Activation of the carotid chemoreflex secondary to muscle metaboreflex stimulation in men

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Submitted 15 October 2013; accepted in final form 20 February 2014

Edgell H, Stickland MK. Activation of the carotid chemoreflex secondary to muscle metaboreflex stimulation in men. Am J Physiol Regul Integr Comp Physiol 306: R693–R700, 2014; First published February 26, 2014; doi:10.1152/ajpregu.00472.2013.—Recent work has shown that the carotid chemoreceptor (CC) contributes to sympathetic control of cardiovascular function during exercise, despite no evidence of increased circulating CC stimuli, suggesting enhanced CC activity/sensitivity. As interactions between metaboreceptors and chemoreceptors have been previously observed, the purpose of this study was to isolate the metaboreflex while acutely stimulating or inhibiting the CC to determine whether the metaboreflex increased CC activity/sensitivity. Fourteen young healthy men (height: 177.0 ± 2.1 cm, weight: 85.8 ± 5.5 kg, age: 24.6 ± 1.1 yr) performed three trials of 40% maximal voluntary contraction handgrip for 2 min, followed by 3 min of postexercise circulatory occlusion (PECO) to stimulate the metaboreflex. In random order, subjects either breathed room air, hypoxia (target S\textsubscript{PO2} = 85%), or hyperoxia (Fi\textsubscript{O2} = 1.0) during the PECO to modulate the chemoreflex. After these trials, a resting hypoxia trial was conducted without handgrip or PECO. Ventilation (Ve), heart rate (HR), blood pressure, and muscle sympathetic nervous activity (MSNA) data were continuously obtained. Relative to normoxic PECO, inhibition of the CC during hyperoxic PECO resulted in lower MSNA (P = 0.038) and HR (P = 0.021). Relative to normoxic PECO, stimulation of the CC during hypoxic PECO resulted in higher HR (P < 0.001) and Ve (P < 0.001). The ventilatory and MSNA responses to hypoxic PECO were not greater than the sum of the responses to hypoxia and PECO individually, indicating that the CC are not sensitized during metaboreflex activation. These results demonstrate that stimulation of the metaboreflex activates, but does not sensitize the CC, and help explain the enhanced CC activity with exercise.

sympathetic nerve activity; ventilation; exercise

EXERCISE is known to involve cardiovascular influences from metaboreceptors, mechanoreceptors, baroreceptors, and central command (25). The carotid chemoreceptor has been shown to be activated/sensitized during exercise (10, 27, 33, 40). Previous work has provided evidence that the carotid chemoreceptor is enhanced with exercise despite no change in circulating carotid chemoreceptor stimuli (K\textsuperscript{+}, lactate, pH, P\textsubscript{CO2}, P\textsubscript{O2}), and that carotid chemoreceptor inhibition with either dopamine or hyperoxia increases cardiac output and/or peripheral blood flow during exercise secondary to a reduction in sympathetic vasoconstrictor outflow (32–34). Similarly, rhythmic handgrip exercise in humans activates/sensitizes the carotid chemoreceptor despite no increase in blood lactate (34); however, the exact mechanism behind this activation/sensitization of the carotid chemoreceptor during exercise is unknown.

Conflicting evidence exists concerning the role of muscle afferents on exercise-induced carotid chemoreceptor activity in animal studies. Biscoe and Purves (4) found that passive limb movement increased carotid chemoreceptor activity in animals, which was abolished by denervation of the muscle or by cutting either the preganglionic cervical sympathetic nerve or the postganglionic branch of the superior cervical ganglion, suggesting that activation of afferent nerves can sensitize the carotid chemoreceptor. However, follow-up studies did not observe this (1, 7). Aggarwal et al. (1) suggest that even though the interactions were not consistently seen in animals, they may still be present in humans. Indeed, species differences in autonomic cardiovascular control are known to exist (20, 21).

Previous human studies have investigated interactions between the metaboreflex and the chemoreflex during exercise (13, 15, 17). These studies found that metaboreceptors, chemoreceptors, and baroreceptors all play a role in the sympathetic response to hypoxic exercise. In particular, they found that both chemoreceptors and metaboreceptors play a role in ventilatory control during exercise, but the metaboreceptors primarily control sympathetic output and blood pressure. However, in these studies inhaled oxygen fraction was modulated either before or during exercise, which could have either influenced other reflexes [i.e., the mechanoreflex and/or central command (reviewed in Ref. 30)], or changed the production of metabolite buildup from exercise. Accordingly, to determine the role of the metaboreflex on the activation/sensitization of the chemoreflex during exercise, we isolated the metaboreflex from central command and the mechanoreflex by using a period of postexercise circulatory occlusion (PECO) and subsequently rapidly adjusted the inhaled oxygen fraction (i.e., normoxia, hyperoxia, or hypoxia) to modulate carotid chemoreceptor activity.

We hypothesized that 1) the carotid chemoreceptor would be sensitized with activation of the metaboreflex, 2) suppression of the carotid chemoreceptor during metaboreflex activation would attenuate sympathetic nerve activity, 3) activation of the carotid chemoreceptor during activation of the metaboreflex would augment sympathetic nerve activity, and 4) similar to the results of Gubic et al. (13), activation of the carotid chemoreceptor with hypoxia would decrease cardiovascular baroreceptor sensitivity (thus increasing heart rate via vagal withdrawal) and that there would be a greater decrease of cardiovascular baroreceptor sensitivity upon concurrent activation of the carotid chemoreceptor and metaboreflex.

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http://www.ajpregu.org 0363-6199/14 Copyright © 2014 the American Physiological Society R693
MATERIALS AND METHODS

Ethical approval. Participants were recruited from the University of Alberta and the general population and each provided written, informed consent. The study was approved by the University of Alberta Health Research Ethics Board (Biomedical Panel) and conformed to the standards set by the Declaration of Helsinki.

Participant description. Fourteen healthy men were recruited (age: 24.6 ± 1.1 yr, height: 177.0 ± 2.1 cm, weight: 85.8 ± 5.5 kg, VO2max: 49.2 ± 3.1 ml/kg^{-1}·min^{-1}). All subjects were free from cardiovascular disease and had normal ECG-blood pressure response during a standard 12-lead ECG during a VO2max test (Vmax system; VIASYS Products).

Protocol. At the beginning of data collection, participants performed a maximum voluntary contraction (MVC) using a handgrip dynamometer (G100; Biometrics, Ladysmith, VA). LabChart sofware was used to calibrate the MVC to 100%. After location of muscle sympathetic nerve activity (MSNA), participants underwent three randomized trials of 40% MVC handgrip exercise for 2 min followed by 3 min of postexercise circulatory occlusion (PECO; occlusion pressure of 200–220 mmHg) of the exercising arm at the level of the biceps muscle. At the onset of PECO, inspired oxygen (O2) remained at room air (n = 13), 2) was increased to Fio2 -1.0 (n = 13), or 3) decreased to achieve a target O2 saturation (SpO2) of 85% for 3 min [end-tidal O2 (PETO2): 47 ± 1 mmHg; n = 11]. Inspired oxygen was modulated using an air-oxygen or air-nitrogen blender system. Trials were separated by at least 5 min of rest allowing cardiorespiratory variables to return to baseline. A resting hypoxia trial followed the 3 handgrip exercise trials (n = 13). For this trial, participants breathed room air for 5 min followed by hypoxic gas to achieve SpO2 of ~85% for 3 min (PETO2: 49 ± 2 mmHg; n = 13). Trials were randomized and participants were blinded toward the inhaled gases.

Cardiorespiratory measurements. All data were recorded and integrated using a Powerlab data acquisition system and analyzed using LabChart 7.2 software (Powerlab 16/30; ADInstruments). In a supine position, participants breathed through a mouthpiece with the nose occluded. Baseline measurements were recorded for 5 min before the onset of handgrip exercise. Inspired gas was humidified (HC 150; Fisher and Paykel Healthcare) and delivered continuously using a flow-through system. Ventilation was measured using a pneumotachometer (3700 series; Hans Rudolph, Shawnee). End-tidal CO2 (PETCO2) and PETO2 were measured using gas analyzers (CD-3A and S-3A; AEI Technologies, Pittsburgh, PA) attached to a port near the mouthpiece. Arterial SpO2 was estimated using pulse oximetry (N-595; Coviden, Mansfield, MA) using a forehead sensor. Heart rate (HR) was determined using a single-lead ECG (BioAmp; ADInstruments). Beat-by-beat blood pressure [mean arterial blood pressure (MAP), systolic blood pressure (SBP), and diastolic blood pressure (DBP)] was determined using finger photoplethysmography (Finometer Midi, Amsterdam, The Netherlands) and was calibrated using LabChart 7.2 (Units Conversion function) at regular intervals using a manual measurement.

As chemosensitivity is traditionally evaluated by the change in ventilation (Ve) or MSNA burst frequency relative to a change in Pao2/SpO2, we compared cardiorespiratory responses to hypoxia alone or hypoxic PECO. The Ve and MSNA burst frequency responses to hypoxia (i.e., ΔVe/ΔSpO2 and ΔMSNA/ΔSpO2) were determined for each individual by calculating the difference between the normoxic baseline of each trial and the hypoxic response (e.g., VeHypoxia) - VeNormoxia (SpO2Normoxia - SpO2Hypoxia, or VMSNAHypoxia - VMSNANormoxia or VMSNAHypoxic PECO - VMSNANormoxic PECO - SpO2Normoxia - SpO2Hypoxia, or SpO2Normoxic PECO - SpO2Hypoxia). These values are conventionally presented as positive values for data presentation.

Autonomic measurements. MSNA was recorded (FE185 NeuroAmp EX; ADInstruments) using microneurography using the right fibular (peroneal) nerve proximal to the bifurcation posterior to the fibular head using tungsten microelectrodes (200 μm, 1–3 μm uninsulated tip; UNA32F2S; FHC, Bowdoin, ME; n = 12). A reference electrode was inserted transeptaneously 3–5 cm from the recording site. The nerve was located using manual palpation. Confirmation of a muscle sympathetic site was determined by the absence of skin paresthesias, an increase of signal in response to a voluntary apnea, and the absence of a response to a loud noise. Raw MSNA was filtered (5 kHz low pass, 300 Hz high pass, 60 Hz notch, and mains) and integrated (absolute value, time constant decay 0.1 s). A peak detection macro was created using LabChart 7.2 (ADInstruments) where a voltage detection threshold was used to detect MSNA bursts. Those bursts that were not detected by the peak detector were manually counted and artifacts were removed. Sympathetic bursts were identified by the characteristic burst shape with rising and falling slopes and had a burst latency of ~1.1–1.4 s. Acceptable recordings had a signal-to-noise ratio of ~2:1. MSNA was quantified as burst frequency (bursts/min), burst incidence (bursts/100 heartbeats), and burst amplitude (arbitrary units). See Fig. 1 for representative data.

Cardiovascular baroreceptor sensitivity was investigated as a measurement of parasympathetic HR control and was determined using the sequence method (3, 5) on 5 min of resting uninterrupted beat-by-beat blood pressure and ECG recording and for 3 min during PECO. Lag 0 data and only those recordings with at least 10 sequences were used (n = 10).

Statistical analysis. One-minute average values were obtained at baseline, the second minute of handgrip exercise, and the third minute of PECO for each trial and used for analysis. Two-way repeated measures ANOVAs were used to compare the responses during exercise trials, and a Wilcoxon Signed Rank Test was used to compare the Ve and MSNA responses to hypoxia (Sigmaplot 12.0, Systat Software, San Jose, CA). Where main or interaction effects were found, Student-Neuman Keuls post hoc tests were used. Significance was set at P = 0.05. Data are presented as means ± SE.

RESULTS

Cardiorespiratory and autonomic responses to handgrip exercise. As expected, in all three trials handgrip exercise increased MAP, Ve, HR, MSNA indices, SBP, and DBP (P < 0.001 for all; Fig. 2, Tables 1 and 2). There were no significant differences between the three handgrip trials with the exception of Ve during the handgrip trial immediately before hypoxic PECO was slightly higher than the other two trials.

Cardiorespiratory and autonomic responses to PECO in normoxia. During normoxic PECO, MAP and MSNA indices remained elevated above baseline (P < 0.01 for all), yet Ve and HR were not different from baseline (Fig. 2, Table 2). When compared with baseline, PECO elevated SBP and DBP (P < 0.001 for both; Table 1), whereas there was no effect of PECO on cardiovascular baroreceptor sensitivity (Fig. 3A).

Cardiorespiratory and autonomic responses to PECO in hypoxia. During hypoxic PECO, MAP, Ve, MSNA indices, SBP, and DBP remained elevated above baseline (P ≤ 0.01 for all; Fig. 2, A–C; Tables 1 and 2), whereas HR was lower than baseline (P = 0.023; Fig. 2D).

Cardiorespiratory and autonomic responses to PECO in hypoxia. During hypoxic PECO, MAP, Ve, HR, MSNA indices, SBP, and DBP were higher than baseline (P < 0.001 for all; Fig. 1, Tables 1 and 2).

Cardiorespiratory and autonomic responses to hypoxia alone. In response to hypoxia alone, Ve, HR, and MSNA burst frequency all increased (P < 0.01 for all) relative to normoxia, whereas SBP, DBP, MAP, MSNA burst incidence, and MSNA burst amplitude did not change (Tables 2 and 3).

Comparisons between conditions. When hypoxic PECO was compared with normoxic PECO, HR and MSNA burst
frequency were lower in hyperoxia than in normoxia \((P < 0.05\) for both; Fig. 2, B and D), whereas no differences were observed in SBP, DBP, or cardiovagal baroreceptor sensitivity (Table 1 and Fig. 2A).

When comparing hypoxic PECO with normoxic PECO, there was no difference in MAP between groups; however, both \(V\dot{E}\) and HR were higher in hypoxic PECO compared with normoxic PECO \((P < 0.001\) for both; Fig. 2, A, C, D). MSNA burst frequency, SBP, and DBP were not different between hypoxic and normoxic PECO (Fig. 2B and Table 1).

The gain of the relationship between systolic blood pressure and R-R interval (i.e., cardiovagal baroreceptor sensitivity) was lower in hypoxic PECO compared with baseline \((P < 0.013)\), normoxic PECO \((P < 0.001)\), and hyperoxic PECO \((P < 0.001;\) Fig. 3A). When comparing cardiovagal baroreceptor sensitivity in resting hypoxia to hypoxic PECO, there was no significant difference between conditions (Fig. 3B).

When the cardiorespiratory responses to hypoxia at rest were compared with the hypoxic responses during PECO, both the ventilatory response to hypoxia \((\Delta V\dot{E}/\Delta S_{PO_2})\) and the MSNA response to hypoxia \((\Delta MSNA/\Delta S_{PO_2})\) were greater during activation of the metaboreflex \((P < 0.05\) Fig. 4, A and B). The change in ventilation during hypoxic PECO was higher than either hypoxia alone or PECO alone \((P < 0.05;\) Fig. 5A), yet the change in MSNA burst frequency during hypoxic PECO was not higher than PECO alone (Fig. 5B). To further investigate these differences, we determined that the ventilatory response to hypoxic PECO compared with baseline \((+9.5 \pm 1.8 \text{ l/min})\) was not significantly different from the sum of the responses to hypoxia alone \((+5.7 \pm 1.4 \text{ l/min})\) and metaboreflex alone \((\pm 2.4 \pm 1.1 \text{ l/min}, P = 0.14)\), and the MSNA response to hypoxic PECO \((+11.8 \pm 2.1 \text{ bursts/min})\) was significantly less than the sum of the responses to hypoxia alone \((+6.7 \pm 1.2 \text{ bursts/min})\) and metaboreflex alone \((+13.1 \pm 1.8 \text{ bursts/min}; P = 0.01)\).

**DISCUSSION**

Metaboreflex activation resulted in higher carotid chemoreceptor activity but did not appear to affect carotid chemoreceptor sensitivity. Simultaneous metaboreflex stimulation and carotid chemoreceptor stimulation with hypoxia resulted in an increase in MSNA and ventilation relative to carotid chemoreceptor stimulation with hypoxia alone, yet the MSNA-ventilatory response to concurrent carotid chemoreceptor and metaboreflex activation was not greater than the sum of each individually, which indicates that carotid chemoreceptor sensitivity is not enhanced by metaboreflex stimulation. However, metaboreflex activation contributed to increased tonic carotid chemoreceptor activity, as suppression of the carotid chemoreceptor with hypoxia during metaboreflex activation decreased MSNA burst frequency to a level lower than the
normoxic response during metaboreflex activation. These results would support a change in central integration of carotid chemoreceptor feedback with metaboreflex activation, which would be consistent with previous evidence of carotid chemoreceptor activation during exercise despite no change in circulating carotid chemoreceptor stimuli (32–34). We had hypothesized that simultaneous activation of the carotid chemoreceptor and metaboreflex would result in greater MSNA relative to activation of either the metaboreflex or chemoreflex alone; however, this was not observed. While breathing hypoxic gas maintained HR and $V_{E}$ above baseline during metaboreflex activation, no potentiation of MSNA was observed. Lower cardiovascular baroreceptor sensitivity with hypoxia indicated that parasympathetic withdrawal was at least partially responsible for the higher HR observed in hypoxia independent of metaboreflex activation. Combined, these results demonstrate that metaboreflex activation by itself can alter basal carotid chemoreceptor activity.

Cardiorespiratory and autonomic responses to metaboreflex activation in hypoxia (carotid chemoreceptor sensitization). The MSNA and ventilatory responses to hypoxia during metaboreflex activation were higher than the responses to hypoxia alone. These results might imply that the carotid chemoreceptor was sensitized with metaboreflex activation; however, when comparing the increase of MSNA with hypoxic PECO to the MSNA response of the metaboreflex alone or hypoxia alone, the response to hypoxic PECO is significantly lower than the sum of those two responses. Similarly, the ventilatory response to hypoxic PECO was not significantly greater than the sum of the responses to hypoxia alone or to metaboreflex alone. These findings indicate that the carotid chemoreceptor is not sensitized with metaboreflex activation, but rather that the ventilatory response is the sum of normal carotid chemoreceptor activation and metaboreflex activation, whereas the MSNA response appears attenuated with both chemoreceptor and metaboreceptor stimulation. The blunted MSNA response to hypoxia PECO relative to the metaboreflex alone may be explained by the greater ventilation with both hypoxia and metaboreflex activation, and the corresponding sympathoinhibitory effects of increased lung stretch (9, 28, 29). The additive nature of the ventilatory response to hypoxia with metaboreflex activation compared with hypoxia alone or metaboreflex activation alone would indicate that both the metaboreflex and chemoreflex play a role in ventilatory control which is consistent with earlier work (15).

Previous work has found that the carotid chemoreceptor is sensitized with exercise (10, 27, 40). Our findings did not support this; however, we isolated the metaboreflex and did not test exercise per se. The exact mechanism(s) for how the carotid chemoreceptor is sensitized by exercise is unclear. Biscoe and Purvis (4) found that direct muscle afferent activity sensitized the carotid chemoreceptor (4); however, these findings were not supported in later studies (1, 7). Some researchers have found that activation of cardiac afferents can also sensitize the carotid chemoreceptor (11), and similarly, that β-adrenergic blockade can decrease ventilation at any metabolic rate (2, 41). Cardiac afferents can be activated by many factors including lactic acid, coronary occlusion (6, 23), and an increase in HR (38). In the current study, HR increased similarly with hypoxia alone (62 ± 3 to 82 ± 4 beats/min) compared with hypoxia during metaboreflex activation (65 ± 3 to 82 ± 3 beats/min), suggesting that the lack of sensitization of the carotid chemoreceptor with metaboreflex activation
Table 1. Cardiovascular and respiratory data during handgrip exercise and postcirculatory occlusion in normoxia, hyperoxia, and hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hyperoxia</th>
<th>Hypoxia</th>
<th>Main effect of trial</th>
<th>Main effect of time</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>PetCO₂, mmHg</td>
<td>38.4 ± 0.9</td>
<td>36.5 ± 1.1</td>
<td>33.2 ± 1.4</td>
<td>§§</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PetO₂, mmHg</td>
<td>104.6 ± 2.4</td>
<td>110.9 ± 4.3</td>
<td>111.0 ± 3.8</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Breathing Frequency, breaths/min</td>
<td>11.9 ± 1.2</td>
<td>15.2 ± 1.8</td>
<td>12.2 ± 1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Svo₂, %</td>
<td>97.9 ± 0.4</td>
<td>97.6 ± 0.5</td>
<td>97.1 ± 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>112.6 ± 2.4</td>
<td>139.0 ± 4.6</td>
<td>138.7 ± 3.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>68.5 ± 2.1</td>
<td>90.7 ± 2.3</td>
<td>85.7 ± 1.6</td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± SE. PetCO₂, end-tidal CO₂; PetO₂, end-tidal O₂; Svo₂, O₂ saturation; SBP, systolic blood pressure; DBP, diastolic blood pressure; HG, handgrip; PECO, postcirculatory occlusion. *HG is significantly different from baseline. †PECO is significantly different from baseline. ‡PECO is significantly different from HG. §difference from all other conditions within PECO.
no evidence of central hypoxia with normoxic handgrip exercise. Prolonged hyperoxia has been shown to be a central stimulant (8); yet, 2–3 min of hyperoxia given at rest does not affect MSNA (32, 34). Therefore, the reduction in MSNA during hyperoxic PECO is likely the direct effect of carotid chemoreceptor inhibition and not a result of changes in central chemoreception. It is hypothesized that the activation of the carotid chemoreceptor with metaboreflex stimulation is secondary to changes in central integration of afferent signals, as we have previously observed evidence of carotid chemoreceptor activation/sensitization without a change in circulating stimuli (32–34) and no increase in circulating carotid chemoreceptor stimuli would be expected to occur during PECO since venous return from the exercised limb is prevented.

While cardiac sympathetic activity does not necessarily equate to MSNA, the observed reduction in MSNA burst frequency with hyperoxia during metaboreflex activation likely plays a role in the reduction of HR in hyperoxic PECO, especially from the finding that hyperoxia did not increase cardiovagal baroreceptor sensitivity (i.e., no vagal activation). Houssiere et al. (16) did not observe a reduction of HR with hyperoxia during handgrip exercise; however, the administration of hyperoxia before and throughout exercise led to a reduction of HR before metaboreflex activation, which could have obscured any reductions due to the metaboreflex itself. Furthermore, prolonged hyperoxia acts as a central stimulant (8), while increasing arterial stiffness and reducing bioavailability of nitric oxide (22), which may have concealed some of the autonomic reflexes.

**Limitations.** Within the current study we did not control breathing frequency or $\text{PETCO}_2$ throughout the experiment. These were not controlled because of the difficulty in determining an appropriate $\text{PETCO}_2$ set point for all exercise/PECO trials, and the practical difficulty of maintaining breathing frequency/$\text{PETCO}_2$ with changes in ventilation. In addition, hyperoxia can have the effect of reducing cardiac output (12, 37, 39), modifying ventilation/perfusion matching in the lung, and increasing alveolar deadspace, resulting in an increase in the alveolar to $\text{PETCO}_2$ difference. Indeed it appears as though this may have occurred during our hyperoxic PECO trials, as ventilation during hyperoxic PECO was similar to the normoxic PECO despite a lower $\text{PETCO}_2$ in hyperoxic PECO.

This table shows a summary of the cardiovascular and respiratory responses to resting hypoxia.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{SpO}_2$, %</td>
<td>97.7 ± 0.5</td>
<td>85.3 ± 1.3*</td>
</tr>
<tr>
<td>$\text{PETCO}_2$, mmHg</td>
<td>99 ± 5</td>
<td>49 ± 2*</td>
</tr>
<tr>
<td>$\text{PETCO}_2$, mmHg</td>
<td>35 ± 2</td>
<td>29 ± 2*</td>
</tr>
<tr>
<td>$\text{VE}$, l/min</td>
<td>9.9 ± 1.3</td>
<td>15.6 ± 2.4*</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>122.1 ± 3.2</td>
<td>124.8 ± 4.5</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>70.7 ± 1.7</td>
<td>70.8 ± 2.4</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>87.8 ± 2.0</td>
<td>88.8 ± 3.0</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>61.5 ± 3.3</td>
<td>81.7 ± 3.8*</td>
</tr>
<tr>
<td>MSNA frequency, bursts/min (n = 9)</td>
<td>20.5 ± 2.8</td>
<td>27.0 ± 4.3*</td>
</tr>
<tr>
<td>MSNA incidence, bursts/100 heartbeats</td>
<td>33.5 ± 4.8</td>
<td>30.7 ± 4.3</td>
</tr>
<tr>
<td>MSNA amplitude, arbitrary units</td>
<td>0.29 ± 0.04</td>
<td>0.24 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE. $\text{VE}$, minute ventilation; MSNA, muscle sympathetic nerve activity. *Significance $P < 0.01$.

Fig. 3. A: cardiovagal baroreceptor sensitivity response to hyperoxia or hypoxia during PECO. B: cardiovagal baroreceptor sensitivity response to hypoxia at rest or during PECO. *Significant effect of hypoxia compared with baseline. †Significant difference between normoxia and hypoxia within PECO.

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**Table 2. Muscle sympathetic nerve activity responses**

<table>
<thead>
<tr>
<th>Burst incidence, bursts/100 heartbeats</th>
<th>Baseline HG PECO</th>
<th>Baseline HG PECO</th>
<th>Baseline HG PECO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burst amplitude, arbitrary units</td>
<td>0.30 ± 0.04</td>
<td>0.47 ± 0.07*</td>
<td>0.44 ± 0.09†</td>
</tr>
</tbody>
</table>

Values are means ± SE. * HG is significantly different from baseline; † PECO is significantly different from baseline; ‡ PECO is significantly different from HG; § difference between normoxia and hypoxia within PECO.
suggesting greater alveolar deadspace with hyperoxia. Despite randomization of trials, we did observe greater \( V_{\text{E}} \) during the handgrip trial immediately before hyperoxic PECO; however, we do not have a clear explanation for this observation as mean handgrip force was not different between trials. The greater \( V_{\text{E}} \) during handgrip exercise may have contributed to greater \( V_{\text{E}} \) during hyperoxic PECO. Of note, if we assume that PETCO\(_2\) approximates arterial CO\(_2\) in hyperoxia, the 6.3 mmHg reduction in PETCO\(_2\) during hyperoxic PECO is unlikely to explain the drop in MSNA relative to normoxia, as similar reductions in PETCO\(_2\) have been shown not to have an effect on MSNA (31). Therefore, while we did not control breathing frequency or PETCO\(_2\), we would suggest that our MSNA findings are unlikely to be explained by changes in CO\(_2\) or central chemoreceptor activity.

Pulse-oximetry on the forehead (SPO\(_2\)) was used as a surrogate measurement of arterial saturation of oxygen (SaO\(_2\)). SPO\(_2\) was used as a target because of our ongoing related work in heart and lung disease whereby end-tidal values do not represent arterial values well. Importantly, end-tidal O\(_2\) values were similar during the resting hypoxia trial (PETO\(_2\): 47 ± 1 mmHg) compared with the hypoxic PECO trial (PETO\(_2\): 49 ± 2 mmHg), indicating that the hypoxic/carotid chemoreceptor stimulus during resting hypoxia and hypoxic PECO was similar.

We did not apply circulatory occlusion during a rest period as a control; however, circulatory occlusion by itself has not demonstrated any cardiorespiratory effects (15). The cardiovascular and sympathetic responses to resting hypoxia were also not examined; however, previous work has shown that short-term hypoxia does not affect HR, blood pressure, \( V_{\text{E}} \), or MSNA (32, 34).

**Summary.** Our findings indicate that activation of the metaboreflex did not change the sensitivity of the carotid chemoreceptor as demonstrated by the ventilatory response to concurrent chemoreceptor and metaboreflex activation being equal to the two responses individually. However, activation of the metaboreflex appears to increase basal carotid chemoreceptor activity, as suppression of the carotid chemoreceptor with hypoxia during activation of the metaboreflex decreased MSNA burst frequency. Unexpectedly, activation of the carotid chemoreceptor with hypoxia during metaboreflex activation did not potentiate the MSNA response, possibly due to the sympathoinhibitory effects of increased ventilation.

**Perspectives and Significance**

We have found that the isolated metaboreflex (i.e., no input from the mechanoreflex or central command) can activate the carotid chemoreceptor. These results support a change in central integration of the carotid chemoreceptor feedback with metaboreflex activation and help explain previous evidence of carotid chemoreceptor activation during exercise despite no increase in circulating carotid chemoreceptor stimuli. Our investigation was only done in young healthy men; however, it would be of interest to investigate the interactions between the metaboreflex and the chemoreflex in young women, particularly considering that the metaboreflex is less sensitive in women (18). It would also be prudent to investigate these interactions in clinical populations such as chronic heart failure as it has been shown to enhance both the metaboreflex (14, 24) and chemoreflex (33, 36).

**ACKNOWLEDGMENTS**

The authors thank Desi Fuhr and Andrea Bui for excellent technical assistance.
GRANTS

This research was supported in part by grants from The Heart and Stroke Foundation of Canada, and the Canadian Institutes of Health Research to M. K. Stickland. M. K. Stickland is supported by a Heart and Stroke Foundation of Canada New Investigator Salary Award.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: H.E. and M.K.S. conception and design of research; H.E. performed experiments; H.E. analyzed data; H.E. and M.K.S. interpreted results of experiments; H.E. prepared figures; H.E. drafted manuscript; H.E. and M.K.S. edited and revised manuscript; H.E. and M.K.S. approved final version of manuscript.

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