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Baroreflex-mediated cardiovascular responses to hyperbaric oxygen

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Demchenko IT, Zhilyaev SY, Moskvin AN, Krivchenko AI, Piantadosi CA, Allen BW. Baroreflex-mediated cardiovascular responses to hyperbaric oxygen. *J Appl Physiol* 115: 819–828, 2013. First published July 3, 2013; doi:10.1152/japplphysiol.00625.2013.—The cardiovascular system responds to hyperbaric hyperoxia (HBO_2) with vasoconstriction, hypertension, bradycardia, and reduced cardiac output (CO). We tested the hypothesis that these responses are linked by a common mechanism—activation of the arterial baroreflex. Baroreflex function in HBO_2 was assessed in anesthetized and conscious rats after deafferentation of aortic or carotid baroreceptors or both. Cardiovascular and autonomic responses to HBO_2 in these animals were compared with those in intact animals at 2.5 ATA for conscious rats and at 3 ATA for anesthetized rats. During O_2 compression, hypertension was greater after aortic or carotid baroreceptor deafferentation and was significantly more severe if these procedures were combined. Similarly, the hyperoxic bradycardia observed in intact animals was diminished after aortic or carotid baroreceptor deafferentation and replaced by a slight tachycardia after complete baroreceptor deafferentation. We found that hypertension, bradycardia, and reduced CO—the initial cardiovascular responses to moderate levels of HBO_2—are coordinated through a baroreflex-mediated mechanism initiated by HBO_2-induced vasoconstriction. Furthermore, we have shown that baroreceptor activation in HBO_2 inhibits sympathetic outflow and can partially reverse an O_2-dependent increase in arterial pressure.

hyperbaric oxygen; baroreflex; hypertension; bradycardia; autonomic nervous system

THE MAMMALIAN CARDIOVASCULAR system responds acutely to hyperoxia with vasoconstriction, hypertension, bradycardia, and decreased cardiac output (CO). Although these effects have been investigated for many years, and several hypotheses have been proposed to explain them individually, no physiological process that links them through a common mechanism is known.

Vasoconstriction, thought to be the primary response to hyperoxia, has been observed in animals, healthy volunteers, and patients (26, 33, 46), particularly in hyperbaric oxygen (HBO_2). It has been variously attributed to direct effects on vascular smooth muscle of O_2 or reactive O_2 species (21, 48); to O_2-mediated alterations in endothelial-derived vasoactive agents, such as nitric oxide (NO) (12, 32, 35) and endothelin-1 (9); or to products of arachidonic acid metabolism (34). Neuronal mechanisms, such as a de-activation of arterial chemoreceptors (15, 24), have also been suggested but not established. The arterial hypertension reported in most animal and human studies involving HBO_2 (1, 3, 6, 22, 44, 47) has been attributed to hyperoxic vasoconstriction, but this remains hypothetical.

Bradycardia is also a consistent effect of hyperbaric hyperoxia and is abolished by muscarinic blockade or vagotomy (3, 16). In addition, power spectrum analysis of heart rate variability (HRV) indicates that HBO_2 slows the heart by increasing parasympathetic activity (25), further implicating vagal efferents; however, no trigger has been identified for this parasympathetic restraint. The reduction in CO observed in HBO_2 has been ascribed to hyperoxic bradycardia, but diminished cardiac contractility may also be a factor (27, 30, 36).

If vasoconstriction is the primary response to hyperbaric hyperoxia, leading to increases in vascular resistance and arterial pressure (AP), and if bradycardia and decreased CO are secondary effects, then the acute cardiovascular responses observed in HBO_2 could be explained by activation of the baroreflex, which is normally the principal, moment-to-moment regulator of blood pressure (19). This hypothesis predicts that elevations in AP due to hyperoxic vasoconstriction activate mechanoreceptors in the aortic arch and carotid sinuses, the major baroreceptors. The resulting afferent discharges would evoke central responses that suppress efferent sympathetic activity and augment parasympathetic outflow, diminishing cardiac rate, contractility, and output, as well as peripheral vascular resistance. Although several investigators have suggested that the baroreflex contributes to bradycardia in normobaric hyperoxia (10, 15), studies do not support this idea conclusively, because the latency of the negative chronotropic response is longer than expected for known neural reflexes, and HR sometimes fails to return to baseline when normoxia is restored. Therefore, hyperoxic bradycardia did not appear to be explained solely by the baroreflex (18, 24). Assessments of baroreflex sensitivity (BRS) in normobaric hyperoxia have also shown conflicting results, with increases (5, 38, 45) and decreases (18) being reported.

In hyperbaric hyperoxia, however, cardiovascular responses to O_2 are more clearly defined, but baroreflex function has not been investigated in these conditions. Therefore, we designed this study to test whether the several cardiovascular responses to HBO_2 are linked by a common physiological mechanism and to determine whether the arterial baroreflex is involved in the resulting alterations in cardiac function and AP.

METHODS AND EXPERIMENTAL DESIGN

Experiments with Anesthetized Rats

Surgical preparation. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), approximately 300–400 g, were

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Table 1. *Experimental design*

Group	*n*	100% O_2, ATA	Denervation, +Antagonist	Cardiovascular Measurements					
				AP	ECG	RSNA	tCBF	CO	BRS
Anesthetized									
1	5	3	VE	+	+				
2	5	3	PB	+	+				
3	5	3	PR	+	+				
4	5	3	TB	+	+				
5	5	3	LN	+	+				
6	7	3	AD	+	+		+		+
7	7	3	CD	+	+		+		+
8	12	3	Complete	+	+	+	+	+	+
9	12	3	Intact	+	+	+	+	+	+
Conscious									
1	12	2.5	Intact	+	+				+
2	8	2.5	AD	+	+				+
3	8	2.5	CD	+	+				+
4	12	2.5	Complete	+	+				+

O_2, oxygen; AP, arterial pressure; RSNA, renal sympathetic nerve activity; tCBF, total cerebral blood flow; CO, cardiac output; BRS, baroreflex sensitivity; VE, vehicle controls, 0.9% sodium chloride; PB, phenoxybenzamine; PR, propranolol; TB, tiotropium bromide; LN, N^G-nitro-L-arginine methyl ester; AD, aortic baroreceptor denervation; CD, carotid baroreceptor denervation; complete, aortic-carotid baroreceptor denervation; intact, baroreceptors intact with sham procedure.

used, according to a protocol approved by the Duke University Institutional Animal Use and Care Committee, using methods reported previously (14).

Baroreceptor denervation. Baroreceptors were denervated as described (8, 37). For selective aortic baroreceptor denervation (AD), the aortic depressor nerves and their communicating branches were severed bilaterally, proximal to the vagus. For selective carotid baroreceptor denervation (CD), each common carotid artery was exposed 2–3 mm above and below its bifurcation, stripped of neuronal tissue, and painted with 10% phenol in ethanol to inactivate any remaining fibers. For animals with complete barodenervation, AD and CD were combined. In sham-operated rats ("intact"), the relevant nerve trunks were exposed but undamaged. The trachea was intubated and mechanical ventilation instituted with room air. Pancuronium bromide (0.5 mg/kg iv) was administered to prevent voluntary respiratory movements and permit control of arterial carbon dioxide partial pressure (PCO_2) by adjusting tidal volume. Arterial blood gases and pH were measured as reported (14). Rectal temperature was monitored with a thermistor and maintained at 37 ± 0.5°C, using a heating pad.

Measurements and calculations. AP was measured continuously using a pressure transducer (Viggo-Spectramed, Oxnard, CA). Total cerebral blood flow (tCBF) was calculated from hydrogen (H_2) washout curves detected by the supradural electrode (13). CO was calculated from transpulmonary thermodilution curves obtained after injection of 75 μl of room-temperature glucose solution (2.5%) and was normalized for body weight ($ml \cdot min^{-1} \cdot 100\ g\ body\ wt^{-1}$). Renal sympathetic nerve activity (RSNA) was averaged over 10 s and expressed as percent change from baselines in animals breathing room air.

AP and HR were recorded in real time with WinDaq software using DI-200 data acquisition hardware (DATAQ Instruments, Akron, OH) or with LabScribe 2 software using iWorx IX-228/S hardware (iWorx Systems, Dover, NH). The SD for mean AP (MAP) and HR was defined as AP variability (APV) and HRV and expressed as percentages. Systemic vascular resistance (SVR) and cerebrovascular resistance (CVR) were computed as MAP/CO and MAP/tCBF.

BRS assessment. BRS, the effect of changes in AP on HR, was assessed pharmacologically (40) or with the sequence method (42). Pharmacologically, BRS was quantified as the slope of the linear regression for the change in HR or R-wave-to-R-wave (RR) interval for a given change in systolic AP (SAP), after bolus injections (0.1 ml iv) of phenylephrine (PHE) or sodium nitroprusside (SNP), and expressed as bpm/mmHg or ms/mmHg. To assess the effectiveness of baroreceptor denervation, doses of PHE (2–6 μg/kg) or SNP (5–15 μg/kg) were adjusted to raise or lower SAP between 20 and 40 mmHg. For BRS assessment during O_2 isopression (the period of constant pressure between the end of compression and the start of decompression), PHE (4 μg/kg) was injected over 3 s, at 10-min intervals. In the sequence method, BRS was assessed during the O_2 compression to 3 ATA, when AP increases spontaneously. A series of consecutive AP pulses was identified, in which SAP and the concurrent RR interval, averaged for 5 s, both increased. A linear-regression slope (ms/mmHg) was determined for each 5-s sequence.

Table 2. *Baseline hemodynamic parameters in anesthetized rats*

	Intact	AD	CD	Complete
MAP, mmHg	96 ± 5	103 ± 7	105 ± 7	119 ± 9*
SAP, mmHg	121 ± 7	128 ± 9	131 ± 8	142 ± 12*
MAPV, %	9.4 ± 1.2	12.9 ± 1.3*	13.3 ± 1.8*	15.1 ± 2.7*
HR, beat/min	362 ± 21	373 ± 18	375 ± 17	409 ± 19*
HRV, %	8.9 ± 0.8	5.8 ± 0.6*	5.9 ± 0.7*	5.6 ± 0.5*
CBF, $ml \cdot 100\ g^{-1} \cdot min^{-1}$	82 ± 6.3	78 ± 6.1	80 ± 6.4	79 ± 5.4
CO, $ml \cdot 100\ g^{-1} \cdot min^{-1}$	38 ± 1.9	40 ± 3.1	39 ± 3.1	43 ± 3.6*
SVR, MAP/CO	2.53 ± 0.12	2.53 ± 0.14	2.69 ± 0.17	2.77 ± 0.21
CVR, MAP/CBF	1.17 ± 0.08	1.28 ± 0.09	1.31 ± 0.1	1.51 ± 0.14*

MAP, mean AP; SAP, systolic AP; MAPV, MAP variability; HR, heart rate; HRV, HR variability; SVR, systemic vascular resistance; CVR, cerebrovascular resistance. Values are means ± SE. *$P < 0.05$ vs. intact (control).

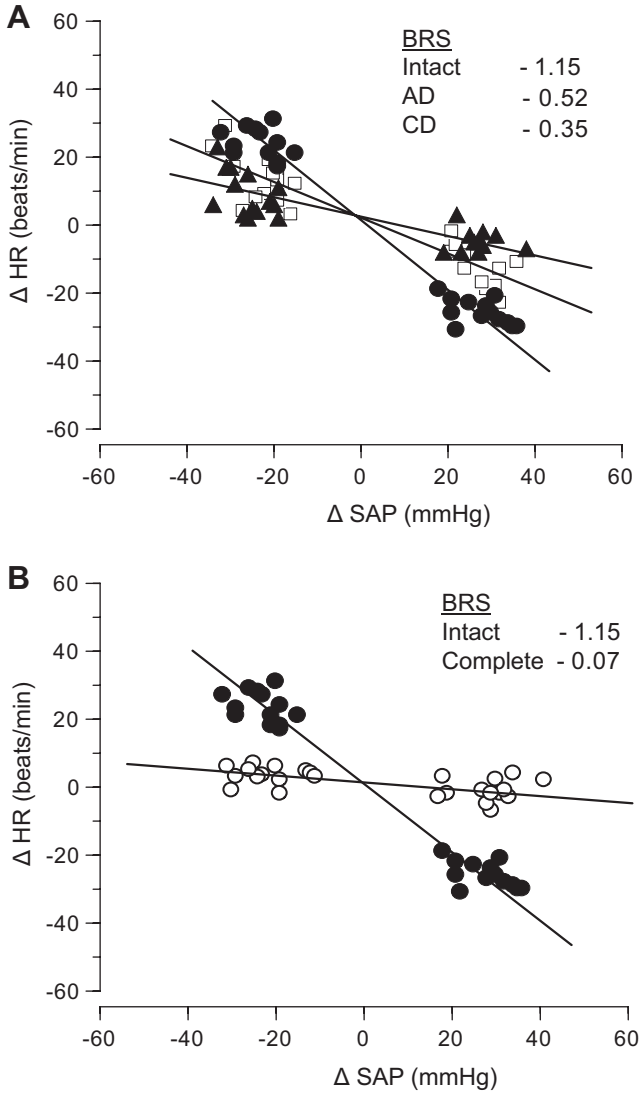

Fig. 1. Baroreflex sensitivity (BRS) in anesthetized rats breathing room air after baroreceptor denervation. *A*: BRS, assessed by bolus injection of nitroprusside (*left* data clusters) or phenylephrine (PHE; *right* data clusters), was diminished in selectively baroreceptor-denervated aortic baroreceptor denervation (AD; open squares) and carotid baroreceptor denervation (CD; closed triangles) rats compared with intact animals (closed circles). *B*: BRS was impaired severely in animals with fully deafferented baroreceptor animals (open circles); the slope of regression line approaches "0." Δ HR, change in heart rate; Δ SAP, change in systolic arterial pressure; intact, baroreceptors intact with sham procedure; complete, AD/CD.

HBO_2 exposures. Baseline physiological parameters were recorded over a 60-min stabilization period, as each rat was ventilated with room air. The ventilator was then supplied with 100% O_2, as the hyperbaric chamber (Duke Center for Hyperbaric Medicine and Environmental Physiology, Durham, NC) was pressurized with air to 3 ATA at 1 ATA/min. Exposures were limited to 60 min. Chamber temperature and relative humidity were maintained at 23 ± 0.5°C and 60 ± 2%. Decompression was accomplished at 0.6 ATA/min, as the rat continued to breathe 100% O_2. Animals were euthanized with Nembutal (250 mg/kg iv) while anesthetized.

Experiments with Conscious Rats

To control for anesthesia, experiments were performed with conscious animals 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.

Chronic catheterization and baroreceptor denervation. Male Wistar rats (Rappolovo animal-breeding facility; Russia), weighing approximately 260–330 g, were anesthetized with pentobarbital (50 mg/kg ip), and a polyethylene catheter (PE-10) was advanced through the right carotid artery into the aortic arch for recording AP. A left jugular vein catheter and ECG electrodes were installed. Baroreceptors were denervated partially or totally or subjected to a sham procedure, as described above. The incision was closed with wound clips. All catheters were secured with ligatures, filled with a solution containing sodium chloride (NaCl; 0.9%), glucose (2.5%), and heparin (300 IU/ml), and together with ECG electrode leads, tunneled subcutaneously to the back of the neck. Animals were given penicillin (30,000 IU/kg) and allowed to recover from surgery for 5–7 days. Catheters were refilled daily with fresh solution.

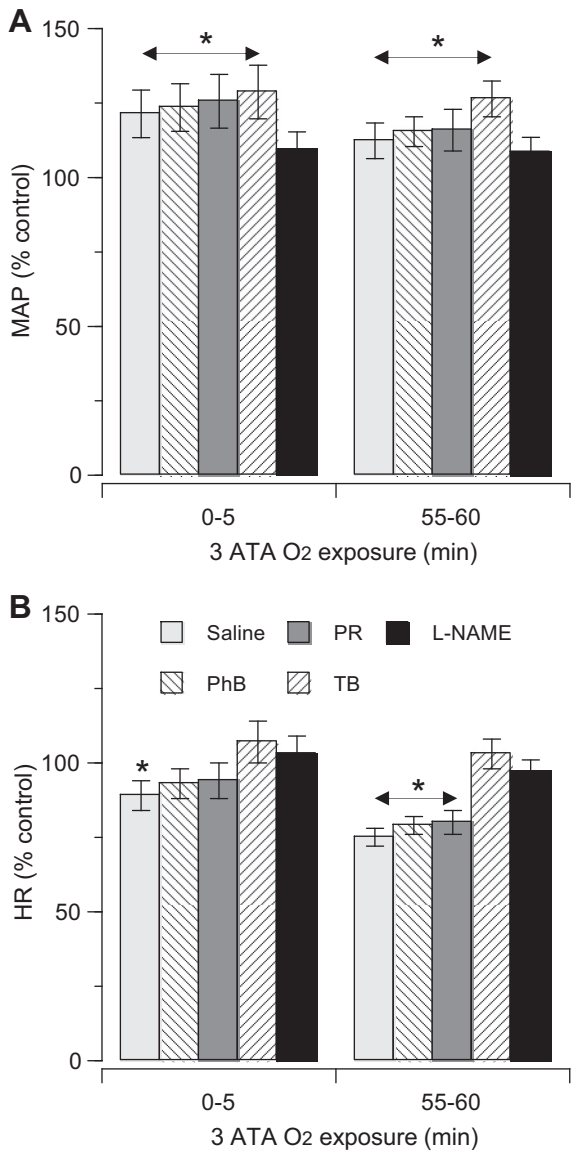

Fig. 2. Cardiovascular responses in anesthetized rats in hyperbaric oxygen (HBO_2) at 3 ATA after adrenergic or cholinergic receptor blockade. *A* and *B*: mean AP (MAP) and HR responses, averaged over 5 min, were assessed at the beginning and end of the isopression phase (0–5 min and 55–60 min) in rats pretreated with vehicle (saline), phenoxybenzamine (PhB), propranolol (PR), tiotropium bromide (TB), or N^G-nitro-L-arginine methyl ester (L-NAME). Values are means ± SE (*$P < 0.05$ vs. pre-exposure levels).

Hemodynamic measurements and baroreflex assessment. Methods for acquiring and analyzing data were similar to those described for anesthetized animals (WinDaq data-acquisition software, WinDaq software and DI-200 data acquisition hardware, DATAQ Instruments). To confirm baroreceptor denervation, BRS was assessed pharmacologically with PHE (4 μg/kg iv), 5–7 days after surgery. During compression, BRS was determined with the sequence method. In isopression, spontaneous changes in AP, mostly associated with voluntary movement, were also used to assess BRS by the sequence method, using four or more consecutive AP pulses in which SAP and RR intervals both increased. A linear-regression slope (ms/mmHg) was determined for selected sequences, as described (42).

HBO_2 exposures. To produce responses comparable with those observed in anesthetized animals at 3 ATA, awake rats were individually exposed to 100% O_2 at 2.5 ATA (60 min) while suspended in a hammock, to which they had been habituated, and AP and ECG were monitored continuously. The chamber was compressed with O_2 at 1 ATA/min and decompressed at 0.6 ATA/min. Chamber temperature and relative humidity were maintained at 23 ± 0.5°C and 60 ± 2%.

Experimental Design

Anesthetized rats (n = 63) in nine experimental groups were individually exposed to HBO_2 at 3 ATA for 60 min (Table 1). *Groups 1–5* were pretreated, 30 min before hyperbaric exposure, with 0.9% NaCl (vehicle controls); the nonselective α-receptor antagonist phenoxybenzamine (0.5 mg/kg ip); the nonselective β-receptor antagonist propranolol (0.5 mg/kg ip); the muscarinic receptor antagonist tiotropium bromide (18 μg/kg ip); or the nonselective inhibitor of NO synthesis, N^G-nitro-L-arginine methyl ester (L-NAME; 40 mg/kg ip). Cardiovascular responses to HBO_2 were compared to delineate the sympathetic or parasympathetic contributions to changes in AP and HR. In *groups 6–8*, aortic or carotid baroreceptors or both were denervated to determine their separate and combined contributions to the baroreflex response in HBO_2. Animals in *group 9* were subjected to a sham procedure and served as controls. CO or BRS was assessed separately in intact rats and in those with complete baroreceptor deafferentation. Conscious rats (n = 40) were exposed to HBO_2 at 2.5 ATA for 60 min and included intact, AD, and CD rats and those with complete baroreceptor deafferentation (Table 1). AP and ECG were monitored continuously, and BRS was assessed.

Statistical Analysis

Data were analyzed using StatView software (SAS Institute, Cary, NC). Absolute or percentage changes in hemodynamic parameters or 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 used. All values were expressed as means ± SE. Comparisons among groups were made using two-way ANOVA, followed by a paired t-test with Bonferroni correction for multiple comparisons. Significance was accepted at $P \leq 0.05$.

RESULTS

Anesthetized Rats

Baseline hemodynamic parameters after baroreceptor denervation. Mean hemodynamic parameters in selectively denervated rats did not differ significantly from those in intact rats, 2 h after surgery, but mean APV (MAPV) was greater, and HRV was less than in the intact group (Table 2). In animals with complete baroreceptor deafferentation, most hemodynamic parameters, including MAPV and HRV, differed significantly from values observed in intact rats (Table 2). As

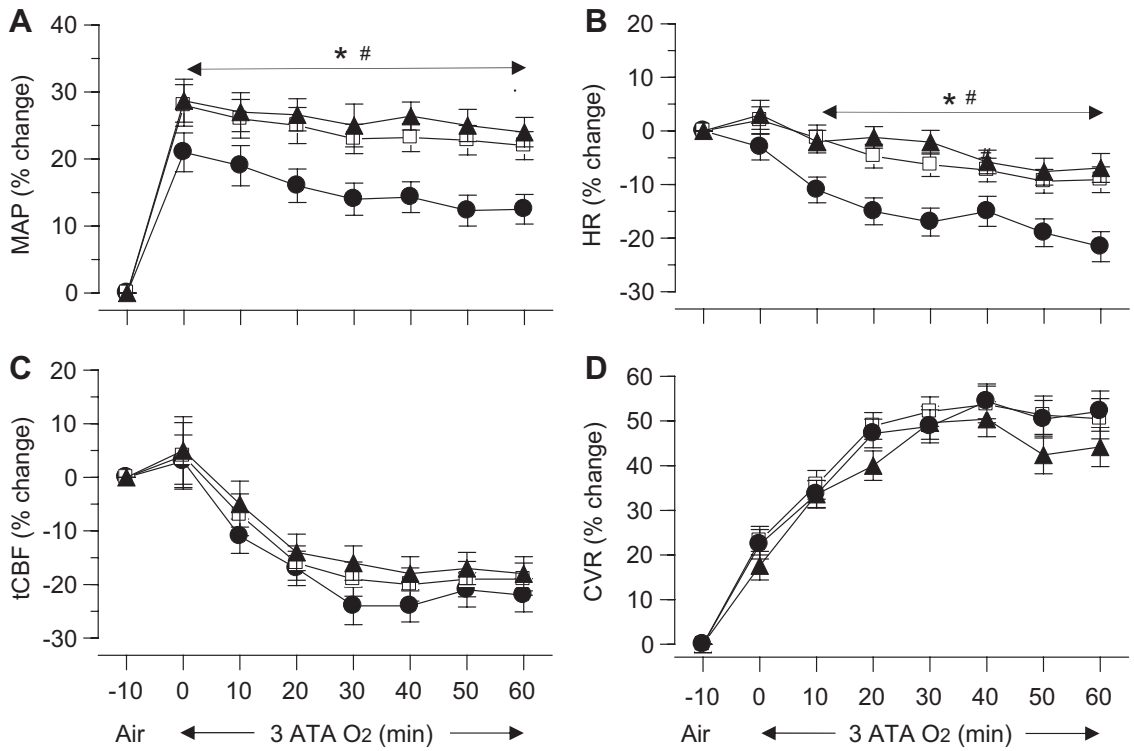

Fig. 3. Hemodynamic responses in selectively baroreceptor-denervated, anesthetized rats in HBO_2 at 3 ATA. *A*: in AD (open squares) and CD (closed triangles) rats, MAP rose to higher levels during compression and declined more slowly during isopression than in intact animals (closed circles) but never returned to baseline in all 3 groups. *B*: HR fell below pre-exposure levels in all 3 groups of rats after compression, most rapidly in intact rats. *C* and *D*: changes in total cerebral blood flow (tCBF) and cerebral vascular resistance (CVR) were similar in all groups (*$P < 0.05$ vs. intact for AD; #$P < 0.05$ vs. intact for CD).

expected, BRS was diminished in AD and CD animals (Fig. 1*A*) and nearly totally impaired in rats with complete baroreceptor deafferentation (Fig. 1*B*). However, of the 14 rats in which complete baroreceptor deafferentation was attempted, three showed cardiovascular responses to PHE and nitroprusside, indicating that baroreceptor deafferentation was incomplete; data from these animals were not used.

Cardiovascular responses to HBO_2 at 3 ATA. In rats pretreated with vehicle, MAP increased during O_2 compression and declined by the end of the 60-min exposure but not to pre-exposure levels (Fig. 2). In rats pretreated with phenoxybenzamine, propranolol, or tiotropium bromide, pressor responses did not differ from those treated with vehicle alone. L-NAME pretreatment caused significantly smaller increases in MAP (Fig. 2). In selectively denervated rats, MAP increased significantly more than in intact rats during compression and remained elevated until decompression (Fig. 3*A*). HR trended downward in intact rats but changed little in AD or CD rats, implicating the arterial baroreceptors in HR responses to HBO_2 (Fig. 3*B*). Changes in tCBF in intact, AD, and CD animals followed similar patterns, decreasing below baseline in the first 30 min and then remaining relatively constant (Fig. 3*C*). Calculated CVR was markedly higher in both intact rats and those with selective baroreceptor denervation, consistent with increased MAP and diminished tCBF and reflecting a powerful hyperoxic vasoconstriction (Fig. 3*D*). Animals with complete baroreceptor deafferentation showed greater increases in MAP and a slight tachycardia compared with the moderate hypertension and significant bradycardia seen in intact animals (Fig. 4, *A* and *B*). In addition, CO, tCBF, and RSNA were significantly greater than in the intact group (Fig. 4, *C–E*), but in both groups, changes in calculated systemic and cerebral vascular resistance (SVR and CVR) tracked closely together (Fig. 4*F*).

Baroreflex function in HBO_2. BRS was assessed in intact and completely deafferented animals by sequence analysis during O_2 compression and by PHE injection during isopression. MAP increased progressively during compression in both groups but was significantly higher in deafferented animals (Fig. 5*A*). The baroreflex functioned properly in the intact rats but was impaired in rats with complete baroreceptor deafferentation, as indicated by BRS, assessed during compression (Fig. 5*B*) and isopression (Fig. 6).

Conscious Rats

Hemodynamic responses and baroreflex function. In rats breathing room air, selective baroreceptor denervation did not alter baseline values of MAP, SAP, and HR, observed 5–7 days

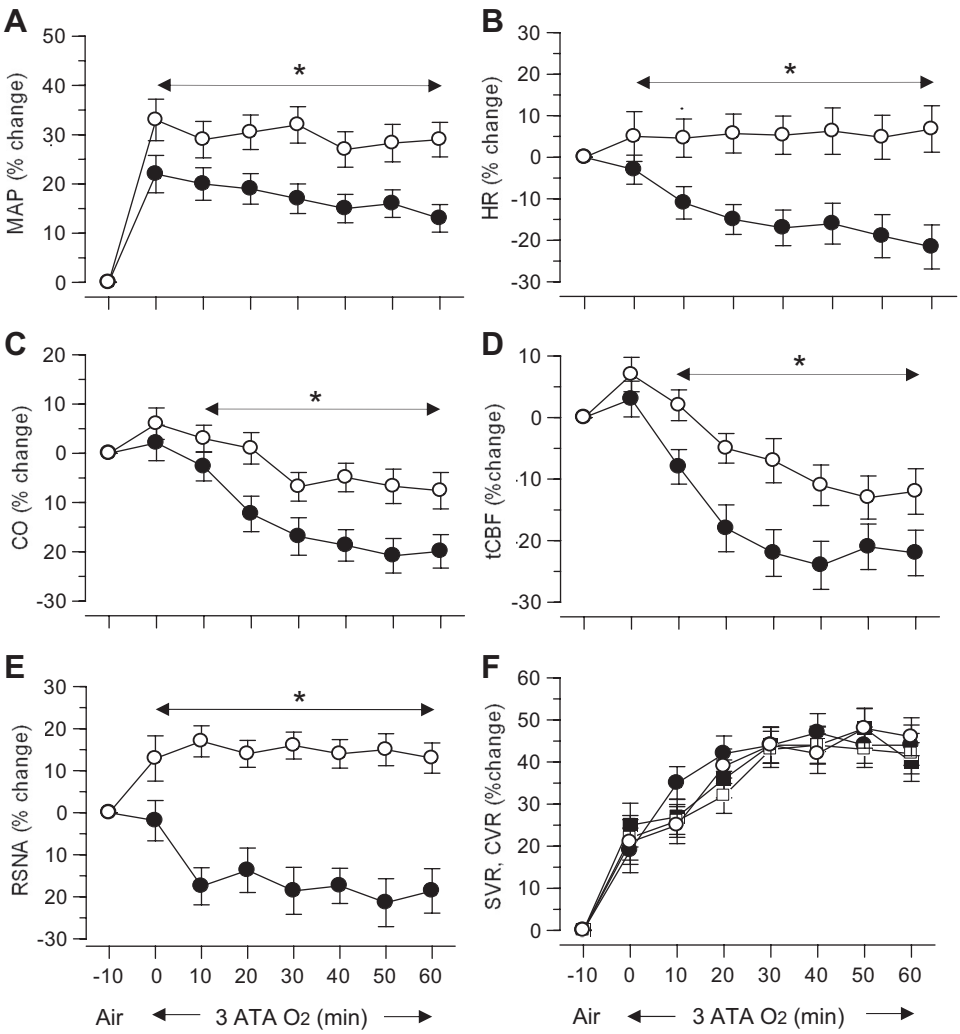

Fig. 4. Hemodynamic responses and renal sympathetic nerve activity (RSNA) in anesthetized rats in HBO_2 at 3 ATA after complete baroreceptor deafferentation. *A* and *B*: MAP and mean HR were significantly higher with complete baroreceptor deafferentation (open circles) than in intact rats (closed circles) and did not decrease with time. *C* and *D*: changes in cardiac output (CO) and tCBF seen in animals with fully impaired baroreflexes tracked with changes seen in intact animals but at more elevated levels. *E*: RSNA rose significantly above baseline in the impaired group compared with a significant decline in intact rats. *F*: temporal profiles for systemic and cerebral vascular resistance (SVR, closed squares; and CVR, closed circles) in intact rats were similar to those for rats with completely deafferented baroreceptors (SVR, open squares; CVR, open circles; $*P < 0.05$ vs. intact).

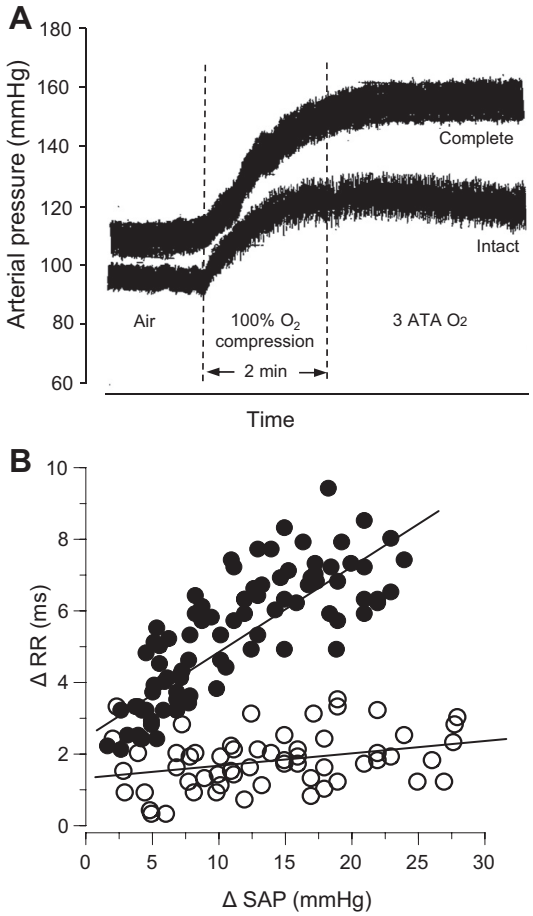

Fig. 5. Arterial pressor responses and BRS in anesthetized rats during O_2 compression. *A*: elevations in MAP during O_2 compression are greater in rats with completely deafferented baroreceptors than in intact rats, as shown in representative recordings of AP during compression to 3 ATA. *B*: BRS (Δ RR/Δ SAP) is robust during the first 2 min of O_2 compression in intact rats, reflecting increases in SAP and RR intervals (decreases in HR). In rats with complete baroreceptor deafferentation, the reduced slope of the regression line indicates baroreflex impairment.

after surgery, but hemodynamic variability was greater (Table 3). By contrast, the same parameters were elevated significantly after complete baroreceptor deafferentation (Table 3). However, the bradycardia and tachycardia elicited by PHE and nitroprusside in intact rats were attenuated in selectively denervated rats and nearly abolished after complete baroreceptor deafferentation (Table 3).

During O_2 compression to 2.5 ATA, MAP increased in intact rats and then fell progressively during isopression but did not reach baseline before decompression. MAP increased even more in selectively denervated rats and was highest in animals in which aortic and carotid baroreceptors were denervated (Fig. 7*A*). In intact rats, bradycardia began immediately with compression and persisted until decompression. Selectively denervated rats showed no significant bradycardia, whereas tachycardia occurred in rats with complete baroreceptor deafferentation (Fig. 7*B*).

Pressor responses to O_2 compression were significantly greater in rats with complete baroreceptor deafferentation than in intact rats (Fig. 8*A*), and baroreflex function was impaired (Fig. 8*B*). During isopression, BRS increased significantly in intact rats compared with pre-exposure levels in the same animals breathing room air but approached zero if baroreceptors were deafferented completely (Fig. 9).

DISCUSSION

We have shown that vasoconstriction, arterial hypertension, bradycardia, and diminished CO—the cardiovascular responses to hyperbaric hyperoxia at 2.5 or 3 ATA—are linked by a baroreflex-mediated mechanism. Furthermore, selective or complete deafferentation of the major arterial baroreceptors significantly increases the hypertension and attenuates or reverses the bradycardia that usually accompany hyperoxia at these pressures.

Baroreflex Function in Normoxia and Hyperoxia

In normoxia, the baroreflex responds to increases in AP that stimulate stretch receptors in the aortic arch and carotid sinuses. Transduction of this mechanical stimulus increases the frequency of impulses relayed through afferent pathways to the nucleus tractus solitarius and ventrolateral medulla, and these signals are processed further in other brain regions (20). Central integration of these inputs shifts autonomic equilibrium so that HR, CO, and SVR all decrease, returning blood pressure to prestimulation levels. These effects involve a vagally mediated reduction in HR and CO and an adrenergically mediated fall in peripheral resistance (19). The main, new findings of the present study are that cardiovascular responses to HBO_2 operate similarly, through the arterial baroreceptor feedback loop, to reduce cardiac rate and output. AP also declines from its initial rise but does not return to baseline.

Arterial hypertension has been reported in most, but not all, animal and human studies during isopression in hyperbaric O_2 (1, 3, 6, 16, 22, 23, 27, 44, 46, 47), but changes in AP during compression have not been investigated previously, because such measurements are technically difficult, especially in awake animals. We were able to measure AP accurately during compression in anesthetized rats by using a paralytic agent to prevent motor or respiratory movements that could alter blood pressure. In conscious rats, light restraint permitted relatively

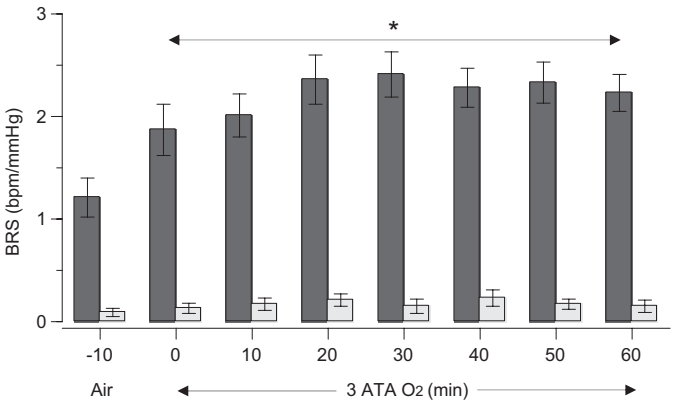

Fig. 6. BRS in anesthetized rats in HBO_2 at 3 ATA. BRS (HR/MAP assessed by PHE injection every 10 min) was elevated in intact rats (black bars) during the 60 min of HBO_2 exposure but was impaired severely in animals with complete baroreceptor deafferentation (gray bars). The 0 time point indicates the beginning of the isopression phase (*$P < 0.05$ for complete baroreceptor denervation vs. intact).

Table 3. *Baseline hemodynamic parameters in conscious rats*

	Intact	AD	CD	Complete
MAP, mmHg	102 ± 6	105 ± 8	107 ± 8	123 ± 9*
SAP, mmHg	125 ± 7	129 ± 9	130 ± 10	147 ± 14*
MAPV, %	10.2 ± 0.9	12.7 ± 1.2*	13.6 ± 1.4*	15.7 ± 2.3*
HR, beats/min	373 ± 22	381 ± 17	387 ± 18	414 ± 19*
HRV, %	13.2 ± 1.8	10.9 ± 2.1*	10.4 ± 1.6*	9.1 ± 1.6*
BRS, bpm/mmHg	−2.18 ± 0.18	−0.78 ± 0.12*	−0.57 ± 0.14*	−0.04 ± 0.1*

Values are means ± SE. *$P < 0.05$ vs. intact (control).

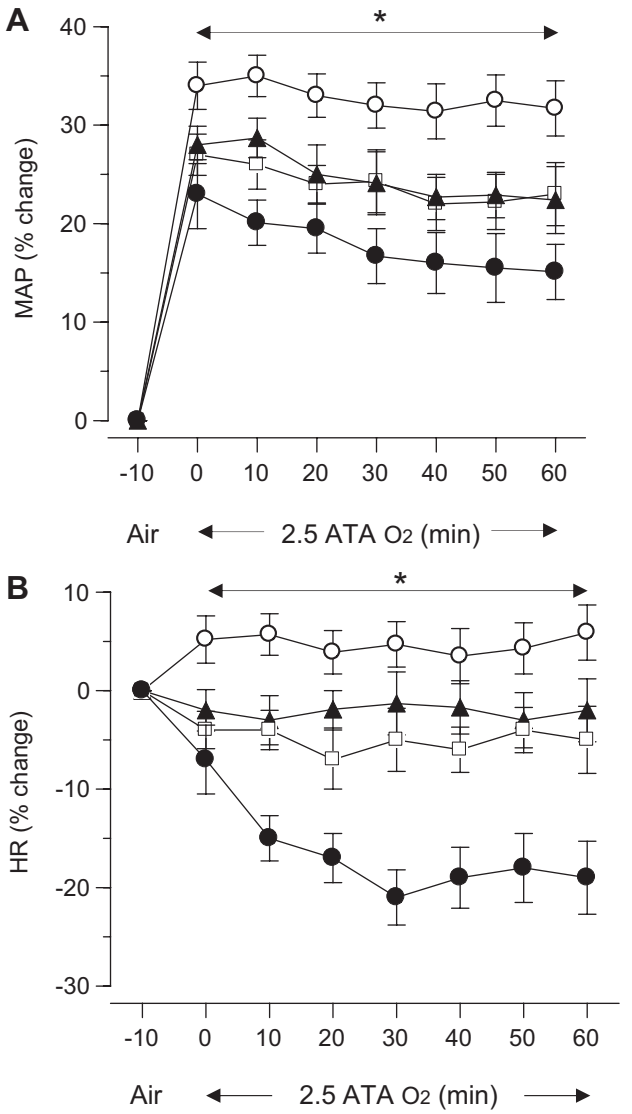

Fig. 7. Temporal profiles of hemodynamic parameters in awake rats in HBO_2. *A*: the increase in MAP was significantly greater in rats with complete baroreceptor denervation (open circles) than in intact animals (closed circles) and did not fall with time. In selectively baroreceptor-denervated rats (AD, open squares; CD, closed triangles), MAP responses to compression were similar to those in intact rats and trended downward within the isopression phase but did not return to baseline. *B*: after compression, HR fell gradually below pre-exposure levels in AD rats but declined profoundly in intact rats. Rats with completely denervated baroreceptors showed no decrease in HR after compression (*$P < 0.05$ for complete baroreceptor denervation vs. intact).

artifact-free recording, although some unidentified intrinsic or environmental factors (noise, changes in temperature, etc.) interfered occasionally. The arterial pressor response began when the inspired gas was changed to 100% O_2 and continued during O_2 compression to 2.5 or 3.0 ATA (Figs. 5 and 8). Although AP responses were similar in both groups, differences between anesthetized and conscious rats included baseline values of MAP, SAP, and HR after baroreceptor denervation, as well as cardiovascular responses to HBO_2. Also, BRS, an O_2-independent index of cardiovascular function, was higher in conscious rats than in anesthetized rats (Fig. 1 and Table 3), but no significant differences were observed between the two strains of rats.

The cause of hyperoxic hypertension has been attributed mainly to the increase in vascular resistance brought about by O_2-induced vasoconstriction (4, 6, 7, 17, 46). Work done in our laboratories has identified a role for endothelium-derived vasoactive products, such as NO (2, 11, 12, 31, 41, 49). In the present study, increases in AP during O_2 compression to 3 ATA were not affected by adrenergic or cholinergic receptor blockade but were significantly less after systemic NOS inhibition (Fig. 2). Thus hyperoxic vasoconstriction and increased vascular resistance, at least partly, reflect a decrease in the availability of NO but not neuronal effects (Fig. 2). However, it is important to note that Mulkey et al. (28) have shown in brain slices that CO_2/H^+-sensitive neurons in the solitary complex are selectively responsive to O_2 at 1,680 or 2,470 Torr, which in the intact brain, are equivalent to higher levels of hyperoxia than we used. At those more elevated levels of HBO_2, it is possible that neuronal effects could influence respiratory responses in spontaneously breathing animals, which in turn, could result in additional vasoconstriction induced by hypocapnia or other mechanisms. This needs further investigation. However, during O_2 compression (and the first few minutes of isopression), levels of the superoxide anion rise and deplete NO by forming peroxynitrite (2, 12, 35). This could account for the elevation of AP in HBO_2 and the baroreflex activation demonstrated here. By definition, the baroreflex responds to changes in AP, including those induced by HBO_2. Thus the pressor responses found here were mitigated when the baroreflex arc was intact and exacerbated when it was not, and complete deafferentation resulted in persistent hypertension (Table 2). Moreover, selective baroreceptor deafferentation provoked immediate hypertension in rats breathing room air, demonstrating that basal barosensory activity holds AP in check, but 2 h later, AP was similar to that in intact animals, suggesting that the remaining baroreceptor function suffices to inhibit increased sympathetic activity.

Although hemodynamic parameters in selectively deafferented rats and intact rats were similar in room air, their

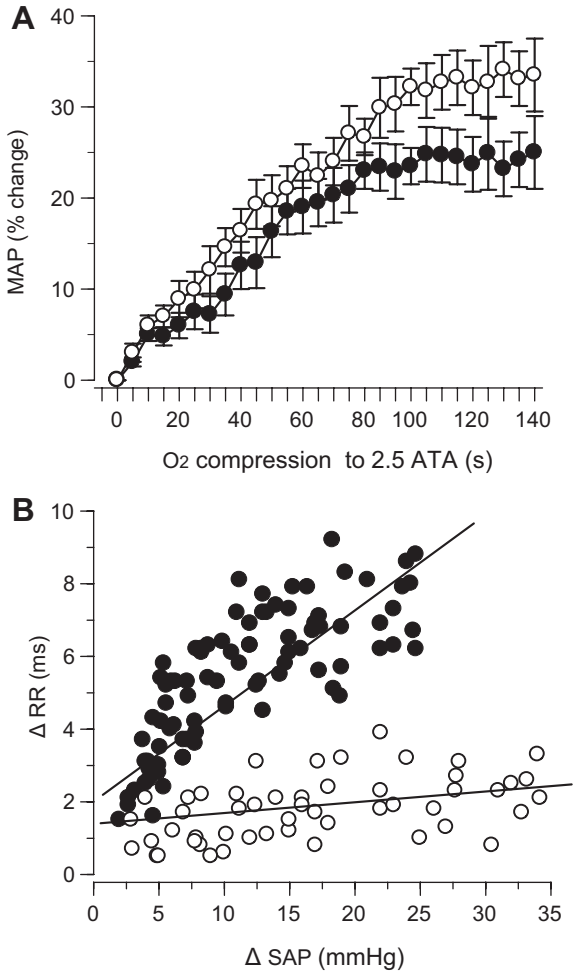

Fig. 8. Arterial pressor responses and BRS in awake rats during O_2 compression. *A*: O_2 compression raises MAP more profoundly in rats with complete baroreceptor deafferentation (open circles) than in intact rats (closed circles). Each point represents responses averaged over 5 s. *B*: complete baroreceptor deafferentation severely impairs BRS (Δ RR/Δ SAP) during O_2 compression, in spite of marked increases in MAP.

responses to O_2 compression were significantly different. Disparities were even greater after complete baroreceptor deafferentation, including a stronger pressor response and relatively persistent elevations in AP during isopression, demonstrating that intact arterial baroreceptors buffer hyperoxic hypertension. Although hyperoxic bradycardia in HBO_2 at 2.5 or 3 ATA is associated with arterial baroreceptor activation, some bradycardia persisted after total baroreceptor deafferentation, suggesting that other mechanisms, located elsewhere in the vasculature, continue to operate. These could include decreased peripheral chemoreceptor activity or direct effects of O_2 or pressure per se on the sinus node (16, 39), but none of these effects could contribute more than a small fraction of the negative chronotropic response observed in intact animals.

Effectiveness of the Baroreflex in HBO_2

Physiologically, the baroreflex responds to stimuli that increase AP abruptly, for example, vasoconstriction, due to sympathetic activation. When this happens, the arterial baroreceptor feedback loop restores AP to control levels through negative chronotropic and inotropic effects, resulting from parasympathetic (vagal) activation, as well as decreases in peripheral vascular resistance as sympathoexcitation is withdrawn. Although the sympathetic limb of the baroreflex is the most effective means for modulating peripheral resistance (43), the overall efficiency of the baroreflex is inferred commonly from the parasympathetic HR response, because it is easier to measure. As we show here, however, the baroreflex is triggered in HBO_2 by a vasoconstriction that is not due to sympathetic activation. In this case, the baroreflex acts mainly through its vagal, parasympathetic limb, as manifested by a profound bradycardia, without significant decreases in regional or systemic vascular resistance (Figs. 3 and 4).

We also made the important observations that HBO_2 increases BRS significantly during the isobaric phase of exposure to hyperbaric O_2, that this is associated with bradycardia and a decrease in RSNA, and that these responses are nearly eliminated by total deafferentation of the arterial baroreceptors, demonstrating the importance of these pathways. Although much of the cardiovascular response to HBO_2 at 2.5 and 3 ATA results from an intact baroreflex responding to peripheral vasoconstriction, hyperoxic hypertension is not fully reversed, in spite of bradycardia and diminished CO. There are at least two reasons for this. First, the more effective limb of the baroreflex, which can decrease peripheral vascular resistance by withdrawing sympathetic tone, is not available in HBO_2, because hyperoxic vasoconstriction observed in this study did not appear to be neuronal in origin. Second, the baroreflex can adapt or "reset," even after a brief hypertensive episode (43), as shown by in vitro studies in which single-fiber aortic baroreceptor activity declines exponentially, with a time constant of 3–4 min, when a step rise in AP is maintained (29). Such resetting can result from changes in the afferent, central, or efferent components of the arterial baroreflex loop (19, 43).

We draw two conclusions from this study. First, the acute cardiovascular changes that occur in moderate levels of hyperbaric O_2 ($\leq$3 ATA) can be explained by a common mechanism—activation of the arterial baroreflex—triggered by a rise in AP due to hyperoxic vasoconstriction. Second, the resulting afferent discharges from the arterial baroreceptors evoke central responses that suppress efferent sympathetic activity and augment parasympathetic outflow. Thus HBO_2 is not only an efficient means for enhancing the delivery of O_2 to tissues but can also be thought of as a means for modulating the activity

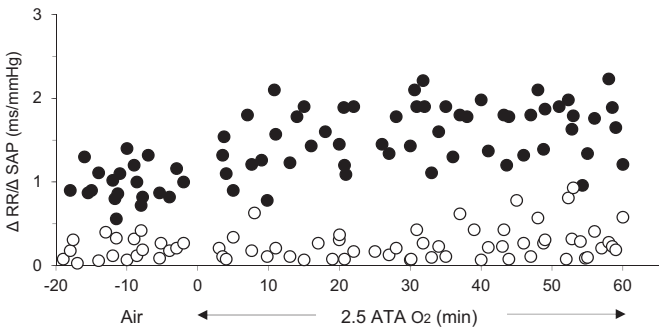

Fig. 9. BRS in awake rats. *A*: BRS (Δ RR/Δ SAP) was elevated in intact rats (closed circles) throughout the 60 min of HBO_2 at 2.5 ATA but was impaired by complete baroreceptor deafferentation (open circles). *Time 0* indicates the beginning of the isopression phase.

of the autonomic nervous system. The implications of this insight have yet to be explored fully and could include new therapies for diseases in which excessive sympathoexcitation is a factor.

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DISCLOSURES

The authors declare no conflicts of interest.

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., S.Y.Z., A.N.M., and A.I.K. performed experiments; I.T.D., S.Y.Z., A.N.M., A.I.K., and B.W.A. 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., C.A.P., and B.W.A. edited and revised manuscript; I.T.D., 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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