Neurocirculatory consequences of intermittent asphyxia in humans

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Xie, Ailiang, James B. Skatrud, David C. Crabtree, Dominic S. Puleo, Brian M. Goodman, and Barbara J. Morgan. Neurocirculatory consequences of intermittent asphyxia in humans. J Appl Physiol 89: 1333–1339, 2000.—We examined the neurocirculatory and ventilatory responses to intermittent asphyxia (arterial O2 saturation = 79–85%, end-tidal PCO2 =3–5 Torr above eupnea) in seven healthy humans during wakefulness. The intermittent asphyxia intervention consisted of 20-s asphyxic exposures alternating with 40-s periods of room-air breathing for a total of 20 min. Minute ventilation increased during the intermittent asphyxia period (14.2 ± 2.0 l/min in the final 5 min of asphyxia vs. 7.5 ± 0.4 l/min in baseline) but returned to the baseline level within 2 min after completion of the series of asphyxic exposures. Muscle sympathetic nerve activity increased progressively, reaching 175 ± 12% of baseline in the final 5 min of the intervention. Unlike ventilation, sympathetic activity remained elevated for at least 20 min after removal of the chemical stimuli (150 ± 10% of baseline in the last 5 min of the recovery period). Intermittent asphyxia caused a small, but statistically significant, increase in heart rate (64 ± 4 beats/min in the final 5 min of asphyxia vs. 61 ± 4 beats/min in baseline); however, this increase was not sustained after the return to room-air breathing. These data demonstrate that relatively short-term exposure to intermittent asphyxia causes sympathetic activation that persists after removal of the chemical stimuli. This carryover effect provides a potential mechanism whereby intermittent asphyxia during sleep could lead to chronic sympathetic activation in patients with sleep apnea syndrome.

Keywords: sympathetic nervous system; hypoxia; hypercapnia

OUR LABORATORY HAS PREVIOUSLY demonstrated that a 20-min steady-state exposure to asphyxia (hypoxia plus hypercapnia) during wakefulness causes marked sympathetic activation that persists after withdrawal of the chemical stimuli (14). This carryover effect may explain the chronic sympathoexcitation observed in patients with sleep-disordered breathing (6); however, our experimental paradigm failed to mimic this clinical entity in at least one important way. Sleep-disordered breathing is characterized by discrete apneas and hypopneas that produce intermittent, rather than sustained, periods of asphyxia.

Because the sympathetic nervous system response to hypoxia is dependent on both the severity and duration of exposure (18), one might predict that intermittent asphyxia would cause less intense sympathetic activation than would steady-state asphyxia. On the other hand, steady-state changes may be less potent than frequent oscillations in stimulating the carotid body, an organ with high dynamic sensitivity to fluctuations in arterial blood-gas tensions (16). The purpose of this experiment was to study the neurocirculatory and ventilatory responses to a 20-min period of intermittent asphyxia in healthy human subjects during wakefulness.

METHODS

Subjects

Seven healthy male volunteers with a mean age of 36 yr (range 27–44 yr) served as subjects. All were free of cardiovascular, pulmonary, and neurological diseases. They were also nonsmokers and were receiving no medication. This study was approved by the University of Wisconsin Health Sciences Human Subjects Committee and the Middleton Memorial Veterans Hospital Human Research Review Committee.

General Procedures

Subjects were studied in the supine position. Room temperature was maintained at 24 ± 1°C. During the experiments, respiratory and cardiovascular variables and sympathetic nerve traffic were measured. All tracings were recorded continuously on paper (Gould, Cleveland, OH) and on magnetic tape (Vetter, Rebersburg, PA).

Respiratory variables. Tidal volume and breathing frequency were monitored with a pneumotachograph (model 5719; Hans Rudolph, Kansas City, MO) attached to a leak-free nasal mask. Minute ventilation (Ve) was calculated by multiplying tidal volume by breathing frequency. End-tidal oxygen (PETO2) and carbon dioxide (PETO2) tensions were measured by using LB-2 and OM-11 analyzers (Beckman, Schiller Park, IL). Arterial oxygen saturation (SaO2) was

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measured by using a pulse oximeter probe placed on the right ear lobe (Biox 3740; Ohmeda, Madison, WI). Heart rate was taken from the electrocardiogram. Arterial pressure was measured at 1-min intervals by using an automated arm-cuff sphygmomanometer (Dinamap; Critikon, Tampa, FL) and also beat by beat by using finger pulse photoplethysmography (Finapres; Ohmeda, Englewood, CO). The finger bearing the photoplethysmograph cuff was positioned at heart level and kept at the same level for the duration of the study.

Cardiovascular variables. Heart rate was directly measured by the microneurography technique (22). The neural signals were passed to a differential preamplifier, an amplifier (total gain = 100,000), a band-pass filter (700–2,000 Hz), and an integrator (time constant = 100 ms). Placement of the recording electrode within a muscle nerve fascicle was confirmed by 1) the presence of muscle twitches, but not paresthesias, in response to electrical stimulation; 2) the pulse synchronous nature of the nerve activity; 3) the appearance of afferent activity in response to tapping or stretching of muscle, but not gentle stroking of skin, in the appropriate receptive fields; and 4) the absence of neural activation in response to a startle stimulus. Once an acceptable neural recording (pulse synchronous activity with signal-to-noise ratio >3:1) was obtained, the subject was instructed to maintain the leg in a relaxed position for the duration of the study. Sympathetic bursts were identified by inspection of the mean voltage neurogram (25). For purposes of quantification, muscle sympathetic nerve activity was expressed as burst frequency (bursts/min), burst amplitude (arbitrary units), and total minute activity (burst frequency times mean burst amplitude in arbitrary units). Changes in all measures of muscle sympathetic nerve activity during the intervention and recovery periods were expressed as a percentage of the baseline level.

Experimental Protocols

After stable baseline measurements of neurocirculatory and ventilatory variables were obtained (at least 5 min), the subject was exposed to a series of repetitive asphyxia challenges that were administered by nasal mask. Nitrogen (95%) was added to the breathing circuit for two to three breaths to quickly lower SaO2. Then, for the remainder of the 20-s asphyxia phase, the nitrogen and carbon dioxide concentrations were adjusted to produce a lowering of SaO2 to 80% and a rise in PetCO2 of +3–5 Torr. For the remaining 40 s of each minute, the subject breathed room air. This intermittent asphyxia cycle was repeated 20 times. The 20-min exposure to intermittent asphyxia was immediately followed by a 20-min recovery period of room-air breathing. In three of the present subjects, time control experiments were performed as part of a previous study (14). In these experiments, the subjects breathed room air through the nasal mask for 40 min.

Data Analysis

To examine the overall response to intermittent asphyxia, 5-min averages of neurocirculatory and ventilatory variables obtained during the baseline, intermittent asphyxia, and recovery periods were compared by one-way ANOVA with repeated measures. Five-minute averages of these variables during time control experiments were compared in the same manner. When overall F tests were statistically significant (P < 0.05), Dunnett’s post hoc tests were used to compare measurements made during the intermittent asphyxia and recovery periods with the baseline period means.

To examine phasic responses to the oscillating chemical stimuli, we subdivided the intermittent asphyxia cycles into phases according to PetO2. The asphyxia phases (e.g., the period between the dashed lines in Fig. 1) began at the end of inspiration of the first hypoxic breath and ended at the end of inspiration of the first room-air breath of each cycle. The interasphyxia phases were defined as the periods of time between two consecutive asphyxia phases. To examine the time course of the phasic neurocirculatory responses over the 20 min of intermittent asphyxia exposure, we performed least squares regression analyses of time vs. Ve and sympathetic nerve activity in both the asphyxia and interasphyxia phases. Analysis of variance of regression was used to determine whether the slopes of the regression lines were statistically different from zero.

RESULTS

Chemical Stimuli During Intermittent Asphyxia

A polygraph record illustrating the oscillating chemical stimuli and resultant physiological responses in one representative subject is shown in Fig. 1. During each minute of the intermittent asphyxia period, the subjects spent an average of 21 ± 2 s breathing the asphyxian gas mixture and 39 ± 2 s breathing room air. The nadir values for SaO2 averaged 82 ± 1% during the asphyxia phases with 7 ± 2 s of each minute spent at SaO2 <85%. In the recovery period, average values for PetCO2 and PetO2 were the same as those for the baseline levels (38 ± 1 vs. 39 ± 2 and 113 ± 2 vs. 110 ± 3 Torr, respectively).

Ventilatory Response to Intermittent Asphyxia

Throughout the 20-min intermittent asphyxia period, Ve was elevated relative to baseline levels (Figs. 1 and 2A). In contrast, Ve did not change in time control experiments conducted in three of the seven subjects (Fig. 2A). During asphyxia-interasphyxia cycles, phasic oscillations in Ve were observed (Figs. 1 and 3A). The Ve during the asphyxia phases increased over time as shown by a positive slope that was statistically different from zero (slope = +0.10 ± 0.04 l·min⁻¹·min⁻¹; P = 0.03; Fig. 3A). In contrast, no progressive increase in Ve was noted during the interasphyxia phases (slope = +0.04 ± 0.04 l·min⁻¹·min⁻¹; P = 0.24). Slopes and regression coefficients for individual subjects are shown in Table 1. During the recovery period, Ve returned to the baseline level within 2 min after return to room-air breathing.

Effects of Intermittent Asphyxia on Muscle Sympathetic Nerve Activity

Total minute activity rose progressively during the intermittent asphyxia period so that in the final 5 min of the intervention, muscle sympathetic nerve activity had risen to 175 ± 12% of the baseline level (Figs. 1 and 2B). This response resulted from increases in both the frequency (141 ± 12% of baseline) and amplitude (127 ± 8% of baseline) of sympathetic bursts. Furthermore, total minute activity remained elevated (150 ±
10% of baseline) at the end of the 20-min recovery period of room-air breathing even though $P_{\text{ETO}_2}$, $P_{\text{ETCO}_2}$, and $V_T$ returned to baseline levels (Figs. 1 and 2B). In contrast, total minute activity remained stable during time control experiments (Fig. 2B).

A closer look at sympathetic activation during the intermittent asphyxia period reveals two major features (Fig. 3B). First, muscle sympathetic nerve activity oscillated during the asphyxia-interasphyxia cycles. Second, total minute activity during successive interasphyxia phases increased progressively during the 20-min exposure period, as shown by a slope that was statistically different from zero (slope $= +2.98 \pm 0.42\%$ of baseline/min; $P < 0.001$; Fig. 3B). In contrast, we did not observe a time-dependent increase in total minute activity during the asphyxia phases (slope $= +0.36 \pm 0.64\%$ of baseline/min; $P = 0.58$). Slopes and regression coefficients for individual subjects are shown in Table 1.

**Cardiovascular Response to Intermittent Asphyxia**

Compared with the baseline period, intermittent asphyxia caused a transient increase in systolic pressure but no change in diastolic pressure (Fig. 4A). During the asphyxia-interasphyxia cycles, systolic and diastolic pressures fluctuated above and below the control value, ascending during the asphyxia and descending during the interasphyxia phases (Fig. 1). Heart rates were higher during the intermittent asphyxia exposure than in the baseline period (Fig. 4B). The increases in

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**Fig. 1.** Original record showing a representative subject’s ventilatory and neurocirculatory responses before, during, and after exposure to intermittent asphyxia. Note that the intermittent asphyxia period tracing shows 1 asphyxia phase (defined by 2 vertical lines) followed by an interasphyxia phase. $V_T$, tidal volume; $P_{\text{ETO}_2}$, end-tidal oxygen tension; $P_{\text{ETCO}_2}$, end-tidal carbon dioxide tension.

**Fig. 2.** Five-minute averages (means ± SE) of minute ventilation ($V_T$; A) and total minute activity (B) before, during, and after 20-min exposure to intermittent asphyxia. Horizontal bars indicate the duration of the intermittent asphyxia period. *$P < 0.05$ vs. baseline (1-way ANOVA).
systolic pressure and heart rate produced by intermittent asphyxia did not persist in the postintervention recovery period.

**DISCUSSION**

The major findings of this study are twofold. First, brief intermittent exposure to asphyxia caused a substantial, progressive increase in sympathetic outflow to skeletal muscle. Second, a statistically significant portion of this asphyxia-induced sympathetic activation persisted even after reestablishment of normoxic and normocapnic conditions and the normalization of VE. The results of this study confirm our previous findings of sustained sympathetic activation caused by steady-state exposure to asphyxia (14) and extend them by demonstrating that a carryover effect also occurs when the asphyxic stimulus is presented in intermittent fashion.

**Table 1. Relationship between time and VE and sympathetic nerve activity in the asphyxia and interasphyxia phases for individual subjects (least squares regression analysis)**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Ventilation</th>
<th></th>
<th>Total Minute Activity</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Asphyxia phase</td>
<td>Interasphyxia phase</td>
<td>Asphyxia phase</td>
<td>Interasphyxia phase</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>r</td>
<td>P</td>
<td>Slope</td>
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<tr>
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<td>-0.07</td>
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</tr>
<tr>
<td>3</td>
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<td>0.54</td>
<td>0.013</td>
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</tr>
<tr>
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<td>0.60</td>
<td>0.006</td>
<td>0.09</td>
</tr>
<tr>
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<td>0.38</td>
<td>0.101</td>
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</tr>
<tr>
<td>6</td>
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<tr>
<td>7</td>
<td>-0.03</td>
<td>-0.03</td>
<td>0.902</td>
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</table>

VE, minute ventilation. P values indicate whether the slopes of the regression lines were statistically different from 0 (analysis of variance of regression).
Critique of Methods

The possibilities that sympathetic outflow increased spontaneously or that the position of the intraneural electrode was not stable over time threaten the validity of our conclusion that intermittent asphyxia caused sustained sympathoexcitation. The former possibility is unlikely because sympathetic nerve activity remained stable during 40 min of room-air breathing in time control experiments. We also consider it unlikely that electrode instability affected our data. Shifts in the position of the electrode within the nerve fascicle produce easily detectable offsets in the baseline of the mean voltage neurogram; we excluded from analysis experiments in which such offsets were detected.

In addition, we recorded sympathetic discharge that is targeted to vascular structures in leg muscle. This discharge is representative of sympathetic outflow to skeletal muscle vascular beds throughout the body (17); however, we cannot extrapolate our findings to other organs or vascular beds.

Sympathetic Neural Response to Intermittent Asphyxia

The stimulant effects of hypoxia and hypercapnia on sympathetic activity are well known (19–21). However, we are aware of only one previous study, conducted in anesthetized cats, that demonstrated a carryover of the sympathoexcitatory effects of hypoxia beyond the exposure period (10). Although the authors did not comment on this aftereffect, several of their figures indicate that 1–10 min of hypoxic exposure caused a significant increase in sympathetic outflow to hindlimb muscle that persisted for several minutes beyond the period of chemical stimulation (10).

The present findings in human subjects indicate that sustained sympathetic activation occurs after a relatively brief (20-min) exposure to intermittent asphyxia. Compared with the steady-state asphyxia intervention utilized in our laboratory’s previous study (14), intermittent asphyxia in the present experiments caused a smaller elevation of sympathetic outflow (175% of baseline level after 20 min of intermittent asphyxia vs. 220% of baseline level after 20 min of steady-state asphyxia). Nevertheless, the carryover effect on muscle sympathetic activity was comparable with the two interventions. This finding is remarkable because the overall duration of chemoreflex stimulation was shorter in the present study (SaO₂ was <85% for 12% of the time during intermittent asphyxia vs. 100% of the time during sustained asphyxia). These findings suggest that intermittent and sustained exposures to asphyxia are equally effective in eliciting a poststimulus elevation in muscle sympathetic nerve activity.

A carryover effect on muscle sympathetic nerve activity was also observed within the intermittent asphyxia cycles, i.e., in the interasphyxia phases when the subjects breathed room air. Muscle sympathetic nerve activity did not return to baseline levels in these brief “recovery” periods between successive asphyxic exposures. In fact, the amount of sympathetic activation present in the interasphyxia phases progressively increased throughout the 20-min intervention period. This finding suggests that asphyxic exposures shorter than 20 min may also induce sympathetic activation that outlasts the chemical stimuli. However, even though PETCO₂ and PETO₂ indicated normalization of blood chemistry in the interasphyxia phases, we cannot rule out the possibility of persistent acidosis in chemosensitive areas of the brain (see below).

Mechanism of Sympathetic Neural Response to Intermittent Asphyxia

Our laboratory has previously hypothesized that asphyxic exposure may produce a facilitatory memory within the central nervous system that requires input from central and/or peripheral chemoreceptors (14). This facilitatory memory may result from enhanced synaptic efficacy (1) and/or persistent increases in concentrations of excitatory neurotransmitters within central nervous system regions that regulate sympathetic outflow. The slowly disappearing postasphyxic effect on sympathetic outflow is analogous to the long-term facilitation that maintains ventilation or phrenic activity above baseline levels for >1 h after episodic carotid sinus nerve stimulation (11, 13). However, the mechanism underlying the observed effect on sympathetic outflow is not identical to that responsible for long-term facilitation of VE, because VE had returned to baseline levels within 2 min after exposure. In contrast to the progressive buildup of sympathetic activity we observed in the interasphyxia phases, there was no concomitant progressive rise in VE. These latter findings are both consistent with short- rather than long-term facilitation of VE (4).

Persistent increases in chemical stimuli in arterial blood or in brain extracellular fluid might explain the persistent elevation in sympathetic nerve activity we observed, but we believe this is unlikely for two reasons. First, our data show that PETCO₂ and PETO₂ returned to baseline levels in the recovery period. Our laboratory has previously demonstrated close correspondence in changes in PETCO₂ and arterial PCO₂ in humans under conditions in which the fraction of inspired CO₂ and VE were raised (24). Second, measurements of medullary surface pH showed a return to baseline levels within 1 min of termination of CO₂ inhalation (2), and arterial blood gases and carotid sinus nerve activity also returned to normal within 15 s after apnea termination (7, 15).

Hemodynamic Responses to Intermittent Asphyxia

In the present experiments, the persistent sympathetic activation after asphyxic exposure was not accompanied by an increase in heart rate, even though heart rate oscillated within the asphyxia-interasphyxia cycles and the average heart rate was higher during intermittent asphyxia than in the baseline pe-
In our study, intermittent asphyxia produced regular arterial pressure fluctuations above and below the baseline level but no sustained increases in systolic or diastolic pressure. Although this minimal effect on arterial pressure seems incongruous with the large increase in sympathetic vasoconstrictor outflow produced by intermittent asphyxia, it is, nevertheless, consistent with data reported by other investigators (12, 18). In humans breathing 8–12% O₂, increases in sympathetic outflow to skeletal muscle were not accompanied by increases in plasma norepinephrine concentrations (18). In addition, the local vasodilatory effects of hypoxia and hypercapnia in many vascular beds are well known (8). It is likely that, during the asphyxic exposure, the vasoconstrictor effects of increased muscle sympathetic nerve activity were not able to offset these local vasodilators sufficiently to raise peripheral vascular resistance and blood pressure.

Why then was there no increase in arterial pressure in the postasphyxia recovery period when sympathetic activation remained high and the local vasodilatory effects of the asphyxic stimuli had presumably dissipated? The reasons for this dissociation between sympathetic vasomotor outflow and blood pressure are unclear. It is possible that the magnitude of the postasphyxic increase in sympathetic vasoconstrictor outflow to skeletal muscle was not sufficient to sustain the rise in arterial pressure, perhaps because of the offsetting of vasodilation in other vascular beds. It is similarly possible that the asphyxic exposure period was too brief to produce long-lasting cardiovascular effects. It is clear from previous studies in experimental animals (9, 23) and humans (3, 5) that longer term hypoxic exposure (days rather than minutes) does elicit a hypertensive effect that persists after removal of the chemical stimulus.

Our intermittent asphyxia intervention mimicked the oscillations in P O₂ and P CO₂ typically experienced by patients with sleep apnea syndrome; however, our model differs from this clinical entity in several important ways. The model we used failed to replicate apnea, upper airway obstruction, the sleep state, and arousal from sleep. Thus parallels between our observation of persistent sympathetic activation after intermittent asphyxia and the chronically elevated sympathetic nervous system activity seen in patients with sleep apnea syndrome must be drawn with caution.

In summary, the major finding of this study is that intermittent exposure to asphyxia causes an elevation in muscle sympathetic nerve activity that outlasts the chemical stimuli. Our findings raise the intriguing possibility that sustained sympathetic activation may contribute to the carryover effect on daytime blood pressure after nocturnal intermittent hypoxia (3).

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REFERENCES