Parabrachial neurons mediate dorsal periaqueductal gray evoked respiratory responses in the rat

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First published October 31, 2003; 10.1152/japplphysiol.00903.2003.—The neural substrates mediating autonomic components of the behavioral defense response reside in the periaqueductal gray (PAG). The cardiovascular components of the defense response evoked from the dorsal PAG (DPAG) have been well described and are dependent, in part, on the integrity of neurons in the region of the parabrachial nucleus as well as the rostral ventrolateral medulla. Descending pathways mediating the ventilatory response associated with activation of DPAG neurons are unknown. The present study was undertaken to test the hypothesis that parabrachial area neurons are also involved in mediating the respiratory response to DPAG stimulation. In urethane-anesthetized, spontaneously breathing rats, electrical stimulation of the DPAG significantly increased respiratory rate, arterial pressure, and heart rate. Changes in respiratory frequency were associated with significant decreases in inspiratory and expiratory durations. After bilateral inhibition of neurons in the lateral parabrachial nucleus (LPBN) region with 5 mM muscimol (n = 6), DPAG-evoked increases in respiration and heart rate were attenuated by 90 ± 6 and 72 ± 13%, respectively. Thepressor response evoked by DPAG stimulation, however, was attenuated by only 57 ± 6%. Bilateral blockade of glutamate receptors with 20 mM kynurenic acid (n = 6) in the LPBN also markedly attenuated DPAG-evoked increases in respiration and heart rate (65 ± 15 and 53 ± 9% reduction, respectively) but only modestly changed the DPAG-evoked pressor response (34 ± 16% reduction). These results demonstrate that LPBN neurons play a significant role in the DPAG-mediated respiratory component of behavioral defense responses. This finding supports previous work demonstrating that the dorsolateral pons plays a significant role in mediating most physiological adjustments associated with activation of the DPAG.

control of breathing; lateral parabrachial nucleus; dorsal periaqueductal gray; hyperventilation; hypertension

The cardiovascular response to DPAG activation has been well characterized. Chemical activation of the DPAG produces marked increases in arterial pressure and heart rate (HR). Associated with the hypertension is a general stereotypic pattern of increased blood flow to skeletal muscle and decreased flow to visceral regions (1, 3, 6). This redistribution of blood flow is also observed when the animal is paralyzed, indicating that these vasomotor changes are not secondary to other ventilatory or somatomotor changes (8, 9, 37). Parallel to the increase in blood pressure, an attenuation of baroreflex function has also been documented during DPAG stimulation (38, 44). Descending pathways involved in mediating cardiovascular changes associated with DPAG stimulation include the ventrolateral PAG (32), the parabrachial nucleus (PBN) (38), and sympathoexcitatory neurons in the rostral ventrolateral medulla (RVLM) (44).

Only recently has the ventilatory response to DPAG stimulation received attention. Data from our laboratory (22) and that of Huang et al. (25) demonstrated that activation of the DPAG induces an increase in respiratory frequency associated with a decrease in inspiratory duration (TI) and expiratory duration (TE) as well as an increase in tonic diaphragmatic electromyographic (dEMG) activity. The descending pathways through which the DPAG mediates these changes in respiratory timing are largely unknown. Huang et al. reported that β-adrenoceptor blockade in the medial nucleus of the solitary tract selectively eliminated DPAG-evoked changes in respiration independent of DPAG-evoked changes in blood pressure and HR. However, direct descending projections from the DPAG to the nucleus tractus solitarius are relatively sparse (4, 14), suggesting that intermediate sites may be involved in relaying input from the DPAG to respiratory control centers.

In the present study, it was hypothesized that DPAG-evoked changes in respiration are mediated through descending projections to the lateral PBN (LPBN). This hypothesis was based in part on previous work from Nosaka and colleagues (38), who demonstrated that the parabrachial region is important in mediating DPAG-evoked modulation of the baroreflex function and blood pressure (38). Additionally, previous work from our laboratory demonstrated that DPAG stimulation induces a significant increase in c-Fos protein expression in the LPBN (21). The role of the LPBN in mediating DPAG-evoked responses was tested after inhibition of LPBN neuronal activity with the inhibitory GABA_A receptor agonist muscimol or chemical blockade of glutamate receptors using the broad-spectrum receptor antagonist kynurenic acid.

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METHODS

All experiments were performed on adult male Sprague-Dawley rats (Harlan, 320–420 g) housed in the University of Florida animal care facility. The rats were exposed to a normal 12:12-h light (6 AM–6 PM)-dark (6 PM–6 AM) cycle. All animal handling and experimental protocols were reviewed and approved by the University of Florida Institutional Animal Care and Use Committee and were in compliance with the Animal Welfare Act and US Public Health Service policy on the humane care and use of laboratory animals.

General preparation. Animals were initially anesthetized with an injection of urethane (1.2–1.4 g/kg ip). No surgical procedures were started until a surgical plane of anesthesia was achieved as evidenced by the absence of a respiratory or motor response to noxious hindlimb pinch. If necessary, supplemental doses of anesthesia (0.1–0.4 g/kg ip) were given to achieve a surgical plane of anesthesia. Next, femoral arterial and venous catheters were inserted for recording of arterial pressure and administration of supplemental anesthesia and intravenous fluids, respectively. While the animals were in the supine position, a midline incision was made on the ventral surface of the neck. A tracheostomy was performed, and the animal was intubated. All animals were spontaneously breathing a mixture of room air mixed with 100% O2. Two small (0.003-mm-diameter) Teflon-coated, stainless steel wires with bare tips were inserted on the right side into the costal region of the diaphragm through the abdominal musculature for measurement of dEMG activity. The animal then was placed in the prone position in a stereotaxic head holder (Kopf Instruments, Tujunga, CA), and the brain regions overlying the PAG and LPBN were exposed bilaterally by a craniotomy and removal of the dura. The need for supplemental anesthesia was evaluated every 30–40 min throughout the experiment by monitoring the response to a noxious pinch of the hindpaw. If fluctuations in blood pressure, HR, or respiration were observed, all experimental procedures were terminated and supplemental anesthesia was administered (0.1 g/kg iv per dose) until a surgical plane of anesthesia was reestablished.

The arterial catheter was attached to a calibrated pressure transducer connected to an amplifier (Stoelting, Wood Dale, IL). The analog output from the blood pressure amplifier was connected to a computer data-sampling system (model 1401 computer interface, Cambridge Electronics Design, Cambridge, UK). The dEMG electrode wires were connected to a preamplifier probe (model H1P5, Grass Instruments, West Warwick, RI) in series with a signal amplifier (model P511). The dEMG signal was amplified (5,000–50,000), band-pass filtered (0.3–3.0 kHz), rectified, and integrated (Paynter filter, 50-ms time constant; BAK Electronics, Rockville, MD). The rectified and integrated dEMG signal was sent to the data-sampling system. Resting rectified and integrated dEMG burst amplitude and arterial pressure were recorded simultaneously. Body temperature was monitored continuously with a rectal temperature probe and maintained within 38 ± 1°C with a heating blanket (Harvard Bioscience, Holliston, MA).

Protocol. The PAG was activated electrically using an insulated, stainless steel wire monopolar (1-MΩ) electrode secured to a micropositioner (model MP-660, Kopf Instruments) for stereotaxic placement in the brain. The stimulating electrode was connected to an isolated voltage stimulator (model DS22A, Digitimer, Hertfordshire, UK), in series with a programmable stimulator (Master8, AMPI, Jerusalem, Israel). The stimulating electrode was positioned into the region of the DPAG at a 45° angle according to stereotaxic coordinates described by Paxinos and Watson (40), such that the tip of the electrode was 7.8–8.0 mm caudal from bregma, 0.2–0.3 mm lateral from midline, and 3.5–4.1 mm ventral to the surface of the brain. Electrical stimulation parameters of the DPAG were set at 70 μA, 0.4-ms pulse width, and 10–40 Hz. Continuous stimulation for 10–15 s was followed by ≥2 min of rest.

After characterization of the cardiorespiratory response to PAG stimulation at different frequencies of stimulation, a single-barrel glass microinjection pipette was positioned first into the left and then the right LPBN. The microinjection pipette was secured to a micromanipulator (Kopf Instruments) and connected to a pressure injection system (model BH2, Medical Systems). One of three solutions was bilaterally microinjected (90 nl per side) into the region of the LPBN. Each animal received only one drug. For chemical inhibition of the LPBN, 0.45 nmol of the GABA, receptor agonist muscimol (5 mM solution) was microinjected bilaterally into the LPBN. The concentration of muscimol used for blockade was determined in preliminary studies examining the minimum dose needed to block DPAG-evoked respiratory responses (2, 5, and 10 mM; data not shown). For selective blockade of glutamatergic input to the LPBN, 1.8 nmol of kynurenic acid (20 mM solution) were microinjected bilaterally into the LPBN. The dose of kynurenic acid was based on previous studies in this laboratory (20). Muscimol and kynurenic acid were diluted in artificial cerebrospinal fluid (aCSF) containing (in mM) 122 NaCl, 3 KCl, 25.7 NaHCO3, and 1 CaCl2, with pH adjusted to 7.4. Finally, to control for the effects of microinjection alone, a separate group of animals underwent bilateral microinjection (90 nl per side) of aCSF into the LPBN. Small amounts of fluorescent latex microspheres (Lumafuor, Naples, FL) were mixed into all injectates to facilitate later identification of the microinjection sites.

The volume of microinj ectate was determined by monitoring the movement of the meniscus in the microinjection pipette with a monocular microscope equipped with a calibrated eyepiece (Titan Tools, Buffalo, NY). At 3 min after stereotaxic placement of the microinjection solution into the second side of the LPBN, the ventilatory and cardiovascular responses to DPAG stimulation were retested. Recovery of DPAG-evoked responses was tested at 60 min after the initial completion of bilateral LPBN microinjection.

At the end of the experiment, the animal was euthanized and an electrolytic lesion was made in the stimulation site (4 mA, 10 s duration). For all animals, the brain was removed and placed in 4% paraformaldehyde solution for 24–72 h. The brains were then frozen and after DPAG stimulation. Peak changes during DPAG stimulation were retested. Recovery of DPAG-evoked responses was tested at 60 min after the initial completion of bilateral LPBN microinjection.

Data analysis. All data were analyzed off-line using Spike2 software (Cambridge Electronics Design) over 1-s time intervals. Mean arterial pressure (MAP) was calculated from the difference between the systolic and diastolic pressures divided by 3, plus the diastolic pressure. HR was calculated from the interval between systolic pressure peaks. Respiratory parameters were calculated from individual bursts in the integrated dEMG signal and then averaged. Ti was measured from the onset of dEMG burst activity to the point at which the peak dEMG activity began to decline. Te was measured from the offset of Ti to the onset of the following inspiration. Respiratory frequency was calculated from the inverse of the sum of Ti and Te. Baseline dEMG was defined as the minimum dEMG measured between bursts. dEMG burst amplitude was measured as the peak amplitude during Ti. The change in integrated dEMG during inspiration, or ∆dEMG, was calculated as the difference between baseline and dEMG peak burst amplitude.

Resting or prestimulus respiratory and cardiovascular values were averaged over a 10-s period just before the onset of DPAG stimulation. After the onset of stimulation, cardiorespiratory parameters were averaged over two successive 5-s periods, which are referred to as Stim-1 and Stim-2 (Fig. 1, left). MAP, HR, Ti, Te, and respiratory frequency were compared before and after DPAG stimulation. Peak changes during DPAG stimulation were calculated as the difference between prestimulus and poststimulus (Stim-1 or Stim-2) values. A two-way ANOVA with repeated
A significant increase in ΔdEMG amplitude (burst amplitude adjusted for changes in baseline) relative to prestimulus levels. Increases in burst amplitude measured during Stim-2 were not significantly different from changes measured during Stim-1.

On the basis of this initial analysis that the DPAG-evoked response was greatest during Stim-2, all subsequent analyses were done comparing prestimulus values with those averaged during Stim-2.

Effect of muscimol blockade in the LPBN on DPAG-evoked responses. Figure 1, right, illustrates the effect of bilateral muscimol microinjection in the LPBN on prestimulus cardiorespiratory function and DPAG-evoked responses. Compared with control responses (before LPBN blockade), bilateral inhibition of LPBN neurons reduced prestimulus respiratory rate and markedly attenuated cardiovascular and respiratory changes evoked by DPAG stimulation.

The average response to DPAG stimulation before and after bilateral microinjection of muscimol in the LPBN from six animals is shown in Figs. 2A and 3A. Bilateral blockade of the LPBN significantly reduced the prestimulus respiratory rate but

measured during Stim-1. DPAG stimulation also induced a significant increase in ΔdEMG amplitude (burst amplitude adjusted for changes in baseline) relative to prestimulus levels. Increases in burst amplitude measured during Stim-2 were not significantly different from changes measured during Stim-1.

On the basis of this initial analysis that the DPAG-evoked response was greatest during Stim-2, all subsequent analyses were done comparing prestimulus values with those averaged during Stim-2.

Effect of muscimol blockade in the LPBN on DPAG-evoked responses. Figure 1, right, illustrates the effect of bilateral muscimol microinjection in the LPBN on prestimulus cardiorespiratory function and DPAG-evoked responses. Compared with control responses (before LPBN blockade), bilateral inhibition of LPBN neurons reduced prestimulus respiratory rate and markedly attenuated cardiovascular and respiratory changes evoked by DPAG stimulation.

The average response to DPAG stimulation before and after bilateral microinjection of muscimol in the LPBN from six animals is shown in Figs. 2A and 3A. Bilateral blockade of the LPBN significantly reduced the prestimulus respiratory rate but
Fig. 2. DPAG-evoked changes in respiration before and after bilateral microinjection into the LPBN. T１, inspiratory duration; Tｅ, expiratory duration; Freq, respiratory frequency. Respiratory parameters before and after muscimol (n = 6; A), artificial cerebrospinal fluid (aCSF, n = 5; B), and kynurenic acid (n = 6; C) microinjection into the LPBN are shown. I, Before LPBN microinjection; I, 3 min after LPBN microinjection; /, 60 min after LPBN microinjection. *Significant difference between baseline and Stim-2 before LPBN microinjection (P < 0.05). **Significant difference between baseline and Stim-2 3 min after LPBN microinjection (P < 0.05). ***Significant difference between baseline and Stim-2 during recovery (P < 0.05). # Significant difference between baseline values measured before and after LPBN microinjection (P < 0.05).

Fig. 3. DPAG-evoked changes in cardiovascular function before and after bilateral microinjection into the LPBN. Mean arterial pressure (MAP) and HR before and after muscimol (n = 6; A), aCSF (n = 5; B), and kynurenic acid (n = 5; C) microinjection into the LPBN are shown. I, Before LPBN microinjection; /, 3 min after LPBN microinjection; /, 60 min after LPBN microinjection. *Significant difference between baseline and Stim-2 before LPBN microinjection (P < 0.05). **Significant difference between baseline and Stim-2 3 min after LPBN microinjection (P < 0.05). ***Significant difference between baseline and Stim-2 during recovery (P < 0.05). # Significant difference between baseline values measured before and after LPBN microinjection (P < 0.05).
did not significantly alter dEMG burst amplitude, MAP, or HR relative to control values (Table 2). The decrease in pre-stimulus respiratory rate was associated with a significant increase in T1 and T2. At 3 min after LPBN blockade (Fig. 2), DPAG stimulation no longer evoked a significant increase in respiratory frequency (80 ± 17 vs. 6 ± 3 breaths/min, control vs. 3 min after drug) or HR (35 ± 2 vs. 11 ± 5 beats/min, control vs. 3 min after drug). Alternatively, DPAG continued to evoke a significant increase in MAP after LPBN blockade (Fig. 3A), but the response was markedly attenuated (42 ± 5 vs. 19 ± 5 mmHg, before vs. 3 min after drug). At 60 min after LPBN blockade with muscimol, there was no recovery of pre-stimulus respiratory parameters (Table 2, Fig. 2) or the respiratory and HR responses to DPAG stimulation. The MAP response to DPAG stimulation remained attenuated (11 ± 3 mmHg at 60 min after drug) but was still significantly different from pre-stimulus MAP (P < 0.02).

In five animals, the effect of repeat DPAG stimulation before and after bilateral microinjection of aCSF into the LPBN was tested. Bilateral microinjection of aCSF into the LPBN did not significantly alter pre-stimulus cardiorespiratory values (Table 2). Furthermore, the cardiorespiratory response evoked by DPAG stimulation was not significantly changed when retested 3 min after aCSF microinjection (Figs. 2B and 3B). At 60 min after aCSF microinjection, there was a significant decline in pre-stimulus MAP (Table 2, P < 0.006). DPAG-evoked changes in MAP, HR, and respiratory timing, however, were not significantly different from those evoked by DPAG stimulation before LPBN microinjection, with the exception of DPAG-induced changes in T1. At 60 min after aCSF microinjection into the LPBN, electrical stimulation of the DPAG continued to decrease T1 relative to before stimulation (206 ± 16 vs. 161 ± 12 ms, pre-stimulus vs. 60 min after LPBN microinjection), but the change was no longer significant (P < 0.07).

Effect of excitatory amino acid receptor blockade in the LPBN on DPAG-evoked responses. The average cardiorespiratory response to DPAG stimulation before and after bilateral microinjection of kynurenic acid in the LPBN for six animals is shown in Figs. 2C and 3C. Similar to muscimol, bilateral glutamate receptor blockade in the LPBN significantly decreased the pre-stimulus respiratory rate (P < 0.003) but did not significantly alter pre-stimulus dEMG burst amplitude, MAP, or HR (Table 2). The decrease in respiratory rate was associated with increases in T1 and T2, but relative to control, these changes were not significant (P < 0.09). At 3 min after glutamate receptor blockade in the LPBN, activation of the DPAG continued to evoke a significant increase in respiratory rate (73 ± 12 vs. 27 ± 9 breaths/min, control vs. 3 min after drug), HR (35 ± 4 vs. 16 ± 4 beats/min, control vs. 3 min after drug), and MAP (42 ± 5 vs. 19 ± 5 mmHg, control vs. 3 min after drug), but the responses were significantly attenuated compared with the pre-blockade responses (P < 0.05). At 3 min after kynurenic acid microinjection, DPAG stimulation evoked a decrease in T1 and T2, but these changes were not significant (P > 0.1).

At 60 min after LPBN blockade, the pre-stimulus respiratory rate returned to near-pre-microinjection levels but remained significantly different from control. In contrast, MAP and HR were unchanged from premicroinjection values (Table 2). At 60 min after LPBN blockade with kynurenic acid, DPAG evoked a significant increase in respiratory rate (51 ± 9 breaths/min) that was significantly greater than that measured 3 min after kynurenic acid microinjection (P < 0.05) but still significantly reduced relative to the DPAG-evoked response recorded before LPBN blockade (P < 0.05). This DPAG-evoked increase in breathing frequency at 60 min was associated with a significant reduction in T2 but not T1 (Fig. 2). At 60 min after LPBN blockade, DPAG-evoked increases in HR (27 ± 3 beats/min) and MAP (41 ± 6 mmHg) were significantly different from responses recorded 3 min after LPBN blockade but were not significantly different from pre-blockade responses (P < 0.1).

In one animal, not included in the data analysis, kynurenic acid was microinjected into the superior colliculus (SC) instead of the LPBN. Electrical stimulation of the DPAG at 40 Hz induced a marked rise in MAP, HR, and respiratory rate that was unchanged 3 min after kynurenic acid microinjection into the SC (38 vs. 37 mmHg, 46 vs. 44 beats/min, and 67 vs. 63 breaths/min).

Reconstructed stimulation and microinjection sites. DPAG stimulation sites were reconstructed from histological sections containing evidence of the electrolytic lesion site (Fig. 4A). Reconstructed stimulation sites from all experiments were distributed throughout the rostral-caudal extent of the DPAG (Fig. 4, B–D). Drug microinjection sites were reconstructed from histological sections containing the highest density of fluorescent beads (Fig. 5A). Reconstructed microinjection sites from all experiments included in the data were distributed throughout the rostral-caudal extent of the LPBN (Fig. 5, B–D). The location of the microinjection site in the SC that was ineffective in modulating the DPAG-evoked response is shown in Fig. 5D.

| Table 2. Pre-stimulus values before and after LPBN microinjection |
|---------------------|-------------------|-------------------|
|                     | Muscimol          | aCSF              | Kynurenic Acid |
| MAP, mmHg           |                   |                   |                |
| Before              | 103±7             | 111±5             | 100±12         |
| 3 min, mmHg         | 102±8             | 107±4             | 106±9          |
| 60 min              | 95±9              | 96±6              | 91±7           |
| HR, beats/min       |                   |                   |                |
| Before              | 410±18            | 401±16            | 421±16         |
| 3 min               | 411±23            | 412±17            | 407±22         |
| 60 min              | 395±28            | 417±24            | 456±22         |
| Freq, breaths/min   |                   |                   |                |
| Before              | 109±6             | 116±7             | 113±6          |
| 3 min               | 79±5*             | 118±5             | 87±8.0*        |
| 60 min              | 70±5*             | 111±2             | 101±4.4*       |
| T1, ms              |                   |                   |                |
| Before              | 190±9             | 206±16            | 201±13         |
| 3 min               | 250±26*           | 209±18            | 241±25         |
| 60 min              | 268±34*           | 201±15            | 207±12         |
| T2, ms              |                   |                   |                |
| Before              | 362±27            | 321±25            | 347±26         |
| 3 min               | 530±37*           | 306±22            | 499±81         |
| 60 min              | 610±62*           | 344±12            | 395±29         |
| Adjusted dEMG burst amplitude, AU | | |
| Before              | 1.4±0.27          | 1.2±0.15          | 1.2±0.37       |
| 3 min               | 1.3±0.2           | 1.2±0.15          | 1.3±0.45       |
| 60 min              | 1.2±0.3           | 1.2±0.2           | 1.2±0.4        |

Values are means ± SE. AU, arbitrary units. *Significantly different from Before, P < 0.05.
DISCUSSION

In the present study, electrical activation of the DPAG evoked decreases in Ti and Te. GABA_A-mediated inhibition of LPBN neurons markedly attenuated DPAG-evoked changes in respiratory timing. Bilateral blockade of glutamatergic input to the LPBN also attenuated DPAG-evoked responses, but the magnitude of the reduction was less. These findings are the first to demonstrate that the LPBN is an important relay in mediating DPAG-evoked changes in respiration. Furthermore, our results suggest that descending input from the DPAG involved in modulating respiratory timing is mediated in part through activation of glutamate receptors in the LPBN. However, the site of breath timing control in the medullary respiratory neural circuit onto which the LPBN mediates DPAG-evoked changes in respiratory timing changes remains unknown.

Effect of DPAG stimulation on cardiorespiratory response. In the present investigation, electrical stimulation was chosen over chemical stimulation as the preferred method for DPAG activation on the basis of the consistency of the response over time. Indeed, when the effects of DPAG stimulation were tested over the course of >1 h (aCSF-microinjected animals), there was remarkably little degradation of the evoked response. The use of electrical stimulation also affords precise identification of stimulus onset and offset times, which facilitates response quantification. Electrical stimulation of the DPAG before LPBN blockade induced a significant increase in respiratory frequency and a small increase in adjusted dEMG burst amplitude (ΔdEMG). The onset of the change in breath timing was immediate, as previously described by our laboratory, occurring within the first breath during DPAG electrical stimulation (22). This change in respiratory timing and dEMG amplitude is similar to that previously reported in response to chemical activation of the DPAG (5, 22, 25). DPAG stimulation also induced a significant increase in MAP and HR that closely matched the changes previously reported to be induced by chemical stimulation (2, 38). This suggests that although electrical stimulation activates neurons and fibers of passage, activation of the DPAG by electrical stimulation primarily mediates responses associated with the local activation of DPAG neurons.

Effect of chemical blockade of the LPBN on DPAG-evoked responses. Nosaka and colleagues (38) first proposed that neurons in the region of the dorsolateral pons may be involved in mediating DPAG-evoked responses. In their study, chemical (kainic acid) blockade or electrolytic lesion of the parabrachial area markedly attenuated DPAG-evoked increases in MAP and DPAG-evoked attenuation of baroreflex function. Yet their study was performed in animals that were paralyzed and...
Fig. 5. Reconstructed LPBN bilateral microinjection sites. A: outline of rostral pons/caudal midbrain (40) and corresponding histological section from an animal illustrating the rostral LPBN viewed with bright-field and fluorescent microscopy. spc, Superior cerebellar peduncle; dashed lines, boundaries of the LPBN; arrow, reconstructed center of the LPBN microinjection site viewed under fluorescence. B–D: reconstructed bilateral LPBN microinjection sites (shaded circles) from all muscimol (n = 6), aCSF (n = 5), and kynurenic acid (n = 6) experiments. Filled rectangles in middle schematic in D illustrate location of bilateral microinjection sites from 1 animal made dorsal to the LPBN and not included in data analysis; this site was not effective in modulating DPAG-evoked responses. Hatched regions represent location of superior cerebellar peduncle. Numbers to the left of LPBN images indicate location of brain section relative to bregma. Schematics of brain regions were adapted from Paxinos and Watson (40). *, Location of central aqueduct.

Artificially ventilated and under continuous β-adrenergic receptor blockade. Consequently, the role of PBN blockade in DPAG-evoked changes in respiration and HR has not been previously studied.

In our study, chemical inhibition of the LPBN essentially eliminated DPAG-evoked changes in respiratory timing and HR. This provides the first evidence that neurons in the region of the LPBN are essential for mediating descending activation of cardiac sympathetic drive and activation of respiratory centers in the medulla during DPAG stimulation. This observation was a little surprising, because direct projections from the PAG to medullary regions specifically involved in modulating cardiac sympathetic preganglionic activity and phrenic motoneuron output have been neuroanatomically identified (15, 24). Our results raise the possibility that although these direct projections exist, the impact of these projections on overall cardiorespiratory function is highly dependent on convergent input from the LPBN.

In contrast to the striking effect of chemical inhibition of the LPBN on DPAG-evoked changes in respiration and HR, changes in MAP were only partially attenuated. This observation suggests that alternate pathways, outside the LPBN, possibly direct projections to the RVLM, are also involved in DPAG-evoked changes in MAP (32, 43, 44). Our observations also support previous work suggesting that changes in HR and MAP evoked from “defense” regions of the brain may travel via separate pathways (17).

Similar to our results, Nosaka and colleagues (38) also reported that DPAG-evoked changes in MAP were attenuated after blockade of the PBN region. In their study, however, the response was attenuated by ~75%, whereas we observed a 50% reduction in the evoked response. Technical differences between the studies may have contributed to this variation. First, in their study, the PBN region was bilaterally blocked using a method of unilateral transection combined with 500 nl of kainic acid on the opposite side. In our study, we bilaterally blocked the LPBN with much smaller volumes (90 nl per side) and did not transect the region. Thus we may not have effectively blocked all regions of the LPBN important in mediating this response. Additionally, in the present study, criteria for identifying a dose of muscimol that was sufficient for blockade were based on the ability of the dose to eliminate the respiratory response. Thus it is possible that microinjection of a higher dose of muscimol might be required to produce more complete blockade of the MAP response. Additional studies are needed to determine whether the threshold for inhibition of the DPAG-evoked MAP response is different from the HR or respiratory responses.

Bilateral blockade of the LPBN with kynurenic acid also markedly attenuated DPAG-evoked responses. This suggests that descending input from the DPAG to the LPBN is mediated in part through the release of glutamate in the LPBN. Additionally, this observation reinforces a recent study from our laboratory demonstrating that c-Fos protein expression, a marker of neuronal activation, increases in select regions of the LPBN after chemical or electrical activation of the DPAG (21). Regions that showed an increase in Fos-like immunoreactivity included the central lateral and superior lateral subnuclei of the rostral LPBN and the central lateral subnuclei of the middle LPBN, but not the medial LPBN or Kölliker-Fuse area. Numerous studies have established that glutamatergic excitation of LPBN neurons yields marked increases in MAP, HR, and
respiratory rate (11–13, 28, 35) and simultaneously attenuates baroreflex function (23, 30, 36, 41), similar to increases evoked by DPAG excitation (2, 6, 10, 33, 38, 39). Yet, because kynurenic acid was less effective than muscimol in attenuating the DPAG-evoked responses, our results raise the possibility that other neurotransmitters are involved in mediating descending excitation of the LPBN. Nevertheless, additional doses of kynurenic acid or other glutamate receptor antagonists should be tested before the role of glutamate release in the LPBN in response to DPAG stimulation can be thoroughly understood.

Effect of LPBN blockade on baseline cardiorespiratory function. Bilateral blockade of the LPBN with muscimol significantly reduced prestimulation or baseline respiratory rate (11–13, 28, 35) and simultaneously attenuates cardiorespiratory responses to glutamate microstimulation of the parabrachial nucleus (21, 26). In conclusion, the results of our study demonstrate for the first time that the respiratory and HR components of DPAG-evoked responses are primarily mediated by neurons in the LPBN. Our findings also suggest that the LPBN has a tonic, steady-state input into the medullary neural respiratory control system involved in modulating breath phase timing and acts on the inspiratory and expiratory phase timing components. Comparisons between the results from muscimol blockade and kynurenic acid suggest that DPAG-evoked changes in cardiorespiratory function are mediated in part through glutamatergic activation of LPBN area neurons and can be inhibited by GABAergic receptor activation. In contrast, DPAG-evoked changes in MAP appear to be mediated only partially through LPBN neurons and probably involve more direct pathways to sympathetic neurons in the RVLM. Together the results of the present study and those from Nosaka and colleagues (39) firmly establish a prominent role for the LPBN in mediating midbrain-elicted physiological changes necessary for the body to defend itself against potentially life-threatening stimuli.

Localization of DPAG stimulation and LPBN microinjection sites. In the present study, all PAG stimulation sites were localized to the DPAG. On the basis of neuroanatomic data (27) and recent c-Fos data from our laboratory (21), we primarily targeted the rostral LPBN for microinjection. Reconstruction of the LPBN microinjection sites indicated that all sites effective in modulating DPAG-evoked responses were localized along the rostral-caudal extent of the LPBN. Similar volume injections into the SC did not modulate DPAG-evoked responses. On the other hand, within the LPBN there appeared to be no specificity of the microinjection site relative to the rostral vs. caudal regions of the LPBN. This generalized effect of LPBN blockade compared with the relatively specific projection pattern of DPAG descending inputs to the rostral LPBN might be explained by the complexity of the interconnections with the different columns of the PAG. For example, the dorsal columns of the PAG are interconnected with the lateral columns of the PAG (26), which in turn has more widespread projections to the LPBN, including rostral, middle, and caudal LPBN (27). Alternatively, subnuclei within the LPBN are interconnected (46). Thus DPAG-evoked responses may involve polysynaptic recruitment of neurons within the LPBN, such that chemical blockade of either rostral or caudal regions sufficiently disrupts output to other brain stem regions. Data from c-Fos studies suggest that both are likely possibilities (21, 26).

In conclusion, the results of our study demonstrate for the first time that the respiratory and HR components of DPAG-evoked responses are primarily mediated by neurons in the LPBN. Our findings also suggest that the LPBN has a tonic, steady-state input into the medullary neural respiratory control system involved in modulating breath phase timing and acts on the inspiratory and expiratory phase timing components. Comparisons between the results from muscimol blockade and kynurenic acid suggest that DPAG-evoked changes in cardiorespiratory function are mediated in part through glutamatergic activation of LPBN area neurons and can be inhibited by GABAergic receptor activation. In contrast, DPAG-evoked changes in MAP appear to be mediated only partially through LPBN neurons and probably involve more direct pathways to sympathetic neurons in the RVLM. Together the results of the present study and those from Nosaka and colleagues (39) firmly establish a prominent role for the LPBN in mediating midbrain-elicted physiological changes necessary for the body to defend itself against potentially life-threatening stimuli.

GRANTS

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REFERENCES


