Effect of repetitive hypoxic apnoeas on baroreflex function in humans

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Baroreflex function is impaired in patients with obstructive sleep apnoea. We tested the hypothesis that short-term exposure to repetitive hypoxic apnoeas (RHA) produces prolonged impairment in baroreflex function. Baroreflex function was determined using the modified Oxford technique in 14 subjects (26 ± 1 years). Baroreflex sensitivity (BRS) was quantified from the R-R interval–systolic blood pressure (BP; cardiovagal BRS), heart rate–systolic BP (HR BRS) and muscle sympathetic nerve activity (MSNA)–diastolic BP (sympathetic BRS) relations. RHA involved subjects performing repetitive end-expiratory apnoeas (20 s) every minute for 30 min during intermittent hypoxia to accentuate oxygen desaturation. After RHA, BP and MSNA at rest were elevated. BRS was measured $\sim$7 (Post 1), $\sim$30 (Post 2) and $\sim$50 min (Post 3) after RHA to provide insight into the temporal pattern of responses. Cardiovagal BRS (16.8 ± 1.3, 16.5 ± 1.6, 17.6 ± 2.0 and 17.4 ± 1.5 ms mmHg^{-1} for Pre, Post 1, Post 2 and Post 3, respectively), HR BRS (-1.1 ± 0.1, -1.1 ± 0.1, -1.3 ± 0.1 and -1.4 ± 0.1 beats min^{-1} mmHg^{-1}) and sympathetic BRS (-4.5 ± 0.6, -4.4 ± 0.7, -3.7 ± 0.5 and -4.7 ± 1.0 arbitrary units (au) beat^{-1} mmHg^{-1}) were unchanged by RHA. In contrast, the operating points of the baroreflexes were shifted rightward (to higher levels of BP) and upward (to higher levels of heart rate and MSNA) after RHA ($P < 0.05$). Time control studies performed in five additional subjects showed no change in any of the measured variables over time. Collectively, these data indicate that short-term exposure to RHA shifts ('resets') the baroreflex stimulus–response curve to higher levels of BP without influencing BRS for extended periods of time.

Obstructive sleep apnoea (OSA) is a prevalent form of sleep-disordered breathing estimated to afflict between 2 and 4% of middle-aged men and women (Young *et al.* 1993). OSA is characterized by the partial or complete obstruction of the upper airway during sleep resulting in intermittent periods of increased respiratory effort and periods of apnoea and/or hypopnoea. These periods of apnoea/hypopnoea are associated with arterial oxygen desaturation and hypercapnia, which are in turn associated with sympathoexcitation and increases in arterial blood pressure (BP) (Imadojemu *et al.* 2002). Individuals with OSA are at elevated risk of developing cardiovascular disease (Hung *et al.* 1990; Peker *et al.* 1999; Leung & Bradley, 2001), particularly hypertension (Guilleminault & Robinson, 1997; Nieto *et al.* 2000) and have increased rates of all-cause mortality (Yaggi *et al.* 2005). Therefore, identifying mechanisms underlying or contributing to the negative consequences associated with OSA are of biomedical significance. In this context the autonomic nervous system may be important (Shamsuzzaman *et al.* 2003).

Previous studies suggest that both basal and reflexive measures of autonomic nervous system function, such as directly recorded muscle sympathetic nerve activity (MSNA) at rest (Carlson *et al.* 1993) and peripheral chemoreflex sensitivity (Narkiewicz *et al.* 1999) are elevated in OSA. Moreover, baroreflex control of cardiovagal and sympathetic outflow appears to be impaired in OSA (Carlson *et al.* 1996; Bonsignore *et al.* 2002). Treatment of OSA with night-time continuous positive airway pressure increases cardiovagal baroreflex function during sleep and wakefulness (Bonsignore *et al.* 2002). These data strongly suggest that periods of sleep-disordered breathing, associated with cyclical exposure to asphyxia (hypoxia and hypercapnia), inspiratory resistance, and apnoeas, may be a critical factor underlying changes in autonomic control in OSA. In healthy humans baroreflex function is impaired acutely by increased inspiratory resistance and/or hypoxia and hypercapnia exposure (Cooper *et al.* 2004). These data support the contention that breathing alterations, which mimic those experienced by OSA patients during

sleep, acutely induce baroreflex dysfunction. However, an important limitation of these previous data is that detrimental effects of inspiratory resistance and/or hypoxia and hypercapnia were only assessed *during* application of the stressors. The fact that baroreflex function is depressed in OSA patients, even in the wakeful hours, suggests that the consequences of stress imposed during sleep persist for extended periods of time after resumption of regular breathing.

Accordingly, in the present study we tested the hypothesis that short-term exposure to intermittent hypoxia and apnoeas impairs cardiac and sympathetic baroreflex function. Moreover, we hypothesized that impairment in baroreflex function persists for an extended period of time after resuming normal breathing. To test these hypotheses, baroreflex function was assessed before and for ~1 h after completing a 30 min period of repetitive apnoeas performed during intermittent hypoxia (RHA).

Methods

Subjects

Nineteen young (18–35 years of age) subjects were studied. All were non-obese (body mass index $< 30 \text{ kg m}^{-2}$), normotensive (resting BP $< 140/90$ mmHg), and non-smokers. Subjects were further screened by medical history and physical examination. The experimental protocol was conducted according to the *Declaration of Helsinki* and was approved by the Institutional Review Board at the Pennsylvania State University College of Medicine. Written informed consent was obtained from all subjects.

Measurements

Subjects were studied supine after an overnight fast (12 h).

MSNA. Recordings of MSNA were obtained using a tungsten microelectrode inserted in the peroneal nerve. This electrode was adjusted until a site with clear spontaneously occurring MSNA bursts was established using standard criteria (Vallbo *et al.* 1979). Raw nerve recordings were amplified (20 000–70 000 times), filtered (700–2000 Hz), full-wave rectified, and integrated (0.1 s time constant) to obtain mean voltage neurograms.

Cardiovagal and sympathetic BRS. Cardiovagal and sympathetic BRS were assessed using the modified Oxford technique (Ebert & Cowley, 1992). Briefly, nitroprusside was injected (50–100 μg) intravenously followed 60 s later by phenylephrine (100–150 μg). Drugs were administered at doses that decreased and increased systolic BP ~15 mmHg from baseline levels (Monahan *et al.* 2004; Monahan & Ray, 2005). For nitroprusside these doses were

50 μg in 5 subjects, 75 μg in 5 subjects, and 100 μg in 4 subjects. For phenylephrine, doses were 100 μg in 2 subjects, 125 μg in 8 subjects, and 150 μg in 4 subjects. Drug doses were the same within a given subject before and after the intervention (see below). Data acquisition began 3 min before nitroprusside infusion and continued for 2 min after phenylephrine infusion. At least 15 min separated consecutive BRS trials. Previously we have established that this interval of time is sufficient to allow for reproducible values of BRS as well as to allow for BP and heart rate (HR) at rest to return to pre-infusion levels (Monahan & Ray, 2005). Cardiovagal BRS was assessed in all subjects. However, due to difficulties in obtaining or maintaining satisfactory MSNA recording sites sympathetic BRS was only measured in 10 experimental and 4 control subjects (see below).

Respiration and oxygen saturation. Partial end-tidal CO_2, arterial oxygen saturation (S_{aO_2}; earlobe pulse oximetry) and minute ventilation were measured with a respiratory gas monitor (Ohmeda RGM 5200).

Arterial blood pressure and heart rate. Resting BP was determined using a semi-automated device (Welch Allyn). Continuous measurements of BP were made using a Finapres photoplethysmograph (Ohmeda). HR was determined from the ECG.

Experimental protocol

A schematic diagram of the experimental protocol is provided in Fig. 1. Three BRS trials were performed at baseline (Pre) and three trials were performed after the intervention (Post). Post-intervention measures were made ~7 (Post 1), ~30 (Post 2) and ~50 min (Post 3) after completing the intervention to gain insight into possible temporal patterns of changes in physiological function. BP and HR were determined in triplicate before the first baseline (Pre) trial and each trial after the intervention (Post 1, Post 2 and Post 3). The intervention consisted of a 30 min period in which subjects performed repetitive hypoxic apnoeas (RHA) ($n = 14$) (Leuenberger *et al.* 2005). RHA involved subjects performing repetitive voluntary end-expiratory apnoeas (20 s duration) every minute for 30 min (30 total apnoeas) while breathing through a 2-way non-rebreathing facemask. To enhance oxygen desaturation during apnoeas hypoxic gas (10.5% O_2) was administered during the period of free breathing. The period of time hypoxic gas was inspired during the 40 s free breathing period was individually adjusted in an attempt to achieve post-apnoeic nadirs in S_{aO_2} in the mid to low 80s (%). At the end of each apnoea subjects were instructed to briefly (1 s) exhale to allow measurement of end-tidal CO_2.

Five additional subjects served as time controls. The experimental protocol in these subjects was identical to those exposed to RHA (Experimental) except that during the period of the intervention subjects were asked to rest quietly for 45 min.

Data analysis

All data were digitally stored on a computer (MacLab 8e, ADInstruments) at a sampling frequency of 400 Hz.

MSNA at rest was quantified as bursts min^{-1} and as the sum of the area under individual bursts per minute (au min^{-1}). The largest burst at rest was assigned an arbitrary amplitude of 1000 and a portion of the neurogram in which neural silence (i.e. no efferent discharges) occurred was used to set the baseline to zero.

Cardiovagal BRS was quantified as the slope of the linear portion of the R-R interval–systolic BP relation (over 2 mmHg pressure ranges or 'bins') from the nadir to peak systolic BP response during each BRS trial (Ebert & Cowley, 1992; Rudas *et al.* 1999). Additionally, a measure of HR BRS was obtained from the HR–systolic BP relation. Points clearly falling in either the threshold or saturation region were removed from the analysis (Halliwill & Minson, 2002). Both measures were made to avoid any potential concerns that may be present if resting HR changes. The operating point of the cardiac arm of the baroreflex in relation to brachial systolic BP, HR and R-R interval was determined as the mean values of these variables measured before each BRS trial (Fig. 5).

Sympathetic BRS was quantified as the slope of the linear portion of the MSNA–diastolic BP relation (over 3 mmHg bins) from the start of the nitroprusside-induced decrease to the peak diastolic BP response during the trial (Ebert & Cowley, 1992; Rudas *et al.* 1999). MSNA was quantified as the integrated area underlying a sympathetic burst. Bursts were visually identified after accounting for conduction latency. Any heartbeat not associated with a sympathetic burst was assigned a value of zero and was included in the analysis (Ebert & Cowley, 1992; Rudas *et al.* 1999). Diastolic BP bins with no sympathetic activity were excluded from the analyses (Rudas *et al.* 1999). The operating point of the sympathetic baroreflex in relation to resting brachial diastolic BP and MSNA was determined as the mean values of these variables measured before each BRS trial.

To determine if baroreflex resetting (shift in the stimulus–response relation) occurred (Pre to Post) we calculated predicted HR, R-R interval and MSNA levels relative to an arbitrary pressure from the respective stimulus–response relation (Fig. 5). The arbitrary pressure used was the resting systolic (for HR and R-R interval) and diastolic BP (for MSNA) determined in the baseline period (Pre).

Cardiovagal BRS trials with linear correlation coefficients (r-value) > 0.70 (Smyth *et al.* 1969; Rudas *et al.* 1999) during the Pre measures were averaged (up to 3) and a single value is reported. Individual trials after the intervention (Post 1, Post 2 and Post 3) were compared on a trial-to-trial basis to baseline levels (Pre) to gain insight into possible temporal patterns of change. The same process occurred for sympathetic BRS except that the criterion for retaining a sympathetic BRS value was an *r* value > 0.50 (Carlson *et al.* 1996; Rudas *et al.* 1999).
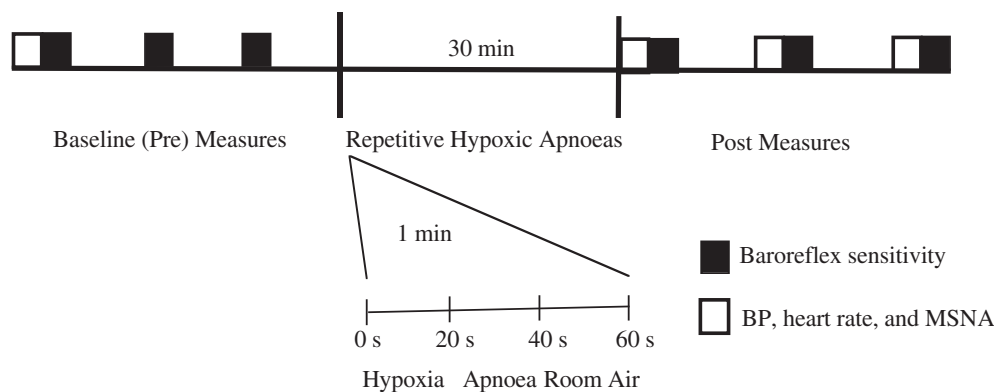

Figure 1. Schematic diagram of the protocol in experimental subjects
Baroreflex sensitivity (BRS) was measured in triplicate at baseline (Pre) and after (Post) the intervention. The intervention consisted of 30 consecutive apnoeas performed during intermittent exposure to hypoxia (10.5% oxygen). The length of intermittent hypoxia exposure before each apnoea was adjusted so that arterial oxygen saturations decreased into the mid to low 80s (%). Apnoeas were voluntarily performed at end-expiration and lasted 20 s. At least 15 min separated each BRS trial. Post measurements of BRS were made at time points corresponding to ~7, ~30 and ~50 min after completion of repetitive hypoxic apnoeas (RHA). Blood pressure (BP), heart rate and muscle sympathetic nerve activity (MSNA) at rest were measured before the first baseline (Pre) trial and before each of the 3 trials performed after RHA. In 5 control subjects the above protocol was followed identically except subjects were not exposed to RHA. The intervention in this group was a 45 min period of rest.

Table 1. Subject characteristics

Variable	Control ($n = 5$)	Experimental ($n = 14$)
Sex	3 male/2 female	7 male/7 female
Age (years)	25 ± 2	26 ± 1
Height (cm)	177.8 ± 4.3	172.8 ± 2.6
Body mass (kg)	77.3 ± 5.8	71.9 ± 4.7
BMI (kg m^{-2})	24.3 ± 1.4	23.8 ± 1.1
Systolic BP (mmHg)	117 ± 3	114 ± 3
Diastolic BP (mmHg)	68 ± 3	69 ± 1
Heart rate (beats min^{-1})	62 ± 3	62 ± 2

Values are mean $\pm$ s.e.m. BMI, body mass index; BP, blood pressure.

An investigator blinded to the experimental condition performed all analyses.

Statistical analysis

Differences in baseline subject characteristics were determined by *t* test. Responses to the intervention were determined using a linear mixed effects model (one-way repeated measures ANOVA). When significant main effects were identified, specific contrasts were then made using Dunnett's *post hoc* tests. Corrections for multiple comparisons were made. Statistical significance was established at $P < 0.05$.

Results

Subject characteristics

Subject characteristics are presented in Table 1.

Effect of apnoeas performed during intermittent hypoxia on S_{aO_2} and end-tidal CO_2

Repetitive apnoeas performed during intermittent hypoxia induced cyclical changes in S_{aO_2} from immediately before apnoea to shortly after the end of apnoea (98.0 ± 0.2 to $83.0 \pm 1.0\%$; $P < 0.05$) (Fig. 2). Additionally, apnoeas
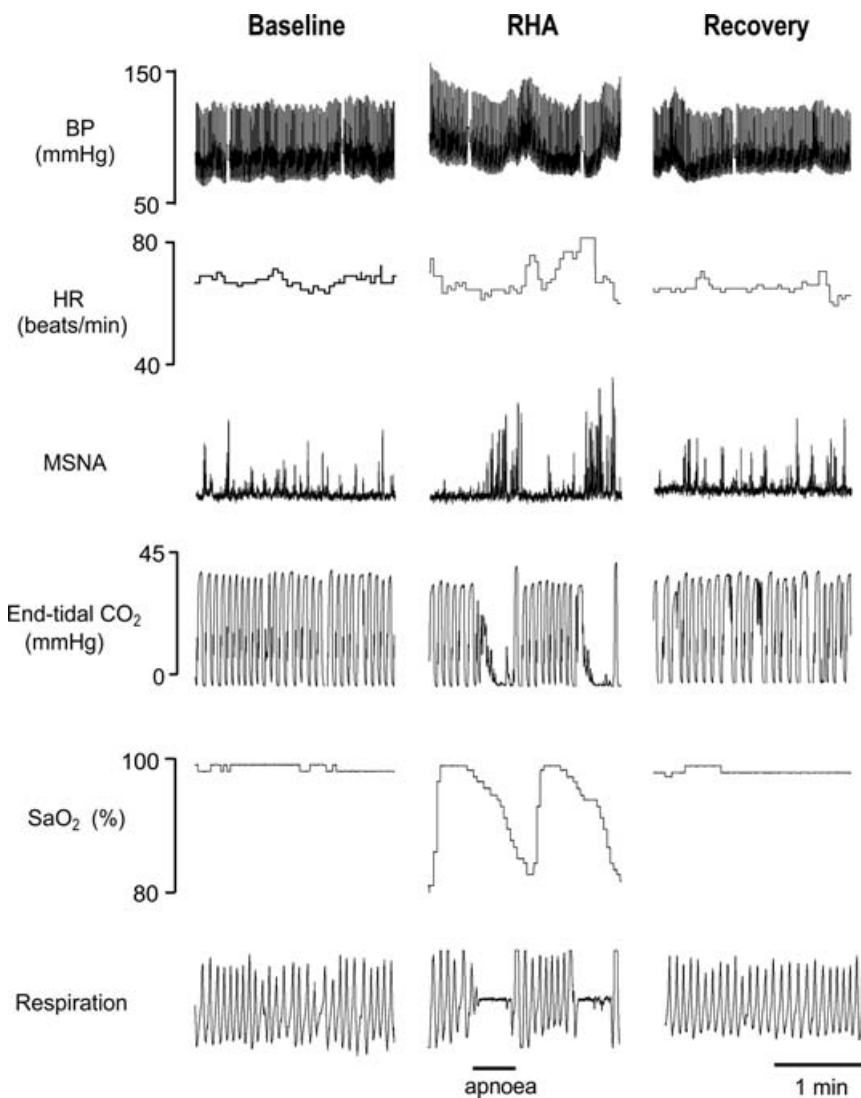

Figure 2. Representative responses to the repetitive hypoxic apnoea (RHA) protocol
Representative recording of BP, heart rate (HR), MSNA, end-tidal CO_2, arterial oxygen saturation (S_{aO_2}) and respiration before (Baseline), during, and after (Recovery) a 30 min period of RHA in a subject in the Experimental group. Segments presented during each phase of the study (Baseline, RHA and Recovery) are 2 min in duration. Performing individual apnoeas (20 s each) during intermittent hypoxia exposure resulted in cyclical changes in S_{aO_2} (from ~98% to ~83%) and surges in BP and MSNA.

increased end-tidal CO_2 from immediately before apnoea to the peak increase after the end of apnoea (35.0 ± 0.4 to 41.0 ± 0.4 mmHg; $P < 0.05$) (Fig. 2). Minute ventilations did not differ in the $\sim$5 min period before ($7.3 \pm 0.4\,\mathrm{l\,min^{-1}}$) and after ($7.3 \pm 0.3\,\mathrm{l\,min^{-1}}$) RHA.

Effect of RHA on BP, HR and MSNA at rest

Systolic, diastolic and mean BP increased from baseline (Pre) levels after RHA. These increases persisted throughout the Post period ($\sim$1 h) (Fig. 3). Similarly MSNA measured at rest increased from baseline (Pre) levels after RHA. These increases persisted throughout the Post period (Fig. 3). HR was unchanged from baseline (Pre) levels after RHA (Fig. 3).

Effect of RHA on baroreflex function

The effects of RHA on indices of BRS are presented in Fig. 4. Cardiovagal BRS was unchanged from baseline (Pre) levels after RHA (16.8 ± 1.3, 16.5 ± 1.6, 17.6 ± 2.0 and 17.4 ± 1.5 ms mmHg^{-1} for Pre, Post 1, Post 2, and Post 3, respectively; $P = 0.92$). Similar responses were noted for both HR BRS (-1.1 ± 0.1, -1.1 ± 0.1, -1.3 ± 0.1 and -1.4 ± 0.1 beats min^{-1} mmHg^{-1}; $P = 0.34$) as well as sympathetic BRS (-4.5 ± 0.6, -4.4 ± 0.7, -3.7 ± 0.5 and -4.7 ± 1.0 au beat^{-1} mmHg^{-1}; $P = 0.20$). In contrast the stimulus–response relations for all measures of

baroreflex function were shifted rightward and upward (HR and sympathetic BRS) to higher levels of BP by RHA suggesting that RHA reset the baroreflexes. These shifts are visually apparent in the group-averaged stimulus–response relations (Fig. 5). To quantify the magnitude of resetting that occurred we calculated HR, R-R interval and MSNA levels at identical levels of systolic (HR and R-R interval) and diastolic (MSNA) BP Pre and Post. The BP level was the systolic or diastolic BP measured at baseline (Pre). These analyses indicated that HR (64 ± 4, 75 ± 5, $79 \pm 5^*$ and $78 \pm 4^*$ beats min^{-1} for Pre, Post 1, Post 2 and Post 3, respectively; $^*P < 0.05$ compared to Pre) and MSNA (1783 ± 378, 2701 ± 544, $4470 \pm 810^*$ and $4153 \pm 1025^*$ au min^{-1}) increased (main effect $P < 0.05$) and R-R interval (959 ± 61, 848 ± 56, $763 \pm 42^*$ and $784 \pm 59^*$ ms) decreased (main effect $P < 0.05$) at a given pressure after RHA.

The ranges over which baroreflex function were determined Pre and Post were slightly greater after RHA ($\Delta 39 \pm 3$ compared to $\Delta 44 \pm 3$ mmHg for Pre and Post, respectively; $P < 0.05$). This difference appears to be the result of a greater decrease in BP after nitroprusside ($\Delta 14 \pm 1$ compared to $\Delta 21 \pm 2$ mmHg; $P < 0.05$) rather than a greater increase in BP after phenylephrine infusion ($\Delta 26 \pm 3$ compared to $\Delta 24 \pm 3$ mmHg; $P = 0.35$). In contrast the rate of change in BP from the nadir to peak change in BP during the BRS trials was identical Pre and Post (0.66 ± 0.06 compared to 0.66 ± 0.09 mmHg s^{-1}; $P = 0.96$).
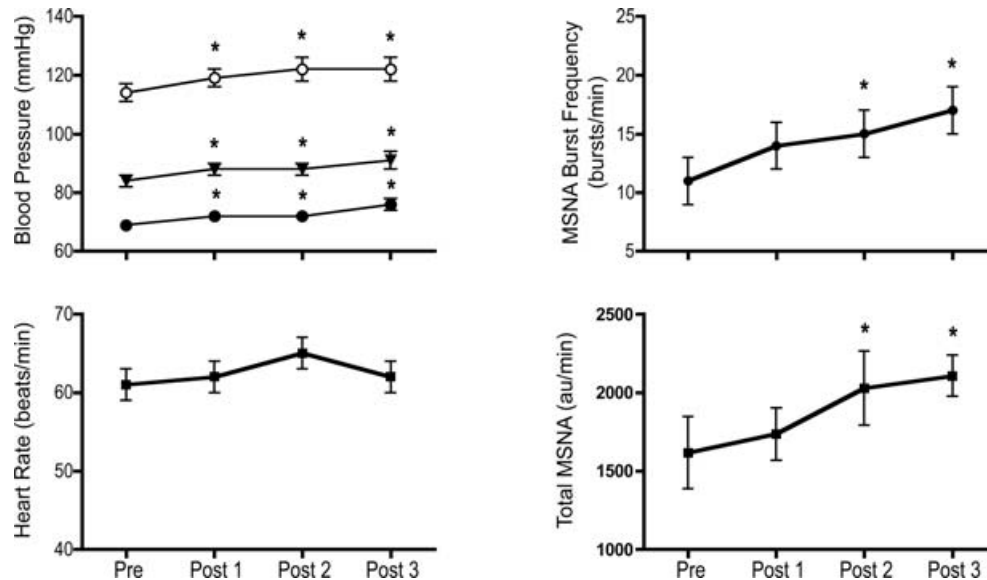

Figure 3. Effect of the intervention on hemodynamics and muscle sympathetic nerve activity (MSNA) at rest
Blood pressure, heart rate, MSNA burst frequency (bursts min^{-1}) and total MSNA (sum of area under individuals' MSNA bursts expressed in au min^{-1}) measured at rest at baseline (Pre) and before each of the 3 baroreflex trials measured after completion of the repetitive hypoxic apnoea protocol (Post 1, Post 2 and Post 3). Systolic (○), diastolic (●) and mean BP (▼), and MSNA, increased after RHA (* $P < 0.05$).

Time control studies

Haemodynamics, MSNA and BRS (cardiovagal, HR and sympathetic) were stable over the duration of the study in control subjects (Table 2).

Discussion

The primary new finding from this study is that short-term exposure to repetitive apnoeas performed during intermittent hypoxia does not impair cardiovagal or sympathetic BRS, but appears to reset the baroreflex stimulus–response curve rightward (to higher levels of BP) and upward (for HR and MSNA). This resetting may help explain why the body does not appear to resist RHA-induced increases in BP by inhibiting sympathetic outflow or decreasing HR.

   The RHA protocol used in the present study provides a complex and variable stimulus to subjects, which acutely mimics the breathing patterns that occur during sleep in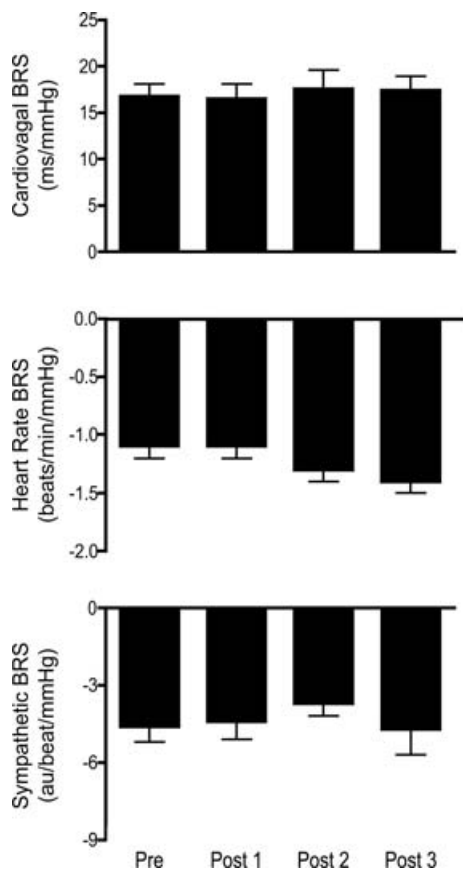 patients with OSA. These stimuli variably include periods of asphyxia, hyperpnoea, hypopnoea, and apnoea. Previous studies in healthy humans have used a similar RHA protocol to acutely study changes in cardio-vascular/autonomic function in humans. These studies have reported that RHA increases MSNA (Cutler *et al.* 2004*b*; Leuenberger *et al.* 2005), BP (Leuenberger *et al.* 2005) and chemoreflex sensitivity (Cutler *et al.* 2004*a*) at
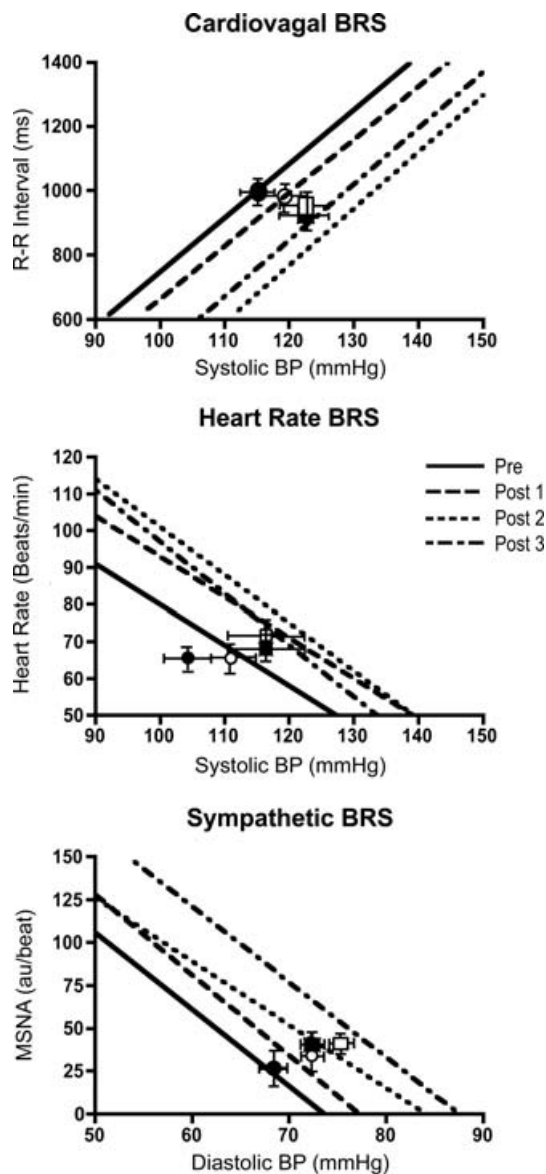

Figure 5. Group average regressions between R-R interval and heart rate *versus* systolic BP and between MSNA and diastolic BP in Experimental group subjects
Operating points are indicated by symbols (●, Pre; ○, Post 1; ■, Post 2; and □, Post 3) and error bars. Note the similar responses, as evidenced by the slope of the respective regressions that were observed before (Pre) and after (Post 1, Post 2, and Post 3) the 30 min period of RHA for each expression of BRS (see also Fig. 4). In contrast the regression lines and operating points are shifted rightward and upward (for heart rate and MSNA) towards higher pressure.

Figure 4. Effects on baroreflex sensitivity (BRS)
Cardiovagal, heart rate and sympathetic BRS at baseline (Pre) and after (Post 1, Post 2, and Post 3) completion of 30 consecutive 20 s apnoeas performed during intermittent hypoxia (RHA). After RHA there were no changes in any of the respective expressions of BRS.

Table 2. Response to intervention

	Pre	Post		
Variable	Baseline	Post 1	Post 2	Post 3
Control subjects (n = 5)				
Systolic BP (mmHg)	118 ± 3	119 ± 2	119 ± 2	119 ± 3
Diastolic BP (mmHg)	68 ± 3	69 ± 3	68 ± 3	70 ± 3
Mean BP (mmHg)	85 ± 3	86 ± 2	85 ± 3	86 ± 3
Heart rate (beats min^{-1})	62 ± 3	62 ± 2	61 ± 2	62 ± 3
MSNA (bursts min^{-1})	17 ± 3	15 ± 3	15 ± 2	14 ± 3
Cardiovagal BRS (ms mmHg^{-1})	15.6 ± 1.3	16.7 ± 4.0	17.9 ± 2.6	15.0 ± 0.09
Sympathetic BRS (au beat^{-1} mmHg^{-1})	−3.7 ± 0.2	−3.8 ± 0.3	−3.6 ± 0.4	−4.0 ± 0.3

Values are mean ± S.E.M. BP, blood pressure; MSNA, muscle sympathetic nerve activity; BRS, baroreflex sensitivity; au, arbitrary units.

rest for extended periods of time (up to 2 h) after RHA. These data establish that RHA is a powerful model to: (1) acutely induce changes in autonomic/cardiovascular function, (2) acutely study the effects of altered breathing on autonomic control in humans, and (3) gain insight into the temporal pattern of changes in physiological function by obtaining multiple measurements after resumption of regular breathing.

Our finding of no change in cardiovagal or sympathetic BRS, but a rightward and upward shift in the operating point of the baroreflexes (for HR and MSNA) *after* RHA is similar to those effects observed *during* acute continuous systemic hypoxia. Specifically, it has been reported that cardiovagal and sympathetic BRS are unaltered by short-term (18 min) continuous systemic hypoxia, but that the baroreflexes are reset to operate around a higher baseline level of BP (Halliwill & Minson, 2002; Halliwill *et al.* 2003). Thus, for a given level of BP both HR and MSNA are elevated by hypoxia. In the present study in addition to exposing individuals to systemic hypoxia (intermittently), repetitive apnoeas (30 in total) were performed. This protocol unlike continuous systemic hypoxia induces cyclical changes in arterial blood oxygenation (cycling saturation/desaturation). Thus, RHA (30 min) may be a more physiologically stressful stimulus than a shorter period of continuous systemic hypoxia (achieving similar decreases in S_{aO_2}). This factor may help explain how RHA induced prolonged periods of baroreflex resetting that persisted beyond the period of RHA. However, we cannot exclude the possibility that hypoxia exposure alone does not elicit prolonged periods of baroreflex resetting as Halliwill *et al.* did not make any measurements after removal of the hypoxic stimulus (Halliwill & Minson, 2002; Halliwill *et al.* 2003). However, as MSNA may remain elevated for extended periods of time after continuous systemic hypoxia exposure (20 min) (Morgan *et al.* 1995; Tamisier *et al.* 2004) we cannot rule out that the resetting persisted.

Previous studies in humans have examined the possible influences that inspiratory resistance, hypoxia, hypercapnia and apnoea acutely exert on baroreflex function. Recently, Cooper and colleagues reported that inspiratory resistance decreased the gain of baroreflex control of vascular resistance and that hypoxia exposure shifted the stimulus–response curve rightward suggesting resetting (Cooper *et al.* 2004). When both physiological stressors were applied simultaneously, baroreflex gain was reduced and the stimulus–response curve was displaced rightward to higher pressures. These data strongly suggest that increased inspiratory resistance and hypoxia alter baroreflex function (impaired BRS as well as resetting the carotid baroreflex). The effects of apnoea on baroreflex function are less clear. A *single* apnoea has been shown not to alter baroreflex control of MSNA during application of neck suction (Muenter Swift *et al.* 2003). However, after completion of the apnoea there was a very transient (< 1 min) impairment in baroreflex control of sympathoexcitation during neck pressure. Collectively, our present findings importantly extend these prior findings by showing that changes in baroreflex function that occur during stressors designed to mimic OSA can induce persistent changes in baroreflex function that extend beyond the exposure to the stressors (intermittent hypoxia, hypercapnia and apnoea).

Most studies that have assessed baroreflex function in humans with OSA have concluded that baroreflex dysfunction occurs. Specifically, cardiovagal BRS has been shown in most (Carlson *et al.* 1996; Bonsignore *et al.* 2002), but not all studies (Narkiewicz *et al.* 1998) to be depressed in OSA. Additionally, baroreflex control of MSNA (i.e. sympathetic BRS) during decreases in BP are blunted in OSA (Carlson *et al.* 1996; Narkiewicz *et al.* 1998). Our present study does not support a role for *short-term* periods of altered breathing (repetitive apnoeas performed during intermittent hypoxia exposure) promoting decreases in cardiovagal or sympathetic BRS. However, our data do

suggest that the operating points of both the cardio-vagal and sympathetic baroreflex are reset to higher levels of BP, HR and MSNA. The resetting of the baroreflex to higher levels of BP is a characteristic feature of hypertension (McCubbin *et al.* 1956). It is unknown how long this resetting persisted in our study as we only acquired measurements for ~1 h after RHA. However, it is possible that even short-term shifts to higher levels of BP may be of physiological relevance. Over time these repetitive exposures to acute hypertension may predispose even normotensive OSA patients to the development of hypertension. This suggestion is supported by data suggesting that OSA may precede the development of chronic hypertension (Peppard *et al.* 2000).

Several limitations are associated with the present study. First, our RHA intervention exposed individuals to only 30 apnoeas performed during intermittent hypoxia. Thus, the level of stress exerted on our subjects during RHA was probably less than that exerted on an OSA patient during an entire nights sleep. Therefore, we cannot exclude the possibility that longer periods of RHA would depress BRS. Second, in our experience the modified Oxford technique does not allow full characterization of the baroreflex stimulus–response curve. This has been reported by others (Halliwill & Minson, 2002). Thus, we cannot determine with certainty if the set point of the baroreflex stimulus–response curve was altered, although our data relating to changes in the operating point pressure, MSNA and HR do suggest that this is the case. This is an inherent limitation in the methods available to assess *integrated* baroreflex responses in intact humans safely. Lastly, measurements were made in healthy young adults. It is possible that different effects would be observed in other populations.

In conclusion, these data suggest that short-term (30 min) exposure to repetitive apnoeas (single 20 s apnoea every minute) performed during intermittent hypoxia does not impair cardiovagal or sympathetic BRS in healthy young humans, but does appear to reset the baroreflexes to operate around a new higher level of BP at rest. This resetting is evident by a rightward (to higher BP) and upward shift (for HR and MSNA) in the stimulus–response curve. Thus both HR and MSNA are increased at a given level of BP after RHA.

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