

Effects of striatal nitric oxide production on regional cerebral blood flow and seizure development in rats exposed to extreme hyperoxia

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Gasier HG, Demchenko IT, Allen BW, Piantadosi CA. Effects of striatal nitric oxide production on regional cerebral blood flow and seizure development in rats exposed to extreme hyperoxia. *J Appl Physiol* 119: 1282–1288, 2015. First published September 3, 2015; doi:10.1152/jappphysiol.00432.2015.—The endogenous vasodilator and signaling molecule nitric oxide has been implicated in cerebral hyperemia, sympathoexcitation, and seizures induced by hyperbaric oxygen (HBO₂) at or above 3 atmospheres absolute (ATA). It is unknown whether these events in the onset of central nervous system oxygen toxicity originate within specific brain structures and whether blood flow is diverted to the brain from peripheral organs with high basal flow, such as the kidney. To explore these questions, total and regional cerebral blood flow (CBF) were measured in brain structures of the central autonomic network in anesthetized rats in HBO₂ at 6 ATA. Electroencephalogram (EEG) recordings, cardiovascular hemodynamics, and renal blood flow (RBF) were also monitored. As expected, mean arterial blood pressure and total and regional CBF increased preceding EEG spikes while RBF was unaltered. Of the brain structures examined, the earliest rise in CBF occurred in the striatum, suggesting increased neuronal activation. Continuous unilateral or bilateral striatal infusion of the nitric oxide synthase inhibitor *N*^ω-nitro-L-arginine methyl ester attenuated CBF responses in that structure, but global EEG discharges persisted and did not differ from controls. Our novel findings indicate that: 1) cerebral hyperemia in extreme HBO₂ in rats does not occur at the expense of renal perfusion, highlighting the remarkable autoregulatory capability of the kidney, and 2) in spite of a sentinel increase in striatal blood flow, additional brain structure(s) likely govern the pathogenesis of HBO₂-induced seizures because EEG discharge latency was unchanged by local blockade of striatal nitric oxide production and concomitant hyperemia.

central autonomic network; renal blood flow; nitric oxide; *N*^ω-nitro-L-arginine methyl ester; neuroexcitation

BREATHING HYPERBARIC OXYGEN (HBO₂) at or above 3 atmospheres absolute (ATA) induces biphasic cardiovascular and autonomic responses that are initially protective, but ultimately contribute to the development of central nervous system (CNS) and pulmonary O₂ toxicity. The initial phase is mediated, in part, by activation and increased sensitivity of the arterial baroreflex, resulting in decreases in heart rate, cardiac output, cerebral blood flow (CBF), and sympathetic drive (19, 22). Eventually, however, impairment in baroreflex function is accompanied by an increase in sympathetic outflow, parasympatholysis, hyperventilation, hypertension, and cerebral hyperemia. The consequences of this second phase can include seizures, lung injury, and death (4–7, 20, 23, 39, 40).

The cerebrovascular and autonomic responses that limit O₂ delivery in putative phase I, yet increase it in phase II, ultimately involve the endogenous vasodilator and signaling molecule nitric oxide (NO). Upon initial exposure to HBO₂, the availability of NO is decreased as it reacts with the rising levels of superoxide to generate peroxynitrite, leading to cerebral vasoconstriction and a protective reduction in CBF (18). However, as the exposure continues, NO produced by both endothelial and neuronal (nNOS) nitric oxide synthases increases, resulting in cerebral hyperemia (18, 20, 31). Also, increased NO production in the CNS (mainly from nNOS) shifts the balance between excitatory (glutamate) and inhibitory [γ -aminobutyric acid (GABA)] neurotransmitters, favoring neuroexcitation and seizures, as well as autonomic activation that leads to left ventricular dysfunction, pulmonary hypertension, and lung injury (20, 21). It remains unknown in which brain structure(s) these changes first occur.

An early study pointed to the cerebral cortex as the site of seizure onset, since decorticated cats did not display seizure activity in HBO₂ (26). This hypothesis, however, was later challenged as cats decerebrated at the mesencephalon (cerveau isolé) did not display seizures, but did so when transection was performed slightly posterior to the midbrain (39). Subsequently, extensive research was conducted to explore the effects of HBO₂ on the CNS in conscious animals by chronically implanting electrodes in cortical and subcortical structures for electroencephalogram (EEG) recordings during exposures to HBO₂ at 4 ATA (40). Two EEG profiles were consistently observed: in one, seizures occurred simultaneously in subcortical and cortical structures; in the other, and most commonly documented, seizure activity first appeared in the subcortical structures and then spread to the cortex, an observation later confirmed by others (35).

We recently demonstrated that increased sympathetic outflow in phase II of the response to HBO₂ precedes EEG spikes, and that activation and enhanced sensitivity of the arterial baroreflex delays this effect (19, 22). Therefore, we propose that brain structures regulating visceral function can be implicated in seizure onset. These structures lie within the cortex, diencephalon, cerebrum, mesencephalon, and rhombencephalon and together comprise the central autonomic network (CAN), an internal regulatory system through which the brain controls visceromotor, neuroendocrine, and behavioral responses (8). Thus, regional hyperemia would be expected here, since neural activation is coupled to perfusion as demonstrated by experiments in which systemic or intracerebroventricular administration of NOS inhibitors blocks or delays neuroexcitation and subsequent excitotoxicity (18, 20). It is not known

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where in the brain the NO responsible for HBO₂-induced hyperemia and neuronal activation is produced.

Our primary objective was to determine whether there is a temporal order of activation by HBO₂ in successive structures within the CAN. Our approach was to monitor CBF responses in discrete regions within the CAN and, based on these observations, to explore whether local inhibition of NO production modifies regional CBF responses to HBO₂ and confers protection against seizures. A secondary objective was to examine whether blood is redistributed from a peripheral organ, the kidney, at the onset of cerebral hyperemia and seizure development. The kidney was selected since it receives ~25% of cardiac output under basal conditions and exhibits autoregulation similar to that in the brain (15).

METHODS AND EXPERIMENTAL DESIGN

Surgical preparation. “The experiments reported herein were conducted in compliance with the Animal Welfare Act and in accordance with the principles set forth in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Research, National Research Council, National Academies Press, 2011.” Male Sprague Dawley rats (Charles River Laboratories) weighing 340 ± 7 g were used for all experiments, according to a protocol approved by the Duke University Institutional Animal Care and Use Committee. Anesthesia was induced with urethane (750 mg/kg ip) and α -chloralose (70 mg/kg ip) before cannulation of the trachea and catheterization of the left femoral artery and vein. Anesthesia was maintained by administering one-fourth of the initial doses, as needed. Pancuronium bromide (0.5 mg/kg iv) was administered to prevent involuntary respiratory movements, and animals were ventilated with 30% O₂ in N₂. Each rat was placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), and platinum needle electrodes (100 μ m diameter) were inserted in one of the following brain regions: frontal cortex, parietal cortex (ventromedial part), striatum, hypothalamus, hippocampus, substantia nigra, cerebellum, or nucleus tractus solitarius (NTS) using published coordinates (33). A platinum disk electrode was placed on the dura mater over the confluence of venous sinuses (18). All electrodes were fixed in position with micromanipulators, and their locations were confirmed postmortem. Two cranial screws were inserted in the skull over the left and right parietal cortexes for EEG recordings. To measure renal blood flow (RBF), a dorsal incision was made to expose the left kidney, and a platinum cuff electrode was placed around the renal vein (MicroProbes, Gaithersburg, MD).

Hemodynamic and EEG recordings. Arterial blood pressure was monitored continuously using a pressure transducer (Viggo-Spectramed, Oxnard, CA). Total CBF (tCBF), regional CBF (rCBF), and RBF were measured by hydrogen clearance using platinum disk, needle, and cuff electrodes, respectively. In brief, 2.5% hydrogen in air was administered through the ventilator for ~40 s, and blood flow ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g tissue}^{-1}$) was calculated from hydrogen washout curves (1, 2). EEG was monitored, and the appearance of the first consistent train of electrical discharges (spikes) marked the onset of CNS O₂ toxicity. Physiological parameters were recorded and analyzed using LabScribe 2 software on iWorx IX-228/S hardware (iWorx Systems, Dover, NH). Mean arterial blood pressure (MAP) and heart rate (HR) were calculated from pulse waves. Cerebral vascular resistance and renal vascular resistance were calculated as MAP/tCBF and MAP/RBF, respectively.

Striatal NOS inhibition. Microdialysis probes (CMA 11, 0.24 mm in diameter and 3 mm membrane length; CMA Microdialysis) were inserted in the left and/or right striatum. Probes were perfused with artificial cerebral spinal fluid [aCSF (in mM): 150 NaCl, 3 KCl, 1.2 CaCl₂, 0.8 MgCl₂, and 31 KH₂PO₄; pH 7.4] at a flow rate of 2.0 μ l/min for 1.5–2 h using a CMA microinjection pump (Carnegie

Medicine, Stockholm, Sweden). Perfusion with aCSF was changed to *N*^ω-nitro-L-arginine methyl ester (L-NAME; 100 μ M) in aCSF 30 min before compression and continued in HBO₂. Platinum needle electrodes were collocated with the microdialysis probes, and rCBF was measured in the left and right striatum during perfusion with either aCSF or L-NAME.

HBO₂ exposures. Following baseline physiological measurements in rats breathing 30% O₂ in N₂, the ventilation gas was switched to 100% O₂, and the animals were compressed in a hyperbaric chamber (Duke Center for Hyperbaric Medicine and Environmental Physiology) with air to 6 ATA at 0.6 ATA/min. At this pressure, we know that 65–80% of the rats will manifest spikes on the EEG within 60 min (19, 20). Temperature and relative humidity were maintained at $23 \pm 0.5^\circ\text{C}$ and $60 \pm 2\%$, respectively. Decompression was performed at 0.6 ATA/min, and the animals were immediately killed with pentobarbital sodium (250 mg/kg iv).

Experimental design. Five groups of anesthetized rats were used. In group 1 ($n = 5$), tCBF and RBF measurements were verified during ventilation with 30% O₂ at 1 ATA in response to hypercapnia (5% CO₂) or hypovolemia (withdrawal of 3–6 ml arterial blood). Group 2 ($n = 6$) was used to determine tCBF and RBF responses in HBO₂ at 6 ATA. Group 3 ($n = 30$) was used to evaluate rCBF during HBO₂ exposures. In groups 2 and 3, EEG and arterial blood pressure were monitored to determine CNS and cardiovascular responses to HBO₂ at 6 ATA. Following compression to 6 ATA and a 10-min stabilization period, initial measurements were performed (designated *time 0*) and used to calculate percent changes for analyses. In group 4 ($n = 14$), L-NAME (either 100 or 500 μ M) was infused in the left striatum in rats breathing 30% O₂ at 1 ATA to determine the spatial distribution of NOS inhibition. Local CBF was measured at distances of 0.5 to ≥ 5 mm from the microdialysis probe. In group 5 ($n = 22$), aCSF or L-NAME (100 μ M) was continuously delivered, unilaterally or bilaterally, to the striatum to explore the effect of striatal NOS inhibition on local blood flow and HBO₂-induced seizure development. Blood flow responses in the cerebellum served as a control due to the distance from the microdialysis probe (13 mm). In group 5, measurements made just before aCSF or L-NAME infusion at 1 ATA were used for calculating percent change and used in analysis.

Statistical analysis. Data were analyzed using SigmaStat 3.5 (Systat Software, San Jose, CA). A one-way ANOVA was used to determine mean significant differences for the following: in tCBF and RBF between 30% O₂, 5% CO₂, and hypovolemic conditions; between rCBF (30% O₂ at 1 ATA) in different brain structures; and in MAP and HR between aCSF, 100 μ M L-NAME, or 500 μ M L-NAME. Pearson's correlation coefficients were calculated to examine associations between rCBF and the distance from the site of L-NAME infusion. To investigate the effects of HBO₂ on tCBF, rCBF (with and without L-NAME infusion), RBF, MAP, and HR, a one-way repeated-measures ANOVA was used. When a significant main effect was observed, post hoc comparisons were performed using the Holm-Sidak method. A *t*-test was used to determine whether L-NAME offered protection against hyperoxic hyperemia and seizure onset. All data are presented as means \pm SE. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Baseline hemodynamics. Mean baseline hemodynamic parameters obtained following a 1-h stabilization period in rats breathing 30% O₂ at 1 ATA are shown in Table 1. Coefficients of variation for baseline tCBF and RBF were 11 and 12%, respectively. Baseline RBF was 3.2 times greater than mean tCBF (Fig. 1). In addition, changing the ventilation gas to 5% CO₂ increased tCBF by 34% but did not change RBF, whereas hypovolemia led to significant reductions in both tCBF (–40%) and RBF (–56%), as expected.

Table 1. Baseline hemodynamic parameters

Parameter	30% O ₂ at 1 ATA
HR, beats/min	428 ± 7
MAP, mmHg	101 ± 3
Total CBF, ml·min ⁻¹ ·g ⁻¹	0.73 ± 0.04
CVR, MAP/CBF	164 ± 10
RBF, ml·min ⁻¹ ·g ⁻¹	2.33 ± 0.11
RVR, MAP/RBF	50 ± 2

Values are means ± SE. ATA, atmosphere absolute; HR, heart rate; MAP, mean arterial blood pressure; CBF, cerebral blood flow; CVR, cerebral vascular resistance; RBF, renal blood flow; RVR, renal vascular resistance.

Measurements of rCBF were low immediately following insertion of the platinum electrodes at 1 ATA, but rose to stable values by 45 min. Of the eight brain structures examined, resting blood flow in the substantia nigra (0.84 ± 0.09 ml·min⁻¹·g tissue⁻¹) was significantly higher than the seven other structures ($P < 0.05$), which ranged from 0.40 to 0.63 ml·min⁻¹·g tissue⁻¹.

Hemodynamic and EEG responses to HBO₂. Compression in HBO₂ to 5–6 ATA is known to cause sudden increases in MAP and arterial PO₂ that trigger baroreflex activation and chemoreceptor impairment (19, 22, 38). By time 0, HR decreased and remained depressed throughout the HBO₂ exposures (Fig. 2). MAP was unaltered for the first 40 min of HBO₂ but then rose continuously until the end of the experiment.

In HBO₂, tCBF was unaltered for the first 50 min and then increased significantly (Fig. 3A), accompanied by a downward trend ($P < 0.08$) in cerebral vascular resistance (Fig. 3B). In contrast, RBF was not influenced by HBO₂ in spite of a significant increase in renal vascular resistance 50 min into the exposure.

Immediately following the stabilization period at 6 ATA, rCBF did not differ from measurements made at 1 ATA in all of the brain structures examined. Sixty minutes into the exposure, significant increases in blood flow were seen in the cerebellum, frontal cortex, and parietal cortex, ranging from 17 to 36% ($P < 0.05$); however, no changes were observed in the substantia nigra (Fig. 4A). More pronounced increases in blood flow (56–163%) were observed in the NTS, hippocampus, and hypothalamus after 60 min ($P < 0.05$) (Fig. 4B). Striatal blood flow, however, displayed the earliest increase, becoming significant 50 min into the exposure ($P < 0.05$).

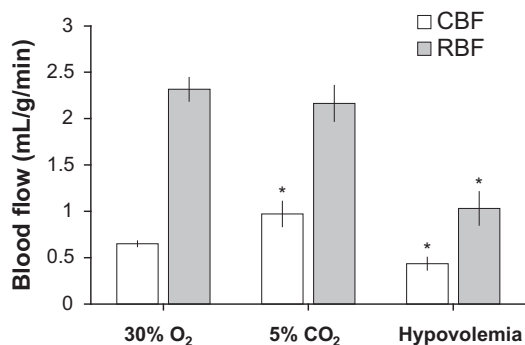


Fig. 1. Total cerebral blood flow (tCBF) and renal blood flow (RBF) responses in rats breathing 30% O₂, 5% CO₂, and following induction of hypovolemia at 1 atmosphere absolute (ATA). Three measurements were performed in each animal for each condition interspersed by a 5-min recovery with 30% O₂. *Significantly different from the 30% O₂ measurements, $P < 0.05$.

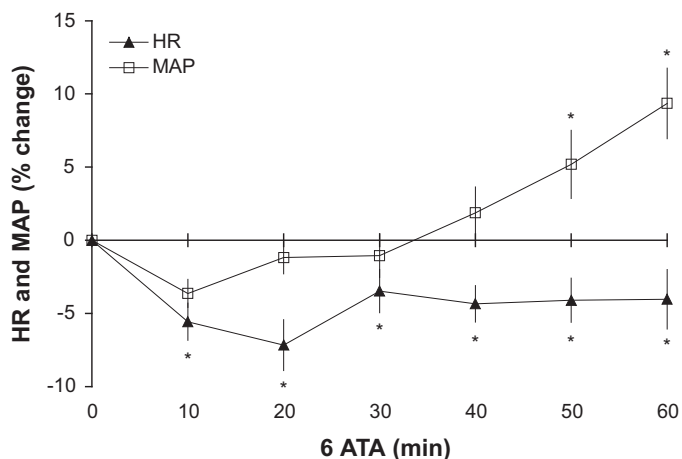


Fig. 2. Cardiovascular responses to hyperbaric oxygen (HBO₂). Heart rate (HR) and mean arterial pressure (MAP) were assessed in 10-min intervals, between cerebral blood flow measurements, throughout the HBO₂ exposure. *Significantly different from 6 ATA baseline (0 min), $P < 0.05$.

EEG patterns consisted of low-amplitude, high-frequency waves within 40–50 min of exposure, which then transformed into high-amplitude, high-frequency spikes in >85% of the rats (Fig. 4C). The mean time of EEG spike occurrence was 2 ± 5 min following the maximal rise in CBF ($165 \pm 23\%$)

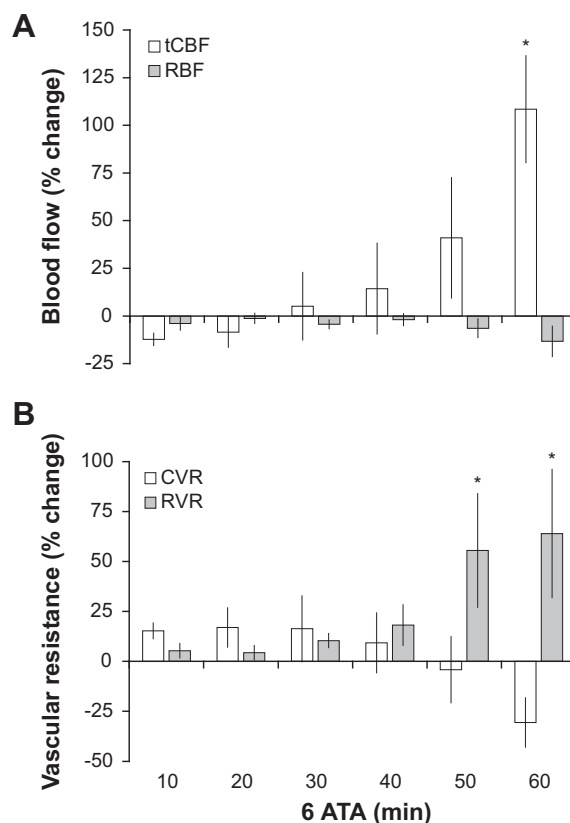


Fig. 3. Cerebral and renal hemodynamics in HBO₂. A: tCBF and RBF were measured every 10 min in HBO₂. B: cerebral vascular resistance (CVR) and renal vascular resistance (RVR) were calculated from MAP recordings and CBF and RBF measurements. *Significantly different from 6 ATA baseline (0 min), $P < 0.05$.

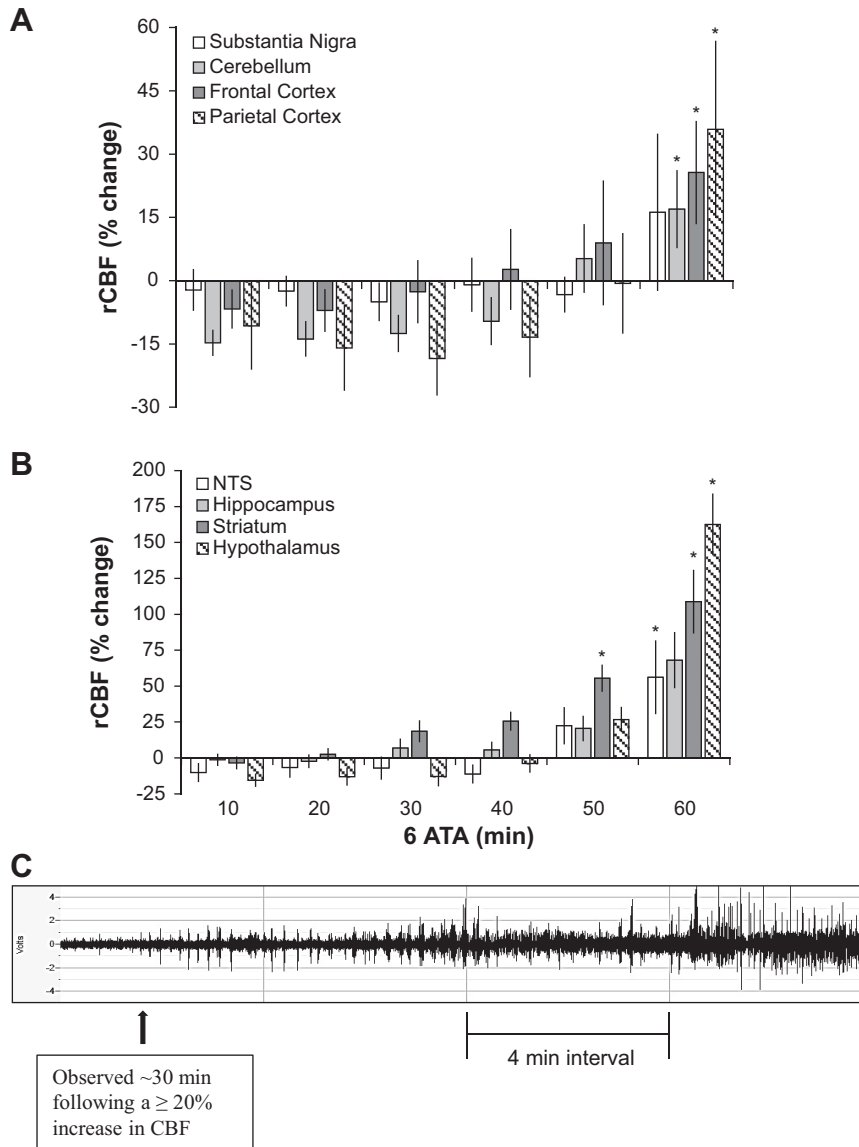


Fig. 4. Regional cerebral blood flow (rCBF) patterns and a representative electroencephalogram (EEG) recording during exposure to HBO₂. Blood flow in the substantia nigra, cerebellum, frontal cortex, and parietal cortex (A) and nucleus tractus solitarius (NTS), hippocampus, striatum, and hypothalamus (B) was measured every 10 min in HBO₂. *Significantly different from 6 ATA baseline (0 min), $P < 0.05$. C: low-amplitude, high-frequency waves preceded high-amplitude ($>100 \mu\text{V}$), high-frequency discharges following an increase in CBF in 86% of the rats.

and 30 ± 7 min following a $\geq 20\%$ increase in CBF, observations that were independent of region.

Effects of NOS inhibition on regional CBF. In animals breathing 30% O₂ at 1 ATA, the change in rCBF was correlated with the distance from the microdialysis probe when 100 μM of L-NAME was infused (Pearson's $r = 0.719$, $P < 0.001$) (Fig. 5). The mean maximal response occurred ~ 0.5 mm from the microdialysis probe ($-21 \pm 3.9\%$) and diminished at distances ≥ 1.5 mm. Continuous infusion of 500 μM of L-NAME resulted in a $25.3 \pm 5.8\%$ reduction in CBF ~ 0.5 mm from the membrane, but there was still a considerable reduction in striatal blood flow ($-8.7 \pm 4.8\%$) at distances ≥ 2 mm from the site of L-NAME delivery. HR and MAP were unaffected by either dose of L-NAME delivered continuously in the striatum (data not shown). Based on these results, 100 μM of L-NAME was selected for the experiments conducted in HBO₂.

In HBO₂, neither unilateral nor bilateral infusion of L-NAME in the striatum significantly altered the blood flow pattern in the cerebellum (Fig. 6A). In fact, cerebellar blood flow tended to increase earlier with L-NAME infusion, reach-

ing significance at 50 min. In the striatum, however, unilateral L-NAME infusion delayed the increase in blood flow by 50 min and reduced its magnitude by 62%, and bilateral infusion further delayed the increase in rCBF until 60 min and attenuated the maximum rise by 80%. Of the animals that received L-NAME (unilaterally or bilaterally), the mean time that striatal blood flow increased by $\geq 20\%$ of baseline was delayed by 23 min compared with control animals (Fig. 6B). However, there were no differences in the percentage of animals that exhibited EEG spikes (80%) or the mean time that they occurred between L-NAME-treated and control rats.

DISCUSSION

There are three novel findings from this study on anesthetized rats. First, increases in MAP and total CBF preceded HBO₂-induced EEG paroxysmal activity (seizures), but without adjustments in RBF, indicating sustained renal autoregulation. Second, cerebral hyperemia associated with oxygen seizures was heterogeneous: maximal in subcortical structures,

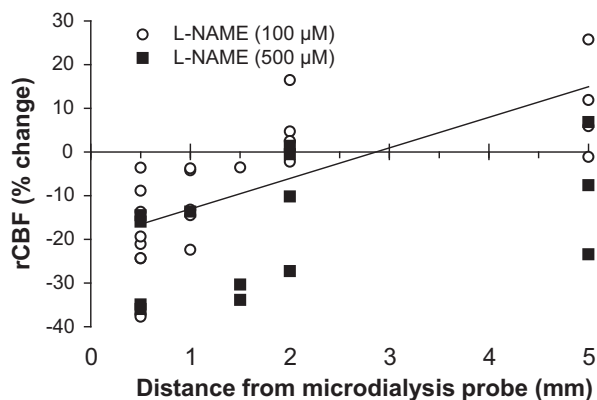


Fig. 5. Changes in rCBF in response to striatal N^G -nitro-L-arginine methyl ester (L-NAME) infusion. L-NAME (100 and 500 μ M) was continuously infused in the striatum in animals breathing 30% O_2 at 1 ATA. Platinum electrodes were implanted 0.5 to \geq 5 mm from the microdialysis probe at a depth of 5.2 mm for measurement of rCBF. At 100 μ M, L-NAME infusion resulted in a reduction in rCBF that was dependent on the distance between the platinum electrodes and microdialysis probe ($r = 0.719$, $P < 0.001$, solid diagonal line). HR and MAP were not affected by striatal L-NAME infusion with either concentration. Values are %change.

moderate in cortical areas and the cerebellum, and minimal in the substantia nigra. Additionally, blood flow in the striatum rose early and in parallel with MAP. Third, local inhibition of NO production in the striatum delayed and attenuated the rise in blood flow in this structure, but did not postpone the onset of CNS O_2 toxicity.

Cerebrovascular responses to HBO₂ clearly depend on inspired oxygen pressure (P_{iO_2}). At moderate levels of HBO₂ (at or below 3 ATA), a decrease in CBF persists (22), independent of an increase in systemic arterial pressure (9, 10, 13, 22, 24). However, at extreme P_{iO_2} values (at or above 5 ATA), arterial pressure abruptly rises and CBF increases, preceding the appearance of EEG spikes (18–20, 22), as also reported here. The documented increase in CBF during exposure to extreme HBO₂ may indicate that cerebral autoregulation is impaired; blood flow is redistributed to the brain from other organs; and/or cerebral vessels are dilated by locally produced endogenous vasodilators (19, 20, 22). As demonstrated here, however, cerebral hyperemia is not supplied by blood flow redistribution from the kidney in HBO₂ at 6 ATA. The validity of using hydrogen clearance to measure RBF was confirmed by adding 5% CO₂ to the inspired gas mixture, which is known to increase CBF but not to influence RBF (11, 29), and by inducing hypovolemia. The preservation of RBF during exposure to HBO₂ at 6 ATA is consistent with results reported from studies of conscious rats and dogs exposed to HBO₂ up to 7 ATA (9, 10, 13, 28, 32, 34, 37). Yet, our findings contrast with those of other investigators who report 30–40% reductions in RBF in anesthetized dogs exposed to HBO₂ at 1–4 ATA for 20-min intervals (34), or at 4 ATA for 90 min (28). Anesthesia, however, should not influence RBF, since renal autoregulation is accomplished by intrinsic mechanisms, maintaining over a wide range of perfusion pressures (80–180 mmHg) (15).

In all but one of the brain regions studied, CBF responded to HBO₂ with similar time-dependent patterns that differed only in magnitude: levels were initially maintained at or slightly below baseline, followed by progressive increases that ultimately preceded EEG spikes. The exception was the substantia

nigra, in which changes were not observed, possibly suggesting that vascular tone in this structure is maintained at 6 ATA. Of the structures within the CAN, the most pronounced increases were evident in the NTS, hippocampus, hypothalamus, and striatum. An early increase in blood flow was observed in the striatum, exceeding baseline levels by 20% at 30 min and 150% after 60 min, consistent with our previous findings (18). Although others have reported rCBF responses to HBO₂ at 3 and 5 ATA, only two measurements were performed during the HBO₂ exposure, one after compression and the second just before decompression (11, 12). In the majority of structures examined by these investigators, CBF decreased, as did HR and cardiac output; however, MAP was stable, and no seizures were reported. These results are all consistent with phase I of our studies (11, 12). Thus, the present data appear to be the first to demonstrate a sentinel rise in striatal CBF in HBO₂ when EEG discharges first ascend.

Although the precise mechanisms of HBO₂-induced regional cerebral hyperemia are unknown, the role of NO in modulating cerebral vascular responses in HBO₂ has been established (18). The biphasic CBF responses (transient vasoconstriction followed by hyperemia) can be explained by an initial decrease in NO availability due to increased O_2^- generation, followed by

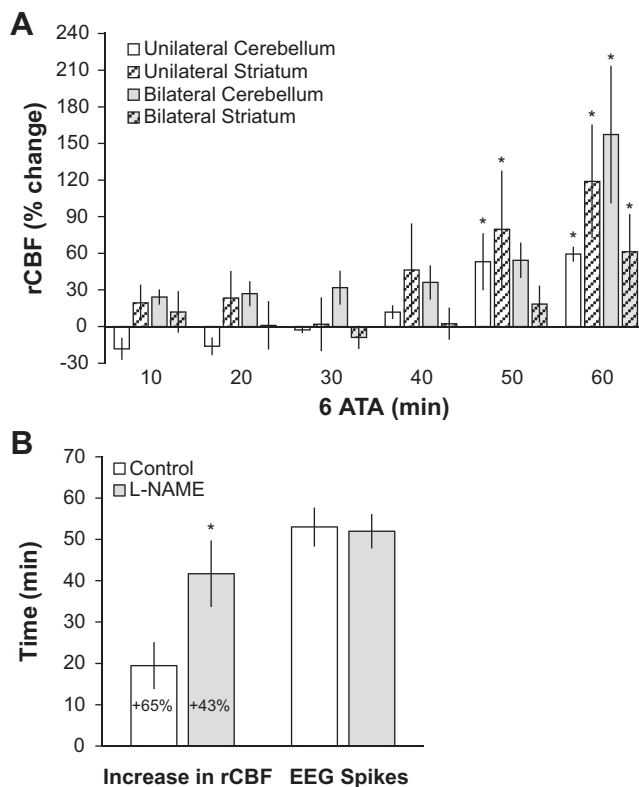


Fig. 6. rCBF responses to striatal nitric oxide synthase inhibition during HBO₂. L-NAME (100 μ M) or aCSF (control) were continuously infused in the left (unilateral) or left and right (bilateral) striatum in animals breathing 30% O_2 at 1 ATA for 30 min and in HBO₂. A: blood flow in cerebellum and striatum was measured before L-NAME and aCSF infusion, and every 10 min in HBO₂. *Significantly different from control measurements made in the same animals breathing 30% O_2 at 1 ATA, $P < 0.05$. B: mean time that a \geq 20% increase in striatal blood flow was observed and the onset of EEG spikes in control and L-NAME animals. *Significantly different from control, $P < 0.05$. Values within each bar represent the actual mean change in striatal blood flow.

an HBO₂-stimulated increase in NO production. The resulting cerebral hyperemia augments oxygen delivery, a precondition for appearance of EEG spikes. In addition to regulating vascular tone, NO has been reported to increase synaptosomal glutamate release via activation of metabotropic glutamate receptors, and to decrease extracellular GABA content in the striatum (3, 14, 36). In HBO₂ at 5–6 ATA, the ratio of glutamate to GABA does in fact increase (21, 41), and dopamine levels decrease (30). By administering the nonspecific NOS inhibitor L-NAME or the nNOS-specific inhibitor 7-nitroindazole (7-NI), either intraperitoneally or intracerebroventricularly, before exposure to HBO₂ at 5–6 ATA, cerebral hyperemia is prevented, the ratio of glutamate to GABA decreases, and seizure onset is significantly delayed or prevented; the occurrence of seizures decreases by 60% (18, 20, 21, 31). In our present study, L-NAME (100 μM) delivered directly in the striatum decreased local blood flow by ~20% in rats breathing air at 1 ATA, and also attenuated the blood flow responses to HBO₂ at 6 ATA. Because this decrease was maintained within 1.5 mm from the site of delivery (the microdialysis membrane), the volume of affected tissue would be ~11 mm³, a substantial portion of the striatum in the rat (33). Also, although striatal CBF responses were highly attenuated by bilateral delivery of L-NAME, this did not prevent HBO₂-induced seizures, since there were no differences in the number of animals that displayed EEG spikes or their time of onset. These data imply that NO was sufficient to dilate cerebral vessels and/or shift the glutamate-to-GABA equilibrium toward excitation in the portion of striatum that did not receive L-NAME. An alternative explanation is that additional brain structures are critical for initiating CNS O₂ toxicity.

In HBO₂, regional cerebral hyperemia can be considered an index of local neuronal activation due to the tight temporal coupling between neuronal activity and vascular oxygen delivery. Thus, the hyperemia appearing first in the striatum probably indicates that neurons within that region were activated before those in other brain formations within the CAN. The largest structure within the basal ganglia, the striatum, is essential for coordinating behavior with motor function (27). It receives glutamatergic inputs from the cerebral cortex and thalamus, dopaminergic inputs from the substantia nigra compacta and tegmentum, and projects GABAergic efferents to the substantia nigra pars reticulata and lateral globus pallidus (27). The mechanisms by which HBO₂ activates neurons in the brain remain to be elucidated, but it is known that reactive oxygen and nitrogen species generated in HBO₂ provoke cerebral excitation by targeting redox-sensitive biomolecules, including membrane lipids and membrane-embedded proteins, ultimately affecting ion channels that regulate neuronal excitability (16, 17, 25). Nevertheless, additional research is needed to better understand the biochemistry and localization of such interactions.

In summary, when signs of CNS O₂ toxicity are present (an increase in CBF and the appearance of EEG spikes), RBF is unaltered, demonstrating sustained autoregulation in extreme hyperoxia. Most of the examined brain structures within the CAN displayed a rise in CBF before the appearance of electrical discharges, but blood flow in the striatum increased earliest. In contrast to our previous experiments that showed neuroprotection when cerebral NO production was globally inhibited by intraperitoneal or intracerebroventricular admin-

istration of L-NAME or 7-NI, local NOS inhibition in the striatum did not prevent or delay the onset of CNS O₂ toxicity, even though striatal hyperemia responses were attenuated. This suggests that hyperoxic seizures in rats are initiated in other brain structures and that exploration with more sensitive techniques, such as functional magnetic resonance imaging or positron emission tomography, will be required to determine the course of critical events.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors. The views expressed in this article are those of the author(s) and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the United States Government. H.G. Gasier is a military service member (or employee of the U.S. Government). This work was prepared as part of Dr. Gasier's official duties. Title 17, USC, §105 provides that "Copyright protection under this title is not available for any work of the U.S. Government." Title 17, USC, §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.

AUTHOR CONTRIBUTIONS

Author contributions: H.G.G., I.T.D., B.W.A., and C.A.P. conception and design of research; H.G.G. and I.T.D. performed experiments; H.G.G., I.T.D., and C.A.P. analyzed data; H.G.G., I.T.D., B.W.A., and C.A.P. interpreted results of experiments; H.G.G. and I.T.D. prepared figures; H.G.G., I.T.D., B.W.A., and C.A.P. drafted manuscript; H.G.G., I.T.D., B.W.A., and C.A.P. edited and revised manuscript; H.G.G., I.T.D., B.W.A., and C.A.P. approved final version of manuscript.

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