In vivo electrophysiological responses of pedunculopontine neurons to static muscle contraction

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Plowey, Edward D., Jeffery M. Kramer, Joseph A. Beatty, and Tony G. Waldrop. In vivo electrophysiological responses of pedunculopontine neurons to static muscle contraction. Am J Physiol Regul Integr Comp Physiol 283: R1008–R1019, 2002.—The pedunculopontine nucleus (PPN) has garnered attention as a potential regulator of cardiorespiratory drive during exercise as a component of the mesencephalic locomotor region (MLR) (11, 43). The MLR of the rat, located in the mesencephalic tegmentum at the lateral extent of the brachium conjunctivum, is an area from which coordinated locomotion can be evoked via electrical stimulation or chemical disinhibition in a nonanesthetized, decerebrate preparation (16). Garcia-Rill and colleagues (17, 18) demonstrated that the MLR of the rat is highly coexistent with the cholinergic, NADPH-diaphorase positive neurons of the PPN. The PPN is known to be active during locomotion, as has been shown by extracellular neuronal recordings in cats (19) and via examination of c-fos expression in the PPN after treadmill exercise in rats (25).

Activation of the MLR in cats produces feed-forward increases in efferent cardiorespiratory drive that parallel concurrent increases in locomotor drive, yet persist in the absence of elevated muscle feedback during fictive locomotion (11). The capacity of the MLR to produce feed-forward increases in cardiovascular drive has been documented in rats as well (4, 8). Given the apparent importance of the PPN as an anatomic component of the MLR (17, 18), it is reasonable to hypothesize that the PPN may play a role in the regulation of the cardiorespiratory adjustments that accompany exercise through a central command mechanism.

To begin to evaluate the possibility that the PPN contributes a potential modulatory influence to the cardiorespiratory responses evoked by muscle contraction, we determined if neurons of the PPN and the surrounding mesencephalic tegmentum respond to

ventrolateral medulla (33), and the dorsal horn of the spinal cord (9). Several authors have hypothesized that orchestration of influences from central command and muscle reflex pathways partly underlies the ability of the central nervous system to evoke alterations in cardiorespiratory drive that are appropriately matched to the metabolic demand of physical activity (32, 33, 35, 43, 44).

The pedunculopontine nucleus (PPN) has been implicated in central command regulation of the cardiorespiratory adjustments that accompany exercise. The current study was executed to begin to address the potential role of the PPN in the regulation of cardiorespiratory adjustments evoked by muscle contraction. Electrophysiological single-unit recording was employed to document the responses of PPN neurons during static muscle contraction. Sixty-four percent (20/31) of neurons sampled from the PPN responded to static muscle contraction with increases in firing rate. Furthermore, muscle contraction-responsive neurons in the PPN were unresponsive to brief periods of hypotension but were markedly activated during chemical disinhibition of the caudal hypothalamus. A separate sample of PPN neurons was found to be moderately activated during systemic hypoxia. Chemical disinhibition of the PPN was found to markedly increase respiratory drive. These findings suggest that the PPN may be involved in modulating respiratory adjustments that accompany muscle contraction and that PPN neurons may have the capacity to synthesize muscle reflex and central command influences.

pedunculopontine nucleus; respiration

CENTRAL COMMAND AND MUSCLE REFLEX mechanisms are thought to be involved in driving cardiorespiratory adjustments observed during the onset of moderate exercise (32, 41, 43). The central command hypothesis states that specific brain areas are responsible for parallel, feed-forward activation of brain stem locomotor and cardiorespiratory loci during exercise (32, 43). Excitation of cardiorespiratory centers is also driven by muscle reflex pathways that are stimulated by the mechanical and metabolic products of active muscles during exercise (26). The capacity of specific brain areas to potentially contribute to both central command and muscle reflex influences has been documented in the caudal hypothalamus (CH; Ref. 44), the

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evoked static contraction of the hindlimb muscles in anesthetized rats using single-unit extracellular recording. We hypothesized that if the PPN modulates the cardiorespiratory adjustments evoked by muscle contraction, then neurons sampled from the PPN will exhibit alterations in firing rate during static muscle contraction. The data presented suggest that the firing rates of PPN neurons are enhanced during evoked muscle contraction in anesthetized rats and that activation of the PPN may, as observed during muscle contraction, have an impact on respiratory drive.

**METHODS**

All of the procedures described in this study were executed under animal experimentation protocols that were approved by the Laboratory Animal Care Advisory Committee of the University of Illinois at Urbana-Champaign. These procedures are in compliance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

**Animal preparation.** Male Sprague-Dawley rats (220–350 g, 63 total animals) were anesthetized with intraperitoneal injections of a mixture of α-chloralose (65 mg/kg) and urethane (800 mg/kg) dissolved in Ringer. Adequate depth of anesthesia was maintained via anesthetic supplements that were administered intravenously upon evidence of a positive foot withdrawal response to noxious pinch or a positive eyelid response to tactile stimulation of the cornea. The trachea was cannulated with PE-205 tubing (Clay Adams, Parsippany, NJ) to maintain a patent upper respiratory tract and facilitate spontaneous ventilation of 100% O2. Catheters (PE-50 tubing; Clay Adams) filled with heparinized saline (75 µg/ml heparin; Sigma, St. Louis, MO) were inserted into the left external jugular vein and left common carotid artery to allow drug administration and measurement of cardiovascular variables, respectively. Pulsatile arterial blood pressure was monitored via a model P23 pressure transducer (Gould, Oxnard, CA) connected to the arterial catheter. Heart rate (HR) was derived from the voltage output of the pressure transducer using a biotachometer (Gould).

Rats were then placed prone in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) to immobilize the cranium. A rectal temperature probe, a radiant heat lamp, and a water-perfused heating pad were employed to maintain the animal's body temperature at 37 ± 1°C. The bite bar of the stereotax was then adjusted to the vertical level at which the lambda and bregma skull coordinates were measured in the same horizontal plane. A parietal craniotomy was performed over the mesencephalon in some subjects. Differentially balanced high-impedance electrodes (3 M Systems, Carlsborg, WA) were inserted into the diaphragm using a 23-gauge hypodermic needle. The electrical activity measured by the diaphragmatic electrodes was amplified (300- to 3,000-Hz bandwidth; P5 Series AC Preamplifier, Grass Instruments, Quincy, MA), full-wave rectified, and integrated (Gould Integrator Amplifier) to yield an electrical correlate of respiratory activity known as integrated diaphragmatic electromyogram activity, or iDEMG. The f' DEMG signal was sent to a biotachometer (Gould) to derive the respiratory rate (f) of the animal. The product of f and the average of the peak f DEMG amplitude, known as minute f DEMG amplitude, was examined as an electrophysiological indicator of relative changes in the minute ventilation.

In 37 animals prepared for muscle contraction experiments, right hindlimb muscle contraction was evoked by electrical stimulation of the right tibial nerve. The tibial nerve was accessed through an incision of the skin of the posterior thigh. The muscles of the posterior compartment were bluntly dissected to expose the origins of the tibial, sural, and peroneal branches of the sciatic nerve. The tibial nerve was dissected from the sural and peroneal nerves and placed on a shielded bipolar platinum electrode. The nerve was covered in a pool of warm mineral oil to prevent desiccation. Limb movement was prevented by placement of a precision clamp about the knee. To evoke static contraction of the hindlimb muscles, the tibial nerve was electrically stimulated (40 Hz, 1-ms square wave pulses) at 2 x motor threshold (MT) for 30 s.

**Data collection.** Single-unit extracellular recordings were made with high-impedance electrodes (3–6 MΩ; FHC, Bowdoinham, ME) stereotaxically placed into the PPN. Recording tracts were executed within the following coordinates according to Paxinos and Watson (34): 0.3–1.7 mm rostral, 1.4–2.2 mm lateral, and 5.5–1.0 mm dorsal to interaural zero. Extracellular activity was amplified (100 K; P5 Series AC Preamplifier, Grass) and filtered (300- to 1,000-Hz bandwidth). Single units were isolated with a window discriminator (FHC). Action potentials that fell within the recording window triggered transistor-transistor logic pulses that were sent to a ratemeter (FHC) and to a digital chart recorder (Windows-based PC running PowerLab v.3.4.4, AD Instruments, Grand Junction, CO). Discriminated action potentials were also sent to a storage oscilloscope to check for consistency of the action potential signature to thus ensure a stable recording of a single unit. Extracellular activity was also sent to a speaker to monitor unit activity auditorily.

Recording tracts were performed in both the right (ipsilateral) and left (contralateral) sides of the mesencephalon. The responses of isolated mesencephalic units were recorded during 30 s of static contraction of the hindlimb muscles. After a 15-min recovery period, response reliability was tested during a subsequent period of muscle contraction. To evaluate the possibility that neuronal responses observed during muscle contraction were evoked by alterations in arterial pressure, the responses of the neurons to brief periods of hypotension or hypertension induced by intravenous injections of sodium nitroprusside (SNP; 5–10 µg; Sigma) or phenylephrine (Phe; 3–5 µg; Sigma), respectively, were documented. To test the possibility that we were directly activating afferents via electrical stimulation to evoke responses in PPN neurons, five muscle contraction-responsive neurons were recorded during electrical stimulation of the tibial nerve at 2 x MT after the nerve was crushed just distal to the stimulation electrode. In all experiments, we made sure that stimulation of the crushed tibial nerve at 2 x MT failed to evoke cardiorespiratory adjustments through direct activation of tibial nerve afferents.

For a subset of six PPN neurons that responded to muscle contraction, the responses to supramesencephalic activation of central command were recorded. Feed-forward increases in cardiorespiratory drive, in the absence of locomotion, were evoked via microinjections of the GABA-B receptors antagonist bicuculline (5 mM in Ringer, 60 nl; Sigma) into the CH (11, 42). A microinjection pipette (20- to 30-µm tip aperture) was pulled from a glass capillary tube (1-mm diameter; World Precision Instruments, Sarasota, FL) with a one-stage, upright pipette puller (Narishige, Tokyo). The pipette was stereotaxically placed in the CH using the following coordinates: rostral +4.8 mm, lateral +0.5 mm, dorsal +1.7 mm relative to interaural zero (34). Microinjections were made with a
PV800 Pneumatic PicoPump (World Precision Instruments) and were measured by monitoring the movement of the meniscus of the injectate through a calibrated microscope reticule (Reichert Scientific Instruments, Buffalo, NY). The firing behavior of PPN neurons was recorded for at least 2 min before the microinjection of bicuculline, throughout the period of CH activation, and for at least 2 min after the return of the cardiorespiratory responses to baseline. 

In separate experiments (9 rats), similar animal preparations, except for preparation of the hindlimb for muscle contraction, were used to document the responses of PPN neurons to systemic hypoxia. Hypoxia was induced for 1-min periods by switching the inspired gas from 100% O2 to a gas mixture composed of 10% O2-90% N2. The effects of the hypotension observed during hypoxia on neuronal firing rate were evaluated via intravenous injections of SNP as described above.

The 17 remaining subjects were prepared similarly to chemically activate the PPN. Single-barrel microinjection pipettes were filled from the tip via suction with bicuculline (5 mM in Ringer, 60 nl; Sigma), vehicle, and 0.5% Chicago sky blue dye (Sigma) in the opposite order of injection. Solutions were separated via thin layers of mineral oil. The microinjection pipettes were stereotaxically placed in the PPN or surrounding mesencephalic tegmentum using the coordinates employed for extracellular recording. Stable baseline cardiorespiratory variables were recorded for 5 min before injection of bicuculline into the PPN or control sites and then for the duration of observed cardiorespiratory responses. Cardiorespiratory variables were also observed after injection of vehicle and/or Chicago sky blue to ensure that responses to bicuculline were specific.

Histology. After the termination of successful recording tracts or injection experiments in the PPN, animals were prepared for histological analyses of recording and injection sites. The positions of recorded neurons were demarcated with direct current (DC) electrolytic lesions (300 µA, 8–10 s). Injection sites in the PPN and CH were marked with 60-nl microinjections of Chicago sky blue dye after the experiments. Animals were then deeply anesthetized with a supplemental injection of α-chloralose-urethane (1/4 initial pre-surgical dose) and perfused transcardially with heparinized saline followed by 4% paraformaldehyde in PBS/1 mM MgCl2. The brain was postfixed in the fixative for 2 h and then infiltrated with 20% sucrose/5 mM MgCl2. Midbrains containing the PPN were sliced on a sliding microtome (American Optical, Buffalo, NY) with a freezing stage (Sensortek, Clifton, NJ) into 30-µm sections. Alternate sections were either mounted on gelatin-coated slides and stained with neutral red or were incubated in 1 mM NADP+, 0.2 mM nitroblue tetrazolium, and 15 mM sodium malate in 0.1 M Tris buffer for 45 min to reveal the NADPH-diaphorase-positive neurons of the PPN (39). Diencephalons containing the CH were sectioned into 50-μm slices and mounted on gelatin-coated slides. Alternate sections were either stained with neutral red (Sigma) or left unstained to allow determination of the position of the microinjection site.

Data analyses. Cardiorespiratory and electrophysiological variables were recorded to the Powerlab digital chart recorder and subsequently analyzed. Cardiorespiratory and electrophysiological responses to muscle contraction were determined by contrasting the means of the variables during the 1-min period immediately before muscle contraction (baseline) with the means of the variables during the entire 30-s period of muscle contraction. Baseline variables were also contrasted to the peak responses observed during muscle contraction. In addition, the SDs of the firing rates from the mean over the entire baseline period were determined. Individual neurons were labeled as responsive (increase or decrease) if the difference between the mean firing rate before muscle contraction and the mean firing rate during muscle contraction exceeded 1 SD. The means of the cardiorespiratory and electrophysiological variables before and during muscle contraction were contrasted using two-tailed, paired Student’s t-tests, with P < 0.05 deemed significantly different. Similar analyses were employed to independently determine the responses of PPN neurons to SNP-induced hypotension and hypoxia and during the cardiorespiratory responses to disinhibition of the CH. Data presented in text and figures are means ± SE.

RESULTS

Cardiorespiratory responses to muscle contraction, SNP, and systemic hypoxia. Cardiorespiratory responses to muscle contraction, intravenous SNP, and systemic hypoxia are summarized in Table 1. Electrical stimulation of the tibial nerve at 2 × MT evoked static contraction of the hindlimb muscles and an increase in tension in the triceps surae muscles and Achilles tendon of 740 ± 50 g. Periods of muscle contraction were associated with decreases in mean arterial pressure (MAP), modest increases in HR, and rapid increases in f (Table 1). In contrast to muscle contraction, intravenous SNP evoked a larger decrease in MAP but no change in f relative to baseline. Hypoxia evoked a similar decrease in MAP compared with muscle contraction, but, in contrast to muscle contraction, mark-

<table>
<thead>
<tr>
<th>Cardiorespiratory Variable</th>
<th>Baseline</th>
<th>Muscle contraction</th>
<th>SNP</th>
<th>Hypoxia</th>
</tr>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>106 ± 2</td>
<td>−20 ± 2*</td>
<td>−41 ± 3†</td>
<td>−19 ± 5*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>380 ± 5</td>
<td>8 ± 1*</td>
<td>5 ± 4</td>
<td>21 ± 5†</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>73.2 ± 1.1</td>
<td>3.2 ± 0.5*</td>
<td>0.7 ± 0.5†</td>
<td>27.3 ± 2.6†</td>
</tr>
<tr>
<td>Minute /DEMG amplitude, breaths-units·min⁻¹</td>
<td>73.2 ± 1.1</td>
<td>2.0 ± 1.2</td>
<td>1.8 ± 0.9</td>
<td>40.9 ± 6.0†</td>
</tr>
<tr>
<td>Muscle tension, g</td>
<td>740 ± 50</td>
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Data are means ± SE; n = 37 (muscle contraction), 19 [sodium nitroprusside (SNP)], and 9 rats (hypoxia). MAP, mean arterial pressure; HR, heart rate; /DEMG, integrated diaphragmatic electromyogram activity; f, respiratory rate. *P < 0.05, significant change compared with baseline; †P < 0.05, significantly different compared with response observed during muscle contraction.
Penniculopontine responses to static muscle contraction, SNP, and systemic hypoxia. Figure 1A depicts the response of one neuron sampled from the PPN during static muscle contraction. This PPN neuron exhibited an immediate, dramatic increase in firing rate that occurred with the changes in arterial pressure and respiration during muscle contraction. The same unit failed to respond to a decrease in arterial pressure evoked by intravenous injection of SNP (Fig. 1B). Figure 2A depicts the responses and locations of all mesencephalic recordings in this study. Figure 2B demonstrates that the histological location of the neuron depicted in Fig. 1, inferred from the lesion made by passing DC current through the tip of the recording electrode, was among the NADPH-diaphorase-positive neurons of the PPN. Some PPN units responded to muscle contraction with more gradual increases in firing rate. One such neuron is depicted in Fig. 3, along with a sample of the raw neurogram of the single-unit recording during muscle contraction.

Table 2 summarizes the responses of PPN neurons to static muscle contraction. Of the entire sample of PPN neurons recorded (n = 31), 20 (64%) responded to muscle contraction with increases in firing rate, 3 (10%) responded with decreases in firing rate, and 8 (26%) PPN neurons were unresponsive to muscle contraction. Of the 20 PPN neurons that responded to muscle contraction with increases in firing rate, 11 of these neurons exhibited immediate increases in firing rate (peak response within 5 s of muscle contraction); the remainder (9 PPN neurons) responded with gradual increases in firing rate (peak response after 5 s of muscle contraction). The basal firing rates of all PPN neurons sampled were contrasted with their firing rates observed during muscle contraction (Fig. 4). The entire sample of PPN neurons (n = 31) exhibited significant increases in mean firing rate and peak firing rate during muscle contraction. In contrast, the preponderance of neurons sampled from the cuneiform nucleus (CnF) and the inferior colliculus (IC) exhibited no changes in firing rate or decreases in firing rate during muscle contraction (Table 2). Neuron samples from the CnF and IC failed to exhibit significant alterations in sample firing rate (peak response and average response) during muscle contraction (Fig. 4).

To test the possibility that the PPN responses observed during muscle contraction were due to deactivation of baroreceptor afferents, 14 of the 20 muscle contraction-responsive PPN neurons were observed during brief bouts of hypotension evoked by intravenous injections of SNP (as demonstrated in Fig. 1B). Only 1 of the 14 PPN neurons tested responded with an increase in firing rate during SNP-evoked hypotension. The sample firing rate of muscle contraction-responsive PPN neurons was unaltered during SNP-induced hypotension (7.4 ± 0.9 Hz at baseline vs. 7.5 ± 0.8 Hz during transient hypotension; P > 0.05). In addition, consistent with the lack of responses to SNP-evoked changes in blood pressure, three PPN neurons in this sample did not exhibit alterations in firing rate when blood pressure was transiently elevated via intravenous injection of phenylephrine.

We were also interested in testing whether the cardiorespiratory and neuronal responses we observed were due to activation of muscle afferents by muscle contraction or due to direct activation of sensory afferents in the tibial nerve by our stimulation electrode. In all experiments, stimulation of the tibial nerve at 2× motor threshold (MT) after the nerve was crushed distal to the stimulation electrode failed to evoke changes in arterial pressure, HR, respiration, and muscle tension. In addition, five muscle reflex-responsive PPN neurons were recorded during electrical stimulation of the tibial nerve after the nerve was crushed distal to the stimulation site (Fig. 5). In all five cases, stimulation of the crushed tibial nerve at 2× MT failed to reproduce the neuronal...
responses observed during muscle contraction, while stimulation at higher voltages did result in activation of PPN neurons.

The responses of 13 PPN neurons during systemic hypoxia, induced by spontaneous ventilation of 10% O₂, were documented in separate animals. Figure 6 depicts the response of one PPN unit during 1 min of exposure to the hypoxic gas mixture. Note that the neuron exhibited an increase in firing rate that coincided with the decrease in arterial pressure and increases in HR and respiration. Eight of the 13 PPN units tested responded with increases in firing rate, two responded with decreases in firing rate, and three failed to respond during 1 min of systemic hypoxia. The basal firing rate of this sample of PPN units was 7.9 ± 1.2 Hz. The firing rate of the entire sample increased to 12.4 ± 1.9 Hz (P < 0.05) at the peak of the response to hypoxia and averaged 10.1 ± 1.6 Hz (P < 0.05) during the period of hypoxic ventilation.

**PPN responses to disinhibition of the CH.** A subset of the muscle contraction-responsive neurons of the mesencephalic tegmentum was also recorded during activation of cardiorespiratory drive evoked via disinhibition of the CH. Figure 7A depicts the response of one muscle reflex-responsive PPN neuron (see Fig. 3 for response to muscle contraction) to microinjection of 60 nl of 5 mM bicuculline into the CH. This treatment resulted in a modest increase in arterial pressure as well as robust increases in HR and respiration. The PPN neuron exhibited a marked, relatively sustained elevation in firing rate during the period of enhanced cardiorespiratory drive induced by disinhibition of the CH. Similar responses to disinhibition of the CH were observed in three other PPN neurons that responded to muscle contraction, all observed in separate animals. Two additional muscle contraction-responsive PPN neurons exhibited triphasic responses consisting of peak increases in firing rates during the onset and
decline of the cardiorespiratory responses to disinhibition of the CH. The peaks in firing rate surrounded periods of increased firing rate variability and general decreases in firing rate from the peaks of the responses, although at all times the firing rates of these neurons remained elevated relative to baseline. Overall, disinhibition of the CH was associated with a marked increase in the firing rate of muscle reflex-responsive PPN neurons from 6.7 ± 2.1 Hz at baseline to 20.4 ± 5.2 Hz ($P < 0.05; n = 6$). Microinjections of identical volumes of vehicle (Ringer) and Chicago blue into the CH failed to evoke similar responses in PPN neurons.

Microinjections of bicuculline into the PPN evoked increases in cardiorespiratory drive. To document the physiological effects associated with activation of PPN neurons, microinjections of 5 mM bicuculline (60 nl, $n = 22$) were executed among the NADPH-diaphorase-positive PPN neurons in anesthetized rats. Figure 8 and Table 3 demonstrate that microinjection of bicuculline into the PPN results in marked, sustained increases in $f$ and minute JDEMG amplitude, as well as moderate increases in MAP and HR. Injections of vehicle (Ringer) and Chicago blue into the PPN failed to reproduce these responses.

DISCUSSION

Responses of PPN neurons to muscle contraction. The principal aim of this study was to document the responses of neurons in the PPN to unilateral evoked static contraction of the hindlimb muscles. A substantial majority of PPN units exhibited increases in firing rate during muscle contraction, and the sample of PPN units as a whole exhibited statistically significant in-

<table>
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<tr>
<th>Region</th>
<th>Increase (%)</th>
<th>Decrease (%)</th>
<th>No response (%)</th>
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<tbody>
<tr>
<td>PPN ($n = 31$)</td>
<td>20 (64)</td>
<td>3 (10)</td>
<td>8 (26)</td>
</tr>
<tr>
<td>CnF ($n = 24$)</td>
<td>5 (21)</td>
<td>7 (29)</td>
<td>12 (50)</td>
</tr>
<tr>
<td>IC ($n = 24$)</td>
<td>7 (29)</td>
<td>5 (24)</td>
<td>12 (50)</td>
</tr>
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</table>

Response profile values are no. of neurons; nos. in parentheses are percentage of total neurons ($n$) sampled in each region. PPN, pedunculopontine nucleus; CnF, cuneiform nucleus; IC, inferior colliculus.

Fig. 3. Single-unit extracellular recording of a PPN unit that responded to muscle contraction with a gradual increase in firing rate. A: unit rate response to muscle contraction. B: side-by-side comparison of extracellular action potential signature from the storage oscilloscope. The action potential at left was observed before muscle contraction; the action potential at right was observed during muscle contraction. C: neurogram activity from bracketed period in A depicting the unit response to muscle contraction.

Fig. 4. Effects of static muscle contraction on the firing rates of neuron samples from the PPN, CnF, and IC during static muscle contraction. PPN neurons exhibited a statistically significant increase in sample firing rate above baseline during muscle contraction ($* P < 0.01; n = 31$). Samples of neurons from the CnF ($n = 24$) and IC ($n = 24$), in contrast, did not exhibit alterations in sample firing rate during muscle contraction.
creases in mean firing rate and peak firing rate during muscle contraction. The majority of responsive PPN units exhibited an immediate increase in firing rate, although many were observed to gradually increase their firing rates during the period of muscle contraction. As demonstrated by local bicuculline injections, PPN activation resulted in significant increases in firing rate and minute DEMG amplitude. On the basis of these observations, the PPN plays a role in the integration of muscle contraction signals.

Fig. 5. Direct electrical stimulation of the crushed tibial nerve did not evoke responses in PPN neurons that were excited during muscle contraction. Left: response of a PPN neuron to static muscle contraction. Middle: stimulation of the crushed tibial nerve at 2× MT failed to evoke muscle contraction and a similar increase in firing rate in this unit. Right: stimulation of the crushed tibial nerve at 8× MT evoked a neuronal response presumably due to direct stimulation of tibial afferents.

Fig. 6. Response of one PPN neuron to 1 min of acute hypoxia (10% O₂). The PPN unit depicted in this trace responded to hypoxia with an increase in firing rate.
results, we conclude that the PPN is activated during evoked static muscle contraction in the anesthetized rat and further hypothesize that the PPN may play a role in the regulation of the respiratory adjustments that accompany muscle contraction in the rat. Further experiments are necessary to conclusively test this hypothesis.

In this study, we conducted two important control procedures to evaluate potential confounding variables of the observed PPN responses during muscle contraction. First, we demonstrated that the PPN responses observed during muscle contraction were not due to the depressor response that accompanies muscle contraction in chloralose-urethane-anesthetized rats. This conclusion is supported by the observation that brief periods of hypotension induced via intravenous injections of SNP were ineffective at altering the firing rates of PPN neurons. Second, we showed that observed responses were not due to direct activation of afferent neurons in the tibial nerve as stimulation of the proximal end of the crushed tibial nerve at 2× MT failed to evoke responses in PPN neurons. Increases in the intensity of the stimulation of the crushed tibial nerve resulted in reproduction of the responses previously observed during muscle contraction, probably due to direct stimulation of tibial afferents. The results of these two control experiments suggest that the increases in PPN activity we observed during muscle contraction were evoked by activation of reflex pathways specific to muscle contraction.

The depressor response observed in chloralose-urethane-anesthetized rats during evoked static muscle contraction in this study and by others from our laboratory (28) contrasts with the pressor responses observed in anesthetized preparations of several species, including the cat (20, 30), the dog (13), the mouse (27), and the chicken (37), as well as the pressor response observed in conscious humans (14). Depressor responses (36) and inconsistent, diminutive pressor responses (38) to static muscle contraction have been reported in separate studies of halothane-anesthetized rats. Rats anesthetized with α-chloralose only exhibit no change in blood pressure during static contraction (40).Smith et al. (36) recently demonstrated that de-
cerebration and withdrawal of anesthetic reverts the depressor response evoked by muscle contraction in halothane-anesthetized rats to an increase in blood pressure. Attenuated pressor responses were observed in decerebrate rats after restoration of halothane anesthesia. Their results suggest that anesthetic alters central neural regulation of the cardiovascular responses that accompany evoked muscle contraction. Because of the integrative nature of cardiovascular and respiratory neural control systems, we acknowledge the possibility that the PPN responses we observed during muscle contraction are not fully representative of those that occur in the absence of anesthetic. Future inquiries into the responses of PPN neurons during evoked muscle contraction in decerebrate, nonanesthetized rats may elucidate this issue.

It is not surprising that the PPN, given its apparent role in regulating coordinated muscular output patterns (15), also receives feedback from contracting muscles. However, at this point we cannot definitively identify the pathway through which muscle reflex activation influences PPN activity. Muscle feedback influences may perhaps be mediated via spinomesencephalic secondary afferent projections known to connect lamina I of the dorsal horn of the spinal cord with the PPN (31). Alternatively, PPN neurons may be influenced through projections from other sites/pathways implicated in the regulation of muscle reflex responses, including the posterior hypothalamus (44, 45) and the ventrolateral medulla (1, 2, 33), the latter of which appears to receive secondary afferent projections that are possibly activated selectively by ergoreceptors (46). Further research is necessary to elucidate these details.

A striking similarity between the data presented here and previous studies (17, 18) is the apparent anatomic specificity of muscle reflex responses and locomotor inducing sites, respectively. Garcia-Rill and colleagues published electrical stimulation (17) and chemical injection data (18) that suggest that the choline acetyltransferase, NADPH-diaphorase-positive PPN is a crucial component, and perhaps the anatomic substrate, of the rat MLR. In our studies, we employed the NADPH-diaphorase staining technique to aid in

Table 3. Cardiorespiratory responses associated with injections of 5 mM bicuculline into the PPN

<table>
<thead>
<tr>
<th>Cardiorespiratory Variable</th>
<th>Baseline</th>
<th>Control mesencephalic injections</th>
<th>PPN injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>125 ± 2</td>
<td>3 ± 3</td>
<td>5 ± 2*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>385 ± 5</td>
<td>2 ± 6</td>
<td>17 ± 2*</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>70.1 ± 1.1</td>
<td>2.4 ± 1.9</td>
<td>15.7 ± 2.5†</td>
</tr>
<tr>
<td>/DEMG amplitude, units</td>
<td>1.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Minute /DEMG amplitude,</td>
<td>70.1 ± 1.1</td>
<td>1.5 ± 3.3</td>
<td>20.4 ± 4.3†</td>
</tr>
<tr>
<td>breaths·units·min⁻¹</td>
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</table>

Data are presented as means ± SE; n = 8 (control mesencephalic injections) and 22 (PPN injections). *P < 0.05 significantly greater increase compared with response to control injections.
the anatomic reconstruction of our extracellular recording sites. The results demonstrated that neurons sampled from the NADPH-diaphorase-positive PPN were largely excited by muscle contraction, whereas the preponderance of neurons sampled from the CnF and the IC were unresponsive or inhibited by muscle contraction. The anatomic specificity of the neuronal responses to muscle contraction we observed in the PPN parallels the uniqueness of the PPN as a mediator of locomotor drive from the mesencephalic tegmentum, or MLR, as described by Garcia-Rill and colleagues (17, 18).

**PPN responses to systemic hypoxia.** Given previous reports of potential involvement of the PPN/peribrachial mesencephalic tegmentum in modulation of respiratory drive (7, 10, 11, 24, 29), we were interested to test the responses of PPN neurons to systemic hypoxia induced by spontaneous ventilation of 10% O2, a stimulus that evokes robust increases in respiration in the anesthetized rat (23). We found that a large percentage of PPN neurons exhibited increases in firing rate during the hypoxic stimulus. The same PPN neurons failed to respond to decreases in arterial pressure evoked by intravenous injections of SNP and thus were not likely affected by the decrease in arterial pressure that accompanies hypoxia in anesthetized rats. These data suggest that the PPN might contribute a modulatory influence to respiratory adjustments evoked by hypoxia and may perhaps be indicative of a more general role for the PPN in the regulation of respiratory adjustments that accompany physiological stressors and behavioral adjustments. Although such roles for the PPN have yet to be thoroughly investigated, it is interesting to note that the PPN has been hypothesized to contribute to the pathologies of obstructive sleep apnea (5) and sudden infant death syndrome (6, 15).

Consideration of PPN responses to systemic hypoxia has also led us to believe that the PPN responses we observed during muscle contraction were not due to changes in pulmonary afferent activity secondary to increases in respiration associated with muscle contraction. Eldridge and Chen (10) reported that bilateral vagotony enhanced the excitatory effect of systemic hypoxia on neurons in the peribrachial mesencephalic tegmentum, an indication that vagal afferents have an inhibitory effect on this neuronal population. It is thus improbable that increases in vagal reflex activation secondary to increases in respiration during muscle contraction are responsible for the excitatory effects of muscle contraction on neurons in the PPN. In addition, if there were a direct, causal relationship between vagal afferent activity and PPN activation, it would stand to reason that a much larger degree of PPN activation would be observed during systemic hypoxia than during muscle contraction since the former stressor induces a much larger increase in respiratory drive than muscle contraction. The data indicate that unilateral static muscle contraction and systemic hypoxia have comparable effects on neuronal firing rate in the PPN. These observations suggest that it is unlikely that pulmonary afferent activation during static muscle contraction is responsible for the PPN responses we observed.

**Responses of muscle contraction-responsive PPN neurons to disinhibition of the CH.** Several authors have suggested that integration of central command and muscle reflex drives may be an important mechanism whereby the central nervous system is able to appropriately match cardiorespiratory adjustments with the intensity of the locomotor task (32, 33, 35, 43, 44). Previous studies have demonstrated the potential to synthesize muscle reflex and central command influences in the CH (11, 12, 45), the ventrolateral medulla (33), and the dorsal horn of the lumbar spinal cord (9). In the current study, we describe muscle reflex-responsive neurons in the PPN that are robustly activated by central command activation via chemical disinhibition of the CH. Feed-forward activation of the PPN from the CH is also supported by anatomic data in which direct, reciprocal projections have been demonstrated between the two nuclei (3). The ability of single PPN neurons to respond to both evoked muscle contraction and disinhibition of the CH suggests that the PPN may have the capacity to synthesize muscle reflex and central command influences on respiratory drive.

**Cardiorespiratory responses to disinhibition of the CH.** To obtain an indication of the potential physiological consequences of increased activity in the PPN, as was qualitatively observed in response to muscle contraction, disinhibition of the CH, and hypoxia, we injected the GABAA receptor antagonist bicuculline into the NADPH-diaphorase-positive PPN. Disinhibition of the PPN evoked robust increases in f and minute f/DEMG amplitude, as well as modest increases in MAP and HR that were not significantly different from those observed in response to injections outside the PPN. These responses were qualitatively similar to those previously documented with electrical stimulation (11, 21, 22, 24). This period of accelerated cardiorespiratory drive eventually returned to baseline in 20–30 min. The observation that activation of the PPN results in an acceleration of cardiorespiratory drive is support for the hypothesis that PPN activation during muscle contraction may play a role in the modulation of the cardiorespiratory adjustments that accompany muscle contraction. The fact that disinhibition of the PPN evoked larger respiratory adjustments in contrast to cardiovascular adjustments could be indicative of a more important role for the PPN in the regulation of respiratory drive. Further experimentation is necessary to fully test these hypotheses.

**Conclusion.** In summary, we sampled the responses of neurons of the NADPH-diaphorase-positive PPN during several stimuli that evoked cardiorespiratory adjustments, including evoked static muscle contraction. PPN neurons were found to respond specifically to muscle reflex activation with increases in firing rate. We also observed robust increases in respiratory drive on chemical disinhibition of the NADPH-diaphorase-positive PPN. Taken together, these observations suggest that the PPN might contribute a modulatory influence to the respiratory adjustments that accompany
muscle contraction in anesthetized rats. Feed-forward activation of cardiorespiratory drive from the CH was associated with robust activation of muscle contraction-responsive PPN neurons. These observations indicate that PPN neurons are influenced by both muscle reflex and central command pathways and therefore may have the capacity to synthesize these influences on exercise-related drives. These results suggest that the PPN warrants future attention regarding potential roles in the modulation of respiratory drive during exercise-related stimuli and physiological stressors/behavioral contexts that involve respiratory adjustments. 

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