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Baroreceptor afferents modulate brain excitation and influence susceptibility to toxic effects of hyperbaric oxygen

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Demchenko IT, Gasier HG, Zhilyaev SY, Moskvin AN, Krivchenko AI, Piantadosi CA, Allen BW. Baroreceptor afferents modulate brain excitation and influence susceptibility to toxic effects of hyperbaric oxygen. *J Appl Physiol* 117: 525–534, 2014. First published July 3, 2014; doi:10.1152/japplphysiol.00435.2014.—Unexplained adjustments in baroreflex sensitivity occur in conjunction with exposures to potentially toxic levels of hyperbaric oxygen. To investigate this, we monitored central nervous system, autonomic and cardiovascular responses in conscious and anesthetized rats exposed to hyperbaric oxygen at 5 and 6 atmospheres absolute, respectively. We observed two contrasting phases associated with time-dependent alterations in the functional state of the arterial baroreflex. The first phase, which conferred protection against potentially neurotoxic doses of oxygen, was concurrent with an increase in baroreflex sensitivity and included decreases in cerebral blood flow, heart rate, cardiac output, and sympathetic drive. The second phase was characterized by baroreflex impairment, cerebral hyperemia, spiking on the electroencephalogram, increased sympathetic drive, parasympatholysis, and pulmonary injury. Complete arterial baroreceptor deafferentation abolished the initial protective response, whereas electrical stimulation of intact arterial baroreceptor afferents prolonged it. We concluded that increased afferent traffic attributable to arterial baroreflex activation delays the development of excessive central excitation and seizures. Baroreflex inactivation or impairment removes this protection, and seizures may follow. Finally, electrical stimulation of intact baroreceptor afferents extends the normal delay in seizure development. These findings reveal that the autonomic nervous system is a powerful determinant of susceptibility to sympathetic hyperactivation and seizures in hyperbaric oxygen and the ensuing neurogenic pulmonary injury.

hyperbaric oxygen; baroreflex sensitivity; afferent stimulation; oxygen seizures; autonomic nervous system

OXYGEN CAN BE ACUTELY TOXIC to the central nervous system when breathed at partial pressures of 2.5 atmospheres absolute (ATA) or above, with manifestations similar to those of generalized epilepsy, including large-scale hypersynchronous neuronal firing in the brain and tonic-clonic motor convulsions (2, 3, 12, 18, 37). The excessive central excitation, manifested as spikes on the EEG, is associated with transpulmonary leakage of protein and focally distributed intra-alveolar hemorrhage (4, 5, 37). This acute neurogenic pulmonary injury is distinct from the diffuse inflammatory lung damage caused by prolonged exposure to hyperoxia at lower partial pressures (24).

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The idea that acute lung injury in hyperbaric oxygen (HBO_2) is related to sympathetic hyperexcitation was formulated more than five decades ago, when it was demonstrated that hypophysectomy, adrenalectomy, or adrenergic blockade could prevent or mitigate pulmonary damage (4). Recently, we have provided direct evidence that a massive sympathetic outflow, reflected in a pronounced increase in renal sympathetic nerve activity (RSNA), precedes oxygen seizures in HBO_2 and is associated with left ventricular (LV) dysfunction and acute pulmonary hypertension (26).

Although the coexistence of spikes on the EEG and sympathetic hyperactivity has been well documented in HBO_2, the mechanisms that initiate central nervous system (CNS) O_2 toxicity and the pathways that link it to acute pulmonary injury are unknown. It is, however, widely accepted that the primary agents that provoke these effects are reactive oxygen and nitrogen species (ROS and RNS), and increases in the production of ROS (15, 19, 38, 48, 60) and RNS (11, 20, 21, 29) have been detected in HBO_2. Thus it is thought that the subsequent oxidation, nitrosation, and nitrosylation of susceptible biomolecules can alter neuronal excitability and synaptic transmission to trigger hypersynchronous firing of neurons across multiple brain structures (13, 14, 18). Indeed, increased neuronal firing has been demonstrated in brain slices (44, 46) directly exposed to oxygen partial pressures as low as 300 mmHg (0.4 ATA), which, in the intact brain, corresponds to an F_{IO2} of ~3 ATA (22). However, in whole animals, we do not see increased central excitability at this level of hyperoxia (25).

Our previous studies of autonomic responses to HBO_2 (23, 24, 26) led us to the idea that signals from the periphery could modify central responses to hyperoxia. Thus we developed the hypothesis that visceral sensory input modulates central excitability and influences susceptibility to seizures in HBO_2. For example, signals ascending from the arterial baroreceptors, in the afferent limb of the baroreflex arc, may be particularly important in preventing or delaying the neurotoxic effects of HBO_2 by opposing brain excitability. Four lines of evidence support this.

First, as recently demonstrated in our laboratory, the well-known protective cardiovascular responses to levels of HBO_2 that normally do not provoke seizures, including an initial systemic vasoconstriction and hypertension followed by decreases in cardiac rate and output and a lower level of hypertension, are linked to activation of the arterial baroreflex (25). From this, we infer that increased afferent traffic resulting from HBO_2-induced activation of arterial baroreflex diminishes excitability in brain regions that regulate sympathetic outflow. Second, bilateral vagotomy hastens the appearance of EEG

patterns associated with CNS toxicity and the subsequent onset of motor convulsions (26). Third, activation of the baroreflex using electrical stimulation of baroreceptor afferent pathways, via the carotid sinus nerve (CSN), has been used clinically to treat conditions caused by excessive sympathetic excitation, such as refractory hypertension (30, 36). Studies with rats have also shown that electrical stimulation of the aortic depressor nerve (ADN) decreases blood pressure (BP) and heart rate (HR) through a centrally mediated increase in parasympathetic drive and sympatholysis (17, 28, 31, 52, 53). Fourth, it has been shown in clinical practice that electrical stimulation of vagal afferents can prevent the seizures of grand mal epilepsy (33, 34, 49).

In the present study, we exposed rats to levels of HBO_2 that can produce seizures (5 and 6 ATA) and used two approaches to test our hypothesis that the arterial baroreflex modulates susceptibility to CNS oxygen toxicity. First, we made physiological measurements after surgically interrupting ascending baroreceptor pathways (the ADN and CSN). Second, we stimulated the ADN electrically in rats with intact baroreceptor afferents. We chose the ADN because it is easier to access and consists almost entirely of baroreceptor afferent fibers (39, 53), whereas the CSN is relatively small in the rat and contains both barosensitive and chemosensitive fibers (32).

METHODS AND EXPERIMENTAL DESIGN

Acute Experiments with Anesthetized Rats

Surgical preparation. Male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 357 ± 17 g were used according to a protocol approved by the Duke University Institutional Animal Use and Care Committee, employing established methods (25). Anesthesia was induced with urethane (750 mg/kg ip) and α-chloralose (75 mg/kg ip) and maintained by intravenous administration of one-fourth of the initial doses, as needed. The left femoral artery and vein were catheterized for monitoring BP and infusing drugs. In some animals, a catheter was inserted in the LV for assessing LV pressure (LVP). In animals in which cardiac output (CO) was to be quantified by thermodilution, a catheter was inserted into the right atrium, and a thermistor (model 511; Yellow Springs Instruments, Yellow Springs, OH) was passed into the ascending aorta. To record RSNA, a pair of platinum electrodes was placed in contact with a branch of the nerve and electrically isolated with SilGel 604 A/B (Wacker Chemie, Munich, Germany) (40). To measure cerebral blood flow (CBF), a platinum disk electrode was positioned on the intact dura mater through a burr hole at lambda. Cranial screws were inserted over the left and right parietal cortices for EEG recording, and ECG electrodes were placed bilaterally under the chest skin.

Baroreceptor deafferentation. For combined aortic (AD) and carotid (CD) baroreceptor deafferentation (ACD), the ADNs and their branches were severed bilaterally, proximal to the vagus, and the CSNs were inactivated by stripping neuronal tissue from each common carotid artery and painting residual fibers with 10% phenol in ethanol (10, 54). In sham-operated rats, the nerve trunks were exposed but left intact (INT). The trachea was intubated and mechanical ventilation begun with room air. Pancuronium bromide (0.5 mg/kg iv) suppressed voluntary respiratory movements and allowed control of tidal volume. Arterial blood gases and pH were measured to confirm normal values; rectal temperature was monitored and maintained at 37 ± 0.5°C, using a heating pad.

Measurements and calculations. Arterial pressure (AP) and EEG were monitored continuously. ECG, RSNA, and total CBF (tCBF) were monitored in selected groups (Table 1). LVP and CO were determined in separate animals to avoid compromising cardiac function. Variables were recorded and analyzed with WinDaq software running on DI-200 data acquisition hardware (DATAQ Instruments, Akron, OH) or with LabScribe 2 software on iWorx IX-228/S hardware (iWorx Systems, Dover, NH). Automatically calculated parameters included systolic, diastolic, and mean arterial pressures (SAP, DAP, MAP), HR, R-wave-to-R-wave (RR) interval, LV end diastolic pressure (LVEDP), and rates of ventricular contraction (+dP/dt) or relaxation (−dP/dt). The standard deviation (SD) for MAP and HR were defined as BP variability (MAPV) and HR variability (HRV) and expressed as percentages. CBF ($ml \cdot min^{-1} \cdot 100\ g\ body\ wt^{-1}$) was calculated from washout curves captured at 10-min intervals by the supradural electrode after 40 s of ventilation with 2.5% H_2 in air (25). CO ($ml \cdot min^{-1} \cdot 100\ g\ body\ wt^{-1}$) was calculated from transpulmonary thermodilution curves obtained at 10-min intervals, after injection of 75 μl of room-temperature glucose solution (2.5%). Systemic vascular resistance (SVR) and regional cerebrovascular resistance (CVR) were computed as MAP/CO and MAP/tCBF. RSNA was integrated over 10 s and expressed as percent change from mean baselines obtained immediately before compression while animals breathed room air. In some animals, bronchoalveolar lavage fluid (BALF) was obtained postmortem to assess transpulmonary protein (57).

Baroreflex function. Baroreflex sensitivity (BRS), the effect of changes in AP on HR (58), was assessed after bolus injections (0.1 ml iv) of phenylephrine (PHE) or sodium nitroprusside (SNP) and quan-

Table 1. *Experimental design*

				Measurements							
Group	*n*	Baroreceptor Afferentation	Stimulation	AP	CO	LVP	BRS	EEG	ECG	RSNA	tCBF
Anesthetized (6 ATA HBO_2)											
1	12	INT		+	+			+		+	+
2	10	INT		+		+	+	+	+		
3	11	ACD		+	+			+		+	+
4	9	ACD		+		+	+	+	+		
5	11	INT	+	+	+			+		+	+
6	8	INT	+	+		+	+	+	+		
Conscious (5 ATA HBO_2)											
1	14	INT		+			+	+*	+		
2	11	AD		+			+	+*	+		
3	12	CD		+			+	+*	+		
4	9	ACD		+			+	+*	+		

AP, arterial pressure; CO, cardiac output; LVP, left ventricular pressure; BRS, baroreflex sensitivity; EEG, electroencephalogram (*in selected awake animals); ECG, electrocardiogram; RSNA, renal sympathetic nerve activity; tCBF, total cerebral blood flow; ATA, atmospheres absolute; HBO_2, hyperbaric oxygen; INT, intact baroreceptor afferents with sham procedure; ACD, aortic-carotid baroreceptor denervation; AD, aortic baroreceptor deafferentation; CD, carotid baroreceptor deafferentation.

tified as the slope of the regression line for the change in HR or RR interval for a given change in systolic arterial pressure: (ΔHR/ΔSAP, bpm/mmHg) or (ΔRR/ΔSAP, ms/mmHg). To verify baroreceptor denervation, doses of PHE (2–6 μg/kg iv) or SNP (5–15 μg/kg iv) were adjusted to raise or lower SAP by 20 to 40 mmHg. To determine BRS during isopression (the period of constant pressure between the end of compression and the start of decompression), PHE (4 μg/kg iv) was infused over 3 s, at 10-min intervals.

Electrical stimulation of baroreceptor afferents. The ADNs were exposed bilaterally below their junctions with the superior laryngeal nerve, placed on bipolar platinum electrodes, isolated with SilGel 604 A/B and connected to an electrical stimulator (Grass Technologies, Warwick, RI). Stimulus intensity needed to decrease HR by about 60 beats/min and BP by 25–45 mmHg was determined, using a 5-s train of 50-Hz pulses (2-ms duration), ranging from 1–3 V. After selection of an optimal voltage, the ADNs were stimulated intermittently (1 s on, 2 s off) for 15 or 30 min to prevent the falloff in response that can occur with sustained stimulation (51).

HBO_2 exposures. Baseline physiological parameters were recorded for a 60-min stabilization period during ventilation with room air. The ventilator was then supplied with 100% O_2, and the hyperbaric chamber (Duke Center for Hyperbaric Medicine and Environmental Physiology) was pressurized with air to 6 ATA at 1 ATA/min. Exposures were limited to 60 min, longer if electrical stimulation was used. All exposures were terminated if seizures occurred. Chamber temperature and relative humidity were maintained at 23 ± 0.5°C and 60 ± 2%. Decompression was accomplished at 0.6 ATA/min, as ventilation continued with 100% O_2. Animals were euthanized with sodium pentobarbital (250 mg/kg iv), and BALF was obtained from selected animals.

Chronic Experiments with Conscious Rats

Experiments were also performed with chronically instrumented conscious animals to determine whether responses to HBO_2 are altered by anesthesia. Hyperbaric facilities were used at the Institute of Evolutionary Physiology and Biochemistry, Russian Academy Sciences, St. Petersburg, Russia, according to a protocol approved by the Ethical Review Board of the Institute, using methods similar to those described above.

Chronic catheterization and baroreceptor deafferentation. Male Wistar rats (Rappolovo animal-breeding facility, Russia), weighing 327 ± 16 g were anesthetized with sodium pentobarbital (50 mg/kg ip), and catheters were passed through the right carotid artery into the aortic arch for recording AP and into the left jugular vein for administering drugs. ECG electrodes were placed under the chest skin, and in some animals cranial screws were inserted for recording cortical EEG. To test whether seizure latency varied with the degree of baroreceptor function, animals were subjected either to a sham procedure as described above (INT), to deafferentation of the AD or CD baroreceptors, or to both (ACD). Catheters were secured, filled with a solution of NaCl (0.9%), glucose (2.5%), and heparin (300 IU/ml), and, together with ECG leads, tunneled subcutaneously to the back of the neck. Animals were given penicillin (30,000 IU/kg) and allowed to recover for 5–7 days. Catheter solutions were refreshed daily.

Hemodynamic measurements and baroreflex assessment. Methods for acquiring and analyzing data were similar to those described above (WinDaq data-acquisition software with DI-200 data acquisition hardware, DATAQ Instruments). AP and ECG were recorded continuously; EEG was recorded in some animals in each group. All animals were observed for CNS toxicity. Seizure latency was defined as minutes after 5 ATA O_2 was reached to the beginning of tonic-clonic convulsions. In animals in which the EEG was recorded, a persistent train of epileptiform spikes marked the start of CNS O_2 toxicity.

To confirm baroreceptor deafferentation, BRS was assessed by PHE injection (4 μg/kg iv), 5–7 days after surgery. During isopression, spontaneous changes in AP, mostly associated with voluntary movement, were used to assess BRS by the sequence method (59). For this, four or more consecutive AP pulses were identified in which SAP and the concurrent RR intervals both increased, and a linear regression (ms/mmHg) was determined.

HBO_2 exposures. To produce responses comparable to those observed in anesthetized animals at 6 ATA, conscious rats habituated to light restraint were monitored visually and exposed to 100% O_2 at 5 ATA for up to 90 min, or until seizures occurred. The chamber was compressed with oxygen at 0.5 ATA/min, and chamber temperature and relative humidity were maintained at 23 ± 0.5°C and 60 ± 2%. Immediately after decompression, at 0.3 ATA/min, arterial blood samples were drawn for norepinephrine assay by HPLC (Coulochem II analyzer; ESA, Chelmsford, MA), as described (62). Animals were euthanized with sodium pentobarbital (250 mg/kg ip), and lung lavage was performed.

Experimental design. Six groups of anesthetized rats were exposed to O_2 at 6 ATA (Table 1). Groups 1 and 2 were subjected to a sham procedure with baroreceptor afferents intact (INT). In groups 3 and 4, both aortic and carotid baroreceptors were deafferentated (ACD). In groups 5 and 6, sham-operated rats with intact baroreceptor afferents were used to test the effect of electrical stimulation of the ADN on physiological parameters and seizure latency. CO was determined in groups 1, 3, and 5; LVP and BRS were determined in groups 2, 4, and

Table 2. *Baseline hemodynamic parameters in anesthetized and conscious rats*

	INT		ACD		AD	CD
Parameters	Anesthetized	Conscious	Anesthetized	Conscious	Conscious	Conscious
MAP, mmHg	97 ± 5	108 ± 6	118 ± 7*	129 ± 10*	111 ± 8	109 ± 9
SAP, mmHg	125 ± 7	129 ± 7	148 ± 11*	149 ± 11*	131 ± 8	134 ± 9
MAPV, %	8.8 ± 0.4	11.3 ± 0.9	15.8 ± 1.5*	14.7 ± 1.8*	12.4 ± 1.3	14.7 ± 1.8*
LVEDP, mmHg	2.7 ± 0.78		7.4 ± 0.89*			
HR, bpm	371 ± 17	397 ± 17	387 ± 9	419 ± 12	388 ± 14	411 ± 12
HRV, %	10.3 ± 2.4	12.7 ± 1.9	6.3 ± 1.1*	9.2 ± 1.7*	10.2 ± 1.1	9.2 ± 1.7*
CO, ml/min per 100 g	37 ± 3.7		40 ± 4.8			
CBF, ml/g per min	89 ± 7.4		87 ± 6.6			
SVR, MAP/CO	2.6 ± 0.23		3.1 ± 0.26			
CVR, MAP/CBF	1.4 ± 0.07		1.5 ± 0.1			
BRS, bpm/mmHg	−1.23 ± 0.02	−2.4 ± 0.18	−0.08 ± 0.004*	−0.03 ± 0.008*	−0.79 ± 0.14*	−0.03 ± 0.008*
$+dP/dt_{max}$, mmHg/s	6342 ± 187		6121 ± 227			
$-dP/dt_{max}$, mmHg/s	7165 ± 213		5236 ± 243*			

Values are means ± SE. MAP, mean AP; SAP, systolic AP; MAPV, MAP variability; LVEDP, left ventricular end diastolic pressure; HR, heart rate; HRV, HR variability; SVR, systemic vascular resistance; CVR, cerebral vascular resistance; BRS, baroreflex sensitivity $+dP/dt_{max}$, maximum rate of LV pressure rise; $-dP/dt_{max}$, maximum rate of LV pressure fall. *$P < 0.05$ vs. INT.

6. BALF, used for analysis of transpulmonary leakage of protein, was taken from some animals in each group and compared with that taken from control animals breathing air at 1 ATA.

Four groups of conscious rats were exposed to HBO_2 at 5 ATA. Group 1 comprised INT rats; in the remaining groups, arterial baroreceptors were deafferentated as follows: AD in group 2, CD in group 3, and ACD in group 4. In all groups, AP and ECG were monitored continuously, and BRS was determined. EEG was monitored in some animals in each group. Lung injury was assessed with bronchoalveolar lavage in some animals in each group and compared with that taken from control animals breathing room air at 1 ATA.

Statistical Analysis

Data were analyzed using StatView software (SAS Institute, Cary, NC), including the Shapiro-Wilk test to determine normality of sample size distribution. Absolute or percentage changes in hemodynamic parameters and BRS were compared with baselines in room air, using repeated-measures ANOVA. When a significant *F*-ratio was obtained, Fisher's least-significant-difference test was applied. All values were expressed as means ± SE. Comparisons among groups were made using two-way ANOVA, followed by a paired *t*-test with Bonferoni's correction for multiple comparisons. Significance was accepted at $P \leq 0.05$.

RESULTS

Effects of Baroreceptor Deafferentation on Baseline Hemodynamic Parameters

In anesthetized ACD rats breathing room air, HR, CO, CBF, and +dP/dt were relatively unchanged 2–3 h after deafferentation, compared with INT animals, but significant changes occurred in other parameters, including increases in MAP, MAPV, SAP, and LVEDP and decreases in HRV, BRS, and −dP/dt max (Table 2).

In conscious rats breathing room air, observed 7 days after surgery, selective baroreceptor deafferentation (AD or CD) resulted in significant reductions in BRS, whereas MAP, SAP,

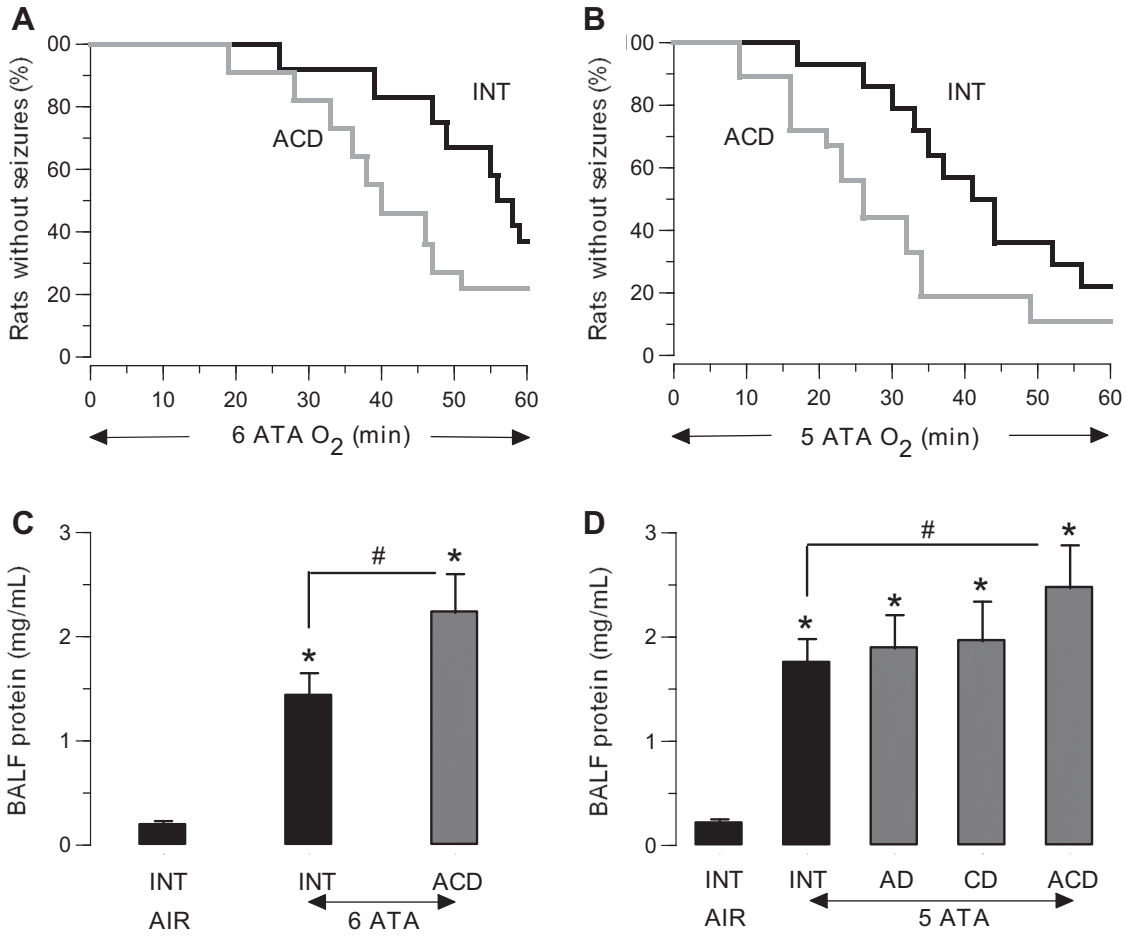

Fig. 1. Hyperbaric oxygen (HBO_2) seizures and pulmonary injury in anesthetized and conscious rats. *A*: in anesthetized rats exposed to HBO_2 at 6 atmospheres absolute (ATA) for 60 min, spikes on the EEG occurred in 8 of 12 intact (INT) rats (67%) and 9 of 11 rats (82%) with combined aortic (AD) and carotid (CD) baroreceptor deafferentation (ACD). Mean seizure latency was 49 ± 4.7 min in INT rats, compared with 38 ± 4.4 min in ACD rats ($P < 0.05$ vs. ACD). *B*: in conscious rats exposed to HBO_2 at 5 ATA for 60 min, motor convulsions were observed in 10 of 14 INT rats (71%) and in 9 of 10 ACD rats (90%). Mean seizure latency was 37 ± 4.7 min in INT rats, compared with 26 ± 4.4 min in ACD rats ($P < 0.05$ vs. ACD). *C*: in anesthetized rats exhibiting spikes on the EEG in HBO_2 at 6 ATA, bronchoalveolar lavage fluid (BALF) protein levels were significantly greater in ACD rats ($n = 7$) than in INT rats ($n = 7$), and levels in both groups were much greater that in INT rats ($n = 7$) breathing room air (*$P < 0.05$ vs. INT, air; #$P < 0.05$ vs. INT, 6 ATA). *D*: in conscious rats exhibiting motor convulsions in HBO_2 at 5 ATA, BALF protein levels were significantly greater in ACD rats ($n = 7$) than in INT rats ($n = 7$), and levels in partially deafferentated animals (AD and CD) were intermediate in value. Values in all groups exhibiting motor convulsions were much greater that in INT animals ($n = 5$) breathing room air (*$P < 0.05$ vs. INT, air; #$P < 0.05$ vs. INT, 5 ATA).

and HR were unchanged. In these animals, only AD produced statistically significant increases in MAPV and decreases in HRV. By contrast, complete baroreceptor deafferentation caused significant increases in MAP, MAPV, and SAP; a marked decrease in HRV; and near abolition of BRS (Table 2).

Effects of Baroreceptor Deafferentation on Responses to HBO_2

CNS and pulmonary responses. In anesthetized animals, ACD curtailed mean seizure latency by ~22%, compared with INT groups (38 ± 4.4 min vs. 49 ± 4.7 min, $P < 0.05$) (Fig. 1*A*). In conscious rats, ACD shortened mean latency for convulsions by ~30% compared with INT animals (26 ± 4.4 min vs. 37 ± 4.7 min, $P < 0.05$) (Fig. 1*B*). However, for conscious animals with selective baroreceptor deafferentation (AD or CD), convulsive latencies (32 ± 6.7 min and 30 ± 5.7 min) did not differ significantly from those in INT animals (37 ± 4.7 min). BALF in ACD animals, both anesthetized and conscious, revealed significantly greater pulmonary protein leakage than in both INT and partially barodeafferentated animals (Fig. 1, *C* and *D*).

Cardiovascular and autonomic responses to HBO_2. In anesthetized animals, MAP rose steeply during compression in both INT and ACD groups, peaking more than 30% above baseline as 6 ATA was reached. For the next 30 min, MAP remained near this level in INT rats but continued to rise slowly in ACD animals (Fig. 2*A*). After 30 min, MAP rose sharply in both groups, nearly converging around 60% above baseline after 50 min. During compression, HR remained unchanged in INT animals and then decelerated for 20 min to almost 10% below preexposure values, but, after ~40 min, tachycardia emerged. In ACD rats, however, tachycardia was observed throughout the hyperbaric exposure. At 60 min, HR for both INT and ACD rats reached levels 15 to 20% above their normalized, preexposure baselines (Fig. 2*B*). CO fell sharply in INT animals but more gradually in ACD animals (Fig. 2*C*).

Changes in tCBF showed an initial plateau or small decrease in INT animals, followed by a steep rise for the remainder of the hyperbaric exposure. In ACD rats, cerebral perfusion increased in two steps (Fig. 2*D*). In both groups, SVR increased roughly in parallel for the first 40 min but then diverged, rising higher in INT groups (Fig. 2*E*). CVR also exhibited a biphasic profile in both groups, but the initial increase was greater in INT rats (Fig. 2*F*). In INT animals, RSNA fell slightly for the first 30–40 min and then increased; however, in ACD rats, both tachycardia and increased sympathetic activity were observed throughout the exposure (Figs. 2*B* and 3*A*). Plasma

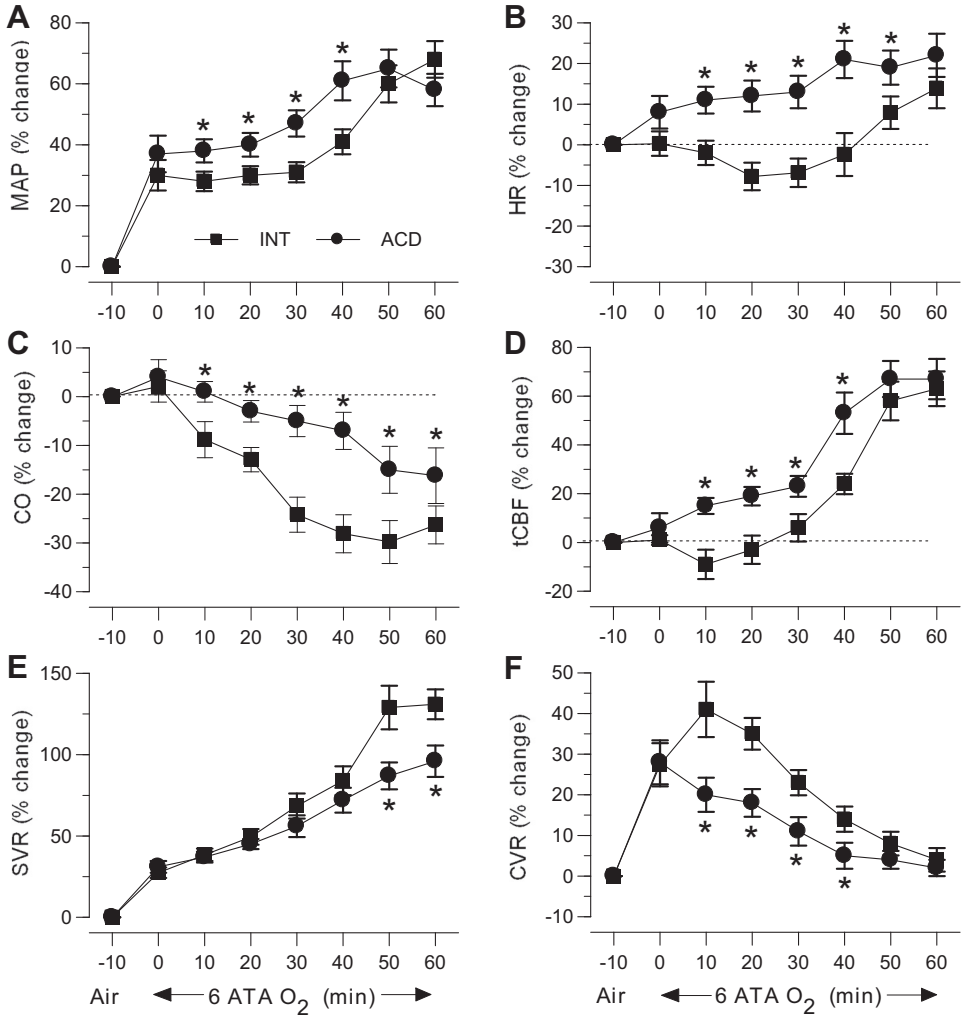

Fig. 2. Cardiovascular responses in anesthetized rats in HBO_2 at 6 ATA. *A*: in INT and ACD animals, mean arterial pressure (MAP) rose in 2 steps separated by an extended period of relative stability; initial, sharp increases to more than 30% above baseline occurred during compression. In INT animals, MAP remained at this plateau for the next 30 min but in ACD animals trended upward. After 30 min, MAP exhibited a second steep rise in both groups, nearly converging around 60% above baseline after 50 min. *B*: heart rate (HR) remained unchanged in INT animals during compression and then decelerated for 20 min to almost 10% below preexposure values; tachycardia emerged at ~40 min. In ACD rats, tachycardia was observed throughout the hyperbaric exposure. After 60 min, HR for both INT and ACD rats reached levels 15 to 20% above their normalized, preexposure baselines. *C*: cardiac output (CO) fell throughout the 60-min exposure in both INT and ACD rats but more sharply in INT animals than in ACD animals. *D*: in INT animals, changes in total cerebral blood flow (tCBF) were biphasic, with a constrictor response followed by hyperemia, but, in ACD rats, CBF increased in 2 steps, with no initial constrictor response. *E*: systemic vascular resistance (SVR) increased roughly in parallel in both groups for the first 40 min but then rose higher in INT groups. *F*: cerebrovascular resistance (CVR) increased in both groups during compression, after which patterns diverged; the increase persisted in INT rats during the first 10 min of isopression, while falling rapidly in ACD rats. CVR ultimately fell in both groups, converging near baseline levels at 60 min (*$P < 0.05$ vs. INT).

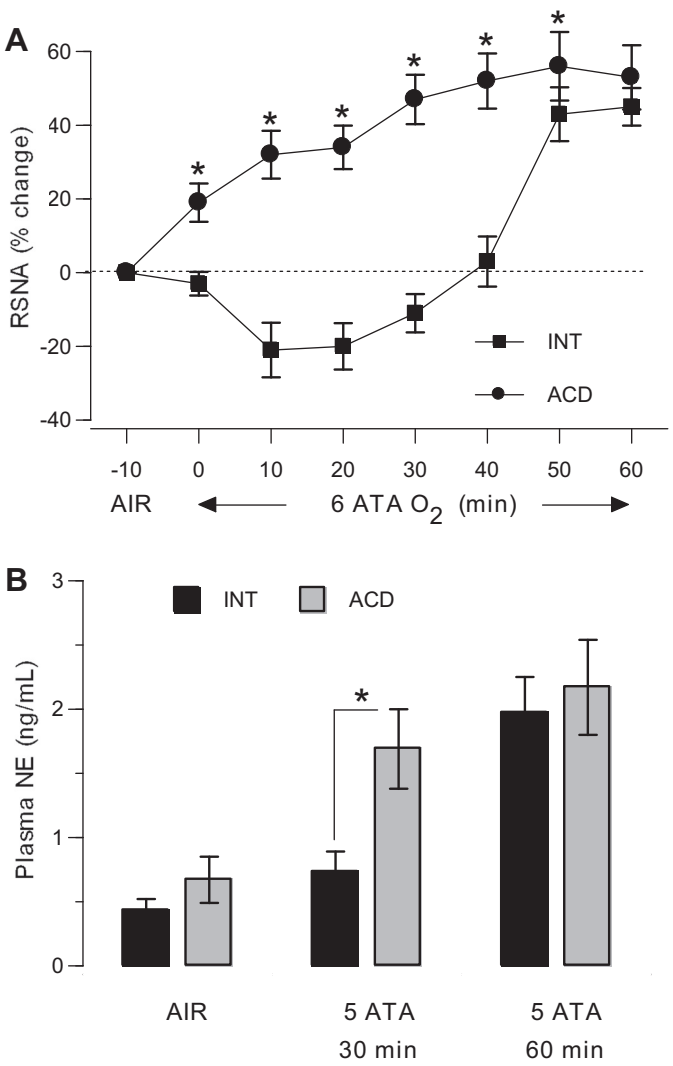

Fig. 3. Sympathetic responses in anesthetized and conscious rats. *A*: renal sympathetic nerve activity (RSNA) in anesthetized rats exposed to HBO_2 at 6 ATA for 60 min depended on the integrity of the arterial baroreflex. Sympathetic activity was depressed in INT groups for the first 30–40 min but in ACD rats rose progressively throughout the HBO_2 exposure. By the end of the exposure, RSNA in both groups converged at levels near 50% above baseline. *B*: plasma norepinephrine (NE) levels in conscious rats exposed to HBO_2 at 5 ATA for 60 min were significantly higher in ACD rats compared with in INT rats after 30 min at 5 ATA, but, after 60 min, both groups showed comparable increases (*$P < 0.05$ vs. INT).

norepinephrine (NE) levels, another index of sympathetic activity, were higher in ACD rats compared with INT animals after 30 min at 5 ATA, but, after 60 min, both groups showed comparable increases (Fig. 3*B*).

BRS in HBO_2. In anesthetized animals exposed to HBO_2 at 6 ATA, BRS increased progressively for the first 30–40 min but then declined abruptly, reflecting baroreflex impairment (Fig. 4*A*) coinciding with equally sudden increases in RSNA, AP, and HR (Figs. 2 and 3). Similarly, in conscious rats exposed to HBO_2 at 5 ATA, BRS increased for approximately the first 30 min but then suddenly declined, demonstrating baroreflex impairment (Fig. 4*B*). AP and HR increased sharply, and EEG spikes and neuromuscular convulsions appeared (data not shown).

Effects of electrical stimulation of ADN in HBO_2. Bilateral electrical stimulation of the ADN in anesthetized INT rats markedly enhanced the protective cardiovascular effects of baroreceptor activation in HBO_2 at 6 ATA; the AP depressor response, bradycardia, and decrease in RSNA were all sustained throughout the period of electrical stimulation (Fig. 5). Stimulation preserved robust baroreflex function and prevented the hyperemia that appeared after ~30 min in unstimulated animals (Fig. 6). Also, seizure latency was delayed and transpulmonary extravasation of protein was diminished (Fig. 7).

DISCUSSION

The novel aspect of this study is the demonstration that CNS, autonomic, and cardiovascular responses to HBO_2 at 5 or 6 ATA occur in two contrasting phases that reflect time-dependent alterations in the functional state of the arterial baroreflex. The first phase is associated with an increase in BRS and confers protection against potentially neurotoxic doses of hyperbaric oxygen. The second phase is characterized by impairment of baroreflex function that is concurrent with the abrupt appearance of cerebral hyperemia, spikes on the

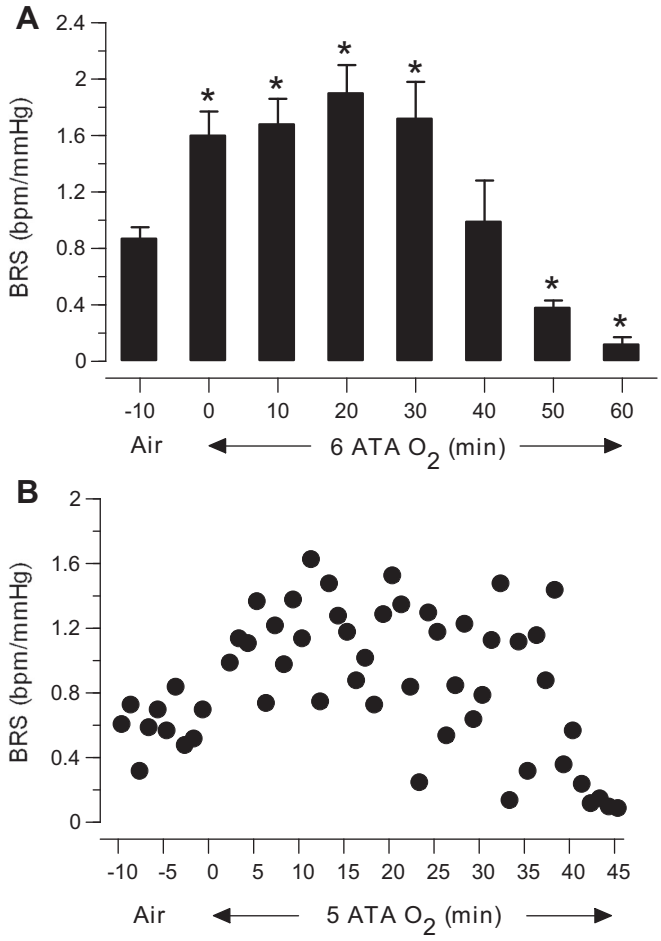

Fig. 4. Baroreflex sensitivity (BRS) in anesthetized and conscious rats. *A*: BRS in anesthetized INT rats exposed to HBO_2 at 6 ATA for 60 min exhibited a clear pattern of increases (for up to 30 min), followed by a gradual decreases leading to baroreflex impairment by 50 min. *B*: BRS in conscious INT rats exposed to HBO_2 at 5 ATA showed a pattern of responses similar to those of anesthetized rats. BRS increased for approximately the first 30 min but then declined abruptly, reflecting baroreflex impairment (*$P < 0.05$ vs. air).

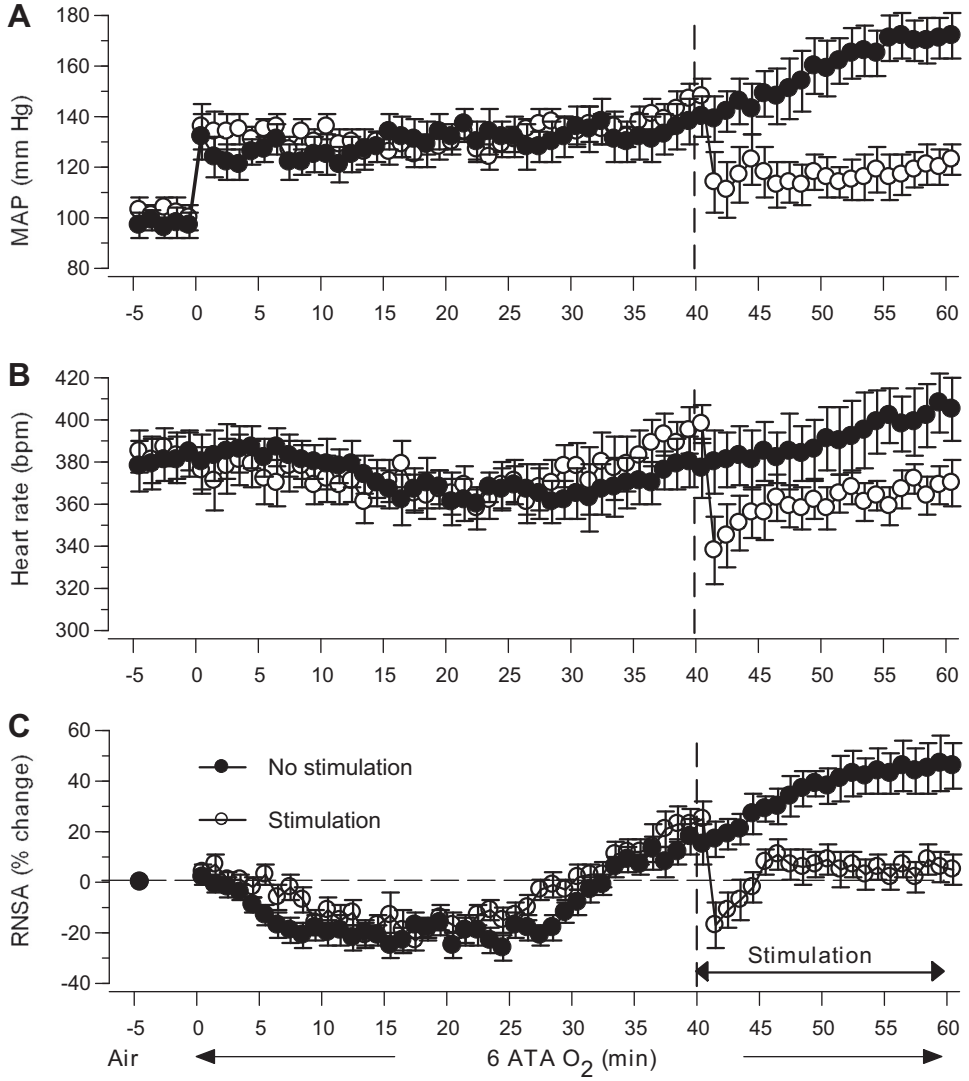

Fig. 5. Autonomic effects of electrical stimulation of baroreflex afferents. In anesthetized INT rats exposed to HBO_2 at 6 ATA, bilateral electrical stimulation of the of aortic depressor nerve (ADN) augmented the beneficial pressor (*A*), cardiac (*B*), and neuronal (*C*) effects of baroreceptor activation, even if initiated after 40 min of exposure, when the protective effect of baroreflex activation begins to decline in unstimulated animals, and these effects persisted throughout the period of stimulation.

EEG, increased sympathetic drive, and parasympatholysis. The central events are followed by neurogenic pulmonary injury characterized by transpulmonary leakage of protein and focally distributed intra-alveolar hemorrhage. Complete baroreceptor deafferentation abolishes the initial protective responses to HBO_2, whereas electrical stimulation of intact baroreceptor afferents augments them.

Changes in baseline cardiovascular parameters after complete baroreceptor deafferentation in both anesthetized and conscious rats breathing room air reveal, most importantly, that BRS was always significantly reduced (Table 2). This finding is essential to establishing the link between baroreflex impairment and CNS oxygen toxicity. However, incomplete baroreceptor deafferentation in conscious rats resulted in insignificant changes, suggesting that a partially intact baroreflex arc can compensate for its missing components, at least in normal environmental conditions. It is also important to note that there were no significant differences between the two strains of rats. This is consistent with the results of DiBona and Jones, who compared cardiovascular responses in Wistar and Sprague Dawley rats and observed comparable coupling between AP and RSNA in both strains, in both conscious and anesthetized animals, immediately after AD or CD (27).

Protective Autonomic and Cardiovascular Responses to HBO_2 Are Baroreflex Mediated

The initial phase that occurs during oxygen compression and the early stages of isopression involves an increase in BRS associated with a slight bradycardia, a decrease in RSNA, and cerebral vasoconstriction that limits oxygen delivery to the brain, which is therefore protective. Furthermore, these responses are nearly abolished by total baroreceptor deafferentation, demonstrating the importance of these afferent pathways. This accords with previous findings, in both animal studies (9, 55, 56, 61) and observations in humans (1, 41–43, 45, 63), that in HBO_2 below 3 ATA, parasympathetic activity predominates, as indicated by decreases in HR, CO, and respiratory frequency. Thus the cardiovascular responses that persist throughout a 60-min exposure to HBO_2 at 3 ATA are similar to those seen only during the initial stages of a 60-min exposure to 5 or 6 ATA. In both cases, the autonomic nervous system regulates cardiovascular and pulmonary functions re-

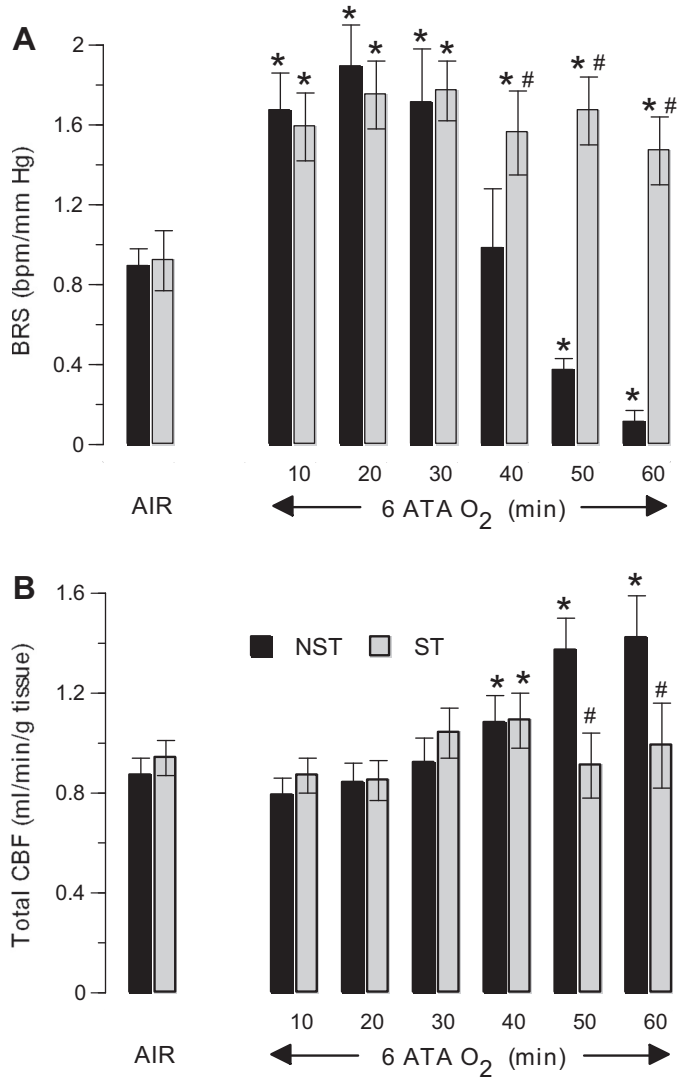

Fig. 6. Cardiovascular responses to electrical stimulation of baroreflex afferents. *A*: in anesthetized INT rats exposed to HBO_2 at 6 ATA, but not stimulated (NST), cerebral hyperemia appeared after ~30 min. In anesthetized INT rats exposed to HBO_2 at 6 ATA, bilateral electrical stimulation (ST) of the ADN nearly abolished the hyperemic response. *B*: in anesthetized INT rats exposed to HBO_2 at 6 ATA, but NST, the initial protective increase in BRS began to decline after 30 min, leading to baroreflex impairment at 60 min. In anesthetized INT rats exposed to the same conditions, bilateral electrical ST of the ADN preserved robust baroreflex function, even if initiated at the time BRS would otherwise begin to decline (*$P < 0.05$ vs. air; #$P < 0.05$ vs. NST).

flexively, mainly through baroreflex activation, as AP rises as a result of hyperoxic vasoconstriction (25). In general, afferent discharges ascend to subcortical and cortical brain regions comprising the central autonomic network (8), for which structures and functions have been anatomically localized (7, 16, 35). It is in these regions that the afferent discharges are integrated to evoke responses that suppress efferent sympathetic activity, augment parasympathetic outflow, and afford short-term protection from hyperoxia. The event that triggers the protective baroreflex response in HBO_2 at 5 or 6 ATA is the vasoconstriction that accompanies oxygen compression and the early stages of isopression and is best explained as loss of the vasodilator activity of nitric oxide (47, 50) as it reacts with superoxide anion to form peroxynitrite (6, 11, 20). Because the resulting increase in systemic vascular resistance is independent of neuronal control, it cannot be completely reversed by the reduction in sympathetic tone that is a normal consequence of baroreflex activation. The heart, however, is also a target of baroreflex efferents, which through negative chronotropic and inotropic responses reduces but does not eliminate hyperoxic hypertension by decreasing CO.

Baroreflex Impairment Precedes Oxygen Seizures

The second phase of cardiovascular responses begins after 30–40 min in HBO_2 at 5 and 6 ATA and is characterized by a shift in autonomic balance that favors sympathetic activation (Figs. 3 and 4). In this phase, BRS decreases rapidly, RSNA increases, tachycardia replaces the initial bradycardia, and

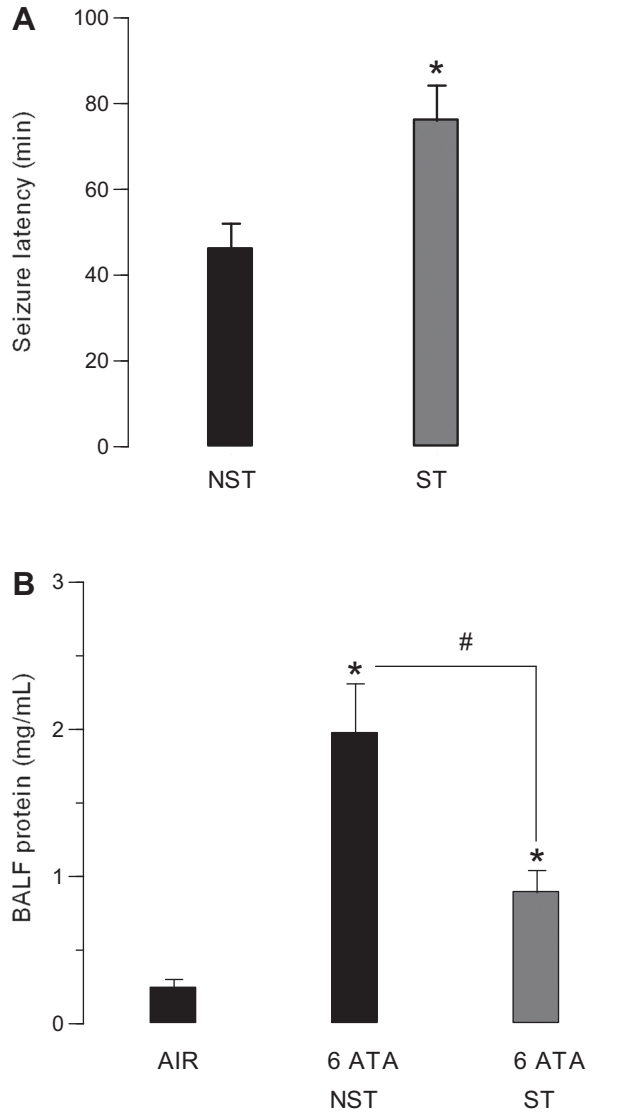

Fig. 7. Central nervous system and pulmonary responses to electrical stimulation of baroreflex afferents. *A*: in anesthetized INT rats exposed to HBO_2 at 6 ATA, bilateral electrical ST of the ADN resulted in a significant increase in mean seizure latency, compared with animals that were NST. *B*: in the same animals, bilateral electrical stimulation of the ADN significantly decreased extravasation of protein into the alveolar space, consistent with a decrease in pulmonary injury (*$P < 0.05$ vs. air; #$P < 0.05$ vs. NST).

cerebral hyperemia supplants the protective vasoconstriction. The mechanism of baroreflex impairment associated with these cardiovascular and autonomic changes is unknown, but it theoretically could occur at any level of the reflex arc. In the present study, the concurrence of an abrupt decrease in BRS with brain activation and increased sympathoexcitation suggests that alterations in central baroreflex control can cause baroreflex impairment. Furthermore, when the normal afferent response of the baroreflex to HBO_2 is supplemented by electrical stimulation, the onset of the second phase is deferred, with the result that hyperoxic hypertension is further reduced, tachycardia is virtually abolished, cerebral hyperemia is diminished, seizure latency is extended, and an important index of lung injury is dramatically lessened (Figs. 5, 6, and 8). The fact that electrical stimulation of the ADN preserves baroreflex responses in neurotoxic levels of HBO_2 may imply that impairment of the reflex results from a loss of sensitivity in the baroreceptors themselves. Future studies are needed to determine where in the reflex arc BRS impairment occurs and what mechanisms are involved.

In a previous study, we demonstrated that interruption of afferents from arterial baroreceptors removes sympathetic restraint in HBO_2 exposures that are not normally neurotoxic ($\leq$3 ATA) (25). Here, in levels of HBO_2 that can be neurotoxic, we have shown that electrical stimulation of baroreceptor afferents delays the onset of CNS toxicity. Taken together, these two studies allow us to draw three conclusions. First, afferent traffic from the arterial baroreflex prevents the development of excessive central excitation that leads to seizures. Second, diminished afferent signaling due to baroreflex inactivation or impairment removes this defense, and seizures may follow. Third, electrical stimulation of baroreceptor afferents enhances the protection provided by the intact baroreflex and delays or prevents seizures that would otherwise occur. These findings support the hypothesis that the functional state of the arterial baroreflex is a powerful determinant of susceptibility to seizures in HBO_2, through its modulation of brain excitation. This suggests that manipulation of afferent signaling, from baroreceptors or other sources of input to the central autonomic network, may provide a novel way to extend operational limits for human divers and others who are exposed to extreme hyperoxia.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

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