Chronic intermittent hypoxia increases sympathetic responsiveness to hypoxia and hypercapnia

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Greenberg, Harly E., Anthony Sica, Deirdre Batson, and Steven M. Scharf. Chronic intermittent hypoxia increases sympathetic responsiveness to hypoxia and hypercapnia. J. Appl. Physiol. 86(1): 298–305, 1999.—We sought to determine whether chronic exposure to intermittent hypoxia (CIH) increases sympathetic responsiveness to subsequent chemoreflex stimulation. Sprague-Dawley rats were exposed to 30 days of CIH: exposure chamber %O2 (fractional concentration of chamber O2 (F\text{\textsubscript{O2}})) nadir 6.5–7% with return to 21% each minute for 8 h/day during the diurnal sleep period (Exp group). Sham controls (SC group) were similarly handled but kept at 21% F\text{\textsubscript{O2}} and compared with unhandled controls (UC group). Rats were then anesthetized with urethan, and preganglionic cervical sympathetic activity (CSA), diaphragm electromyogram, arterial pressure, and electrocardiogram were recorded while the rats were spontaneously breathing 100% O2, room air, 10% O2, 12% CO2, and 10% O2-12% CO2. CSA and heart rate were also recorded during phenylephrine infusion to assess baroreceptor function. Mean arterial pressure was significantly greater in Exp than in SC and UC rats during all conditions (P < 0.05). A vasopressor response to 10% O2-12% CO2 was observed only in Exp rats. CSA was greater in Exp than in SC and UC rats during 10% O2, 12% CO2, and 10% O2-12% CO2 but not during room-air exposure. A significant increase in CSA compared with room air was noted during 10% O2, 12% CO2, and 10% O2-12% CO2 in Exp but not in SC or UC rats. No differences in baroreceptor function were observed among groups. We conclude that CIH leads to increased sympathetic responsiveness to chemoreflex stimulation.

sympathetic nervous system; intermittent hypoxia; chemoreflex; sleep apnea

OBSTRUCTIVE SLEEP APNEA (OSA) has been associated with several cardiovascular complications that increase morbidity and mortality, including hypertension, myocardial ischemia, and stroke (18–20, 24, 37). In particular, acute elevation of systemic arterial pressure has been observed at the termination of obstructive apneas, and OSA has been shown to be an independent risk factor for sustained diurnal systemic hypertension (18, 29). OSA might lead to these adverse cardiovascular consequences by increasing sympathetic nervous system activity. In support of this premise, prior investigations have demonstrated elevated urinary and plasma catecholamine levels in OSA as well as increased muscle sympathetic nerve discharge occurring acutely in association with obstructive apneas (12, 35). The recent observation of elevated muscle sympathetic nerve activity and an abnormal vasopressor response to hypoxia in awake OSA patients suggests that elevated sympathetic activity might persist beyond the sleep period (2, 17, 31). Thus evidence for an increased level of sympathetic nerve discharge in OSA is strong, although its relationship to the development of sustained systemic hypertension has not been clearly elucidated.

Long-term exposure to intermittent hypoxia, such as that occurring in association with obstructive apneas, may be one factor leading to increased sympathetic activity in OSA. Prior investigations in rats have shown that chronic exposure to continuous hypoxia leads to increased sympathetic activity (21). To explore the effects of long-term exposure to intermittent hypoxia, similar to that occurring in OSA, Fletcher et al. (14) developed a model exposing rats for 30 days to intermittent hypoxia for 8 h each day during their sleep period. Sustained diurnal systemic hypertension developed (14) that was dependent on carotid sinus chemoreceptor and sympathetic nervous system activity (11, 13). Thus chronic intermittent exposure to hypoxia is a stimulus capable of leading to sustained diurnal systemic hypertension, and this effect is mediated, at least in part, by the sympathetic nervous system.

Whereas the studies of Fletcher et al. (11, 13, 14) suggest that chronic intermittent hypoxia results in increased baseline sympathetic nervous system activity, several questions remain: 1) Is the gain of the response of sympathetic circuits to subsequent hypoxia altered by prior exposure to chronic intermittent hypoxia? 2) If so, is this alteration in gain specific to hypoxia or is a nonspecific alteration in gain of the sympathetic chemoreflex response also induced to other stimuli, such as hypercapnia? 3) Because arterial baroreceptor reflexes are important in modulating sympathetic activity (30), does chronic exposure to intermittent hypoxia alter baroreceptor modulation of sympathetic activity?

These issues are important in understanding the role of long-term exposure to intermittent hypoxic stimulation in OSA. Increased sympathetic responsiveness to hypoxia or hypercapnia may alter the physiological response to apneas and may lead to progressive increases in sympathetic activity in this chronic disease.

METHODS

Experiments were performed on 2-mo-old male Sprague-Dawley rats that weighed 150–200 g at entry into the protocol. All procedures were approved by the institutional animal care and use committee and were consistent with the National Institutes of Health guidelines. Three groups of
animals were studied for hypoxic and hypercapnic challenge studies: 1) an experimental group that underwent 30 days of intermittent hypoxic exposure as described below (n = 8); 2) a sham control group that underwent identical handling during the 30-day exposure protocol but remained in room-air conditions at all times (n = 9); and 3) an unhandled control group that remained in their usual cages throughout the 30-day period (n = 5). A second series of rats was used for baroreceptor function studies, which included an experimental group (n = 5) and a sham control group (n = 5), exposed as described above.

Intermittent Hypoxia Exposure Protocol

During the daily 8-h exposure protocol, both experimental and sham control animals were housed in identical plastic chambers placed side by side. Animals were unrestrained and freely mobile within the exposure chambers. Baffles were utilized to dissipate the incoming airstream to ensure adequate gas mixing. Continuous measurement of fractional chamber O2 concentration (FCO2) was performed by using an O2 analyzer (Beckman). With the use of timed solenoid valves, the exposure chamber was flushed with 100% N2 for 30 s of each minute, alternating with a low flow of 100% O2. Gas flows were adjusted such that the chamber FCO2 declined over the first 30 s of each minute to 6.5–7%, stabilizing at this level for 5–7 s and then gradually increasing to 21% over the remainder of each minute. This cycle was repeated for 8 h/day during the animals’ sleep period. An example of the change in the FCO2 during the animals’ sleep period is shown in Fig. 1. Sham control animals were placed in a similar chamber flushed with room air in an identical manner. The animals quickly acclimated to the chamber and were observed to be asleep throughout much of the time while in the chamber. Rats were returned to their standard cages for the remainder of the day, with food and water freely available, and were maintained on a 12:12-h light-dark cycle.

Assessment of Sympathetic Nerve Activity

After completion of the 30-day exposure protocol, animals were anesthetized with urethan (1 g/kg ip). This anesthetic was chosen as it has been shown to induce a surgical plane of anesthesia with only a minimal effect on neurotransmission in various subcortical areas of the central nervous system and in the peripheral nervous system (22). Thus it is a suitable general anesthetic for studying neural function with preservation of most autonomic reflex responses (22). A cervical incision was made, and a tracheostomy was performed to maintain airway patency and for delivery of test inspiratory gases. Animals breathed spontaneously throughout the protocol. Supplemental O2 was provided during the surgical preparation. The external jugular vein was cannulated to provide intravenous access for fluid and drug administration. The femoral artery was isolated, cannulated, and connected to a pressure transducer (Gould) for measurement of arterial pressure and blood-gas determination. Electrodes were applied to the chest wall for continuous recording of the electrocardiogram (band pass 0.3–1 kHz). The preganglionic portion of the cervical sympathetic nerve was then isolated, cut, and the central end was placed on a bipolar platinum electrode and immersed in a pool of mineral oil to prevent desiccation. The preganglionic portion of this nerve was used for recording, because it is the most accessible sympathetic nerve for recording in these animals. The same electrode and recording technique was used in all animals. The signal was amplified and band-pass filtered at 0.1–3 kHz and passed through a Paynter filter (100-ms time constant) for integration (integrated cervical sympathetic activity (CSi)). A patch electrode was applied to the abdominal surface of the diaphragm, with cotton pledgets placed between the electrode and the hepatic surface to ensure consistent contact, to record the diaphragm electromyographic (EMGdi) signal (band pass 10 Hz–1 kHz). This signal was also integrated by Paynter filter, 100-ms time constant (EMGdi). All signals were displayed on a polygraph for continuous monitoring during experiments and stored on analog magnetic tape for off-line analysis. After completion of the experiment, the raw cervical sympathetic and EMGdi signals previously recorded on tape were band-pass filtered at 0.1–3 kHz and passed through a Paynter filter (100 ms) to an analog-to-digital converter and acquired on a computer (128-Hz sampling rate) for further analysis.

Experimental Protocol

Recordings were made while animals were spontaneously breathing 100% O2, room air, 10% O2-balance N2 (10% O2), 12% CO2-30% O2-balance N2 (12%CO2), and 10% O2-12% CO2-balance N2 (10%O2+12%CO2). Each gas-exposure run was 5 min in duration and was followed by 15 min of recovery breathing 100% O2. The order of these runs was randomly varied between animals. Using signal-averaging software, CSi was averaged over 30 breaths for each run. Respiratory phase-related cervical sympathetic activity was quantified as the amplitude (in arbitrary units) of the CSi signal. The integrated EMGdi was similarly signal averaged over 30 breaths, and its amplitude was recorded for each run.

The absolute value of the amplitude of an electroneurographic or EMG signal cannot be compared among animals because the amplitude of the signal depends on variable factors such as electrode contact, the number of fibers actually sampled, etc. Whereas the same electrode and similar technique were used in all experiments, these factors cannot be completely eliminated. The signal can, however, be compared among runs in any given animal, as the above factors remain constant in each preparation. Thus, to compare responses across animals and among groups, each animal’s data must be expressed as the percent change in the electroneurogram or EMG signal with stimulation from a baseline value. 100% O2 was chosen as the baseline condition, as sympathetic activity was at its lowest level, presumably because of minimal chemoreceptor input. CSi and EMGdi, expressed as a percentage of the 100% O2 value, were calculated during room air, 10% O2, 12% CO2, and 10% O2-12% CO2 conditions and were expressed as means ± SD. Because the data were not normally distributed, data were compared within each group between room air and 10% O2, 12% CO2, and 10% O2-12% CO2 stimulation runs by the Mann-Whitney
test. In addition, data were compared among groups during each condition by the Mann-Whitney test.

Mean arterial pressure (MAP), heart rate (HR), and respiratory rate were determined for each run. These data were compared between runs and among groups by two-way analysis of variance with post hoc multiple pairwise comparisons performed with the Student-Newman-Keuls method.

Assessment of Baroreceptor Function

In a separate series of rats, baroreceptor modulation of cervical sympathetic activity was examined during infusion of phenylephrine (0.01% solution), titrated to achieve an increase in systolic arterial pressure of at least 50 mmHg over a 60-s period. Arterial pressure, electrocardiogram, and CS1 were continuously recorded and digitized, as described above during the phenylephrine infusion. The amplitude of the digitized CS1 signal was continuously calculated and averaged over each second of the 60-s run. Similarly, systolic arterial pressure was calculated and averaged over each second. The linear regression line relating arterial pressure to CS1 amplitude was determined for each run. The slopes of these regressions were compared between control and experimental groups by t-test analysis.

The vagal component of the baroreceptor reflex was defined as the slope of the regression relating systolic pressure to HR, which was determined over each run for each animal. The slopes of these regressions were similarly compared among groups.

RESULTS

No significant differences in weight were noted among groups. At the end of the 30-day exposure protocol, animal weight was 302 ± 19 g in the experimental group vs. 307 ± 27 g in the sham control animals and 304 ± 21 g in the unhandled control rats.

Table 1 shows arterial blood gases obtained during 100% O2, room air, 10% O2, 12% CO2, and 10% O2+12%CO2 runs. Arterial blood gases did not differ significantly among groups, either at baseline or while the rats were breathing any of the experimental gases.

MAP

Figure 2 shows MAP in each group during each run. Under 100% O2 conditions, MAP was significantly greater in the experimental group than in the sham control (P < 0.0002) and unhandled control (P < 0.0006) groups. Similarly, under room-air conditions, MAP was significantly greater in the experimental group than in either the sham control (P < 0.0001) or unhandled control groups (P < 0.05).

During the 10% O2 run, MAP remained higher in the experimental than in the sham control group (P < 0.0001), although no differences were noted compared with the unhandled control animals. A 31.8 ± 15.9% fall in MAP was observed in the experimental animals during hypoxia compared with 100% O2, P < 0.005. Similarly, a 51.1 ± 19.6% decline in MAP was observed in sham control animals compared with 100% O2, P < 0.0002. The degree of decline in MAP was not statistically different among groups. No significant change in MAP was noted with hypoxia in the unhandled control group.

During 12%CO2 stimulation, MAP was again greater in the experimental than in both the sham (P < 0.01) and unhandled control (P < 0.02) groups. An increase in MAP was observed in each group during 12% CO2 stimulation compared with the 100% O2 value (P < 0.05), with no significant differences in the magnitude of the increase noted among groups (20.0 ± 10.6% increase in MAP in the experimental group, 25.5 ± 20.0% in sham controls, and 21.9 ± 26.0% in unhandled controls).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Experimental</th>
<th>Sham control</th>
<th>Unhandled control</th>
<th>Experimental</th>
<th>Sham control</th>
<th>Unhandled control</th>
<th>Experimental</th>
<th>Sham control</th>
<th>Unhandled control</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% O2</td>
<td>34.8 ± 7.9</td>
<td>34.8 ± 5.2</td>
<td>41.4 ± 6.1</td>
<td>425.6 ± 74.5</td>
<td>371.4 ± 94.4</td>
<td>426.6 ± 12.7</td>
<td>100.1 ± 12.9</td>
<td>91.3 ± 32.8</td>
<td>12.9 ± 91.3</td>
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<tr>
<td>Room air</td>
<td>29.0 ± 8.7</td>
<td>30.1 ± 8.3</td>
<td>34.8 ± 4.4</td>
<td>110.9 ± 23.9</td>
<td>100.1 ± 12.9</td>
<td>91.3 ± 32.8</td>
<td>117.4 ± 35.6</td>
<td>117.4 ± 35.6</td>
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<tr>
<td>10% O2-bal N2</td>
<td>22.2 ± 6.8</td>
<td>26.3 ± 6.3</td>
<td>25.0 ± 4.9</td>
<td>44.8 ± 6.1</td>
<td>43.7 ± 12.9</td>
<td>42.0 ± 9.6</td>
<td>123.3 ± 23.8</td>
<td>174.7 ± 35.6</td>
<td>123.3 ± 23.8</td>
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<tr>
<td>12% CO2 + 30% O2-bal N2</td>
<td>69.5 ± 11.7</td>
<td>71.3 ± 13.8</td>
<td>75.5 ± 3.7</td>
<td>129.1 ± 8.8</td>
<td>123.3 ± 23.8</td>
<td>174.7 ± 35.6</td>
<td>123.3 ± 23.8</td>
<td>174.7 ± 35.6</td>
<td>174.7 ± 35.6</td>
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<tr>
<td>10% O2 + 12% CO2-bal N2</td>
<td>73.4 ± 18.3</td>
<td>68.7 ± 17.5</td>
<td>69.3 ± 39.4</td>
<td>40.2 ± 15.2</td>
<td>33.4 ± 5.0</td>
<td>32.9 ± 6.2</td>
<td>32.9 ± 6.2</td>
<td>32.9 ± 6.2</td>
<td>32.9 ± 6.2</td>
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</tbody>
</table>

Values are means ± SD. Arterial blood-gas values were not significantly different among groups for any condition.
With the 10% O₂ + 12% CO₂ protocol, MAP was again significantly greater in the experimental rats than in the sham control (P < 0.001) and unhandled control (P < 0.02) groups. Furthermore, an increase in MAP in response to combined hypoxic and hypercapnic challenge was observed in the experimental animals (20.2 ± 11.7% increase in MAP compared with 100% O₂, P < 0.05), with no significant change in MAP noted in either the sham or unhandled control groups.

No significant difference was noted in MAP between the sham control and unhandled control groups during any condition.

The HR for each group during each condition is shown in Table 2. No significant differences in HR were noted among groups, and differences in HR between conditions were not statistically different.

Respiratory Phase-Related Cervical Sympathetic Activity

An example of a raw cervical sympathetic nerve activity and the corresponding EMGdi signal is shown in Fig. 3. Cervical sympathetic activity was predominantly related to respiratory phase, as been previously reported in rats (3), with peak activity occurring during the late inspiratory and early expiratory phases of the respiratory cycle. Both EMGdi and CS increased with ventilatory stimulation. An example of concurrent recordings of respiratory phase-related CS and EMGdi in each group is shown in Fig. 4.

Figure 5 shows CSI, expressed as a percent of the 100% O₂ value, in each group during each run.

Within-group analysis. In the experimental group, compared with room-air conditions, a significant increase in CSI, expressed as percent of the 100% O₂ value, was observed during 10% O₂ (P < 0.03), 12% CO₂ (P < 0.01), and 10% O₂ - 12% CO₂ (P < 0.01) stimulation. Small but nonsignificant increases were observed in both control groups during 10% O₂ and 12% CO₂ stimulations. The increase with 10% O₂ - 12% CO₂ stimulation in the unhandled control group approached statistical significance (P < 0.06), with no significant change in the sham control group.

Between-groups analysis. CSI, expressed as a percent of the 100% O₂ value, was significantly greater in the experimental than in the sham and unhandled control groups during 10% O₂ (P < 0.03 and P < 0.02, respectively), 12% CO₂ (P < 0.01 and P < 0.05, respectively), and 10% O₂ - 12% CO₂ (P < 0.006 and P < 0.05, respectively). No significant differences among groups were observed during room-air conditions. No significant differences were observed between the sham and unhandled control groups.

Baroreceptor Function

An example of simultaneous recordings of CSI and arterial pressure during phenylephrine infusion is shown in Fig. 6.

The slopes of the regression relating systemic arterial pressure to CSI during phenylephrine infusion were similar in the experimental and sham control groups: −0.59 ± 0.35 and −0.92 ± 0.58, respectively (P = not significant).

The slopes of the regression between systemic arterial pressure and HR during phenylephrine infusion were also similar for the experimental and sham control groups: −1.99 ± 0.94 and −1.62 ± 0.64, respectively (P = not significant).

Ventilatory Parameters

Figure 7 shows EMGdi amplitude, expressed as a percent of the 100% O₂ value, during room air, hypoxia, hypercapnia, and combined hypoxia-hypercapnia in all groups. Significant increases in EMGdi were noted with hypoxic, hypercapnic, and combined hypoxic-hypercapnic challenge in each group. No differences in the degree of augmentation of EMGdi with hypoxic,

Table 2. Heart rate values for the experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>100% O₂</th>
<th>Room Air</th>
<th>10% O₂</th>
<th>12% CO₂ + 30% O₂</th>
<th>10% O₂ + 12% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental, beats/min</td>
<td>453 ± 38</td>
<td>450 ± 52</td>
<td>480 ± 46</td>
<td>432 ± 16</td>
<td>445 ± 32</td>
</tr>
<tr>
<td>Unhandled control, beats/min</td>
<td>444 ± 39</td>
<td>490 ± 46</td>
<td>481 ± 51</td>
<td>456 ± 32</td>
<td>430 ± 62</td>
</tr>
<tr>
<td>Sham control, beats/min</td>
<td>426 ± 41</td>
<td>443 ± 71</td>
<td>460 ± 52</td>
<td>437 ± 41</td>
<td>455 ± 49</td>
</tr>
</tbody>
</table>

Values are means ± SD. No significant differences in heart rate were noted among groups or between conditions.
hypercapnic, and combined hypoxic and hypercapnic stimulation were observed among groups. Respiratory rate in each group during each condition is shown in Table 3. No significant differences in respiratory rate were noted among groups during any condition. In addition, the percent increase in respiratory rate during the stimulation runs was similar among groups.

DISCUSSION

The present data confirm previous results that chronic exposure to intermittent hypoxia produces sustained systemic hypertension in a rat model (14). Our findings extend understanding of this phenomenon by demonstrating that animals exposed to chronic intermittent hypoxia exhibited greater facilitation of sympathetic discharge in response to chemoreflex stimulation than did control animals. Interestingly, this phenomenon was observed not only with hypoxic challenge, a stimulus that had been repetitively presented over the previous 30 days, but also with hypercapnic and combined hypoxic-hypercapnic challenge, stimuli to which the animals had not received prior exposure or conditioning. In conjunction with this, whereas arterial pressure increased with hypercapnic stimulation in both experimental and control groups, only animals chronically exposed to intermittent hypoxia exhibited a vasopressor response to combined hypoxic-hypercapnic challenge.

These data indicate that prior long-term exposure to intermittent hypoxia increased sympathetic responsivity to chemoreflex stimulation. Such changes may involve alteration of brain stem neural circuits involved in the integration or regulation of sympathetic chemoreflex responses. One potential site where such modulation of sympathetic responsiveness might occur is the medullary sympathoexcitatory neurons located in the rostral ventrolateral medulla (RVLM). These reticulospinal neurons receive afferent input from arterial chemoreceptors and baroreceptors and excite spinal sympathetic neurons. They are critical for maintenance of resting arterial pressure and are important components of the arterial chemoreflex and baroreceptor reflex responses (27). RVLM neurons also serve as central O2 sensors (27), as brief (5- to 7-s) episodes of hypoxia in the rat have been shown to reversibly excite the RVLM, increasing sympathetic discharge (32). Evidence for plasticity of brain stem neurons in response to chronic exposure to hypoxia comes from previous find-
ings demonstrating increased tyrosine hydroxylase mRNA levels in caudal nucleus tractus solitarii neurons after 14 days of continuous hypoxia in rats (8). We speculate that chronic intermittent hypoxic exposure in our protocol may have altered the function or responsiveness of sympathoexcitatory RVLM neurons, or neuronal circuits involved in integration or modulation of their response, thereby increasing sympathetic responsiveness to chemoreflex stimulation.

Several methodological issues merit discussion. Larger increases in MAP were observed in our experimental animals after 30 days of chronic intermittent hypoxia than was previously reported by Fletcher et al. (14). Several possibilities may explain these differences. First, our blood pressure measurements were made from an indwelling arterial catheter after surgery. Arterial pressure measurements in the Fletcher et al. (14) study were performed in awake animals breathing room air. Thus the greater MAP values observed in our protocol may reflect intercurrent effects of surgery. However, since all groups underwent identical surgical procedures and daily animal handling, surgical stress or environmental effects cannot be responsible for the differences in systemic arterial pressure and sympathetic activity observed among groups. Second, our chronic intermittent hypoxia protocol differed somewhat from that of Fletcher et al. (14). Our technique resulted in a nadir O₂ concentration of 6.5–7% once each minute, whereas the latter exposure protocol reduced the O₂ concentration to 3–5% twice per minute. These differences in the degree and duration of hypoxic exposure might also explain differences in the magnitude of the changes in MAP. Last, differences in the strain of rats utilized in these studies, Sprague-Dawley in the present investigation and Wistar in the study of Fletcher et al. (14), may have contributed to this difference.

The present protocol necessitated data collection from rats under anesthetized conditions. Conceivably, this may have affected chemo- and baroreflex responses. However, urethan was chosen as the anesthetic because prior studies have shown a minimal effect of this agent on neurotransmission and spontaneous firing rates at various subcortical levels (1, 5). Furthermore, central nervous system neurons have been shown to remain responsive to a variety of peripheral stimuli, with preservation of many autonomic reflex responses during urethan anesthesia (5). In particular, the vasopressor and HR responses to hypoxic and hypercapnic stimulation remain intact with urethan (4). Similarly, the ventilatory response to hypercapnic stimulation is unaffected by urethan (3, 26). Other autonomic reflex responses have also been shown to be preserved during urethan anesthesia (22). Furthermore, while both experimental and control animals were subjected to the same anesthetic conditions, a distinct difference in the vasopressor and sympathetic response to hypoxia and hypercapnia was noted among groups. Thus, although we cannot exclude an effect of urethan anesthesia on our findings, we believe that the observed sequelae of chronic exposure to intermittent hypoxia are not entirely an artifact of anesthesia.

Activity from the preganglionic portion of the cervical sympathetic nerve was recorded and used as an index of central sympathetic discharge. The pattern of sympathetic nerve activity that was observed exhibited significant respiratory phase modulation, with peak activity occurring predominantly in late inspiration and early expiration. Such respiratory modulation of sympa-
hypothetic activity has frequently been observed in whole nerve and single-fiber recordings in both conscious humans (9) and anesthetized rats (6, 15). Respiratory phase-related sympathetic discharge is likely to be of central origin, since its temporal pattern is not altered by vagotomy, carotid sinus nerve section, or variation of respiratory frequency (7). Further evidence for a central origin of this activity comes from observations showing similar respiratory phase-sympathetic coupling in rat RVLM neurons, which provide direct synaptic excitation to spinal sympathetic preganglionic neurons (15). Thus we feel that our recordings are representative of central sympathetic discharge. As we did not perform postganglionic recordings, we cannot comment on the role of the peripheral ganglia in modulating this response.

As previously observed in normal rats under urethan anesthesia (7), hypoxic stimulation with 10% O₂ caused a decrease in systemic arterial pressure. This may be related to a predominance of local vasodilatory effects of hypoxia (33) producing a decline in overall systemic vascular resistance despite the observed increase in sympathetic activity. It is conceivable that this decline in arterial pressure may have contributed to the increase in sympathetic activity observed during hypoxia by decreasing baroreceptor inhibition of sympathetic discharge. However, we do not believe that this accounts for the differences observed among groups in the level of sympathetic discharge observed with hypoxic stimulation. First, a greater percent decrease in arterial pressure was observed during hypoxia in the sham control than in the experimental group, yet the increase in sympathetic activity with hypoxic challenge was greater in the experimental group. Furthermore, during hypercapnic and combined hypoxic-hypercapnic challenge, when arterial pressure increased, further increases in sympathetic activity were observed in experimental animals. If baroreceptor function was the sole factor regulating sympathetic discharge, a decline in sympathetic activity, rather than an increase, should have been observed when arterial pressure increased during these stimulation runs. Thus we do not believe that baroreceptor activity was solely responsible for our findings.

It is conceivable, however, that resetting or alteration in the gain of arterial baroreceptor function may have occurred as a consequence of exposure to chronic intermittent hypoxia (36). This could diminish baroreceptor-mediated inhibition of sympathetic discharge. However, we found no evidence of alteration of baroreceptor function in our model, as no differences were observed among groups in the slope of the regression relating systemic arterial pressure to cervical sympathetic activity during phenylephrine infusion. We also assessed the vagal component of the baroreceptor reflex by monitoring the change in HR with increasing systemic arterial pressure. There were no demonstrable differences among groups. Thus we could not find evidence indicating alteration of baroreceptor regulation of sympathetic activity in this model.

No differences in the ventilatory response to hypoxia or hypercapnia, as assessed by change in EMGdi and respiratory rate, were observed as a result of prior chronic intermittent exposure to hypoxia. These findings are in contradistinction to the exaggerated ventilatory response to hypoxia observed in spontaneously hypertensive rats (25) and in humans with mild hypertension (34), who have also been shown to exhibit an exaggerated sympathetic nerve response to hypoxia (23). Several factors may be responsible for the absence of an observable effect in our study. First, the 30-day intermittent-hypoxia-exposure period may have been of insufficient duration to alter ventilatory control. Second, fundamental differences may exist in the mechanisms leading to hypertension in these different models. Our model is one of acquired hypertension, whereas spontaneously hypertensive rats and humans with mild hypertension may have a preexisting genetic predisposition for hypertension that may also affect ventilatory control. Last, our ventilatory measurements were made under urethan anesthesia. Whereas we and others have demonstrated that urethan does not significantly alter baseline minute ventilation and the ventilatory response to hypercapnia (3, 26), this does not preclude an effect of anesthesia on our ventilatory measurements.

In summary, the present findings indicate that chronic exposure to intermittent hypoxia increases the sympathetic response to subsequent hypoxic, hypercapnic, and combined hypoxic and hypercapnic challenge. It is possible that sympathetic responsiveness to other stimuli might also have been altered, although not necessarily in a uniform fashion. These results suggest plasticity of neural mechanisms regulating the sympathetic response to chemoreflex stimulation and indicate that long-term exposure to intermittent hypoxia might be one factor capable of altering responsiveness of these neuronal circuits. Thus chronic exposure to intermittent hypoxia is a potential factor that should be considered in the pathogenesis of increased sympathetic activity in OSA.

Table 3. Respiratory rate values for the experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>100% O₂</th>
<th>Room Air</th>
<th>10% O₂</th>
<th>12% CO₂ + 30% O₂</th>
<th>10% O₂ + 12% CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental, breaths/min</td>
<td>90.4 ± 22.1</td>
<td>117.4 ± 6.7</td>
<td>119.9 ± 10.7</td>
<td>98.6 ± 18.7</td>
<td>94.8 ± 23.8</td>
</tr>
<tr>
<td>Unhandled control, breaths/min</td>
<td>97.8 ± 34.8</td>
<td>125.6 ± 42.9</td>
<td>133.2 ± 50.8</td>
<td>130.2 ± 60.4</td>
<td>113.9 ± 2.9</td>
</tr>
<tr>
<td>Sham control, breaths/min</td>
<td>94.7 ± 14.6</td>
<td>111.7 ± 16.2</td>
<td>120.7 ± 21.5</td>
<td>107.6 ± 27.8</td>
<td>98.6 ± 34.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. No significant differences in respiratory rate were noted among groups or between conditions.
REFERENCES


