ACUTE EPISODES OF SLEEP-DISORDERED BREATHING PRODUCE HYPOXIA, HYPERCAPNIA, AND AROUSAL FROM SLEEP. Recent studies have demonstrated that sleep-disordered breathing in humans is associated with the presence of hypertension (23, 32) and other cardiovascular diseases such as congestive heart failure and stroke (7, 25). Intermittent airway obstructions and episodic hypoxia (both hallmarks of sleep-disordered breathing) cause sustained elevations of blood pressure in dog and rat models (4, 8, 9). The key component responsible for raising blood pressure in these experimental models appears to be hypoxia rather than hypercapnia or sleep disruption, because the addition of CO₂ does not augment the hypertensive effects of intermittent hypoxia (9) and repetitive arousal from sleep does not cause sustained increases in blood pressure (4). The mechanisms by which intermittent hypoxia causes hypertension are not known, although one potential contributor is endothelial dysfunction. Attenuated endothelium-dependent vasodilation has been observed in patients with sleep-disordered breathing (5, 16) and in rats exposed to intermittent hypoxia, concurrent with an elevation in arterial pressure (30).

Impaired endothelial function in the vessels that lie immediately proximal to the microcirculation could increase vascular resistance and promote the development of hypertension. On the other hand, it has been hypothesized that elevated blood pressure could produce endothelial dysfunction (20). Impaired relaxation or paradoxical vasoconstriction in response to ACh and blunted responses to physiological dilator stimuli such as reduced oxygen availability and increased shear stress have been observed in several experimental models of hypertension (13, 14, 20); however, it is not clear whether this endothelial dysfunction is a cause or a consequence of the elevation in arterial pressure.

To our knowledge, no studies have evaluated the effect of intermittent hypoxia per se on endothelial function in the absence of hypertension. Therefore, the goal of the present study was to test the hypothesis that chronic intermittent hypoxia (CIH), applied for a relatively brief duration that does not result in a sustained increase in arterial pressure, impairs responses to vasodilator stimuli in resistance arteries of the cerebral and skeletal muscle circulations.

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 provided drinking water ad libitum during exposure to either intermittent normoxia or hypoxia. 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 cerebral and skeletal muscle resistance arteries (see Preparation of isolated vessels).

Hypoxic exposure. Rats were exposed to CIH for 12 h/day (from 1800 hours to 0600 hours) for 14 days. 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). Oxygen concentrations in the chamber were monitored using a heated zirconium sensor (Fujikura America; Pittsburgh, PA) connected to solenoid valves that controlled the flow of oxygen and nitrogen. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Chronic intermittent hypoxia impairs endothelium-dependent dilation in rat cerebral and skeletal muscle resistance arteries

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Phillips, Shane A., E. B. Olson, Barbara J. Morgan, and Julian H. Lombard. Chronic intermittent hypoxia impairs endothelium-dependent dilation in rat cerebral and skeletal muscle resistance arteries. Am J Physiol Heart Circ Physiol 286: H388–H393, 2004. First published September 25, 2003; 10.1152/ajpheart.00683.2003.—The goal of the present study was to evaluate the effects of relatively short-term chronic intermittent hypoxia (CIH) on endothelial function of resistance vessels in the skeletal muscle and cerebral circulations. Sprague-Dawley rats were exposed to 14 days of CIH (10% fraction of inspired oxygen for 1 min at 4-min intervals, 12 h/day, n = 6). Control rats (n = 6) were housed under normoxic conditions. After 14 days, resistance arteries of the gracilis muscle (GA) and middle cerebral arteries (MCA) were isolated and cannulated with micropipettes, perfused and superfused with physiological salt solution, and equilibrated with 21% O₂-5% CO₂ in a heated chamber. The arteries were pressurized to 90 mmHg, and vessel diameters were measured via a video micrometer before and after exposure to ACh (10⁻⁷–10⁻⁴ M), sodium nitroprusside (10⁻⁴ M), and acute reduction of PO₂ in the perfusate/superfusate (from 140 to 40 mmHg). ACh-induced dilations of GA and MCA from animals exposed to CIH were greatly attenuated, whereas responses to nitroprusside were similar to controls. Dilations of both GA and MCA in response to acute reductions in PO₂ were virtually abolished in animals exposed to CIH compared with controls. These findings suggest that exposure to CIH reduces the bioavailability of nitric oxide in the cerebral and skeletal muscle circulations and severely blunts vasodilator responsiveness to acute hypoxia.

acetylcholine; gracilis artery; middle cerebral artery; vascular reactivity

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valves were operated by a microprocessor-controlled timer. This system was set to provide hypoxic exposures at 4-min intervals. The first minute of each exposure was transitional, with nitrogen 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. Oxygen was then introduced at a rate sufficient to achieve a FiO2 of 0.209 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). Figure 1 provides an illustration, obtained via a mass spectrometer, of the FiO2 profile produced by our intermittent hypoxia protocol. The temperature of the chamber was maintained at 22 ± 1°C, and the relative humidity was maintained between 30 and 70%.

Normoxic exposure. Three rats were maintained in standard housing conditions of the animal care facility, whereas three rats were subjected to the same noises and airflow perturbations experienced by the hypoxia-exposed rats.

Preparation of isolated vessels. The small muscular branch of the femoral artery supplying the gracilis muscle was freed from surrounding tissue and allowed to equilibrate in situ for 30 min with the application of warm physiological salt solution (PSS). After the equilibration period, the artery was carefully excised. The anesthetized rats were then decapitated, and the brain was quickly removed after the application of warm physiological salt solution.

The proximal and distal ends of the MCA and gracilis artery were cannulated with glass micropipettes (100–150 μm, FHC; Brunswick, ME) and secured to the pipettes using 10-0 nylon sutures in a superfusion-perfusion chamber (10, 21). The vessels were stretched to the in situ length, and side branches were singly ligated with small strands teased from a 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 significant levels of active tone (as evidenced by a substantial increase in resting diameter upon exposure to Ca2+−free PSS) was not used in this study. The level of resting tone in the vessel was calculated as follows: T = [ (ΔD × Dmax) / 100], where T is resting tone (in %), ΔD is the diameter increase in the maximally relaxed vessel, and Dmax represents the maximum diameter of the vessel at that pressure.

Response to reduced P O 2. After the initial control period in PSS equilibrated with 21% O2, the responses of gracilis arteries and MCA to reduced P O 2 were assessed in each group. P O 2 reduction was achieved by simultaneous perfusion and superfusion of the arteries for 25 min with PSS equilibrated with 0% O2-5% CO2-95% N2 as previously described (10). Under these conditions, control values for P O 2 during 21% O2 perfusion and superfusion are ~140 Torr, whereas equilibration of the PSS reservoirs with 0% O2 reduces both the luminal and extraluminal P O 2 to 35–45 Torr (10). After exposure of the vessels to hypoxia, the perfusate and superfusate were reequilibrated with 21% O2 for 20 min, and recovery from reduced P O 2 was verified by measuring the vessel diameter.

Response to vasodilator agents and Ca2+−free solution. The responses of resistance arteries to the endothelium-dependent vasodilator Ach (10−7−10−4 M) and the nitric oxide (NO) donor sodium nitroprusside (10−4 M, Sigma; St. Louis, MO) were assessed in cerebral and skeletal muscle vessels in each group of rats. When these drugs were administered, the superfusion was stopped in the bath, the vessel was pressurized by clamping the outflow pipette, and an appropriate amount of drug was added to the tissue chamber to achieve the final desired concentration in the superfusion solution. Vessel diameter was monitored continuously and was measured at the point of its maximum value after the addition of the dilator agent.

After the response of the arteries to the various vasodilator stimuli had been determined, vessel diameter was determined after the vessels were maximally dilated with Ca2+−free relaxing solution containing the following constituents (in mM): 92.0 NaCl, 4.7 KCl, 1.17 MgSO4, 1.17 NaH2PO4, 1.18 MgSO4, 24.0 NaHCO3, 0.026 EDTA, 2.0 EGTA, and 5.5 dextrose.

Statistical analysis. All data are expressed as means ± SE. Responses to incremental doses of Ach were analyzed with two-way repeated-measures ANOVA. Significance differences between the individual means after two-way ANOVA were determined using a Student-Newman-Keuls post hoc test. Differences in the responses to hypoxia, nitroprusside, and maximal dilation in calcium-free solution between groups were determined utilizing an unpaired t-test.

RESULTS

Mean arterial pressure, body weight, and vessel diameter. Table 1 summarizes data describing the rats exposed to normoxia or intermittent hypoxia for 14 days. Although resting diameter (Table 1) and maximum diameter of the vessels in}

![Figure 1](https://example.com/figure1.png)

**Table 1. Characteristics of rats exposed to normoxia or CIH**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, wk</th>
<th>Body Weight, g</th>
<th>MAP, mmHg</th>
<th>Resting Diameter, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Gracilis artery</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MCA</td>
</tr>
<tr>
<td>Normoxia</td>
<td>6</td>
<td>11.5 ± 0.1</td>
<td>397 ± 34.0</td>
<td>127 ± 5.5</td>
<td>147 ± 8.0</td>
</tr>
<tr>
<td>CIH</td>
<td>6</td>
<td>11.8 ± 0.1</td>
<td>406 ± 14.0</td>
<td>126 ± 8.3</td>
<td>134 ± 9.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, total number of normoxic or chronic intermittent hypoxia (CIH) rats used in the study. Numbers of vessels for individual studies are indicated in the figures summarizing the results of those experiments. The characteristics of rats exposed to normoxia or CIH include body weight, mean arterial pressure (MAP), and the internal diameters of the isolated gracilis arteries and middle cerebral arteries (MCA) in physiological salt solution.
Ca²⁺-free solution tended to be reduced in arteries of animals receiving hypoxic exposure, the difference between animals exposed to normoxia (gracilis: 178 ± 6.0 μm; MCA: 192 ± 13.4 μm) and those exposed to CIH (gracilis: 169 ± 11.9 μm; MCA: 164 ± 8.6 μm) was not statistically significant. In addition, there was no significant difference in body weight or mean arterial pressure between groups.

Response of resistance arteries to ACh and sodium nitroprusside. Figures 2 and 3 summarize the response of resistance arteries to ACh in animals exposed to normoxia or intermittent hypoxia. In these experiments, cerebral and skeletal muscle arteries from animals maintained under normoxic conditions for 14 days demonstrated a pronounced dilation to incremental doses of ACh (10⁻⁷–10⁻⁴ M), similar to previous results (10). There were no significant differences in the responses to ACh (10⁻⁵ M) in vessels of animals exposed to standard normoxic conditions (gracilis: 17 ± 1.7 μm; MCA: 19 ± 1.3 μm) or housed under normoxic conditions in the hypoxia chamber (gracilis: 24 ± 2.0 μm; MCA: 21 ± 0.5 μm). However, dilation of skeletal muscle and cerebral arteries to ACh was significantly reduced in animals exposed to intermittent hypoxia for 14 days (Figs. 2 and 3). The responses of skeletal muscle and cerebral resistance arteries from rats administered normoxia and intermittent hypoxia to the NO donor sodium nitroprusside are summarized in Fig. 4. In contrast to vessel responses to ACh, exposure to CIH had no effect on the vascular relaxation in response to sodium nitroprusside in either skeletal muscle or cerebral resistance arteries.

Responses of resistance arteries to acute reductions in PO₂. Figure 5 summarizes the responses of skeletal muscle (A) and cerebral resistance arteries (B) to acute hypoxia in rats exposed to normoxia or intermittent hypoxia for 14 days. Arteries from rats exposed to normoxia demonstrated a significant increase in diameter in response to reduced PO₂, as previously reported in both skeletal muscle (10) and cerebral resistance arteries (21). However, gracilis arteries (Fig. 5A) and MCA (Fig. 5B) from rats receiving intermittent hypoxia for 14 days failed to dilate in response to an acute reduction in perfusate and superfusate PO₂ from ~140 to ~35–45 Torr. As in the case of ACh, there was no significant difference in the vasodilator responses to acute hypoxia in vessels from animals exposed to standard normoxic conditions (gracilis: 16 ± 1.2 μm; MCA: 15 ± 2.5 μm) or housed under normoxic conditions in the hypoxia chamber.

Fig. 2. Response to 10⁻⁷–10⁻⁴ M ACh in gracilis arteries of rats exposed to normoxia or chronic intermittent hypoxia (CIH) for 14 days (n = 6 rats/group). Data are presented as mean changes in diameter (in μm) ± SE from control measured before the application of ACh. *Significant difference from the response of vessels from normoxic control animals, P < 0.05.

Fig. 3. Response to 10⁻⁷–10⁻⁴ M ACh in middle cerebral arteries of rats exposed to normoxia and CIH for 14 days (n = 6 rats/group). Data are presented as mean changes (in μm) ± SE from the diameter measured before application of ACh. *Significant difference from the response of vessels from normoxic control animals, P < 0.05.

Fig. 4. Responses to sodium nitroprusside (10⁻⁶ M) in isolated gracilis arteries (A) and middle cerebral arteries (B) from animals exposed to normoxia or CIH for 14 days (n = 5–6). Data are presented as means ± SE. There was no significant difference in the response of vessels to nitroprusside in normoxic control animals and animals exposed to CIH.
However, gracilis arteries (Fig. 5A) and MCA (Fig. 5B) from rats receiving intermittent hypoxia for 14 days failed to dilate in response to an acute reduction in perfusate and superfusate PO$_2$ from 140 to 35–45 Torr.

Maximal relaxation in calcium-free PSS. Figure 6 shows the responses to calcium-free PSS in skeletal muscle (A) and cerebral resistance arteries (B) from rats administered normoxia or CIH for 14 days. Maximal dilation of both vessel types was unaltered by exposure to intermittent hypoxia. There was also no significant difference in the resting active tone of gracilis arteries from rats administered normoxia (%tone: 16 ± 2.9, n = 5) and intermittent hypoxia (%tone: 23 ± 2.8, n = 6) or in the resting active tone of MCA from animals administered normoxia (%tone: 23 ± 7.2, n = 5) or intermittent hypoxia (%tone: 19 ± 3.5, n = 5).

**DISCUSSION**

In the present study, exposure to CIH for 14 days significantly impaired endothelium-dependent vasodilator responses to ACh (Figs. 2 and 3) and acute hypoxia (Fig. 5) in resistance arteries from the skeletal muscle and cerebral vascular beds but did not alter vessel responses to the NO donor sodium nitroprusside (Fig. 4) or maximal dilation of the arteries in Ca$^{2+}$-free PSS (Fig. 6). These observations suggest that impaired vasodilator responses in resistance arteries are due to altered mechanisms of endothelium-dependent vascular relaxation after exposure to intermittent hypoxia rather than altered sensitivity to NO or structural impairments in the arterial wall that restrict the vessels’ ability to increase their diameter in response to dilator stimuli. This impairment of endothelial function was evident before the development of hypertension in this experimental model (known to raise blood pressure), because mean arterial pressures were nearly identical in the hypoxia- and normoxia-exposed rats (Table 1).

Previous studies of animal models indicate that the primary prohypertensive component of CIH is transient blood oxygen desaturation (4, 9, 26, 31) rather than the hypercapnia and sleep disruption that occurs during sleep-disordered breathing (3, 4). The present findings, obtained in a model that produces hypocapnic hypoxia, suggest that hypoxia is also the primary stimulus that impairs endothelial function, because rats exposed to the same noise and cage environment during normoxia did not demonstrate similar alterations in their responses to dilator stimuli.

The sensitivity of blood vessels to vasoactive stimuli has been extensively studied during hypertension and sleep-disordered breathing (2, 16, 22, 28). For example, a previous study...
by Tahawi et al. (30) reported impaired responses to ACh in cremasteric arterioles of rats exposed to chronic episodic hypoxia, concomitant with a significant elevation in blood pressure. However, virtually no studies to date have determined the effect of systemic exposure to intermittent hypoxia on vascular reactivity in the absence of hypertension. Thus the present study is the first to document the effects of exposure to CIH on response of isolated skeletal muscle and cerebral resistance arteries to vasodilator stimuli and to demonstrate that CIH can lead to impaired vascular reactivity in the absence of elevated arterial pressure.

Effect of intermittent hypoxia on NO-dependent vasodilation. As noted above, previous studies have demonstrated impaired responses to ACh in arterioles of Sprague-Dawley rats treated with intermittent hypoxia for 35 days (30). ACh-induced dilation is normally mediated via NO but also may involve the release of several different vasoactive compounds (1, 6, 15, 17, 18), depending on the specific vessel studied. Previous studies in our laboratory have determined that the vascular response to ACh is mediated by NO released from the endothelium in rat skeletal muscle and cerebral resistance arteries (29) and that ACh-induced vascular relaxation in both of these arteries is impaired in hypertension (12, 20). In the present study, skeletal muscle and cerebral resistance arteries of rats administered normoxia for 14 days exhibited a significant dilator response to ACh (10^{-7}–10^{-8} M), similar to previous reports in this laboratory (10) (Figs. 2 and 3). However, vasodilator responses to the same doses of ACh were significantly impaired in arteries of rats exposed to intermittent hypoxia (Figs. 2 and 3). These findings suggest that vasodilation to ACh is impaired in CIH due to reductions in the synthesis and/or release of NO by the endothelium, because the response of the vessels to sodium nitroprusside, an endothelium-independent, NO-mediated dilator, were unaltered by CIH (Fig. 4, A and B).

Effect of CIH on maximal relaxation of skeletal muscle and cerebral resistance arteries. In the present study, there was no difference in the maximal relaxation occurring in response to Ca^{2+}-free PSS in CIH versus control rats (Fig. 6). The latter observation demonstrates that 14 days of CIH exposure does not cause structural narrowing of the vessels and that the diminished ability of these arteries to respond to vasodilator stimuli is not due to constraints resulting from structural alterations in the vessel wall, which can contribute to the impaired ability of arteries to respond to vasodilator stimuli in hypertension.

Effect of CIH on acute hypoxia-induced dilation in skeletal muscle and cerebral resistance arteries. In the present study, isolated cerebral and skeletal muscle resistance arteries from normoxic rats demonstrated a significant increase in diameter in response to a simultaneous reduction in superfusion and perfusion PO_{2} (Fig. 5A), similar to observations reported in previous studies (10, 20). However, dilator responses to acute hypoxia were completely abolished in arteries from rats subjected to CIH (Fig. 5B). This alteration in the ability of arteries to dilate in response to hypoxia may be important in the development and progression of hypertension caused by sleep-disordered breathing. The finding that gracilis arteries and MCA fail to dilate during acute exposure to hypoxia is important because it demonstrates that short-term exposure to intermittent hypoxia can lead to alterations in the signaling mechanisms that mediate hypoxic dilation of resistance arteries, independent of elevations in arterial blood pressure.

Although the mechanism by which intermittent hypoxia causes impairments in the ability of resistance arteries to respond to endothelium-dependent vasodilator stimuli is unknown, other investigators have determined that elevated levels of reactive oxygen species deplete vascular NO during hypertension and exposure to sustained hypoxia (11, 19, 27, 31). Coupled with studies suggesting that patients with obstructive sleep apnea have increased superoxide generation (24), additional studies investigating the role of reactive oxygen species in the vasculature of this model of sleep-disordered breathing seem to be warranted. Future studies of the effect of CIH on vascular control mechanisms may be important in determining factors that cause individuals to develop hypertension as a consequence of sleep-disordered breathing. It is possible that endothelial dysfunction arising from CIH may impair blood flow regulation and compromise tissue perfusion and oxygen delivery during stresses such as hemorrhage, exercise, and episodes of hypoxemia caused by sleep-disordered breathing, even in the absence of a significant elevation of arterial blood pressure.

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