Chemoreflex and metaboreflex control during static hypoxic exercise

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Houssiere, Anne, Boutaina Najem, Agniezka Ciarka, Sonia Velez-Roa, Robert Naeije, and Philippe van de Borne. Chemoreflex and metaboreflex control during static hypoxic exercise. Am J Physiol Heart Circ Physiol 288: H1724–H1729, 2005. First published December 16, 2004; doi:10.1152/ajpheart.01043.2004.—To investigate the effects of muscle metaboreceptor activation during hypoxic static exercise, we recorded muscle sympathetic nerve activity (MSNA), heart rate, blood pressure, ventilation, and blood lactate in 13 healthy subjects (22 ± 2 yr) during 3 min of three randomized interventions: isocapnic hypoxia (10% O2) (chemoreflex activation), isometric handgrip exercise in normoxia (metaboreflex activation), and isometric handgrip exercise during isocapnic hypoxia (concomitant metaboreflex and chemoreflex activation). Each intervention was followed by a forearm circulatory arrest to allow persistent metaboreflex activation in the absence of exercise and chemoreflex activation. Handgrip increased blood pressure, MSNA, heart rate, ventilation, and lactate (all P < 0.001). Hypoxia without handgrip increased MSNA, heart rate, and ventilation (all P < 0.001), but it did not change blood pressure and lactate. Handgrip enhanced blood pressure, heart rate, MSNA, and ventilation responses to hypoxia (all P < 0.05). During circulatory arrest after handgrip in hypoxia, heart rate returned promptly to baseline values, whereas ventilation decreased but remained elevated (P < 0.05). In contrast, MSNA, blood pressure, and lactate returned to baseline values during circulatory arrest after hypoxia without exercise but remained markedly increased after handgrip in hypoxia (P < 0.05). We conclude that metaboreceptors and chemoreceptors exert differential effects on the cardiorespiratory and sympathetic responses during exercise in hypoxia.

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ACCUMULATION OF METABOLITES (e.g., lactic acid, H+, diprototated phosphate) during exercise stimulate group III and IV afferent neurons within the active skeletal muscle, which evokes a reflex increase in muscle sympathetic nerve activity (MSNA), known as the muscle metaboreflex (12, 13, 16). As a result, during exercise, a metaboreflex-mediated rise in cardiac output and vasoconstriction of the nonischemic vascular beds will increase perfusion pressure and correct blood flow deficits (14, 15).

Both dynamic and static exercise are associated with a metaboreflex activation of sympathetic activity, with increases in heart rate, blood pressure, and ventilation, with the increases in ventilation and heart rate being most prominent during dynamic exercise and the increase in sympathetic activity and blood pressure most prominent during static exercise (1, 3, 12, 13, 20). Arterial hypoxemia is associated with a peripheral chemoreflex-mediated increase in sympathetic activity, together with increases in ventilation and heart rate, but no change in blood pressure (8, 19, 20, 22, 25). Although an increase in systolic blood pressure has been reported in subjects who performed dynamic exercise in hypoxic conditions (30), the effects of static exercise on blood pressure in hypoxia are not well documented.

There is evidence that muscle afferents and chemoreceptors contribute directly to cardiovascular and ventilatory adjustments to static exercise in hypoxic conditions. It has also been suggested, in three previous studies, that these two mechanisms may interact in producing the autonomic responses (8, 20, 22). However, in the first study, chemoreflex activation was limited and did not increase MSNA (22). Thus without chemoreflex-mediated sympathetic excitation, it was difficult to analyze chemoreflex and metaboreflex interaction during exercise in hypoxia. In the second study, exercise was performed during hypocapnic hypobaric hypoxia (20). Possible chemoreflex inhibition by hypocapnia during this study prevents an accurate determination of the contribution of peripheral chemoreflex activation during exercise in hypoxia. In the last study, post-handgrip ischemia was performed under hypoxia, i.e., when both chemoreceptors and metaboreceptors remained activated (8). Posthandgrip ischemia during local circulatory arrest is used to maintain metaboreflex stimulation after exercise cessation. Thus this study does not allow differentiation of the chemoreflex and metaboreflex contributions to sympathetic activation during exercise. However, all three studies indicate that the sympathetic response to exercise during hypoxia is greater than to exercise or to hypoxia separately (8, 20, 22).

We tested the hypothesis that metaboreceptors make important contributions to sympathetic and blood pressure responses during static exercise in hypoxia. Accordingly, we recorded peroneal nerve sympathetic traffic (MSNA) during several interventions designed to determine the contribution of metaboreceptors to sympathetic activation during static exercise in hypoxia. Hypoxia was achieved by subjects breathing a low-O2 mixture while CO2 was titrated to maintain isocapnia in the presence of hyperventilation. We hypothesized that handgrip exercise in hypoxia would markedly increase anaerobic muscle metabolism and lactic acid production. As a result, we anticipated that sympathetic activity and blood pressure would remain markedly elevated during a local circulatory arrest despite handgrip cessation and return to normoxia. This

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would reveal the importance of the stimulation of metaboreflex muscle afferents in the absence of any residual chemoreflex activation. In addition, subjects performed longer handgrips than in the study of Saito and al. (20) because more prolonged handgrip results in larger MSNA responses (16), which could unmask interactions between muscle afferents and chemoreceptors.

**METHODS**

**Subjects.** We studied 13 healthy subjects (11 men and 2 women) with a mean age of 22 yr (range 20–24 yr). The Ethical Committee of the Erasme University Hospital approved the study protocol, and informed written consent was obtained from each subject.

**Measurements.** We obtained continuous recordings of the electrocardiogram (Siemens), ventilation (pneumotachometer), arterial O₂ saturation (SaO₂; Nellcor N-100 C Pulse Oxymeter) in the finger, and end-tidal CO₂ (PetCO₂, Capnometer, Datex Normocap). Blood pressure (Physiocontrol Colin MBP-880 sphygmonanometer) was measured every minute during each intervention. Breathing was performed via a mouthpiece with the use of a nose clip to ensure exclusive mouth breathing. Blood lactate, taken in a vein of the exercising arm, was determined after each intervention in 11 of 13 subjects.

MSNA was recorded continuously by obtaining multiunit recordings of postganglionic sympathetic activity, measured from a nerve fascicle in the peroneal nerve posterior to the fibular head (5) in 10 of 13 subjects. Electrical activity in the nerve fascicle was measured using tungsten microelectrodes (shaft diameter 200 μm, tapering to a noninsulated tip of 1–5 μm). 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 amplified, filtered, rectified, and integrated to obtain a mean voltage display of sympathetic nerve activity.

Variables were recorded on a Power Macintosh Computer (Apple Computer; Cupertino, CA).

**Protocol and interventions.** The subjects were studied in the supine position under carefully standardized conditions. The maximal voluntary contraction of their dominant forearm was determined in triplicate with a handgrip dynamometer.

A 3-min baseline period of stable ventilation, with volunteers breathing room air, was followed by 3 min of three interventions performed in random order, namely, 1) isocapnic hypoxia (10% O₂ in N₂, with CO₂ titrated to maintain isocapnia), 2) isometric handgrip exercise of the dominant arm at 30% of maximum voluntary contraction in normoxia, and 3) isometric handgrip exercise during isocapnic hypoxia. All these interventions were followed by 3 min of local circulatory arrest to the upper arm without handgrip while subjects breathed room air. Local circulatory arrest was produced by inflating a standard blood pressure cuff at 240 mmHg on the upper arm, 5 s before the end of each intervention. The subjects were instructed to relax their grip after the cuff was inflated.

We decided to use a low grip force that allows muscle chemoreflex activation (22) while minimizing muscle fatigue. Subjects was requested to eliminate or minimize any muscle contraction in their resting muscles, especially in leg muscles, during handgrip. Subjects were not allowed to hold their breath during the handgrip exercise.

We also observed responses to inflation of the pneumatic cuff on the upper arm for 3 min without a preceding handgrip. This was performed to exclude the possibility that inflation of the pneumatic cuff alone produced a reflex response.

Each intervention was followed by a 10-min recovery period and a further 3-min baseline period of stable ventilation, with volunteers breathing room air, before the next intervention was started.

**Data analysis.** Sympathetic bursts were identified by careful inspection of the mean voltage neurogram. 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 the percent increase from baseline values.

**Statistical analysis.** Results are expressed as means ± SE. The statistical analysis consisted of a repeated-measures ANOVA. When
the F-ratio of the ANOVA revealed a $P < 0.05$ level of significance, post hoc modified t-tests were used to compare specific situations (32).

RESULTS

Effects of chemoreflex activation by isocapnic hypoxia. The effects of chemoreflex activation by isocapnic hypoxia are shown in Figs. 1 and 2. Three minutes of isocapnic hypoxia, in the absence of handgrip, did not change mean blood pressure (from $83 \pm 2$ to $86 \pm 3$ mmHg, $P = 0.35$) or lactate (from $1.08 \pm 0.21$ to $1.13 \pm 0.33$ mmol/l, $P = 0.59$). MSNA increased by $141 \pm 10\%$ ($P < 0.0001$). Isocapnic hypoxia also increased heart rate (from $64 \pm 3$ to $83 \pm 3$ beats/min, $P < 0.0001$) and ventilation (from $6.5 \pm 0.2$ to $10.8 \pm 1.1$ l/min, $P < 0.0001$), whereas $\text{SaO}_2$ decreased from $97 \pm 0.2$ to $84 \pm 0.9\%$ ($P < 0.0001$), and $\text{PETCO}_2$ was maintained at baseline values ($38 \pm 1$ mmHg).

Effects of metaboreflex activation by handgrip. The effects of metaboreflex activation by handgrip are shown in Figs. 1 and 3. Three min of handgrip increased mean blood pressure from $87 \pm 2$ to $105 \pm 4$ mmHg ($P < 0.0001$), heart rate from $65 \pm 3$ to $84 \pm 3$ beats/min ($P < 0.0001$), MSNA by $251 \pm 43\%$ ($P < 0.0001$), ventilation from $6.8 \pm 0.2$ to $14.2 \pm 1.1$ l/min ($P < 0.0001$), and lactate from $1.08 \pm 0.21$ to $2.96 \pm 1.34$ mmol/l ($P < 0.0001$) while the subjects were breathing room air.

Effects of concomitant chemoreflex and metaboreflex stimulation by handgrip during hypoxia. The effects of concomitant chemoreflex and metaboreflex stimulation by handgrip during hypoxia are shown in Figs. 1 and 2. Three minutes of local circulatory arrest after hypoxia, the inflation of a pneumatic cuff on the upper arm for handgrip exercise enhanced the blood pressure ($P < 0.0001$), heart rate ($P < 0.0001$), MSNA ($P < 0.0003$), and ventilatory ($P < 0.03$) responses to hypoxia.

Contribution of metaboreflex and chemoreflex activation to the cardiorespiratory and sympathetic responses to handgrip in hypoxia. The contribution of metaboreflex and chemoreflex activation to the cardiorespiratory and sympathetic responses to handgrip in hypoxia are shown in Figs. 2 and 3. This was investigated by the 3 min of local circulatory arrest after hypoxia, handgrip, and the combination of hypoxia and handgrip while subjects breathed room air. Without a preceding hypoxia, the inflation of a pneumatic cuff on the upper arm for 3 min did not modify blood pressure, heart rate, MSNA, ventilation, or lactate.

$\text{SaO}_2$ returned to baseline values at the second minute of local circulatory arrest ($P = 0.7$) and stabilized at $98 \pm 1\%$ during both the third minute of local circulatory arrest and baseline ($P = 0.8$). Thus, during this third minute, metaboreflex stimulation was maintained in the absence of chemoreflex activation, and this allowed determination of the contribution of metaboreflex activation to the cardiorespiratory and sympathetic response to handgrip in hypoxic conditions.

During local circulatory arrest after handgrip in hypoxia, handgrip alone, and hypoxia alone, heart rate returned promptly to baseline values (Fig. 3). Ventilation decreased during the local circulatory arrest but remained higher during the third minute of local circulatory arrest after handgrip, normoxia and handgrip in hypoxia than during the corresponding baselines (both $P < 0.05$). In contrast, MSNA and blood pressure returned to baseline values during local circulatory arrest after hypoxia without handgrip but remained markedly increased after handgrip in hypoxia and handgrip alone (both $P < 0.05$). Lactate returned to baseline values after hypoxia alone ($1.17 \pm 0.41$ vs. $1.08 \pm 0.21$ mmol/l at baseline) but remained elevated during the third minute after handgrip with...
Sympathetic nerve activity and blood pressure. Exercise and hypoxia are two potent stimuli to sympathetic activity. In our study, we used microneurographic recordings to study the mechanisms responsible for sympathetic neural activation during handgrip performed in hypoxic conditions. We demonstrate that moderate isometric handgrip enhances the MSNA and blood pressure responses elicited by hypoxia. These findings agree with previous observations that MSNA is activated by hypoxia (18, 24–26) and by static muscular contraction under normoxic conditions (12, 29, 31).

During hypoxia, our subjects decreased their SaO2 at the third minute of hypoxia to 84%, which corresponds to an arterial PO2 of about 50 mmHg. The stimulus-response curve of carotid body chemoreceptor afferent fibers reveals that a PO2 of about 50 mmHg is a large stimulus to arterial chemoreceptors. Indeed, the rate of discharge of chemoreceptor afferent fiber increases progressively from 0.5 to 5 impulses/s at an arterial PO2 of 180 mmHg and becomes maximal (20–25 impulses/s) at 25–50 mmHg (2).

Interestingly, handgrip increased the sympathetic response to hypoxia, but the response to the combined stimulation was not significantly larger than the response elicited by handgrip alone. Indeed, sympathetic activation during the combined stimulation was well below the sum of the individual stimulations. These results speak against an enhanced metaboreflex sympathetic response in the presence of hypoxia. Hypoxia markedly enhanced the spontaneous tonic activity of the metaboreceptors in a previous study (11). Hypoxia seemed, however, to have little effects on the metaboreflex activation during exercise in two other studies (6, 10). It did not increase the oxidative stress in resting and contracting muscle in humans (6). Moreover, hypoxia, in the absence of exercise, did not affect hydrogen and lactate ion concentrations in the femoral venous blood. Accordingly, hypoxia with the muscles at rest appeared to have little effects on the discharge of group III and IV muscles afferents in cats (10). These studies could explain why sympathetic activation during exercise in hypoxia was not higher than the sum of the responses during exercise alone and hypoxia alone in our study.

We observed that exercise alone was a more potent stimulus for sympathetic activation than chemoreflex activation alone (P < 0.05). This is in good accordance with our finding that metaboreceptors are the main determinants of sympathetic control during exercise in hypoxia.

Our results also differ from those of a previous study (22) using muscle sympathetic nerve activity recordings, where combined stimulation resulted in a larger response than when individual stimulations were taken separately and summed. However, hypoxia did not result in MSNA activation in that study (22). Thus it may be that the sympathetic response to isometric exercise is enhanced only during very low levels of chemoreflex activation but that this clear-cut augmentation disappears as soon as chemoreflex activation becomes more pronounced. The onset of sympathetic activation in resting muscle coincides with the development of cellular acidification in active muscle (23, 27, 28), and increases in MSNA have been correlated closely with decreases in intracellular pH (27). Comparable lactate levels during handgrip and handgrip during hypoxia could explain our findings of comparable MSNA responses during both interventions.

This study reveals that metaboreceptors and chemoreceptors exert differential effects on the cardiorespiratory and sympathetic responses to static exercise in hypoxic conditions.
MSNA, blood pressure, and lactate remained elevated only after postnormoxic and posthypoxic handgrip local circulatory arrest but returned to baseline values when the local circulatory arrest occurred after isocapnic hypoxia without exercise. This persistent rise in MSNA cannot be explained by the inflation of the pneumatic cuff per se, because inflation of the cuff in the absence of exercise did not increase MSNA. In addition, subjects were not uncomfortable during the study, and it is unlikely that the rise in MSNA during posthandgrip local circulatory arrest was due to an unnoticed painful stimulus. Persistent MSNA elevation during metaboreflex activation in the absence of chemoreflex stimulation reveals that metaboreceptors play an important role in the sympathetic responses to handgrip in hypoxia. The larger MSNA response to hypoxic exercise than to hypoxia alone may have a functional significance (22). Indeed, a greater central sympathetic outflow to skeletal muscle resistance vessels results in a heightened α-adrenergic vasoconstrictor tone. This heightened vasoconstrictor tone may in turn maintain arterial perfusion pressure by offsetting hypoxia-induced vasodilatation (17). This heightened sympathetic neural outflow may also play an important role in the preservation of submaximal exercise capacity during systemic hypoxia (22). In agreement with this hypothesis, it has been shown that the markedly augmented MSNA with combined hypoxemia and exercise does not cause local skeletal muscle vasoconstriction or suppressed oxygen uptake in the resting or exercising limbs (8). This suggests that compensatory mechanisms in resting and contracting skeletal muscle are capable of overriding the elevated vasoconstrictor stimuli. Blood pressure did not increase during isocapnic hypoxia alone, although there were distinctive increases in heart rate, ventilation, and MSNA. This could be explained by the short-lasting duration of hypoxia in our study. We choose 3 min of hypoxia because it was identical to the duration of the handgrips, which lasted also 3 min. This allowed standardizing the metaboreflex and chemoreflex responses for stimulus duration.

During hypoxia, the sympathetic premotoneurons are inhibited by lung stretch afferents (4). It seems, however, unlikely that lung stretch afferents hampered the rise in MSNA and mean blood pressure during our study. This is because the reduction in ventilation during local circulatory arrest after handgrip in hypoxia was higher (4.7 ± 3.1 liters) than after handgrip alone (1.9 ± 2.0 liters, P = 0.047) and hypoxia alone (2.8 ± 2.5 liters, P = 0.01). MSNA and mean blood pressure were comparable during handgrip alone and handgrip in hypoxia. In addition, MSNA and mean blood pressure during postcirculatory arrest did also not differ after handgrip alone and after handgrip in hypoxia, despite the fact that ventilation decreased more after handgrip in hypoxia. These results indicate that the rise in blood pressure during handgrip in hypoxia is mainly due to metaboreflex activation.

Heart rate and ventilation. We observed a greater increase in heart rate during handgrip in hypoxia than during hypoxia alone or handgrip alone. This is in agreement with previous studies (7, 8, 20, 22) and with the observation that cardiac output is higher during the same level of exercise in the presence of hypoxia (9). During posthandgrip local circulatory arrest, that is when metaboreflex stimulation is maintained and chemoreflex stimulation is ended, heart rate returned promptly to baseline values. This indicates that metaboreceptors do not play an important role in the rise in heart rate during handgrip in hypoxia.

This suggests that both chemoreceptors and metaboreceptors contribute to the rise in ventilation during handgrip in hypoxia.

Previous observations that heart rate and ventilation are closely related to decreased arterial Po2 and thus to chemoreflex control, instead of MSNA, also support the concept that differential control mechanisms regulate these variables (8).

In our study, during the local circulatory arrest, metaboreflex activation increased ventilation by 0.9 ± 1.4 l/min (P = 0.047, data not shown). In previous studies, metaboreflex effect on ventilation in control subjects ranged between 0.4 l/min (21) and 3.3 l/min (16) after dynamic handgrip. This confirms our results that both metaboreflex and chemoreflex play a role in the increased in ventilation during exercise in hypoxia.

Study limitations. Central command could have affected the presently reported responses to hypoxic isometric handgrip. However, whereas there is evidence that central command plays an important role in the heart rate increase during voluntary exercise in humans, it plays a role in the activation of MSNA only during intense isometric muscle contractions in humans (12, 29). This view is strengthened by our results that increases in heart rate produced by normoxic or hypoxic isometric handgrip always returned promptly to baseline values during posthandgrip local circulatory arrest, i.e., when central command ended and the metaboreflex-induced increase in MSNA was maintained.

We did not perform local circulatory arrest while subjects were breathing the hypoxic gas mixture in our study. This would have allowed further analysis of the interaction between metaboreceptors and chemoreceptors without central command and mechanoreceptor stimulation. However, this would have also required longer periods of hypoxia. Moreover, we controlled for potential confounding effects of central command and mechanoreceptor activation by asking our subjects to perform identical exercises during normoxia and hypoxia.

Finally, MSNA recordings do not provide an overall index of sympathetic discharge, and further studies are needed to assess sympathetic responses to other vascular beds. In conclusion, our study shows that metaboreceptors and chemoreceptors exert differential effects on the cardiorespiratory and autonomic responses during handgrip in hypoxia. Metaboreceptors play little role in the increase in heart rate. Both chemoreceptors and metaboreceptors contribute to the increase in ventilation, but metaboreceptors are the major determinants of the rise in MSNA and blood pressure.

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REFERENCES


