Interactions between breathing rate and low-frequency fluctuations in blood pressure and cardiac intervals

H. M. Horsman, K. C. Peebles, and Y. C. Tzeng

1Cardiovascular Systems Laboratory and 2Centre for Translational Physiology, University of Otago, Dunedin, New Zealand; and 3Department of Physiology, University of Otago, Wellington, New Zealand; and 4Department of Health Professions, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia

Submitted 22 June 2015; accepted in final form 22 July 2015

The cardiovagal baroreflex plays an important role in the dynamic regulation of blood pressure via reflex changes in heart rate (6). Reduced cardiovagal baroreflex sensitivity (BRS) is associated with increased risk and poor prognosis in a range of cardiovascular diseases including hypertension, chronic heart failure, and stroke (16, 18). Consequently, there has been considerable interest in identifying and adapting methods that can be clinically applied to preserve or restore BRS (1, 2, 14). To this end, slow breathing at 6 breaths/min augments BRS. However, increases in BRS associated with slow breathing may simply reflect the frequency-dependent nature of the baroreflex rather than the modulation of baroreflex function by changes in breathing rate per se. To test this hypothesis we employed a crossover study design (n = 14) wherein breathing rate and systolic arterial blood pressure (SAP) oscillation induced via the application of oscillating lower body negative pressure (OLBNP) were independently varied at fixed frequencies. Breathing rate was controlled at 6 or 10 breaths/min with the aid of a metronome, and SAP oscillations were driven at 0.06 Hz and 0.1 Hz using OLBNP. The magnitudes of SAP and R-R interval (cardiac period) oscillations were quantified using power spectral analysis, and the transfer function gain between SAP and R-R interval was used to estimate BRS. Linear mixed-effects models were used to examine the main effects and interactions between breathing rate and OLBNP frequency. There was no statistical interaction between breathing and OLBNP frequency (P = 0.59), indicating that the effect of breathing rate on BRS did not differ according to OLBNP frequency (and vice versa). Additionally, there was no main effect for breathing rate (P = 0.28). However, we observed a significant main effect for OLBNP frequency (P = 0.01) consistent with the frequency-dependent nature of baroreflex. These findings suggest that increases in spectral indices of BRS reflect the frequency dependence of the baroreflex and are not due to slow breathing per se.

One potential explanation that has not been fully examined relates to the frequency-dependent nature of the baroreflex. The baroreflex is sensitive to both magnitude and rate of BP change (3), and BRS assessed from systolic arterial blood pressure oscillations (SAP) driven by repeated squatsit-to-stand maneuvers, has been shown to exhibit frequency-dependent characteristics (12, 30). A known disadvantage of the modified Oxford method is that it affords no control over the time course of the rise and fall of BP, and therefore the method cannot account for the frequency-dependent effects of the baroreflex response. In light of this consideration, we considered the possibility that increases in spontaneous measures of BRS assessed at slow breathing frequencies may actually reflect the frequency-dependent nature of the baroreflex rather than a global enhancement of baroreflex function per se.

To explore this possibility, we propose a crossover-design experiment in which the effects of breathing rate on BRS can be isolated from the frequency-dependent nature of the baroreflex. For this purpose we applied oscillating lower body negative pressure (OLBNP) to generate controlled SAP perturbations at two distinct frequencies (0.1 Hz and 0.06 Hz), and calculated the transfer function gain between SAP and R-R interval fluctuations to derive frequency-dependent estimates of BRS. At each OLBNP frequency, participants were instructed to pace breathe at 6 breaths/min (0.1 Hz) or at 10 breaths/min (0.16 Hz), in random sequence. Data were then tested for two hypothetical propositions using linear mixed-effects models. First, we argued that if slow breathing at 0.1 Hz were able to globally enhance baroreflex function, then slow breathing should increase BRS at both OLBNP frequencies. Conversely, if increases in BRS associated with 0.1 Hz breathing were due in part to the frequency of blood pressure oscillations, then BRS would be greater at 0.1 Hz OLBNP regardless of breathing rate.

METHODS

Participants. Fourteen healthy participants (7 male, aged 25 ± 6 years, body mass index 23 ± 3 kg·m⁻²) were recruited. All participants were nonsmokers and free from respiratory, cardiovascular, neurological, and endocrine disease. Females were not pregnant and were in...
the early follicular phase of their menstrual cycle or in their contra-
ceptively pill-free days at the time of the study. This study was approved by the Central Regional Ethics Committee and conformed to the standards set by the Declaration of Helsinki. All participants gave written informed consent.

Instrumentation. Heart rate was recorded using a three-lead ECG, (AD Instruments, Colorado Springs, CO). Estimates of SAP, diastolic arterial pressure (DAP), and mean arterial pressure (MAP) were made using finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). The finometer cuff was positioned on the middle finger and referenced to heart level. Finger photoplethysmography BP measurements were cross-referenced immediately prior to each experimental protocol, against manual oscillometric BP measurements (Seimens Electronics, United Kingdom). An infrared CO₂ gas analyzer (model ML206; AD Instruments) measured the partial pressure of end-tidal CO₂ (PtETCO₂) sampled from a nasal cannula. Data signals were captured at 1 kHz using an analog-to-digital converter (Powerlab/16SP ML795; AD Instruments), and offline analysis was performed using custom written software (LabView 8.2; National Instruments, Austin, TX).

Experimental design. All studies commenced at 9:00 AM, in a temperature-controlled (≈22°C) laboratory. Each participant was as-
sessed to confirm they had: (i) consumed a light breakfast (e.g., cereal or toast) at least 1 h earlier and were normally hydrated; (ii) refrained from strenuous exercise for at least 24 h; and (iii) abstained from caffeine and alcohol intake for at least 12 h prior to testing. After instrumentation, 10 min was allowed for acclimatization. BRS was then assessed at two different breathing frequencies (6 and 10 breaths/ 
minute) during two different OLBNP frequencies (0.06 Hz and 0.1 Hz). A 5-min recovery period was allowed between each trial. The order of breathing rate and OLBNP frequency was randomized, and all participants completed the entire protocol within 3 h. The slow breathing rate of 6 breaths/min, and OLBNP frequency of 0.1 Hz, were selected because previous studies have shown this is the frequency at which the greatest augmentation of BRS occurs. The upper limit of frequency of 10 breaths/min (0.16 Hz) was chosen, as it represents a physiologically relevant breathing rate, and the second OLBNP frequency (0.06 Hz) was chosen to be clearly distinct from respiratory frequencies but still within timescales that approximate orthostatic challenges.

OLBNP. Participants lay supine with their lower body (up to their iliac crest) sealed in the chamber. Fifteen oscillations/cycle of moderate LBNP (~40 mmHg) were applied at two frequencies in random order: (i) 0.06 Hz (for 15 cycles), and (ii) 0.1 Hz (for 15 cycles). Each OLBNP frequency was run twice, once while breathing was controlled at 6 breaths/min, and once while breathing was controlled at 10 breaths/min. An electronic metronome was used to guide the participant to breathe at the correct frequency. Participants were instructed to breathe as normally as possible during OLBNP and to minimize breath holding. They were allowed to adjust their tidal volume to remain comfortable. PtETCO₂ levels were monitored to ensure they remained close to baseline levels.

Data analysis. All data analysis was conducted using custom written software in LabView 8.2 (National Instruments).

Spectral and transfer function analysis. BRS was estimated from the transfer function between SAP and R-R interval oscillations evoked by OLBNP (13, 23). Briefly, ECG and SAP recordings were visually checked for the presence of artifacts, and any erroneously detected or missed R waves were corrected by linear interpolation. For all data, SAP and R-R interval series were filtered, resampled at 4 Hz, and then linearly detrended. These series then passed through a Hanning window and underwent fast Fourier Transformation. The power spectral density (PSD) was calculated for SAP and R-R intervals (SAP PSD and R-R interval PSD, respectively) at each OLBNP frequency. The SAP and R-R interval PSDs reflect the variability of SAP and R-R interval oscillations. Transfer function gain, phase, and coherence were derived from the cross-spectrum of the SAP signal (input) and R-R interval (output). Transfer function gain represents the change in output for a given change in input and reflects BRS (13, 23). Transfer function phase represents the lag between input and output signals and is representative of the physiological latency of the baroreflex response. Transfer function coherence represents the correlation between the input and output signals and indicates the precision of the transfer function.

Statistical analysis. Changes in heart rate, R-R interval, SAP, DAP, MAP, and PtETCO₂, during each condition, were examined using a one-way repeated-measures ANOVA (Greenhouse-Geisser corrected). A Bonferroni-Dunn test was used for post hoc analysis if a significant main effect was found. A priori defined comparisons examining the main effects and interactions between OLBNP (0.06 Hz vs. 0.1 Hz) and breathing rate (6 breaths/min vs. 10 breaths/min) for PSD and BRS were assessed using linear mixed-effects models. To account for the precision of transfer function gain measurements, all statistical tests of main effects and interactions were weighted for coherence. Where a significant interaction between OLBNP frequency vs. breathing rate was detected, paired t-tests (Sidak corrected) were used to compare the breathing rate and OLBNP frequency. All analyses were conducted using SPSS 20 (SPSS). Data are presented as means ± SD, except for inferential statistics associated with linear mixed-effects models (specified in text), which are presented as means ± SE. Significance was defined at an alpha level of P < 0.05 for all comparisons.

RESULTS

Data analysis. All data analysis was conducted using custom written software in LabView 8.2 (National Instruments).

Spectral and transfer function analysis. BRS was estimated from the transfer function between SAP and R-R interval oscillations evoked by OLBNP (13, 23). Briefly, ECG and SAP recordings were visually checked for the presence of artifacts, and any erroneously detected or missed R waves were corrected by linear interpolation. For all data, SAP and R-R interval series were filtered, resampled at 4 Hz, and then linearly detrended. These series then passed through a Hanning window and underwent fast Fourier Transformation. The power spectral density (PSD) was calculated for SAP and R-R intervals (SAP PSD and R-R interval PSD, respectively) at each OLBNP frequency. The SAP and R-R interval PSDs reflect the variability of SAP and R-R interval oscillations. Transfer function gain, phase, and coherence were derived from the cross-spectrum of the SAP signal (input) and R-R interval (output). Transfer function gain represents the change in output for a given change in input and reflects BRS (13, 23). Transfer function phase represents the lag between input and output signals and is representative of the physiological latency of the baroreflex response. Transfer function coherence represents the correlation between the input and output signals and indicates the precision of the transfer function.

Statistical analysis. Changes in heart rate, R-R interval, SAP, DAP, MAP, and PtETCO₂, during each condition, were examined using a one-way repeated-measures ANOVA (Greenhouse-Geisser corrected). A Bonferroni-Dunn test was used for post hoc analysis if a significant main effect was found. A priori defined comparisons examining the main effects and interactions between OLBNP (0.06 Hz vs. 0.1 Hz) and breathing rate (6 breaths/min vs. 10 breaths/min) for PSD and BRS were assessed using linear mixed-effects models. To account for the precision of transfer function gain measurements, all statistical tests of main effects and interactions were weighted for coherence. Where a significant interaction between OLBNP frequency vs. breathing rate was detected, paired t-tests (Sidak corrected) were used to compare the breathing rate and OLBNP frequency. All analyses were conducted using SPSS 20 (SPSS). Data are presented as means ± SD, except for inferential statistics associated with linear mixed-effects models (specified in text), which are presented as means ± SE. Significance was defined at an alpha level of P < 0.05 for all comparisons.

RESULTS

All participants completed the study, were able to follow the breathing protocols, and contributed data to the analysis. Table 1 shows that heart rate, R-R interval, SAP, and MAP did not differ across each of the study conditions. However, compared with baseline DAP, values were slightly higher when subjects were breathing at 6 breaths/min under both the 0.06 Hz and 0.1 Hz OLBNP conditions. Additionally, PtETCO₂ was marginally

Table 1. Cardiovascular parameters during controlled breathing and OLBNP

<table>
<thead>
<tr>
<th>Cardiovascular Parameters</th>
<th>Baseline</th>
<th>6 Breaths/min</th>
<th>10 Breaths/min</th>
<th>6 Breaths/min</th>
<th>10 Breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>62 ± 6.0</td>
<td>63 ± 5.5</td>
<td>63 ± 5.4</td>
<td>63 ± 5.4</td>
<td>63 ± 5.0</td>
</tr>
<tr>
<td>R-R interval, s</td>
<td>0.98 ± 0.1</td>
<td>0.96 ± 0.08</td>
<td>0.97 ± 0.08</td>
<td>0.97 ± 0.08</td>
<td>0.96 ± 0.08</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>107 ± 14</td>
<td>117 ± 15</td>
<td>113 ± 17</td>
<td>118 ± 17</td>
<td>116 ± 18</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>86 ± 7</td>
<td>93 ± 10</td>
<td>91 ± 11</td>
<td>94 ± 11</td>
<td>92 ± 13</td>
</tr>
<tr>
<td>DAP, mmHg</td>
<td>67 ± 7</td>
<td>73 ± 10*</td>
<td>71 ± 10</td>
<td>74 ± 10*</td>
<td>72 ± 12</td>
</tr>
<tr>
<td>PtETCO₂, mmHg</td>
<td>38 ± 2.9</td>
<td>36 ± 2.9</td>
<td>34 ± 2.8</td>
<td>37 ± 3.5†</td>
<td>35 ± 3.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. OLBNP, oscillating lower body negative pressure; SAP, systolic arterial pressure; MAP, mean arterial pressure; DAP, diastolic arterial pressure; PtETCO₂, partial pressure of end-tidal carbon dioxide. *P < 0.05 vs. baseline; †P < 0.05 vs. 0.06 Hz at 10 breaths/min and 0.1 Hz at 10 breaths/min.

J Appl Physiol • doi:10.1152/japplphysiol.00525.2015 • www.jappl.org
higher while breathing at 6 breaths/min during 0.1 Hz OLBNP, although the absolute values were still within normal physiological limits.

Figure 1 shows an example of the SAP PSD and R-R interval PSD traces during each condition, in 1 individual. Irrespective of OLBNP frequency, slow breathing at 6 vs. 10 breaths/min increased SAP and R-R interval PSD. Figure 1, A (0.06 Hz OLBNP during 6 breaths/min), B (0.06 Hz OLBNP during 10 breaths/min), and D (0.1 Hz OLBNP during 10 breaths/min), show two distinct peaks. These peaks show SAP PSD (left) and R-R interval PSD (right) focused at i) the OLBNP frequency (dashed lines), and ii) the breathing rate (dotted lines). In contrast, Fig. 1C shows a single dominant peak centered at 0.1 Hz (dashed-dotted lines), where the SAP and R-R interval spectral components at the OLBNP and breathing frequencies are synchronized at 0.1 Hz.

Table 2 shows the effect of breathing and OLBNP frequency on SAP PSD and R-R interval PSD, calculated at the OLBNP frequencies. An interaction was observed for SAP PSD, indicating that the effect of breathing on SAP PSD varied according to the OLBNP frequency. Specifically, SAP PSD was enhanced when participants breathed at 6 vs. 10 breaths/min during 0.1 Hz OLBNP. However, there were no differences in SAP PSD between breathing rates during 0.06 Hz OLBNP. There was no interaction for R-R interval PSD, indicating that the effect of breathing on R-R interval PSD was the same at each OLBNP frequency. Specifically, the R-R interval PSD was greater at 6 vs. 10 breaths/min, regardless of OLBNP frequency.

Fig. 1. Example of systolic arterial blood pressure (SAP) power spectral density (PSD; left) and cardiac period (R-R interval) PSD (right) traces during each condition, from one individual. A: 0.06 Hz oscillating lower body negative pressure (OLBNP) during 6 breaths/min. B: 0.06 Hz OLBNP during 10 breaths/min. C: 0.1 Hz OLBNP during 6 breaths/min. D: 0.1 Hz OLBNP during 10 breaths/min. Broken lines indicate the frequency components at each OLBNP frequency (dashed lines) and each breathing rate (dotted lines), except during 0.1 Hz OLBNP and 6 breaths/min (dashed-dotted lines), where the OLBNP and breathing rate components are synchronized.
DISCUSSION

Main findings. We sought to determine whether the apparent BRS augmentation associated with slow breathing is due to global enhancement of BRS or a product of the frequency-dependent nature of the baroreflex. While we found that slow breathing increased SAP PSD and R-R interval PSD at 0.06 Hz and 0.1 Hz OLBNP, we did not observe consistent increases in BRS with slow breathing. Rather, we found that BRS was consistently higher at 0.1 Hz than 0.06 Hz OLBNP, irrespective of breathing rate. Taken together, these findings suggest that apparent improvements in BRS associated with slow breathing may simply reflect the frequency of blood pressure oscillations (i.e., the frequency-dependent nature of the baroreflex). While these results do not preclude the possible benefits of slow breathing in reducing sympathetic activity and BP (8, 14), they do challenge the notion that slow breathing per se enhances BRS.

Comparison to previous studies. Previous studies have shown that slow breathing augments spontaneous measures of BRS, and this has led to the suggestion that a simple breathing technique could offer therapeutic benefits in patients with cardiovascular disease such as hypertension (1, 2, 14). However, our findings do not support these conclusions and suggest that at least two potential confounders warrant consideration. First, previous studies have largely been based on spontaneous indices of BRS, whereas our study derived BRS from experimentally driven oscillations of SAP at specific frequencies. Spontaneous assessment of BRS at the breathing frequency is controversial (9, 19, 20), and it remains unclear whether BP and R-R interval fluctuations coincident with respiration are linked by the baroreflex or arise from a central neural mechanism (4, 20, 26). Second, when spontaneous methods are conducted at the breathing frequency, it is not possible to uncouple the effects of breathing rate on BRS from the effects of BP oscillation frequency.

Mindful of this, the present study took an alternative approach of examining the influence of baroreflex frequency dependence during slow breathing. As in previous studies (1, 2, 14), breathing rate was controlled, but in addition the application of OLBNP was used to drive BP oscillation at specific frequencies, discrete from the prevailing breathing frequency. The application of lower body negative pressure causes blood to pool in the lower limbs, evoking a fall in central blood volume, a fall in venous return, and ultimately a decrease in BP, which in turn unloads the baroreceptors (11, 25). By introducing controlled fluctuations in the chamber pressure, the resultant BP oscillation can be uncoupled from the effects of breathing to determine whether i) slow breathing augmented baroreflex function globally, regardless of the frequency of BP oscillations at which BRS was assessed; ii) the increase in BRS associated with slow breathing was due to the frequency of BP oscillations.

The influence of breathing rate and OLBNP on cardiovascular oscillations and BRS. Spectral analyses of SAP PSD showed an interaction between breathing rate and OLBNP frequency, where a significant increase in SAP variability was only observed when breathing rate and OLBNP frequency were synchronized at 0.1 Hz. These changes suggest that the effects of OLBNP and breathing activity on cardiac output and
SAP are likely additive. Corresponding analysis of R-R interval PSD showed that slow breathing increased R-R variability regardless of the frequency of OLNBP, which is consistent with a large body of literature indicating that slow breathing is a powerful modulator of heart rate (7, 24). However, BRS analysis did not reveal an interaction effect (breathing × OLNBP), or a main effect for breathing rate, suggesting that the changes in R-R interval PSD did not translate to an increase in BRS. Therefore, our findings do not support the notion that slow breathing globally enhances baroreflex function. Rather, our observation of a significant main effect for OLNBP frequency indicates that BRS is dependent on the frequency of BP oscillations.

In this study we used OLNBP to define the frequency of BP oscillations for subsequent examination of baroreflex frequency-dependent effects. It is possible that these frequency-dependent properties could also be revealed by other maneuvers capable of entraining BP oscillations such as cyclic sit-to-stand maneuvers (12) or, as we postulate here, the act of breathing. In relation to the latter, the mechanical effects of inhaling and exhaling alternately decrease and increase intrathoracic pressure, which in turn increases and decreases venous return and stroke volume that ultimately translate into arterial pressure oscillations (5, 28). Such mechanical entrainment of BP oscillations to the prevailing breathing frequency can potentially give the false impression that breathing rate modulates BRS. This notion is supported by our current observation that slow breathing does not enhance BRS when BP oscillations are driven at 0.06 Hz via OLNBP. The notion also corroborates previous observations where BRS assessed by the Oxford method showed no respiratory modulation (29).

Practical implications. An accumulating body of clinical research has linked slow breathing to a wide range of favorable physiological outcomes, such as BP reduction in essential hypertension (14), chemoreflex stabilization in congestive heart failure (1), and sympathetic inhibition in patients with chronic obstructive airways disease (22). Of these, baroreflex potentiation appears to be the cardinal change that potentially explains the concomitant reductions in chemoreflex sensitivity and sympathetic tone. It is important to recognize that our results do not disqualify these apparent correlations demonstrated in patient groups (1, 2), and it remains entirely possible that slow breathing can have favorable physiological effects. However, our findings suggest that the underlying mechanism(s) may be unrelated to BRS augmentation as previously suggested. In the context of our findings, pulmonary stretch and direct central inhibitory mechanisms warrant further investigation.

Methodological considerations. The findings from this study need to be interpreted in view of several methodological considerations. First, the application of OLNBP generates large and coherent oscillations in BP which are of sufficient magnitude to partially open the reflex loop and ensure the engagement of the baroreflex without the use of drugs (11, 27). Additionally, OLNBP can be applied at specific frequencies to enable the frequency-dependent characteristics of the baroreflex to be examined using transfer function analysis (TFA). It is acknowledged that there are a number of methods available that can control the frequency of BP oscillations, including the sit-to-stand method. However, the need to follow distinct cues for paced breathing and for sit-stand meant this approach was practically difficult for participants to execute.

Second, an inherent assumption of TFA is that SAP and R-R interval are linearly related. In practice this means that BRS estimates are most reliable when the cross-spectral coherence is sufficiently high. To help ensure this assumption was generally met, we applied OLNBP at −40 mmHg to ensure large and coherent oscillations in BP of sufficient magnitude to stimulate the baroreceptors (11, 27). Additionally, rather than apply an arbitrary cut-off point (21), all coherence values were incorporated into our statistical model so that BRS estimates were weighted according to their individual precision.

Finally, the pharmacological modified Oxford method is a popular approach for assessing BRS because of its ability to partially open the baroreflex loop (4, 15, 17). With the use of this technique, we have previously found that slow breathing did not augment BRS (29). In common with the OLNBP method, the modified Oxford method experimentally generates large and clear perturbations in BP. However, it does not allow for the effects of BP oscillation and breathing rate to be isolated, or account for the frequency-dependent nature of BRS. Hence, our current study was intended to overcome these methodological weaknesses.

In conclusion, we examined the hypotheses that suggested an elevated BRS, calculated from spontaneous measures at the slow breathing rate of 0.1 Hz, was not due to a global effect of slow breathing per se but was associated with the frequency-dependent nature of the baroreflex. Using OLNBP to isolate the effects of breathing rate and BP oscillation frequency, we found that respiration modulated SAP PSD and R-R interval PSD during OLNBP. However, these changes did not appear to simply translate to an increase in BRS, and we were unable to confirm that slow breathing specifically mediated the enhancement of BRS. In contrast, we found that BRS was clearly dependent upon the frequency of BP oscillation. These results suggest that the increase in spontaneous BRS metrics, when slow breathing, is a consequence of a breathing-related mechanical entrainment of BP oscillations that reveals the frequency-dependent nature of the baroreflex.

ACKNOWLEDGMENTS

We thank the participants for their assistance in this study and Dr. James Stanley for his statistical advice.

GRANTS

K. C. Peebles was supported by a National Heart Foundation Fellowship (Ref. 1421). Y. C. Tzeng was supported by the New Zealand Health Research Council and holds the council’s Sir Charles Hercus Fellowship (Ref. 11/125). This work was supported by the Wellington Medical Research Fund (No. 2012/22).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES