Estimation of Peripheral Chemoreceptor Contribution to Exercise Hyperpnea in Man

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Abstract Nine normal male subjects were studied at three levels of exercise (0, 40, and 80 W). Single vital capacity breath test was applied at rest and during exercise (phases 2 and 3). Minimum minute ventilation found within 4 breaths following the test was compared to the control value. Significant depression in minute ventilation was invariably observed. The minute ventilation was depressed more and more with increasing intensity of exercise. A significant difference was found between exercise and rest. However, the relative contribution of chemoreceptor activity remained the same 10–20% at all exercise levels. The magnitude of ventilatory depression (ΔV.resp) in phase 2 was larger than that in phase 3, when work rate increased to 80 W, both relative and absolute.

A significant part of the exercise hyperpnea is due to peripheral chemoreceptor activity. The peripheral chemoreceptor activity is greater in phase 2 than in phase 3 at work rates of light to moderate intensity.

Key words: exertion, exercise, ventilation, chemoreceptors.

Following the onset of dynamic muscular activity of moderate intensity, ventilation (V̇) abruptly increases from the first up to a few breaths. Ventilation remains approximately constant at this higher level for a period of 15–20 s and then increases exponentially towards its steady state. The time course of ventilation during exercise is shown in Fig. 1; it is convenient to characterize the rapid initial increases and short subsequent plateau as phase 1, the slow rise to steady state as phase 2, and the steady state itself as phase 3.

When the level of exercise is below the anaerobic threshold, ventilation is linearly related to both O2 consumption (V̇O2) and CO2 production (V̇CO2) during...
steady state (phase 3). Therefore, arterial $P_{O_2}$, $P_{CO_2}$, and pH levels remain fairly constant, although the mixed venous blood values may be altered.

On the other hand, since the body stores for $O_2$ are relatively small compared to $CO_2$ (Farhi and Rahn, 1955, 1960), then the time course of relative $\dot{V}_{O_2}$ change is faster than that of $\dot{V}_{CO_2}$ during the transient phase 2 period (Linnarsson, 1974; Whipp, 1981; Hughson and Morrissey, 1982; Miyamoto et al., 1982). The relative hypoxemia resulting from the time lag kinetics between $\dot{V}_{O_2}$ and $\dot{V}$ must effectively stimulate the peripheral chemoreceptors. Therefore, augmented peripheral chemosensitivity plays an important role in phase 2 exercise hyperpnea. The importance of the carotid body during phase 2 is shown in patients with resected carotid bodies. These patients slow ventilatory kinetics compared to controls (Wasserman et al., 1975; Honda et al., 1979). Normal subjects during hypoxia were found to show faster, phase 2 exercise hyperpnea compared with subjects breathing 100% $O_2$. These latter subjects can be assumed physiologically denervated of their peripheral chemosensitivity (Whipp, 1981; Ward et al., 1987).

Peripheral chemoreceptors must also play a significant role in steady state (phase 3) exercise hyperpnea. Utilizing the abrupt and surreptitious substitution of $O_2$ for air, the magnitude of ventilatory depression at phase 3 was greater than at rest (Ekblom et al., 1975; Stockley, 1978; Young and Woolcock, 1978).

The object of the present investigation was to estimate the contribution of peripheral chemoreceptor activity to exercise hyperpnea and to compare that of phases 2 and 3 in the same subject. Rest, light, and moderate exercise was compared. Vital capacity (VC) breath test (which effectively accomplished physiological chemodenervation by a single $O_2$ breath) was used.

METHODS

Nine normal male subjects, whose mean ($\pm$ S.D.) age, weight, and height were respectively $41 \pm 11$ year, $68 \pm 6$ kg, and $172 \pm 4$ cm, were studied. They were non-athletes and had no history of cardiopulmonary disease. All subjects agreed to participate in the study after the experimental procedure had been fully explained. However, they were unaware of the purpose of the study. All subjects were healthy without lung and heart disease.

Exercise was performed on a bicycle ergometer. The subjects were pedaling at the rate of 40 rpm with three loads, 0, 1, and 2 kp, resulting in a work rate of 0, 40, and 80 W, respectively. The subjects rested for about 5 min until their heart rate and ventilation returned to the resting level between each work rate. The three exercise runs were regarded as one series of experiment and repeated three times (i.e., three series).

Subjects were seated on a bicycle ergometer and breathed room air through a mouthpiece with a one-way respiratory valve. Inspiratory airflow was monitored by a hot-wire flowmeter (Minato, RF-2). Inspiratory tidal volume ($V_T$), inspiratory and expiratory duration ($T_I$ plus $T_E$) were electrically computed from the flowmeter.
signal. Inspiratory minute ventilation ($\dot{V}_i$) was calculated from the $V_{Ti}$ and the respiratory cycle duration ($T_i + T_e$). Respiratory gas was sampled continuously by drawing through a heated sampling tube from the respiratory valve, and the gas fractions ($F_{O_2}$ and $F_{CO_2}$) monitored by a rapid response $CO_2$ and $O_2$ analyzer (San-ei Expired Gas Monitor 1H21).

ECG was monitored continuously during all three series of experiments.

Expired air was introduced into an elastic bag connected to the outlet of a one-way valve just before each test, and minute $O_2$ uptake ($\dot{V}_{O_2}$) and $CO_2$ production ($\dot{V}_{CO_2}$) were calculated.

Monitored variables were displayed on a stripchart recorder (San-ei RECTI-HORIZ-8K). Subsequently, the following variables were determined breath by breath.

The signal VC breath test was performed at rest and during phases 2 and 3 exercise. This method was originally developed by GABEL et al. (1973). The subject first fully expired to the residual volume, rapidly inspired the test gas from the bag to total lung capacity, and immediately expired passively to functional residual capacity. Room air and $O_2$ were used in the test. Both test gases contained 5% $CO_2$ in order to prevent a fall in alveolar $P_{CO_2}$ due to the VC maneuver. Room air served as a control (air test), and $O_2$ was used to abolish peripheral chemoreceptor activity ($O_2$ test). Both air and $O_2$ tests were applied at rest and during phase 3, but only one $O_2$ test was possible during phase 2. Air test was applied first and $O_2$ test was conducted after the ventilation returned to the control level. Profile of these experimental procedures is schematically represented in Fig. 1.

Minimum minute ventilation found within 4 breaths following the test ($\dot{V}_{min}$) was identified. Average minute ventilation, calculated from 10 breaths just before the test, was taken as the control $\dot{V}_i$, both at rest and during phase 3. Difference

![Fig. 1. Experimental protocol. Arrows indicate the time of the test trial. Air and $O_2$ show the gases used in the test and these contents are described in the methods.](image-url)
between control $\dot{V}_1$ and $\dot{V}_{\text{min}}$ was defined as the test response ($\Delta\dot{V}_{\text{resp}}$). Ventilation during phase 2 progressively increased with time, so reference ventilation could not be determined (as was the case at rest and at phase 3). Ventilation in phase 2 has the kinetic characteristics of a first-order exponential system. We used the following formula to express this ventilatory profile:

$$\Delta\dot{V}_1(t') = \Delta\dot{V}_1 \times (1 - e^{-t'/\tau})$$

where $\Delta\dot{V}_1(t')$ is the increment at any time ($t'$) after the exercise onset during phase 2, $\Delta\dot{V}_1$ is the difference between the prior plateau state (phase 1) and the final steady state (phase 3), and $\tau$ is the time constant of the response. We determined the time needed, and calculated the time constant, $\tau$, as indicated in Fig. 2. The time period was found to be 62–70 s, which appeared to be in accordance with the magnitude generally found in the literature (65–75 s). The amount of ventilatory response to the single VC breath test ($\Delta\dot{V}_{\text{resp}}$) was calculated as illustrated in Fig. 2. $\Delta\dot{V}_{\text{resp}}$ was measured as the vertical distance from the $\dot{V}_{\text{min}}$ to the exponential phase 2 curve.

The level of significance was chosen as two-sided $\alpha=0.05$. The distribution of the decrements in minute ventilation ($\Delta\dot{V}_{\text{resp}}$) was, with good approximation, shown to be normal. Accordingly, statistical comparisons were performed using a paired $t$-test; $p<0.05$ was considered statistically significant.

RESULTS

Metabolic rates ($\dot{V}_{\text{O}_2}$ and $\dot{V}_{\text{CO}_2}$) and minute ventilation ($\dot{V}_1$) at rest and during steady state exercise are given in Table 1. Ventilation could be regarded as a good linear function of metabolic rate and $\dot{V}_1$ appeared well below the anaerobic threshold even when work rate was elevated to maximal intensity (80 W).
The ventilatory responses to an air and an O₂ test at rest are shown in Fig. 3. The arrows indicate the points where inhalation of the VC volume was performed. Since inspiratory minute volume (\(\dot{V}_i\)) was calculated as the product of inspiratory tidal volume (\(V_T\)) and the successive ventilatory cycle duration (\(T_i + T_e\)), one breath delay appeared in the \(\dot{V}_i\) display (Fig. 3). The 4 breaths, among which the smallest minute ventilation represents the test response, are indicated with closed circles (Fig. 3). No apparent ventilatory depression was seen following the air test, whereas a fall in \(\dot{V}_i\) was observed following the O₂ test (Fig. 3).

Results of the single VC breath tests (control \(\dot{V}_i\) and \(\dot{V}_{\text{min}}\)) at rest and during exercise are presented in Table 1. The table shows the metabolic rate and ventilation at rest and during exercise.

Table 1. Metabolic rate and ventilation at rest and during exercise.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>0 W</th>
<th>40 W</th>
<th>80 W</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\dot{V}_{O_2}) (l/min)</td>
<td>0.310 ± 0.051</td>
<td>0.403 ± 0.061</td>
<td>0.830 ± 0.126</td>
<td>1.347 ± 0.131</td>
</tr>
<tr>
<td>(\dot{V}_{CO_2}) (l/min)</td>
<td>0.257 ± 0.027</td>
<td>0.368 ± 0.051</td>
<td>0.684 ± 0.083</td>
<td>1.226 ± 0.106</td>
</tr>
<tr>
<td>(\dot{V}_i) (l/min)</td>
<td>9.904 ± 1.330</td>
<td>13.794 ± 2.376</td>
<td>21.214 ± 3.603</td>
<td>32.079 ± 4.726</td>
</tr>
</tbody>
</table>

Values are mean ± S.D.

Fig. 3. Examples of ventilatory response to single vital capacity breath test at rest, showing breath-by-breath change of airway \(F_{O_2}\) and \(F_{CO_2}\), and inspiratory tidal volume (\(V_T\)), inspiratory and expiratory time (\(T_i\) and \(T_e\)) and inspiratory minute ventilation (\(\dot{V}_i\)). Arrows indicate the time of test application. Four breaths used to estimate the ventilatory response are shown by 4 closed circles.
Table 2. Ventilatory response at rest and during exercise with single vital capacity breath test.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>0 W</th>
<th>40 W</th>
<th>80 W</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phase 2</td>
<td>Phase 3</td>
<td>Phase 2</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>O₂</td>
<td>O₂</td>
<td>O₂</td>
</tr>
<tr>
<td>±0.928 ±0.954</td>
<td>±2.131</td>
<td>±2.068 ±2.065</td>
<td>±2.477</td>
<td>±3.436</td>
</tr>
<tr>
<td>(p)</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
<td>NS</td>
</tr>
<tr>
<td>Time to (\dot{V}_{min}) (s)</td>
<td>12.0</td>
<td>15.5</td>
<td>11.0</td>
<td>11.1</td>
</tr>
<tr>
<td>±6.7 ±6.8</td>
<td>±5.4</td>
<td>±5.6 ±4.7</td>
<td>±6.2</td>
<td>±4.8</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. \((n=9)\). Control \(\dot{V}_1\), the average 10 breaths before application of the single VC breath test at rest and during phase 3, or the estimated control \(\dot{V}_1\) in phase 2, which was calculated by the method illustrated in Fig. 2. \(\dot{V}_{min}\), minimum minute ventilation found within 4 breaths following the single VC breath test. Time to \(\dot{V}_{min}\), time delay from onset of the test to appearance of \(\dot{V}_{min}\). \(p\) values refer to probability of difference between control \(\dot{V}_1\) and \(\dot{V}_{min}\).
exercise (0, 40, 80 W) are shown in Table 2. No significant difference was seen between two control values observed before the air and before the O<sub>2</sub> test (Table 2). This was the case not only at rest, but also at phase 3 exercise (Table 2). Minute ventilation was reduced at both the air and the O<sub>2</sub> test (Table 2). However, the ventilatory depression at the air test was not statistically significant. This suggests that our single VC breath test did not in itself affect the subsequent ventilation.

Nevertheless, significant depression in minute ventilation by O<sub>2</sub> tests was invariably observed in all series. Time delay from onset of the test to appearance of response ($\dot{V}_{\text{min}}$) was in the range of 8 to 16 s. No significant difference was found among different experimental conditions, whereas the time delay did shorten with increasing intensity (Table 2).

![Fig. 4. Ventilatory response to the single VC breath test. Asterisk indicates that the difference between 80 W and rest is significant ($p < 0.05$). Vertical bars show the standard error from the mean (S.E.M.).](image1)

![Fig. 5. Ventilatory response to the single VC breath test expressed in percent to the total ventilation. Asterisk indicates that the difference between phases 2 and 3 is significant ($p < 0.05$). Vertical bars represent S.E.M.](image2)
The air test did not reduce ventilation significantly. The average ventilatory response to the O₂ test (ΔV resp) is illustrated in Fig. 4. The response was the largest at the highest exercise intensity. The magnitude of ΔV resp was significantly larger during 80 W exercise than at rest (Fig. 4).

The absolute size of ventilation was augmented with increasing work rate. Ventilatory responses to the O₂ test were also evaluated in relative terms, i.e., in percent of control values (Fig. 5). Contrary to the absolute results presented in Fig. 4, the relative responses did not differ significantly (Fig. 5). However, at a work rate of 80 W the response obtained in phase 2 was significantly larger than in phase 3 (Fig. 5).

DISCUSSION

Following the abrupt elevation (phase 1) of ventilatory and metabolic exercise variables, they increase further (phase 2) toward a steady state, phase 3 (Fig. 1). The time course of the above variables during phase 2 is generally represented by a simple first-order exponential function. However, due to the different capacity of the O₂ and the CO₂ stores (FARRI and RAHN, 1955, 1960), V O₂ changes faster than V, and V CO₂ follows slightly behind (LINNARSSON, 1974; WHIPP, 1981; HUGHSON and MORRISSEY, 1982; MIYAMOTO et al., 1982). Thus the time constant of the exponential function for V O₂, V, and V CO₂ increases in this order. Difference in the V O₂ and V CO₂ kinetics elicits transient hypoxia (BJURSTEDT and WIGERTZ, 1971; PEARCE and MILHORN, 1977; YOUNG and WOOLCOCK, 1978; ODENBURG et al., 1979), and difference in the V and V CO₂ kinetics elicits transient hypercapnia (WHIPP, 1981).

Occurrence of such a transient hypercapnic hypoxia during phase 2 has repeatedly been proposed by Wasserman and his colleagues (WASSERMAN, 1983). As the peripheral chemoreceptors are located to detect blood gas changes within seconds after disproportionate gas exchange in the lung, ventilation in phase 2 must be affected by these receptors, particularly the carotid bodies. In fact, evidence in a number of studies supports this view (CUNNINGHAM, 1974; CASABURI et al., 1978; WASSERMAN et al., 1975).

Functional chemodenervation by inhaling 100% O₂ or 30% O₂ induces a slowing of ventilatory kinetics following the onset of exercise (CUNNINGHAM et al., 1968; LINNARSSON, 1974). WASSERMAN et al. (1975) demonstrated slower attainment of steady state exercise hyperpnea in patients with bilateral carotid body resection. GRIFFITHS et al. (1980) and WHIPP and WASSERMAN (1980) found the time constant in phase 2 inversely proportional to carotid body responsiveness. The role of the carotid bodies was evidently enhanced during steady state exercise. Hereby hypoxemia and/or hypercapnia were absent in phase 3 in contrast to phase 2. WEL et al. (1972) found increased hypoxic chemosensitivity in normal man. HONDA et al. (1979) reported significantly less steady state ventilation during moderate exercise in patients with bilateral carotid body resection compared to control patients with comparable limitation of pulmonary function.
Despite ample evidence so far reported, proving the carotid bodies involvement in exercise hyperpnea, no attempt has even been made to compare phases 2 and 3 in the same subject. Possible physiological mechanisms will be different between these two phases. We evaluated carotid body activity by single VC breath test. As expected, the carotid body activity increased with increasing work rate. However, the relative contribution to the total ventilation remained at about the same level, 10–20%. Similar observations were reported by Stockley (1978). Using the oxygen breath test, he found that the reflex hypoxic drive did not increase with exercise. Carotid chemosensitivity in our study appeared significantly larger in phase 2 than in phase 3 at a work rate of 80 W. This may suggest that transient hypoxia and/or hypercapnia becomes stronger with increasing exercise intensity.

Recently, possible involvement of the carotid body in steady state exercise hyperpnea appears to be explained by a potassium (K\(^+\)) release mechanism. It is well documented that the exercising muscle releases potassium and causes a rise in arterial plasma potassium in man (Laurell and Pernow, 1966; Kilburn, 1966; Coester et al., 1973; Van Beaumont et al., 1973; Lim et al., 1981). Using the potassium-selective electrode, the magnitude and time course of the changes in arterial plasma potassium produced by moderate exercise have been demonstrated (Band et al., 1982; Linton et al., 1984). Band et al. (1982) have shown that arterial potassium increased 3.8 mmol, pre-exercise level, to 5.4 mmol at 100 W and 4.9 mmol at 50 W. Therefore, there must be an appreciable increase in potassium with our work intensities, 40 and 80 W, although we have not measured it. In response to a K\(^+\) injection in cats (corresponding to the dose released in moderate exercise), an enhanced sinus nerve discharge was found (Band et al., 1985; Linton and Band, 1985; Band and Linton, 1986). Whether or not this potassium effect is affected by the \(P_{O_2}\) level is controversial. Burger et al. (1986) and Band and Linton (1987) reported hypoxic potentiation, whereas Sneyd et al. (1988) observed no difference among hypoxia, normoxia, and hyperoxia. If hypoxic potentiation is a reality, a larger response to our \(O_2\) test in phase 2 at a work rate of 80 W is conceivable. On the other hand, if the K\(^+\) effect cannot be abolished by \(O_2\) administration, our estimation of the carotid body activity may be underestimated.

Finally, it must be noted that we used a special single VC breath test. To provide a sufficient dose of \(O_2\) with one inspiration, and to evaluate the response without involvement of central chemoreceptors, we preferred this method. The method is particularly applicable during transient periods, such as phase 2. It will take at least 10–15 s for humoral information to reach the central chemosensitive regions (Whipp, 1981). Thus the time period to \(\dot{V}_{\text{min}}\) (Table 2) after high oxygen inspiration was short enough to exclude the influence of central chemoreceptor activity. Therefore, we consider our results to be a relevant contribution for an evaluation of the carotid body activity.

To perform a maximal breathing effort the intention and cooperation of the subject is necessary; this possibly distorts the spontaneous breathing pattern (Cunningham, 1974). Since we used the VC test during exercise, such a distortion

Vol. 38, No. 5, 1988
by the VC maneuver was felt subjectively less than at rest. Moreover, the effect of air administration instead of O₂ in our test revealed no significant ventilation change (Table 2, Fig. 3). Thus the single VC breath test is a useful tool to detect peripheral chemoreceptor activity in exercise.

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Vol. 38, No. 5, 1988
