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RESEARCH ARTICLE

Adrenoceptor blockade modifies regional cerebral blood flow responses to hyperbaric hyperoxia: protection against CNS oxygen toxicity

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Gasier HG, Demchenko IT, Zhilyaev SY, Moskvin AN, Krivchenko AI, Piantadosi CA. Adrenoceptor blockade modifies regional cerebral blood flow responses to hyperbaric hyperoxia: protection against CNS oxygen toxicity. *J Appl Physiol* 125: 1296–1304, 2018. First published July 19, 2018; doi:10.1152/japplphysiol.00540.2018.—Exposure to extreme hyperbaric oxygen (HBO_2) >5–6 atmospheres absolute (ATA) produces baroreflex impairment, sympathetic hyperactivation, hypertension, tachycardia, and cerebral hyperemia, known as phase II, culminating in seizures. We hypothesized that attenuation of the effects of high sympathetic outflow would preserve regional cerebral blood flow (rCBF) and protect against HBO_2-induced seizures. To explore this possibility, we tested four adrenoceptor antagonists in conscious and anesthetized rats exposed to HBO_2 at 5 and 6 ATA, respectively: phentolamine (nonselective α_1 and α_2), prazosin (selective α_1), propranolol (nonselective β_1 and β_2), and atenolol (selective β_1). In conscious rats, four drug doses were administered to rats before HBO_2 exposures, and seizure latencies were recorded. Drug doses that provided similar protection against seizures were administered before HBO_2 exposures in anesthetized rats to determine the effects of adrenoceptor blockade on mean arterial pressure, heart rate, rCBF, and EEG spikes. All four drugs modified cardiovascular and rCBF responses in HBO_2 that aligned with epileptiform discharges, but only phentolamine and propranolol effectively increased EEG spike latencies by ~20 and 36 min, respectively. When phentolamine and propranolol were delivered during HBO_2 at the onset of phase II, only propranolol led to sustained reductions in heart rate and rCBF, preventing the appearance of epileptiform discharges. The enhanced effectiveness of propranolol may extend beyond β-adrenoceptor blockade, i.e., membrane stability and reduced metabolic activity. These results indicate that adrenoceptor drug pretreatment will minimize the effects of excessive sympathetic outflow on rCBF and extend HBO_2 exposure time.

NEW & NOTEWORTHY Blocking adrenergic receptors with phentolamine (nonselective α_1 and α_2), prazosin (selective α_1), propranolol (nonselective β_1 and β_2), and atenolol (selective β_1) modified cardiovascular and regional cerebral blood flow (rCBF) responses in hyperbaric oxygen (HBO_2) at 6 atmospheres absolute (ATA); however, only phentolamine and propranolol extended EEG spike latencies. When these two agents were delivered at the onset of sympathetic hyperactivation, only propranolol reduced heart rate and rCBF throughout the exposure and prevented epileptiform discharges. These data validate the strong role of adrenergic control of cardiovascular function and rCBF in extreme HBO_2 and the potential use of antiadrenergic drugs to prevent seizures.

α- and β-blockers; cerebral blood flow; oxygen toxicity

INTRODUCTION

Breathing hyperbaric oxygen (HBO_2) is used in controlled clinical settings, but divers may also be exposed to HBO_2, particularly when using closed-circuit oxygen rebreathers that serve to prevent inert gas accumulation and decompression illness. During HBO_2 therapy and during closed-circuit oxygen rebreather diving, the clinical protocols and diving tables are preventive since the consequences of HBO_2 toxicity include acute lung injury, seizures, and death. Effective strategies for preventing pulmonary and central nervous system (CNS) oxygen toxicity would, therefore, increase HBO_2 treatment options and extend depth and time limits for divers.

The toxic effects of oxygen on the CNS and lungs are well known (3), and a plethora of studies have advanced our understanding of the pathophysiology of oxygen toxicity. Although explicit molecular biological mechanisms remain under investigation, the physiological responses to HBO_2 have been documented and include two contrasting phases linked to baroreflex sensitivity (18, 20). In so-called phase I, sympathetic output, cardiac output (because of bradycardia), and cerebral blood flow decrease, alterations that serve to protect the organism from HBO_2 (8, 20, 27, 40). In phase II, sympathoexcitation and parasympatholysis accompany tachycardia, hypertension, and cerebral hyperemia, ultimately culminating in seizures and pulmonary injury if exposure to HBO_2 is not interrupted or discontinued (5, 18, 21). Stimulation of baroreceptors maintains sympathovagal balance and reduces sympathetic outflow, effectively delaying the onset of HBO_2-induced seizures and attenuating the magnitude of acute lung injury (18). In humans, baroreflex-activation therapy has demonstrated efficacy in restoring sympathetic-parasympathetic balance and reducing arterial blood pressure in patients with resistant hypertension (29). Implanting electrodes for HBO_2 therapy or in divers is unrealistic, thus pharmacological ap-

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proaches are favored for preventing CNS and pulmonary oxygen toxicity.

The notion that the sympathetic nervous system contributed to the genesis of HBO_2-induced seizures and pulmonary damage developed from initial experiments conducted in rats that were injected with epinephrine and/or that underwent adrenalectomy before HBO_2 at ~6 atmospheres absolute (ATA) (5). Confirmatory studies were subsequently performed in mice and rats pretreated with sympathetic blocking agents [i.e., tetraethylammonium chloride and hexamethonium (ganglionic blockers), chlorpromazine (sympathetic outflow inhibition), bis(4-methyl-l-homopiperazinyl-thiocarbonyl)disulfide (dopamine β-hydroxylase inhibitor), and oxidopamine (selectively destroys dopaminergic and noradrenergic brain neurons)] and exposing the animals to HBO_2 ranging from 4 to 7 ATA (4, 31, 34). The aforementioned experiments were able to confirm that the etiology of the CNS and pulmonary oxygen toxicity was linked to a neurogenic sympathetic component and led to subsequent testing of adrenoceptor antagonists in HBO_2. Perhaps the first to be tested, propranolol (nonselective β-adrenergic receptor antagonist) provided to mice before HBO_2 at 4 ATA resulted in a 3.5-fold decrease in the incidence of seizures and extended seizure latencies by a factor of 2 (34). In rats exposed to HBO_2 at 6 ATA, pretreatment with propranolol or practolol (selective β_1-adrenergic receptor antagonist) increased seizure latencies by ~69% and ~49%, respectively, and propranolol reduced gross lung injury (12). Remaining unknown, however, is whether α-adrenoceptor blockade offers similar antiseizure protection in HBO_2 and whether efficacy of adrenoceptor antagonists is attributed to cerebrovascular adjustments.

The purpose of this research was to determine whether attenuating sympathetic hyperactivation by adrenoceptor blockade preserves regional cerebral blood flow (rCBF) and delays HBO_2-induced seizures. Here, we tested four subtypes of Food and Drug Administration-approved adrenoceptor antagonists used in the treatment of hypertension: phentolamine (nonselective α_1 and α_2), prazosin (selective α_1), propranolol (nonselective β_1 and β_2), and atenolol (selective β_1). Phentolamine and prazosin reduce blood pressure, primarily by decreasing vascular resistance, whereas propranolol and atenolol reduce heart rate and cardiac output (30, 33). Upon establishing drug doses that resulted in similar antiseizure activity in rats exposed to HBO_2 at 5 ATA, subsequent experiments were performed to primarily determine rCBF, cardiovascular secondary, responses to HBO_2 at 6 ATA, and their antiseizure potential. Neuronal activity and oxygen delivery are tightly coupled, thus alterations in rCBF are considered to reflect neuronal activation in HBO_2 (17, 19). Blood flow in striatum, substantia nigra, and hypothalamus were monitored because of their involvement in motor control and autonomic regulation (26) and sensitivity to extreme HBO_2 (24). Final experiments were conducted to test the most effective adrenoceptor antagonists, phentolamine and propranolol, to determine if they could reestablish rCBF and prevent neuronal excitability when delivered at the onset of phase II in HBO_2.

METHODS

Experimental design. Three different experiments were performed using male Sprague Dawley (Charles River Laboratories, Wilmington, MA) and Wistar (Rappolovo animal-breeding facility, Russia) rats weighing 317–373 g. In *experiment 1*, to examine the antiseizure efficacy of α- and β-adrenergic receptor antagonists, conscious Wistar rats (n = 166) received an intraperitoneal injection of either 0.9% NaCl or 4 different doses of the following α- and β-adrenergic receptor antagonists (Sigma-Aldrich) 30 min before HBO_2 exposures at 5 ATA for 90 min: phentolamine in 0.9% NaCl (1, 2, 5, or 10 mg/kg), prazosin in 0.9% NaCl (0.1, 0.5, 1, or 5 mg/kg), propranolol in 0.9% NaCl (0.5, 1, 5, or 10 mg/kg), or atenolol in DMSO (5, 10, 20, or 40 mg/kg). Seizure latency was considered the time that the animals exhibited tonic-clonic convulsions. In *experiment 2*, to determine the effects of α- and β-adrenergic receptor antagonists on rCBF responses to HBO_2, anesthetized Sprague Dawley rats (n = 38) received an intraperitoneal injection of either 0.9% NaCl or equally effective doses of phentolamine (5 mg/kg), prazosin (1 mg/kg), propranolol (1 mg/kg), or atenolol (20 mg/kg) 15 min before exposure to HBO_2 at 6 ATA for 90 min. Electroencephalogram (EEG) recordings were continuously monitored for the appearance of epileptiform discharges (>100 μV) consistent with seizures. Baseline measurements were made 5 min following 0.9% NaCl and drug administration. In *experiment 3*, to determine whether the most effective adrenoceptor antagonists could reestablish rCBF and prevent neuronal excitability when delivered at the onset of phase II in HBO_2 at 6 ATA, phentolamine (5 mg/kg) and propranolol (1 mg/kg) were delivered to anesthetized Sprague Dawley rats (n = 25) using a CMA microinjection pump (Carnegie Medicine, Stockholm, Sweden).

Experiment 1 (conscious animals) was conducted at the Institute of Evolutionary Physiology and Biochemistry, Russian Academy Sciences, St. Petersburg, Russia in compliance with a protocol approved by the Institute's Ethical Review Board. *Experiments 2* and *3* (anesthetized animals) were conducted at the Duke Center for Hyperbaric Medicine and Environmental Physiology using a protocol approved by the Duke University Institutional Animal Care and Use Committee.

Surgical procedures. Rats were anesthetized with urethane (750 mg/kg) and α-chloralose (70 mg/kg) intraperitoneally before trachea cannulation and catheterization of the left femoral artery and vein. Anesthesia was maintained by providing one-fourth of the initial doses as necessary. Rats were ventilated mechanically with 30% O_2 in N_2 using a small animal respirator (Edco Scientific Inc., Chapel Hill, NC). Tidal volume and ventilatory rate were maintained at 2 ml and 80 breaths/min, respectively. Pancuronium bromide (0.5 mg/kg) was delivered via the femoral vein to prevent involuntary respiratory movements and to maintain a normal pulmonary gas exchange, thus preventing hypercapnia (15). Rats were positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), and burr holes were drilled to the dura over the striatum, hypothalamus (paraventricular nucleus), and substantia nigra using published stereotaxic coordinates (36). Platinum electrodes (100 μm diameter) were inserted into these structures by a micromanipulator with location placement confirmed by postmortem examination (15). Two additional burr holes were drilled to the dura over the left and right parietal cortexes, and cranial screws were implanted for EEG recordings.

Hemodynamic and EEG recordings. A pressure transducer (Viggo Spectramed, Oxynard, CA) to the left femoral artery continuously monitored mean arterial pressure. Blood flow ($ml \cdot min^{-1} \cdot g\ tissue^{-1}$) in the striatum, hypothalamus, and substantia nigra was determined by changing the ventilation gas to 2.5% hydrogen in air for ~40 s and computing hydrogen washout curves (1, 15). EEG was monitored continuously for changes in electrocortical activity. Mean arterial pressure, heart rate, rCBF, and EEG were recorded and analyzed using LabScribe 2 software on iWorx IX-228/S hardware (iWorx Systems, Dover, NH). Mean arterial pressure and heart rate were calculated from pulse waves.

HBO_2 exposures. In *experiment 1*, conscious animals were compressed in a hyperbaric chamber with oxygen at 0.5 ATA/min to 5 ATA for 90 min, or until seizures occurred. Decompression was performed at 0.3 ATA/min. In *experiments 2* and *3*, anesthetized rats were exposed to HBO_2 at 6 ATA for 90 min. The physiological

Table 1. *Effects of α- and β-adrenergic receptor blockade compared with vehicle in HBO_2 at 5 ATA*

Drug and Vehicle	Dose, mg/kg	*n*	Mean Seizure Latency, min ± SE	*P* (vs. control)
Control (0.9% NaCl)		16	35.1 ± 3.5	
Nonselective α-adrenergic receptor antagonist				
Phentolamine, 0.9% NaCl	1	9	37.7 ± 4.2	ns
	2	9	42.9 ± 4.9	ns
	5	10	57.0 ± 5.8	0.006
	10	10	61.1 ± 6.3	<0.001
Selective α_1-adrenergic receptor antagonist				
Prazosin, 0.9% NaCl	0.1	10	38.7 ± 2.8	ns
	0.5	8	40.6 ± 5.0	ns
	1	10	54.7 ± 5.1	0.005
	5	9	59.3 ± 5.6	<0.001
Nonselective β-adrenergic receptor antagonist				
Propranolol, 0.9% NaCl	0.5	10	43.7 ± 5.3	ns
	1	9	58.9 ± 5.7	0.004
	5	10	76.8 ± 6.9	<0.001
	10	9	85.3 ± 3.9	<0.001
Selective β_1-adrenergic receptor antagonist				
Atenolol, DMSO	5	9	37.7 ± 4.2	ns
	10	8	43.6 ± 5.2	ns
	20	10	57.3 ± 5.5	0.005
	40	10	50.2 ± 6.3	ns

Control (0.9% NaCl) or drugs (propranolol, atenolol, phentolamine, or prazosin) were administered intraperitoneally 30 min before exposing conscious rats to hyperbaric oxygen (HBO_2) at 5 atmospheres absolute (ATA) for 90 min.

responses to HBO_2 are comparable between anesthetized rats exposed to 6 ATA and conscious rats exposed 5 ATA because the use of anesthetics and paralytics in HBO_2 can depress cardiovascular function and prevent hypercapnia, extending seizure latencies (18, 19, 21). Following a 60-min stabilization period in rats breathing 30% O_2 in N_2, baseline parameters were recorded, and the ventilation gas was changed to 100% O_2 before compressing the chamber with air at 0.6 ATA/min to 6 ATA. Decompression was accomplished at 0.6 ATA/min. Temperature and relative humidity within the hyperbaric chambers (Institute of Evolutionary Physiology and Biochemistry and Duke Center for Hyperbaric Medicine and Environmental Physiology) were maintained at 23 ± 0.5°C and 60.2%. Rats were euthanized after the studies using pentobarbital sodium (250 mg/kg) administered intraperitoneally or intravenously.

Statistical analysis. Data were analyzed using SigmaPlot 13.0 (Systat Software, Inc., San Jose, CA). A one-way ANOVA was used to compare seizure latencies between drugs and vehicle. A two-way repeated measures ANOVA was used to determine the effects of HBO_2 within the drug groups and vehicle (time) and between drugs and vehicle (time × group). Post hoc comparisons were performed using Bonferroni *t*-tests. All data are presented as means ± SE. $P < 0.05$ was accepted as being statistically significant.

RESULTS

Seizure latencies in conscious rats exposed to HBO_2 at 5 ATA following pretreatment with vehicle and α- and β-adrenergic antagonists. Mean seizure latencies for rats receiving 0.9% NaCl, phentolamine, prazosin, propranolol, and atenolol 30 min before exposure to HBO_2 at 5 ATA for 90 min are displayed in Table 1. In comparison with control rats exposed to HBO_2, the lowest drug doses tested did not increase seizure latencies. Neuroprotection was, however, afforded with higher drug doses. Atenolol was an exception, where 20 mg/kg offered greater antiseizure protection than 40 mg/kg.

From *experiment 1*, drug doses that delayed seizure latency by ~50% (ED_{50}) were used in subsequent studies aimed at determining the effects of adrenoceptor blockade on rCBF and cardiovascular responses to HBO_2 and their antiseizure potential. The drug doses were as follows: phentolamine (5 mg/kg), prazosin (1 mg/kg), propranolol (1 mg/kg), and atenolol (20 mg/kg).

Baseline hemodynamic parameters. Providing α- and β-adrenergic antagonists at doses that provided similar antiseizure activity had no effect on heart rate in the rat's breathing room air at 1 ATA (Table 2). Phentolamine and prazosin resulted in an ~15% reduction in mean arterial pressure 10 min after injection, whereas β-adrenergic blockade had no effect. None of the adrenergic antagonists altered blood flow in the striatum, hypothalamus, or substantia nigra at sea level.

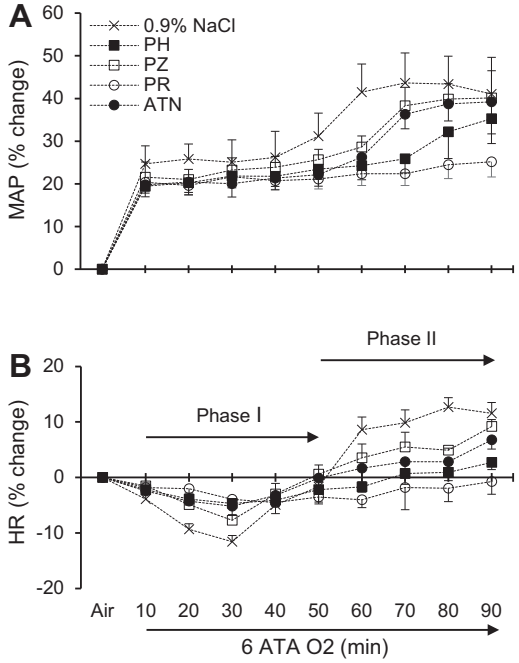
Cardiovascular and rCBF responses, and seizure latencies in anesthetized rats exposed to HBO_2 at 6 ATA pretreated with α- and β-adrenergic antagonists. Upon exposure to HBO_2, mean arterial pressure immediately increased in all rats and remained elevated during the 90 min exposures (Fig. 1*A*). Sixty minutes into HBO_2, a second increase in mean arterial pressure occurred, and this was attenuated with atenolol, phentolamine, and propranolol (in order of effectiveness). Heart rate also followed a biphasic response, decreasing from 20 to 30 min and then increasing from 60 min until the end of the exposures (Fig. 1*B*). Only phentolamine and propranolol prevented the initial bradycardia and subsequent tachycardia during HBO_2.

In control rats pretreated with 0.9% NaCl, striatal and hypothalamic blood flow increased 50 min into HBO_2 (Fig. 2, *A* and *B*). In the substantia nigra, the hyperemic response was seen 10 min later, and the magnitude of increase was less than the other structures (Fig. 2*C*). Prazosin, atenolol, phentolamine, and propranolol, from least to most effective, delayed hyperemia in the striatum and hypothalamus. In the substantia

Table 2. *Baseline hemodynamic parameters*

Parameter	0.9% NaCl	Phentolamine	Prazosin	Propranolol	Atenolol
Heart rate, beats/min	408 ± 15	378 ± 8	377 ± 9	377 ± 17	388 ± 10
MAP, mmHg	117 ± 5	99 ± 2*	99 ± 3*	105 ± 4	103 ± 3
rCBF, $ml \cdot min^{-1} \cdot g\ tissue^{-1}$					
Striatum	0.73 ± 0.05	0.75 ± 0.04	0.74 ± 0.09	0.83 ± 0.07	0.77 ± 0.09
Hypothalamus	0.79 ± 0.06	0.70 ± 0.04	0.70 ± 0.08	0.83 ± 0.07	0.85 ± 0.10
Substantia nigra	0.89 ± 0.04	0.81 ± 0.07	0.89 ± 0.07	0.81 ± 0.05	0.89 ± 0.06

Values are means ± SE (n = 6–9/group). Anesthetized rats breathing 30% O_2 in N_2 at 1 atmospheres absolute were administered 0.9% NaCl (controls), phentolamine (5 mg/kg), prazosin (1 mg/kg), propranolol (1 mg/kg), or atenolol (20 mg/kg) intraperitoneally 10 min before measurement of baseline parameters. MAP, mean arterial pressure; rCBF, regional cerebral blood flow. *Significantly different from controls, $P < 0.05$.



Time (P < 0.001)
0.9% NaCl: 10+ min
PH: 10+ min
PZ: 10+ min
PR: 10+ min
ATN: 10+ min

Time x Drug (P < 0.001)
60 min: PH, PR, ATN
70 min: PH, PR
80+ min: PR

Time (P < 0.001)
0.9% NaCl: 20-30 min & 60+ min
PZ: 30 & 90 min
ATN: 90 min

Time x Drug (P < 0.001)
20 min: PR
30 & 70 min: PH, PR, ATN
80 min: PH, PZ, PR, ATN
90 min: PH, PR

Fig. 1. Mean arterial pressure (MAP) and heart rate (HR) in anesthetized rats exposed to hyperbaric oxygen (HBO_2) at 6 atmospheres absolute (ATA) pretreated with saline, phentolamine (PH), prazosin (PZ), propranolol (PR), or atenolol (ATN). MAP (*A*) and HR (*B*) was measured in air (30% O_2 at 1 ATA) 10 min before and every 10 min in HBO_2. NaCl (0.9%) and drugs were administered intraperitoneally 15 min before compression. Values are mean changes from air ± SE (n = 6–9/group). Significant main effects of time and time × drug interaction are displayed right of figures.

nigra, only propranolol prevented an increase in blood flow during HBO_2; however, the overall blood flow response did not differ from control rats.

EEG recordings revealed high-amplitude, high-frequency spikes (i.e., seizures) in all of the rats provided vehicle ~50 min into the HBO_2 exposures (Fig. 3). Only phentolamine and propranolol significantly extended seizure latency, ~38% former and ~69% latter.

From these data, phentolamine and propranolol were the most effective in maintaining mean arterial pressure, heart rate, and rCBF, and in delaying the onset of epileptiform discharges. A final experiment was conducted to determine whether delivery of these adrenoceptor antagonists at the onset of phase II could reestablish blood flow in the striatum and hypothalamus and prevent neuroexcitation. These structures were monitored because of their increased sensitivity to HBO_2 at 6 ATA.

Cardiovascular and rCBF responses, and seizure latencies in anesthetized rats administered phentolamine or propranolol during HBO_2 at 6 ATA. Phase II hypertension occurred 50 min into HBO_2 and was unaffected by phentolamine or propranolol (Fig. 4*A*). The increased heart rate that follows the initial bradycardia and mirrors the subsequent rise in mean arterial pressure and rCBF was largely unaltered by phentolamine, yet completely abolished with propranolol Fig. 4*B*).

When phentolamine (5 mg/kg) was infused into the venous circulation 50 min into HBO_2, a sudden drop in both striatal and hypothalamic blood flow occurred but rebounded and rose thereafter (Fig. 5, *A* and *B*). Infusion of propranolol (1 mg/kg) led to a steady decline in striatal and hypothalamic blood flow that significantly differed from vehicle-infused rats 70 min until the end of the exposures, dropping to ~1/2 of maximum values of both saline and phentolamine.

EEG spikes were documented in all of the controls and rats that received intravenous phentolamine 50 min into HBO_2, yet the time of onset was significantly delayed by >20 min (Fig. 6). Provision of propranolol at 50 min, however, prevented EEG spikes in all of the animals tested.

DISCUSSION

In extreme HBO_2, impaired arterial baroreflex afferent traffic and parasympathetic withdrawal results in sympathetic hyperactivation and seizures. Inhibiting, or attenuating, adrenergic nerve activity should thus serve as a viable strategy to prevent CNS oxygen toxicity. By testing nonselective (α_1 and α_2 and β_1 and β_2) and selective (α_1, β_1) adrenoceptor antagonists in conscious and anesthetized animals exposed to HBO_2 at 5 and 6 ATA, we not only determined their antiepileptic potential but also improved our understanding of the cellular mechanisms related to adrenergic nerve transmission. In addition to a description of the rCBF and cardiovascular responses to each of four adrenoceptor antagonists in HBO_2, we report two novel findings. First, only phentolamine and propranolol modified rCBF and cardiovascular responses in HBO_2 that extended EEG spike latencies, and only propranolol effectively prevented hypertension, tachycardia, and regional cerebral hyperemia, reducing the percentage of rats that exhibited epileptiform discharges by ~71%. Second, the delivery of propranolol during HBO_2 at the onset of phase II reduced heart rate and rCBF, and EEG spikes were absent.

Cardiovascular and rCBF responses to adrenoceptor blockade in phase I of HBO_2. The early cardio- and cerebrovascular responses to extreme hyperoxia include an increase in systemic and cerebral vascular resistance, hypertension, bradycardia, reduced cardiac output, and unaltered cerebral blood flow (9, 18, 43). Here, the cardiovascular effects recorded in the first 50 min of HBO_2 were consistent with phase I. Delivery of adrenergic antagonists before HBO_2 did not prevent hypertension, consistent with our previous observations in rats after nonselective α- (phenoxybenzamine) or β-adrenoceptor blockade 30 min before HBO_2 at 3 ATA (20). The new data strengthen the view that systemic vascular resistance in HBO_2 is not primarily mediated via adrenergic mechanisms. Instead, hyperoxic-induced vasoconstriction is, in part, attributed to a decrease in the vasodilatory action of nitric oxide ($NO^{\cdot}$) (20, 38), specifically

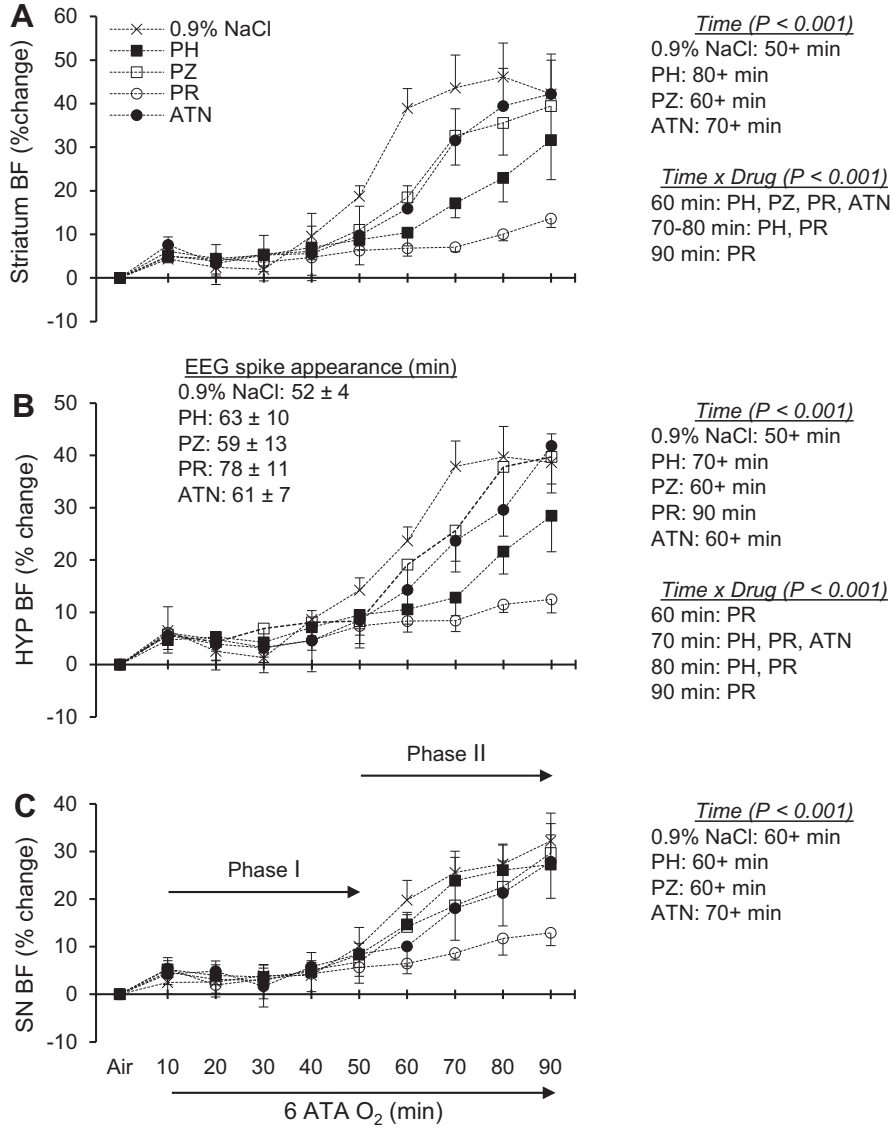


Fig. 2. Regional cerebral blood flow in anesthetized rats exposed to hyperbaric oxygen (HBO_2) at 6 atmospheres absolute (ATA) pretreated with 0.9% NaCl, phentolamine (PH), prazosin (PZ), propranolol (PR), or atenolol (ATN). Blood flow (BF) in the (*A*) striatum, (*B*) hypothalamus (HYP), and (*C*) substantia nigra (SN) was measured in air (30% O_2 at 1 ATA) 10 min before and every 10 min in HBO_2. NaCl (0.9%) and drugs were administered intraperitoneally 15 min before compression. Values are mean changes from air ± SE (n = 6–9/group). Significant main effects of time and time × drug interaction are displayed right of figures.

($NO^{\cdot}$) and the increased superoxide anion ($^{\cdot}O_2^-$) form peroxynitrite ($ONOO^-$) (11, 14, 35). As a consequence, the baroreflex is stimulated, activating the parasympathetic limb while inhibiting efferent sympathetic traffic, resulting in bradycardia and a lower cardiac output without significantly affecting vascular resistance (18, 20). The initial bradycardia was prevented with phentolamine, atenolol, and propranolol and may have reflected the small nonsignificant reduction in arterial blood pressure (3%–6%) and subsequent reduction in afferent stimulation to the vasomotor center of the medulla. The scale of this attenuated heart rate response is minor and unlikely to significantly alter blood flow. Indeed, adrenergic blockade did not alter blood flow in the striatum, hypothalamus, and substantia nigra during the early period of isopression. These data imply that baroreflex activation during phase I of extreme hyperoxia is not triggered primarily by adrenergic mechanisms.

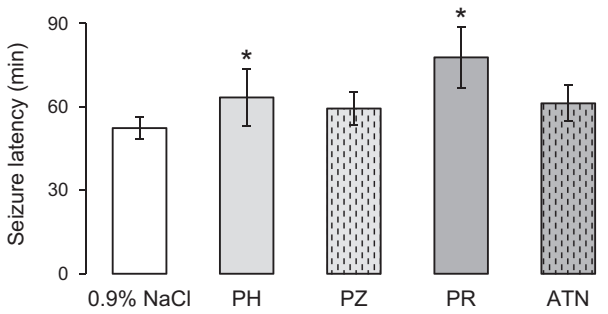
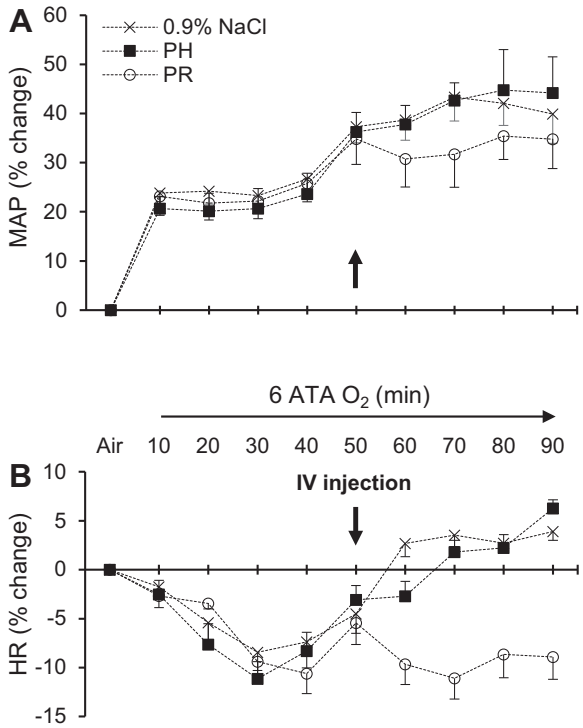


Fig. 3. Seizure latencies in anesthetized rats exposed to hyperbaric oxygen (HBO_2) at 6 atmospheres absolute pretreated with 0.9% NaCl (controls), phentolamine (PH), prazosin (PZ), propranolol (PR), or atenolol (ATN). Values are the mean appearance times of high-amplitude (>100 μV), high-frequency waves ± SE (n = 6–9/group). *Significantly different from controls, $P < 0.05$.

Cardiovascular and rCBF responses to adrenoceptor blockade in phase II of HBO_2. Phase II is characterized by a secondary increase in arterial blood pressure, a declining cardiac output despite tachycardia, and cerebral hyperemia, responses attributed to baroreflex impairment and sympathoexcitation (18). A secondary increase in mean arterial pressure and tachycardia occurred 50–60 min into HBO_2. The cerebral circulation is under tight autoregulatory control and changes



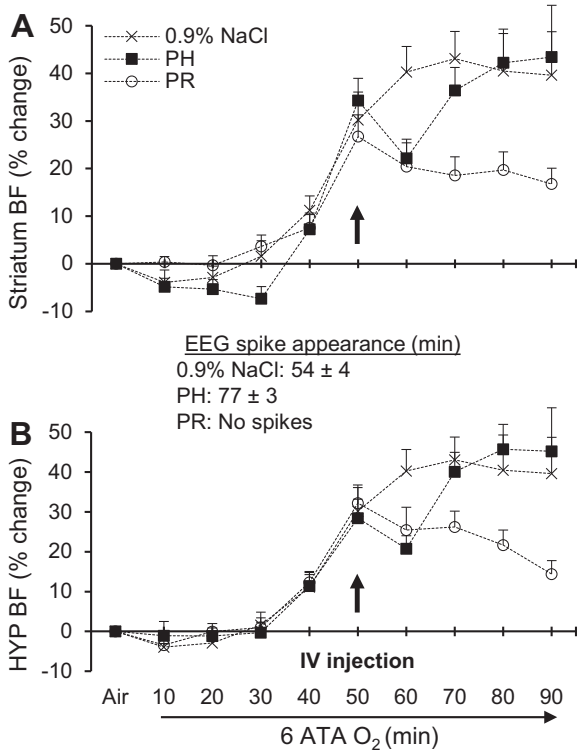
Time ($P < 0.001$)
0.9% NaCl: 10+ min
PH: 10+ min
PR: 10+ min

Time ($P < 0.001$)
0.9% NaCl: 20-40 min
PH: 20-40 & 90 min
PR: 30+ min

Time x Drug ($P < 0.001$)
60 min: PH, PR
70+ min: PR

Fig. 4. Mean arterial pressure (MAP) and heart rate (HR) in anesthetized rats administered saline, phentolamine (PH), or propranolol (PR) during hyperbaric oxygen (HBO_2) exposures at 6 atmospheres absolute (ATA). MAP (*A*) and HR (*B*) were measured in air (30% O_2 at 1 ATA) 10 min before and every 10 min in HBO_2. Saline and drugs were administered intravenously 50 min into the HBO_2 exposures. Values are mean changes from air ± SE (n = 8–9/group). Significant main effects of time and time × drug interaction are displayed right of figures.

directly with alterations in mean arterial pressures outside of ~75–150 mmHg (41); thus, hyperemia in the striatum, hypothalamus, and substantia nigra paralleled HBO_2-induced hypertension. Pretreatment with α- and β-adrenoceptor antagonists extended the time of phase II onset; however, the cardiovascular and rCBF responses differed between drugs. Most notably, selectively blocking α_1- and β_1-adrenoceptors with prazosin and atenolol, respectively, were essentially ineffective in preventing an increase in mean arterial pressure and marginally effective in thwarting tachycardia and regional cerebral hyperemia. In contrast, nonselective blockade of α- and β-adrenoceptors with phentolamine and propranolol, respectively, maintained blood pressure, heart rate, and prevented the surge in rCBF. If the drugs were delivered at the onset of phase II,



Time ($P < 0.001$)
0.9% NaCl: 50+ min
PH: 50+ min
PR: 50+ min

Time x Drug ($P < 0.001$)
60 min: PH, PR
70+ min: PR

Time ($P < 0.001$)
0.9% NaCl: 50+ min
PH: 50+ min
PR: 50-80 min

Time x Drug ($P < 0.001$)
60 min: PH, PR
70+ min: PR

Fig. 5. Regional cerebral blood flow in anesthetized rats administered saline, phentolamine (PH), or propranolol (PR) during hyperbaric oxygen (HBO_2) exposures at 6 atmospheres absolute (ATA). Blood flow (BF) in the striatum (*A*) and hypothalamus (HYP) (*B*) was measured in air (30% O_2 at 1 ATA) 10 min before and every 10 min in HBO_2. NaCl (0.9%) and drugs were administered intravenously 50 min into the HBO_2 exposures. Values are mean changes from air ± SE (n = 8–9/group). Significant main effects of time and time × drug interaction are displayed right of figures.

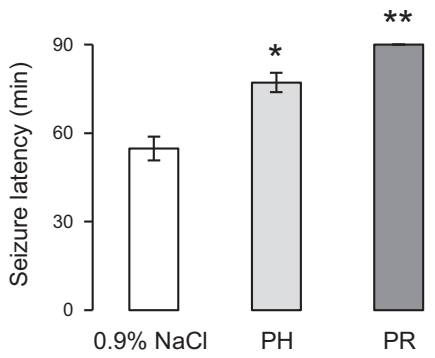


Fig. 6. Seizure latencies in anesthetized rats administered 0.9% NaCl (controls), phentolamine (PH), or propranolol (PR) during hyperbaric oxygen exposures at 6 atmospheres absolute. Values are the mean appearance times of high-amplitude (>100 μV), high-frequency waves ± SE (n = 8–9/group). *Significantly different from controls; **significantly different from controls and PH-treated rats; $P < 0.05$.

only propranolol effectively prevented tachycardia and reduced rCBF throughout the experiment.

These results may be explained by the drug's mode of action. Phentolamine reduces vascular resistance by blocking postsynaptic α_1- + α_2-adrenoceptors on vascular smooth muscle and blocks postganglionic presynaptic α_2-adrenoceptors leading to dysregulated norepinephrine release (10, 22). Propranolol reduces heart rate and contractility by blocking cardiac β_1- and β_2-adrenoceptors and inhibits vasodilation by blocking postsynaptic β_2-adrenoceptors on vascular smooth muscle (2, 10, 44). Our data seem to imply that the adrenergic control of cardio- and cerebrovascular responses in phase II of HBO_2 are primarily mediated via α_2- and β_2-adrenoceptors. However, with continued exposure to extreme HBO_2 or onset of sympathetic hyperactivation, the activation threshold is reached; because the slope of the stimulus response does not change, arterial blood pressure increases, and the $NO^{\cdot}$-mediated increase in rCBF ensues (16, 17, 19). Yet, even in the presence of sympathoexcitation, β-adrenoceptor blockade prevents tachycardia (21) and preserves rCBF.

Antiseizure activity of adrenergic antagonists. Normally, HBO_2 increases brain oxygenation and, if uninterrupted, culminates in epileptiform discharges (EEG) and tonic-clonic seizures (6, 7). The antiseizure effectiveness of the antihypertensive drugs tested may, therefore, be ascribed to their ability to modify cerebral blood flow. Here, when the adrenoceptor antagonists were administered before HBO_2 exposures, the EEG spike onset aligned with the time-dependent regional cerebral hyperemic responses, supporting this notion.

Prazosin and atenolol attenuated the magnitude of rise in rCBF for ~10–20 min; however, this response did not extend EEG spike onset. Phentolamine and propranolol, however, increased EEG spike latencies, whether administered before or after HBO_2, at the transition from phase I to phase II. The immediate decrease in rCBF following phentolamine may be due to a breakthrough of cerebral autoregulation, as suggested to occur with acute hypertension at sea level (41). The eventual or rebound regional cerebral hyperemia observed with phentolamine, however, suggests that excessive sympathetic outflow and increased $NO^{\cdot}$ production overrides this temporary relief and predominates in regulating cerebral blood flow in phase II of HBO_2 (19). Propranolol, in contrast, maintains or reduces rCBF in HBO_2 and further delays or prevents the consequent epileptiform discharges and motor convulsions (12, 39). Unanswered from this study, however, is how propranolol is able to prevent or reverse $NO^{\cdot}$-mediated regional cerebral hyperemia and/or neuroexcitation. One possibility is that propranolol decreases membrane excitability in the heart and brain by inhibiting voltage-gated sodium channels (23, 42). Presumably, this would attenuate the reduction in neuronal GABA (γ-aminobutyric acid) ergic synaptic transmission that occurs in HBO_2 (25, 45–47) and reduce the cerebral metabolic rate (12, 28), both serving to prevent epileptiform discharges and motor convulsions. In addition, reduced metabolic requirements would reduce CO_2 production (37) and prevent hypercapnic-induced cerebral hyperemia (8, 24, 32). The fact that propranolol has no effect on rCBF at sea level (13) supports this attractive, yet untested, hypothesis.

Conclusions. To our knowledge, this is the first investigation to collectively examine the effects of nonselective (α_1 and α_2 and β_1 and β_2) and selective (α_1, β_1) adrenoceptor antagonists on rCBF and cardiovascular responses and antiseizure activity in HBO_2. In phase I of HBO_2 at 6 ATA, adrenoceptor blockade has minimal effects on cardiovascular and rCBF responses, suggesting that baroreflex activation is maximal, and vascular tone is mediated primarily via nonadrenergic mechanisms, i.e., $NO^{\cdot}$. In phase II, α_2- and β_2-adrenoceptors, versus α_1- and β_1-adrenoceptors, contribute more to adrenergic control of mean arterial pressure, heart rate, and regional cerebral hyperemia. Of the four drugs, only propranolol abated the cardiovascular and rCBF responses during phase II of HBO_2, affording the most antiseizure activity in extreme HBO_2. Although the mechanisms of propranolol's effectiveness may extend beyond β-adrenoceptor antagonism and include inhibition voltage-gated sodium channels in the heart and brain and/or reduced metabolic activity and CO_2 production, we did not test these possibilities. Collectively, our results do demonstrate the contribution of α- and β-adrenoceptors in regulating mean arterial pressure, heart rate, and rCBF in HBO_2 and the potential use of selective adrenoceptor antagonists to extend the duration of HBO_2 exposures.

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DISCLAIMERS

The views expressed are those of H. G. Gasier and do not reflect the official position of the Uniformed Services University of the Health Sciences or United States Department of Defense.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

I.T.D. and C.A.P. conceived and designed research; I.T.D., S.Y.Z., A.N.M., and A.I.K. performed experiments; H.G.G. analyzed data; H.G.G., I.T.D., S.Y.Z., A.N.M., A.I.K., and C.A.P. interpreted results of experiments; H.G.G. prepared figures; H.G.G. drafted manuscript; H.G.G., I.T.D., and C.A.P. edited and revised manuscript; H.G.G., I.T.D., S.Y.Z., A.N.M., A.I.K., and C.A.P. approved final version of manuscript.

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