Group III and IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in humans

Markus Amann,1,2 Gregory M. Blain,1 Lester T. Proctor,3 Joshua J. Sebranek,3 David F. Pegelow,1 and Jerome A. Dempsey4

1John Rankin Laboratory of Pulmonary Medicine, University of Wisconsin, Madison, Wisconsin; 2Department of Internal Medicine, University of Utah, Salt Lake City, Utah; and 4Department of Anesthesiology, University of Wisconsin, Madison, Wisconsin

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Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA. Group III and IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in humans. J Appl Physiol 109: 966–976, 2010. First published July 15, 2010; doi:10.1152/japplphysiol.00462.2010.—We investigated the role of somatosensory feedback on cardioventilatory responses to rhythmic exercise in five men. In a double-blind, placebo-controlled design, subjects performed the same leg cycling exercise (50/100/150/325 W, 3 min each) under placebo conditions (interspinous saline, 20 ml) and with lumbar intrathecal fentanyl impairing central projection of spinal opioid receptor-sensitive muscle afferents. Quadriceps strength was similar before and after fentanyl administration. To evaluate whether a cephalad migration of fentanyl affected cardioventilatory control centers in the brain stem, we compared resting ventilatory responses to hypercapnia (HCVR) and cardioventilatory responses to arm vs. leg cycling exercise after each injection. Similar HCVR and minor effects of fentanyl on cardioventilatory responses to arm exercise excluded direct medullary effects of fentanyl. Central command during leg exercise was estimated via quadriceps electromyogram. No differences between conditions were found in resting heart rate (HR), ventilation [minute ventilation (V̇e)], or mean arterial pressure (MAP). Quadriceps electromyogram, O2 consumption (V̇O2), and plasma lactate were similar in both conditions at the four steady-state workloads. Compared with placebo, a substantial hypoventilation during fentanyl exercise was indicated by the 8–17% reduction in V̇O2/C02 production (V̇CO2) secondary to a reduced breathing frequency, leading to average increases of 4–7 Torr in end-tidal PCO2 (P < 0.001) and a reduced hemoglobin saturation (−3 ± 1%; P < 0.05) at the highest workload (~90% maximal V̇O2) with fentanyl. HR was reduced 2–8%, MAP 8–13%, and ratings of perceived exertion by 13% during fentanyl vs. placebo exercise (P < 0.05). These findings demonstrate the essential contribution of muscle afferent feedback to the ventilatory, cardiovascular, and perceptual responses to rhythmic exercise in humans, even in the presence of unaltered contributions from other major inputs to cardioventilatory control.

hyperpnea; control of breathing; neural feedback

OUR AIM WAS TO ASSESS the contribution of group III and IV afferent neurons innervating contracting locomotor muscles to circulatory and ventilatory control during rhythmic cycling exercise in healthy humans. There is substantial evidence supporting significant cardiovascular and ventilatory effects of this feedback pathway when studied in isolation. These findings include 1) increased impulse activity of group III and IV locomotor muscle afferents during walking in the decerebrate cat (2) and during and after fatiguing muscular contractions in anesthetized rabbits (21, 22); 2) increases in heart rate (HR) following electrical stimulation of hindlimb group III and IV muscle afferents in anesthetized vagotomized cats (64); 3) normal ventilatory responses to limb muscle electrical stimulation (which were subsequently prevented via spinal cord lesioning) in anesthetized dogs whose humoral influences on ventilatory control were controlled via cross-circulation techniques (41); 4) similar ventilatory responses during human voluntary limb exercise compared with those measured when the limb muscle contractions were evoked via electrical motor nerve stimulation sufficient to increase CO2 production (V̇CO2) about twofold from resting level (9); 5) significant impact of local anesthetic blockade, or denervation of the dorsal roots, on the cardiovascular and ventilatory responses to isometric muscle contractions evoked by electrical spinal ventral root stimulation in anesthetized and decerebrate cats (18, 51) and to dynamic muscle contractions evoked by electrical nerve stimulation in anesthetized dogs (72); and 6) in vitro evidence in the isolated brain stem-spinal cord preparation showing that lumbar locomotor networks can rhythmically entrain medullary respiratory neurons (56).

Despite these important observations, which firmly established the existence of limb afferent activity in response to muscle contraction and their significant cardioventilatory effects, we are unsure of their relative importance in the intact exercising human largely because of the coexistence of strong feedforward influences from the higher central nervous system (CNS), i.e., so-called “central command” (25, 49). Furthermore, it is controversial as to whether afferent feedback from exercising muscles contributes significantly to cardiovascular responses to normal rhythmic exercise or whether the functional significance of this input is limited only to conditions of muscle ischemia as produced in sustained isometric muscle contractions with compromised blood flow (63).

To address the relative importance of the feedback influence from contracting muscles to cardioventilatory responses in the exercising human, local anesthetics (e.g., lidocaine) have been injected into the lumbar epidural space to block significant amounts of sensory feedback from the lower limbs (3, 27, 36, 55). Unfortunately, local anesthetics also reduce efferent nerve activity to the limbs, thereby “weakening” the locomotor musculature. As a consequence of the weakened musculature and in order to achieve the identical work rate, subjects are required to increase central motor command. In turn, augmented central command will exert powerful “feedforward” influences on cardiovascular and ventilatory regulation during exercise (25, 73) as demonstrated experimentally in humans.
via curare-induced (partial) muscle paralysis studies (8, 31, 57). Accordingly, epidural anesthesia creates a condition during constant-load exercise of reduced feedback in the face of increased feedforward influences and is therefore unsatisfactory to assess the potential contribution from limb feedback mechanisms.

We have chosen an approach using lumbar intrathecal injection of the selective μ-opioid receptor agonist fentanyl to attenuate the central projection of lower limb muscle afferent nerve fibers, most of which synapse on cells in the lumbar dorsal horn of the spinal cord (39, 53). These dorsal horn cells, in turn, relay to neural circuits in both the ventral lateral medulla and the nucleus tractus solitarii, where they influence breathing as well as both sympathetic and parasympathetic cardiovascular control (19). Stimulation of spinal opioid receptors, as found in the superficial dorsal horn (12, 13) and on primary afferent fibers (28), is known to inhibit transmission of nociceptive, mechanosensitive, and metabosensitive input from group III and IV afferents to the spinal cord (28, 40, 52, 78–80) without affecting the force-generating capacity of skeletal muscle (4, 33, 68). Intrathecal injection of opioid receptor agonists has been shown to reduce the group III/IV afferent-dependent HR, blood pressure, and ventilatory responses to isometric limb contractions in anesthetized cats (35, 52) and to ischemic muscle exercise in awake dogs (59). We recently reported (4) the effects of intrathecal fentanyl on central motor drive and locomotor muscle fatigue during the performance of a cycling time trial test. In this study we observed, serendipitously, a substantial fentanyl-induced hyperventilation during the time trial.

On the basis of these background findings, we have used lumbar intrathecal fentanyl in humans to achieve partial blockade of sensory afferents from contracting muscle in order to assess the contribution of these afferents to the cardioventilatory response to rhythmic lower limb cycling exercise over a wide range of intensities. To account for potential secondary actions of opioids on the central nervous control of ventilation (45) [e.g., via cephalad migration of fentanyl within the cerebrospinal fluid (CSF) to the medulla (70)] we also measured I) the ventilatory response to hypercapnia at rest; 2) the cardioventilatory response to upper versus lower body exercise; and J) circulating levels of fentanyl. Our findings support a significant contribution of muscle afferents to the control of ventilation, HR, and blood pressure during rhythmic steady-state exercise in the intact human.

METHODS

Subjects

Seven moderately trained male cyclists volunteered to participate in these studies [age 23.9 ± 3.6 yr, body mass 74.0 ± 8.3 kg, stature 1.80 ± 0.04 m, maximal O2 consumption (V̇O2max) 66.0 ± 2.4 ml·kg⁻¹·min⁻¹]. Written informed consent was obtained from each participant. The investigation consisted of a lower body and an upper body exercise component; the same set of subjects participated in both parts. All procedures were approved by the Institutional Review Board and conformed to the Declaration of Helsinki.

Protocol

Lower body exercise. During preliminary visits, all participants were thoroughly familiarized with hypercapnic ventilatory response (HCVR) measurements and bicycling exercise. Furthermore, each subject performed a maximal incremental exercise test [20 W + 25 W/min (6)] on a bicycle ergometer (Velotron, Elite Model, Racer Mate, Seattle, WA) for the determination of peak power output (Wpeak) and V̇O2max. On two separate days, double blind and in random order, all subjects performed constant-load bicycle exercise either under placebo conditions including a sham injection of neutral saline into the ligaments between the processus spinosi of the vertebral column or under experimental conditions including intrathecal fentanyl applied through the same vertebral interspace. The bicycle ergometer exercise was identical on both days and consisted of 50, 100, and 150 W for 3 min each, followed by 4 min against 80% of Wpeak (325 ± 19 W). There was no break between each workload. At exercise onset subjects were instructed to slowly pick up their pace, and the recording period started after the individual target pedal cadence (101 ± 5 rpm), as determined during the practice session, was reached (<10 s). To determine whether fentanyl migrated within the CSF to reach the medulla, HCVR measurements were conducted in each subject before and after 10–25 min after the fentanyl/placebo injection. As previously described (4), we also tested the effects of intrathecal fentanyl on the resting maximal voluntary contraction force (MVC) of the quadriceps by comparing MVCs measured before to those measured 5–10 min after the injection of fentanyl/placebo (but before exercise).

Upper body exercise. During preliminary visits, the same participants were thoroughly familiarized with upper body ergometry (arm cycling; Pro 2, SciFit, Tulsa, OK). On two separate days, double blind and in random order, all subjects performed constant-load exercise under either placebo or fentanyl conditions (see above). The exercise was identical on both days and consisted of 25, 50, and 75 W for 3 min each. There was no break between the first and the second workload, but subjects rested for ~3 min before the final workload. The target cadence was set at 60 rpm, and the recording period started after this cranking frequency was reached (<10 s).

The exercise sessions in each component of the study were separated by at least 48 h and were completed at the same time of day. In both parts of the study, the duration from the fentanyl/placebo injection to the end of exercise was <60 min. Subjects were instructed to refrain from caffeine for 12 h and from stressful exercise for 48 h before each exercise trial. Ambient temperature and relative humidity were not different between conditions.

Exercise Responses

Ventilation and pulmonary gas exchange were measured breath by breath at rest and throughout exercise with an open-circuit system including two pneumotachographs (Hans Rudolph, model 3800) (inspiration, expiration) and two Perkin-Elmer mass spectrometers (model 1100) for the analysis of mixed expired and end-tidal gases. Arterial O2 saturation (SpO2) was estimated with a pulse oximeter (Nellcor N-595, Pleasanton, CA) with adhesive forehead sensors. HR was measured from the R-R interval of an electrocardiogram with a three-lead arrangement. Ratings of perceived exertion (RPE) were obtained at rest and during the final 30 s of each workload (leg exercise only) with Borg’s modified CR10 scale (14). Arterialized (Fingal, Boehringer Ingelheim, Germany) capillary blood samples were collected from the earlobe at rest and during the final 30 s of each workload for determination of whole blood lactate concentration ([La]b) with an electrochemical analyzer (YSI 1500 Sport). During the lower body exercise part of the study, systolic and diastolic blood pressure were manually measured on the left arm at rest and during the last minute of each workload. Mean arterial pressure (MAP) was calculated as diastolic pressure + 1/3 × (systolic pressure − diastolic pressure). To determine a potential diffusion of fentanyl into the systemic circulation, venous blood samples were drawn ~40 min after the placebo/fentanyl injection and again immediately after the exercise, i.e., ~60 min after injection. The blood samples were stored and
later analyzed for fentanyl content via liquid chromatography-tandem mass spectrometry (37).

**Hypercapnic Ventilatory Response Measurement**

CO$_2$ rebreathing tests were performed before and 10–25 min after the fentanyl/placebo injection. Participants sat comfortably in a chair while breathing through a mouthpiece. Upon reaching a steady state of eupneic air breathing (<3 min), a valve close to the mouthpiece was turned to switch the subjects into a rebreathing circuit. The rebreathing circuit was initially filled with a gas mixture consisting of 3% CO$_2$, 27% N$_2$, and 70% O$_2$, and the bag volume was set at 0.5 liters above the subject’s vital capacity. Subjects rebreathed the gas mixture until end-tidal PCO$_2$ (PETCO$_2$) reached ~55 Torr. The measurements were repeated three times, and the tests were separated by at least 5 min of exposure to room air to allow ventilatory variables to return to baseline levels. The HCVR response slope for each trial [change in minute ventilation (ΔVE)/ΔPETCO$_2$] was determined by applying a best-fit regression line across the linear range of response of VE vs. PETCO$_2$ throughout the rebreathing period.

**Surface electromyography.** During the leg exercise high workload, quadriceps electromyogram (EMG) was recorded from the right vastus lateralis with electrodes with full-surface solid adhesive hydrogel (Kendall H59P, Mansfield, MA) and on-site amplification (5). Raw EMG signal from the vastus lateralis corresponding to each muscle contraction was recorded for later analysis. The EMG signal was amplified and filtered by a Butterworth band-pass filter (BMA-830, CWE, Ardmore, PA) with a low-pass cutoff frequency of 10 Hz and a high-pass cutoff frequency of 1 kHz. The slope of the filters was ~6 dB/octave. The filtered EMG signals were sampled at 2 kHz by a 16-bit A/D converter (PCI-MIO-16XE-50, National Instruments, Austin, TX) with custom software (Labview 6.0, National Instruments). A computer algorithm identified the onset of activity where the rectified signal crossed the moving average of 30 ms. Each EMG burst was inspected to verify the timing identified by the computer algorithm identified the onset of activity where the rectified signal crossed the moving average of 30 ms. Each EMG burst was inspected to verify the timing identified by the computer algorithm.

A two-way analysis of variance (ANOVA) with repeated measures was performed to evaluate differences between the placebo and fentanyl trials. A least significant difference test identified the means that were significantly different with P < 0.05. Results are expressed as means ± SE.

**RESULTS**

**Resting Ventilatory Responses to CO$_2$, Cutaneous Hypoaesthesia, and Venous Fentanyl Concentrations**

Table 1 shows individual effects of placebo and fentanyl on HCVR slopes measured before and 10–20 min after the injection. Because of the substantial impact of fentanyl on the HCVR slope of subjects 6 and 7, the exercise data sets of both individuals were excluded from our analyses of fentanyl effects on the group mean exercise responses (see data on these subjects at the end of RESULTS).

Neurological examinations just before the start of the exercise revealed cutaneous hypoaesthesia to pinprick and cold perception below T2–T3 in five of the seven subjects. This was evident by sensory changes on the torso at— or below— T2 and by the absence of sensory changes on the upper limbs.

**Table 1. Individual effects of placebo and fentanyl on HCVR slopes measured before and 10–20 min after injection**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Preplacebo</th>
<th>Postplacebo</th>
<th>% Change</th>
<th>Prefentanyl</th>
<th>Postfentanyl</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.59</td>
<td>2.44</td>
<td>-6</td>
<td>1.75</td>
<td>1.64</td>
<td>-6</td>
</tr>
<tr>
<td>2</td>
<td>1.78</td>
<td>1.71</td>
<td>-4</td>
<td>1.71</td>
<td>1.72</td>
<td>+1</td>
</tr>
<tr>
<td>3</td>
<td>2.17</td>
<td>1.91</td>
<td>-12</td>
<td>1.60</td>
<td>1.52</td>
<td>-5</td>
</tr>
<tr>
<td>4</td>
<td>1.32</td>
<td>1.70</td>
<td>+29</td>
<td>1.93</td>
<td>2.02</td>
<td>+5</td>
</tr>
<tr>
<td>5</td>
<td>3.71</td>
<td>3.91</td>
<td>+6</td>
<td>5.28</td>
<td>4.79</td>
<td>-9</td>
</tr>
<tr>
<td>Mean ± SE*</td>
<td>2.31 ± 0.41</td>
<td>2.33 ± 0.42</td>
<td>2.45 ± 0.71</td>
<td>2.35 ± 0.62</td>
<td>4.53</td>
<td>+6</td>
</tr>
<tr>
<td>6</td>
<td>4.43</td>
<td>4.70</td>
<td>+6</td>
<td>2.71</td>
<td>2.73</td>
<td>-40</td>
</tr>
<tr>
<td>7</td>
<td>2.76</td>
<td>2.73</td>
<td>-1</td>
<td>1.81</td>
<td>1.81</td>
<td>-33</td>
</tr>
</tbody>
</table>

HCVR, hypercapnic ventilatory response; VE, minute ventilation; PETCO$_2$, end-tidal PCO$_2$. *P = 0.87 for preplacebo vs. postplacebo and P = 0.32 for prefentanyl vs. postfentanyl.

**Intrathecal Fentanyl**

A 20-gauge intravenous catheter was inserted, and a bolus of ~500 ml of normal saline was infused as a precaution to correct any potential drop in blood pressure (66). The subjects were placed in the flexed sitting position, and the skin and subcutaneous tissue were anesthetized at the L$_1$–L$_4$ vertebral interspace with 2–4 ml of 1% (10 mg/ml) lidocaine. In the experimental trial (fentanyl) a 25-gauge, 3.5-in. Pecan needle was advanced to the subarachnoid space. Free-flowing CSF confirmed subarachnoid positioning of the needle tip. A small amount of CSF was aspirated and 1 ml of fentanyl (0.05 mg/ml) injected. The needle was then removed, and the subjects remained in an upright sitting position to minimize the potential risk of cephalad movement within the CSF. The participants were observed for 5 min with repeated sampling of vital signs. In the placebo trial the needle was advanced (L$_4$–L$_4$), but just before entering the subarachnoid space 1 ml of preservative-free normal saline was injected. The needle was then removed, and the subjects were observed for 5 min with repeated sampling of vital signs. To minimize the potential risk of a cephalad spread within the CSF, subjects remained in an upright sitting position during the remainder of the experiment.

Cutaneous hypoaesthesia to pinprick and cold perception on the torso and upper limbs was examined before exercise.

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Effects of Fentanyl on Resting Quadriceps MVC

Quadriceps MVCs were similar before and after the administration of lumbar intrathecal fentanyl (566 ± 52 N and 570 ± 50 N, respectively; P = 0.65), suggesting that the force-generating capacity of the quadriceps was not affected by the drug.

Lower Body Cycling Exercise

Metabolic rate. Fentanyl had no effect on resting VO2, VCO2 at any of the work rates, or the capillary plasma lactate concentrations during leg cycling exercise (Fig. 1). Individual target pedal cadence (101 ± 5 rpm) was the same during all work rates in the placebo and fentanyl trials.

Ventilation. At rest breathing room air, fentanyl had no effect on VE, breathing pattern, PETCO2, or arterial hemoglobin saturation (Figs. 2 and 3 and Table 2). During all but the lowest workload with fentanyl, VE and VE/VCO2 were attenuated (8–17%) compared with placebo exercise. This reduction in VE/VCO2 resulted in 4–7-Torr average increases in PETCO2 across all workloads. The lower VE during the fentanyl exercise was mainly caused by a reduction in breathing frequency since tidal volumes at various workloads were nearly identical during both trials. Compared with placebo exercise, the reduction in VE during the fentanyl trial also resulted in a significant reduction in VE/VCO2 and end-tidal PO2 (PETO2) at most work rates but had no significant impact on reducing hemoglobin O2 saturation until the highest workload. Figure 3, A and B, illustrate individual subject responses to fentanyl and placebo exercise at rest and at various work rates. Whereas there was no difference between placebo and fentanyl at rest, VE/VCO2 at each work rate was lower in all five subjects during the fentanyl exercise. This consistent hypoventilation caused an increase in PETCO2, at all workloads and in all subjects. These fentanyl-induced lower ventilatory responses and CO2 retention obtained in the steady-state after 3 min of exercise were already present over the initial 15 s of exercise at each work rate (data not shown).

Heart rate and mean arterial blood pressure. At rest, fentanyl had no effect on HR and MAP (Fig. 4). Throughout the exercise, fentanyl had a significant overall main effect on HR. The difference failed to reach significance at 50 W (−6 ± 3%, P = 0.11) and at 325 W (−2 ± 1%, P = 0.32), but HR was significantly lower during the fentanyl exercise at 100 W (−7 ± 2%) and 150 W (−8 ± 2%). MAP was significantly lower at each workload during the fentanyl trial (8–13%), and this difference was present in all subjects. Figure 3, C and D, illustrate individual subject responses for HR and MAP, respectively. MAP at each work rate was lower during the fentanyl exercise in all five subjects (Fig. 3D), and HR was similarly reduced, although there were a few individual exceptions where HR was unchanged.

Integrated EMG. During the high constant-load leg cycling trial, iEMG of vastus lateralis rose significantly from the first to the final minute of exercise in both conditions (P < 0.05; Fig. 5). The change in vastus lateralis iEMG was similar in both trials; there was no overall main effect of fentanyl (P = 0.3).

Rate of perceived exertion. There was a significant overall main effect of fentanyl on dyspnea RPE (P = 0.005), which was consistently rated between 20% and 40% lower during the fentanyl exercise (Table 2). Furthermore, there was also a significant overall main effect of fentanyl on limb RPE (P = 0.003). Although the difference in limb RPE was not significant between placebo and fentanyl at the three lower workloads...
During the high workload (87 ± 2% \(V_\text{O}_2\text{max}\)), the rating for limb discomfort in the fentanyl trial was 13% lower.

Upper Body Exercise

Metabolic rate. Fentanyl had no effect on resting or exercise \(V_\text{O}_2\) or capillary lactate concentrations during arm cycling exercise (Table 3).

Ventilation. At rest, fentanyl had no effect on \(V_\text{E}\), \(V_\text{E}/V_\text{CO}_2\), \(P_\text{ETCO}_2\), breathing pattern, and arterial hemoglobin saturation (Table 3). Throughout the exercise, there was no significant overall main effect of fentanyl on \(V_\text{E}\) and \(V_\text{E}/V_\text{CO}_2\). \(V_\text{E}/V_\text{CO}_2\) during exercise with fentanyl was between 1% and 5% lower than placebo during each workload, and this hypoventilation caused significant increases in \(P_\text{ETCO}_2\) at 50 and 75 W, respectively. In both cases, four of the subjects had a 2-Torr and one subject had a 3-Torr increase in \(P_\text{ETCO}_2\) with fentanyl. The slightly lower \(V_\text{E}\) during fentanyl exercise was caused by a reduction in breathing frequency.

Heart rate. Mean HR at rest and during each workload was identical in the placebo and fentanyl trials (all \(P > 0.6\); Table 2). The HR in one of the five subjects was consistently between 5 and 8 beats/min lower during the fentanyl exercise.

It should be noted that the two subjects with a fentanyl-induced reduction in resting ventilatory response to hypercapnia (Table 1) showed the greatest \(CO_2\) retention of any of the remaining five subjects during leg cycling (+10 to +12 Torr \(\Delta P_{ETCO}_2\), fentanyl vs. placebo) and also during upper body exercise (+5 to +11 Torr \(\Delta P_{ETCO}_2\)) (data not shown).

DISCUSSION

The goal of this investigation was to evaluate the relative importance of muscle afferent feedback to cardioventilatory control in rhythmically exercising humans. Lumbar intrathecal fentanyl was used to block the cortical/medullary projection of \(\mu\)-opioid receptor-sensitive lower limb muscle afferents at rest and during bicycling exercise of various intensities ranging from \(25\%\) to \(90\%\) of \(V_\text{O}_2\text{max}\). The results from additional experiments conducted to test for a cephalad spread and thus a potential direct effect of the drug on medullary opioid receptors of the cardiovascular and respiratory control centers in the brain stem provide evidence in favor of a segmental effect of the drug at or below the cervical level. Furthermore, in light of the identical MVC force of the quadriceps muscles, the similar levels of plasma lactate and \(V_\text{O}_2\) at each exercise load under both fentanyl and placebo conditions, and the similar output from the locomotor muscle motoneuron pool (as estimated via...
quadriceps EMG) during exercise, we concluded that feed-
forward influences on cardioventilatory regulation were
similar during placebo and fentanyl exercise. Although
having no effect at rest, breathing room air or CO2, the
blockade of \mu\textsuperscript{-}opioid receptor-sensitive lower limb muscle
afferents resulted in a substantial respiratory frequency-
related reduction in exercise hyperpnea during bicycling
exercise, which caused average increases in PETCO2 of 4 –7
Torr at the various work rates. Furthermore, HR and blood
pressure were also not affected at rest but were reduced
10 –15% when the identical bicycle exercises were per-
formed with attenuated neural feedback from the lower
limbs. We interpret this evidence to mean that opioid recep-
tor-sensitive afferent feedback from rhythmically contract-
ing limb muscles is required for normal cardiovascular and
ventilatory control during steady-state rhythmic exercise in
healthy humans.

\textbf{Evaluation of Potential Medullary Effects of Lumbar}
\textbf{Intrathecal Fentanyl}

A cephalad movement of fentanyl within the CSF to the brain
stem, or passage of fentanyl to the systemic circulation and then
across the blood-brain barrier (65), would negate the implications
of our findings for exercise hyperpnea since a direct effect of the
drug on medullary opioid receptors is known to impact medullary

\textbf{Table 2. Physiological response to last minute of lower body leg cycling exercise at four different workloads}

<table>
<thead>
<tr>
<th></th>
<th>Rest 50 W</th>
<th>Placebo</th>
<th>Fentanyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise time, min</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>% of W\textsubscript{peak}</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>% of V\textsubscript{O2max}</td>
<td>27 ± 2</td>
<td>27 ± 2</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>PETCO\textsubscript{2}, Torr</td>
<td>98.0 ± 2.4</td>
<td>97.9 ± 2.1</td>
<td>96.0 ± 1.5</td>
</tr>
<tr>
<td>RPE (dyspnea)</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. W\textsubscript{peak}, peak power output; V\textsubscript{O2max}, maximal O2 consumption (V\textsubscript{O2}); PETCO\textsubscript{2}, end-tidal PO2; RPE, rating of perceived exertion. P value indicates the overall main effect of fentanyl. *P < 0.05 vs. placebo.
neurons involved in cardiovascular (16, 61, 69) and ventilatory (44, 45) control. These central effects of opioids are manifested in a reduced rate and depth of resting ventilation and an attenuated tidal volume and ventilatory response to imposed hypercapnia in humans (10, 45, 76).

Limited evidence on cephalad migration of fentanyl following lumbar injections has shown: 1) in sheep an appearance of trace amounts of fentanyl in cisternal CSF (70); 2) in recumbent humans significant movement of fentanyl from lower to higher lumbar segments (24); and 3) after epidural application of fentanyl detectable amounts appearing in the cervical CSF in recumbent humans (32). In these animal and human studies there was a striking degree of between-subject variability in the degree of cephalad migration of fentanyl, and the investigators speculated that this might be explained, at least in part, by variations in axial spinal length (24, 32, 70).

We have several lines of evidence to suggest that the effects of lumbar intrathecal fentanyl we observed on the cardioventilatory response to exercise were attributable to blockade of spinal μ-opioid receptor-sensitive afferents rather than to any effects of the drug at the medullary level. First, neither resting eupneic ventilation and PETCO2 nor ventilatory responsiveness to hypercapnia at rest was affected by intrathecal fentanyl, suggesting that the drug remained below medullary neuronal sites concerned with the chemical control of breathing. We have confirmed these results, using a steady-state CO2 response test in nine subjects before and after lumbar intrathecal fentanyl while maintaining an upright posture (unpublished findings). In two of the initial group of our seven subjects the tidal volume and ventilatory response to CO2 were substantially attenuated after the drug injection (Table 1)—a result that is consistent with the individual variability in fentanyl migration reported within the spinal column. These two subjects were excluded from any further analysis of their exercise response. Second, no detectable level of fentanyl was found in systemic venous blood samples obtained before and after exercise. We also note that the reduced exercise ventilation with lumbar intrathecal fentanyl was due to a reduced breathing frequency, whereas the reduced CO2 response reported after intravenous fentanyl was due exclusively to a reduced tidal volume response (10). Third, neurological examinations immediately before exercise revealed cutaneous hypoaesthesia to pinprick and cold perception only below T2–T3. Finally, compared with placebo exercise, both HR and ventilation were attenuated during leg fentanyl exercise (Fig. 2), whereas during arm exercise HR was unchanged and differences in Ve/VO2 were relatively small for the placebo versus fentanyl trials. For the arm exercise comparisons, despite the absence of a significant difference in absolute ventilation, a small degree of alveolar hypoventilation during fentanyl exercise at 50 and 75 W was suggested by the 1–2 Torr higher average PETCO2 and reduced breathing frequency (Table 3); however, these relatively small differences might be explained by the attenuated afferent feedback at the thoracic or cervical spinal level from various trunk muscles involved in upper body exercise.

In summary, we did not directly address the question of how cephalad fentanyl migrated after lumbar injection. However, we can state with certainty that the cardioventilatory responses to rhythmic leg exercise were significantly attenuated while ventilation, blood pressure, and HR at rest—during room air breathing or in response to hypercapnia—were not. These contrasts between the effects of fentanyl at rest and during exercise imply that the exercise effects were not likely to have been mediated by the same CNS influences that are purported to depress ventilation in the resting subject while breathing room air or CO2.

Fig. 4. HR (A) and MAP (B) response during the final minute of leg cycling exercise at 4 different workloads without (Placebo) and with (Fentanyl) partially blocked somatosensory neural feedback from locomotor muscles. The P value indicates the overall main effect of fentanyl. *P < 0.05.

Fig. 5. Myoelectrical activity [integrated EMG (iEMG)] of vastus lateralis muscle used to illustrate the similarity in the feedforward component (central neural drive) during the high constant-workload (325 ± 19 W) leg cycling trial with intact (Placebo) and partially blocked (Fentanyl) somatosensory afferent feedback. iEMG during each muscle contraction (cycle revolution) was calculated, averaged over each 60-s period, and normalized to the 1st minute of exercise. Pedal cadence was held constant (101 ± 5 rpm) throughout the exercise.
Relative Contribution of Muscle Afferents to Exercise Hyperpnea

Our data allow us to approximate the relative amount of the exercise hyperpnea attributable to muscle afferents. First, we observed at each of 100, 150, and 325 W that absolute $V_e$ was reduced 8–10 l/min with the $\mu$-opioid receptor agonist. Coincidently, PetCO$_2$ also rose an average of 4–7 Torr at each of these work rates, and these levels of systemic hypercapnia—presumably acting via feedback chemoreceptor stimulation—would be expected to have masked a greater fall in $V_e$ (11). On the basis of measured CO$_2$ responsiveness of our subjects at rest (on average a 2.4 l/min increase in $V_e$ per 1 Torr increase in PetCO$_2$; $\Delta V_e/\Delta$PetCO$_2$, Table 1) we approximated that the rising PetCO$_2$ prevented a further 15, 18, and 12 l/min reduction in $V_e$ during the 100-, 150-, and 325-W exercise, respectively. Thus, in total, we estimate that the blockade of afferent input resulted in reductions in $V_e$ at 100, 150, and 325 W that averaged 49% (23 in 47 l/min), 43% (26 in 60 l/min), and 15% (22 in 150 l/min), respectively. Similarly, in our previous study employing 5-km cycling time trial tests (4), we observed a fentanyl-induced hypoventilation and CO$_2$ retention throughout the race that averaged +3–8 Torr PetCO$_2$, but the relative reductions in $V_e$/PetCO$_2$ and levels of CO$_2$ retention were greatest at the beginning of the trial and subsided as peripheral fatigue, metabolic acidosis, and arterial hypoxemia progressed over the latter half of the time trial. Therefore, both the present and our previous findings with $\mu$-opioid receptor agonists suggest thatafferent feedback from contracting muscle may have its greatest influence on exercise hyperpnea during moderate exercise intensities when other potentially overriding humoral and feedforward neural inputs are minimal.

Our estimates are only approximations (and likely minimal estimates of the actual contribution) because we do not know the completeness of our afferent blockade; nor can we be certain that our CO$_2$ responses (as measured at rest) remain unaltered during all exercise intensities (7, 17, 54) or that our afferent blockade did not compromise any “interactive” effects of peripheral feedback with ongoing central command (74). We have also not accounted for the ventilatory equivalent of the mild hypoxemia (plus hypercapnia) accompanying the hypoventilation during the heavy exercise intensity. Furthermore, the magnitude of the reduced HR response to exercise with fentanyl is likely underestimated in the face of baroreceptor compensation for the lower MAP. More conservatively, we believe our results most clearly permit the interpretation that muscle afferent feedback is essential to the normal cardioventilatory response to exercise in the healthy human and that the relative strength of this contribution is likely exercise intensity (and/or duration) dependent.

Epidural Anesthesia vs. Intrathecal Opioids

Local anesthetics applied to the lumbar epidural space have been used to investigate the influence of lower limb muscle afferents on the cardioventilatory response in exercising humans. These studies, including our own, found reduced, similar, or increased cardioventilatory responses during exercise with partially blocked spinal neurotransmission to and from the working muscle (3, 27, 29, 30, 36, 38, 55, 67). However, epidural lidocaine attenuates efferent as well as afferent nerve activity (see introduction), and this drug-induced “muscle weakening” inevitably requires an increase in central motor command (i.e., feedforward) in order to achieve a given external workload. Thus partial afferent blockade with local anesthetics creates a condition of reduced feedback in the face of increased feedforward, and this increase in central command, by itself, augments the cardioventilatory response to exercise as demonstrated in curarization experiments (8, 31, 57). With this in mind, the resultant, net effect on ventilatory and/or circulatory responses during exercise with partially blocked feedback via local anesthetics depends upon the degree to which the increase in central motor command/feedforward response balances the reduced feedback from the working limb muscle. To circumvent these confounding effects of local epidural anesthetics we used fentanyl, a $\mu$-opioid receptor agonist that has no effect on the force-generating capacity of the lower limb muscles (4, 33, 68), suggesting that efferent motor activity was not compromised after the intrathecal injection of the drug. Unchanged plasma lactate levels during exercise also suggest that relative exercise intensities were similar in fentanyl versus placebo trials at all work rates. These similarities combined with similar quadriceps muscle EMG at the highest workload

### Table 3. Physiological response to last minute of upper body arm exercise at three different workloads

<table>
<thead>
<tr>
<th>Workload</th>
<th>Placebo</th>
<th>Fentanyl</th>
<th>Placebo</th>
<th>Fentanyl</th>
<th>Placebo</th>
<th>Fentanyl</th>
<th>Placebo</th>
<th>Fentanyl</th>
<th>Placebo</th>
<th>Fentanyl</th>
<th>ANOVA P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>65 ± 3</td>
<td>66 ± 4</td>
<td>88 ± 4</td>
<td>89 ± 4</td>
<td>105 ± 6</td>
<td>104 ± 5</td>
<td>130 ± 7</td>
<td>132 ± 7</td>
<td>0.907</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_e, l/min</td>
<td>13 ± 2</td>
<td>14 ± 1</td>
<td>33 ± 2</td>
<td>31 ± 3</td>
<td>45 ± 2</td>
<td>43 ± 2</td>
<td>64 ± 4</td>
<td>62 ± 3</td>
<td>0.321</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f_b, breaths/min</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
<td>25 ± 2</td>
<td>23 ± 2</td>
<td>28 ± 3</td>
<td>26 ± 3</td>
<td>32 ± 5</td>
<td>33 ± 5</td>
<td>0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_t, liters</td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>2.0 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>0.136</td>
<td></td>
<td></td>
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<tr>
<td>VCO2, l/min</td>
<td>0.69 ± 0.03</td>
<td>0.68 ± 0.03</td>
<td>0.92 ± 0.04</td>
<td>0.93 ± 0.05</td>
<td>1.26 ± 0.05</td>
<td>1.24 ± 0.05</td>
<td>1.70 ± 0.05</td>
<td>1.71 ± 0.05</td>
<td>0.431</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ve/PetCO2</td>
<td>19.1 ± 0.3</td>
<td>20.1 ± 0.2</td>
<td>35.7 ± 1.4</td>
<td>33.6 ± 1.1*</td>
<td>35.6 ± 1.7</td>
<td>34.8 ± 1.5</td>
<td>37.6 ± 1.6</td>
<td>36.4 ± 1.8</td>
<td>0.182</td>
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<tr>
<td>Vt/PetCO2</td>
<td>26.7 ± 0.3</td>
<td>27.9 ± 0.8</td>
<td>38.7 ± 1.8</td>
<td>36.5 ± 1.0*</td>
<td>33.7 ± 1.5</td>
<td>32.9 ± 0.8</td>
<td>32.8 ± 1.6</td>
<td>32.4 ± 1.4</td>
<td>0.187</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PetCO2, Torr</td>
<td>102.8 ± 21.0</td>
<td>102.3 ± 1.7</td>
<td>104.1 ± 2.7</td>
<td>99.7 ± 2.5</td>
<td>103.9 ± 1.5</td>
<td>101.3 ± 1.6*</td>
<td>108.4 ± 2.2</td>
<td>106.9 ± 2.1*</td>
<td>0.033</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PetCO2, Torr</td>
<td>36.4 ± 1.0</td>
<td>36.7 ± 1.2</td>
<td>36.2 ± 1.7</td>
<td>37.4 ± 1.4</td>
<td>37.5 ± 1.2</td>
<td>39.2 ± 1.1*</td>
<td>36.4 ± 1.7</td>
<td>38.1 ± 1.5*</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SpCO2%</td>
<td>98.3 ± 0.2</td>
<td>97.8 ± 0.2</td>
<td>98.7 ± 0.3</td>
<td>98.4 ± 0.3</td>
<td>99.1 ± 0.2</td>
<td>99.0 ± 0.3</td>
<td>97.2 ± 0.3</td>
<td>97.1 ± 0.3</td>
<td>0.901</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[La$^-$]_b, mmol/l</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>2.8 ± 0.3</td>
<td>2.7 ± 0.2</td>
<td>4.8 ± 0.5</td>
<td>4.8 ± 0.5</td>
<td>0.935</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. HR, heart rate; f_b, respiratory frequency; V_t, tidal volume; VCO2, CO2 production; SpCO2, arterial CO2 saturation by pulse oximetry; [La$^-$]_b, whole blood lactate concentration. ANOVA P value indicates the overall main effect of fentanyl. *P < 0.05 vs. placebo.
form the basis for our presumption that any influence from feedforward central motor command was also similar during placebo and fentanyl exercise (Fig. 5). Accordingly, we believe the use of lumbar intrathecal fentanyl provided a suitable means of evaluating the influence of reduced locomotor muscle afferent feedback, per se, on the cardioventilatory response in exercising humans.

Role of Muscle Afferents in Cardiovascular and Ventilatory Responses and Effort Perceptions: Past vs. Present Findings

What is the significant advance provided by our findings concerning the relative contribution of muscle afferents to exercise hyperpnea in the rhythmically exercising human and in the presence of all other known major influences on ventilatory control? First, we recognize that the century-long examination of the exercise hyperpnea question has revealed that it likely requires multiple major mechanisms—including neural and humoral components as well as memory-like poststimulus potentiation effects and perhaps even a “learned” feedforward effect linked to past experience (25, 34, 50, 60, 71). Our study has examined only one of these important inputs, demonstrating that the afferent feedback mechanism was essential to the production of a normal exercise hyperpnea as well as for normal pressor and tachycardic responses to rhythmic exercise. This demonstration is consistent with the well-established importance of group III and IV muscle afferents in ventilatory control when studied in isolation, but it uniquely extends previous findings by showing that partial blockade of the afferent input from rhythmically contracting muscle of varying intensities prevented the normal cardiovascular and ventilatory responses even in the presence of other known and presumably unaltered major inputs to circulatory and ventilatory control.

Our conclusion is not in agreement with most studies using epidural local anesthetics (see above), or some studies using local muscle ischemia (to enhance local metabolic stimuli), which reported no effect on ventilatory recovery following exercise (23, 62). Furthermore, our findings in humans are not consistent with those in anesthetized animals in which the rapid hyperpnea evoked via hindlimb electrical stimulation was unaffected by spinal cord transection (20, 46, 47, 75) or with the normal ventilatory response to electrical stimulation of limb muscles and relatively small increments in \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) in paraplegic humans (1, 15). On the basis of findings from many of these studies that have reported no or little effect of eliminating a single sensory input on exercise hyperpnea, some have postulated the emergence of redundant control mechanisms that are able to compensate for—and thereby mask the effects of—the absent sensory input (81). Perhaps, then, our findings with partial muscle afferent blockade and no accompanying muscle weakness may represent a minimal—if any—upregulation of the remaining controllers of the cardiovascular and ventilatory responses to exercise.

There is also skepticism concerning any role for muscle afferents on circulatory and ventilatory control during rhythmic exercise—as opposed to static ischemic exercise—based primarily on negative findings obtained during very light rhythmic exercise in the presence of “complete” sensory blockade (via epidural lidocaine) sufficient to prevent the pressor response to muscle ischemia in humans (29, 43) and with the use of opioid agonists in dogs undergoing mild-intensity exercise employing a small muscle mass (59). In contrast, our present results obtained during rhythmic leg cycling showed a substantial effect of partial blockade of muscle afferents across the range of moderate to heavy exercise intensities (requiring 3- to 8-fold increases in resting metabolic rate and 4- to 15-fold increases in resting \( \dot{V}E \)) and over the 3-min time course at each work rate. These findings are in agreement with the significant activity of group III and IV muscle afferents observed in the rhythmically exercising cat with normal limb blood flow (2), with the significant impact of cold blockade of group III/IV afferents on the cardiovascular response to rhythmic electrical stimulation of limb muscle in the anesthetized dog (72), and with recent evidence in humans showing that muscle afferent blockade during rhythmic exercise significantly attenuated “baroreceptor resetting” (67). Pan et al. (58) also reported that bilateral spinal ablation of the dorsolateral sulcus and funiculus at L2 in the pony altered the transient changes in arterial \( P_{CO_2} \) at exercise onset—but had no effect on arterial \( P_{CO_2} \) in the steady state of exercise. Furthermore, Galbo et al. (31) implicated an important role for afferent feedback in heavy rhythmic cycling exercise in humans with their observation of a markedly attenuated ventilatory response following partial curarization when comparisons with controls were made at maximal exercise intensity and presumably at comparable levels of central command. As previously suggested (63), the relative importance of muscle afferents on cardioventilatory control during exercise is likely to be critically dependent upon the mass of muscle involved as well as the intensity of the exercise and perhaps even the magnitude of the intact cardiorespiratory response.

Finally, it has been argued that “perception of effort” is independent of afferent feedback from skeletal muscles (48). Certainly, the awareness of central motor command plays an important role in determining the perception of effort (31, 77); however, a critical role of peripheral muscle afferents is also likely to be important in this process (3, 77). Data from the present study confirm this latter notion. In the face of similar central command and blocked spinal opioid receptor-sensitive afferent feedback from the locomotor muscles, the RPE was lower compared with the placebo trial performed with an intact somatosensory feedback system (Table 2).

Summary

Our findings show that although only a portion of the locomotor muscle afferents, namely, the \( \mu \)-opioid receptor-sensitive fibers, were blocked via lumbar intrathecal fentanyl, ventilation, HR, and blood pressure were significantly reduced compared with placebo exercise. This evidence supports the necessity of muscle afferents in regulating the normal steady-state circulatory and ventilatory responses in rhythmically exercising humans across a wide range of exercise intensities—even in the presence of apparently normal contributions from other major effectors of the cardiorespiratory response.

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