HIGHLIGHTED TOPIC | Oxygen Sensing in Health and Disease

Cerebrovascular inflammation after brief episodic hypoxia: modulation by neuronal and endothelial nitric oxide synthase

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Altay, Tamer, Ernesto R. Gonzales, T. S. Park, and Jeffrey M. Gidday. Cerebrovascular inflammation after brief episodic hypoxia: modulation by neuronal and endothelial nitric oxide synthase. J Appl Physiol 96: 1223–1230, 2004; 10.1152/japplphysiol.00798.2003.—Obstructive sleep apnea, apnea of prematurity, and sudden infant death syndrome are associated with a high risk of morbidity and mortality secondary to the neuronal and cerebrovascular consequences of the associated intermittent hypoxia. We hypothesized that episodic hypoxia (EH) promotes inflammation in the cerebral microcirculation and that nitric oxide (NO) produced by the endothelial and neuronal isoforms of NO synthase (eNOS and nNOS, respectively) modulates this response. Anesthetized and ventilated Swiss-Webster ND4 mice, wild-type mice, and NO synthase knockout mice were subjected to a 1-h period of EH (twelve 30-s periods of hypoxia every 5 min). Four, 24, or 48 h later, mice were reanesthetized for imaging of leukocyte dynamics in the cortical venular microcirculation by epiﬂuorescence videomicroscopy through closed cranial windows. In Swiss-Webster ND4 mice, leukocyte adherence increased 2.1-fold at 4 h, 3.4-fold at 24 h, and 1.8-fold at 48 h relative to time-matched, normoxic controls; there was no evidence of delayed hippocampal CA1 pyramidal cell death. A similar response was noted in wild-type mice. However, in eNOS knockouts, leukocyte-endothelial cell adhesion was elevated to 4.4-fold over baseline 24 h after EH, and a signiﬁcant fraction of these animals showed evidence of delayed CA1 cell death. Conversely, in nNOS knockouts, no increase in adherence was noted at 24 h and CA1 viability remained unaffected. We conclude that NO derived from nNOS promotes an inﬂammatory response in the cerebrovascular microcirculation after short-term EH and that NO produced by eNOS blunts the extent of this response and exerts neuroprotective effects.

intermittent hypoxia; cerebral microcirculation; leukocytes; endothelium; hippocampus

INTERMITTENT OR EPISODIC HYPOXIA (EH) has emerged in recent years as a research topic of considerable clinical interest because of its suspected role in triggering the pathophysiological changes associated with obstructive sleep apnea, apnea of prematurity, and sudden infant death syndrome. Cardiovascular dysfunction (32), neurocognitive disorders (53), and stroke (7, 15) contribute importantly to the morbidity and mortality associated with these syndromes. It is likely that the brain is uniquely vulnerable to EH-based disorders and exhibits a distinct spatial and temporal injury proﬁle, on the basis of the ﬁnding that more severe injury occurs after repetitive global ischemia relative to a single period of sustained ischemia of equivalent duration (36, 58); moreover, the pattern of neuronal death after episodic ischemia, which is predominantly striatal, differs from the parasagittal cortical damage caused by a sustained ischemic insult (39). However, laboratory investigations of EH-dependent cerebrovascular or parenchymal dysfunction are few. Although sustained periods (several weeks) of multiple daily episodes of EH cause neuronal cell loss in developing (14) and adult (12) rats, we are aware of no studies that have examined the threshold for such dysfunction or injury or addressed the possibility that even brief periods of EH might affect cerebral physiology in a deleterious manner.

Experimental studies indicate that sustained periods of cerebral hypoxia or ischemia trigger an inﬂammatory response in brain, characterized in part by neutrophil recruitment and inﬁltration into brain parenchyma, and that this inﬂammatory response contributes importantly to secondary brain injury in these settings (20). Interestingly, individuals that suffer from sleep apnea exhibit hematological proﬁles indicative of chronic inﬂammation, including elevated plasma levels of proinﬂammatory cytokines, purine catabolites, soluble adhesion molecules, and markers of oxidative stress (34). We hypothesized that short periods of EH trigger leukocyte adherence to venular endothelium in brain that may ultimately lead to cerebrovascular and/or neuronal injury. To date, this hypothesis has not been explored in preclinical models.

Nitric oxide (NO) is a water- and lipid-soluble free radical synthesized by the oxidation of L-arginine by three unique NO synthase (NOS) enzymes: endothelial NOS (eNOS) and neuronal NOS (nNOS) are constitutively expressed, calcium-dependent enzymes, for the most part expressed in endothelium and neurons, respectively, whereas inducible NOS (iNOS), expressed in astrocytes and circulating leukocytes, is induced by a variety of stimuli in a calcium-independent manner. Because NO negatively modulates various aspects of the inﬂammatory response, including microvascular permeability (31), platelet activation/aggregation (49), and leukocyte-endothelial cell interactions (13), we hypothesized that EH-mediated inﬂammatory responses in the brain would be modulated by NO. Indeed, several lines of evidence indicate that endothelial dysfunction is manifested in several ways in the sleep apnea patient and that diminished NO availability may underlie this pathology (34). In brain, NO exerts dual roles in the setting...
of hypoxic or ischemic stress: eNOS-derived NO exerts anti-inflammatory effects similar to those mentioned earlier (11, 19, 37, 51) and improves tissue perfusion (18, 48), whereas nNOS-derived NO is implicated in neuronal injury (9, 45). Whether similar modulatory actions of NO occur in brain after EH has not been examined.

Thus, we undertook the present study to identify whether EH of short duration might promote neutrophil adherence in brain and, in turn, the possible modulation of such a response by eNOS- and/or nNOS-derived NO. We introduce herein a clinically relevant murine EH model, characterized by periods of true apnea and associated hypercapnia and acidosis, as opposed to continuous normocapnic ventilation during periods of reduced ambient \( \text{PO}_2 \) that is typical of most experimental models. Because we used mice, we could elucidate the modulatory role of NO in the cerebrovascular inflammatory response by examining isoform-specific NOS knockout animals.

**MATERIALS AND METHODS**

**Animal preparation and episodic hypoxia protocol.** In this study, Swiss-Webster ND4 (SW), wild-type, and NOS knockout (−/−) male mice weighing 25–32 g, from 12–16 wk of age, were used. The nNOS and eNOS knockout mice we used, obtained from Dr. Paul Huang at the Massachusetts General Hospital, were characterized previously in detail (18, 25, 42, 45). Age- and sex-matched 129SvEv (Taconic) and C57BL/6 (Harlan) mice both served as the wild-type control groups, as reported previously (18, 25, 28, 45, 46, 43).

All experimental procedures were approved by our institutional animal studies committee. Briefly, mice were anesthetized with chlоро hydrate (350 mg/kg ip) and xylazine (4 mg/kg ip). On a 45°-angled platform, mice were intubated with a 22-gauge cannula under a surgical microscope and ventilated (Harvard Apparatus, model 845). For control (sham EH) animals, ventilatory parameters used were a stroke volume of 5 ml/kg and a respiratory rate of 110 breaths/min. To maintain arterial blood gases within normal ranges during and after EH, animals subjected to EH were ventilated with a stroke volume of 7 ml/kg and 130 breaths/min. Room air was used to ventilate all animals. A femoral arterial catheter was placed in subgroups of animals for blood-gas analyses. Core temperature was maintained at 37°C in all animals with a customized thermoregulated heating pad under the animal.

After establishing mechanical ventilation for 10 min, we subjected animals to 12 cycles of EH by turning off the ventilator for 30 s every 5 min for a duration of 1 h. Equal hemithorax expansion on inhalation was the criterion used to identify a sufficient ventilation, which was confirmed by blood-gas measurements during the same time in a subgroup of animals. At the end of the 1-h period of EH, animals were ventilated normally again (for ~5–10 min) until they regained their spontaneous breathing effectively enough to keep arterial blood gases within the normal range. Thereafter, the animals were disconnected from the ventilator, placed in an incubator at 33°C for 30 min until fully ambulatory, and then returned to their cages. Another group of animals was anesthetized and ventilated with normoxic room air in an uninterrupted fashion for 1 h, followed by 30 min in the incubator, and served as a sham-EH control group.

**Cranial window and cerebral blood flow determinations.** Four or 24 h after EH (or sham EH), animals were reanesthetized, tracheo-tomized through a midline ventral neck approach, and ventilated starting at 5 ml/kg and 110 breaths/min. Anesthesia was maintained throughout the surgical preparation and imaging procedures by administering chloral hydrate-xylazine as needed. A femoral arterial line was placed for the measurement of blood gases and blood pressure and rhodamine 6-6-6 administration. A blood-gas sample was obtained before placement of the cranial window, and minor adjustments in tidal volume and/or respiratory rate were made to keep these values within physiological ranges (Table 1). In the prone position, the animal’s head was stabilized in a custom stereotaxic apparatus. The right parietal bone was exposed through a vertical scalp incision, and relative measures of cerebral blood flow were obtained by laser Doppler flowmetry by direct, via a micromanipulator, the tip of the flow probe through the bone at five different medial and five different lateral locations relative to where the cranial window would subsequently be placed. Flow measurements were made under constant ambient light conditions after a 10- to 20-s period of stabilization. A 3-mm diameter craniotomy was then performed, leaving the dura intact, and over the next 15 min a Plexiglas window was mounted over the opening and allowed to seal to the cranium laterally by dental acrylic. Over the next 5 min, the animal was repositioned on a microscope stage, and regions of the cortical surface exhibiting a heterogeneous pattern of secondary and tertiary arterioles and venules were identified for imaging. An additional 10-min stabilization period was then invoked before the intravital leukocyte imaging was initiated; thus the total duration of time between the craniotomy and the imaging sessions was on the order of 30 min.

**Epifluorescence videomicroscopy for leukocyte dynamics.** As described previously for similarly instrumented piglets (11), intravascular leukocyte dynamics in cortical venules (flowing, rolling, and adherence) could be visualized by using epifluorescence videomicroscopy after in situ labeling with rhodamine-labeled WBCs (lymphocytes and neutrophils) with rhodamine 6-6 (0.007% in PBS, administered intra-arterially at a rate of 150 \( \mu \)l/min). Video images of leukocyte dynamics were recorded to videotape in real time with the use of a charge-coupled device camera (Olympus, 110) mounted on an epifluorescence microscope (Olympus, BHM1) using a \( \times 10 \) water immersion lens (1.3 numerical aperture).

During offline playback of the video recording, leukocyte adherence to the endothelium of the pial venular wall was quantified manually by counting the number of leukocytes adherent to the vessel within a user-defined venular network that included only secondary and tertiary (20–60 \( \mu \)m diameter) postcapillary branches. We empirically defined adherent leukocytes as those remaining stationary within the venule for longer than 10 consecutive seconds. The results reported herein represent the average adherence value measured over both lateral and medial microvascular regions and are expressed as the number of leukocytes per square millimeter of total endothelial vessel surface (11).

**Histopathology.** The effect of EH on neuronal injury in the vulnerable hippocampal CA1 pyramidal cell region was studied in each genotype in separate groups of EH-treated animals and age- and sex-matched controls, 1 wk after EH or sham EH. Animals were euthanized 1 wk after EH or sham EH by halothane overdose and transcardially perfused with heparinized saline. Brains were removed, embedded in plastic, and cryoprotected for 1 wk at –20°C before sectioning at 40 \( \mu \)m.

**Table 1. Physiological and hemodynamic variables in SW mice 4, 24, and 48 h after a 1-hour period of episodic hypoxia, relative to matched normoxic controls**

<table>
<thead>
<tr>
<th>Control</th>
<th>n</th>
<th>Age, wk</th>
<th>( p_{O_2} ) Torr</th>
<th>( p_{CO_2} ) Torr</th>
<th>MABP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td>9</td>
<td>13±0.2</td>
<td>7.26±0.02</td>
<td>24±4</td>
<td>121±17</td>
</tr>
<tr>
<td>24 h</td>
<td>12</td>
<td>13±0.4</td>
<td>7.30±0.02</td>
<td>34±2</td>
<td>86±4*</td>
</tr>
<tr>
<td>48 h</td>
<td>11</td>
<td>12±0.2</td>
<td>7.36±0.02*</td>
<td>32±2</td>
<td>117±8†</td>
</tr>
</tbody>
</table>

**Episodic hypoxia**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age, wk</th>
<th>( p_{O_2} ) Torr</th>
<th>( p_{CO_2} ) Torr</th>
<th>MABP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td>10</td>
<td>14±0.2</td>
<td>7.28±0.02</td>
<td>29±4</td>
<td>91±13†</td>
</tr>
<tr>
<td>24 h</td>
<td>12</td>
<td>13±0.3</td>
<td>7.28±0.02</td>
<td>33±3</td>
<td>129±15†</td>
</tr>
<tr>
<td>48 h</td>
<td>11</td>
<td>12±0.3</td>
<td>7.31±0.03</td>
<td>28±2</td>
<td>104±7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. SW, Swiss-Webster N4 mice; \( p_{O_2} \), arterial \( p_{O_2} \); \( p_{CO_2} \), arterial \( p_{CO_2} \); MABP, mean arterial blood pressure. *\( P < 0.05 \) vs. 4 h; †\( P < 0.05 \) vs. 24 h; ††\( P < 0.05 \) vs. control group at same time point.
frozen on dry ice, and stored at −80°C until the time of sectioning. Coronal sections of 16-μm thickness through the hippocampus were taken starting 1 mm posterior to the bregma and were subsequently Nissl stained. Ischemic neuronal injury was evaluated by light microscopy in three nonadjacent sections from each brain by counting viable pyramidal cells present over a 0.1-mm length in the CA1 sector by an observer blinded to the identity of the mouse genotype and experimental condition. Each hemisphere was examined independently.

Statistical analysis. Differences in physiological and hemodynamic variables, leukocyte-endothelial cell adherence, and the histopathological data within and between groups were assessed by nonparametric repeated-measures ANOVA by using Mann-Whitney’s rank sum test. A P value <0.05 was considered significant.

RESULTS

Physiological and hemodynamic variables. Blood samples obtained in a separate group of SW mice during the periods of intervening normoxia spaced throughout the 1-h period of EH revealed a progressive fall in pH from 7.31 to 7.25, with a rise to 45 Torr (n = 14). As shown in Table 1, when measured 4, 24, or 48 h after EH, blood-gas values and blood pressures did not vary outside of physiological ranges for chloral hydrate-anesthetized SW control mice or those subjected to a 1-h period of EH, although the animals studied at 48 h were borderline hypotensive. Blood cell counts obtained from SW mice subjected 24 h earlier to EH did not differ significantly from animals subjected to sham EH in terms of total numbers of white blood cells, red blood cells, and platelets, and the relative percentage of neutrophils to lymphocytes (n = 5; data not shown). Similarly, blood-gas and blood pressure values remained within expected ranges in the wild-type and NO knockout mice subjected to EH 24 h previously, as shown in Table 2; a previous study using mice from the same founder line reported no differences in circulating neutrophil counts between wild-type and eNOS and nNOS mice (35). Data from the C57Bl/6 (n = 6) and 129SvEv (n = 6) wild-type animal groups were indistinguishable with respect to both physiological and hemodynamic values, as well as their cerebrovascular inflammatory response to EH; hence, data from these two animal groups were merged and presented as the general “wild-type” group (n = 12) for Table 2 and in the graphical presentations. No significant changes in systemic physiological and hemodynamic variables occurred in response to mechanical ventilation with or without EH, although the eNOS- and nNOS-null mice subjected to EH tended to lose 10 ± 1% of their body weight over the 24-h post-EH period relative to ventilated, sham-EH controls (5 ± 1%).

Table 3. Relative cortical cerebral blood flow in 2 locations across the cranial window 24 h after episodic hypoxia or sham episodic hypoxia in mice of the different genotypes shown

<table>
<thead>
<tr>
<th>Cerebral Blood Flow, relative units</th>
<th>Swiss-Webster</th>
<th>Wild Type</th>
<th>eNOS−/−</th>
<th>nNOS−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Lateral</td>
<td>Median</td>
<td>Lateral</td>
</tr>
<tr>
<td>Control</td>
<td>38±4</td>
<td>16±1</td>
<td>28±3</td>
<td>16±2</td>
</tr>
<tr>
<td>Episodic hypoxia</td>
<td>38±6</td>
<td>20±3</td>
<td>30±2</td>
<td>16±1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12–13 mice per group. No significant differences were found between episodic hypoxia and control groups, or between any of the genotypes.
DISCUSSION

Several important results derive from the present study of acute EH in adult mice. First, a relatively brief, 1-h period of EH was sufficient to trigger a rapid and prolonged inflammatory response in the cerebral microcirculation. Within 4 h, leukocytes became adherent to cortical venular endothelium, and the magnitude of this response elevated significantly 24 h later; by 48 h, the response was subsiding. Second, nNOS-derived NO modulates leukocyte-endothelial cell interactions triggered by EH, because EH-induced leukocyte-endothelial cell adherence was completely absent in nNOS knockout mice. Third, a marked augmentation of EH-induced microvascular leukostasis in eNOS-null mice indicates that NO produced by the eNOS isoform normally serves to limit the extent of this EH-induced cerebrovascular inflammatory response; eNOS may also prevent injury of vulnerable neurons from EH insults, because evidence of delayed hippocampal pyramidal cell death was noted only in the eNOS-mutant animals. These results are discussed further in the context of EH as an inflammatory stimulus and the modulation of leukocyte adherence by NO.

To our knowledge, ours is the first investigation of tissue inflammatory responses to EH in a preclinical model. Our documentation of venular leukostasis in the brains of mice subjected to a short period of EH extends and supports accumulating clinical evidence that inflammation is a primary response to conditions like sleep apnea that are characterized by more chronic EH (3, 8, 44). Although inflammatory responses to EH remain unexplored in animal models, it is well established that a single period of hypoxia, as well as the reoxygenation period that follows it, can trigger endothelial cell and leukocyte activation (21, 26, 57, 65, 66), resulting in selectin- and integrin-mediated leukocyte-endothelial rolling and adherence. In brain, rapid increases in endothelial and leukocyte adhesion molecule expression occurs in several species in response to a single global ischemic event (11, 19, 23, 54, 59). However, the microvascular leukostasis induced by global ischemia is very transitory (54, 59), resolving after 4–7 h. In contrast, we found adherence increased between 4 and 24 h after the single period of EH. Although there are significant differences between these interventions, our observation of leukostasis extending 48 h after a short period of EH suggests that more sustained inflammatory responses may...
Fig. 3. Representative photomicrographs of fluorescently labeled leukocytes in the mouse cortical microcirculation in wild-type (A, B), nNOS-null mice (C, D), and eNOS-null mice (E, F) under normoxic control conditions (A, C, E) or 24 h after a 1-h period of episodic hypoxia (B, D, F). Scale bar = 100 μm.

Fig. 4. Representative thin sections of Nissl-stained dorsal hippocampal CA1 pyramidal cells from Swiss-Webster ND4 mice (A, B), wild types (C, D), nNOS-null mice (E, F), and eNOS-null mice (G–I) under normoxic control conditions (A, C, E, G) or 1 wk after a 1-h period of episodic hypoxia (B, D, F, H, I). eNOS-null mice exhibiting moderate (H) and more severe (I) cell injury are shown. Scale bar = 50 μm.
result from the intermittent hypoxia-reoxygenation that characterizes this stimulus. Indeed, brain injury resulting from repetitive episodes of transient global cerebral ischemia is more extensive than that after a single period of sustained ischemia of equivalent cumulative duration (36, 58). Additional laboratory studies are warranted to examine across brain and other tissues whether EH is actually a more potent stimulus for vascular inflammation than comparable periods of sustained hypoxia or ischemia to begin to understand the morbidity and mortality associated with EH-based clinical syndromes.

To initiate the process of uncovering the mechanistic basis of this response, we examined whether EH affects NO homeostasis and the inhibition of leukocyte-endothelial cell interactions that NO exerts normally in many tissues (31, 35). Evidence indicates that this inhibition results from an NO-mediated block of the activation of the transcription factor NFκB (46), thereby reducing the surface expression of endothelial selectins (10, 24) and integrins (6). NO also inhibits neutrophilic NADPH oxidase (4, 50) and negatively affects platelet-endothelial interactions (2). The source of NO responsible for these intravascular effects in brain has long been assumed to be the nearby endothelial cell. However, in our study, under baseline, resting conditions, both eNOS and nNOS knockout mice exhibited slight but significant elevations in the level of leukocyte-endothelial cell adherence relative to wild types. Assuming no compensatory increases in nNOS activity are realized in eNOS-null mice, and vice versa, these results indicate that nNOS-derived NO also contributes to basal vascular leukocyte homeostasis in brain, similar to its participatory role in cerebral dilation under a variety of circumstances (42, 54, 62).

The complete lack of EH-induced microvascular leukostasis in nNOS-null mice indicates that, after EH, NO derived from nNOS exerts proinflammatory actions in the cerebral vasculature. Brief severe hypoxia (41), sustained mild hypoxia (47), and global ischemia (9, 63) are known to activate nNOS gene expression, nNOS activity, and NO production in brain, so it is not unlikely that EH triggers a similar activation of this enzyme. We are unaware of any published reports on the direct measures of cerebral nNOS or eNOS activity after EH, however, NOS isoform-dependent effects of EH in brain have been implicated in some studies. In particular, nNOS or eNOS gene deletion affected the ventilatory response to EH and the magnitude of the increase in brain stem cGMP levels after exposure of mice to three 5-min periods of hypoxia at 20-min intervals (28, 29).

In addition to its proinflammatory effects, nNOS-derived NO may contribute to neuronal injury in the setting of EH, much as it does after global ischemia, in a manner independent of cerebrovascular anatomy or hemodynamics (45). Mechanistically, this injury is thought to result from the formation of the potent oxidant peroxynitrite (9, 16) from concomitantly produced superoxide and nNOS-derived NO (13). Additional injury may occur from NO binding to iron-sulfur complexes and to the heme moiety of proteins and the DNA damage that results from NO-dependent nucleotide base deamination and activation of poly(ADP-ribose) polymerase (43). Despite the evidence for nNOS-mediated injury in ischemic brain, and the fact that sustained periods (2 wk) of EH lead to hippocampal CA1 pyramidal cell injury (12), results of the present study do not allow us to say with certainty that nNOS-derived NO contributed to the loss of CA1 cells observed in a significant percentage of our EH-treated eNOS-null mice. Two possibilities exist: the loss of eNOS-mediated protective effects in the eNOS knockouts, including improved perfusion (17, 38), reduced platelet aggregation (33, 51), maintenance of blood-brain barrier integrity (43, 60), and enhanced release of the inhibitory neurotransmitter GABA (25), “unmasked” the injurious effects of nNOS-derived NO. A reduction in leukocyte-endothelial cell interactions, as documented in the present study, may also contribute to eNOS-mediated neuroprotection, or a number of other nNOS-independent mechanisms may account for the selective vulnerability of CA1 pyramidal neurons after a short period of EH. Indeed, unique biochemical pathways leading to CA1 pyramidal cell injury may be triggered by EH, given that episodic ischemia results in striatal damage, whereas sustained ischemia causes parasagittal cortical damage (39). In any event, our results do indicate that eNOS-derived NO is neuroprotective and that some of this benefit may derive from its anti-inflammatory actions.

Our observation of a robust exacerbation of leukocyte-endothelial cell adherence after EH in eNOS-null mice indicates that NO derived from a still functional eNOS isoform attenuates or blocks the cerebrovascular inflammatory response that follows brief EH. Studies in other vascular beds have shown that this antiadhesive effect of NO occurs independently of changes in microvascular blood flow (30), consistent with our measurements showing no differences in cerebral blood flow between baseline and post-EH recovery time points and between genotypes. Importantly, in the setting of more severe, or more chronic, EH, this anti-inflammatory effect of eNOS-derived NO may no longer be realized. After global ischemia, for example, eNOS activity is reduced for at least 24 h (61, 63), which is manifested as impaired eNOS-dependent dilation (5, 52), hypoperfusion secondary to a loss of NO-mediated vasodilation (17, 38), increased platelet aggregation (33, 51), and increased leukocyte adherence (11, 19, 23). This impairment in eNOS activity is thought to be secondary to ischemia-induced increases in the production of superoxide radical by both endothelial cells (64) and neutrophils (1, 40), which in turn upregulates the expression of adhesion molecules on both neutrophils and endothelium (13), effectively quenches available NO, and directly impairs eNOS function (13). Significant elevations in superoxide may also occur in more chronic EH disorders, given the repetitive return of reoxygenated blood to previously hypoxic tissue in what might be considered as multiple bouts of “reperfusion injury” (13, 17, 26). Indeed, leukocytes from sleep apnea patients produce elevated levels of superoxide (8, 55), and an increased concentration of soluble adhesion molecules (3, 44) and C-reactive protein (56) is found in their plasma. These patients also exhibit reduced levels of circulating NO (22) and impairments in endothelium-dependent vascular relaxation (27). Thus our findings are consonant with the increasingly supported hypothesis advanced in clinical studies that interrelated changes in free radical and NO homeostasis underlie the pathological inflammatory sequelae of chronic apnea (see Ref. 34 for review).

In summary, we have demonstrated that a brief period of EH leads to a protracted period of cerebrovascular inflammation that is uniquely modulated in an isoform-dependent manner by NO. NO produced by the neuronal isoform promotes EH-induced endothelial-leukocyte adherence, whereas NO gener-
ated from the endothelial isoform exerts anti-inflammatory actions that mitigate this response. In our model, the “net” effect observed in the cerebral circulation of wild-type animals is proinflammatory. Neuronal injury resulting from EH is also abrogated by eNOS-derived NO by mechanisms that may extend beyond anti-inflammatory effects.

GRANTS

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REFERENCES


1230

EPISODIC HYPOXIA, NO, AND CEREBROVASCULAR INFLAMMATION


