Cardiovascular response to arousal from sleep under controlled conditions of central and peripheral chemoreceptor stimulation in humans

Denise M. O'Driscoll,1,3 Guy E. Meadows,1,3 Douglas R. Corfield,1,2 Anita K. Simonds,1,2 and Mary J. Morrell1,3

1Clinical and Academic Unit of Sleep and Breathing, National Heart and Lung Institute, Imperial College, London SW3 6LY; 2MacKay Institute of Communication and Neuroscience, School of Life Sciences, Keele University, Keele ST5 5BG; and 3Sleep and Ventilation Unit, Royal Brompton Hospital, London SW3 6NP, United Kingdom

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O'Driscoll, Denise M., Guy E. Meadows, Douglas R. Corfield, Anita K. Simonds, and Mary J. Morrell. Cardiovascular response to arousal from sleep under controlled conditions of central and peripheral chemoreceptor stimulation in humans. J Appl Physiol 96: 865–870, 2004. First published October 24, 2003; 10.1152/japplphysiol.00749.2003.—The cardiovascular response to an arousal occurring at the termination of an obstructive apnea is almost double that to a spontaneous arousal. We investigated the hypothesis that central plus peripheral chemoreceptor stimulation, induced by hypercapnic hypoxia (HH), augments the cardiovascular response to arousal from sleep. Auditory-induced arousals during normoxia and HH (>10-s duration) were analyzed in 13 healthy men [age 24 ± 1 (SE) yr]. Subjects breathed on a respiratory circuit that held arterial blood gases constant, despite the increased ventilation associated with arousal. Arousals were associated with a significant increase in mean arterial blood pressure at 5 s (P < 0.001) and with a significant decrease in the R-R interval at 3 s (P < 0.001); however, the magnitude of the changes was not significantly different during normoxia compared with HH (mean arterial blood pressure: normoxia, 91 ± 4 to 106 ± 4 mmHg; HH, 91 ± 4 to 109 ± 5 mmHg; P = 0.32; R-R interval: normoxia, 1.12 ± 0.04 to 1.02 ± 0.05 s; HH, 1.09 ± 0.05 to 0.92 ± 0.04 s; P = 0.78). Mean ventilation increased significantly at the second breath postarousal for both conditions (P < 0.001), but the increase was not significantly different between the two conditions (normoxia, 5.35 ± 0.40 to 9.57 ± 1.69 l/min; HH, 8.57 ± 0.63 to 11.98 ± 0.70 l/min; P = 0.71). We conclude that combined central and peripheral chemoreceptor stimulation with the use of HH does not interact with the autonomic outflow associated with arousal from sleep to augment the cardiovascular response.

hypoxia; hypercapnia; blood pressure; heart rate

IN HEALTHY YOUNG PEOPLE, AROUSALS from sleep are associated with acute surges in blood pressure and heart rate (11, 29, 36). In patients with obstructive sleep apnea (OSA), the cardiovascular response to a postapneic arousal is almost double that to a spontaneous arousal (1, 21, 27). The repeated arousals from sleep that occur in OSA may contribute to the increased risk of developing hypertension (25, 28), with the mediating factor being the frequent, acute cardiovascular insults. The aim of the present study was to elucidate the mechanisms responsible for the augmentation of the cardiovascular response to arousal at the termination of an obstructive apnea.

In patients with OSA, arousal from sleep occurs at the termination of an apnea under conditions of hypercapnic hypoxia (HH). Central hypercapnic chemoreceptor stimulation is a potent stimulus for ventilation, sympathetic nerve activity, and arousal (8, 34). Hypoxic stimulation of peripheral chemoreceptors also increases ventilation and causes systemic vasoconstriction, with both acute and chronic increases in sympathetic nerve activity (19). Combined hypercapnic and hypoxic stimulation, independent of arousal, produces a synergistic increase in sympathetic nerve activity that is more than additive (34). Arousal, in the absence of hypercapnia or hypoxia, has been reported to be associated with an acute increase in sympathetic activity (16). The influence HH has on the cardiovascular response to arousal from sleep has not been previously investigated. Therefore, we reasoned that central and peripheral chemoreceptor stimulation (HH) may potentially contribute to the arousal-related sympathetic outflow at the termination of an apnea.

Some studies (10, 31), but not all (32, 37), have shown that hypoxia alone does not alter the cardiovascular response to arousal. However, hypoxia at the termination of an apnea does not occur in isolation but in association with hypercapnia (20). It is unknown whether arousal from sleep during HH results in an augmented cardiovascular response. We have tested the hypothesis that central plus peripheral chemoreceptor stimulation of autonomic outflow interacts with the stimulus associated with arousal from sleep to augment the cardiovascular response. To achieve this, we compared the cardiovascular response to auditory-induced arousals (matched for duration), in healthy male subjects under conditions of controlled eucapnic normoxia (normoxia) and HH. During the testing period, subjects breathed on an experimental respiratory circuit designed to hold arterial blood gases constant despite increases in ventilation associated with arousal.

METHODS

Subjects. Twenty-two healthy male subjects recruited from the general population were studied. Sufficient data for within-subject comparison were collected from 13 subjects [age, 24 ± 1 (SE) yr; body mass index, 23.7 ± 0.19 kg/m2]. No subject had a history of cardiovascular disease, snoring, or other sleep pathology associated with excessive daytime somnolence (Epworth sleepiness scale, 4.5 ± 1.0) (18). All were normotensive (office blood pressure, systolic, 120 ± 3 mmHg; diastolic, 68 ± 3 mmHg) and had normal lung function as determined by forced spirometry (ratio of forced expiratory volume in 1 s to forced expiratory vital capacity >75%). All subjects had normal ventilatory responses to hypercapnia (HCVR; 1.72 ± 0.25 l·min⁻¹·Torr⁻¹) and hypoxia (−0.19 ± 0.04 arterial
oxygen saturation (SaO2) measured by a constant-flow, steady-state technique (14, 38). Data from 9 of the 22 subjects were excluded because of insufficient sleep (5 subjects were excluded because they were unable to remain asleep with the respiratory circuit) or insufficient arousals of similar duration under the two conditions of normoxia and HH (4 subjects were excluded for not achieving at least 1 arousal of similar duration during both normoxia and HH). There were no anthropometric differences between subjects included and excluded. The Brompton and Harefield Ethics Committee approved this study, and all subjects gave written, informed consent.

Protocol. Subjects were asked to limit their total sleep time the night before the study (<4 h) and to abstain from caffeine and alcohol consumption on the day of testing. Sleep restriction was employed the night before testing in an effort to promote consolidated sleep in the laboratory despite the unfamiliar setting and instrumentation. Each subject arrived at the sleep laboratory at 1900, and the monitoring equipment was attached for overnight polysomnography. During stable stage 2 non-rapid eye movement (NREM) sleep, an auditory stimulus was sounded to induce arousals under the two conditions of normoxia and HH.

The auditory stimulus consisted of a tone delivered remotely via an audio speaker placed at the end of the subject’s bed (75 dB measured at subject’s ear, 1-s duration). Each recorded tone was swept linearly over a 60-Hz bandwidth, with the ambient room noise measured as 50 dB. During sleep, the subjects breathed via a respiratory “clamping” circuit that allowed the experimenter to not only control the mixture of inspired gas but also utilize variable rebreathing to maintain the subject’s end-tidal PCO2 (PETCO2) to within ±2 Torr of the baseline, irrespective of rapid increases in ventilation (6, 22).

Physiological measurements. EEG (C3/A2, O1/A2), electrooculograms (EOG; F7/F8), and an electromyogram (EMG; submental muscle) were recorded (Grass Instrument) to determine sleep state according to standard criteria (30). Continuous ECG was recorded (Lifetrak, HME), and blood pressure was measured via finger plethysmography (Finapres, Ohmeda). SaO2 was measured by using pulse oximetry (model N-200E, Nellcor). Respirated gases were sampled via a probe placed within the nostril. PETCO2 and end-tidal PO2 were measured by using rapidly responding gas analyzers (models CD3A and S-3A, AEI Technologies). Ventilation was measured via a pneumotachograph (model 4700A, Hans Rudolph) and differential pressure transducer (model MC1-3, Validyne) attached to a full-face mask (B&D Electro-medical). All physiological parameters were recorded by using a computerized data system (CED 1401, Spike 2, Cambridge Electronic Design).

Sleep studies. Subjects slept between 2200 and 0700. The interventional study began at 0030 in an effort to ensure all arousals were induced during consolidated stage 2 sleep. After 5 min of stable stage 2 sleep, while the subjects were breathing medical air, the respiratory circuit was adjusted to clamp PETCO2 at the sleep eucapnic level. The clamping was maintained for 4 min before the auditory stimulus was sounded. This served as the normoxia condition. The HH condition involved mixing air with 100% CO2 and 9% O2 to increase PETCO2 by 3 Torr above sleep eucapnia and simultaneously reduce oxygen saturation by 5%, relative to measurements made in the previous 5 min of stable sleep. The respiratory circuit was then adjusted to clamp PETCO2 at the hypercapnic level, and the clamping was maintained for 4 min before the auditory stimulus was sounded. The auditory stimulus was sounded at end expiration provided there were no spontaneous arousals in the preceding 30 s. The testing cycle involved two normoxia conditions followed by 2 HH conditions, all in stage 2 sleep, repeated until morning wake time.

Data analysis. Cardiovascular and ventilatory responses were analyzed for each induced arousal. Blood pressure and R-R interval were analyzed from 14 s pre to 14 s post the presentation of the auditory stimulus. The blood pressure and R-R interval data points were resampled at 1-s intervals, by using linear interpolation, to enable comparison between interventions. Breath-by-breath analysis of tidal volume (VT), total breath duration (Tt), ventilation, PETCO2, and SaO2 was performed for six breaths before and after the presentation of the auditory stimulus.

Sleep stage and induced arousals were scored from the EEG, EOG, and chin EMG channels from 30-s epochs by a researcher blinded to other physiological data. Arousal were graded into one of the following five categories on the basis of type and duration of the EEG frequency shift: 1) no discernible change; 2) an increase in the amount of slow-wave activity; 3) an abrupt shift in EEG frequency lasting between 1.5 and 3 s (including any combination of theta, alpha, or other activity >16 Hz but not spindle or delta frequencies); 4) an abrupt shift in EEG frequency lasting between 3 and 10 s; and 5) an abrupt shift in EEG frequency lasting >10 s. Similar categories have been used previously to assess the autonomic response to arousal during sleep (9, 29).

Statistical analysis. The difference in the increase in systemic blood pressure after a spontaneous arousal compared with that which occurs at the end of an apnea (with HH) has been reported as ~19 ± 16 mmHg (27, 36). To detect a similar difference between arousals induced during normoxia and HH, we calculated that we needed to study 15 subjects (power, 0.9; rejection rate α = 0.05). Therefore, we recruited 22 subjects to the study and obtained data in 13 (see discussion) normoxia and obtained data in 13 (see discussion) normoxia arousals; 19 normoxia arousals (1–5 per subject) and 16 HH arousals (2–4 per subject).

The mean SaO2 remained constant pre- and postarousal in both conditions (normoxia: prearousal, 97.9 ± 0.3%; postarousal, 97.9 ± 0.3%; HH: prearousal, 92.9 ± 0.4%; postarousal, 93.1 ± 0.4%). The mean PETCO2 also remained constant pre- and postarousal in both conditions (normoxia: prearousal, 45.6 ± 0.6 Torr, postarousal, 45.3 ± 0.6 Torr; breath 1, 45.1 ± 0.6 Torr; breath 2, 44.9 ± 0.6 Torr; breath 3, 45.0 ± 0.6 Torr); HH: prearousal, 48.2 ± 0.7 Torr, postarousal, 48.4 ± 0.7 Torr (breath 1, 48.5 ± 0.7 Torr; breath 2, 48.6 ± 0.7 Torr; breath 3, 48.4 ± 0.7 Torr). Figure 1 shows original traces in one subject, when category 5 arousals were induced during normoxia and HH.

Cardiovascular parameters. There was no significant difference in the group data mean arterial blood pressure (MAP) during normoxia, compared with the HH measured 1 s before the arousal (normoxia, 91 ± 4 mmHg; HH, 91 ± 4 mmHg; P = 0.91). Arousal were associated with a significant increase in MAP at 5 s (normoxia, 106 ± 4 mmHg; HH, 109 ± 5 mmHg; P < 0.001). At 14 s postarousal, MAP had declined (normoxia, 92 ± 4 mmHg; HH, 95 ± 4 mmHg), with the increase in MAP during HH having a tendency to be more sustained than during normoxia. However, the magnitude and time course of the arousal-induced increase in MAP were not
significantly different during normoxia compared with HH ($P = 0.32$; Fig. 2).

The mean R-R intervals measured 1 s before the arousal were not significantly different between the normoxia and HH trials (normoxia, $1.12 \pm 0.04$ s; HH, $1.09 \pm 0.05$ s; $P = 0.42$). Arousals were associated with a significant decrease in the R-R interval at 3 s (normoxia, $1.02 \pm 0.05$ s; HH, $0.92 \pm 0.04$ s; $P < 0.001$). However, the magnitude of the decrease in R-R interval was not significantly different between the two conditions ($P = 0.42$; Fig. 2).

**Respiratory parameters.** Mean ventilation measured at the breath immediately preceding the arousal was significantly increased during HH compared with normoxia (normoxia, $5.35 \pm 0.40$ l/min; HH, $8.57 \pm 0.63$ l/min; $P < 0.001$). Ventilation significantly increased at the second breath postarousal for both conditions (normoxia, $9.57 \pm 1.69$ l/min; HH, $11.98 \pm 0.70$ l/min; $P < 0.001$). However, there was no significant difference in ventilation between the two conditions ($P = 0.71$; Fig. 3). Mean VT at the breath immediately preceding the arousal was significantly increased during HH compared with normoxia (normoxia, $0.40 \pm 0.02$ liter; HH, $0.60 \pm 0.04$ liter; $P < 0.001$). VT significantly increased at the second breath postarousal for both conditions (normoxia, $0.73 \pm 0.15$ liter; HH, $0.90 \pm 0.12$ liter; $P < 0.001$). However, there was no significant difference in VT between the two conditions ($P = 0.94$). Mean TR measured at the breath immediately preceding the arousal was significantly decreased during HH compared with normoxia (normoxia, $4.7 \pm 0.2$ s; HH, $4.3 \pm 0.2$ s; $P < 0.001$). TR did not change significantly after arousal ($P = 0.29$; Fig. 2).

**Cardiovascular and ventilatory response to category 4 arousals.** The magnitudes of the cardiovascular and ventilatory responses to category 4 arousals were similar to those that occurred to category 5 arousals. MAP significantly increased (normoxia, $90 \pm 6$ to $103 \pm 8$ mmHg; HH, $89 \pm 6$ to $103 \pm 6$ mmHg; $P < 0.001$) and the R-R interval significantly decreased (normoxia, $1.14 \pm 0.05$ to $0.90 \pm 0.03$ s; HH, $1.06 \pm 0.06$ to $0.83 \pm 0.04$ s; $P < 0.001$) postarousal, but the magnitudes were not significantly different between normoxia and HH (MAP, $P = 0.44$; R-R interval, $P = 0.90$). Ventilation also significantly increased postarousal (normoxia, $6.26 \pm 0.79$ to $10.66 \pm 2.11$ l/min; HH, $9.32 \pm 1.00$ to $12.13 \pm 1.29$ l/min; $P < 0.001$), but, again, this increase was not significantly different between conditions ($P = 0.43$).

**DISCUSSION**

The key finding of this study is that combined central and peripheral chemoreceptor stimulation with the use of HH does not augment the cardiovascular response to arousal from sleep. Arousals were induced in healthy subjects via an auditory tone during stage 2 NREM sleep under conditions of normoxia and HH. Any arousal-related ventilatory response associated with changes in $PaCO_2$ was minimized by the use of the clamping circuit. The magnitude and time course of the blood pressure and heart rate response (measured by R-R interval) were not different when arousals were induced during HH compared...
with normoxia. This is the first demonstration of the role of HH in the cardiovascular response to arousal from sleep.

All induced arousals were associated with acute surges in blood pressure that peaked at 5 s and heart rate that peaked at 3 s. The profile of this cardiovascular response confirms that described in previous studies for arousals that have been induced from sleep under normoxic conditions (11, 29, 36). We have also extended the findings of Catcheside et al. (10). These investigators found no significant difference in the heart rate, pulse transit time, or skin blood flow response to arousal from sleep induced by an auditory tone during mild hypoxia compared with normoxia. Our findings obtained during HH support the notion that hypoxic stimulation, in the presence or absence of hypercapnia, does not augment the cardiovascular response to arousal from sleep.

The levels of HH used in the present study (+3-Torr increase in PETCO2 and −5% reduction in SaO2) were less than those that can occur at the termination of an obstructive apnea. However, they do fulfill the criteria commonly used to define respiratory events in sleep-disordered breathing (i.e., ≥4% oxygen desaturation) (23), and they are representative of

Fig. 2. Group data of mean arterial BP (A), systolic and diastolic BP (B), and R-R interval (C) associated with category 5 arousals (>10 s) induced by auditory tone. Values are means ± SE for 13 subjects. Vertical solid line (time 0), start of the 1-s auditory tone. All values interpolated into 1-s intervals. HH, hypercapnic hypoxia.

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Fig. 3. Group data of breath-by-breath ventilation (A), tidal volume (B), and total breath duration (C) for category 5 arousals (>10 s) induced by auditory tone. Values are means ± SE for 13 subjects. Vertical solid line, start of the 1-s auditory tone.
events seen in mild OSA-hypopnea syndrome. The hypoxic gas mixture, plus carbon dioxide, in the HH trials resulted in a 60% increase in the level of ventilation before the arousal. This increase in ventilation is likely to be the result of chemoreceptor stimulation, although the synergistic effect of the hypercapnia and hypoxia at the level of the peripheral chemoreceptor could be independent of that which occurs at the central chemoreceptors by hypercapnia. Although the low level of hypoxia most likely did not stimulate the peripheral chemoreceptors independently, because hypoxia and hypercapnia have a synergistic effect, it would have enhanced the effect of hypercapnia on the peripheral chemoreceptors (7, 33).

For example, in the present study, the HCVR measured during wakefulness was 1.72 l·min⁻¹·Torr⁻¹. During stage 2 sleep, the HCVR is reduced by ~57% (12). Therefore, we would predict the HCVR during sleep in our study to be ~0.74 l·min⁻¹·Torr⁻¹. However, with a 3-Torr increase in PETCO₂, ventilation increased by 3.22 l/min with concomitant hypoxia, rather than the 2.22 l/min we would have calculated for hypercapnia alone. We conclude that we were increasing PETCO₂ and decreasing SaO₂ sufficient to stimulate chemoreceptors within the physiological range and that the failure to demonstrate an increased cardiovascular response during HH could not have been due to insufficient stimulation of the central and peripheral chemoreceptors. Although much larger levels of HH could have been used, healthy subjects would have certainly woken from the ventilatory stimulus itself, before the 4-min protocol with auditory tone could have been implemented (8).

Episodic hypoxia has been shown to elicit long-term facilitation (LTF) of ventilation in sleeping humans (3). The most consistent markers for the development of LTF are snoring and/or flattening of inspiratory flow (i.e., flow limitation) (4). The subjects used in this present study were screened to exclude snorers and subjects with upper airway resistance. For this reason, we do not think that LTF is likely to have occurred in our study. Furthermore, we do not believe that these present negative findings are a result of type II statistical error. Using our sample size, subject standard deviations, and a significance level of 0.05, we could have detected differences in blood pressure and R-R interval of 20 mmHg and 0.25 s, respectively, between the two conditions with a power of 90%. Given the differences actually detected in the study, we would have needed to study 597 subjects to demonstrate statistical significance. Furthermore, the mean difference of 3 mmHg, even if of statistical significance, may not be of substantial physiological significance. A further limitation of this present study may be the use of an auditory tone to induce arousal. Auditory arousals have been used in many studies investigating the physiological response to arousal in humans (5, 10, 11, 24, 29). However, the auditory stimulus is unlike the stimuli that occur during an apnea because it has an abrupt onset compared with the build up of endogenous stimuli (e.g., hypercapnia, hypoxia, negative intrathoracic pressure). Nevertheless, once the arousal is induced, regardless of the stimulus, the interaction between the autonomic and respiratory system is likely to occur in a similar fashion. In the present study, our aim was to induce arousals under controlled ventilatory conditions. Therefore, we chose to use an auditory stimulus because it was a means of inducing cortical arousal during conditions of normoxia vs. HH.

It has been suggested that the cardiovascular surges that occur during an arousal from sleep are associated with a withdrawal of parasympathetic activity and with an acute increase in sympathetic activity that is beyond functional requirements (16). Several studies indicate that both hypercapnia and hypoxia (induced in isolation) are associated with an increase in sympathetic nerve activity during wakefulness (19, 34). Interestingly, the findings from Somers et al. (34) indicate that combined HH stimulation has a synergistic effect on sympathetic nerve activity and ventilation during wakefulness. In the present study, we did not find a synergistic effect of HH on the cardiovascular system during arousal from sleep when the arousal-related increase in respiratory motor output was similar. Because the arousal-related increase in VT was similar between conditions, the lung volume contribution and the influence of pulmonary stretch receptors on the cardiovascular system, which could mask the effects of peripheral chemoreceptor stimulation (2) and are thought to be the dominant determinants of respiratory modulation of sympathetic nerve activity in humans (35), were also likely to be similar.

The factor(s) that specifically lead to the increased cardiovascular response at the termination of an apnea remains unclear: potential mechanisms include stimulation of pulmonary stretch receptors and negative intrathoracic pressure swings. O'Donnell et al. (26) assessed the impact of airflow obstruction on the cardiovascular response to arousal by using peripheral arterial tonometry as a marker of sympathetic nerve activity (SNA). They found that SNA increased according to the level of obstruction and that it further increased with an associated arousal. Gleeson et al. (15) found that arousals from sleep occurred at the same level of increasing ventilatory effort irrespective of the level of arterial blood gases. It is possible that increasing negative pressure may contribute to the arousal-related increase in sympathetic activity that augments the cardiovascular response at the termination of an apnea.

On the other hand, Eastwood et al. (13) found that stimulation of upper airway pressure sensitive receptors, in dogs, did not contribute directly to the increase in blood pressure during a simulated obstructive apnea in the absence of arousal from sleep. This finding is consistent with results from human studies (35). In humans, the negative intrathoracic pressure did not modulate SNA when central respiratory motor output was controlled for by matching VT during active and passive ventilation. However, this study did not take into account the interaction between negative pressure and arousal from sleep.

It has been suggested that the cardiovascular response to spontaneous arousal is caused by the change in conscious state per se. This is supported by evidence that a transient arousal is a unique state of heightened preparedness immediately after awakening, which is functionally different from later quiet wakefulness (17, 36). The fact that the cardiovascular response during this unique state can be augmented at the termination of an apnea suggests that there may be a neural interaction, possibly at the level of the brain stem, between autonomic and arousal-related mechanisms. Alternatively, the arousal-related cardiovascular response may be determined by changes in negative intrathoracic pressure and stimulation of pulmonary stretch receptors. Whether the arousal-related cardiovascular response is determined by feedback from pulmonary receptors or feedforward from central respiratory outflow during arousal requires further investigation.
Conclusion. This study does not support the hypothesis that combined central and peripheral chemoreceptor stimulation augments the cardiovascular response to arousal. Concomitant HH did not augment the magnitude or time course of the arousal-related surges in blood pressure and heart rate. We conclude that, in OSA, the enhanced cardiovascular response at the termination of an apnea is most likely due to a combination of factors specific to the cessation of airflow, such as increased negative intrathoracic pressure and/or stimulation of the pulmonary stretch receptors.

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