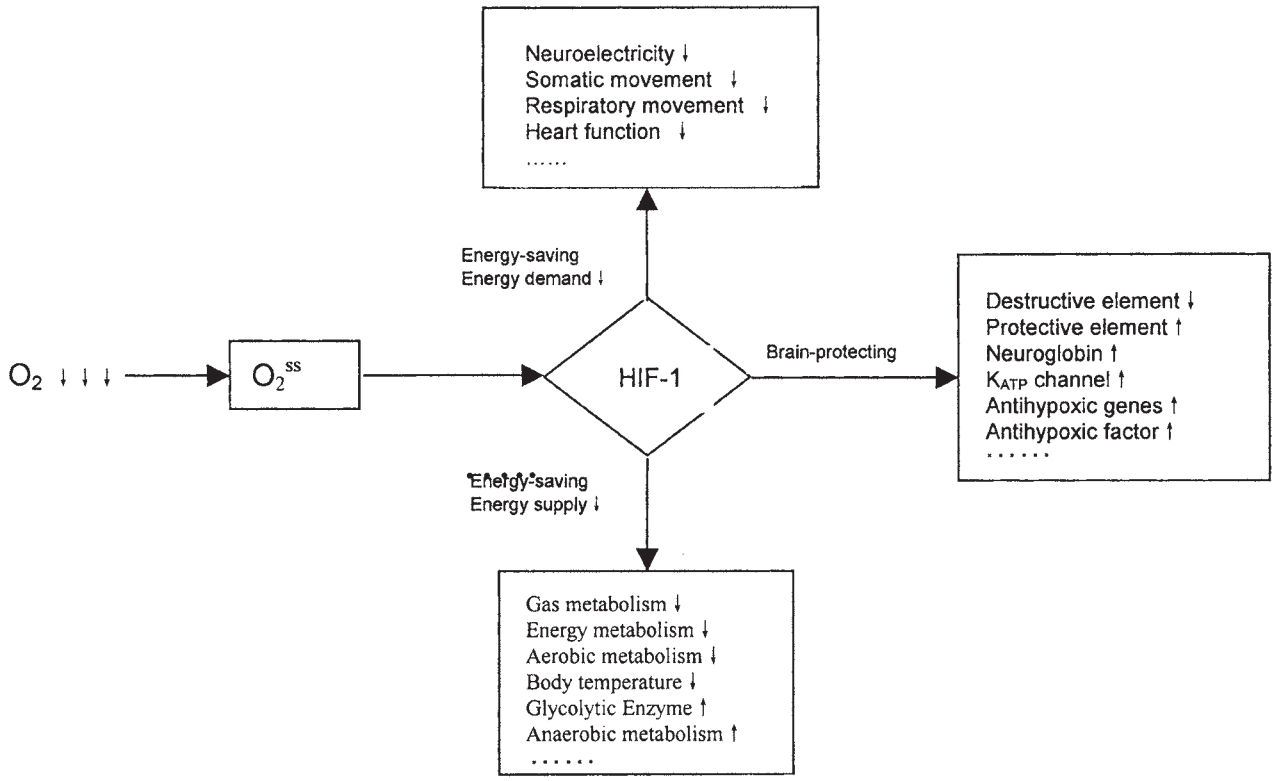

Fig. 10. A framework of mechanisms underlying hypoxic preconditioning. O_2 ↓↓↓, repetitive exposure to hypoxia; O_2^{ss}, oxygen-sensing/signal transduction pathways.

Practical Implications *(1,7,9,10,96–100)*

Oxygen therapy is almost the sole treatment for fighting against hypoxia-induced insults. It could provide 100% effective efficacy in the case of atmosphere and hypoventilation hypoxia and hypoxia induced by impaired alveolar diffusion. However, in hypoxia caused by anemia, abnormal transport of oxygen, and circulatory deficiency, it would be of much less value because normal oxygen is available already in the alveoli. The therapy might also be hardly any measurable benefit in different types of hypoxia caused by inadequate tissue use of oxygen, because neither abnormality of oxygen pickup by the lungs nor transport to the tissues appears and even tissue metabolic enzymes are incapable of using the delivered oxygen.

A strategy completely different from traditional oxygen therapy for the prevention/ treatment of hypoxic diseases and conditions might thus be determined based on the theoretical framework. Hypoxic/ischemic preconditioning seems to be a powerful endogenous protective strategy against hypoxic injury in many situations via various approaches.

Repetitive Hypoxic Exposure or Training

Procedures such as repetitive compression of carotid, coronary artery, and other arteries before surgery on the brain, heart, and other organs should be helpful in promoting patients' tolerance to hypoxia/ischemia. It has been successfully applied in neurosurgery and cardiosurgery. It would be beneficial to increase hypoxic tolerance of human populations living or working in high altitude, under deep water,

and in spacecraft by repetitive breath-holding exercise or training. The procedure might also be used as an approach for training sportsmen, flyers, and divers. A technique called interval hypoxic training was already developed in the former Soviet Union.

Pharmacologico-Chemical Approaches to Initiate Preconditioning and Affect Oxygen-Sensing/Signal Transduction Systems

Application of chemicals affecting mitogen-activated protein kinase (MAPK) systems, such as ERK1/2, JNK, p38, and MEK5/ERK5 pathways initiate the preconditioning exogenously. It was demonstrated preliminarily in our laboratory that tolerance of animals to hypoxia was significantly increased when phenylmethyl sulfoxide, an inhibitor of phopholipase C, was given to animals.

Reduction/Promotion of Brain Destructive/Protective Elements

Examples of these elements are administration of no inhibitor/adenosine, local hypothermia of the brain, and artificial hypothermia and should be effective in the clinic. Tolerance times under the hypoxic condition were significantly shortened and prolonged when preadministration of L-arginine and its analog was made respectively in our lab. Similar results were also shown when agonist/antagonist such as L-glutamate/ketamine and antagonist/agonist such as AMT/ adenosine were given before hypoxic exposure.

Cloning of Antihypoxic Genes or Synthesis of Antihypoxic Neuroactive Chemicals

This should be very helpful in the rescue and cure of patients suffering from severe hypoxia. To apply these products to the clinic in the future, it would depend on further clarification of cellular and molecular mechanisms underlying the preconditioning. Gene transfer and metabolic modulation to increase voltage-gated potassium channel function in the pulmonary artery, as a new therapy for pulmonary hypertension in rats and humans, has recently been reported to be feasible and beneficial.

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