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Mechanism of ischemic tolerance induced by hyperbaric oxygen preconditioning involves upregulation of hypoxia-inducible factor-1α and erythropoietin in rats

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Gu G-J, Li Y-P, Peng Z-Y, Xu J-J, Kang Z-M, Xu W-G, Tao H-Y, Ostrowski RP, Zhang JH, Sun X-J. Mechanism of ischemic tolerance induced by hyperbaric oxygen preconditioning involves upregulation of hypoxia-inducible factor-1α and erythropoietin in rats. *J Appl Physiol* 104: 1185–1191, 2008. First published January 3, 2008; doi:10.1152/japplphysiol.00323.2007.—We studied the effect of hyperbaric oxygen (HBO) preconditioning on the molecular mechanisms of neuroprotection in a rat focal cerebral ischemic model. Seventy-two male Sprague-Dawley rats were pretreated with HBO (100% O_2, 2 atmospheres absolute, 1 h once every other day for 5 sessions) or with room air. In *experiment 1*, HBO-preconditioned rats and matched room air controls were subjected to focal cerebral ischemia or sham surgery. Postinjury motor parameters and infarction volumes of HBO-preconditioned rats were compared with those of controls. In *experiment 2*, HBO-preconditioned rats and matched room air controls were killed at different time points. Brain levels of hypoxia-inducible factor-1α (HIF-1α) and its downstream target gene erythropoietin (EPO) analyzed by Western blotting and RT-PCR as well as HIF-1α DNA-binding and transcriptional activities were determined in the ipsilateral hemisphere. HBO induced a marked increase in the protein expressions of HIF-1α and EPO and the activity of HIF-1α, as well as the expression of EPO mRNA. HBO preconditioning dramatically improved the neurobehavioral outcome at all time points (3.0 ± 2.1 vs. 5.6 ± 1.5 at 4 h, 5.0 ± 1.8 vs. 8.8 ± 1.4 at 8 h, 6.4 ± 1.8 vs. 9.7 ± 1.3 at 24 h; $P < 0.01$, respectively) and reduced infarction volumes (20.7 ± 4.5 vs. 12.5 ± 3.6%, 2,3,5-Triphenyltetrazolium chloride staining) after cerebral ischemia. This observation indicates that the neuroprotection induced by HBO preconditioning may be mediated by an upregulation of HIF-1α and its target gene EPO.

ischemic preconditioning; transcriptional factors; cerebral ischemia; brain injury; DNA-binding activity

ALMOST ALL INJURIOUS STIMULI, when applied below the threshold of damage, activate endogenous protective mechanisms that significantly decrease the degree of injury after subsequent major injurious stimuli. For ischemic stimuli, this phenomenon has been termed ischemic preconditioning or ischemic tolerance (IT) (26). IT was first identified in the heart (28), and it was subsequently found to occur in the brain (24). It could be induced by various stimuli (i.e., ischemia, hypoxia, hyperbaric oxygen, chemical agents, cortical spreading depression, sleep deprivation, dietary restriction, and both hyperthermia and hypothermia, etc.) and in vital organs (i.e., brain, heart, etc.) (50). An analogous process is also believed to occur in human beings (42, 45), and induction of brain ischemic preconditioning has been suggested as a promising clinical strategy to prepare the brain to situations where ischemia could be anticipated (i.e., during surgery of the heart and brain and in patients with high risk for stroke) (10, 31, 35).

Hyperbaric oxygenation (HBO) has been widely used as a primary therapy in patients with carbon monoxide poisoning, decompression sickness, and arterial gas embolism, and it has been used as an adjunctive therapy for the treatment of various diseases accompanied by impaired oxygen delivery (40, 43). Interestingly, HBO has also been tested to produce IT in stroke models (31, 44, 47) and in organs such as spinal cord (4), heart (23), and liver (49), suggesting that HBO produces a wide-scale protective effect, and it may be a safer preconditioning stimulus compared with other stimuli such as hypoxia (16). Yet the mechanisms underlying its neuroprotective effects remained poorly defined.

The transcription factor hypoxia-inducible factor-1 (HIF-1) is a key regulator responsible for the induction of genes that facilitate adaptation and survival of cells and the whole organism under hypoxic conditions (36, 46). It has been found to be an important mediator of hypoxia-induced IT (2, 3, 21) and cross-tolerance (25, 39). In this regard, HIF-1 appears to be a universal molecular master switch, controlling cellular survival, glucose metabolism and transport, and metabolic adaptation. Recently, studies showed that HBO increased reactive oxygen species (ROS) generation (1, 5, 17) and that increased ROS levels upregulated HIF-1 expression (18, 22, 30). It is possible that HBO preconditioning induced neuroprotection may involve the upregulation of HIF-1 and its downstream genes.

To date, there are more than 100 HIF-1 downstream genes identified with varying functions (34). One of the downstream genes regulated by HIF-1 is erythropoietin (EPO), which is a hematopoietic growth factor that regulates red blood cell proliferation, differentiation, and maturation by binding to the surface of erythroid cells and preventing their apoptosis. Only recently it was found that EPO is also expressed in the central nervous system and that it exerts potent neuroprotective effects (12, 20, 27). EPO transcription as well as EPO translation have been found in preconditioned brains (3, 21, 31). In addition, there is solid evidence that exogenously applied EPO is neu-

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roprotective in vitro (27, 32) and in vivo (31, 33, 41). The neuroprotective effect of EPO, a HIF-1 target gene, taken together with the possibility that HBO may upregulate HIF-1 expression, led us to further investigate the involvement of this pathway in the HBO-induced IT. Thus the purpose of the current study was twofold: *1*) to establish whether HBO preconditioning is neuroprotective against subsequent ischemia-reperfusion injury and *2*) to examine the effect of HBO on the expression of HIF-1α and EPO.

MATERIALS AND METHODS

Animals and Groups

All surgical procedures were approved by the Ethics Committee for Animal Experimentation and were conducted according to the Guidelines for Animal Experimentation of our institutes.

A total of 72 male Sprague-Dawley rats weighing 280–320 g were used. They were allowed free access to food and water before and after treatment. The animals were divided into two groups randomly: control (Con group), treated with normobaric room air (NBA); and HBO preconditioned (HBO group), pretreated with HBO (100% O_2, 2 ATA) of 1-h duration once every other day for five sessions (24).

HBO Administration

For HBO treatment, pure oxygen was supplied continuously at a pressure of 2 ATA for 1 h once every other day for five sessions. Compression was performed at 1 $kg \cdot cm^{-2} \cdot min^{-1}$, and decompression was performed at 0.2 $kg \cdot cm^{-2} \cdot min^{-1}$. No seizures were observed in any animal during any procedure. The animals in the NBA group were placed in the chamber, which was not pressurized for sham treatment, with the same schedule as the HBO group. Chamber temperature was maintained between 22 and 25°C. Accumulation of carbon dioxide was prevented by using a small container with calcium carbonate crystals. To minimize the effects of diurnal variation, all exposures were started at 8:00 AM.

Brain Ischemia

In the present study, we used the endothelin-1 (ET-1)-induced focal cerebral ischemia model that was originally developed by Sharkey et al. in 1993 (38). Compared with other focal ischemia models, this model involves simpler surgical techniques and is not associated with postsurgical complications (e.g., feeding difficulties) (37) or with surge reperfusion and hyperemia (19). The resulting ischemia is highly representative of clinical vasospasm (14), in that blood flow is reduced for a prolonged period of time and that reperfusion of the compromised tissue is gradual (6). Rats were anesthetized with pentobarbital sodium (40 mg/kg ip) and placed in a stereotaxic apparatus. Rectal temperature was regulated at 37–38°C by means of a thermostatically controlled heating blanket. The femoral arteries and veins were cannulated for the monitoring of arterial blood pressure and arterial blood-gas status. Middle cerebral artery (MCA) occlusion was induced by microinjection of ET-1 (120 pmol in 3 μl saline) over 90 s via a 20-gauge needle adjacent to the MCA at stereotaxic coordinates +9 mm anterior, −5.2 mm lateral, and −8.7 mm ventral relative to bregma. The cannula was left in situ for 5 min after final drug injection, before being slowly withdrawn. Animals were then placed in an incubator to maintain normothermia until full recovery from anesthesia. At the outset of the study, the mean arterial blood pressure was 101 ± 7 mmHg, arterial pH was 7.44 ± 0.06, arterial P_{CO_2} was 38 ± 5 Torr, and arterial P_{O_2} was 247 ± 28 Torr; these parameters were maintained within these ranges for the duration of the experiment.

Neurobehavioral Function Scoring

To examine the effect of HBO preconditioning on functional recovery after cerebral ischemia, we used a neurobehavioral function scoring system proposed by Dean et al. (9) and Ohlsson and Johansson (29) and selected some appropriate and available tests of them in our laboratory. All testing was done in each rat at 4, 8, and 24 h after full recovery from anesthesia by a single observer without knowledge of the treatment group, using the following tests (15, maximum possible score, namely, healthy rat).

Symmetry in the movement of four limbs. The rat was held in the air by the tail to observe symmetry in the movement of the four limbs. Scores indicate the following: 3, all four limbs extended symmetrically; 2, limbs on left side extended less or more slowly than those on the right; 1, limbs on left side showed minimal movement; and 0, forelimb on left side did not move at all.

Forepaw outstretching. The rat was brought up to the edge of the table and made to walk on forelimbs while being held by the tail. Symmetry in the outstretching of both forelimbs was observed while the rat reached the table and the hindlimbs were kept in the air. Scores indicate the following: 3, both forelimbs were outstretched, and the rat walked symmetrically on forepaws; 2, left side outstretched less than the right, and forepaw walking was impaired; 1, left forelimb moved minimally; and 0, left forelimb did not move.

Beam-walking test. Coordination and integration of motor movement was tested with a beam-walking test and walking on a rotating pole. The beam was 1,750 mm long and 19 mm wide and was placed 700 mm above the floor. A wall was alternately placed 13 mm to the left or the right of the beam. (Rats are more willing to walk when a wall is placed next to the beam.) Scoring was as follows: 0, the rat falls down; 1, the rat is unable to traverse the beam but remains sitting across the beam; 2, the rat falls down while walking; 3, the rat can traverse the beam, but the affected hindlimb does not aid in forward locomotion; 4, the rat traverses the beam with more than 50% foot slips; 5, the rat crosses the beam with a few foot slips; and 6, the rat crosses the beam with no foot slips.

The limb-placement test. The limb-placement test was shortened and modified after De Ryck et al. (8). The forelimb and hindlimb placements were evaluated by an observer blinded to group designation. Each test was scored as follows: 0, no placing; 1, incomplete and/or delayed (>2 s) placing; and 2, immediate and correct placing.

2,3,5-Triphenyltetrazolium Chloride Staining

Animals were killed in deep anesthesia by perfusion through the left ventricle with 200 ml of ice-cold isotonic saline 24 h after cerebral ischemia. Brains were removed and cut into five 2-mm coronal slices starting 1 mm from the frontal pole. Histological staining was performed using 2% 2,3,5-triphenyltetrazolium chloride (TTC) in 0.2 mol/l PBS (pH 7.4). The stained sections were then fixed in 4% phosphate-buffered paraformaldehyde. After 24 h, the sections were photographed and infarction volumes were determined using ImageJ software (http://www.quickvol.com/) and expressed as a mean percent gray-scale value over the whole region of interest (striatum or surrounding cortex).

Western Blot

At 24 h after HBO preconditioning, nuclear extracts (for HIF-1α) or whole cell extracts (for EPO) were obtained using a nuclear extraction kit (Active Motif) and following the manufacturer's protocol. Equal amounts of the protein samples were loaded per lane. The primary antibodies were mouse polyclonal antibody against HIF-1α (1:1,000; Sigma), goat polyclonal antibody against EPO (1:200; Santa Cruz Biotechnology), mouse polyclonal antibody against β-actin (1:10,000; Sigma). Western blots were performed by means of horseradish peroxidase (HRP)-conjugated immunoglobulin G and the use of enhanced chemiluminescence detection reagents (Pierce). Bands were

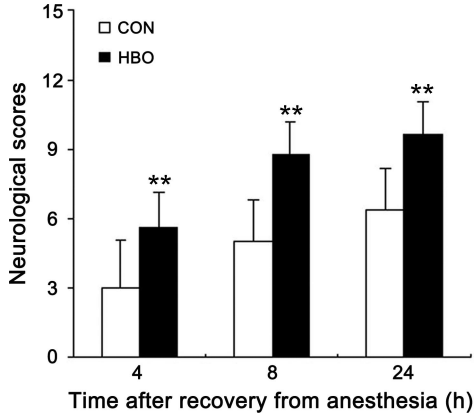

Fig. 1. Beneficial effect of hyperbaric oxygen (HBO) preconditioning on neurobehavioral recovery after focal cerebral ischemia. Neurological scores were significantly higher in HBO-preconditioned animals compared with control (Con) animals at 4, 8, and 24 h after cerebral ischemia. Values are ± means SD; $n = 10$ per group. $^{**}P < 0.01$.

scanned using a densitometer (model GS-700, Bio-Rad Laboratories), and quantification was performed using Quantity One 4.5.2 software (Bio-Rad).

HIF-1α DNA-Binding Assay

To measure HIF-1 DNA-binding activity, nuclear extracts were obtained 1 h after the last HBO pretreatment using a nuclear extraction kit (Active Motif) and following the manufacturer's protocol. HIF-1α DNA-binding activity of the nuclear fraction was determined by using an ELISA-based kit (Active Motif) according to the manufacturer's specifications. The assay uses a 96-well plate on which oligonucleotide containing the hypoxia response element has been immobilized. HIF dimers contained in nuclear extracts bind specifically to this oligonucleotide and are detected through the use of an antibody directed against HIF-1α. Addition of a secondary antibody conjugated to horseradish peroxidase (HRP) provides sensitive colorimetric readout that is easily quantified by spectrophotometry. The COS-7 ($CoCl_2$) nuclear extract is provided as a positive control for HIF-1 activation. The specificity of the assay was confirmed by adding wild-type competitor oligonucleotides (20 pmol) to positive-control nuclear extracts. The precision of the assay was determined by statistical analysis of the quantification results for wild-type competitor oligonucleotides that were measured three times in threefold, respectively.

Quantitative Real-Time PCR

For evaluation of EPO gene expression, real time TaqMan RT-PCR was performed. RNA was isolated 1 h after the last HBO pretreatment using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. First-strand cDNA was synthesized using Superscript II (Invitrogen). cDNA was quantified in duplicate on a Rotor-Gene RG3000 (Corbett Research, Sydney, Australia) using a SYBRgreen core reagent kit (Molecular Probes) according to the manufacturer's instructions. Expression of each sample was normalized on the basis of its β-actin mRNA content. PCR reactions were performed in 25-μl volumes with 2.5 μl of the appropriate RT reaction mixture. The following sequence-specific primers were used in RT-PCR. For β-actin, forward, 5′-CCTCTATGCCAACACAGTGC-3′; reverse, 5′-GTACTCCTGCTTGCTGATCC-3′. For EPO, forward, 5′-GCTCCAATCTTTGTGGCATCT-3′; reverse, 5′-TGGCTTCGTGACCCTCTGT-3′. Reactions were run in duplicate, and real-time data were analyzed with Rotor-Gene Real-Time Analysis Software 6.0.

Statistical Analysis

Results are expressed as means ± SD. Statistical significance was verified by analysis of variance performed in one-way ANOVA followed by the Tukey test for multiple comparisons. A value of $P < 0.05$ was considered to denote statistical significance.

RESULTS

HBO Preconditioning Improved Neurobehavioral Function

To examine whether HBO preconditioning exerts a beneficial effect on cerebral ischemia, neurobehavioral function was scored. Animals subjected to sham surgery ($n = 10$) showed no neurobehavioral functional deficit in both groups (data not shown). Neurological scores of stroked HBO-preconditioned rats ($n = 10$) were compared with those of stroked Con group ones ($n = 10$) (Fig. 1). After ischemia, HBO-preconditioned animals showed better neurological function recovery at all time points (3.0 ± 2.1 vs. 5.6 ± 1.5 at 4 h, 5.0 ± 1.8 vs. 8.8 ± 1.4 at 8 h, 6.4 ± 1.8 vs. 9.7 ± 1.3 at 24 h; $P < 0.01$ respectively).

HBO Preconditioning Reduced Infarction Volume

HBO preconditioning significantly reduced the size of brain tissue damage on TTC staining at 24 h after the MCA occlusion (20.7 ± 4.5 vs. 12.5 ± 3.6%) (Fig. 2).

HBO Preconditioning Increased HIF-1α and EPO Proteins Expression

To determine how HBO would affect the level of HIF-1α and EPO proteins, Western blot analysis was performed on the brains of the experimental groups 24 h after the last HBO pretreatment (Fig. 3). After normalization with β-actin, Western blot analysis showed a 1.52 ± 0.13-fold increase in HIF-1α level ($n = 5$ per group; $P < 0.05$; Fig. 3*A*) and a 1.43 ± 0.12 fold increase in EPO level ($n = 5$ per group; $P < 0.05$; Fig. 3*C*)

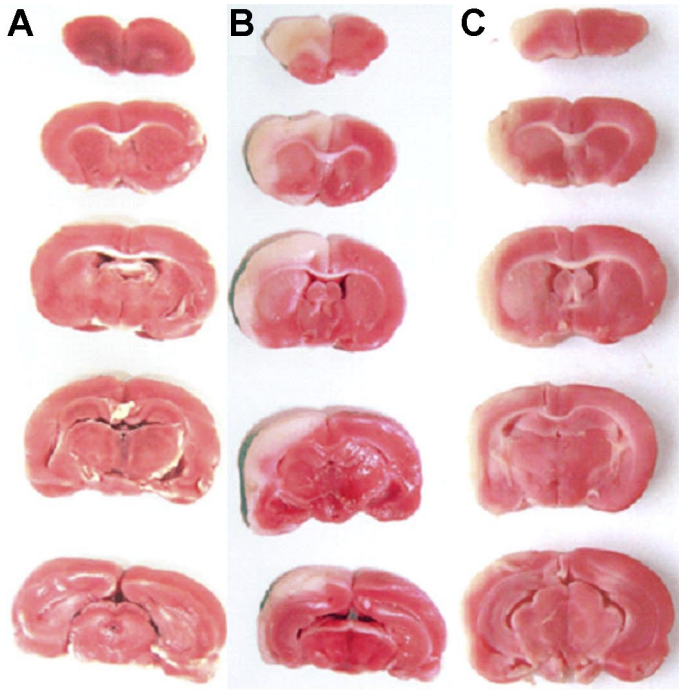

Fig. 2. Effect of HBO preconditioning on ischemic brain damage measured by 2,3,5-triphenyltetrazolium chloride staining at 24 h after middle cerebral artery occlusion. The damaged area in the HBO group (*B*) is significantly decreased compared with the Con group (*C*) ($P < 0.01$).

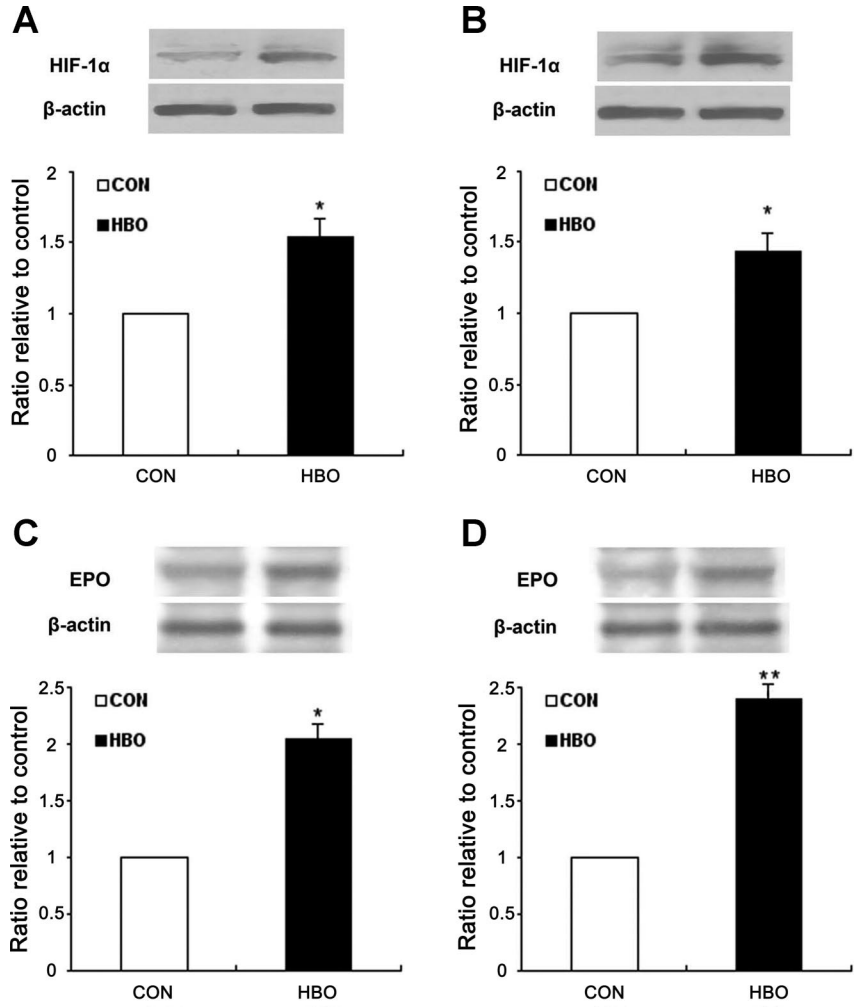

Fig. 3. Western blot analysis of hypoxia-inducible factor-1 (HIF-1α) and erythropoietin (EPO) protein expression. *Top* panels show HIF-1 (*A* and *B*) and EPO (*C* and *D*) protein bands and corresponding β-actin bands representative for each experimental group. *Bottom* panels include levels of HIF-1 and EPO protein expression measured by densitometry using samples obtained from the 2 experimental groups 24 h after preconditioning. HIF-1 expressions increased significantly in the cortex (*A*) and hippocampus (*B*) 24 h after HBO preconditioning compared with Con. EPO protein levels were also significantly increased in the cortex (*C*) and hippocampus (*D*) 24 h after HBO preconditioning. Values are means ± SD; $n = 5$ per group. *$P < 0.05$ vs. Con. **$P < 0.01$ vs. Con.

in the cortex compared with Con group rats. In addition, there were a 2.01 ± 0.13-fold increase in HIF-1α level ($P < 0.05$, Fig. 3*B*) and 2.42 ± 0.09-fold increase in EPO level ($P < 0.05$, Fig. 3*D*) in the hippocampus compared with Con group rats as well.

HBO Preconditioning Increased HIF-1α DNA-Binding Activity

To see whether HBO may activate HIF-1 in vivo, we examined HIF-1α DNA binding activity in nuclear protein extracts derived from cortical and hippocampus tissues. HBO preconditioning dramatically increased HIF-1α DNA-binding activity in nuclear extracts from both cortex (0.051 ± 0.020 vs. 0.165 ± 0.072; $n = 6$ per group; $P < 0.01$) and hippocampus (0.070 ± 0.013 vs. 0.188 ± 0.065; $n = 6$ per group; $P < 0.01$) (Fig. 4). Specificity of HIF-1 DNA binding was confirmed by competition experiments. Addition of 20 pmol wild-type competitor oligonucleotide to positive-control nuclear extracts decreased the signal up to 36%. The coefficient variations of repetition and stability for positive-control nuclear extracts were 2.76 and 3.87%, respectively.

HBO Preconditioning Increased EPO Gene Expression

To determine whether the increase in HIF-1α DNA-binding activity is associated with an increase in the expression of downstream target genes, we measured the mRNA levels of EPO in brain tissues ($n = 5$ per group) by RT-PCR. As shown in Fig. 5, HBO preconditioning resulted in an increase in the mRNA levels of EPO both in the cortex (2.20 ± 0.25-fold; $P < 0.01$) and hippocampus (3.13 ± 0.51-fold; $P < 0.01$) respectively.

DISCUSSION

In this study, we confirmed that HBO preconditioning improves functional recovery and reduces the brain infarction volumes after focal cerebral ischemia. We also showed that HBO preconditioning increased HIF-1α DNA-binding activity and the mRNA expression of EPO, a downstream gene of HIF-1, followed by the increased protein expressions of HIF-1α and EPO both in the cortex and hippocampus. The documented evidence on EPO as a neuroprotective agent and the causal evidence between better clinical recovery and the upregulation of these factors in HBO-preconditioned rats suggest their possible involvement in neuroprotection.

Here we used the modified model to induce ischemia injury in HBO and Con group rats and compare their postinjury functional recovery and infarction volumes. We established the neuroprotective effect of HBO preconditioning against ischemia in rats by showing that recovery of motor ability in HBO rats is improved compared with the recovery in CON rats at 4, 8, and 24 h after ischemia. In addition, we have also showed

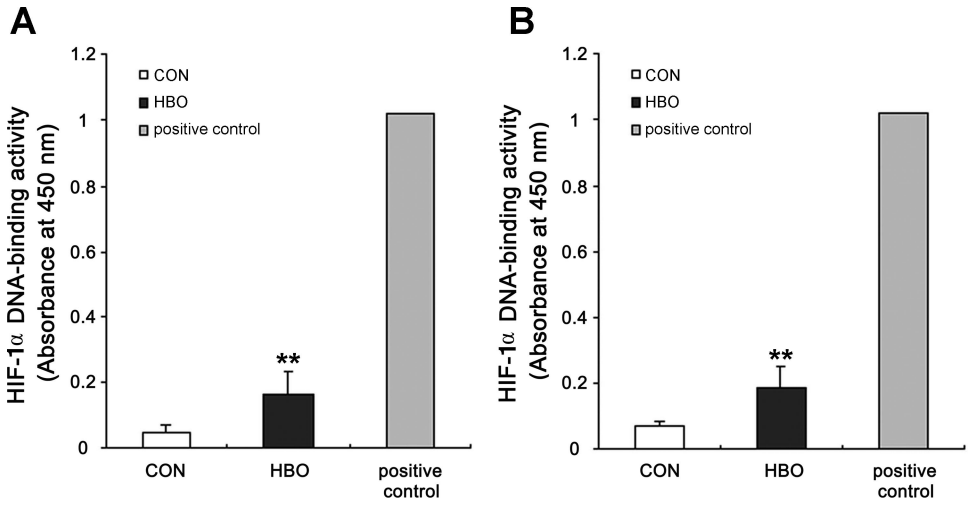

Fig. 4. Effect of HBO preconditioning on HIF-1 DNA-binding activity. Rats were pretreated with room air or HBO as described previously. Nuclear extracts were prepared 1 h after the last HBO pretreatment, HIF-1 DNA-binding activity was detected using an ELISA-based kit. Nuclear extract from $CoCl_2$-treated cells provided by the manufacturer were used as positive control. We found that HBO preconditioning increased HIF-1 DNA-binding activity both in cortex (*A*) and hippocampus (*B*). Values are means ± SD; $n = 6$ per group. $^{**}P < 0.01$ vs. Con.

that infarction volumes are significantly decreased in the preconditioned rats, as showed by the TTC staining. These findings are in agreement with results from previous experiments performed using other ischemic models in mice (31) and rabbits (15), which also showed a beneficial effect of HBO preconditioning on the outcome of ischemia-reperfusion injury.

It has been previously suggested that exposure to nondamaging stress can induce protection against subsequent more severe exposure to a second stressor of a different kind. This phenomenon is known as cross-tolerance and has been shown in a variety of experimental models for a variety of stressors and in various organs (50). However, significant changes in gene transcription/translation have been documented following focal stroke that consist of well-defined sequential expression of genes with diverse functions that may bear on tissue remodeling and resolution of the ischemic brain (26).

HIF-1 is a transcription factor that is found to be a crucial regulator in the hypoxia-induced IT model (3), as well as in heat acclimation (39). Besides hypoxia, other stimuli such as growth factors, cytokines, vascular hormones, and viral proteins can induce HIF-1 (11). Recently, Salhanick et al. (34) found that HIF-1 also could be induced after HBO exposure in the rat liver. In this study, we found that after HBO preconditioning, HIF-1α protein level in the brain was significantly increased both in the cortex and hippocampus, followed by the enhanced expression of EPO protein levels.

The neuroprotective effect of EPO has been previously mentioned, although the precise mechanism by which EPO fosters neuroprotection is not entirely clear.

EPO exerts its effects through the activation of the EPO receptor (EPOR), part of the cytokine-receptor type I superfamily. To confer protection, EPO must be given before the insult, which is consistent with the notion that it induces and expresses antideath proteins. The effect lasts for at least 3 days without the continued presence of EPO. Erythropoietin binding to EPOR causes homodimerization and autophosphorylation of Janus tyrosine kinase-2 (JAK-2) (48). JAK-2 phosphorylation and activation leads to phosphorylation of several downstream signaling pathways, such as the pathway involving phophatidylinositol 3-kinase (PI3K)-Akt/protein kinase B (PKB) and renin-angiotensin system RAS-mitogen-activated protein kinase (MAPK) and the transcription factor STAT 5. Recent studies suggest that erythropoietin activation of the EPOR-RAS-MAPK and EPOR-PI3K-Akt/PKB pathways have important roles in EPO-induced neuroprotection because specific inhibitors of the MAPK and PI3K pathways abolish EPO-induced protection (41). The action of EPO may extend beyond direct inhibition of cell death because it also seems to have anti-

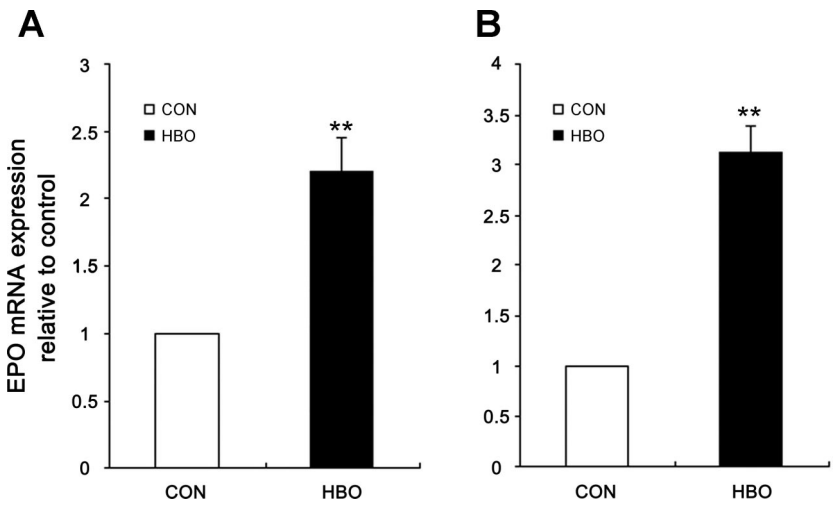

Fig. 5. Effect of HBO preconditioning on EPO mRNA expression. Total RNA was isolated 1 h after the last pretreatment with room air or hyperbaric oxygen (100% O_2, 2 ATA, 1 h once every other day for 5 sessions). Using a quantitative competitive RT-PCR approach and the housekeeping gene β-actin as an internal standard, we determined EPO mRNA expression. We found a 2.20 ± 0.25-fold increase of EPO mRNA expression in the cortex and a 3.13 ± 0.51-fold increase in the hippocampus. Data were obtained from 3 independent experiments. Values are means ± SD; $n = 5$ per group. $^{**}P < 0.01$ vs. Cont.

inflammatory effects; pretreatment with erythropoietin was shown to decrease the inflammation accompanying cortical trauma and ameliorate experimental autoimmune encephalitis (7). Now Digicaylioglu and Lipton (13) have described an alternative and/or additional mechanism by which EPO exerts its neuroprotective actions (13). EPO seems to trigger cross-talk between two, probably independent, signaling pathways of JAK-2 and nuclear factor-κB (NF-κB). EPOR-mediated activation of JAK-2 leads to the phosphorylation of the inhibitor of NF-κB (IκB). NF-κB is typically kept in an inactive state outside of the nucleus, being bound to its inhibitory protein IκB. Phosphorylation of IκB on two serine residues leads to its inactivation through subsequent ubiquitin-mediated degradation followed by the activation and nuclear translocation of NF-κB.

In conclusion, HBO preconditioning reduced brain injury after focal cerebral ischemia, probably by upregulation of HIF-1α and its target genes EPO, which prevents ischemic neuron change, and leads to the decrease of apoptosis. This mechanism may underlie HBO-induced neuroprotection, resulting in improved neurological function and reduced infarction volumes after focal cerebral ischemia.

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GRANTS

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