Chronic intermittent hypoxia alters NE reactivity and mechanics of skeletal muscle resistance arteries

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Phillips, Shane A., E. B. Olson, Julian H. Lombard, and Barbara J. Morgan. Chronic intermittent hypoxia alters NE reactivity and mechanics of skeletal muscle resistance arteries. J Appl Physiol 100: 1117–1123, 2006. First published December 15, 2005; doi:10.1152/japplphysiol.00994.2005.—Although arterial dilator reactivity is severely impaired during exposure of animals to chronic intermittent hypoxia (CIH), few studies have characterized vasoconstrictor responsiveness in resistance arteries of this model of sleep-disordered breathing. Sprague-Dawley rats were exposed to CIH (10% inspired O2 fraction for 1 min at 4-min intervals; 12 h/day) for 14 days. Control rats were housed under normoxic conditions. Diameters of isolated gracilis muscle resistance arteries (GA; 120–150 μm) were measured by television microscopy before and during exposure to norepinephrine (NE) and angiotensin II (ANG II) and at various intraluminal pressures between 20 and 140 mmHg in normal and Ca2+-free physiological salt solution. There was no difference in the ability of GA to constrict in response to ANG II (P = 0.42; not significant; 10–10–10–6 M). However, resting tone, myogenic activation, and vasoconstrictor responses to NE (P < 0.001; 10–10–10–6 M) were reduced in CIH vs. controls. Treatment of rats with the superoxide scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (tempol; 1 mM) in the drinking water restored myogenic responses and NE-induced constrictions of CIH rats, suggesting that elevated superoxide production during exposure to CIH attenuates vasoconstrictor responsiveness to NE and myogenic activation in skeletal muscle resistance arteries. CIH also leads to an increased stiffness and reduced vessel wall distensibility that were not correctable with oral tempol treatment.

Interestingly, diminished vasoconstrictor responsiveness has also been demonstrated in patients with SDB (21). In rats, exposure to continuous hypoxia for as few as 48 h diminishes responsiveness to vasoconstrictor agents and stimuli in the mesenteric circulation (13, 14), whereas others report no difference in constrictor reactivity in experimental models of intermittent hypoxia (50). The purpose of this study was to determine whether CIH (12 h/day for 14 days) causes alterations in skeletal muscle reactivity to vasoconstrictor stimuli, vascular tone, and/or vascular wall distensibility. Because recent literature suggests that oxidative stress is a contributor to the progression of cardiovascular pathology related to intermittent hypoxia (38, 46, 52), a second goal of our study was to determine whether scavenging of reactive oxygen species (ROS) with orally administered 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (tempol) prevents these hypoxia-induced impairments in arteriolar reactivity and mechanics during CIH.

MATERIALS AND METHODS

Animals. Age-matched, male Sprague-Dawley rats (Harlan Teklad, Madison, WI) weighing 250–400 g at the time of arrival were used for all experiments. Each rat was fed standard rat chow (Purina) and administered normal or tempol (1 mM)-treated drinking water (24) ad libitum during exposure to either intermittent hypoxia or normoxia. All rats were housed in an animal care facility at the University of Wisconsin-Madison, which is approved by the American Association for the Accreditation of Laboratory Animal Care, and all protocols were approved by the Medical School’s Animal Care and Use Committee. On the day of the study, rats exposed to normoxic and hypoxic conditions were weighed and anesthetized with an injection of pentobarbital sodium (50 mg/kg ip, Abbott Laboratories, Chicago, IL), and a carotid artery was cannulated with polyethylene tubing for arterial pressure measurement before the isolation of skeletal muscle resistance arteries (see Preparation of isolated vessels).

Hypoxic exposure. Rats were exposed to CIH for 12 h/day (from 1800 to 0600) for 14 days as previously described (40). In the hypoxia chamber, rats were housed three per cage, in accordance with space recommendations set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1985). Briefly, nitrogen was flushed into the chamber at a rate sufficient to achieve a fraction of inspired oxygen (FIO2) of 0.10 within 60 s and to maintain this level of FIO2 for an additional minute. Then, oxygen was introduced at a rate sufficient to achieve an FIO2 of 0.20 within 30 s and to maintain this level of FIO2 for the remainder of the 4-min interval. Daily checks of chamber oxygen concentrations during hypoxia and normoxia were made using a TED60T oxygen sensor (Teledyne, City of Industry, CA). The temperature of the chamber was maintained at 22 ± 1°C, and the relative humidity was maintained between 30 and 70%.

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Norepinephrine (NE; 10 \(^{-4}\) M) was used to determine the maximum diameter of the vessel at that pressure. All study. The level of resting tone in the vessel was calculated as follows:

**Proportion of significant levels of active tone (as evidenced by a substantial increase in vessel diameter)** was defined as the ratio of the active diameter to the mean contractile diameter (17). The vessels were stretched to in situ length, and side branches were singly ligated with small strands teased from 6-0 silk suture (Ethicon, Somerville, NJ) to ensure optimal pressurization. The inflow pipette was connected to a reservoir perfusion system that allowed the intraluminal pressure and luminal gas concentration to be controlled. Vessel diameter was measured using television microscopy and a video micrometer. Any vessel that did not exhibit significantly active tone (as evidenced by a substantial increase in resting diameter on exposure to Ca\(^{2+}\)-free PSS) was not used in this study. The level of resting tone in the vessel was calculated as follows:

\[ T = \left( \frac{\Delta D}{\Delta D_{max}} \times 100 \right) \]

where \( T \) represents the diameter increase in the maximally relaxed vessel, and \( \Delta D_{max} \) represents the maximum diameter of the vessel at that pressure. All measurements of vessel diameter were made by investigators blinded to the animal’s treatment group (CIH vs. normoxia and/or tempol vs. ordinary drinking water).

**Evaluation of vascular reactivity.** Reactivity of gracilis arteries to norepinephrine (NE; \( 10^{-3} \)–\( 10^{-6} \) M), angiotensin II (ANG II; \( 10^{-10} \)–\( 10^{-7} \) M) (Sigma; St. Louis, MO), and altered intravascular pressure (myogenic activation) were assessed in gracilis arteries of all rats. Myogenic activation, vessels were pressurized randomly to pressures ranging from 0 to 140 mmHg (20-mmHg intervals). Vessel diameter was monitored continuously and measured at the point of its minimum value after the addition of the constrictor agent or pressure change.

Active tension development in vessels exposed to different levels of intraluminal pressure was calculated as:

\[ \Delta T = -1.333 \times P_{in} \times \left[ (0.5 \times \text{ID}_{\text{active}}) - (0.5 \times \text{ID}_{\text{resting}}) \right] \times 0.0001 \]

where \( \Delta T \) (dyn/cm) represents the difference in wall tension between passive conditions (Ca\(^{2+}\)-free PSS) vs. active conditions (normal PSS) at a given intraluminal pressure (\( P_{in} \); mmHg) as described previously (41). ID represents arterial inner diameter (\( \mu \)m) under “active” (calcium present) or “passive” (zero calcium) conditions. The constants 1.333 and 0.0001 are factors for converting pressure in millimeters of mercury to dynes per centimeters squared and from micrometers to millimeters, respectively. Therefore, \( \Delta T \) represents the absolute value describing the amount by which the passive tension in the vascular wall would be increased if all active contractile mechanisms were inhibited at a given intraluminal pressure.

**Determination of passive arterial wall mechanics.** After completion of the above procedures, the perfusate and superfusate were replaced with Ca\(^{2+}\)-free PSS. Intraluminal pressure within the isolated vessel was changed, in 20-mmHg increments, between 0 and 140 mmHg, and the inner and outer diameter of the arteries was determined at each pressure. All calculations of passive arterial wall mechanics (used as indicators of structural alterations in the individual vessel) were based on those described previously (2, 41).

Vessel wall thickness was calculated as:

\[
WT = \frac{(OD - ID)}{2}
\]

where WT represents wall thickness (\( \mu \)m) and OD and ID represent artery’s outer and inner diameter, respectively (\( \mu \)m).

The arterial cross-sectional wall area (CSWA; in \( \mu \)m\(^2\)), assuming the artery is round, was calculated as:

\[
CSWA = \left[ \pi \left( \frac{OD}{2}\right)^2 \right] - \left[ \pi \left( \frac{ID}{2}\right)^2 \right]
\]

Incremental arteriolar distensibility (DIST\(_{\text{INC}}\); % change in arteriolar diameter/mmHg) was calculated as:

\[
DIST_{\text{INC}} = \frac{\Delta ID}{ID \times \Delta P_{in}} \times 100
\]

where \( \Delta ID \) represents the change in internal arteriolar diameter for each incremental change in intraluminal pressure (\( \Delta P_{in} \)). In addition, these vessels met the thin-walled assumption as previously described (39).

For the calculation of circumferential stress, intraluminal pressure was converted from millimeters of mercury to newtons per meter squared, where 1 mmHg = 1.334 × 10\(^{-5}\) N/m\(^2\). Circumferential stress (\( \sigma \)) was then calculated as:

\[
\sigma = \frac{P_{in} \times \pi ID}{2WT}
\]

Circumferential strain (\( \varepsilon \)) was calculated as:

\[
\varepsilon = \frac{ID - ID_{10}}{ID_{10}}
\]

where ID\(_{10}\) represents the internal arteriolar diameter at the lowest intraluminal pressure. In these studies 10 mmHg was used as the lowest pressure to prevent passive collapse of the vessel.

**Statistical analysis.** Arterial vasococontractor reactivity data to all pharmacological agonists as well as the data describing the passive diameter and incremental distensibility of the vessel were analyzed using repeated-measures ANOVA. From each experiment, data describing the change in active tension with increasing intraluminal pressure were fit with a linear regression equation \( y = \alpha + \beta x \); where \( \alpha \) represents arterial diameter at a specific intraluminal pressure, \( \beta \) is an intercept term, and \( x \) represents intraluminal pressure. The rate of change in active tension for an incremental change in intraluminal pressure. Circumferential stress vs. strain curves were fit with exponential equations and statistically significant differences in the slope coefficients were evaluated using Student’s \( t \)-test. Differences in the means after ANOVA were determined with post hoc analysis using a Student-Newman-Keuls test.

**RESULTS**

**General characteristics of normoxic and CIH rats.** Table 1 represents data describing the rats exposed to normoxia or...
intermittent hypoxia for 14 days. Consistent with previous studies, there was no significant difference in mean arterial pressure between CIH and normoxic rats administered normal drinking water (40). Body weight was significantly higher in the normoxic group of rats treated with tempol for 14 days.

Responses of resistance arteries to constrictor agonists and myogenic activation. Data describing vessel responses to ANG II and NE are described in Figs. 1 and 2, respectively. In these studies, there was no difference between vasoconstrictor responsiveness to ANG II in gracilis arteries from rats administered normoxia or CIH, and no effect of tempol on angiotensin II-induced constriction of arteries from rats exposed to CIH.

Fig. 1. Response to 10^{-10} M–10^{-7} M angiotensin II in gracilis arteries of rats exposed to normoxia (n = 4) or chronic intermittent hypoxia (CIH; n = 7) for 14 days, with and without tempol. Values are means ± SE. There was no significant difference in the response of vessels to angiotensin II in normoxic control animals and animals exposed to CIH, and no effect of tempol on angiotensin II-induced constriction of arteries from rats exposed to CIH.

Fig. 2. Changes in arterial diameter in response to norepinephrine (10^{-9}–10^{-6} M) in isolated gracilis arteries from normoxic and CIH-treated rats in the absence (n = 5) and presence (B; n = 7) of tempol. Values are means ± SE. *P < 0.05 vs. responses in normoxic control rats.

Tempol treatment completely restored reactivity to NE in gracilis arteries from CIH rats (Fig. 2).

Figure 3A describes myogenic responses of gracilis arteries from animals exposed to normoxia and CIH. Arterial diameter was significantly greater during increases in incremental pressure (>80 mmHg) in vessels from CIH vs. normoxic rats. Tempol treatment restored myogenic activation during increases in intraluminal pressure in CIH rats (data not shown). There was also a significant reduction in the slope of the curve describing the development of active tension in gracilis arteries from rats exposed to CIH (β = 3.0 ± 0.5; P = 0.023) vs. normoxia (β = 5.7 ± 0.8; Fig. 3B). Tempol treatment in CIH rats prevented alterations in active tension development (β = 7.2 ± 0.7; Fig. 3B).

Passive wall mechanics. Inner diameters during incremental increases in intraluminal pressure above 40 mmHg in Ca^{2+}-free PSS were similar between control and CIH-treated rats (Fig. 3A). However, inner diameter was significantly lower at pressures <40 mmHg in the CIH group compared with control.
(Fig. 3A), resulting in a significant reduction in incremental distensibility at 20-mmHg intraluminal pressure in gracilis arteries from rats exposed to CIH vs. normoxic controls (Fig. 4).

There was a left shift in the passive stress vs. strain relationship in the CIH group vs. normoxia group (Fig. 5). In these studies, the slope coefficient of the stress vs. strain curve was significantly increased in CIH vs. control rats ($P = 0.04$). Tempol treatment in animals administered CIH had no significant effect on the left shift in the stress vs. strain curve ($P = 0.25$; Fig. 5).

There was no difference in arterial wall-to-lumen ratio, wall thickness, or cross-sectional wall area between normoxia- and CIH-exposed rats (Fig. 6).

**DISCUSSION**

The purpose of this study was to test the hypothesis that administration of intermittent hypoxia for 14 days impairs vasoconstrictor responses and alters vascular wall mechanics in small resistance arteries. In addition, we sought to determine whether these alterations were prevented by scavenging superoxide radicals with tempol, a superoxide dismutase mimetic. In the present study vasoconstrictor reactivity to NE, myogenic activation, and increases in active tension with elevated transmural pressure were significantly attenuated by exposure to CIH. There was also a significant increase in vascular wall stiffness in gracilis arteries from rats exposed to CIH for 14 days compared with normoxic controls. In line with previous studies in patients with obstructive sleep apnea where forearm vascular responses to ANG II were augmented (32), there was some tendency for enhanced ANG II constriction in the present study, but this difference was not significant in gracilis arteries.

Acute episodes of hypoxia appear to be an important component of the pathological sequelae related to SDB, exposure to high altitude, and pulmonary disease (4, 15). Similar to studies by Fletcher and colleagues (50), we have recently employed a rat model of chronic intermittent hypoxia to mimic the intermittent nature of hypoxia during SDB (40). However, in our model, Sprague-Dawley rats are exposed to intermittent hypoxia for 14 days and do not develop a significant elevation in arterial blood pressure, thereby removing the confounding nature of elevated blood pressure on peripheral vasculature function (40).

Multiple studies indicate that continuous hypoxia alters vascular control. Gonzales and Walker (20) reported that isolated mesenteric arteries of rats exposed to 48 h of continuous hypoxia exhibited impaired vasoconstrictor responsiveness to phenylephrine and myogenic activation. In addition, renal artery vasoconstriction to $\alpha_1$-adrenergic stimulation was reduced in conscious rats exposed to hypobaric hypoxia, indicating the effect of hypoxia on attenuated constrictor responses may not be limited to the mesenteric circulation (28, 37). Taken together, those studies indicate that continuous hypoxia leads to impaired vascular responsiveness to adrenergic agonists and other constrictor stimuli.

In the present study, skeletal muscle resistance arteries demonstrated reduced vasoconstrictor reactivity to NE after exposure to intermittent hypoxia. Myogenic vasoconstriction was also significantly reduced in CIH vs. normoxic rats, resulting in reduced active tone development during incremental increases in intraluminal pressure (Fig. 3). These findings, taken together with those of previous investigators (9, 12, 13, 20), suggest that attenuated vasoconstriction may be a generalized response to hypoxia and not dependent on the pattern of exposure (i.e., continuous vs. intermittent). Our observations are not consistent with a previous report that 35 days of intermittent hypoxia had no effect on constrictor reactivity to endothelin-1 and NE in the rat cremaster muscle microcirculation (50). This inconsistency may exist because our intermittent hypoxia paradigm was more moderate than the previous one in terms of frequency, severity, and duration, and our rats did not develop significant hypertension. The discordant results may also reflect differences in vessel size and branching order within the circulation, because the present study focuses on resistance artery function (49).
Mechanisms of blunted vasoconstriction. Recent evidence suggests that elevated vascular superoxide production is responsible for reduced vasodilation and decreased nitric oxide (NO) bioavailability during systemic hypoxia (46, 51). In addition, it appears that patients with obstructive sleep apnea demonstrate increased generation of ROS in vascular cells (11, 48).

In the present study, we sought to determine the role of ROS generation in contributing to the impaired reactivity of isolated gracilis arteries to vasoconstrictor stimuli. There was no effect of tempol on responses of gracilis arteries to ANG II in rats exposed to normoxia or CIH (Fig. 1B). However, tempol treatment during CIH exposure prevented the development of impaired constrictor responses to NE (Fig. 2) and impaired myogenic activation (Fig. 3A), and it eliminated differences in the slope coefficient of the curve describing active tension development during incremental increases in intraluminal pressure (Fig. 3B). Taken together, these observations provide evidence that impaired vasoconstrictor responses during CIH may be related to increased ROS generation.

The specific mechanistic difference between the effect of CIH on vascular ANG II and NE responses is unknown. However, previous studies of disease models associated with elevated oxidative stress suggest that ROS mediates G protein uncoupling, an effect that impairs agonist-dependent vasodilation (1, 33). Therefore, the present observation that CIH impairs NE- but not ANG II-mediated constriction may be related to an increased susceptibility of adrenergic receptor G-protein coupling vs. angiotensin AT1 receptor G-protein coupling to ROS-induced dysfunction.

The restored vasoconstriction in CIH rats receiving tempol treatment may be confounded by the effect of tempol on sympathetic nervous system activity, especially because elevated sympathetic nervous system (SNS) activation is thought to contribute to the cardiovascular sequelae of obstructive sleep apnea and CIH (6, 16). In previous studies, chronically elevated sympathetic activity altered different indices of cardiovascular structure and function, leading to reduced α-adrenergic responsiveness (25, 44) and smooth muscle cell hypertrophy (8, 45). In addition, NO has an inhibitory effect on SNS activity, whereby improved NO bioavailability during intravenous tempol treatment reduces central and peripheral SNS activity in the rat (5). The effect of tempol on SNS activity in that study was related to an improvement in NO bioavailability and a reduction in the ability of ROS to accentuate SNS activity.

Although the measurement of peripheral SNS activity was beyond the scope of the present study, alterations in peripheral SNS activity cannot be ruled out as a mechanism by which CIH attenuates vasoconstrictor responsiveness in gracilis arteries. The ability of tempol to partially prevent this impaired NE constriction may be related to its inhibitory effect on chronic SNS activity and/or the effects of tempol directly on vascular NO bioavailability. In this regard, our conclusion that these vascular alterations are superoxide dependent is somewhat limited because direct vascular ROS measurements were not made during tempol treatment.

Effect of CIH on vascular wall mechanics. Increased stiffness and increased vascular wall thickness occurs in models of hypertension (19, 42, 43) and diabetes (18). In the present study, there was a minimal reduction in passive internal diameter and incremental distensibility evident only at intraluminal pressures <40 mmHg in individual gracilis arteries. In contrast, there was virtually no difference in arterial wall thick-
ness, cross-sectional wall area, or wall-to-lumen ratios (Fig. 6) after 14 days of CIH vs. normoxia exposure. However, arteries of CIH animals demonstrated a leftward shift in the stress-strain relationship, indicating that the passive vessels of CIH-treated rats are stiffer and less deformable than those of normoxic rats. Taken together, these data suggest that exposure to systemic hypoxemia per se can lead to alterations in the wall mechanics of individual vessels, because these changes occur in the absence of significant elevations in blood pressure. However, it is unlikely that the changes in NE and myogenic constrictions during CIH are related to altered wall distensibility, because this effect was only observed at intraluminal pressures of 20 mmHg, when the vessel is essentially flaccid (Fig. 4). Rather, a stiffer arterial wall may reduce passive dilation and active constriction to myogenic responses, with minimal effects on wall distensibility.

In contrast, there was virtually no difference in the stress vs. strain curves of arteries from CIH-exposed rats drinking normal and tempol-treated water, ruling out the possibility that these changes are directly related to elevated ROS generation in this model of SDB. Further studies are necessary to elucidate the mechanism of this unique vascular remodeling that occurs without the simultaneous increase in wall thickness or cross-sectional wall area that typically occurs during the development of hypertension and diabetes mellitus (3, 30, 34, 43). The unique changes in arterial wall stiffness without wall hypertrophy in CIH-exposed rats may result from increased wall collagen deposition and/or orientation of collagen fibers (19).

In addition, this apparent eutrophic remodeling of the individual vessel occurs in the context of a reduced passive inner vascular vessel occurs in the context of a reduced passive inner diameter in the absence of hypertension, in contrast to the dramatic inward hypertrophy that occurs during hypertension (36). Further studies are needed to determine whether these changes are related to the relatively short duration of exposure to CIH, whereas longer durations of exposure may be necessary to induce vascular wall hypertrophy. Clinical significance. Studies in human subjects have determined that patients with SDB have a reduced responsiveness to NE (21). This finding was attributed to a downregulation in the vasoconstrictor effects of NE (21). The finding of altered vascular wall stiffness during CIH is consistent with clinical data indicating arterial stiffness in the radial artery increases acutely during obstructive events in patients with obstructive sleep apnea (27). However, in contrast to studies indicating that peripheral arterial remodeling occurs in patients with diagnosed obstructive sleep apnea (47), the findings of the present study also indicate that structural alterations during CIH can occur without wall hypertrophy (Fig. 6). Taken together, these observations indicate that exposure to CIH results both in complex patterns of vascular regulation and changes in vessel structure that are unique to the development of SDB as an independent risk factor for cardiovascular disease.

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Hypoxia alters vascular reactivity and mechanics


