Periodic breathing in healthy humans at exercise in hypoxia*

Eric Hermand,1 Aurélien Pichon,1 François J. Lhuissier,1,2 and Jean-Paul Richalet1,2

1Université Paris 13, Sorbonne Paris Cité, Laboratoire Hypoxie et poumon, Bobigny, France; and 2Assistance Publique-Hôpitaux de Paris, Hôpital Avicenne, Service de Physiologie, explorations fonctionnelles et médecine du sport, Bobigny, France

Submitted 15 September 2014; accepted in final form 10 November 2014

Periodic breathing in healthy humans at exercise in hypoxia. J Appl Physiol 118: 115–123, 2015. First published November 13, 2014; doi:10.1152/japplphysiol.00832.2014.—Periodic breathing is frequent in heart failure or ventilatory disorders during sleep, and common during sleep at high altitude, but has been rarely studied in wakefulness and during exercise. A retrospective analysis of ventilation from hypoxia exercise tests was realized in 82 healthy subjects separated into two groups with either high or low ventilatory response to hypoxia at exercise (HVRe). A fast Fourier transform spectral analysis of the breath-by-breath ventilation (Ve) signal, O2 saturation, and end-tidal PCO2 evidenced a periodic pattern with a period of 11.1 to 12.0 s. The peak power of the Ve spectrum was higher in the high HVRe group (P < 0.001). A prospective study (25 subjects) was performed to evaluate the influence of cardiorespiratory factors on the amplitude and period of oscillations in various conditions of exercise (20 to 40% maximal aerobic power) and hypoxia (0 to 4,000 m altitude). The period of Ve was shorter at exercise (vs. rest, P < 0.001) and hypoxia (vs. normoxia, P < 0.001), and inversely related with cardiac output and Ve (P < 0.001). Ve peak power was higher at exercise (P < 0.001) and hypoxia (P < 0.001), and was positively related with cardiac output and Ve (P < 0.001). Ve peak power in hypoxia was positively related with the ventilatory response to CO2 (HCVR). This novel observation suggests that healthy subjects demonstrate a spontaneous periodic breathing, not clearly observable at rest and in normoxia, but triggered by hypoxic exercise. The periodic pattern is enhanced in subjects with high HVRe and high HCVR, suggesting that oxygen and CO2 play a synergistic role in the modulation of these oscillations.

hypoxia; control of ventilation; periodic breathing; hypoxic ventilatory response; hypercapnic ventilatory response; exercise

THE CONTROL OF VENTILATION involves three main components: the respiratory centers, which are responsible for the genesis of the respiratory rhythm; the effector system (the ventilatory muscles); and the receptors (mainly the chemoreceptors), which inform the centers of the status of the entire system. Central chemoreceptors are sensitive to the level of arterial PCO2 with gain adjustments from peripheral chemoreceptors (5, 25), and although these interactions have been debated in humans (16), the response to hypoxia is mainly due to peripheral chemoreceptors (carotid bodies) (23, 26). All these components are involved in a closed loop system of control that in most usual circumstances produces a relatively stable level of ventilation adapted to the needs of the organism. However, in some cases, the system shows a marked degree of instability with periodic breathing or interruption of ventilation. Sleep apneas or hypopneas might be of obstructive or central origin and are accompanied by oscillations in the level of end tidal PCO2 (PETCO2) and arterial O2 saturation (2, 46). Indeed, patients with cardiac failure may show a periodic breathing or Cheyne-Stokes pattern of ventilation at rest (7, 35, 37, 41) and during exercise (13, 14). Normal subjects may also show a periodic breathing pattern during sleep at high altitude (1, 3, 24, 29, 30). The factors affecting breathing instability have been extensively discussed (11, 22). However, studies carried out in awake subjects at high altitude are very scarce (8, 10, 17, 31, 42), and only one observation of periodic breathing during exercise at high altitude in field conditions has been reported (20).

The instability of the ventilation control system in patients is related to three main factors: the low reserve of O2 and CO2 in the lungs and tissues, the delay of signal transmission between the lungs and the central/peripheral chemoreceptors, and a high gain of the chemoreceptor response to O2 or CO2 or of the central response to input signals (11, 34). A physiological or environmental stress can reveal the unstable nature of the ventilatory control system. This can be initiated by a mechanical (obstruction of airways), chemical (rapid variation of O2 or CO2 pressures), or central (sigh) constraint (12).

When ventilation was recorded breath-by-breath, periodic breathing was observed in healthy subjects seeking an outpatient mountain medicine consultation at Avicenne hospital in Bobigny, France. The ventilation signal showed a periodic breathing pattern, which increased when the subject started to exercise in hypoxic conditions and was subsequently maintained during normoxic exercise. During this consultation, subjects performed a hypoxia exercise test, which allowed a good prediction of their risk factors for severe high-altitude illnesses (9, 39).

Therefore, our objective was to confirm this observation of periodic breathing in healthy subjects in a retrospective study of 82 subjects who performed this hypoxia exercise test. We hypothesized that subjects with a high ventilatory response to hypoxia might show a more pronounced periodic pattern of ventilation due to a higher gain of the chemoreceptor feedback loop. Then, in a prospective study conducted in 25 subjects, we determined the influence of cardiorespiratory parameters (ventilation, cardiac output) on the observed periodic pattern at different levels of exercise intensity and hypoxic stress. We also conducted a CO2 rebreathing test on each subject to assess the potential role of central chemoreceptors (15, 38) on the genesis of ventilation oscillations.

If the hypothesis were confirmed, periodic breathing would not be considered as a rare phenomenon observed in some patients or in normal subjects in hypoxic conditions, but as a common characteristic of the breathing pattern in humans, exacerbated in some pathological or physiological conditions. The observation of this phenomenon in high-risk patients may

* Clinical trial NCT02201875.

Address for reprint requests and other correspondence: J.-P. Richalet, Laboratoire Hypoxie et poumon, EA2363, 74 rue Marcel Cachin, 93017 Bobigny Cedex, France (e-mail: richalet@univ-paris13.fr).

http://www.jappl.org

8750-7587/15 Copyright © 2015 the American Physiological Society

First published November 13, 2014; doi:10.1152/japplphysiol.00832.2014.
help the early detection of patients susceptible to developing apneic syndromes.

SUBJECTS AND METHODS

Retrospective Study

Subjects. Among the population seeking an outpatient consultation in mountain medicine at Avicenne hospital in 2012, 82 subjects (38 women, 44 men) were retrospectively randomly selected and separated in two groups of 41 high and 41 low responders to hypoxia according to the median value of the hypoxic ventilatory response to hypoxia at exercise (HVRe) derived from the hypoxic exercise test (inspired fraction of O2 0.115, exercise intensity of 30% of maximal aerobic power), as previously described and defined in the Procedure section below. Characteristics of the two groups are shown in Table 1.

Procedure. The hypoxic exercise test consists of four successive phases of 3 to 4 min each with the following sequence: rest in normoxia (RN), rest in hypoxia (RH), exercise in hypoxia (EH), and exercise in normoxia (EN) (Fig. 2). Minute ventilation (VE, liter/min) was measured through a metabograph (Vmax Encore; SensorMedics, Yorba Linda, CA). Tidal volume (VT), total respiratory cycle time (Ttot), and inspiratory time (Ti) were derived from the ventilation signal. Pulse O2 saturation (SpO2, %) was measured by transcutaneous oximetry (Nellcor N-595; Nellcor, Pleasanton, CA) on a prewarmed ear lobe. End tidal PCO2 (PETCO2) was measured by infrared thermopile (Vmax Encore). As previously defined, ventilatory response to hypoxia at exercise was measured as the ratio of increase in VE over the decrease in SpO2 between normoxic and hypoxic conditions at exercise (6, 9, 27, 28, 39) as follows: HVRe = (VEEH - VEEN)/(SpO2EH - SpO2EN)/body wt × 100 in liter·min⁻¹·kg⁻¹.

During the entire test, VE, SpO2, and PETCO2 were recorded breath-by-breath (Fig. 2). Data were transferred to a computer for further variability analysis. A fast Fourier transform (FFT) was then applied to the breath-by-breath ventilation signal and extracted from the raw data (Fig. 1) in sequences of 128 points (one point per second) of a steady-state interval at the end of each phase of the test. This method allowed us to detect the presence of peaks in the frequency domain of the ventilation signal (Fig. 3). Two main parameters were derived from the FFT: the frequency in hertz (or period in seconds) of the larger peak and its power estimated as the area under the peak at 0.02 Hz around the peak (in liter²·min⁻², %², and mmHg², respectively, for VE, SpO2, and PETCO2 spectra). This method allowed us to precisely quantify the presence of oscillations in the signals that are not observable in the standard protocol routinely used in the hypoxic exercise test in which the signals are averaged every 20 s (39).

Prospective Study

To determine the main factors likely to influence ventilatory oscillations, we designed two separate protocols: one was centered on the effect of intensity of power output at exercise (group intensity), the second on the effect of the level of hypoxia (group altitude).

Subjects. For both protocols, healthy and nonsmoking male subjects volunteered and were given exhaustive information about the successive tests. All were in good physical condition, with a medium level of regular physical activities (from 2 to 10 h per wk). They showed no evidence of cardiovascular or pulmonary disease. Subject characteristics are presented in Table 2.

Procedure. All subjects were first asked to perform a standard ramp test protocol on a cycloergometer to determine their maximal aerobic power (MAP, Table 2). After a 3-min warm-up at 60 W, power output was increased by 30-W steps every 2 min until exhaustion.

GROUP INTENSITY. Six tests were randomly performed with a minimum 2-day interval. After 1 min of rest for material habituation...
and stabilization of cardiorespiratory parameters, subjects were first asked to keep a resting, sitting position on the ergometer for 6 min, and then to pedal for 6 min at around 65 rpm pedaling cadence at an exercise intensity of 20, 30, and 40% of MAP. The entire test was executed either in normoxia or in normobaric hypoxic condition simulating an altitude of 4,000 m.

GROUP ALTITUDE. Four tests were randomly conducted at different simulated altitudes: sea level, 2,000 m, 3,000 m, and 4,000 m, with fractions of inspired oxygen of 0.209, 0.168, 0.145, and 0.127, respectively. A 6-min rest/6-min exercise test was performed and exercise intensity was set at 30% of MAP.

The ventilatory response to CO2 (HCVR) was determined using a modified Read’s rebreathing method (38). After breathing room air through a mouthpiece device to establish a baseline, the valve was switched to a 10-liter rebreathing bag containing a mixture of 93% O2 and 7% CO2. After two deep breaths to speed up the gas mixing in the lungs, the subject was asked to spontaneously breathe into the bag until ventilation reached 60 liters/min. Collected data were then compiled into a $V\dot{E}$-PETCO2 graph to calculate HCVR from the slope of the linear regression between $V\dot{E}$ and PETCO2.

The same data acquisition and analysis as in the retrospective study was performed in the prospective study. In addition, instantaneous cardiac output was measured using a transthoracic impedance technique (Physioflow; Manatec Biomedical, France) throughout the different phases.

The protocols were approved by the Ile-de-France Ethics Committee (CPP-IDF2) and written informed consent was collected from each subject.

Statistical Analysis

Results are presented as means ± SD. In the retrospective study, a two-way ANOVA was performed to evaluate the difference in period and peak power of $V\dot{E}$, SpO2, and PETCO2 spectra between groups (low and high HVRe) and between conditions (rest, exercise, hypoxia, normoxia). A Bonferroni post hoc test was then used when applicable. In the prospective study, regarding the expected difference in mean period (3 s), the standard deviation (2.4 s), a level of significance at 0.05%, and a statistical power at 80%, the minimum number of subjects to be included was seven for each protocol. Because spectra peak power showed a high standard deviation and a nonnormal distribution, we performed a logarithmic transformation of raw data and calculated a minimum number of 10 subjects to include. A two-way ANOVA was then performed to evaluate the difference in period and power of the $V\dot{E}$, SpO2, and PETCO2 spectra between conditions (rest and exercise, hypoxia, and normoxia). A multivariate regression was carried out to establish potential correlations between period and peak power of $V\dot{E}$, SpO2, and PETCO2 on one hand, and cardiorespiratory parameters on the other hand: $V\dot{E}$, SpO2, PETCO2, $T_{tot}$, $T_i$, VT, heart rate, and cardiac output ($Q\dot{c}$).

Table 2. Characteristics of the subjects of the intensity and altitude studies

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>Maximal aerobic power, watts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity</td>
<td>12</td>
<td>28.6 ± 6.7</td>
<td>175.1 ± 7.4</td>
<td>75.1 ± 14.7</td>
<td>270.0 ± 67.0</td>
</tr>
<tr>
<td>Altitude</td>
<td>13</td>
<td>27.2 ± 6.5</td>
<td>173.8 ± 9.0</td>
<td>69.4 ± 12.4</td>
<td>235.8 ± 47.9</td>
</tr>
</tbody>
</table>

Mean ± SD.

Fig. 2. Breath-by-breath output of minute ventilation ($V\dot{E}$), pulse O2 saturation (SpO2), and end tidal PCO2 (PETCO2) during a hypoxia exercise test. Top: subject with a high ventilatory response to hypoxia at exercise (HVRe) (0.93 liter·min$^{-1}$·kg$^{-1}$). Bottom: subject with a low HVRe (0.60 liter·min$^{-1}$·kg$^{-1}$) RN, rest in normoxia; RH, rest in hypoxia; EH, exercise in hypoxia; EN, exercise in normoxia.
RESULTS

Retrospective Study

Typical recordings of a hypoxic exercise test with breath-by-breath output are shown in Fig. 2. In the subject with a high HVRe (bottom), oscillations of VE, especially in the hypoxic exercise phase, are clearly shown. Note that the oscillations are also observable on the SpO2 and PETCO2 signals. Oscillations are less apparent in the subject with low HVRe (top).

The corresponding frequency spectrum of the ventilation signal during the four phases of the hypoxic exercise test in a given subject (high HVRe) is presented in Fig. 3 as an example. Note that a peak was observable in all phases but it was particularly sharp in exercise in hypoxia (EH). Mean values of VE and SpO2 signals, their period of oscillation, and power of the corresponding peak in the four phases of the test are presented in Fig. 4. VE and SpO2 were significantly higher in the high HVRe group during exercise in hypoxia (P < 0.001 and P < 0.01). Two-way ANOVA showed that VE, SpO2, and PETCO2 periods were lower at exercise (P < 0.001) and similar in both groups. VE and SpO2 peak powers were higher at exercise (P < 0.001) and higher in the high HVRe group (P < 0.001). Because a higher peak power in the high HVRe group could be due to a higher mean VE, we calculated the ratio VE peak power/VE. A two-way ANOVA still showed a significant difference of this ratio between the two groups (high and low HVRe, P < 0.01) and between phases (P < 0.001). Detailed differences are showed in Fig. 4 for VE and SpO2 (PETCO2 not shown). For the entire population, the average period was around 12.5 s at rest and significantly decreased to 11.1 s at exercise (P < 0.001), without difference between the two HVRe groups. The mean period of the VE signal (11.1 ± 1.8 s) was slightly shorter than the period for SpO2 (12.0 ± 1.8 s, 118 Intrinsic Periodic Breathing • Hermand E et al. J Appl Physiol • doi:10.1152/japplphysiol.00832.2014 • www.jappl.org
Prospective Study

The same breath-by-breath recording methods were used in the prospective study. Varying altitude and exercise intensities allowed us to identify some of the factors influencing period and peak power. The following data are presented by combining the results of the intensity and altitude groups.

As expected, $V_E$ increased with exercise and from normoxia to hypoxia, $SpO_2$ decreased with hypoxia and with exercise (in hypoxia), and $PETCO_2$ decreased with hypoxia and increased with exercise (Fig. 5, left).

As in the retrospective study, a peak in $V_E$, $SpO_2$, and $PETCO_2$ spectra was clearly noticeable, especially during exercise in the hypoxic phase. Mean values for period and peak power are presented in Fig. 5. The period of oscillations during exercise was shorter than in rest for all altitudes ($P < 0.001$), decreased with increasing exercise intensity ($P < 0.001$), and was not influenced by altitude. Peak power of $V_E$ oscillations increased with altitude ($P < 0.001$) and from rest to exercise ($P < 0.001$), but it was not influenced by the level of exercise intensity. A similar trend was shown in the $SpO_2$ and $PETCO_2$ signals, although the variations were less marked (Fig. 5).

Multivariate regressions evidenced a shorter $V_E$ period associated with increased $V_E$ ($P < 0.001$), increased $q_c$ ($P < 0.001$), and more severe hypoxia ($P < 0.01$). Moreover, a greater $V_E$ peak power was also associated with increased $V_E$ ($P < 0.001$), increased $q_c$ ($P < 0.001$), and more severe hypoxia ($P < 0.001$). Regressions for $V_E$ period and $V_E$ peak power for each condition (normoxia and hypoxia) are shown in Fig. 6. The $V_E$ period was negatively related to the intensity of $V_E$ and $q_c$ in hypoxic conditions. $V_E$ peak power was positively related with $V_E$ and $q_c$ both in normoxic and hypoxic conditions (Fig. 6). For a given level of $V_E$ (or $q_c$), $V_E$ peak power and period were greater in hypoxia than in normoxia ($P < 0.001$). Similar correlations were found for the $PETCO_2$ peak power with $V_E$ and $q_c$ (results not shown). No significant correlation was found for the $SpO_2$ signal.

Interestingly, relating $V_E$ period with $T_{tot}$, we found a very significant correlation both at rest and exercise (Fig. 7). Two-factor regression analysis of $V_E$ period as a function of $T_{tot}$ and exercise showed a significant correlation with $T_{tot}$ ($P < 0.001$) and with exercise ($P < 0.05$).

When pooling the individual values of oscillation periods at exercise in hypoxic conditions, the mean period of the $V_E$ signal was 10.9 ± 1.3 s, slightly shorter than the $SpO_2$ period (11.6 ± 1.5 s, $P < 0.001$) and $PETCO_2$ period (11.6 ± 1.3 s, $P < 0.001$), similarly to what was found in the retrospective study.

Fig. 5. Mean values of $V_E$, $SpO_2$, and $PETCO_2$ and corresponding periods and peak powers extracted from the prospective study. Mean ± SD. Condition vs. rest +$P < 0.05$, ++$P < 0.01$, +++$P < 0.001$. Condition vs. sea level *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

J Appl Physiol • doi:10.1152/japplphysiol.00832.2014 • www.jappl.org
Relating $\dot{V}E$ peak power to HCVR, we found a significant correlation in hypoxic (3,000 m) conditions but not in normoxia (Fig. 8, left). Thus the intensity of $\dot{V}E$ oscillations is higher in subjects with a higher ventilatory response to $CO_2$, but only in hypoxic conditions. A similar relationship was found for $PETCO_2$ peak power (Fig. 8, right) but not for $SpO_2$ (results not shown). No correlation was found between $\dot{V}E$, $SpO_2$, or $PETCO_2$ period and HCVR (results not shown).

**DISCUSSION**

To our knowledge, this is the first description of a spontaneous oscillation of ventilation in awake, normal subjects at rest and during exercise in normoxic and hypoxic conditions. We observed a period of oscillations between 8 and 16 s, meaning that spontaneous ventilation oscillates between a maximum and a minimum every three to five breaths at rest and four to seven breaths during exercise. No such observation has been made in previous studies of ventilation in hypoxic exercise conditions, probably because it requires a breath-by-breath recording of the ventilation signal and, in most cases, a spectral analysis to evidence the periodicity of the signal.

Both retrospective and prospective studies showed that the period of oscillations is shorter at exercise when cardiac output and ventilation are higher. In addition, the oscillation is more pronounced in subjects with a higher sensitivity to hypoxia of their peripheral chemoreceptors. These findings provide novel evidence that support the theory of the closed loop system of control of ventilation involving the sensitivity of the central and peripheral chemoreceptors and the time delay of the $O_2$/$CO_2$ signals between the receptors and the respiratory control centers (19, 21, 29). The lung-to-carotid body circulation time may also affect the oscillation period (43). Exercise may shorten the delay through increased blood velocity and therefore decrease the period of oscillation. The high gain of the feedback control system in high responders to hypoxia may
favor the appearance of oscillations. This spontaneous periodic breathing was probably uncovered by the brisk stimulation of the closed loop control system by imposing a hypoxic exercise to the subject, probably inducing rapid variations in the O2 and CO2 signals. However, neither O2 and CO2 stores nor circulation delays were directly measured to further assess the factors involved in the oscillatory process.

The combination of exercise and hypoxia may trigger the instability of the system because exercise by itself induces a further drop in O2 arterial pressure and saturation in hypoxic conditions as the ventilatory response to CO2 (HCVR), extracted from the prospective study.

Our results indicate that VE peak power rises with ventilatory response to CO2 in hypoxia. This suggests that the well-established synergistic effect of hypoxia and hypercapnia on the control of ventilation (15, 32) is involved in the genesis of ventilatory oscillations.

Oscillations were found in the ventilation signal and in SpO2 and PETCO2, which are indirect markers of the two main physiological variables controlling ventilation (i.e., arterial PO2 and PCO2). The fact that these three signals were obtained through completely independent techniques reinforces the validity of the observed periodic pattern. The slight but highly significant difference in the period for VE, SpO2, and PETCO2 is surprising, but could be in fact explained by either a nonlinearity of the control system involving O2 and CO2 signals, or the existence of the lung O2/CO2 stores that would act as a low-pass filter reducing the frequency at which the peak power is maximum by suppressing part of the high-frequency spectrum of the SpO2 and PETCO2 signals.

The mean period of VE oscillation (11 s) observed at exercise in the present study is shorter than the one observed at rest at high altitude (20 s at 3,050 m) (8), during sleep at high altitude (15 s at 8,000 m) (19), or in patients suffering from cardiac failure (around 1 min) (12, 36, 37) or obstructive sleep apneas (>38 s) (12, 40). It stands in the large range of oscillations (5 to 100 s) observed in uncontrolled conditions in subjects climbing at high altitude (20). It is clearly out of the range of the periods observed in the present hypoxia exercise test for breathing frequency (3 to 5 s), heart rate (0.4 to 1.0 s), or pedaling rate (1 s). It is interesting to note that in the present study, an increase in cardiac output (as expected during exercise) exacerbated the oscillations, whereas in patients with cardiac failure, the predominant hypothesis to explain the Cheyne-Stokes breathing pattern is an increase in time delay due to low cardiac output (11, 18). A phase shift between the various physiological signals involved in the control loop might contribute to the instability of the system as observed with heart rate and blood pressure, with arterial O2 saturation, or tidal volume in normal subjects during sleep in hypoxia (3, 29), or in patients with cardiac failure (4, 36). The observation that exercise appears to increase ventilation oscillations suggests that whatever effect enhances oscillations is strong enough to offset the stabilizing effect of the reduced phase shift. The tight correlation between VE period and Ttot (Fig. 7) suggests that the duration of the respiratory cycle also contributes to this phase shift, probably because the VE response to a chemical stimulus does not happen before the previous cycle is complete. In obstructive sleep apneas, an initial apnea may trigger oscillations in an already unstable system, and airway obstructions (permanent or intermittent) may promote hypoxia and make the system more unstable. Our data also confirm that periodic breathing is associated with high HVRe and high HCVR, as observed during sleep at high altitude (30, 33).

In conclusion, a spontaneous periodic breathing pattern with a period of around 11 s at exercise has been evidenced in normal subjects, uncovering the intrinsic oscillatory structure of the ventilation control loop. The intensity of these oscillations depends on the ventilatory response to hypoxia and hypercapnia, suggesting that peripheral chemoreceptors play a
role in the genesis or modulation of this phenomenon. Further studies will be necessary to determine the respective influence of O₂ and CO₂ signals, sensitivity of the central and peripheral chemoreceptors, and intensity of cardiac output on the period and power of this observed phenomenon. This observation may influence our knowledge of central or obstructive apneas and periodic breathing in diseases and aid early detection in patients at high risk of having a periodic breathing pattern.

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

AUTHOR CONTRIBUTIONS

REFERENCES

