Regulation of the Brain’s Vascular Responses to Oxygen

Ivan T. Demchenko, Tim D. Oury, James D. Crapo, Claude A. Piantadosi

Abstract—The mechanism of oxygen-induced cerebral vasocostriction has been sought for more than a century. Using genetically altered mice to enhance or disrupt extracellular superoxide dismutase (EC-SOD, SOD3), we tested the hypothesis that this enzyme plays a critical role in the physiological response to oxygen in the brain by regulating nitric oxide (NO) availability. Cerebral blood flow responses in these genetically altered mice to changes in PO2 demonstrate that SOD3 regulates equilibrium between superoxide (-O2-) and NO, thereby controlling vascular tone and reactivity in the brain. That SOD3 opposes inactivation of NO is shown by absence of vasoconstriction in response to PO2 in the hyperbaric range in SOD3+/+ mice, whereas NO-dependent relaxation is attenuated in SOD3−/− mutants. Thus, EC-SOD promotes NO vasodilation by scavenging -O2- while hyperoxia opposes NO and promotes constriction by enhancing endogenous -O2- generation and decreasing basal vasodilator effects of NO. (Circ Res. 2002;91:1036-1047)

Key Words: cerebral blood flow • hyperoxia • superoxide dismutase • nitric oxide • peroxynitrite

A physiological role for extracellular superoxide dismutase (EC-SOD, SOD3), which catalyzes superoxide (-O2-) dismutation to hydrogen peroxide and oxygen (O2) extracellularly, has never been demonstrated experimentally.1–5 Because of relatively low content in most tissues and present at roughly the same activity as SOD1,8,9 in arterial wall is approximately 10-fold higher than in other microenvironment concentrations of ·O2. Hence, we have proposed a hypothesis that this enzyme plays a critical role in the physiological response to oxygen in the brain by regulating nitric oxide availability. Using mice that overexpress (+/+ or lack (−/−) SOD3, we performed experiments to determine whether the enzyme regulates the balance between -O2- and NO in the brain, and how the two radicals affect basal tone and vasoconstrictor responses to increasing O2 concentrations. SOD3+/+ and SOD3−/− mice were compared for blood flow changes in specific brain regions (rCBF) in response to PO2 changes. rCBF was correlated by cerebral microdialysis with NO release (NOx), and a biochemical marker, 3-nitrotyrosine, to assess ONOO− as a product of SOD3-controlled reactions between -O2- and NO.

Materials and Methods

Mice Strains

The SOD3 mutant strains have been described and characterized.15,21 SOD3+/+ mice were generated by microinjecting nuclei of fertilized oocytes from (C57BL/6) (C3H) F1 female mice with cDNA of human SOD3 containing a β-actin promoter. Mice carrying the transgene were identified by polymerase chain reaction (PCR) amplification of the human SOD3 gene and backcrossed into (C57BL/6×C3H) F1 mice. SOD3 transgenic mice were out-bred to improve fertility. The EC-SOD gene was disrupted in Neo−-generated SOD3-deficient mice (SOD3−/−). Null mice were bred into WT C57BL/6 mice to yield heterozygotes, which were crossed to produce homozy-
TABLE 1. Blood Pressure and rCBF After L-NAME

<table>
<thead>
<tr>
<th>Group</th>
<th>MABP (mm Hg)</th>
<th>PC</th>
<th>HC</th>
<th>CP</th>
<th>SN</th>
<th>MABP</th>
<th>PC</th>
<th>HC</th>
<th>CP</th>
<th>SN</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>91 ± 6</td>
<td>78 ± 7</td>
<td>73 ± 6</td>
<td>79 ± 8</td>
<td>91 ± 9</td>
<td>109 ± 10</td>
<td>60 ± 5</td>
<td>57 ± 6</td>
<td>59 ± 6</td>
<td>69 ± 6</td>
</tr>
<tr>
<td>SOD3&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>93 ± 9</td>
<td>77 ± 6</td>
<td>68 ± 6*</td>
<td>74 ± 7*</td>
<td>87 ± 9</td>
<td>711 ± 10</td>
<td>61 ± 5</td>
<td>55 ± 7</td>
<td>60 ± 5</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>SOD3&lt;sup&gt;+/−&lt;/sup&gt;</td>
<td>89 ± 8</td>
<td>84 ± 8</td>
<td>81 ± 7</td>
<td>88 ± 9</td>
<td>96 ± 10</td>
<td>115 ± 11</td>
<td>64 ± 6</td>
<td>63 ± 8</td>
<td>61 ± 6</td>
<td>74 ± 8</td>
</tr>
</tbody>
</table>

MABP indicates mean arterial BP (mm Hg) before and 30 minutes after L-NAME; rCBF (mL/100g per min) in parietal cortex (PC), hippocampus (HC), caudate putamen (CP), and substantia nigra (SN) before and 30 minutes after L-NAME. WT is averaged data set for two strains (n=14).

Values are mean ± SE; *P<0.05 vs SOD3<sup>−/−</sup>. All MABP and rCBF values after L-NAME are significantly different than before L-NAME (no symbols).

Animal Studies

The Institutional Animal Care and Use Committee approved the protocol. At 10 to 12 weeks of age, mice were anesthetized with urethane (800 mg/kg, IP) followed by continuous infusion of 1/4 initial dose/hour by IP pump. Anesthetized mice underwent tracheotomy and ventilation (tidal volume=0.4 mL, rate 110/min) with 30% O<sub>2</sub> (balance N<sub>2</sub>) and paralysis (pancuronium 0.1 mg/kg, IV). The head was fixed in a stereotaxic frame, and hydrogen/oxygen-sensitive microelectrodes inserted through a burr hole into substantia nigra (SN), caudate putamen (CP), hippocampus (HC), and/or parietal cortex (PC). Electrodes were made of platinum wire of 100-μm diameter insulated with epoxy except 4.0 mm at the pointed tip (15 to 20 μm) covered by Nafion to prevent fouling. In some mice, a microdialysis probe (CMA/10: 2-mm membrane, 0.24-mm OD, CMA/AB, Sweden) was inserted into CP and perfused with artificial CSF at 2 μL/min using a microinjection pump. For EEG recording, stainless steel screws were inserted into the skull symmetrically over parietal cortex.

Regional Cerebral Blood Flow and Brain PO<sub>2</sub>

Regional cerebral blood flow (rCBF) was measured by a H<sub>2</sub> clearance method. Platinum electrodes were inserted into brain and reference Ag–AgCl electrodes attached to the base of the tail. H<sub>2</sub> (2.5%) was added via the respirator for 60 seconds then switched to the base breathing gas and the H<sub>2</sub>-washout curve recorded. Absolute values of CBF (mL · g<sup>−1</sup> · min<sup>−1</sup>) were calculated by initial slope using a WINDAQ data system (DATAQ Inst) and Mathematica 3.0 software (Wolfram Research, Inc) with minor modification. CBF data from the two WT strains, C57BL/6 (n=6) and C57BL/6CH/F1 (n=9), were compared for baseline flow and responses to HBO<sub>2</sub>, CO<sub>2</sub>, or L-NAME. There were no significant differences in the mean values; therefore, nontransgenic C57BL/6CH/F1 littermates were used as WT unless indicated otherwise.

Brain PO<sub>2</sub>, during HBO<sub>2</sub> exposures was measured continuously in CP of rats using the same microelectrodes as for rCBF measurements. PO<sub>2</sub> electrode calibration was performed with 100% N<sub>2</sub> and 20% O<sub>2</sub> at 1 ATA and 100% O<sub>2</sub> at 5 ATA. Absolute values for brain PO<sub>2</sub> were calculated from a calibration curve for each electrode.

Measurement of Interstitial NO<sup>·</sup> and Peroxynitrite

Interstitial NO was estimated from the microdialysis yield of nitrite and nitrate (NO<sub>x</sub>). After system equilibration, dialysate was collected every 15 minutes before and during HBO<sub>2</sub>. Samples were assayed for NO<sub>x</sub> after catalytic reduction to NO gas and chemiluminescence detection. Nitrite and nitrate signals, quantitatively linear between 25 and 500 pmol/25 μL, were used for calibrations. Recovery efficiency for the dialysis probes were 31±6% and 29±8% for NO<sub>2</sub> and NO<sub>3</sub>, respectively. The data were expressed as [μmol/L] nitrite and nitrate (NO<sub>x</sub>). The amount of 3-nitrotyrosine (3-NTyr) in dialysate was measured relative to tyrosine by HPLC and electrochemical detection in timed samples and retention time compared with authentic standards. 3-NTyr values were expressed as 3-NTyr/Tyr ×100.

Hyperbaric O<sub>2</sub> Exposures

Hyperbaric O<sub>2</sub> (HBO<sub>2</sub>) exposures were used to maximize O<sub>2</sub>-induced vasoconstriction in the brain, which in mice occurs at 4 to 5 atmospheres absolute (ATA). Mice were instrumented and placed in a chamber containing the respirator, EEG amplifier, pressure transducer and amplifier, heating pad, and microdialysis system. After placing electrodes in the brain, the animals were recovered for 60 minutes. For microdialysis studies, the recovery period was 2 hours. Once the mice were stable and blood gases and temperature in the physiological range, three control H<sub>2</sub> clearance curves were recorded and three dialysate samples collected. The respirator was connected to 100% O<sub>2</sub> and the chamber compressed to a maximum pressure of 5 ATA at 0.6 ATA per min. During HBO<sub>2</sub>, rCBF was measured every 15 minutes using a remotely operated pneumatic system for H<sub>2</sub> delivery. Brain PO<sub>2</sub>, EEG and blood pressure were monitored continuously. Body temperature was maintained between 36° and 37°C. Dialysate was collected every 15 minutes and NO<sub>x</sub> and 3-NTyr measured later. Arterial blood was taken at 1 ATA to determine PaO<sub>2</sub>, PaCO<sub>2</sub>, and pH while the mice breathed 100% O<sub>2</sub>.

Experimental Design

The hypothesis was tested first by evaluating differences in basal rCBF and responses to pharmacological agents known to alter brain NO and O<sub>2</sub> availability. These studies were conducted in mice breathing 30% O<sub>2</sub> (balance N<sub>2</sub>) at sea level. Then, time-dependent measurements of rCBF, EEG, and physiological variables were made in mice exposed to HBO<sub>2</sub> at 3 and 5 ATA. Timed accumulation of NO<sub>x</sub> and 3-NTyr was correlated with rCBF responses and EEG activity.

Statistical Analysis

Group values are given as mean ± SE. Group sizes for mutant strains were 6 to 9 animals each. Changes in rCBF and physiological variables were compared using ANOVA followed by post hoc comparison (Fisher’s exact test) or unpaired t test using computer software. A value of P<0.05 was accepted as significant.

Results

Modulation of Basal CBF by EC-SOD

Using hydrogen clearance, rCBF was measured in WT, SOD3<sup>−/−</sup>, or SOD3<sup>+/−</sup> mice before and after interventions designed to unbalance the equilibrium between ·O<sub>2</sub>·<sup>−</sup> and NO. Resting rCBF in WT and mutant strains were similar in the same brain regions, but lower absolute values were observed in SOD3<sup>−/−</sup> than SOD3<sup>+/−</sup> mice in CP and HC (Table 1). CBF responses were statistically identical in the inbred and out-
bred WT mice (data not shown); therefore, these values were pooled into one control group of 14 animals. To determine whether SOD3 modulates NO-mediated basal tone, we measured rCBF after treating mice with NOS inhibitor, L-NAME to block endothelial and neuronal NO synthesis. rCBF was reduced significantly in a L-arginine–dependent manner in all groups of L-NAME–treated mice, but SOD3+/− mice showed larger decreases in rCBF in selected brain regions (Table 1). These data indicate that basal tone in brain vessels depends on both NO availability and SOD3 activity. To confirm this assertion we measured changes in rCBF after infusing mice intravenously with a high concentration of SOD1 (10 000 U/kg body weight) to reduce ·O₂⁻ at the endothelial surface. Preliminary studies showed that active but not inactive SOD1 produced dose- and time-dependent increases in rCBF in WT mice: rCBF responses were reproducible and statistically significant after SOD1 infusion and reached maximal amplitude within 10 minutes (Figures 1a and 1b). This SOD1 dose and time was used for rCBF measurement in mutant mice. In mice treated with SOD1, rCBF in SN, CP, and PC increased significantly in WT, SOD3+/−, and SOD3−/− mice, but significantly less in SOD3+/+ mice than SOD3+/− mice (Figure 1c). Because these increases in rCBF after SOD1 treatment could reflect either an increase in NO availability in vessels due to ·O₂⁻ removal or a dilator effect of the enzyme, we tested these possibilities by treating the mice with L-NAME to inhibit endothelial and neuronal NOS. Infusion of SOD1 into WT and mutant mice pretreated with L-NAME had no effect on rCBF (Figure 1d), suggesting SOD1 altered rCBF by preventing ·O₂⁻–mediated inactivation of NO. SOD3−/− mice have higher extracellular levels of ·O₂⁻ compared with SOD3+/− mice, thus SOD1 infusion has less influence on the equilibrium between ·O₂⁻ and NO in SOD3+/− animals.

**Figure 1.** CBF in CuZn-SOD in WT and SOD3 mutant mice. **a,** Dose-response curve of rCBF in caudate of WT mice after CuZn-SOD injection (0.1 to 100 U/g body weight IV). *P < 0.05 vs pretreatment.** **b,** Time-course of rCBF in substantia nigra (SN), caudate (CP), and parietal cortex (PC) of WT mice after CuZn-SOD (10 U/g, IV). *P < 0.05 vs pretreatment. **c and d,** CuZn-SOD and rCBF in SN, CP, and PC in pooled WT, SOD3−/−, and SOD3+/− mice. **c,** CBF responses to CuZn SOD are greater in SOD3+/− mice than SOD3+/− mice. **P < 0.05 vs pretreatment;** +P < 0.05 vs SOD3−/−. **d,** rCBF responses to CuZn-SOD after NOS inhibition with L-NAME (30 mg/kg, IP) were attenuated equally in both mutants and WT mice.

**Functional Implications of EC-SOD in Endothelium-Derived Relaxation**

Endothelium-derived relaxation is a physiological response of vessels to stimuli that activate NO signals. If ·O₂⁻ opposes endothelium-derived relaxation, then NO-mediated cerebrovascular responses will depend critically on vascular SOD3 activity. To determine the extent to which SOD3 contributes to endothelium-derived vasodilation, we compared rCBF responses in SOD3+/− and SOD3−/− mice to an NO donor and to other stimuli that operate via NO-dependent mechanisms. Representative data are shown in Figure 2.

The NO donor PAPA NONOate (at ≈0.1 mg/kg) increased rCBF in WT and both genetic strains of mice, but rCBF responses in SOD3+/− mutant were more pronounced and significantly greater than that of SOD3−/− mice (representative data in Figure 2a). At higher NONOate concentrations, SOD−/− mice also were found to have attenuated CBF responses relative to the WT, but these infusions caused hypotension in SOD−/− mice (data not shown).

The role of EC-SOD in vascular CO₂ reactivity was examined by measuring the rCBF responses in WT and mutant mice to inhaled CO₂. The use of 5% CO₂ produced rCBF increases on the flat part of the CO₂ response curve and relatively rapid reversal of the CBF effect allowed multiple interventions per animal (Figure 2b). WT and both genetically altered mouse strains showed rCBF increases of 17% to 87% to 5% CO₂ relative to baseline, but the greatest increases (in striatum) were observed in SOD3+/− mice. This response was greater than that of SOD3−/− mutants (Figure 2b). Increases in rCBF to inhaled CO₂ were attenuated significantly and to approximately the same extent in WT and both mutant strains pretreated with L-NAME (data not shown). In mice given acetylcholine, arterial blood pressure decreased slightly and transiently for about 5 minutes followed by a peak rCBF response within 10 minutes. The peak response was...
augmented significantly when a constant dose of acetylcholine (0.05 mg/kg) was administered to SOD3$^{+/+}$ mice, whereas it was attenuated in SOD3$^{−/−}$ mice (Figure 2c). WT controls had similar responses to acetylcholine (not shown). NOS inhibitor, L-NAME completely blocked the rCBF responses to acetylcholine (Figure 2d). The data of Figure 2 collectively support the interpretation that EC-SOD in mouse brain modulates NO-dependent vasodilation via ·O$_2^−$-dependent regulation of NO availability.

Role of EC-SOD in regulating cerebrovascular responses to hyperoxia. We assessed regulation of the vasoconstrictor response to O$_2$ by SOD3 by exposing mice to O$_2$ during HBO$_2$ to increase brain ·O$_2^−$ production. We report here rCBF in WT and mutant mice exposed to HBO$_2$ that showed stable, normal arterial BP, P$_{CO_2}$, and pH values for 60 minutes at 3 and 5 atmospheres absolute (ATA) (Tables 1 and 2). HBO$_2$ altered rCBF in an O$_2$ dose- and time-dependent manner unrelated to the change in hydrostatic pressure per se (data not shown). Mean blood pressure in mice at 3 or 5 ATA rose during the first few minutes of HBO$_2$ exposure and remained elevated until decompression. At 3 ATA, EEG discharges of HBO$_2$ toxicity were not apparent. At 5 ATA, HBO$_2$ produced marked differences in EEG pattern in all strains consisting of paroxysmal EEG discharges of single or multiple spiking activity of amplitude >100 μV. These changes were observed after HBO$_2$ exposures of 40±4 minutes in SOD3$^{+/+}$ mice, 49±5 minutes in SOD3$^{−/−}$ mice, and 56±6 minutes in WT mice consistent with the enhanced susceptibility reported for both mutant strains (Figure 3a).

At 3 ATA, the rCBF flow in cortex and deep brain structures decreased significantly over 60 minutes of HBO$_2$ in WT and SOD3$^{−/−}$ mice. At 30 to 45 minutes, vasoconstriction reached maximum where it was maintained until decompression (Figure 3b). In contrast, rCBF responses in SOD3$^{+/+}$ mice to HBO$_2$ were minimal and not statistically different from preexposure levels (Figure 3b). At 5 ATA, HBO$_2$ decreased rCBF in WT and SOD3$^{−/−}$ mice over the initial 30 minutes, but then rCBF gradually rose. By 45 minutes, rCBF had returned to preexposure levels and over the next 15 minutes increased above baseline (Figure 3c). This secondary increase in rCBF always preceded abnormal EEG discharges. SOD3$^{+/+}$ mice did not show significant cerebral vasoconstriction at 5 ATA of HBO$_2$, and within 30 minutes rCBF had increased significantly. After 60 minutes of HBO$_2$, rCBF had increased 57% above control values (Figure 3c).

### TABLE 2. Arterial Blood Gases After HBO$_2$ at 3 and 5 ATA

<table>
<thead>
<tr>
<th>Group</th>
<th>PaO$_2$ (mm Hg)</th>
<th>PaCO$_2$ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ATA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>112±11/412±21</td>
<td>38±2/42±2</td>
<td>7.43/7.29</td>
</tr>
<tr>
<td>SOD3$^{−/−}$</td>
<td>109±8/397±17</td>
<td>36±1/40±2</td>
<td>7.39/7.36</td>
</tr>
<tr>
<td>SOD3$^{+/+}$</td>
<td>106±10/421±22</td>
<td>36±2/41±2</td>
<td>7.42/7.31</td>
</tr>
<tr>
<td>5 ATA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>107±9/398±19</td>
<td>36±2/40±2</td>
<td>7.42/7.39</td>
</tr>
<tr>
<td>SOD3$^{−/−}$</td>
<td>105±8/383±18</td>
<td>37±1/41±2</td>
<td>7.41/7.29</td>
</tr>
<tr>
<td>SOD3$^{+/+}$</td>
<td>111±11/411±24</td>
<td>37±2/39±2</td>
<td>7.35/7.25</td>
</tr>
</tbody>
</table>

PaO$_2$ (mm Hg), PaCO$_2$ (mm Hg), and pH measured before and immediately after HBO$_2$ exposure at 3 or 5 ATA. Mice breathed 30% O$_2$ before and 100% O$_2$ after HBO$_2$.

Blood gases are mean±SE; pH is mean (SE<0.02 units).
PO$_2$ values in the striatum of WT mice and SOD3 mutant strains breathing 30% O$_2$ at 1 ATA ranged from 24 to 45 mm Hg. During compression, PO$_2$ progressively rose and after one minute at 5 ATA ranged from 497 to 738 mm Hg. During HBO$_2$ exposure, PO$_2$ increased gradually and immediately before EEG spikes reached values of 1276 to 2197 mm Hg. No significant differences in PO$_2$ responses to HBO$_2$ were observed among strains of mice.

The rCBF responses to HBO$_2$ in these mice collectively are consistent with the SOD3 mechanism of O$_2$- dependent regulation of NO availability. This was confirmed by measuring the effects of HBO$_2$ on CBF after treating mice with L-NAME. L-NAME decreased rCBF equally in all strains of mice breathing air (data not shown). During exposure to HBO$_2$ at 5 ATA, rCBF remained at or near the control level during HBO$_2$ exposure, P O$_2$ values in the striatum of WT mice and SOD3 mutant mice overexpressing or lacking SOD3 exposed to 5 ATA (Figure 4a) indicating HBO$_2$-mediated increases in ONOO$^-$ related differences in NO varied with EC-SOD expression in different strains.

Brain interstitial 3-NTyr content, unlike NO$_x$, accumulated steadily at different rates during HBO$_2$ in WT and SOD3 mutant mice exposed to 5 ATA (Figure 4b). Marked increases in 3-NTyr were detected in WT and SOD3$^{-/-}$ mice at 5 ATA consistent with an NO-O$_2$-dependent mechanism of ONOO$^-$ formation. This was confirmed by less accumulation of 3-NTyr in striatum of mice pretreated before HBO$_2$ with L-NAME. Thus, HBO$_2$-mediated increases in ONOO$^-$ production at 5 ATA were blocked by NOS inhibition to a similar degree in both mutants (Figure 4c).

EC-SOD Regulates Interstitial NO and O$_2^-$ Equilibrium in the Brain

Intracerebral microdialysis experiments revealed dynamic changes in equilibrium between NO and O$_2$- in brain extracellular fluid as a function of EC-SOD expression. We measured interstitial NO$_x$ to assess NO release and 3-NTyr to assess ONOO$^-$ formation in mice at 5 ATA. Basal interstitial NO$_x$ in the striatum of WT mice was stable at 3.9±0.4 μmol/L, in SOD3$^{-/-}$ and SOD3$^{+/+}$ mice these values were 3.8±0.5 μmol/L and 4.3±0.6 μmol/L, respectively. Within 30 minutes at 5 ATA, brain interstitial NO$_x$ decreased significantly. Thereafter, NO$_x$ gradually increased to above control levels (Figure 4a). The recovery of NO$_x$ in brain interstitial fluid at 5 ATA was remarkably different in SOD3$^{+/+}$ and SOD3$^{-/-}$ mice. In SOD$^{+/+}$ mice, NO$_x$ levels gradually rose during 60 minutes of HBO$_2$ (Figure 4a). In SOD$^{-/-}$ mice, NO$_x$ decreased significantly over 30 minutes at 5 ATA and then stabilized (Figure 4a) indicating HBO$_2$-mediated increases in ONOO$^-$ related differences in NO varied with EC-SOD expression in different strains.

These physiological measurements in genetically altered mice provide definite evidence that SOD3 regulates rCBF in vivo by controlling the equilibrium between NO and O$_2^-$ in the extracellular brain space. Furthermore, SOD3 regulates both basal tone and NO-mediated rCBF responses during increases in PO$_2$. The different rCBF responses to raising the PO$_2$ of mice that overexpress or lack SOD3 supports the central hypothesis of the study: oxygen alters the equilibrium between O$_2^-$ and NO, which regulates vessel tone and blood flow. Enhanced -O$_2^-$ scavenging by SOD3 augments the relaxing effect of NO on vascular smooth muscle. Because NO is a central regulator of vascular tone, extracellular -O$_2^-$ production functionally reduces the influence of eNOS on guanylate cyclase in smooth muscle. As a major vascular sink for extracellular -O$_2^-$, SOD3 activity moderates the vasodilator effect of NO on cerebral vessels. Evidence for this premise...
which allowed SOD1 entry into parenchyma. However, ·O2 blood brain barrier (BBB) damage by electrode insertion, inactivated SOD1. This response could be related in part to ·O2 inactivation of SOD1 is consistent with less ·O2 toxicity than WT animals. When inactivation of NO by ·O2 scavenging were increased. Notably, rCBF responses to SOD3 mice first show less interstitial NOx in HBO2 at 5 ATA followed by an increase in NOx to above control. By comparison, SOD3 mice consistently show elevated brain NO levels in HBO2 at 5 ATA. The directional changes in NO metabolites are appropriate to the O2-dependent rCBF responses, and these measurements are the first in vivo evidence that O2 causes cerebral vasconstriction by interfering with NO.

Oxygen may inactivate NO because, in addition to heme and thiol interactions, NO residence time shows dependence on Po2 and rate of O2 generation. At the onset of HBO2, hemoglobin and thiol pools are stable while brain Po2 and ·O2 production rate increase in proportion to Po2. If [O2] greatly exceeds [NO], NO and NOx are produced by autoxidation of NOx. If autoxidation had been a major factor in NOx formation, high instead of low NOx would have been expected. On the other hand, the transient decrease in NOx most readily explained by a decrease inextracellualr NO from rapid inactivation by ·O2. This effect is also qualitatively independent of any effect of CBF on recovery of the compounds by dialysis because decreases in CBF tend to increase NOx recovery and vice versa.

NO and ·O2 also produce ONOO− in a nearly diffusion limited reaction, and at steady ·O2 flux, ONOO− is formed almost as rapidly as NO is released. This chemistry predicts NO activity is regulated directly by ·O2 rather than by independent and opposite effects of NO and ·O2 on tone. The lack of difference in O2-dependent vasconstriction between WT and EC-SOD−/− mice and the lack of a greater fall in rCBF in HBO2 after L-NAME in EC-SOD mice support this concept because HBO2 generates ·O2. It is not possible to assess the reaction of NO and ·O2 directly in brain, but the initial decline in NOx with HBO2 suggests NO inactivation by increased ·O2 production. HBO2-mediated decreases in rCBF were not observed in SOD3−/− mice, which together with increased NOx and decreased 3-NT are compatible with SOD3 opposition of NO inactivation.

One difference between this study and several earlier ones is that basal rCBF in these mice responded to active and not inactivated SOD1. This response could be related in part to blood brain barrier (BBB) damage by electrode insertion, which allowed SOD1 entry into parenchyma. However, ·O2 and NO flux are regulated on both sides of a thin endothelium, and a large sink for ·O2 within vessels might well increase NO flux on the other side of the barrier. NO released by RBCs also might have a greater dilator effect if ·O2 scavenging were increased. Notably, rCBF responses to SOD1 were abolished completely in mice pretreated with L-NAME, suggesting the vasoactivity of SOD1 was NO-dependent and did not require BBB damage. In any event, rCBF response to SOD1 infusion in SOD3−/− mice was significantly greater than in SOD3−/− animals.

A consistent finding in mice exposed to HBO2 is that seizure onset is accelerated in SOD3−/−, and SOD3−/− mice brought forth by interventions to alter the equilibrium between NO and ·O2. These interventions yield the predicted differences in rCBF response in the mutants. SOD3−/− mice treated with an NO donor show more pronounced rCBF responses than in SOD3−/− mutants, which is consistent with less ·O2 bioactivity in cerebral vessels of SOD3−/− and a lower tendency for NO inactivation, thus, we surmise that SOD3−/− mice have higher steady-state vascular ·O2 levels, which allow a more substantial increase in the NO effect when SOD1 is administered.

One difference between this study and several earlier ones is that basal rCBF in these mice responded to active and not inactivated SOD1. This response could be related in part to blood brain barrier (BBB) damage by electrode insertion, which allowed SOD1 entry into parenchyma. However, ·O2 and NO flux are regulated on both sides of a thin endothelium, and a large sink for ·O2 within vessels might well increase NO flux on the other side of the barrier. NO released by RBCs also might have a greater dilator effect if ·O2 scavenging were increased. Notably, rCBF responses to SOD1 were abolished completely in mice pretreated with L-NAME, suggesting the vasoactivity of SOD1 was NO-dependent and did not require BBB damage. In any event, rCBF response to SOD1 infusion in SOD3−/− mice was significantly greater than in SOD3−/− animals.

A consistent finding in mice exposed to HBO2 is that seizure onset is accelerated in SOD3−/−, and SOD3−/− mice brought forth by interventions to alter the equilibrium between NO and ·O2. These interventions yield the predicted differences in rCBF response in the mutants. SOD3−/− mice treated with an NO donor show more pronounced rCBF responses than in SOD3−/− mutants, which is consistent with less ·O2 bioactivity in cerebral vessels of SOD3−/− and a lower tendency for NO inactivation.

One difference between this study and several earlier ones is that basal rCBF in these mice responded to active and not inactivated SOD1. This response could be related in part to blood brain barrier (BBB) damage by electrode insertion, which allowed SOD1 entry into parenchyma. However, ·O2 and NO flux are regulated on both sides of a thin endothelium, and a large sink for ·O2 within vessels might well increase NO flux on the other side of the barrier. NO released by RBCs also might have a greater dilator effect if ·O2 scavenging were increased. Notably, rCBF responses to SOD1 were abolished completely in mice pretreated with L-NAME, suggesting the vasoactivity of SOD1 was NO-dependent and did not require BBB damage. In any event, rCBF response to SOD1 infusion in SOD3−/− mice was significantly greater than in SOD3−/− animals.

A consistent finding in mice exposed to HBO2 is that seizure onset is accelerated in SOD3−/−, and SOD3−/− mice brought forth by interventions to alter the equilibrium between NO and ·O2. These interventions yield the predicted differences in rCBF response in the mutants. SOD3−/− mice treated with an NO donor show more pronounced rCBF responses than in SOD3−/− mutants, which is consistent with less ·O2 bioactivity in cerebral vessels of SOD3−/− and a lower tendency for NO inactivation.

One difference between this study and several earlier ones is that basal rCBF in these mice responded to active and not inactivated SOD1. This response could be related in part to blood brain barrier (BBB) damage by electrode insertion, which allowed SOD1 entry into parenchyma. However, ·O2 and NO flux are regulated on both sides of a thin endothelium, and a large sink for ·O2 within vessels might well increase NO flux on the other side of the barrier. NO released by RBCs also might have a greater dilator effect if ·O2 scavenging were increased. Notably, rCBF responses to SOD1 were abolished completely in mice pretreated with L-NAME, suggesting the vasoactivity of SOD1 was NO-dependent and did not require BBB damage. In any event, rCBF response to SOD1 infusion in SOD3−/− mice was significantly greater than in SOD3−/− animals.

A consistent finding in mice exposed to HBO2 is that seizure onset is accelerated in SOD3−/−, and SOD3−/− mice brought forth by interventions to alter the equilibrium between NO and ·O2. These interventions yield the predicted differences in rCBF response in the mutants. SOD3−/− mice treated with an NO donor show more pronounced rCBF responses than in SOD3−/− mutants, which is consistent with less ·O2 bioactivity in cerebral vessels of SOD3−/− and a lower tendency for NO inactivation.

One difference between this study and several earlier ones is that basal rCBF in these mice responded to active and not inactivated SOD1. This response could be related in part to blood brain barrier (BBB) damage by electrode insertion, which allowed SOD1 entry into parenchyma. However, ·O2 and NO flux are regulated on both sides of a thin endothelium, and a large sink for ·O2 within vessels might well increase NO flux on the other side of the barrier. NO released by RBCs also might have a greater dilator effect if ·O2 scavenging were increased. Notably, rCBF responses to SOD1 were abolished completely in mice pretreated with L-NAME, suggesting the vasoactivity of SOD1 was NO-dependent and did not require BBB damage. In any event, rCBF response to SOD1 infusion in SOD3−/− mice was significantly greater than in SOD3−/− animals.

A consistent finding in mice exposed to HBO2 is that seizure onset is accelerated in SOD3−/−, and SOD3−/− mice brought forth by interventions to alter the equilibrium between NO and ·O2. These interventions yield the predicted differences in rCBF response in the mutants. SOD3−/− mice treated with an NO donor show more pronounced rCBF responses than in SOD3−/− mutants, which is consistent with less ·O2 bioactivity in cerebral vessels of SOD3−/− and a lower tendency for NO inactivation.
In WT mice at 5 ATA, the initial decrease in interstitial NOx was followed by an increase to above control in concert with increases in rCBF. This observation suggests that prolonged HbO2 exposure promotes NO production, which augments CBF.22 Because NOS requires O2 it is possible that [O2] is limiting,32 and HbO2 stimulates NOS activity by increasing cerebral Po2. HbO2 also elevates brain l-arginine, the substrate for NOS.33 L-arginine synthesis is stimulated by ·O222 augments CBF. Because NOS requires O2 it is possible that NOx was followed by an increase to above control in concert with increases in rCBF. This work was supported by grants from NHLBI and the Office of Naval Research.

In summary, O2 vasoconstriction defines a physiological role for SOD3 in regulating CBF in vivo. SOD3 is present in high concentration in vessels where NO is important for vascular relaxation. Inactivation by O2− interferes with NO-dependent basal tone and vasorelaxation. Scavenging of ·O222 by EC-SOD plays a critical role in NO CBF function as shown by predictable differences in rCBF response to high Po2 in mice with varying enzyme expression. These findings have implications in vascular diseases where extracellular ·O222 production exceeds SOD3 function, diminishing responsiveness to NO, and allowing production of oxidative/nitrosative stress. The strategic location of vascular SOD3 in the brain between endothelium and smooth muscle preserves and regulates the endogenous dilator function of NO.

Acknowledgments

This work was supported by grants from NHLBI and the Office of Naval Research.

References

Regulation of the Brain's Vascular Responses to Oxygen
Ivan T. Demchenko, Tim D. Oury, James D. Crapo and Claude A. Piantadosi

Circ Res. published online October 24, 2002;
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/early/2002/10/24/01.RES.0000043500.03647.81.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/