Hyperoxia enhances metaboreflex sensitivity during static exercise in humans

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Hyperoxia enhances metaboreflex sensitivity during static exercise in humans. Am J Physiol Heart Circ Physiol 291: H210–H215, 2006; doi:10.1152/ajpheart.01168.2005.—Peripheral chemoreflex inhibition with hyperoxia decreases sympathetic nerve traffic to muscle circulation [muscle sympathetic nerve activity (MSNA)]. Hyperoxia also decreases lactate production during exercise. However, hyperoxia markedly increases the activation of sensory endings in skeletal muscle in animal studies. We tested the hypothesis that hyperoxia increases the MSNA and mean blood pressure (MBP) responses to isometric exercise. The effects of breathing 21% and 100% oxygen at rest and during isometric handgrip at 30% of maximal voluntary contraction on MSNA, heart rate (HR), MBP, blood lactate (BL), and arterial O2 saturation (SaO2) were determined in 12 healthy men. The isometric handgrips were followed by 3 min of postexercise circulation (muscle sympathetic nerve activity (MSNA)). Hyperoxia also markedly increases the activation of sensory endings in skeletal muscle with hyperoxia, this may not prevail over activation of chemoreflex inhibition. This occurs despite a reduced lactic acid production.

There are several reasons to believe that hyperoxia may affect sympathetic regulation during exercise.

First, hyperoxia decreases blood and muscle lactate accumulation (1, 9, 18), which stimulates group III and IV chemosensitive afferents. This phenomenon is observed despite evidence that high levels of oxygen are present in the cytosol of lactate producing muscles (9) and that oxygen-limited metabolism is not the main mechanism responsible for blood lactate (BL) increase during exercise. Decreased lactate levels with hyperoxia may be due to decreased lactate production, secondary to reduced glycolysis, glycolysis and pyruvate production, and/or increased lactate clearance (1, 18).

Second, hyperoxia reduces resting muscle sympathetic nerve activity (MSNA), BP, and heart rate (HR). This is because hyperoxia suppresses the resting chemoreflex drive in humans (8, 26, 38). The carotid and aortic chemoreceptors increase MSNA in response to reductions in arterial oxygen content (19, 27, 42). Short periods of 3–4 min of hyperoxia (38) or hyperoxia with a 50% inspired O2 fraction (FIO2) (12, 21) do not decrease BP. However, more sustained reductions in sympathetic nerve traffic during longer periods of hyperoxia with a 100% FIO2 are accompanied by a fall in BP (8, 26).

Finally, there is evidence in animal studies that hyperoxia markedly increases the activation of sensory endings in skeletal muscle and enhances the discharge of group IV muscle afferents (4, 20). However, in healthy young subjects, short periods of hyperoxia did not affect sympathetic activation to rhythmic exercise (38). Effects of longer periods of hyperoxia and of isometric handgrip are, however, unknown. This is important because longer periods of hyperoxia are accompanied by reductions in resting MSNA and BP as a result of chemoreflex inhibition (8, 26, 38). Moreover, isometric exercise produces a greater increase in BL than rhythmic exercise (5, 36).

Reduced BL production during hyperoxia could blunt the MSNA and BP response to exercise. However, in skeletal muscle with hyperoxia, this may not prevail over activation of sensory endings (4, 20) because BL is only one of numerous mediators of the metaboreflex response (40). We decided, therefore, to test the hypothesis that prolonged hyperoxia enhances the MSNA and BP responses to isometric exercise even in the presence of reduced BL production. The study protocol was randomized, placebo controlled, and utilized a crossover study design.

METHODS

Subjects. We studied 12 healthy young men with a mean age of 23 yr (range, ±2). The Ethical Committee of Erasme University Hospital approved the study, and all subjects gave written consent.

MUSCLE METABORECEPTORS regulate sympathetic activation during exercise (19, 25). This reflex is activated by metabolites released from exercising skeletal muscle. Several substances, such as lactic acid, phosphate, K+, H+, adenosine, prostaglandins, and bradykinin, are now identified as stimulators of this pressor reflex (35, 37, 40).

These metabolites stimulate group III and IV chemosensitive afferents in the working muscles (32). These afferent fibers can also be activated by injection of lactic acid or a hyperosmolar solution of potassium chloride, and their activity is modulated by endogenous nitric oxide in resting and contracting muscle (3, 6, 11, 13, 16). This activation in both nonexercising and exercising limbs (32) provokes a rise in cardiac output and vasoconstriction of the nonischemic vascular beds. As a result, blood pressure (BP) and perfusion pressure increase and correct blood flow deficits during exercise (32, 35, 40, 43).

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approved the study protocol, and informed written consent was obtained from each subject.

Measurements. We obtained continuous recordings of the electrocardiogram (Siemens Medical, ECG Monitoring, Erlangen, Germany), minute ventilation (Ve) (pneumotachometer, Medical Electronic Equipment, Brussels, Belgium), oxygen saturation (Nellcor N-100 C Pulse Oximeter, Pleasanton, CA) and end-tidal CO2 (Nor- mocap 200 Capnometer, Datex-Ohmeda, Hatfield, UK). BP (Physio- control Colin BP-880 sphygmomanometer, Colin Press Mate, Colin, Komaki City, Japan) was measured every minute during each inter- vention. Breathing was performed via a mouthpiece with the use of a nose clip to ensure exclusive mouth breathing. BL, taken from a vein of the exercising arm, was determined after each intervention for 10 of the 12 subjects.

MSNA was recorded continuously by obtaining multiunit measure- ments of postganglionic sympathetic activity from a nerve fascicle in the peroneal nerve posterior to the fibular head (14) in all the subjects. Electrical activity in the nerve fascicle was measured by using tungsten microelectrodes (shaft diameter, 200 μm, tapering to a noninsulated tip of 1–5 μm; Frederick Haer). A subcutaneous reference electrode was inserted 2–3 cm away from the recording electrode, which was inserted into the nerve fascicle. The neural signals were sent to the Nerve Traffic Analysis System of the University of Iowa. This equipment amplified, filtered, rectified, and integrated the nerve traffic recordings to obtain a mean voltage display of sympa- thetic nerve activity. Acceptable recordings met the following four criteria: spontaneous bursts of neural discharge synchronous with HR, no response to arousal stimuli or skin stroking, an increase in nerve burst frequency with apnea, and a signal-to-noise ratio of 3 to 1. These recordings were displayed on a Power Macintosh Computer (Apple Computer, Cupertino, CA) using the MacLab 8/s data acquisition system (ADInstruments, Castle Hill, Australia).

Protocol and interventions. The subjects were studied in the supine position under carefully standardized conditions. The maximal voluntary contraction (MVC) of the dominant forearm was determined in triplicate with a handgrip dynamometer. Isometric handgrip exercise of the dominant arm was performed at 30% MVC in all subjects.

Handgrips were performed at 30% MVC for 3 min to induce fatigue and the release of associated inflammatory mediators. Most studies (7, 34) using handgrip exercise have employed 30% MVC as the work load because muscle contraction of 30% MVC interferes with intramuscular blood perfusion due to increased intramuscular pressure. Moreover, the time to fatigue during isometric handgrip at 30% MVC ranges between 2.5 and 4 min (15, 30, 31).

During handgrip, each subject was requested to eliminate or min- imize any muscle contraction in his resting muscles, especially in the leg muscles. Subjects were not allowed to hold their breath during the handgrip exercise.

We compared the effects of 21% and 100% oxygen administration on MSNA, HR, mean BP (MBP), and arterial O2 saturation (SaO2) responses to isometric handgrip using a randomized and crossover study design (Fig. 1). The gases were administered via a nonrebreathing mask at identical flow rates.

The normoxic trial consisted of a 5-min baseline recording while room air was breathed without a mask (baseline), followed by 15 min of normoxia (normoxic preexercise rest) and 3 min of isometric handgrip at 30% MVC in normoxia (metaboreflex activation, normoxic exercise). The same sequence was performed in hyperoxia after a 10-min rest period.

All isometric handgrip exercises were followed by 3 min of postexercise circulatory arrest (PE-CA). This procedure is used to trap metabolites released by the muscle contraction and to dissociate the mechanics of the muscle contraction (mechanoreceptor reflex) and volitional effects (central command) from stimulation of metabolically sensitive muscle afferents (metaboreflex). This technique allows a determination of the contribution of metaboreflex activation to the hemodynamic and sympathetic changes during exercise in the absence of other reflex mechanisms that can mask, attenuate, or inhibit metaboreflex activation (22, 33, 44). Indeed, a previous study (33) suggests that during exercise, mechanoreceptors activation can attenuate or inhibit metaboreceptors responses in humans.

PE-CA was produced by inflating a standard blood pressure cuff at 240 mmHg on the upper arm, 5 s before the end of handgrip. The subjects were instructed to relax their grip after the cuff was inflated. Subjects continued to breathe 21% or 100% O2 through the mask during these periods.

Data analysis. Measurements were averaged during the last 3 min of the baseline periods, during the last 5 min of the 15-min hyperoxic or normoxic preexercise resting period, and during the last minute of the 3 min of handgrip and PE-CA. Sympathetic bursts were identified by careful inspection of the mean voltage neurogram by a single trained observer blinded to the interventions. The amplitude of each burst was determined, and sympathetic activity was calculated as bursts per minute, multiplied by mean burst amplitude (in arbitrary units) and expressed as percent increase from baseline values. Burst amplitude depends on neural signal amplification, which varies from one subject to another but is kept constant throughout each experiment. Therefore, we used the percent increase from baseline and preexercise resting values to permit the comparison of changes in sympathetic nerve activity between different subjects. The increases in HR, MBP, and BL were expressed in absolute unit changes from baseline values.

Statistical analysis. Results are expressed as means ± SD. Statistical analysis was performed with Statview 5.0 (SAS). The effects of breathing 100% oxygen on cardiovascular and sympathetic responses to exercise were examined using an ANOVA for repeated measures.
When the F-ratio reached the significance level, pairwise comparisons were performed by using a modified paired two-tailed Student’s t-test (27). A \( P < 0.05 \) was considered significant.

**RESULTS**

There were no differences in any of the variables between the two baseline periods.

**Effects of hyperoxia on preexercise resting values.** The rise in \( \text{SaO}_2 \), during hyperoxia was accompanied by a reduction in resting MSNA, HR, MBP, and BL, whereas normoxia had no effect on any variable (Table 1). The changes elicited by hyperoxia were significantly different from the values achieved in normoxia for all variables except for BL.

**Effects of hyperoxia on variables during handgrip exercise compared with preexercise rest.** MSNA increased more when exercise was performed in hyperoxia than in normoxia (\( P = 0.04 \)). MBP increased by 33 ± 9 mmHg during hyperoxic exercise and by 26 ± 10 mmHg during normoxic exercise (\( P = 0.03 \)). However, there was a lesser increase in BL during exercise in hyperoxia than in normoxia (1.4 ± 0.8 vs. 1.6 ± 0.9 mmol/l; \( P < 0.05 \)). (See Table 2 and Figs. 2 and 3.)

**Effects of hyperoxia on variables during PE-CA, compared with preexercise rest.** This was investigated during the third minute of PE-CA after normoxic and hyperoxic exercise. During this third minute, metaboreflex stimulation was maintained while subjects were at rest and breathing 21% or 100% \( \text{O}_2 \). MSNA and MBP decreased from end-handgrip levels but remained elevated above preexercise resting values. MSNA was greater during PE-CA in hyperoxia than in normoxia (\( P < 0.04 \)). Moreover, MBP was increased by 21 ± 9 mmHg during hyperoxic exercise compared with an increase of 16 ± 10 mmHg during normoxic exercise (\( P < 0.05 \)). BL also remained elevated above preexercise resting values. HR after handgrip in normoxia and hyperoxia returned to preexercise resting values.

**SA-\( \text{O}_2 \) remained more elevated under hyperoxia than in normoxia.** (See Table 2 and Figs. 2 and 3.)

**DISCUSSION**

The main new finding of our study is that hyperoxia increases the sympathetic and BP responses to isometric handgrip in the presence of reduced lactic acid production.

**Effects of hyperoxia on sympathetic activity and BP regulation.** Hyperoxia suppresses resting carotid afferent sinus nerve activity in animal studies (29). Our study confirms the sympathoinhibitory, bradycardic, and BP lowering effects of sustained peripheral chemoreflex inhibition (8, 26, 38). The fall in MSNA and HR elicited by 100% oxygen was expected even though BP also decreased. This suggests that reductions in MSNA, HR, and BP are related because baroreflex deactivation through reductions in BP elicit reflex increases in MSNA and HR (26).

The effects of hyperoxia on BP are, however, variable (9, 26, 29, 38). They likely depend on the combined effects of reductions in sympathetic activity, which are more marked when resting chemoreflex drive is elevated (8, 26), and increases in peripheral resistance due to an attenuation of the endothelium-dependent relaxation of vascular smooth muscle (17, 41).

We observed the new finding that metaboreflex activation overruled autonomic control mechanisms responsible for the sympathoinhibitory and BP lowering effects of chemoreflex inhibition. Although limited, the increases in MSNA and MBP were more important during exercise in hyperoxia than in normoxia. These larger changes compensated for the reductions in MSNA and BP induced by hyperoxia.

Sympathetic activation during exercise is believed to improve perfusion of the contracting muscle by constricting vessels in resting muscle and increasing BP (32, 35, 40, 43). This notion is supported by the observation that the increase in sympathetic activity is correlated to the rise in BP during exercise (39). Thus one explanation of the larger sympathetic response to exercise in hyperoxia could be that during many types of acute stress, arterial BP is the key variable being regulated, and MSNA, along with possible other regional vasconstrictor activity, will be adjusted as necessary to achieve the appropriate level of arterial BP (28).

Another explanation could be that hyperoxia elicits neuromuscular hyperexcitability (20). Greater MSNA and MBP responses were observed not only during hyperoxic exercise but also during PE-CA in hyperoxia, when metaboreceptors are activated in the absence of other reflex mechanisms. This argues strongly in favor of metaboreflex sensitization by hyperoxia.

**Table 2. Effects of hyperoxia on variables during exercise and PE-CA compared with preexercise resting values**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Preexercise Rest</th>
<th>Exercise</th>
<th>Postexercise Circulatory Arrest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normoxia</td>
<td>Hyperoxia</td>
<td>Normoxia</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>23±9</td>
<td>19±7*</td>
<td>41±13‡</td>
</tr>
<tr>
<td>MSNA, %</td>
<td>100</td>
<td>100</td>
<td>211±80§</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>63±6</td>
<td>58±4*</td>
<td>80±14‡</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>88±6</td>
<td>84±5*</td>
<td>112±12‡</td>
</tr>
<tr>
<td>BL, mmol/l</td>
<td>1±0.2</td>
<td>0.9±0.3</td>
<td>2.6±1*‡</td>
</tr>
<tr>
<td>( \text{SaO}_2 ), %</td>
<td>97±1</td>
<td>99±0*</td>
<td>97±1*</td>
</tr>
</tbody>
</table>

Values are means ± SD. PE-CA, postexercise circulatory arrest; *\( P < 0.05 \), normoxia vs. hyperoxia; †\( P < 0.05 \), vs. exercise; ‡\( P < 0.05 \), vs. preexercise rest.
A previous study (4) in rabbits, which examined the consequences of moderate hyperoxia on the activation of sensory nerve endings in skeletal muscle, showed that group IV muscle afferents were stimulated about fivefold more during hyperoxia than during normoxia. In the same way, it has also been documented that hyperoxia increases the production of oxygen free radicals in the rat diaphragm (2), which could also enhance metaboreflex stimulation during hyperoxia. Pharmacological blockade of the production of inflammatory mediators by injections of acetylsalicylic acid, a cyclooxygenase blocker, decreased the activation of group IV afferents during low-rate electrically induced fatigue stimulation (10). This highlights the importance of inflammatory mediators in the activation of group IV muscle afferents. This was also suggested in another study (4) where marked activation of group IV muscle afferents occurred in hyperoxia and persisted during a subsequent period of hypoxia. Prolonged periods of hyperoxia (>48 h) are accompanied by an increased release of oxygen free radicals in hyperoxic muscles (2). Whether this mechanism activated muscle afferents during the shorter period of hyperoxia in our study will need further investigation.

Our results differ from those of a previous study (38) on healthy, young subjects where chemoreflex deactivation by hyperoxia lowered sympathetic nerve activity at rest but did not affect sympathetic activation to rhythmic handgrip. The increase in MSNA tended to be greater during exercise in hyperoxia in this previous study (38). However, subjects were in hyperoxic conditions for only 3 min, and this may not have been sufficient to inhibit the resting chemoreflex drive and/or to induce metaboreflex hyperexcitability (38).

Arterial baroreflex control of vascular sympathetic nerve activity is greatly modified during static handgrip exercise in humans: the baroreceptor-vascular sympathetic response relationship is rightward shifted to a higher pressure (resetting), and the gain in the reflex is markedly increased (23). The muscle metaboreflex appears to be involved in this modification (23). Thus our observation of larger increases in both blood pressure and MSNA during exercise in hyperoxia, despite evidence of more sensitive baroreceptors, provides a strong argument for metaboreflex sensitization.

Enhanced metaboreceptor sensitivity could contribute to the beneficial effect of hyperoxia on exercise tolerance. However, other mechanisms are likely implicated in this phenomenon,
such as a lower muscle metabolic acidosis that protects contractile ability during exercise (24).

Effect on BL. We observed a decrease in lactate concentrations at rest and during exercise in hyperoxia. This is in agreement with previous studies (1, 9, 18) where hyperoxia decreased blood and muscle lactate accumulation during exercise. The decreased lactate production did not, however, restrain metaboreflex activation during isometric handgrip. Thus our results emphasize that BL is not the main trigger of the metaboreflex response. This was also observed in a previous study (37) on heart failure patients where only prostaglandins correlated with the exaggerated metaboreflex activity.

Effect on HR. Chemoreflex inhibition reduced HR below baseline levels, whereas sustained sympathoexcitiation and BP elevation provided clear-cut evidence of metaboreflex activation during hyperoxic PE-CA. The reduction in HR during simultaneous metaboreflex activation and chemoreflex inhibition was identical to that observed during chemoreflex inhibition alone. This highlights remarkable differential effects of metaboreflex activation on peripheral and cardiac autonomic control. Metaboreflex activation selectively suppressed the peripheral sympathoinhibitory effects of chemoreflex inhibition but left the cardiac inhibitory effects of hyperoxia unaffected. These results further extend our observation that MSNA and HR are regulated by different mechanisms during exercise (19).

In conclusion, our study reveals that hyperoxia enhances the sympathetic and BP reactivity to metaboreflex activation. This is due to an increase in metaboreflex sensitivity by hyperoxia that overrules the sympathoinhibitory and BP lowering effects of chemoreflex inhibition. This occurs despite a reduced lactate acid production.

GRANTS

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