Effects of acute hypoxia and hyperthermia on the permeability of the blood-brain barrier in adult rats

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Natah SS, Srinivasan S, Pittman Q, Zhao Z, Dunn JF. Effects of acute hypoxia and hyperthermia on the permeability of the blood-brain barrier in adult rats. J Appl Physiol 107: 1348–1356, 2009. First published July 30, 2009; doi:10.1152/japplphysiol.91484.2008.—Acute mountain sickness (AMS) develops within a few hours after arrival at high altitude and includes headache, anorexia, nausea, vomiting, and malaise. This affliction 15–25% of the general tourist population at moderate altitudes. High-altitude cerebral edema (HACE) is considered to be the end stage of severe AMS and has been suggested to be a vasogenic edema, raising the possibility that acute hypoxia may increase blood-brain barrier (BBB) permeability. At present, there are no good small-animal models to study this syndrome. We hypothesize 1) that acute hypoxia can damage the BBB and 2) that rat can be used as a model to study hypoxia-induced changes in BBB permeability, especially if hypoxia-induced hypothermia could be minimized with high ambient temperature (HAT). Male Wistar rats were exposed to 1, 2, and 7 days of hypobaric hypoxia (equivalent to 0.5 atm), and changes in the temperature and BBB permeability were studied. The extravasation of endogenous immunoglobulin G, a large molecule, did not increase during room temperature hypoxia but did increase when hypoxia was combined with HAT. Hypoxia caused a significant increase in the leakage of sodium fluorescein (mol wt 376 Da). The expression of endothelial barrier antigen (EBA), a protein associated with the BBB, was reduced to 50% between 24 and 48 h after exposure to hypoxia, and the loss was exacerbated by HAT. The values almost returned to control levels by 7 days, showing adaptation to hypoxia. Hypoxic rats exhibited sodium fluorescein leakage mainly in focal areas in the brain parenchyma. In conclusion, it is possible to have transient BBB damage through exposure to acute hypoxia, and this damage is exacerbated by increasing body temperature to more of a normothermic value.

The unique characteristics of the blood-brain barrier (BBB) include tight intercellular junctions, a complex glycocalyx, a paucity of pinocytic vesicles, and an absence of fenestrae. These properties enable the endothelium to exclude proteins, ions, nonelectrolytes, and water and allow for the selective exchange of substances between the systemic circulation and the interstitial fluid of the brain (15). In addition, the endothelial cells possess transport systems for hexoses, amino acids, choline, purines, and amines, many of which are located exclusively within central nervous system (CNS) vessels (9). Acute mountain sickness (AMS) develops within a few hours after arrival at high altitude >3,000 m and includes headache, anorexia, nausea, vomiting, lack of energy, and malaise (3, 19). Honigman et al. (21) reported AMS in 15–25% of general tourist population at moderate altitudes of 2,000–3,000 m. Currently millions of people are being exposed to the risk of AMS due to rapid travel to high altitudes (13). Although AMS has been recognized over the past two centuries, little is known about the fundamental causes of these symptoms (33).

High-altitude cerebral edema (HACE) is characterized by disturbances of consciousness that may progress to confusion, deep coma, psychiatric changes, and ataxia of gait (19). HACE is considered to be the end stage of severe AMS. Cerebral vasogenic edema has been implicated in HACE (18, 28), suggesting that BBB permeability may play a role in AMS. The corollary is that hypoxia may disrupt the BBB (19). After a 24-h simulated altitude exposure, the T2 signal in the white matter on magnetic resonance imaging brain scans increased in four of seven patients, with the most severe symptoms of AMS indicating vasogenic edema (28).

Other experimental studies have revealed that the brain swells during the ascent to high altitude (22, 23, 30), presumably from vasodilatation and irrespective of AMS. However, the link between BBB disruption, hypoxia, and AMS remains uncertain (3, 22). For instance, another study found no evidence of edema or total increases in brain volume within the first 6–10 h of exposure to hypoxia despite symptoms of AMS (3, 11). Rats exposed to acute hypoxia show a marked hypothermic response that may be protective (16, 17). At present, there are no good small animal models to study this syndrome. However, we believe that the rat could be used as a model to study the effect of hypoxia on permeability of the BBB.

We hypothesize that hypoxia affects the function of the BBB, and ambient temperature (Ta) changes could modify the effects of hypoxia on the BBB. The primary goal of this study was to determine whether acute hypobaric hypoxia will disrupt the permeability of the BBB, and whether the degree of disruption will vary depending on the Ta and duration of hypoxia. The other goal of this study was to examine the possible site of disruption of the BBB following acute exposure to hypoxia. The BBB was assessed histologically using endogenous IgG and sodium fluorescein tracer (NaF) (which are normally excluded from the brain by the BBB) (20, 26), as well as the endothelial barrier antigen (EBA) immunoreactivity (39), to visualize BBB disruption.

MATERIALS AND METHODS

Animals

Male Wistar rats (n = 111) weighing 250–300 g (Charles River, Quebec, Canada) were maintained in a temperature-controlled room with a 12:12-h light-dark cycle. Animals were kept under standard laboratory conditions with access to water and food ad libitum and allowed to adjust to their environment for at least 3 days before starting any experiment. All experimental procedures were approved...
Experimental Groups

The body temperature study consisted of three groups: normoxic control, maintained at room temperature; room temperature hypoxia (RT-hypoxia at 22°C); and high ambient temperature (HAT) hypoxia control, maintained at room temperature; room temperature hypoxia in OCT embedding medium (Sakura Finetek, Torrance, CA). Consecutively coronal sections (50-μm thickness) of brain were cut using a cryostat, and the slices were stored in PBS until further processing of the tissue.

Body-Temperature Recording and Surgery

Adult male rats were anesthetized with isoflurane (induced at 3%, maintained at 2%, and silicone-coated temperature data loggers (SubCue, Calgary, Canada) were surgically implanted into the abdomen. After a 4-day recovery, the animals were placed in a 0.5-atm hypobaric chamber for 1 and 2 days as previously described (10). The chamber was followed for 10 days in one group to see if core temperatures would return to baseline. For the HAT hypoxia group, the body temperature was followed for 10 min because previous studies have observed that peak fluorescence in animal brains occurred in a time range between 5 and 15 min following intravascular injection (20, 27). After 10 min of NaFl circulation, cardiac perfusion with cold normal saline and 4% paraformaldehyde was performed to wash out all intravascular marker.

Animal Perfusion and Tissue Preparation

The animals were anesthetized with intraperitoneal ketamine/xylazine at a dose of 10 mg/100 g body wt (Bimed-MTC Animal Health, Cambridge, Ontario, Canada). The chest was rapidly opened, the right atrium was incised. Perfusion through the cannula was carried out with 250 ml of cold normal saline followed by 300 ml of cold 4% paraformaldehyde fixative in 0.1 M PBS (pH 7.4). Absence of color was confirmed by the Animal Care Committee of the University of Calgary and conformed to the guidelines established by the Canadian Council on Animal Care.

Sodium Fluorescein Permeability Study

Twenty-eight rats were injected intravenously with 1 ml of 2% NaFl tracer (Sigma, St. Louis, MO) dissolved in 0.9% saline (20 mg/ml, mol wt 376 Da). The tracer was allowed to circulate for 10 min because previous studies have observed that peak fluorescence in animal brains occurred in a time range between 5 and 15 min following intravascular injection (20, 27). After 10 min of NaFl circulation, cardiac perfusion with cold normal saline and 4% paraformaldehyde was performed to wash out all intravascular marker. The brains were removed, and 50-μm serial cryostat sections were collected on coated slides, cover-slipped, and examined using an Olympus fluorescence microscope fitted with suitable optical filters.

Immunohistochemistry Studies for IgG, EBA, and EBA/Laminin Double Labeling

Brain sections were incubated for 1 h at room temperature in a cyanine-3 dye (CY3)-conjugated donkey anti-rat IgG antibody (1:100, Jackson Immuno-Research Laboratories, West Grove, PA) or with the primary mouse monoclonal antibody to rat blood-brain barrier (anti-EBA, SMI 71, IgM, 1:1,000, Sternberger Monoclonals) overnight in a small humid chamber at 4°C.

For EBA staining, the antigen-antibody reaction was detected by incubation with fluorescein (CY3)-conjugated goat anti-mouse IgM (Jackson Lab, 1:100) for 30 min. Sections were washed with PBS (3 × 10 min) and mounted in bicine-buffered glycerol (pH 8.6). Omission of the secondary antibody from the IgG staining or the use of normal mouse serum in place of the primary antibody served as the staining controls.

Double labeling for EBA/laminin was performed in a sequential manner. Tissue was first incubated with the primary and secondary antibody for the EBA as indicated above. Samples were then washed in PBS and subsequently incubated with the primary (rabbit polyclonal anti-laminin, 1:50) and secondary [FITC-conjugated donkey anti-rabbit (Jackson Lab, 1:100)] antibodies. Tissue was mounted in bicarbonate-buffered glycerol at pH 8.6 and visualized either with an Olympus fluorescence microscope (BX61) or an Olympus Fluoview (FV300) confocal microscope.

Image Analysis and Quantification

A digital camera (Microfire A/R, Optronics) interfaced for Windows digital imaging system (StereoInvestigator 7.50.4, MicroBright Field) was used to convert the microscopic images of different brain regions into digitized images. To measure the immunoreactivity of NaFl and EBA labeling in the brain, five sections from each of the frontal cortex, cingulated cortex, parietal cortex, striatum, and the hypothalamic regions in each animal were imaged using a 10× objective. Measurements of NaFl or EBA labelings were conducted across brain regions starting at bregma (corresponds to the point that the sagittal and coronal sutures of the skull meet): 5.64 mm for frontal cortex, 2.28 mm for cingulated cortex and striatum, 1.20 mm for parietal cortex, and 0.60 mm for the hypothalamus. Every third section was counted from the frontal cortex, whereas every fifth section was analyzed in cingulated, parietal cortices, striatum, and the hypothalamus. Morphometric analyses of NaFl labeling were performed by measuring the average pixel intensity of each image using the StereoInvestigator program. The results are expressed as the mean average pixel intensity of NaFl in each of the different regions of the brain.

For IgG labeling, five sections through each of the above brain regions were employed for the measurements of the average pixel intensity. The mean values for the studied brain regions were com-
Body Temperature Responses to Hypoxia

Body temperature responses to hypoxia at room temperature. With the onset of hypoxia, the body temperature (Tb) circadian rhythm was abolished, and Tb, which had been rising toward its normal nocturnal peak at the onset of hypoxia, not only reversed (Fig. 1) but also fell 3.5°C in 3.25 h. Tb, after reaching its hypothermic nadir, reversed again and over the next 3–4 days rose 2.41°C at a rate of 0.03 ± 0.01°C/h to a peak of 36.20 ± 0.14°C. During this phase, the circadian rhythm was disrupted and erratic waveform changes appeared (Fig. 2). When the hypoxic period was extended to more than 2 days, core temperature reached an average maximum value of 36.61°C (the maximum value was defined as the highest 5 points in 1 day/night cycle), a value significantly different (P < 0.01) from that of the control animals at the same point in time. The core temperature during hypoxia was greatly depressed (Fig. 2), and the observed cycles were reduced in amplitude (averaging only ~0.4°C). On return to normoxia, Tb rapidly increased and over the next 2 days of normoxia, the cycles appeared to return to their prehypoxic values (data not shown).

Body temperature responses to hypoxia at HAT. The Tb in the group exposed to hyperthermia was at most 1.0°C higher than the other groups at the start of the hypoxia and showed a sharp decline over the first 2 h (Fig. 1). During the first 2 days of hypoxia, the mean Tb of the HAT rats was actually 1.9°C above that of the RT-hypoxic group (P < 0.05, Kruskal-Wallis ANOVA). The Tb in the HAT-hypoxic group also declined rapidly upon onset of hypoxia. The Tb returned to a higher value, close to that of the normoxic controls, within 24–48 h. Although the Tb values of the HAT-hypoxic group and controls during the last 48 h of hypoxia were close, statistical analysis showed that the HAT-hypoxic group was significantly higher compared with the control (P < 0.05, Kruskal-Wallis ANOVA, Fig. 2). The normal temperature cycle was reestablished after 2 days of return to normoxia (data not shown).

IgG Extravasation

Exposure to hypoxia alone for 1, 2, and 7 days, did not produce significant extravasation of IgG in the brain, except in the circumventricular (CVO) regions that are normally permeable (12). In contrast, hypoxic HAT animals showed few scattered focal leakages of IgG in the brain parenchyma during the observation period of 1–2 days (Fig. 3). The changes we
Observed were transient, because at 7 days, IgG leakage in the hypoxic HAT group was only observed in permeable CVO regions.

Sodium Fluorescein Extravasation

In brains of normal control rats, no signs of extravasation of NaFl could be seen, except in the CVO regions that are normally permeable (12).

RT-hypoxia rats exhibited NaFl leakage in focal areas in the brain parenchyma 1 day after the induction of hypoxia, but these were not widespread. These areas included the frontal cortex (Fig. 4), the cingulate cortex, periventricular zones around the lateral ventricles, and the hypothalamus. On the other hand, hypoxic HAT rats had increased BBB permeability at 24 h compared with both normoxic control and RT-hypoxic rats (Figs. 4 and 5).

After 2 days of hypoxia, RT-hypoxic rats exhibited NaFl leakage mainly in a few isolated well-defined spots scattered throughout the brain tissue (Fig. 4). In the hypoxia plus HAT group, the fluorescein took the shape of multifocal staining that was mainly distributed in frontoparietal cortices, striatum, hypothalamus, and subcortical parenchyma (Fig. 4). Both hypoxia groups showed significantly higher levels of NaFl pixel intensity in the brain (50–100%, \( P < 0.05 \)) than normoxic control animals (Fig. 5). NaFl did not leak out of the cerebral vessels in any of the hypoxia-treated rats at 7 days. Recovery from the hypoxic changes was complete in treated animals after 7 days of hypoxia.

EBA Immunoreactivity

In all brains, EBA immunostaining was seen exclusively on blood vessels. Neurons and glia were not immunostained, and there was no background staining of the neuropil. In control animals, EBA-positive microvessels were evenly distributed in all parts of the brain but were absent in the areas without a functional BBB (e.g., subfornical organ and median eminence).

No significant differences in length or length per volume were found between normoxic controls and normoxic HAT controls (Table 1).

After 12 h of exposure to hypobaric hypoxia, no reduction in EBA staining was present in the brain. At 1 day there was a moderate reduction in the number of immunostained vessels of the brain, and 2 days after hypoxia started, there was a marked reduction of immunostained vessels in brain parenchyma (Fig. 6). After 7 days of hypoxia, the EBA staining had almost returned to the same level as in the controls. However, some of the microvessels were not as markedly stained as those in the controls. Exposure to RT-hypoxia induced a moderate reduction in the EBA expression, whereas exposure to hypoxia + HAT led to a greater decrease in EBA expression (\( P < 0.05 \) in frontal and parietal regions when HAT hypoxia compared with RT-hypoxia, Table 1); however, it was not statistically significant when HAT hypoxia was compared with RT-hypoxia in striatum or hypothalamic regions.

EBA expression in rats that received hypoxia + HAT treatment for 2 days exhibited a 58% reduction in the frontal cortex (\( P < 0.01 \)), 49% reduction in the parietal cortex (\( P < 0.01 \)), 45% reduction in the striatum (\( P < 0.01 \)), and 52% reduction in the hypothalamus (\( P < 0.01 \)) compared with HAT controls (Table 1).
The hypoxia-induced hypothermia (Fig. 1) was not unexpected. Many species of small animals, along with human infants, have a lower $T_b$ value when exposed to hypoxia (2, 12, 40). The hypothermic component of the hypoxic response of $T_b$ is thought to be a result of thermal regulatory suppression (16) or an override of thermogenesis generated by the hypoxia-induced increase in ventilation (36). The hypothermic response may also be neuroprotective (6). The decline of temperature in RT hypoxia in this study was less than that observed previously in Sprague-Dawley rats (5). This can be attributed to a difference in average prehypoxic temperatures as well as to a different room temperature (21°C vs. 22°C). HAT was used to prevent hypoxia-induced hypothermia and to minimize or eliminate any protective effects of the hypothermic response on the BBB. The HAT group did have a $T_b$ that was closer to that of normoxic controls. At the end of hypoxia, the $T_b$ value of the RT hypoxia group increased transiently above the normal value, which is consistent with earlier reports (4, 31). The transitory nature of the hypoxic-induced hypothermia may protect the organs until more slowly recruited adaptive mechanisms of ventilatory acclimation and increased $O_2$ delivery capacity can counter the continuing stresses imposed by hypoxia.

In this study we chose a half-reduction of the ambient atmosphere as this is commonly used in hypobaric hypoxia studies. Calgary is 1,000 m above sea level with an average pressure of 670 mmHg; a half-reduction corresponds to ~330 mmHg of chamber pressure, which is equivalent to the altitude on Mount Everest, 6,750 m (41). However, compared with 1 absolute atmosphere (sea level) the hypobaric hypoxia in this study was conducted at 0.43 atm. When comparisons are made to other studies done at 0.5 atm at sea level, the animals in this study were exposed to a greater level of hypoxia.

In control animals, plasma protein IgG cannot cross BBB so there is almost no presence of IgG in the brain parenchyma. IgG is only found in some structures of the brain, like CVOs, which have fenestrated capillaries that allow the exchange of hydrophilic substances of high molecular weight between plasma and brain tissue (32). Our findings showed that the endogenous IgG entered the brain parenchyma via BBB only when animals were exposed to combination of hypoxia and HAT, and the disruption of the BBB recovered after exposure to 7 days of hypoxia (Fig. 3).

In terms of molecular size, sodium fluorescein (mol wt 376 Da) is one of the smallest tracers, and it cannot cross BBB (20, 26, 27). In control rats in which NaFl was injected, there was no visible extravasation of the fluorescein in the brain parenchyma, except in midline structures containing CVOs. The leakage of the NaFl dye in CVOs is expected, since it has no BBB (14).

The NaFl data indicate that BBB leakage to small molecules occurs in a focal fashion and does not occur in all vessels at the same time. This focal disruption may make it difficult to observe BBB disruption using in vivo methods such as MRI as the leakage occurs in volumes that are much less than that of an MRI voxel. The time course indicates that BBB permeability to NaFl remained intact for at least 12 h after induction of hypoxia. Our results are in agreement with a previous study that showed a twofold increase in fluorescence intensity in hypoxic mice brains, indicative of significant vascular leakage during hypoxia (34).

Although the EBA is not yet fully characterized, it appears to be an important constituent of the BBB (39). The observation that this antigen was selectively reduced in the hypoxic rat brain supports the hypothesis that changes in the BBB may be a correlate of AMS and HACE. This is the first report to show an alteration in EBA following exposure to acute hypobaric...
Animals did not show significant EBA reductions in any of the above brain regions, indicating that temperature did not cause reductions in EBA. While the tracers showed focal leakage, the above brain regions, indicating that temperature did not cause reductions in EBA. While the tracers showed focal leakage, the BBB network revealed with EBA in this region of the brain. Fig. 6. Endothelial barrier antigen (EBA) immunohistochemistry as a marker of BBB disruption. Periventricular regions are shown. A: controls. Note the extensive endothelial (vascular) network revealed with EBA in this region of the brain. B represents EBA immunoreactivity in HAT-hypoxic rat brain. Note the marked reduction in EBA expression in HAT-hypoxic brains. RT-hypoxic rats also show a reduction in EBA (data not shown) which appears to be greater in the HAT vs. the RT groups (see Figs. 7 and 8). Scale bar, 200 μm.

Table 1. Average length and length per volume of EBA-positive blood vessels in four different regions of brain after 1, 2, or 7 days of RT-hypoxia and HAT hypoxia compared with normoxic control and normoxic HAT control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Frontal Cortex</th>
<th>Parietal Cortex</th>
<th>Striatum</th>
<th>Hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length, mm/mm²</td>
<td>Length Per Volume, mm³</td>
<td>Length, mm/mm²</td>
<td>Length Per Volume, mm³</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day (n = 5)</td>
<td>19.573 ± 1.425</td>
<td>1.376 ± 100</td>
<td>21.936 ± 2.128</td>
<td>1.543 ± 1.250</td>
</tr>
<tr>
<td>2 days (n = 5)</td>
<td>20.105 ± 1.855</td>
<td>1.408 ± 136</td>
<td>22.363 ± 2.390</td>
<td>1.573 ± 1.168</td>
</tr>
<tr>
<td>HAT control</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 day (n = 5)</td>
<td>19.919 ± 1.850</td>
<td>1.401 ± 130</td>
<td>19.408 ± 3.653</td>
<td>1.365 ± 0.257</td>
</tr>
<tr>
<td>2 days (n = 5)</td>
<td>20.437 ± 1.787</td>
<td>1.399 ± 186</td>
<td>20.542 ± 3.089</td>
<td>1.445 ± 0.217</td>
</tr>
<tr>
<td>RT-hypoxia</td>
<td></td>
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<tr>
<td>1 day (n = 5)</td>
<td>11.090 ± 1.703†</td>
<td>0.780 ± 120†</td>
<td>13.590 ± 2.345†</td>
<td>0.956 ± 1.65†</td>
</tr>
<tr>
<td>2 days (n = 5)</td>
<td>10.983 ± 1.843†</td>
<td>0.772 ± 130†</td>
<td>12.674 ± 2.411†</td>
<td>0.892 ± 1.70†</td>
</tr>
<tr>
<td>7 days (n = 4)</td>
<td>17,623 ± 3,787§</td>
<td>1,239 ± 266§</td>
<td>18,428 ± 4,651§</td>
<td>1,295 ± 327§</td>
</tr>
<tr>
<td>HAT hypoxia</td>
<td></td>
<td></td>
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<tr>
<td>1 day (n = 5)</td>
<td>9.129 ± 2.174†‡</td>
<td>0.642 ± 153‡</td>
<td>10.600 ± 2.850‡‡</td>
<td>0.745 ± 200‡‡</td>
</tr>
<tr>
<td>2 days (n = 5)</td>
<td>8.624 ± 2.262‡‡</td>
<td>0.606 ± 159‡‡</td>
<td>10.441 ± 2.470†‡</td>
<td>0.753 ± 169†‡</td>
</tr>
<tr>
<td>7 days (n = 4)</td>
<td>18,065 ± 2,580§</td>
<td>1.270 ± 181§</td>
<td>18,310 ± 4,811§</td>
<td>1.288 ± 338§</td>
</tr>
</tbody>
</table>
| Values are means ± SD. Note the significant reduction of endothelial barrier antigen (EBA) expression in the hypoxia treated groups compared with normoxic controls. HAT, high ambient temperature; RT, room temperature. One way ANOVA, Tukey’s post hoc procedure was performed for comparing the means of the groups. *Significant difference: P < 0.05 vs. normoxic control group. †Significant difference: P < 0.01 vs. normoxic control group. §Significant difference: P < 0.05. HAT hypoxia vs. RT hypoxia. ‡Nonsignificant: P > 0.05 vs. control groups.
transport across cells, this would suggest that BBB disruption may be more widely spread than indicated by the tracer leakage data.

Similar to the tracer leakage data, hypoxic brain vessels following exposure to hypoxia reduced or lost their expression for EBA transiently (less than 7 days); this was in tandem with the leaking of IgG and NaFl from blood into brain parenchyma. Such reduction was then followed by restitution of EBA immunostaining at 7 days after acute hypoxia. It is possible that EBA is involved with the expression of a type of transporter or certain degradative mechanisms in cerebral endothelia. The factors causing the disappearance and restitution of the antigen are unknown. It appears that BBB disruption in many areas of the brain following a combined exposure to hypoxia and HAT in this study reflects additive interaction of hypoxia and heat stress.

In this study, we used double labeling of laminin and EBA to investigate the time- and hypoxia-induced changes in endothelial density in the rat brain. Laminin (approximate mol wt 200 kDa) is a glycoprotein of the extracellular matrix and a major component of the vascular basement membrane (1). It is present in normal as well as proliferating blood vessels (8) and is commonly used to visualize the presence and condition of blood vessels in paraffin or frozen tissue sections (7). These specimens showed that the downregulation of EBA found in hypoxic rats (Figs. 7 and 8) was not attributable to a loss of microvessels, because laminin-positive vessels were evenly distributed within the hypoxic brains even in areas that lacked...
EBA staining (Figs. 7 and 8). Also, chronic hypoxia stimulates new microvessel formation (10, 24), making it unlikely that one would see a transient decline in microvessel density at this time.

The altered BBB permeability may relate to hypoxia-induced free radical formation (25, 35). Hypoxia also causes hypoxia-inducible factor-1α (HIF-1α) stabilization and an increase in VEGF. This would link the BBB disruption to the known increase in angiogenesis. Since VEGF increases BBB permeability, it is very reasonable to assume that regions with angiogenesis would also have transient increases in BBB permeability (29).

The observation that hypoxia alone can cause disruption of the BBB may help with discerning the pathophysiology of AMS and HACE. It also has widespread relevance, since hypoxia is known to exist in many conditions such as stroke and cancer. BBB disruption occurs in other conditions as well such as multiple sclerosis plaques (37), leading to the possibility that hypoxia and focal ischemia may be involved. Migraine headaches may have a transient ischemic component (38), which in turn could generate sufficient hypoxia to disrupt the BBB.

A limitation of this study is that EBA is only specific for the rat and its function is not fully understood (39). This may mean that a loss of EBA relates to other factors in addition to impaired BBB.

These data indicate that HAT exacerbated the BBB disruption elicited by hypoxia. Therefore this study suggests that hypoxia + HAT is a good model of hypoxia-induced BBB disruption and that enhanced BBB permeability occurs during acute hypoxia.

In the future, it is important to investigate the mechanisms that may be causing the BBB disruption. Also, it is important to establish whether AMS itself relates to this observed disruption in the BBB. Based on the fact that climbers tend to recover quickly from AMS when supplementary oxygen is administered or they are taken to a lower altitude (3, 19, 42), one might argue that more extensive acclimation to hypoxia before a severe hypoxic insult such as climbing or stroke may improve outcome by reducing the hypoxia-induced BBB disruption.

In summary, this study reports that hypoxia caused a subtle disruption of the BBB, and this disruption was transient and reversible. Our study also indicates that EBA immunoreactivity serves as a sensitive marker of intact BBB and can be used to detect sites of barrier dysfunction and recovery in the brain after acute hypoxia. In addition, HAT administered with hypoxia exacerbated the increases in BBB permeability to large and small molecules, suggesting that hypothermia is protective and that HAT + hypoxia provides a novel model of transient BBB disruption. Acute disruption of the BBB caused by hypoxia may be one of the mechanisms of AMS and HACE.

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GRANTS

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