Dichotomous Effects of Chronic Intermittent Hypoxia on Focal Cerebral Ischemic Injury

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Background and Purpose—Obstructive sleep apnea, a condition associated with chronic intermittent hypoxia (CIH), carries an increased risk of stroke. However, CIH has been reported to either increase or decrease brain injury in models of focal cerebral ischemia. The factors determining the differential effects of CIH on ischemic injury and their mechanisms remain unclear. Here, we tested the hypothesis that the intensity of the hypoxic challenge determines the protective or destructive nature of CIH by modulating mitochondrial resistance to injury.

Methods—Male C57Bl/6J mice were exposed to CIH with 10% or 6% O2 for ≤35 days and subjected to transient middle cerebral artery occlusion. Motor deficits and infarct volume were assessed 3 days later. Intrainschemic cerebral blood flow was measured by laser-Doppler flowmetry and resting cerebral blood flow by arterial spin labeling MRI. Ca2+-induced mitochondrial depolarization and reactive oxygen species production were evaluated in isolated brain mitochondria.

Results—We found that 10% CIH is neuroprotective, whereas 6% CIH exacerbates tissue damage. No differences in resting or intrainschemic cerebral blood flow were observed between 6% and 10% CIH. However, 10% CIH reduced, whereas 6% CIH increased, mitochondrial reactive oxygen species production and susceptibility to Ca2+-induced depolarizations.

Conclusions—The influence of CIH on the ischemic brain is dichotomous and can be attributed, in part, to changes in the mitochondrial susceptibility to injury. The findings highlight a previously unappreciated complexity in the effect of CIH on the brain, which needs to be considered in evaluating the neurological effect of conditions associated with cyclic hypoxia. (Stroke. 2014;45:1460-1467.)

Key Words: free radicals ■ mitochondria ■ sleep apnea syndromes ■ stroke

Obstructive sleep apnea (OSA) is a common form of sleep disordered breathing, affecting ≈4% of men and 2% of women.1 It is characterized by cyclic periods of airflow cessation (apneas), increased intrathoracic pressure, intermittent hypoxia, hypercapnia, and sleep deprivation.2 OSA is well established as an independent risk factor for stroke, increasing stroke risk by 2- to 3-fold.3,4 an effect that seems to be related to the number of apnea-hypopnea episodes per hour of sleep.5 Yet, the mechanisms by which OSA increases susceptibility to cerebral ischemia remain to be defined.

Chronic intermittent hypoxia (CIH) reproduces selected features of OSA and is commonly used as a model of the human disease.6 In CIH, as in OSA, there is an impairment in cerebrovascular regulation, consisting of attenuation of endothelial-dependent responses and of the increases in cerebral blood flow (CBF) induced by neural activity.7-10 Although these deleterious cerebrovascular effects may reduce cerebrovascular reserves and contribute to the increase in the risk of stroke in patients with OSA,4,5 there is experimental evidence that CIH can also reduce cerebral ischemic brain injury.11 Furthermore, mortality is reduced in older patients with OSA,12 possibly because of a preconditioning effect of CIH.13 This raises the intriguing possibility that the influence of CIH on the ischemic brain may, in fact, be dichotomous and that the fate of the ischemic tissue may depend on factors other than cerebrovascular reserves.

The intensity of the hypoxic episodes and the period of CIH exposure are likely to influence reaction of the brain tissue to ischemic injury. Indeed, studies in the heart have indicated that the duration of CIH exposure can determine the outcome of myocardial ischemia,14-16 an effect in part related to changes in the susceptibility of mitochondria to damage.17,18 However, it is unclear how the duration of CIH and the intensity of the episodic hypoxia influence the outcome of cerebral ischemia and whether mitochondria may play a role in determining the fate of the ischemic tissue.

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The key aim of this study was to test the hypothesis that intensity and duration of CIH are critical factors determining the effect of CIH on the postischemic brain. We found that CIH with cyclic hypoxia induced by 10% O₂ for 35 days is neuroprotective in a mouse model of middle cerebral artery occlusion (MCAO), whereas CIH with 6% O₂ exacerbates the injury. These effects could not be attributed to differences in cerebrovascular reserves, but to changes in mitochondrial function modulating mitochondrial depolarization and production of reactive oxygen species (ROS). Our findings support the hypothesis that the effects of CIH on the ischemic brain can be either protective or destructive, and that CIH-induced changes in mitochondrial resistance to injury have a key role in determining the fate of the ischemic tissue.

Methods

Mice

All procedures were approved by the institutional animal care and use committee of Weill Cornell Medical College. Experiments were performed in male C57Bl/6 mice (7–13 weeks; Jackson Laboratory).

Chronic Intermittent Hypoxia

CIH was used to reproduce the cyclic hypoxia experienced by patients with OSA. Although all OSA models have limitations, CIH is frequently used to investigate the cerebral and extracerebral effects of cyclic hypoxia. CIH was induced using a custom system designed by Dr Delagrammatikas and described previously. Briefly, mice were randomly assigned to CIH (10% or 6% O₂) or sham (room air) groups. In CIH groups, oxygen levels within the animals’ cage were changed from normal (21±1%) to low (10±1% or 6±1%) O₂ for 90 s at 90-s intervals, resulting in 20 hypoxic episodes per hour. The cages of sham-treated mice were infused at the same rate with room air. Hypoxia/sham cycling was induced throughout the light (sleep) phase (8:00 AM to 4:00 PM; 8 hours). During the remaining 16 hours of the day (4:00 PM to 8:00 AM), both CIH and sham cages were infused with room air. CIH and sham protocols were repeated for 14, 21, or 35 days, during which time mice had free access to food and water. The length of the CIH period was determined based on previous studies, demonstrating that CIH for 35 days was sufficient to induce alterations in N-methyl-D-aspartic acid receptor trafficking and function or cerebrovascular regulation. Systolic blood pressure was measured using a noninvasive tail cuff system (Model MC4000; Hatteras Instruments), as previously described.

Transient Focal Cerebral Ischemia

Transient MCAO was induced using an intraluminal filament, as described. Briefly, mice were anesthetized with isoflurane (1.5%–2%), and rectal temperature was maintained at 37°C. A heat-blunted suture was inserted into the right external carotid artery and advanced along the internal carotid artery until it obstructed the MCA. The common carotid artery was simultaneously ligated for the duration of the ischemic period (30–35 minutes). In all studies, CBF was monitored using laser-Doppler flowmetry (Periflux System 5010; Perimed) in the ischemic territory (2 mm posterior and 5 mm lateral to bregma), as determined in preliminary studies. Three days after MCAO functional impairment was assessed using the hanging-wire test, and infarct volume was quantified in cresyl violet–stained sections and corrected for swelling, as previously described. Blood glucose and hematocrit were determined at the time of euthanasia.

Real-Time Polymerase Chain Reaction

Total RNA was isolated from a 1-mm-thick coronal slice collected at the center of the MCA territory in nonischemic mice. Quantitative determination of gene expression was performed on a Chromo 4 detector (Peltier Thermal Cycler; MJ Research) using a 2-step cycling protocol, as described. Primers have been previously described. Relative expression levels were calculated according to Livak and Schmittgen. Quantities of all targets in test samples were normalized to the housekeeping gene, and values were correlated to sham-treated samples.

Mitochondria Isolation and Measurements of Ca²⁺-Induced Mitochondrial Depolarization

Brain mitochondria were isolated and purified from the forebrain of CIH and sham naïve mice that were not subjected to MCAO, as described previously. Ca²⁺-induced mitochondrial depolarization was assessed using the fluororescent mitochondrial membrane potential indicator Safranin O (Molecular Probes). Briefly, 100 μg of purified mitochondria were suspended in KCl buffer containing glutamate (5 mmol/L), malate (5 mmol/L), and ADP (0.2 mmol/L). Safranin O (5 μm) was added, and fluorescence intensity was measured spectrophotometrically. Boluses of 50 μm CaCl₂ were added sequentially until mitochondrial Ca²⁺ uptake declined, as indicated by the mitochondrial depolarization resulting from the mitochondrial permeability transition induced by Ca²⁺ overload. The amount of Ca²⁺ needed to cause mitochondrial depolarization was estimated from standard curves.

Measurement of Mitochondrial ROS

In isolated and purified brain mitochondria from CIH and sham mice (see above), ROS emission was measured by Amplex Red (Invitrogen) fluorescence, as described. Briefly, 100-μg mitochondrial suspensions were added to 1-mL incubation buffer. Standard curves were used to calculate H₂O₂ emission rates after sequential addition of substrate (5 mmol/L glutamate and 2 mmol/L malate), the complex I inhibitor rotenone (1 μmol/L), and the complex III inhibitor antimycin A (1.8 μmol/L).

Statistical Analysis

Mice were randomly assigned to the experimental groups, and analyses were performed by an investigator blinded to the treatment protocol. Data are expressed as mean±SE. Intergroup differences were analyzed using a Student unpaired t test or 1-way ANOVA with Bonferroni post hoc. Differences were considered statistically significant for P<0.05. Group sizes were calculated based on power analysis. We used a power of 0.8 and significance levels of 0.05.

Results

Influence of CIH on Physiological Parameters and Hypoxia-Inducible Factor-1α Target Genes

Exposure to CIH with 10% O₂ (10% CIH) for 14 to 35 days had no effect on body weight, plasma glucose, or hematocrit (Table in the online-only Data Supplement). Exposure to 6% CIH transiently increased hematocrit and reduced plasma glucose at 21 days and decreased body weight both at 21 and 35 days (Table in the online-only Data Supplement). Systolic blood pressure tended to increase, but the effect did not reach statistical significance (Table in the online-only Data Supplement). Next, we assessed mRNA expression of the hypoxia-inducible factor-1α target genes erythropoietin, vascular endothelial growth factor-A, and glucose transporter 1, which are induced by CIH. Both 10% and 6% CIH increased forebrain erythropoietin mRNA expression at 35 days (Figure I in the online-only Data Supplement). The induction was more pronounced with 6% than with 10% CIH, in accordance with the intensity of the hypoxic challenge. Increases (<2-fold) in vascular endothelial growth factor-A at 10% CIH and glucose transporter 1 at 6% CIH were also observed (Figure I in the online-only Data Supplement).
Opposing Effects of CIH on Ischemic Brain Injury

Exposure to 6% or 10% CIH for 14 days had no effect on the size of the infarct (Figure 1A and 1B). Exposure to 6% CIH for 21 days exacerbated ischemic brain injury, increasing infarct size by ≈50%, whereas 10% CIH was without effect (Figure 2A and 2B). Surprisingly, exposure to 10% CIH for 35 days reduced infarct volume by ≈50%, whereas 6% CIH increased infarct volume and functional impairment at the hanging-wire test (Figure 3A and 3B). The reduction in infarct volume with 10% CIH was not associated with motor improvement at the hanging-wire test, perhaps reflecting the ability of this test to detect more readily worsening than improvement in this model.

CIH Does Not Affect Resting or Intraischemic CBF

Because CIH alters cerebrovascular reactivity and may reduce cerebrovascular reserves,7,10 we examined whether the differences in tissue outcome between 6% and 10% CIH could be related to changes in intraischemic CBF. However, no differences in the reduction in CBF produced by MCAO were observed among the groups (Figure IIA in the online-only Data Supplement; \( P > 0.05 \)). Because laser-Doppler flowmetry cannot measure resting CBF quantitatively, we used arterial spin labeling-MRI to examine whether the worsening of injury with 6% CIH could be related to reductions in resting CBF, which could result in more severe ischemia after MCAO. However, resting CBF did not differ between mice exposed to sham treatment or CIH (CIH 10%: sham 146±2 and CIH 144±10; CIH 6%: sham 170±6 and CIH 181±5 mL/100 g per minute) at 35 days (Figure IIB in the online-only Data Supplement; \( P > 0.05 \)).

Effects of CIH on Proinflammatory Gene Expression in Brain

Next, we examined whether CIH could influence the outcome of cerebral ischemia by modulating inflammatory gene expression. Exposure to 6% CIH for 35 days increased expression of regulated and normal T-cell expressed and secreted/CCL5, monocyte chemotactic protein-1, inducible nitric oxide synthase, intercellular adhesion molecule-1, vascular cell adhesion molecule-1 (Figure 4). There was a trend for proinflammatory gene expression to be downregulated in mice exposed to 10% CIH, particularly inducible nitric oxide synthase, which was significantly reduced (Figure 4). These changes in inflammatory gene expression were not associated with the evidence of tissue injury both with 6% or 10% CIH, as assessed by fluoro jade-B (Figure IIIA and IIIB in the online-only Data Supplement). As a positive control, fluoro jade-B was able to detect brain damage after MCAO (Figure IIIC in the online-only Data Supplement).

Opposing Effects of CIH on Mitochondrial Susceptibility to Ca²⁺-Induced Depolarization

Mitochondria are critical modulators of ischemic brain injury and are influenced by intermittent hypoxia.17,18,34,35 Therefore, we tested the hypothesis that CIH-induced changes in mitochondrial resistance to injury play a role in the opposing effects on postischemic brain damage. Mitochondria were isolated from nonischemic mice subjected to sham treatment, 10% or 6% CIH for 35 days and their susceptibility to Ca²⁺-induced loss of membrane potential (\( \Delta \Psi \)) tested. Sham-treated mice maintained \( \Delta \Psi \) in response to \( \leq 200 \) nmol CaCl₂. Beyond this point, administration of an additional aliquot of CaCl₂ (40 nmol) resulted in a complete loss of \( \Delta \Psi \), consistent with mitochondrial permeability transition pore (mPTP) opening (Figure 5A and 5B). In contrast, mitochondria isolated from mice exposed to 10% O₂ CIH had an increased Ca²⁺ capacity, maintaining \( \Delta \Psi \) in response to \( \leq 240 \) nmol CaCl₂ (Figure 5A), whereas mitochondrial Ca²⁺ capacity of mice exposed to 6% O₂ CIH was reduced, with loss of \( \Delta \Psi \) occurring in response to 120 nmol CaCl₂ (Figure 5B).
Effect of CIH on Mitochondrial ROS Production

ROS promote mPTP opening and subsequent loss of membrane potential. To gain an insight into the mechanism by which CIH alters susceptibility of mitochondria to Ca^2+ -induced depolarization, we evaluated mitochondrial ROS production with substrate (malate and glutamate), rotenone (complex I inhibitor), and antimycin A (complex III inhibitor) in nonischemic mice exposed to 10% and 6% CIH for 35 days. Consistent with a reduced susceptibility to Ca^2+ -induced membrane depolarization and mPTP transition, exposure to 10% CIH attenuated mitochondrial ROS production when compared with sham-treated mice (Figure 6A). In contrast, ROS production was similar in mice exposed to either sham treatment or 6% CIH (Figure 6B). Although there was a tendency for ROS production in response to substrate to increase in 6% CIH mice relative to control, this effect did not reach statistical significance ($P>0.05$).

Discussion

We have demonstrated that CIH can either ameliorate or worsen ischemic brain injury depending on the intensity of the hypoxic challenge. Thus, 10% CIH induces neuroprotection, whereas 6% CIH exacerbates ischemic brain injury. Differences in the effects of 10% and 6% CIH on outcome were independent of alterations in CBF, because both basal cerebral perfusion, measured using arterial spin labeling-MRI, and the degree of blood flow reduction in response to MCAO were similar in 10% and 6% CIH mice. However, the expression of key pro-inflammatory mediators was altered by CIH, increasing with 6% CIH and showing a tendency to decrease with 10% CIH,

Figure 2. Influence of 21-day chronic intermittent hypoxia (CIH) on focal cerebral ischemic injury. A, Exposure to 10% CIH for 21 days before induction of ischemia had no effect on infarct volume or functional impairment (hanging-wire test) evaluated at 72 hours (n=8–10 per group). B, Exposure to 6% CIH significantly increased infarct volume when compared with sham-treated mice, whereas functional impairment was not affected (n=7–9 per group; *$P<0.05$, t test).

Figure 3. Influence of 35-day chronic intermittent hypoxia (CIH) on focal cerebral ischemic injury. A, Exposure to 10% CIH for 35 days before induction of ischemia resulted in a significant reduction in infarct volume (n=8–10 per group; *$P<0.05$, t test). Functional impairment (hanging-wire test) was not affected (n=8–10 per group). B, Exposure to 6% CIH increased both infarct volume and functional impairment 72 hours after induction of cerebral ischemia (n=8–10 per group; *$P<0.05$, t test).
CIH Exposure Exerts Opposing Effects on the Ischemic Brain

Decreasing the intensity from 10% to 6% CIH resulted in a switch from beneficial to detrimental effects of CIH on the ischemic brain. These opposing effects of 10% and 6% CIH were first observed after 21 days exposure, when 6% CIH increased susceptibility to ischemic brain injury. By 35 days, however, the dichotomy was fully developed, with 10% CIH decreasing and 6% CIH exacerbating the injury. These effects could not be attributed to changes in systolic blood pressure, blood glucose, hematocrit, or CBF, because changes in these parameters did not correlate with the postischemic outcome. Interestingly, while the pathogenic effects of CIH developed more rapidly, neuroprotective effects of CIH on the ischemic brain took >21 days to develop. This observation is consistent with the idea that exposure to CIH induces delayed cerebral ischemic tolerance.33 Indeed, others have shown that hypoxic episodes can induce short-term36–39 or long-lasting11 preconditioning in the brain. However, our results indicate that the effects of CIH are not uniformly beneficial, and that their protective or destructive character depends on the intensity and the duration of the recurring hypoxic challenge. Clinical data suggest that older patients with moderate OSA may have reduced mortality when compared with the general population,12 attesting to a potential preconditioning effect of mild CIH.33 Therefore, it would be important to test whether the dichotomous effect of CIH also occurs in older animals.

CIH-Induced Vascular Dysfunction Does Not Influence the Outcome of Cerebral Ischemia

Postischemic cerebral perfusion has profound effects on the outcome of MCAO.40 Thus, alterations in CBF regulation reduce intraischemic CBF and exacerbate focal ischemic brain injury,41–43 whereas counteracting postischemic cerebrovascular dysregulation ameliorates the damage.26 Because 10% CIH alters critical vasomotor responses of the cerebral circulation,7 we anticipated that intraischemic CBF would be reduced because of the impaired vasodilatory capacity of cerebral blood vessels and failure of collateral flow. Contrary to this prediction, however, we found that 10% CIH does not exacerbate the CBF reduction induced by MCAO and results in less damage because of its beneficial parenchymal effects. Collectively, these observations indicate that the preconditioning effect of CIH may protect the postischemic brain from the deleterious effects of cerebrovascular dysfunction.

CIH Influences Inflammation and the Tolerance of Mitochondria to Ca2+-Induced Membrane Depolarization

Proinflammatory markers were slightly upregulated in the brain of mice exposed to 6% CIH in the absence of changes in tissue injury and tended to be reduced in mice exposed to 10% CIH. This supports the idea that inflammation contributes to the CIH-induced changes in susceptibility ischemic brain injury and is in agreement with previous studies reporting alterations in mediators and markers of inflammation in the brain after CIH11,44 and in the blood of patients with OSA.45,46 However, the significance of these relatively minor changes in
inflammatory gene expression remains to be established and a more detailed analysis of the cellular and molecular features of postischemic inflammation would be needed to assess the effect of CIH-induced immunomodulation on the outcome of cerebral ischemia.

A more striking and novel finding of the present study is that CIH exerts a profound influence on cerebral mitochondria. Mitochondria play a central role in cell death and survival and are critical for buffering excessive postischemic intracellular Ca²⁺ levels. Here, we found that cerebral mitochondria isolated from mice exposed to 10% CIH had a significant reduction in susceptibility to Ca²⁺-induced membrane depolarization, consistent with a protection from injury, whereas 6% CIH increased susceptibility to Ca²⁺-induced membrane depolarization. Although changes in mitochondrial function have been reported after CIH, we found, for the first time, that the effects of CIH on mitochondrial Ca²⁺ buffering capacity can be either protective or harmful depending on the intensity of the cyclic hypoxia.

Induction of mPTP and loss of mitochondrial membrane potential occurs because of excessive accumulation of Ca²⁺ and results in impaired ATP production and cell death. This loss of mitochondrial membrane potential can be promoted by oxidative stress. Indeed, we observed a reduction in mitochondrial ROS production in mice exposed to 10% CIH, in which susceptibility to Ca²⁺-induced membrane depolarization is attenuated. This is consistent with changes in mitochondrial ROS production in response to CIH, altering the stability of the mPTP and the susceptibility of the brain to ischemic injury. In agreement with this hypothesis, 6% CIH did not increase mitochondrial ROS production relative to control, whereas mitochondrial ROS levels were higher in 6% than in 10% CIH. This supports the idea that mitochondrial ROS play an important role in the effects of CIH on cerebral mitochondria and subsequent injury development. Interestingly, we previously reported that cerebrovascular ROS production is increased by 10% CIH, an effect mediated

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**Figure 5.** Effect of chronic intermittent hypoxia (CIH) on mitochondrial Ca²⁺-induced membrane depolarization in nonischemic mice. A, Representative trace and group data illustrating exposure to 10% CIH decreased susceptibility of mitochondria to Ca²⁺-induced loss of membrane potential (n=9 per group; *P<0.05, t test). B, In contrast, exposure to 6% CIH increased susceptibility to Ca²⁺-induced membrane depolarization (n=9 per group; *P<0.05, t test). Additions of Ca²⁺ are indicated by arrows.
by a NOX2-containing NADPH oxidase. In contrast, in the present study, we observed that mitochondrial ROS are reduced by the same CIH regimen. Although cell-type–specific effects cannot be ruled out, these observations raise the intriguing possibility that CIH has differential effects on ROS production, depending on the enzymatic system and the cellular compartment generating the radicals. Additional studies targeting specifically mitochondrial and extramitochondrial sources of ROS would be required to address this issue.

Conclusions

This study demonstrates that CIH can have both beneficial and deleterious effects on the ischemic brain. Neuroprotection was observed after exposure to 10% CIH, whereas injury exacerbation was seen with 6% CIH, suggesting that the intensity of the hypoxic insult is critical in determining the effect of CIH on tissue outcome. At the mechanistic level, this study provides novel evidence for a key role of mitochondria in the effects of CIH on the ischemic brain, with alterations in the susceptibility to Ca²⁺-induced membrane depolarization and mitochondrial ROS production observed. Although additional studies are required to define the mechanisms of these mitochondrial effects, the dichotomous nature of the effect of CIH on the postischemic brain unveils a novel and intriguing aspect of the pathobiology of CIH. This may be of great relevance to clinical conditions associated with cyclic changes in oxygenation levels.

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Disclosures

None.

References


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SUPPLEMENTAL MATERIAL

Dichotomous effects of chronic intermittent hypoxia on focal cerebral ischemic injury

Supplemental Methods

Resting CBF by ASL-MRI
CBF was assessed quantitatively using arterial spin labeling magnetic resonance imaging (ASL-MRI), performed on a 7.0 Tesla 70/30 Bruker Biospec small animal MRI system with 450 mT/m gradient amplitude and a 4500 T/m/s slew rate, as previously described1. Briefly, a volume coil was used for transmission and a surface coil for reception. Anatomical localizer images were acquired to find the transversal slice at the level of bregma. One axial slice was acquired with a field of view of 15 × 15 mm, spatial resolution of 0.117 × 0.117 × 1 mm, TE of 5.368 ms, effective TE of 48.32 ms, recovery time of 10 s, and a RARE factor of 72. 22 TIR values ranging from 30 to 2300 ms were used, and the inversion slab thickness was 4 mm. For computation of CBF, the Bruker ASL perfusion processing macro was used. The masked CBF images were exported and further processed using customized software. Data was spatially despiked and the average value over the slice is reported as CBF (ml/100g/min).

Fluoro-Jade B staining for degenerating neurons
Fluoro-Jade B (FJB) is a polyanionic fluorescein derivative that specifically binds degenerating neurons. FJB staining was performed in coronal sections (14 μm) isolated from the forebrains of paraformaldehyde (4%) perfused naïve mice. Briefly, sections were placed in 1% sodium hydroxide/80% ethanol solution, followed by 70% ethanol, 0.06% potassium permanganate solution and 0.0004% FJB staining solution. Fluorescence (excitation 495 nm; emission 519 nm) was visualised on a confocal laser microscope (Leica) equipped with an argon laser in the region of the cortex. For the positive control, the ipsilateral cortex of a mouse subjected to cerebral ischemia (MCAO) was stained with FJB and imaged.
Supplemental Tables

Table I: Physiological parameters

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* *p<0.05
Supplemental Figures & Figure Legends

Figure I

![Graphs showing mean fold change for EPO, VEGF-A, and GLUT-1 across different conditions.](image-url)
Figure II

A. Intra-ischemic perfusion

14 days

10% O₂

21 days

10% O₂

35 days

10% O₂

B. Resting perfusion

10% O₂

6% O₂
Figure III

A. 10% O₂

B. 6% O₂

C. Postive control
Figure I: CIH induced changes in HIF-1-dependent gene expression. mRNA expression of HIF-1 mediated genes EPO, VEGF-A and Glut-1 tended. Both 10% and 6% CIH increased EPO mRNA expression (35 days; n=5-15/group; *p<0.05 from sham, ANOVA). In addition, modest increases in VEGF-A at 10% CIH and Glut-1 at 6% CIH were observed.

Figure II: Influence of CIH on resting and intra-ischemic CBF. A. The degree of CBF reduction and reperfusion is similar in the ischemic territory in mice exposed to either sham, 10% or 6% CIH (14, 21 and 35 days; n=7-10/group). B. Resting CBF (ml/100g/min), measured using ASL-MRI, is similar in sham, 10% and 6% CIH exposed mice (35 days; n=5/group; p>0.05, t-test).

Figure III: A, B. Representative images of cerebral cortex stained with FJB illustrating no evidence of neuronal degeneration in mice exposed to either sham, 10% or 6% CIH. C. Cortex from mouse subjected to MCAO (positive control) confirms that FJB detects degenerating neurons. CIH, chronic intermittent hypoxia; FJB, Fluoro-Jade B; MCAO, middle cerebral artery occlusion.
Supplemental References