Nitric oxide and cerebral blood flow responses to hyperbaric oxygen

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Nitric oxide and cerebral blood flow responses to hyperbaric oxygen. J. Appl. Physiol. 88: 1381–1389, 2000.—We have tested the hypothesis that cerebral nitric oxide (NO) production is involved in hyperbaric O2 (HBO2) neurotoxicity. Regional cerebral blood flow (rCBF) and electroencephalogram (EEG) were measured in anesthetized rats during O2 exposure to 1, 3.4, and 5 ATA with or without administration of the NO synthase inhibitor (Nω-nitro-ω-arginine methyl ester), L-arginine, NO donors, or the N-methyl-D-aspartate receptor inhibitor MK-801. After 30 min of O2 exposure at 3 and 4 ATA, rCBF decreased by 26–39% and by 37–43%, respectively, and was sustained for 75 min. At 5 ATA, rCBF decreased over 30 min in the substantia nigra by one-third but, thereafter, gradually returned to preexposure levels, preceding the onset of EEG spiking activity. Rats pretreated with Nω-nitro-ω-arginine methyl ester and exposed to HBO2 at 5 ATA maintained a low rCBF. MK-801 did not alter the cerebrovascular responses to HBO2 at 5 ATA but prevented the EEG spikes. NO donors increased rCBF in control rats but were ineffective during HBO2 exposures. The data provide evidence that relative lack of NO activity contributes to decreased rCBF under HBO2 but, as exposure time is prolonged, NO production increases and augments rCBF in anticipation of neuronal excitation.

O2 breathing under pressure in excess of 1.6 ATA induces O2 toxicity of the central nervous system (CNS), which can lead to convulsions and to death (1, 2). The mechanisms responsible for CNS O2 toxicity are not well understood. Aldosterone, though excessive ROS production in the brain in response to HBO2 has been reported (11) and alterations in oxidative processes do occur (6), specific biochemical mechanisms involving ROS are not well understood.

Hyperbaric hyperoxia also produces a variety of other metabolic responses in the brain, including alterations in oxidative energy (32) and glucose metabolism (31). Most of the known biochemical effects of hyperoxia occur only after prolonged exposures or at higher O2 pressures, when visible manifestations of CNS O2 toxicity, such as motor convulsions, are observed (1). HBO2-induced convulsions are the final expression of cellular dysfunction in the brain, but the actual metabolic changes triggered by the O2-dependent processes remain poorly defined.

It is well known that HBO2 induces vasoconstriction in systemic organs including the brain (1, 4). In the brain, HBO2-induced vasoconstriction and a decrease in total and regional cerebral blood flow (rCBF) have been found in humans (19, 22) and in various animal species (4, 16). Despite thorough documentation of HBO2-mediated vasoconstriction, the biochemical mechanisms and mediators by which HBO2 reduces cerebral blood flow (CBF) are not clear.

Among the possible targets for the toxic effect of O2 are O2-sensitive neurotransmitters such as catecholamines (35). In addition to catecholamines, both inhibitory and excitatory amino acids have been implicated in CNS O2 toxicity (7). More recently, nitric oxide (NO) production has also been implicated in the pathogenesis of CNS O2 toxicity (23, 34, 35). Inhibition of the constitutive NO synthases (NOS) dramatically protects against hyperoxic convulsions (5, 23, 35). The mechanisms of such protection from CNS O2 toxicity are not well understood, because NO plays several key roles in the brain. NO acts both as a mediator of glutaminergic neurotoxicity, acting via N-methyl-D-aspartate (NMDA) receptors (9, 14), and a powerful dilator of cerebral vessels (15, 33), and inhibition of NOS prevents vasodilator responses to CO2 (15). Inhibition of NOS also decreases CBF under basal conditions (15), and this effect may protect against CNS O2 toxicity during HBO2, because the level of tissue PO2 is a function of blood flow (O2 delivery) and O2 consumption. Hyperoxic vasoconstriction helps reduce O2 delivery to the brain, partially protecting it from excessive tissue O2 tensions.
Cerebral NO is synthesized in vascular nerves and neuronal and endothelial cells and diffuses rapidly in all directions. The diffusion constant is \( \sim 3.8 \times 10^{-5} \) cm²/s, close to that of O₂ (20). Extracellular NO concentrations have been proposed to be stable, in the nanomolar range, at steady-state conditions (3). Because NO is a relatively long-lived molecule, it can diffuse away from its site of synthesis and reach critical targets in the brain at distances up to 200 µm (21). Hence, NO can participate in a variety of biochemical reactions, depending on its biological half-life after synthesis. Physiologically, NO half-life depends on its rate of autooxidation, its reaction with O₂, scavenging by metal-containing compounds, e.g., Hb and other hemoproteins, and its binding to cellular thiols (30).

NO that escapes degradation in the brain can activate guanylate cyclase, which catalyzes the formation of cGMP and triggers a number of cellular responses, including smooth muscle relaxation (28). Although the effect is concentration dependent, only 5 nM of NO are needed to activate guanylate cyclase (3). In principle, HBO₂ exposure should change NO kinetics in the brain. For instance, HBO₂ exposure elevates brain Po₂ and production of ROS such as O₂⁻. These hyperoxic events can decrease the half-life of NO, modify its diffusion, and interrupt basal relaxation of cerebral vessels, which may lead to vasoconstriction. It has been found in vitro that superoxide-generating agents rapidly neutralize endothelium-derived relaxing factor, whereas superoxide dismutase increases the half-life of NO and prevents this inactivation (8).

The present study was designed to two related test hypotheses: 1) that HBO₂ decreases CBF in vivo by transient interruption of NO-mediated basal relaxation of cerebral vessels, and 2) NO effects on CBF are restored at the onset of excessive neuronal excitation after prolonged HBO₂ exposures.

**MATERIALS AND METHODS**

**Drugs and Chemicals**

All drugs and chemicals were obtained from Sigma Chemical (St. Louis, MO) unless otherwise indicated. N-nitro-L-arginine methyl ester (L-NAME) and L-arginine also were purchased from Sigma Chemical. S-nitrosohemoglobin (SN0-Hb) was synthesized by using Hb(Fe₃⁺) and excess S-nitrosocysteine and was provided as a gift by Dr. Jonathan Stuehr. S-nitrosoglutathione (GSNO) was prepared with a thiold/SNO/Hb exchange by using a procedure described by Jia et al. (18).

**Animal Preparation**

This study was approved by the Duke University Animal Care and Use Committee. Experiments were performed on Sprague-Dawley rats (Charles River, NC) weighing 290–320 g. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip). The trachea was cannulated, and animals were ventilated mechanically with 30% O₂ in air by using a small animal respirator (Edco, Chapel Hill, NC). Both femoral arteries and one vein were cannulated with polyethylene tubing (ID 0.58 mm, length 25 cm) for monitoring blood pressure, taking blood samples, and injecting drugs, respectively. The arterial blood pressure was measured continuously by using a pressure transducer (Viggo-Spectramed, Oxnard, CA). The signals were integrated to obtain mean arterial blood pressure (MAPB). Arterial Po₂, Paco₂, and pH were determined before and after HBO₂ exposure by using an automatic blood-gas analyzer calibrated before each experiment (IL 1306 blood gas/pH analyzer, Lexington, MA). A femoral vein catheter was connected to an injection pump (model 351, Sage Instruments, Cambridge, MA) to give supplementary pentobarbital (15 mg·kg⁻¹·h⁻¹) during hyperbaric exposure. For the actual experiments, the rats were paralyzed with tubocurarine (0.5 mg/kg, iv) to prevent respiratory motion during rCBF measurements to ensure precise control of blood gases. Supplemental injections of tubocurarine (0.1 mg·kg⁻¹·h⁻¹) during hyperbaric exposure were also performed by pump. Depth of anesthesia with this regimen was assessed for adequacy by testing corneal reflexes in anesthetized animals during surgical preparation and the blood pressure responses to toe pinch in paralyzed rats before and after HBO₂ exposure. Depth of anesthesia under the hyperbaric condition in preliminary studies indicated that our infusion regimen of supplemental pentobarbital during HBO₂ exposure maintained adequate anesthesia. Body temperature was monitored continuously by a thermal probe inserted into the rectum of the rat and was maintained at 37 ± 0.5°C by a heating pad.

The animals were placed in a stereotaxic frame (David Kopf Instruments), and burr holes were drilled to the dura over two brain structures: the substantia nigra (SN) and the caudate putamen nucleus (CPN) by using published stereotaxic coordinates (24). Platinum electrodes were inserted into these structures by using a micromanipulator. The anatomic locations of the brain electrodes were confirmed by postmortem examinations. For electroencephalogram (EEG) recording, two stainless steel screws were inserted into the bone of the skull over the left and right parietal cortex symmetrically.

**Measurement of CBF**

Blood flow in the SN and the CPN during HBO₂ exposure was measured by the hydrogen clearance method, as described previously (10). Platinum electrodes, 0.1 mm in diameter, were inserted into the brain and polarized to a voltage of +0.6 V with respect to a reference Ag-AgCl electrode attached around the base of the tail after the hair was removed. The platinum and reference electrodes were connected through the wall of the hyperbaric chamber to a specially designed dual-channel current-measuring amplifier (direct current measurements: 5–50 nA, input resistance < 50 kΩ, temperature coefficient < 2 pA/°C). For rCBF measurements, H₂ (2.5% in air) was added via the ventilating pump for 60 s. The breathing gas was then switched immediately to the baseline (H₂-free) gas, and the washout curves were recorded.

Hydrogen clearance curves and systemic parameters were recorded on a computer via analog-to-digital conversion (DATAQ Institute, Akron, OH). Calculation of rCBF values was performed by the initial slope method (12). To circumvent artifacts due to recirculated arterial H₂, the first 20 s of the clearance data were discarded, and the next 2-min segment of the curves was used to calculate CBF according to the equation: rCBF = \( \frac{\lambda}{T_{1/2}} \) \( \ln \frac{2}{T_{1/2}} \), where \( \lambda \) is the brain-blood flow partition coefficient for the indicator (\( \sim 1 \) for H₂), 0.693 is the value of ln 2, and T₁₂ is the time in minutes required for the H₂ clearance curve to diminish to one-half of its maximum amplitude relative to baseline. With the formula rCBF = \( \frac{\lambda}{T_{1/2}} \), where T₁₂ is in seconds and 4.158 is the value obtained from 0.693 × 60 × 100, CBF (in ml·100 g⁻¹·min⁻¹) was obtained.
HBO2 Exposure

Animals were placed into the hyperbaric chamber (volume = 39 m³) together with the respirator, blood pressure transducer, EEG amplifier, heating pad, and injection pump. Output from the amplifiers was connected through the wall of the hyperbaric chamber to a computer located outside. After placement of platinum and reference electrodes and application of the polarizing potential, a 60-min recovery period was allowed. When arterial blood gases and body temperature were determined to be in the physiological range, control H2 clearance curves were recorded. The respirator circuit was then connected to a supply of 100% O2, and chamber compression was performed with air to a total pressure of 4 and 5 ATA at a rate of 0.6 ATA/min. During hyperbaric exposures, EEG, MABP, and body temperature were monitored continuously. Blood flow in the SN and the CPN was measured every 15 min. The last H2 clearance curve was recorded after 75 min of HBO2 exposure and lasted for 10 min. A special pneumatic system for H2 breathing under hyperbaric conditions was designed so that it could be operated remotely from outside the chamber (10). The heating pad, respirator, and injection pump also were operated outside the chamber.

Experimental Design

Sixteen independent groups of rats were studied as outlined below.

rCBF responses to HBO2 exposure. In group 1 (n = 7), rCBF was measured in rats breathing 30% O2 (balance N2) at normal atmospheric pressure (1 ATA 30% O2 group). This group served as a time control to establish reproducibility of rCBF values measured in anesthetized rats for 75 min. In rats in groups 2 (1 ATA 100% O2, n = 9), 3 (3 ATA, n = 9), 4 (4 ATA, n = 8), and 5 (5 ATA, n = 9), blood flow in the SN and the CPN as well as physiological variables were measured during 75-min O2 exposures at 1, 3, 4, and 5 ATA, respectively. The purpose of these experiments was to evaluate O2 pressure-dependent rCBF responses and the development of CNS O2 toxicity. To control for the effects of hydrostatic pressure, rCBF was measured in seven additional animals in group 5 (5 ATA) while the rats breathed normoxic helium-O2 (HeO2). Control rCBF measurements at 1 ATA were made by using 30% O2 (balance He), and then the chamber was pressured while the rats breathed 6% O2 (balance He) to provide a normoxic P02 at 5ATA.

rCBF responses to HBO2 exposure after NOS inhibition. Group 6 (1 ATA 30% O2 + L-NAME, n = 7) animals were used for time and pressure controls wherein changes in rCBF were measured in response to NOS inhibition with L-NAME (20 mg/kg ip) and L-arginine (160 mg/kg ip) were treated 30 min before hyperbaric exposure. rCBF responses to HBO2 exposure after NMDA receptor inhibition. The effect of O2 at 5 ATA on rCBF and EEG was evaluated in group 10 rats (5ATA + MK-801, n = 6) treated with MK-801 (1 mg/kg iv) given 30 min before hyperbaric oxygenation. The purpose of these experiments was to determine whether rCBF and EEG responses to HBO2 exposure were changed by inhibition of glutamate-mediated neurotransmission.

rCBF responses to HBO2 exposure in the presence of NO donors. Six separate groups of rats were used to evaluate the cerebrovascular effects of two NO donors: SNO-Hb and GSNO. SNO-Hb (1 µM/1 kg iv) was given to nine rats in each of the following groups: group 11A, 30% O2 at 1 ATA; group 11B, 100% O2 at 1ATA; and group 11C, 100% O2 at 3ATA. For nine rats each in groups 12A, 12B, and 12C, rCBF changes were measured in response to administration of GSNO (1 µM/1 kg iv) during exposure to 30% O2 at 1ATA, 100% O2 at 1ATA, and 100% O2 at 3ATA, respectively.

Data Analysis

Blood flow and physiological variables under normal conditions over time were compared by using a repeated-measures ANOVA. To examine the effects of HBO2 exposure on rCBF, an ANOVA was also used. For comparison of the absolute and percent changes of rCBF values in the absence and presence of NOS inhibitor or NO donor, statistical analysis was performed with the use of paired t-tests. All values are expressed as means ± SD. A value of P < 0.05 was considered significant.

RESULTS

In anesthetized rats breathing 30% O2, physiological variables were similar for animals in the different groups (Table 1). Arterial Pco2 and pH values were stable throughout the exposures at 3, 4, and 5 ATA as shown by blood-gas measurements made before compression and immediately after decompression of the chamber (Table 1). In addition, arterial PO2 values during hyperoxia at 1 ATA indicated that normal pulmonary gas exchange was preserved throughout the experiments. Arterial blood-gas measurements could not be obtained under hyperbaric conditions for technical reasons that involved calibrating the blood-gas analyzer at 4 and 5 ATA.

The rCBF values under control conditions were similar in all groups of rats. Considering all animals together, the control value for blood flow in the SN was 84 ± 8 ml·100 g−1·min−1 (n = 51), and in the CPN it was 65 ± 7 ml·100 g−1·min−1 (n = 54). To evaluate the reproducibility of rCBF measurements, we calculated the coefficient of variation of the blood flow values measured in control animals every 15 min for 75 min. The mean coefficients of variation for rCBF values were for the SN 9.8 ± 0.9% and the CPN 8.4 ± 0.8%.

Patterns of rCBF Responses to HBO2 Exposure

HBO2 exposure produced changes in rCBF that were pressure and time dependent. The average decline in rCBF after 30 min at each of the tested pressures is
shown in Fig. 1A. In general, maximum declines in rCBF were reached at 4 ATA and occurred between 30 and 45 min of hyperoxia. The decline in rCBF was attributable entirely to HBO2 exposure, because animals that breathed normoxic HeO2 at 5 ATA showed no decrease in rCBF in either brain region (Fig. 1B).

The actual time course of rCBF for O2 exposure to 4 ATA is presented in Fig. 2A. Blood flow in both deep brain structures decreased significantly over the first 30 min. By 45 min, vasoconstriction had approached its maximum and was maintained close to this level until decompression. Arterial blood pressure rose during compression and significantly increased within the first minutes of HBO2 exposure. Arterial Pco2 after decompression was slightly higher than control values, but these changes were still within the physiological range for the rat. In this group of animals, visible EEG discharges such as paroxysmal spiking activity suggesting O2 toxicity were not observed for the period of hyperbaric exposure.

Exposure to 5 ATA O2 (group 3) caused a decrease in rCBF over the first 30 min, after which the blood flow gradually increased. By 60 min, rCBF had reached preexposure levels and increased over the next 15 min by 35–52% compared with control values (Fig. 2B). In almost all cases, the secondary flow increase preceded the first EEG spiking activity (Fig 3). Such EEG discharges were observed after 45–75 min of hyperbaric hyperoxia. In four of nine animals, visible electrical discharges were not observed, but rCBF still increased after 60 min. Postexposure arterial Pco2 in this group of rats did not change significantly compared with control values (see Table 1).

**Table 1. Physiological variables in rats exposed to hyperbaric oxygen**

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>MABP</th>
<th>PaO2</th>
<th>PaCO2</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 ATA (30%)</td>
<td>120 ± 6</td>
<td>105 ± 17</td>
<td>36 ± 2</td>
<td>7.40 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>1 ATA</td>
<td>121 ± 6</td>
<td>101 ± 17</td>
<td>37 ± 2</td>
<td>7.42 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>3 ATA</td>
<td>121 ± 12</td>
<td>101 ± 12</td>
<td>37 ± 1</td>
<td>7.42 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>4 ATA</td>
<td>125 ± 17</td>
<td>110 ± 10</td>
<td>37 ± 2</td>
<td>7.41 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>5 ATA</td>
<td>130 ± 9</td>
<td>104 ± 10</td>
<td>37 ± 3</td>
<td>7.41 ± 0.01</td>
</tr>
<tr>
<td>6</td>
<td>1 ATA (30%) + L-NAME</td>
<td>127 ± 6</td>
<td>108 ± 11</td>
<td>37 ± 3</td>
<td>7.42 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>4 ATA + L-NAME</td>
<td>123 ± 6</td>
<td>116 ± 19</td>
<td>36 ± 3</td>
<td>7.42 ± 0.02</td>
</tr>
<tr>
<td>8</td>
<td>5 ATA + L-NAME + L-arginine</td>
<td>121 ± 6</td>
<td>113 ± 16</td>
<td>36 ± 3</td>
<td>7.41 ± 0.02</td>
</tr>
<tr>
<td>9</td>
<td>5 ATA + L-NAME + L-arginine</td>
<td>120 ± 5</td>
<td>99 ± 19</td>
<td>37 ± 2</td>
<td>7.40 ± 0.01</td>
</tr>
<tr>
<td>10</td>
<td>5 ATA + MK-801</td>
<td>124 ± 8</td>
<td>103 ± 13</td>
<td>36 ± 3</td>
<td>7.41 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SD. MABP, mean arterial blood pressure (in mmHg); PaO2, arterial PO2 (in Torr); PaCO2, arterial PCO2 (in Torr); L-NAME, Nω-nitro-L-arginine methyl ester. *P < 0.05 compared with pre-hyperbaric oxygen exposure.

**Fig. 1. Regional cerebral blood flow (rCBF) responses in rats exposed to hyperbaric pressure and O2. A: rCBF responses to 100% O2 at 1, 3, 4, and 5 ATA. Blood flow in substantia nigra and in caudate putamen in rats of each group was measured after 30-min O2 exposure and compared with rCBF values in rats breathing 30% O2 (balance N2) at atmospheric pressure (control). *P < 0.05 compared with control expressed as 100%. B: stable rCBF values during 60-min exposures to normoxic HeO2 (6% O2) at 5 ATA. In B, rCBF responses to normoxic HeO2 at 5ATA in the 2 brain regions show a absence of vasoconstriction. Values are means ± SD.**

**Fig. 2.**
animals breathing 100% O₂ at 1 ATA and abolished in rats exposed to HBO₂ at 3 ATA (Fig. 4B).

NO-mediated rCBF Responses

The administration of L-NAME at 20 mg/kg ip produced a small but significant increase in MABP, which gradually returned toward normal within 30 min (data not shown). In contrast, the effect of L-NAME treatment on the rCBF in control rats breathing 30% O₂ was quite pronounced (Fig. 5). The blood flow in the SN and the CPN decreased significantly within 30 min after L-NAME injection, and rCBF was maintained to close to that level for 75 min.

Blood flow in the deep brain structures fell dramatically in L-NAME-treated rats during O₂ exposure at 5 ATA (Fig. 6A). Within 45 min, blood flow in the SN had decreased by 48 ± 4% (P < 0.01) and in the CPN by 65 ± 5% (P < 0.01) compared with the preexposure values. The extent of the cerebrovascular response to L-NAME in animals exposed to 5 ATA (group 5) was more pronounced than in animals exposed to 5 ATA without treatment with L-NAME (group 3); however, the difference in rCBF responses between these two groups of rats was not statistically significant. MABP in L-NAME-treated animals during hyperbaric oxygenation reached 160–170 mmHg, and these changes were similar to those in group 3 animals. EEG discharges as a manifestation of O₂ toxicity in the L-NAME-treated rats were not observed. The rCBF responses to L-NAME + HBO₂ were reversible when rats in group 6 were given L-arginine (Fig. 6B). EEG spike activity was observed in this group of rats (in 65% of animals), and its pattern was similar to that of animals exposed to 5 ATA without L-NAME + L-arginine. Although the onset of EEG spiking activity was variable among animals, the interval between the increase in rCBF and the first electrical discharges was consistently in the range of 10–20 min.

Effect of NMDA Inhibition on rCBF Responses

Fig. 7 illustrates the effect of HBO₂ at 5 ATA on the CBF in MK-801-treated animals. The pattern of rCBF responses was very similar to the time course of the blood flow in untreated rats at 5 ATA (group 3). The rCBF decreased in the first 30 min, but after that rose secondarily, reaching preexposure level within 60 min and increasing by 24–29% at the end of hyperbaric exposure. EEG spikes as a sign of O₂ toxicity were not observed in this group of animals, despite an increase in rCBF after the initial vascular responses.

DISCUSSION

The results of the present study provide four major findings: 1) prolonged O₂ exposure in anesthetized rats...
to 5 ATA, sufficient to induce EEG responses of CNS O2 toxicity, was associated with reversal of initial decreases in rCBF, leading to an increase in blood flow, presumably via reversal of vasconstriction; 2) the secondary increase in rCBF and development of O2-related EEG spikes were not observed after NOS inhibition with L-NAME, but these responses appeared again after concurrent treatment with L-arginine; 3) rCBF responses to the NO-based vasodilators GSNO and SNO-Hb were abolished by HBO2 at 3 ATA; 4) inhibition of NMDA receptor activity by MK-801 did not alter rCBF responses to HBO2, but EEG spiking activity was not observed.

It has been known for a long time that relatively short periods of O2 exposure at pressures of 1–5 ATA induce cerebral vasoconstriction in experimental animals (32) and in human subjects (19). Bean et al. (2) first demonstrated that rCBF responses in conscious rats during prolonged O2 exposure at 5 ATA were biphasic. The initial reduction in rCBF was often followed by secondary elevation of the blood flow to control levels and above. HBO2-induced vasoconstriction failed before the appearance of convulsive EEG discharges. Another study in rats exposed to O2 at 5 ATA reported an initial decrease in rCBF, which returned to control levels before the onset of the first preconvulsive EEG discharges (31, 32). Our results in anesthetized rats are completely consistent with these data. The secondary increase in rCBF in our study was observed within 45–75 min of hyperbaric oxygenation at 5 ATA and always preceded the onset of EEG spikes. The motor convulsions were not observed in our study because the animals were anesthetized with pentobarbital. Nonetheless, almost three-fourths of the rats (72%) exposed to 5 ATA showed visible EEG spiking activity after 60 min.

The NOS inhibitor L-NAME dramatically reduced rCBF and protected against the secondary increase in rCBF. This treatment also inhibited the EEG discharges at 5 ATA. These protective effects of L-NAME were completely reversed by L-arginine given before hyperbaric exposure. Moreover, hyperoxia-induced spiking EEG activity in L-arginine-treated animals was very similar to that seen in rats exposed to 5 ATA. These results were obtained at relatively low doses of L-NAME (15) and despite the presence of barbiturate anesthesia, which may blunt NO-dependent vascular responses. Thus cerebral NO metabolism is strongly implicated in the rCBF responses and subsequent development of CNS O2 toxicity. The data also suggest HBO2 leads to enhanced NO production that increases rCBF and facilitates the appearance of acute manifestations of O2 toxicity.

Several plausible explanations are possible for NO production in the brain in response to hyperoxia. These possibilities are as follows. 1) Constitutive NOS (either or both isoforms) synthesizes NO from L-arginine by using increased availability molecular O2 and other
substrates and cofactors. Because NO synthesis requires O₂ as a substrate, and the apparent Michaelis constant for O₂ of NOS in brain tissue is ~14 µM (25), it is logical to hypothesize that hyperoxia stimulates NOS enzyme activity, increasing NO production in the brain. 2) HBO₂ significantly elevates brain levels of L-arginine (35), the precursor for NO production by NOS. This O₂-dependent biochemical reaction depends on oxidative deamination of catecholamines by monoamine oxidase to produce H₂O₂ and NH₃. The latter metabolite can be used to produce L-arginine. Production of H₂O₂ and NH₃ by monoamine degradation during hyperoxia is also associated with altered GABA/glutamate metabolism, which contributes to acute manifestations of CNS O₂ poisoning (7). 3) Neurons (9), perivascular nerve terminals (33), and glia (27) contain constitutive NOS localized to the cytosol. This neuronal NOS is Ca²⁺-calmodulin dependent and produces NO in response to brief postsynaptic receptor activation. HBO₂ increases brain electrical activity in the preconvulsive period and stimulates O₂ and glucose utilization (6, 31, 32). The present study, however, does not distinguish the cell types or intracellular compartments responsible for NO production in response to HBO₂, because administration of L-NAME inhibits NO production in all cell types in the brain.

The results of this study lead to the postulate that NO plays at least a dual role in the development of CNS O₂ toxicity. One mechanism, involving NO as a dilator of cerebral vessels, is evident from our results demonstrating biphasic rCBF responses to hyperbaric hyperoxia. Thus HBO₂ exposure would induce initial vasoconstriction, presumably protecting the brain from excess tissue oxygenation. This vascular response, as we have shown, is related in part to interruption of NO release from SNO-Hb under hyperoxic conditions (29). In addition, attenuation of the vascular effects of NO by its interaction with O₂ is well known in vitro (26). The lack of blood flow responses to GSNO and SNO-Hb at 3 ATA in this study is consistent with, but not proof of, this hypothesis.

After the initial fall in rCBF, the production of NO appears to compensate and recruit additional blood flow to the brain during prolonged hyperoxic exposure. The rCBF response to this NO production may result in delivery of toxic amounts of O₂ to the brain. The source of such NO production could be either endothelial or parenchymal, because NO is freely diffusible (13). Of note, the secondary rCBF increase that was observed at 5 ATA in this study always preceded the first brain electrical manifestation of O₂ poisoning.

NO also has been reported to contribute to neurotoxicity through an excitatory mechanism, and the present data suggest this may also occur in CNS O₂ toxicity. NO is a mediator of glutamate excitotoxicity in rat cortical cell culture where it plays a detrimental role by participating in the intracellular events leading to cell death after NMDA receptor activation (9). In support of this mechanism of toxicity, inhibition of excitatory amino acid neurotransmitters (7) or NMDA receptors by MK-
Among the biochemical mechanisms by which NO could participate in CNS O₂ toxicity are its reduct reactions with thiols or metals in the cell (30). NO reacts rapidly with superoxide to form peroxynitrite anion (ONOO⁻) and slowly with molecular O₂ to form nitrogen dioxide (NO₂). These secondary products are strong oxidants and potent toxins in biological systems (8). Superoxide generation is known to increase as a by-product of metabolism during hyperoxia. At a NO concentration of only 3 nM, a concentration close to that encountered in physiological conditions, the NO-superoxide reaction rate equals the sum of the rates of the other known superoxide degradation reactions. Based on the chemistry of NO, it seems likely that its half-life is regulated by ROS that increase in proportion to brain PO₂. Observations in isolated vessels strongly suggest that superoxide decreases the concentration of NO, favoring an increase in arterial tone (16). Also, transgenic mice overexpressing extracellular superoxide dismutase in the brain are more sensitive to CNS O₂ toxicity (23); the latter effect can be overcome by NOS inhibition (23). The rCBF data reported in the present in vivo study are completely consistent with such a mechanism of O₂-dependent vasoconstriction. Elevated production of superoxide during HBO₂ exposure under the conditions of our study may have been responsible for the attenuated rCBF responses to NO donors at 100% O₂ and the loss of response in blood flow at 3 ATA. If NO released from GSNO or Hb-SNO was inactivated directly, because NO and O₂ react so rapidly with each other.

In conclusion, our results provide new observations regarding the extent to which endogenous NO synthesis contributes to CNS O₂ toxicity. The data support the hypothesis that the protective effects of inhibition of NO synthesis on CNS O₂ toxicity reflect a multifaceted role of NO on blood flow and neurotransmission in the brain. These include the regulatory effects of NO on cerebral vascular tone and perhaps its role in modulating the excitatory effects of amino acid neurotransmitters in CNS O₂ toxicity.

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