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Jianguo Zhuang
Lovelace Respiratory Research Institute

Fadi Xu
Lovelace Respiratory Research Institute

Cancan Zhang
Lovelace Respiratory Research Institute

Donald T. Frazier
University of Kentucky, donald.frazier@uky.edu

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Passive limb movement augments ventilatory response to CO₂ via sciatic inputs in anesthetized rats

Jianguo Zhuang^a, Fadi Xu^{a,*}, Cancan Zhang^a, and Donald T. Frazier^b

^aPathophysiology Program, Lovelace Respiratory Research Institute, Albuquerque, NM 87108

^bDepartment of Physiology, University of Kentucky, Lexington, KY 40536

Abstract

Passive limb movement (PLM) in humans induces a phasic hyperpnea, but the underlying physiological mechanisms remain unclear. We asked whether PLM in anesthetized rats would produce a similar phasic hyperpnea associated with an augmented ventilatory ($\dot{V}_E$) response to CO₂ that is dependent on sciatic afferents. The animals underwent 5 min threshold PLM, 3 min hypercapnia (5% CO₂), and their combination (CO₂ exposure at the end of 2nd min of 5-min PLM) before and after bilateral transection of the sciatic nerves. We found that a threshold PLM evoked a phasic hyperpnea, similar to that denoted in humans, and an augmented ($\dot{V}_E$) response to CO₂. Both responses were greatly diminished by sciatic nerve transection. Moreover, similar responses were also evoked by electrically stimulating the central end of the transected sciatic nerve. Our findings suggest an ability of the sciatic afferents to augment the ($\dot{V}_E$) response to CO₂ that likely contributes to the PLM-induced hyperpnea.

Keywords

passive exercise; chemosensitivity; hypoxia; blood pressure; heart rate

1. Introduction

Passive exercise is achieved by passively moving individual's limbs in an exercise-like manner. Passive limb movement (PLM) for 5–10 min has been applied in humans with a resultant phasic hyperpnea characterized by an abrupt increase in minute ventilation ($\dot{V}_E$) (phase I) at the onset of exercise followed by a decline to a steady level higher (phase II) than that during rest (Dejours et al., 1959; Gozal et al., 1996; Bell and Duffin, 2003; Noah et al., 2008). The hyperpnea was achieved by increasing both tidal volume (V_T) and respiratory frequency (f_R) (Gozal et al., 1996) with the latter reported to be closely correlated to the passive movement frequency (Noah et al., 2008). It is well documented that the hyperpnea by PLM is associated with hypocapnia in both awake humans (Dejours, 1964; Bell and Duffin, 2003) and anesthetized animals (Agostoni and D'Angelo, 1976). Because hypocapnia is definitely inhibitory to $\dot{V}_E$, these results raise a fundamental physiological question as to why there is a hyperpnea during PLM-induced hypocapnia.

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*Correspondence: Fadi Xu, MD, Lovelace Respiratory Research Institute, 2425 Ridgcrest Dr SE, Albuquerque, NM 87108, Phone: 505-348-9565, Fax: 505-348-8567, Email: fxu@lrri.org.

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In contrast to active exercise, passive exercise causes less change in neurohumoral release, metabolism, and cardiovascular activities. Thus, passive exercise allows investigators to focus on effects of afferent feedback mechanisms during exercise. Several studies have examined neural components involved in passive exercise-induced hyperpnea, and concluded that in the absence of central volitional drive-induced neurohumoral modulation, afferent inputs from working limbs are responsible for the hyperpnea (Comroe and Schmidt, 1943; Gozal et al., 1996). It is generally accepted that similar to PLM, active exercise also causes hyperpnea. Interestingly, although a number of studies have been conducted to test the presence of active exercise-induced augmentation in the $\dot{V}_E$ response to CO_2 (Dejours, 1964; Forster, 2000; Dempsey et al., 2006; Poon et al., 2007), it has not been systematically studied whether PLM affects this $\dot{V}_E$ response, thereby contributing to the hyperpnea. In addition, previous studies demonstrated that limb movements elicited by stimulating muscle nerves, such as electrical stimulation of the sciatic nerve, evoked hyperpnea in anesthetized dogs, rabbits, and cats (Comroe and Schmidt, 1943; Lamb, 1968; Agostoni and D'Angelo, 1976). An early report further indicated that selective activation of the sciatic afferents by electrical stimulation of its central end also evokes hyperpnea in dogs (Henderson, 1910). It remains unclear whether the sciatic afferents play an important role in the augmented $\dot{V}_E$ response to CO_2 , if the augmented CO_2 response occurs during PLM.

In the present study, we tested the hypothesis that, as in the case of humans, PLM in anesthetized rats produces phasic $\dot{V}_E$ responses, and further, that PLM evokes an augmented $\dot{V}_E$ response to CO_2 . We also tested the hypothesis that the sciatic afferents is critical to generate the augmented $\dot{V}_E$ response to CO_2 during PLM.

2. Methods

Three series of experiments were carried out on a total of 22 rats. All procedures described in this study were conducted under protocols approved by the Institutional Animal Care and Use Committee at Lovelace Respiratory Research Institute. The institute is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

2.1. Animal preparations

The experiments were conducted in anesthetized, tracheotomized, and spontaneously breathing male Sprague-Dawley rats (body weight: 350 – 450 g). Anesthesia was induced using a mixture of chloralose and urethane (100 mg kg^{-1} and 500 mg kg^{-1} ; intraperitoneally), and supplemented as needed via the left femoral vein cannula to suppress corneal and withdrawal reflexes. The animals were exposed to rich-oxygen air (30% O_2) as the baseline control throughout the experiment unless otherwise stated. The right femoral artery was cannulated for monitoring and recording arterial blood pressure (ABP) and heart rate (HR) through a blood pressure transducer (ADInstruments MLT0380, Castle Hill, Australia). The trachea was cannulated below the larynx and connected to a pneumotachograph to measure the respiratory flow via a differential pressure transducer (ADInstruments ML141). The stainless-steel pneumotachograph had a linear flow-pressure relationship in the range of $0 - 20 \text{ ml s}^{-1}$ with a flow resistance of $0.046 \text{ cmH}_2\text{O s ml}^{-1}$ and a dead space of $\sim 0.2 \text{ ml}$. End-tidal CO_2 partial pressure (P_{ETCO_2}) was monitored via an infrared CO_2 analyzer (MicroCapStar, Model 15–10000, CWE Inc. Ardmore, PA). The core temperatures of the animals were monitored with a rectal probe and maintained at $\sim 37.5^\circ\text{C}$ by a heating pad and radiant heat.

2.2. Experimental protocol

Study series I was designed to determine the threshold PLM to evoke hyperpnea. Six rats were fixed in prone position by clamping the vertebrate column to a frame leaving the hind

limbs free for passive movement. PLM was achieved by passively pedaling the hind limbs (8 cm in distance) via an electrically driven “backward-forward” exercise device for 5 min. The PLM device consists of a custom made “forward-backward” ergocycle (Frank Mfg. Co. Albuquerque, NM) that is equipped with an electrically driven engine with pedaling frequencies from 0 – 250 rpm and a moving distance of 6 – 10 cm. Three levels of PLM at 100, 150, and 200 pedaling revolutions per min (rpm) were applied, and each level repeated once. These levels of PLM were utilized, since in our pilot study, 150 rpm was the threshold at which a detectable increase in $\dot{V}_E$ constantly occurred. Cardiorespiratory activities were continuously recorded throughout the experiment.

Study series II was conducted in eight other rats to investigate the PLM-induced augmented $\dot{V}_E$ response to CO_2 . The control $\dot{V}_E$ response to CO_2 was determined by exposing the rat to a gas mixture, containing 5% CO_2 mixed with 30% O_2 and balanced with N_2 , for 3 min. With respect to the $\dot{V}_E$ response to CO_2 during PLM, the protocols were similar to those described in *Study series I* with some modifications. Briefly, at the end of the 2nd min of threshold PLM (150 rpm), the animals were exposed to hypercapnia (~6% CO_2) for the following 3 min of PLM defined as PLM + CO_2 . The purpose of using this slightly higher CO_2 during PLM + CO_2 was to maintain the PET_{CO_2} at levels similar to those during CO_2 alone, since PLM caused hypocapnia as denoted in our pilot experiments. Two minutes of PLM before CO_2 application was chosen because hyperpnea became relatively stable thereafter.

Study series III was conducted in two groups of rats. The first group was used to verify whether the hyperpnea elicited by first 2 min PLM prior to application of CO_2 and the augmented $\dot{V}_E$ response to CO_2 were achieved mainly by stimulation of sciatic afferents. We compared the $\dot{V}_E$ response to the first 2 min of threshold PLM and the $\dot{V}_E$ response to PLM + CO_2 before and after bilateral transection of sciatic nerves in 5 of 8 rats used in *Series II*, and after subsequent bilateral transection of femoral nerves in 2 rats. The second group of rats ($n = 8$) was used to test whether electrical stimulation of the sciatic nerve (ESN) could also produce hyperpnea and the augmented $\dot{V}_E$ response to CO_2 . The right sciatic nerve was isolated via the dorsal route, transected, and placed on a bipolar electrode. Electrical stimulation for 5 min was applied to the central end of the transected nerve for 2 trials, while recording cardiorespiratory activities. Stimulating pulses were delivered from a stimulator (Grass, Model S88, Quincy, MA) with a current of 30 – 50 μA , and a pulse width of 0.2 ms at stimulating frequencies of 5 – 10 Hz. After determining the threshold ESN to evoke hyperpnea, the stimulating parameters were fixed throughout the experiment. Subsequently, the same CO_2 stimulation protocols used above were applied and repeated with ESN (ESN + CO_2). Finally, the hypercapnic protocols were replaced by 3 min of hypoxia (10% O_2 balanced with N_2) to define the specificity of the augmented $\dot{V}_E$ response to CO_2 .

2.3. Data acquisition and analysis

Baseline cardiorespiratory variables and their responses to PLM, ESN, 5% CO_2 , and 10% O_2 , and their combined stimulations were monitored and recorded continuously throughout the experiment. Raw signals of respiratory flow, ABP, inspired and expired CO_2 fractions, and rectal temperature were digitized and recorded using a PowerLab/8sp (ADInstruments) connected to a computer employing the PowerLab Chart 5 software (ADInstruments). Mean arterial blood pressure (MABP), HR , V_T , f_R , and $\dot{V}_E$ were derived by the on-line calculation functions of the software. The production of CO_2 $\dot{V}_{\text{CO}_2}$ was calculated from the expired CO_2 concentration and the corresponding respiratory flow rate. After the cardiorespiratory activities stabilized for at least 5 min, baseline values were collected for a period of 30 s immediately before stimulations. The response values were collected and averaged from a period of 10 s around the peak response. The baseline cardiorespiratory variables and their

responses to chemical challenges were expressed by absolute values and percentage change from values immediately before application of the given chemical stimulation ($\Delta\%$), respectively.

2.4. Statistics

All grouped data are presented as mean $\pm$ standard error (SE). One-way analysis of variance (ANOVA) with repeated tests were used to evaluate the cardiorespiratory responses ($\Delta\%$) to 5 min of PLM or ESN at the different time-points. Two-way ANOVA with repeated tests were applied to determine the significant differences of the responses ($\Delta\%$) to: (1) different degrees of PLM (over 5 min); (2) the threshold PLM or ESN with and without chemical challenge (5% CO₂ or 10% O₂) or their combination; and (3) threshold PLM/ESN alone or coupled with CO₂ before and after bilateral transection of sciatic nerves. If an overall ANOVA test indicated a significant difference, the data were then analyzed using Fisher's post hoc test for the difference between individual groups. P-values less than 0.05 were considered significant.

3. Results

3.1. PLM produces a phasic hyperpnea in anesthetized rats with threshold PLM at 150 rpm

In the present study, the baseline values of $\dot{V}_E$, V_T , f_R , P_{ETCO_2} , $\dot{V}_{CO_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ were 151 ± 9 ml min⁻¹, 2.1 ± 0.1 ml, 70 ± 3 breath min⁻¹, 38 ± 2 torr, 12.9 ± 0.3 ml kg⁻¹ min⁻¹, and 29 ± 2 , respectively, that are comparable to those previously reported in anesthetized rats by other investigators (Cragg and Drysdale, 1983). Fig. 1 compares the responses of $\dot{V}_E$, P_{ETCO_2} , $\dot{V}_{CO_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ to three levels of PLM stimulations at 100, 150, and 200 rpm in the anesthetized and spontaneously breathing rats. As shown, PLM at 100 rpm hardly altered these values, however, PLM at 150 and 200 rpm significantly increased $\dot{V}_E$ associated with a decreased P_{ETCO_2} and no change in $\dot{V}_{CO_2}$, leading to a significant elevation in $\dot{V}_E/\dot{V}_{CO_2}$. The $\dot{V}_E$ responses to 150 and 200 rpm were characterized by two phases: an abrupt $\dot{V}_E$ increase at the onset of PLM (phase I) followed by a transient decline (completed within 1 min) to a steady level above the baseline value (phase II). In addition, the increases of $\dot{V}_E$ and $\dot{V}_E/\dot{V}_{CO_2}$ in phase I (46% and 42%) and II (31% and 32%) elicited by PLM at 200 rpm were markedly greater than those (32% and 27%; 17% and 17%) evoked by 150 rpm ($P < 0.01$). These data indicate that the evoked hyperpnea is stimulation frequency-dependent, but mainly independent of any induced metabolic changes. It is noteworthy that the changes in P_{ETCO_2} mirror the changes in $\dot{V}_E$. Compared to control (before PLM), PLM at 200 and 150 rpm reduced P_{ETCO_2} by 5.3 and 3.8 torr during phase I, and by ~ 3.8 and ~ 2.7 torr during phase II, respectively.

3.2. Threshold PLM causes hyperpnea via increasing V_T and f_R and is associated with tachycardia

To further clarify the respiratory pattern and cardiovascular responses to the threshold PLM stimulation, we analyzed the responses of V_T , f_R , ABP, and HR. The typical experimental recordings obtained from an anesthetized rat undergoing PLM at 150 rpm and corresponding group data are exhibited in Fig. 2A and 2B, respectively. We found that the hyperpnea was primarily due to a significant elevation in f_R , particularly during phase II. HR initially was not significantly affected by the PLM, but it gradually increased reaching a significant plateau 3 min after the onset of PLM, while MABP did not show any remarkable change. After PLM termination, the evoked cardiorespiratory responses returned to pre-PLM baseline levels within 2–3 min.

3.3. PLM augments the $\dot{V}E$ response to hypercapnia

Cardiorespiratory responses to CO_2 and PLM alone, and PLM + CO_2 were compared and depicted in Fig. 3A (representative samples) and Fig. 3B (group data). As presented, compared to control (baseline), CO_2 and PLM increased $\dot{V}E$ by 118% and 15%, respectively, while PLM + CO_2 elevated the $\dot{V}E$ by 216%. Clearly, the $\dot{V}E$ response to PLM + CO_2 was significant higher (62%) than the summation of the responses to CO_2 and PLM alone. Meanwhile, the increases in $P_{ET}CO_2$ during CO_2 or PLM + CO_2 were not significantly different. It should be emphasized that both the V_T and f_R responses to CO_2 were amplified by PLM ($P < 0.05$) with a greater effect on the f_R response, while cardiovascular responses to hypercapnia (increased MABP and decreased HR) were not significantly altered by PLM.

3.4. PLM-induced hyperpnea and the augmented $\dot{V}E$ response to CO_2 depend on sciatic afferents

As presented above, 2 min of PLM significantly increased the $\dot{V}E$ response to CO_2 ; thus, we asked whether the hyperpnea within this time-frame of PLM was dependent on afferent nerves emanating from the moving limbs. The cardiorespiratory responses to 2 min PLM before and after bilateral transection of the sciatic nerves are illustrated in Fig. 4A. As shown, the PLM-induced hyperpnea was greatly attenuated by bilateral transection of the sciatic nerves, and essentially eliminated by subsequent transection of the femoral nerves (not shown). We also compared the $\dot{V}E$ response to PLM + CO_2 before and after bilateral transection of the sciatic nerves, and found that the augmented response was markedly diminished by the transection (Fig. 4B). Transection of the sciatic nerves results in a reduction of the $\dot{V}E$ response to PLM + CO_2 through a decrease in both f_R and V_T with a greater effect on the former than the latter. In contrast, the transection did not substantially alter the cardiovascular responses to PLM + CO_2 .

3.5. ESN also evokes hyperpnea

Hyperpnea was induced by unilateral electrical stimulation of the central end of the transected sciatic nerve (30 – 50 μA , 5–10 Hz) for 5 min (Fig. 5). Interestingly, the hyperpnea was also characterized by an abrupt increase at the onset of the electrical stimulation followed by a decline to a plateau higher than that at the resting state. Moreover, the presence of a gradual increase in HR with no change in MABP was also observed in response to ESN. As ESN was only applied to the central end of the transected sciatic nerve in the present study, no detectable muscular movement was observed in the anesthetized rats.

3.6. ESN elevates the $\dot{V}E$ response to hypercapnia but not to hypoxia

We compared the cardiorespiratory responses to CO_2 , ESN, and ESN + CO_2 (Fig. 6). Hypercapnia and ESN elevated $\dot{V}E$ by 135% and 16%, whereas ESN + CO_2 increased $\dot{V}E$ by 244%, indicating that the $\dot{V}E$ response to ESN + CO_2 was higher (61%) than the sum of the responses to CO_2 and ESN alone. This amplification of the $\dot{V}E$ response was primarily achieved by an enhanced f_R response associated with an increase in MABP. The V_T , HR, and $P_{ET}CO_2$ responses to hypercapnia were not significantly affected by ESN. Surprisingly, the $\dot{V}E$ responses to hypoxia were not markedly affected by ESN (Fig. 7). Hypoxia caused an increase in $\dot{V}E$, V_T , f_R , and decrease in $P_{ET}CO_2$, and all of these responses were not significantly changed by ESN.

4. Discussion

There are two new findings reported in this PLM study conducted in anesthetized and spontaneously breathing rats. We found that PLM for 5 min produced not only a phasic

hyperpnea correlated with the stimulating frequency, but also an augmented $\dot{V}E$ response to CO_2 . We further found that the PLM-induced augmentation of the $\dot{V}E$ response to CO_2 was largely dependent on the integrity of the sciatic afferents.

4.1. The major features of hyperpnea observed in anesthetized rats are qualitatively similar to those denoted in conscious humans

Previous studies in humans have shown that passive movement for 5 – 10 min brings about phasic $\dot{V}E$ responses characterized by an abrupt increase in $\dot{V}E$ followed by a gradual (several min) decline to a relatively constant level above the baseline (Dejours et al., 1959; Gozal et al., 1996; Bell and Duffin, 2003; Noah et al., 2008). With respect to the respiratory pattern, the passive movements increased $\dot{V}E$ by elevating both V_T and f_R (Gozal et al., 1996). A recent study further pointed out that this hyperpnea primarily resulted from an elevation in f_R since the f_R but not V_T responses were positively correlated with the passive moving frequency (Noah et al., 2008). PLM was first used by Harrison, et al. (1932) and subsequently by others (Flandrois et al., 1967) in anesthetized dogs. Unfortunately, the duration of PLM in these studies was very short (less than 1 min) that only produced phase I response featured by an immediate increase in $\dot{V}E$ (~30%) through enhancing both V_T and f_R . In agreement with the results from humans, we found that in anesthetized rats 5 min of PLM at 150 and 200 rpm produced an abrupt increase in $\dot{V}E$ (32–46%) predominantly via increasing f_R within several seconds after the onset of the stimulation. This phase I response was followed by a quick (within 1 min) decline to a relatively constant level (17–30%) above the baseline (phase II). In comparison, although the pattern was similar, the peak response during phase I was higher (~60%) and decline duration longer (several minutes) in humans (Gozal et al., 1996; Bell and Duffin, 2003; Noah et al., 2008). This quantitative discrepancy may be due to the differences in species, exercise modes (e.g., parallel moving vs. cycling), states (anesthetized vs. awake), and the stimulating frequency of the passive movements. In addition, we are aware that a 2.5-fold higher rpm is required to produce the detectable hyperpnea in the rat (150 rpm) than that in humans (60 rpm). The fact that a higher rpm is required for rats seems reasonable because rats have faster pace of limb movement, compared to the humans, owing its much shorter pace distance. Another potential variable is the use of anesthesia, a potent inhibitor to ventilatory chemoreflexes (Nattie, 2001), that could increase the threshold needed for evoking the detectable hyperpnea. Collectively, our data, for the first time, show that the major features of hyperpnea noted in conscious humans and anesthetized rats are qualitatively similar. The importance of this result is to establish an animal model, by which we can further elucidate the physiologic mechanisms underlying PLM-induced hyperpnea.

4.2. Sciatic afferents play an important role in the PLM-induced hyperpnea in anesthetized rat

Several lines of evidence from this study suggest the involvement of afferent nerves from working limbs in the genesis of the hyperpnea. First, the PLM-induced hyperpnea was markedly attenuated by bilateral transection of the sciatic nerves and almost abolished by subsequent transection of femoral nerves. Our data are similar to the previously reported results in which transection of the nerves innervating the exercising muscles (Comroe and Schmidt, 1943; Flandrois et al., 1967; Russo et al., 1977; Tallarida et al., 1985) or spinal lesions substantially attenuated or even abolished the exercises-induced hyperpnea in dogs (Harrison et al., 1932), cats (Weissman et al., 1980) and humans (Weissman et al., 1980; Brice et al., 1988). Second, ESN was applied in this study to activate only sciatic afferent fibers without muscle contraction and it produced a phasic hyperpnea, consistent with an early report conducted in dogs (Henderson, 1910). Indeed, the limb movements elicited by electrical stimulation of nerves innervating limb muscles, such as the sciatic and

gastrocnemius nerves, have been reported to cause hyperpnea in rabbits (Raimondi et al., 1996), cats (Parrish et al., 1991), and dogs (Bennett, 1984; Haxhiu et al., 1984). Third, PLM-evoked an immediate increase in $\dot{V}_E$ that was not associated with a significant change in $\dot{V}_{CO_2}$ indicates little or no effect on metabolism. In support, several investigators have indicated that PLM induces no alteration in O_2 consumption (Russo et al., 1977) or in volitional drive-induced neurohumoral changes (Gozal et al., 1996). In this study and other studies conducted in humans and animals, PLM produced tachycardia with little effect on MABP (Fisher and Nutter, 1974; Hansen et al., 1994; Miyamura et al., 1997; Smith et al., 2001). However, the hyperpnea observed in this study is not secondary to the associated changes in HR. We found that the threshold PLM-induced $\dot{V}_E$ increase occurs earlier than the HR elevation, and the pattern of the $\dot{V}_E$ response (an abrupt elevation followed by a decline) differs from that observed in HR (gradual increase).

4.3. PLM augments the $\dot{V}_E$ response to CO_2 dependent on the integrity of the sciatic afferents

A major finding in this study is that PLM significantly augments the $\dot{V}_E$ response to hypercapnia (62% greater than the sum of the responses to CO_2 and PLM alone), suggesting a multiplicative effect of PLM + CO_2 on the $\dot{V}_E$ response. Our observation that the augmented $\dot{V}_E$ response to CO_2 is greatly reduced by bilateral transection of the sciatic nerves clearly suggests that PLM increases $\dot{V}_E$, at least partially, via a central reflex triggered by PLM activation of sciatic afferent fibers. This assumption is further supported by our data that the ESN also produces an augmented $\dot{V}_E$ response to CO_2 . PLM-strengthened CO_2 -driven breathing is also observed in the clinic. Children with congenital central hypoventilation syndrome (CCHS) present with ineffective CO_2 -driven breathing and severe hypoventilation. Such patients have equivalent spontaneous $\dot{V}_E$ similar to normal subjects during PLM (Shea et al., 1993a; Shea et al., 1993b; Gozal et al., 1996). In contrast, one report indicated that passive movement produced a hyperpnea associated with hypocapnia (reduction of $P_{ET}CO_2$ by 2 – 3 torr) in humans, and this hyperpnea was not strikingly changed during isocapnic passive movement (Bell and Duffin, 2003). This absence of an augmented $\dot{V}_E$ response to CO_2 was most likely related to the relative small hypercapnic stimulation (increasing $P_{ET}CO_2$ by 2 – 3 torr) compared to ours (increasing $P_{ET}CO_2$ by ~12 torr). Consistent with our finding, previous studies have pointed out a regulatory influence of peripheral inputs on CO_2 sensitivity. For example, the $\dot{V}_E$ (respiratory-related motor) response to CO_2 was greatly enhanced by stimulation of vagal afferents in bullfrogs (Kinkead et al., 1994) and carotid body in rats (Takakura et al., 2006). Revealing the interaction between neural inputs from working limbs and CO_2 sensitivity adds to our basic understanding of the physiological mechanisms involved in the control of breathing. Because the hyperpnea by PLM is associated with hypocapnia in the present study similar to the previous results (Dejours, 1964; Agostoni and D'Angelo, 1976; Bell and Duffin, 2003), the role of an augmented CO_2 sensitivity in the $\dot{V}_E$ response becomes particularly important. We believe that the augmented CO_2 sensitivity not only offsets the weakened CO_2 stimulation (hypocapnia during PLM), but also contributes to hyperpnea. In addition, while different from PLM, active exercise increases $\dot{V}_E$ in the presence of a constant arterial CO_2 tension in humans. Both types of exercise produce hyperpnea that is dependent on the nerve afferents from working limbs. Therefore, our data show that similar to PLM, the increased CO_2 sensitivity is partially involved in the hyperpnea by active exercise. In fact, some investigators have demonstrated an increased $\dot{V}_E$ response to CO_2 elicited by active exercise, although this view is not agreed by the others (Dejours, 1964; Forster, 2000; Dempsey et al., 2006; Poon et al., 2007).

4.4. What is the possible central mechanism underlying the PLM-induced augmentation of the $\dot{V}_E$ response to CO_2 ?

It remains unknown how PLM centrally amplifies the $\dot{V}_E$ response to CO_2 . It has been well-documented that some central nuclei containing CO_2/H^+ chemoreception responsible for the $\dot{V}_E$ response to CO_2 receive inputs from the limbs. For example, the cerebellar deep nuclei, such as the fastigial nucleus, contain CO_2/H^+ chemosensitive neurons and participate in the $\dot{V}_E$ response to CO_2 (Xu et al., 2001; Xu and Frazier, 2002). Moreover, these structures also receive heavy inputs from limbs and are closely related to exercise (Xu and Frazier, 2002; Holschneider et al., 2007). Thus, it is plausible that the peripheral inputs from moving muscles are able to sensitize cerebellar local chemosensitive neurons and/or to facilitate the ascending pathways to or the descending pathways from cerebellar chemosensitive neurons, leading to the augmented $\dot{V}_E$ response to CO_2 . This assumption is indirectly supported by Macey and colleagues who indicated that the impairment of $\dot{V}_E$ responses to CO_2 in patients with *cchs* is associated with abnormal neuronal activity in the cerebellar cortex and fastigial nucleus (Macey et al., 2004).

In summary, our data showed that in anesthetized rats, PLM yields a stimulation frequency-dependent phasic hyperpnea and an augmented ventilatory responsiveness to CO_2 primarily via activating the sciatic afferents. These results suggest that PLM enhances CO_2 -chemoreflex via activation of limb afferents, especially the sciatic afferents, contributing to the resultant hyperpnea.

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Fig. 1.

The ventilatory responses elicited by 5 min passive limb movement (PLM) at 100, 150 and 200 rpm. The traces from the top to bottom are the responses of $\dot{V}_E$ (minute ventilation), P_{ETCO_2} (end-tidal CO_2 partial pressure), $\dot{V}_{\text{CO}_2}$ (CO_2 production), and $\dot{V}_E / \dot{V}_{\text{CO}_2}$ (CO_2 -ventilatory equivalent); $n = 6$; Mean $\pm$ SE; * $P < 0.05$, compared with the baseline values (0%); † $P < 0.05$, 200 rpm vs. 150 rpm. Note: the data, except $\dot{V}_{\text{CO}_2}$, obtained from PLM at 200 and 150 rpm were significantly different from that from PLM at 100 rpm ($P < 0.01$).

Fig. 2.

The respiratory patterns and cardiovascular activities in response to passive limb movement (PLM) at 150 rpm. Typical recordings of cardiorespiratory responses elicited by 5 min of PLM in an anesthetized rat are presented in panel A. The traces from the top to bottom in panel A are arterial blood pressure, ABP; heart rate, HR; minute ventilation, $\dot{V}_E$; tidal volume, V_T ; respiratory frequency, f_R ; and end-tidal CO_2 partial pressure, P_{ETCO_2} . The corresponding group data are illustrated in panel B (see also Fig. 1). $n = 6$; Mean $\pm$ SE; * $P < 0.05$, compared with the baseline values (0%).

Fig. 3.

The augmented $\dot{V}_E$ responses to 5% CO₂ elicited by PLM. Panel A shows the typical experimental recordings of the cardiorespiratory responses to 5% CO₂ PLM or PLM + CO₂ in one rat and panel B presents the corresponding group data. The traces from the top to bottom in panel A are arterial blood pressure, ABP; heart rate, HR; minute ventilation, $\dot{V}_E$; tidal volume, V_T ; respiratory frequency, f_R ; and end-tidal CO₂ partial pressure, P_{ETCO_2} . $n = 8$; Mean $\pm$ SE; * $P < 0.05$, stimulation-evoked responses vs. the baseline values expressed by 0%; † $P < 0.05$, PLM + CO₂ vs. CO₂ or PLM alone; # $P < 0.05$, PLM + CO₂ vs. the sum of CO₂ and PLM alone. MABP, mean arterial blood pressure; HR, heart rate.

Fig. 4.

Group data showing the effects of bilateral transection of sciatic nerves (SNX) on the PLM-induced hyperpnea (panel A) and the cardiorespiratory responses to 5% CO₂ for 3 min (B). The traces from the top to bottom in panel A are mean arterial blood pressure, MABP; heart rate, HR; minute ventilation, $\dot{V}_E$; tidal volume, V_T ; respiratory frequency, f_R ; and end-tidal CO₂ partial pressure, P_{ETCO_2} . $n = 5$. * $P < 0.05$, compared with the control values (0%) that are the baseline values for CO₂ alone (Panel A) and the variables collected at the end of 2 min PLM prior to CO₂ exposure (B); † $P < 0.05$, SNX vs. SN intact. SNX, bilateral sciatic nerves transected; PLM, passive limb movement.

Fig. 5.

Group data showing the effects of unilateral electrical stimulation of the central end of the transected sciatic nerve (ESN) on cardiorespiratory activity in anesthetized rats. The traces from the top to bottom are mean arterial blood pressure, MABP; heart rate, HR; minute ventilation, $\dot{V}_E$; tidal volume, V_T ; respiratory frequency, f_R ; end-tidal CO_2 partial pressure, P_{ETCO_2} ; CO_2 production, $\dot{V}_{CO_2}$; and CO_2 -ventilatory equivalent, $\dot{V}_E/\dot{V}_{CO_2}$. $n = 8$; Mean $\pm$ SE; * $P < 0.05$, evoked response vs. baseline "0%".

Fig. 6.

Comparison of the cardiorespiratory responses to 5% CO₂ ESN or ESN + CO₂. ESN, unilateral electrical stimulation of the central end of the severed sciatic nerve; n = 8; Mean ± SE; * P < 0.05, stimulation-evoked responses vs. baseline values (0%); † P < 0.05, ESN + CO₂ vs. CO₂ or ESN alone; # P < 0.05, PLM + CO₂ vs. the sum of CO₂ and PLM alone. Mean arterial blood pressure, MABP; heart rate, HR; minute ventilation, $\dot{V}_E$; tidal volume, V_T ; respiratory frequency, f_R ; end-tidal CO₂ partial pressure, $P_{ET}CO_2$.

Fig. 7.

Comparison of the cardiorespiratory responses to 10% O₂, ESN or ESN + 10% O₂. ESN, unilateral electrical stimulation of the central end of the severed sciatic nerve; $n = 8$; Mean $\pm$ SE; * $P < 0.05$, stimulation-evoked responses vs. baseline values (0%). Mean arterial blood pressure, MABP; heart rate, HR; minute ventilation, $\dot{V}_E$; tidal volume, V_T ; respiratory frequency, f_R ; end-tidal CO₂ partial pressure, P_{ETCO_2} .