Heart rate variability and spontaneous baroreflex sequences in supine healthy volunteers subjected to nasal positive airway pressure

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Valipour, Arschang, Frank Schneider, Wolfgang Kössler, Sleman Saliba, and Otto Chris Burghuber. Heart rate variability and spontaneous baroreflex sequences in supine healthy volunteers subjected to nasal positive airway pressure. J Appl Physiol 99: 2137–2143, 2005. First published July 7, 2005; doi:10.1152/japplphysiol.00003.2005.—To determine the dynamic effects of short-term nasal positive airway pressure (nPAP) on cardiovascular autonomic control, continuous recordings of noninvasively obtained hemodynamic measurements and heart rate variability (HRV) were obtained in 10 healthy subjects during frequency-controlled breathing (between 0.20 and 0.24 Hz) in supine posture under different pressures of nPAP ranging from 3 to 20 cmH2O. HRV was assessed using spectral analysis of the R-R interval. The slope of the regression line between spontaneous systolic blood pressure and pulse interval changes was taken as an index of the sensitivity of arterial baroreflex modulation of heart rate (sequence method). Application of nPAP resulted in a pressure-dependent decrease of cardiac output and stroke volume (P < 0.05, ANOVA) and in an increase in total peripheral resistance (P < 0.03, ANOVA). Hemodynamic changes under increasing nPAP were accompanied by a decrease in total power of HRV despite mean R-R interval remaining unchanged. The overall decrease in HRV was accompanied by a reduction across all frequency bands when absolute units were used (P < 0.01). When the power of low frequency and high frequency was calculated in normalized units, a diminished high frequency and an increased low-to-high frequency ratio were observed (P < 0.05). Compared with low levels of nPAP, pressure levels of >10 cmH2O were associated with a significant decline in the mean slope of spontaneous baroreceptor sequences (P < 0.04). These findings indicate that short-term administration of nPAP in normal subjects exerts significant alterations in R-R interval variability and spontaneous baroreflex modulation of heart rate.

METHODS

The study was randomized in which each subject was tested during frequency-controlled spontaneous breathing and at five different levels of nPAP. The study population consisted of 10 (5 men/5 women) healthy, nonsmoking volunteers (medical students). All volunteers underwent physical and neurological examination as well as routine laboratory tests, lung function testing, a 12-channel ECG recording, and a chest X-ray before the study. There was no evidence of heart or pulmonary disease in any of the subjects. None of the subjects were receiving acute or chronic medication. They were carefully informed about the study and gave written consent. The experiments were approved by the institutional Ethical Committee.

Experimental setting. Experiments always began at the same time of day (3:00 PM) in the same semidarkened room at ~22°C room temperature and at least 2 h after a light meal. Alcohol, coffee, and tea were prohibited for at least 6 h before the study. Subjects were asked to refrain from heavy exercise for at least 24 h before the test. Studies were prohibited for at least 6 h before the study. Subjects were asked to refrain from heavy exercise for at least 24 h before the test.

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were performed by the same investigators under quiet conditions with the subjects in a 30° head-up tilt position.

nPAP (Pegasus Nasal CPAP, Viasys Healthcare, Germany) was applied using a tightly but comfortably fitted nasal mask. To minimize external resistance and work of breathing, large-bore flexible tubing was used for the breathing circuit. Rebreathing was prevented by a low-resistance unidirectional valve. Each subject was familiarized with nPAP at different pressure levels at a separate visit before the study day.

After an adaptation period of 10 min, data acquisition was started for a 10-min baseline recording, which was followed by measurements during each of the following ventilatory settings in random sequence: 1) breathing via nasal mask with the pressure set at 3 cmH\textsubscript{2}O, 2) nPAP with the pressure set at 5 cmH\textsubscript{2}O, 3) nPAP at 10 cmH\textsubscript{2}O, 4) nPAP at 15 cmH\textsubscript{2}O, and 5) nPAP at 20 cmH\textsubscript{2}O (nPAP20). nPAP20 was tolerated by only seven subjects. To minimize the effects of variations in respiratory rate on HRV, each subject was trained to maintain breathing frequency between 0.2 and 0.24 Hz by following a respiratory-pacing stimulus displayed on an oscilloscope (frequency-controlled breathing). Subjects were instructed to maintain this breathing frequency throughout the different ventilatory settings. Short-term laboratory recordings using frequency-controlled breathing protocols avoid artifacts in the low-frequency (LF) range of HRV from irregular slow breaths (15). Each PAP level was sustained for 10 min. Each ventilatory setting was separated from the following by a 15-min resting period of frequency-controlled breathing without PAP.

Cardiovascular parameters. For monitoring and automatic online calculation of all hemodynamic parameters as well as HRV, we used the Task Force Monitor (CNSystems, Graz, Austria): continuous measurements of systolic (BP\textsubscript{sys}), diastolic, and mean beat-to-beat BP were obtained by using the vascular unloading technique on the finger (15). These BP values were automatically and continuously corrected to oscillometric BP values obtained at the contralateral arm (brachial artery). An estimate of real-time beat-to-beat SV was derived using an improved method of transthoracic impedance cardiography (ICG) (15, 16, 18). ICG utilizes changes in thoracic electrical impedance to estimate changes in blood volume in the aorta and changes in fluid volume in the thorax (16). By measuring the maximum of the electrical impedance in the thorax during ventricular ejection, dividing it by the base impedance multiplying by the left ventricular ejection time and a volume constant of the chest (determined by age, weight, height, and body surface area), SV of the left ventricle can be calculated (49). The total peripheral resistance index was calculated according to Ohm’s law: total peripheral resistance index = mean BP/cardiac index.

A six-channel ECG was included for R-R interval determination whose beat-to-beat values were used for real-time calculation of HRV by an autoregressive model, displayed as three-dimensional sliding whose beat-to-beat values were used for real-time calculation of HRV.

Results are given as means ± SD and are expressed as absolute values or changes from baseline. Mean values for hemodynamic variables, measurements of R-R interval variability, and spontaneous baroreflex were calculated as the average of the corresponding time series. Changes in cardiovascular parameters under increasing nPAP levels were examined by repeated-measures ANOVA. If trends with nPAP reached statistical significance, a Tukey’s honestly significant difference post hoc test correction was performed to determine differences between the respective ventilatory settings. The null hypothesis was rejected at the 5% level.

RESULTS

We studied 10 subjects (5 men/5 women) with a mean age of 25.2 ± 1.5 yr, height of 173.2 ± 7.8 cm, and body weight of 63.0 ± 10.3 kg. Results of lung function testing, ECG, and laboratory analysis were unremarkable.

Hemodynamic responses to nPAP. Figure 1 gives an example of an original recording of hemodynamic parameters during frequency-controlled breathing and at two different pressure levels of nPAP (nPAP at 10 cmH\textsubscript{2}O and nPAP20). Overall, nPAP resulted in a pressure-dependent decrease of noninvasively obtained CO and SV (Table 1). The maximum decrease in CO and SV per subject was on average 2.0 ± 0.5 l/min and 52.0 ± 9.0 ml, respectively, or 28% of baseline for CO and 38% of baseline for SV. Changes in CO and SV paralleled those of cardiac index and stroke index and decreased with each increase in pressure level (P < 0.05 for both, ANOVA). In conjunction with these changes, total peripheral resistance index increased progressively from 23.2 ± 4.3 during controlled breathing to 29.2 ± 5.1 mmHg·l/min during nPAP20 (P < 0.03). Post hoc analysis revealed that changes in the above-mentioned cardiovascular variables reached significance at a nPAP of ≥15 cmH\textsubscript{2}O. nPAP had no significant effect on beat-to-beat BP\textsubscript{sys}, diastolic BP, mean BP, or HR.

Effects of nPAP on HRV using power spectral analysis. Table 2 summarizes the indexes of spectral analysis of the R-R interval variability under different ventilatory settings. nPAP levels ≥15 cmH\textsubscript{2}O were associated with a significantly lower total power of R-R interval variability (P < 0.01) than nPAP levels below 15 cmH\textsubscript{2}O. The overall decrease in total HRV was accompanied by a reduction across all frequency bands when using absolute units. When the power was calculated in normalized units, a dominant increase in very low frequency (P < 0.05, ANOVA) and a decrease in HF (P < 0.02, ANOVA) components of HRV were observed under increasing nPAP levels (Fig. 2). As a result, we observed a continuous increase in the LF-to-HF ratio from 0.89 ± 0.61 during controlled spontaneous breathing to 2.71 ± 0.69 during nPAP20 (P < 0.01). The latter changes were significant at a nPAP of ≥10 cmH\textsubscript{2}O.

Effects of nPAP on spontaneous baroreflex control of HR. An example of the relationship between PI and BP\textsubscript{sys} sequences recorded from a subject under three different ventilatory settings is demonstrated in Fig. 3. The total number of baroreceptor sequences was not significantly different during
controlled spontaneous breathing and nPAP (P > 0.2). However, there was a significant reduction in the mean slope of PI+/BPsys+ and PI−/BPsys− sequences (P < 0.04, ANOVA) under increasing nPAP levels (Table 2).

**DISCUSSION**

**Hemodynamic effects of nPAP.** In the present report, ICG was used to assess hemodynamic changes under increasing nPAP levels. Although ICG may have limits regarding the accuracy of the absolute values of SV and CO, it is a noninvasive tool being increasingly used for physiological and clinical studies when relative changes of hemodynamic recordings are of primary interest (47). In fact, recent work suggests that measurements as obtained by ICG are less variable and more reproducible than by the thermodilution technique (51). Using ICG, we observed a reduction in both SV and CO under increasing nPAP levels. The application of PAP during invasive ventilation has long been known to suppress CO (43); however, results of previous experiments using noninvasive PAP ventilation have been inconsistent (28, 37). PAP results in a reduction in volume in both the left and right ventricles, as has been shown by echocardiography (23) and magnetic resonance imaging (29). This reduction in volume appears to be secondary to decreased venous return that is caused by the increase in ITP (13). PAP also evokes a heart-lung interaction characterized by an increase in right ventricular afterload that may suppress right ventricular output and flatten the ventricular septum (23).

Peters et al. (41) have shown that nPAP results in shifting of blood from the intrathoracic to the abdominal compartment. Under normal conditions, momentary changes in CO can be regulated by changes in HR, vascular tone, or blood volume. In the present report, blood volume and HR were unaffected and maintenance of a constant perfusion pressure at high nPAP levels was obtained by an increase in systemic vascular resistance (5, 41). Blevins and coworkers (5) have shown that the increase in vascular resistance observed in conjunction with hemodynamic changes during positive end-expiratory pressure

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**Table 1. Effects of controlled breathing and nPAP at different pressure levels on beat-to-beat hemodynamic measurements**

<table>
<thead>
<tr>
<th>Cardiovascular Variable</th>
<th>Ventilatory Settings</th>
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<tr>
<td></td>
<td>CB (n = 10)</td>
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<tr>
<td>Stroke volume, ml</td>
<td>105.2 ± 20.6</td>
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<tr>
<td>Stroke index, ml/m²</td>
<td>62.3 ± 8.74</td>
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<tr>
<td>Cardiac output, l/min</td>
<td>7.1 ± 1.2</td>
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<tr>
<td>Cardiac index, 1/min⁻¹·m⁻²</td>
<td>4.24 ± 0.69</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>68 ± 11.2</td>
</tr>
<tr>
<td>BPsys, mmHg</td>
<td>122.8 ± 7.6</td>
</tr>
<tr>
<td>BPdia, mmHg</td>
<td>79.6 ± 6.9</td>
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<tr>
<td>BPmean, mmHg</td>
<td>92.4 ± 7.0</td>
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<tr>
<td>TPRi, mmHg/1/min</td>
<td>23.2 ± 4.3</td>
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</table>

Values are means ± SD. CB, controlled breathing; shamPAP, nasal positive airway pressure (PAP) with the pressure set at 3 cmH₂O; nPAP5/10/15/20, nasal PAP with the pressure set at 5, 10, 15, or 20 cmH₂O, respectively; BPsys, dia, mean, beat-to-beat systolic, diastolic, or mean blood pressure, respectively; TPRi, total peripheral resistance index. *P ≤ 0.05 vs. CB and shamPAP; †P ≤ 0.05 vs. CB, shamPAP, and nPAP5. ‡P < 0.01 vs. CB, shamPAP, and nPAP5 (Tukey’s honestly significant difference post hoc test).
largely depends on the afferent inputs from the carotid arterial baroreceptors and from the vagus nerves. Although spenic contraction has been reported to compensate partially for the effects of nPAP (44), intra-abdominal blood accumulation seems to overwhelm any compensation by the baroreceptor reflexes that would serve to maintain CO.

Interpretation of changes in HRV. The efferent vagal activity is a major contributor to the HF component of HRV, as seen in clinical and experimental observations of autonomic maneuvers such as electrical vagal stimulation, muscarinic receptor blockade, and vagotomy (1, 33, 42). Whether the LF component of R-R interval variability reflects sympathetic modulation remains a subject of vigorous debate. In humans, an increased LF component in R-R variability has been documented in various conditions known to increase sympathetic outflow such as tilting, mental stress, infusions with sodium nitroprusside, or exercise (32, 48). Other reports, however, suggest that LF R-R interval is a parameter that results from changing levels of both the sympathetic and parasympathetic inputs to the sinoatrial node (45). Studies that investigated the relationship between more direct measures of sympathetic nerve firing and HRV produced conflicting results. Although some reports found no significant correlation between changes in cardiac or muscle sympathetic nerve activity (MSNA) and changes in LF R-R interval power (20, 26), others reported that spectral analysis of MSNA and R-R interval variability share almost identical oscillatory components with a high correlation between both measures in the human subject (9, 36, 38, 50). LF R-R interval oscillations may also be related to cardiac sympathetic modulation resulting from the baroreflex response to LF BP oscillations (8). Studies on sinoaortic denervation, which results in a consistent reduction in the LF power of R-R variability, support a considerable contribution of the baroreflex (11). However, a significant amount of variability is still present in R-R, organized in a definite LF peak, suggesting that the baroreflex may not be the only determinant of the LF rhythm. Importantly, the residual strength at this frequency appears to be sympathetic in origin as it is eliminated with ganglionic blockade. Other findings suggest that LF R-R interval variability may originate centrally from oscillatory neural activity in the medulla and/or in the spinal cord (34).

Factors that may affect R-R interval variability during nPAP. In contrast to findings from negative-pressure breathing (7), which were associated with significant increases in all spectral power components of R-R, increasing nPAP resulted in a significantly decreased total HRV. The overall decrease in HRV was accompanied by a reduction across all frequency bands when absolute units are used; however, when the power of LF and HF was calculated in normalized units, a diminished HF and an increased LF-to-HF ratio were observed. This is of particular importance since interpretation of changes in the total power of HRV may affect spectral components of LF and HF expressed in absolute units in the same direction and may prevent the appreciation of the fractional distribution of the energy (48). The observed decrease in HF and increase in LF/HF-R-R interval under increasing nPAP levels may suggest a pressure-dependent change in the sympathovagal balance of sinoaortal discharge toward a reflex reduction in parasympathetic modulation (1, 4, 21, 32). These findings are further supported by the observed reduction in the mean slope of spontaneous baroreceptor sequences (4, 21, 40), which indi-

<table>
<thead>
<tr>
<th>Components of R-R Interval Variability</th>
<th>Ventilatory Settings</th>
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<tbody>
<tr>
<td>VLFnu-RR</td>
<td>CB (n = 10)</td>
</tr>
<tr>
<td>14.4±6.2</td>
<td>14.3±8.7</td>
</tr>
<tr>
<td>0.08±0.06</td>
<td>0.05±0.04</td>
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<tr>
<td>35.8±11.8</td>
<td>34.9±11.8</td>
</tr>
<tr>
<td>0.15±0.12</td>
<td>0.19±0.16</td>
</tr>
<tr>
<td>50.2±13.8</td>
<td>51.6±15.6</td>
</tr>
<tr>
<td>0.28±0.25</td>
<td>0.20±0.21</td>
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<tr>
<td>0.89±0.61</td>
<td>0.82±0.51</td>
</tr>
<tr>
<td>0.53±0.29</td>
<td>0.48±0.36</td>
</tr>
<tr>
<td>35.2±22.9</td>
<td>34.7±27.6</td>
</tr>
<tr>
<td>25.0±7.8</td>
<td>24.9±9.5</td>
</tr>
<tr>
<td>12.0±5.4</td>
<td>13.5±5.3</td>
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Values are means ± SD. VLFnu-RR, LFnu-RR, HFnu-RR, normalized units of very low, low, and high frequency R-R interval variability; LF/HF-R-R, low-frequency to high-frequency ratio of R-R interval variability; PI/BPsys, mean slope of sequences in which RR and BPsys concurrently increased (‡) or decreased (†) over ≥3 beats. *P ≤ 0.05 vs. CB and shamPAP. ‡P ≤ 0.05 vs. CB, shamPAP, and nPAP5. †P < 0.01 vs. CB, shamPAP, and nPAP5 (Tukey’s honestly significant difference post hoc test).
cates that the ability of the arterial baroreflex to respond to alterations in R-R interval to given changes in BPsys is reduced as vagal activity is withdrawn under high levels of nPAP. A similar change in the phase relationship between BPsys and R-R interval was observed when reducing venous return to the heart by using lower body negative pressure (4).

Can noninvasive PAP influence sympathovagal activity in healthy subjects? With nPAP levels above 10 cmH2O, ITP remains positive throughout the entire breathing cycle (25). Increased ITP can produce significant hemodynamic alterations (such as reductions in SV or CO), which are sensed by the carotid sinus and aortic baroreceptors, and fluctuations in cardiac filling, sensed by cardiac baroreceptors (30). Macefield (31) suggested that increasing ITP during static lung inflation maneuvers increases sympathetic activity due to unloading of baroreceptors via a reduction in cardiac filling pressures. Similarly, findings were observed when ITP was elevated using noninvasive PAP. Both Ikeda et al. (22) and Heindl et al. (19) observed increased MSNA during the short-term application of nPAP in healthy subjects.

Whether elevated PAP levels can exert influences on spectral components of R-R interval variability in healthy volunteers has not been studied yet. Floras and coworkers (14) found similar changes in the frequency components of HRV in response to "15 mmHg lower body negative pressure associated with a significant reduction in SV and CO. These findings suggest arterial baroreceptor unloading due to a detectable hemodynamic stimulus, resulting in a relative increase in cardiac sympathetic modulation of HR (32, 48). It has to be acknowledged, however, that, in contrast to MSNA, HRV has limited utility when changes in autonomic outflow due to unloading of low-pressure cardiopulmonary baroreceptors are investigated (14). These receptors, which are located in the atria, ventricles, and pulmonary veins, are unloaded by a reduction in venous return and/or lowering of cardiac filling pressure even in the absence of significant changes in SV or CO (35, 46).

Limitations of the present study. One of the main limitations in the present report is that we have not measured ventilation under different nPAP levels. Any interpretation of measurements of HRV and baroreflex gain must acknowledge the influences of respiration. When the breathing rate is at frequencies below ~12 breaths/min, LF and HF R-R interval oscillations tend to merge, making it impossible to evaluate parasympathetic neural outflow without pharmacological blockade (10). In the assessment of relative power distribution of HRV, it is therefore important to ensure that the respiratory pattern is limited to the HF component (6). Although we have attempted to minimize the effects of changes in the respiratory frequency by following a respiratory-pacing stimulus under the different

![Fig. 3. Original record of the relationship between pulse interval (PI) and systolic blood pressure (BPsys) under 3 different ventilatory settings in a healthy subject: CB, nasal positive airway pressure at 5 cmH2O (nPAP5), and nPAP20. n, Total no. of identified spontaneous baroreceptor sequences.](http://jap.physiology.org/)
ventilatory settings, we cannot entirely rule out a change in breathing pattern under nPAP. Calabrese et al. (7) have shown that adding resistive loads throughout the entire breathing cycle, hence increasing negative ITP swings, resulted in an increase in total power of HRV. Noteworthy, the increase in HRV appeared to be correlated to an increase in the respiratory period during resistive breathing rather than due to changes in ITP. nPAP may affect the ventilatory pattern in a manner similar to that described for resistive breathing (increased tidal volume and decreased respiratory frequency) (24); however, we observed that changes in R–I interval variability occurred in the opposite direction. These findings suggest that mechanisms unrelated to changes in the breathing pattern may have influences on HRV under increasing nPAP levels. This is in consistency with a recent report from Bartels and coworkers (2), who questioned the importance of changes in ventilation on spectral analysis of cardiovascular autonomic modulation in healthy subjects.

In conclusion, we have shown that, in healthy human subjects, application of nPAP induces significant hemodynamic changes, which are associated with alterations in spectral power of HRV, toward a reflex reduction in parasympathetic modulation of HR. These findings may contribute to the understanding of the physiological effects of nPAP and should be considered when treating patients with cardiopulmonary conditions.

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