Respiratory response to activation or disinhibition of the dorsal periaqueductal gray in rats

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Hayward, Linda F., Camille L. Swartz, and Paul W. Davenport. Respiratory response to activation or disinhibition of the dorsal periaqueductal gray in rats. J Appl Physiol 94: 913–922, 2003. First published November 15, 2002; 10.1152/japplphysiol.00740.2002.—The neural substrates mediating autonomic components of the behavioral defense response have been shown to reside in the periaqueductal gray (PAG). The cardiovascular components of the behavioral defense response have been well described and are tonically suppressed by GABAergic input. The ventilatory response associated with disinhibition of the dorsal PAG (dPAG) neurons is unknown. In urethane-anesthetized, spontaneously breathing rats, electrical stimulation of the dPAG was shown to decrease the expiration time and increase respiratory frequency, with no change in time of inspiration. Baseline and the change in diaphragm electromyograph also increased, resulting in an increase in neural minute activity. Microinjection of bicuculline methobromide, a GABA<sub>A</sub>-receptor antagonist, into the dPAG produced a similar response, which was dose dependent. Disinhibition of the dPAG also produced a decrease in inspiration time. These results suggest that GABA<sub>A</sub>-mediated suppression of dPAG neurons plays a role in the respiratory component of behavioral defense responses. The respiratory change is due in part to a change in brain stem respiratory timing and phasic inspiratory output. In addition, there is an increase in tonic diaphragm activity.

THE DORSAL PERSIQUEDUCTAL GRAY (PAG; dPAG) nucleus is a relay from various central neural pathways to the limbic cortex. Stimulation of the dPAG is known to induce autonomic reflexes that have a strong sympathetic component. The behavioral response to dPAG stimulation is defensive behavior, typically characterized by an increase in aggressive or escape actions of the animal (1–3, 8). Stimulation of this region of the PAG also elicits fear (15), anxiety (5), and vocalization (23, 35). Part of the autonomic response for these behaviors is an increase in muscular, cardiovascular, and ventilatory activity to prepare the animal for high-intensity exercise. This defensive control system is inhibited by GABAergic neurons. Injection of GABA<sub>A</sub> agonists increases the threshold for eliciting defensive behavior, whereas GABA<sub>A</sub> antagonist injection into the dPAG elicits defensive-like behavior (6, 19, 31, 32). Obstruction of breathing, hypercapnia, bronchoconstriction, and hypoxia elicit well-characterized respiratory reflexes, and they also elicit defensive affective responses. The affective components of respiratory behavioral responses to threatening ventilatory conditions are unknown. It has been hypothesized that the dPAG is one component of the defensive behavioral response to ventilatory challenges (9).

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, 5). 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 (11, 12, 25). Parallel to the increase in blood pressure, an attenuation of baroreflex function has also been documented during dPAG stimulation (27, 33). Descending pathways involved in mediating cardiovascular changes associated with dPAG stimulation include the ventrolateral PAG (21), the parabrachial nucleus (27), and sympatoexcitatory neurons in the rostral ventrolateral medulla (33).

The ventilatory response to dPAG stimulation has received very little investigation. Huang et al. (18) used an excitatory amino acid receptor agonist, dl-homocysteic acid (DLH), to stimulate the dPAG while recording diaphragm electromyographic (dEMG) activity. They reported an increase in respiratory frequency associated with a decrease in both time of inspiration (T<sub>i</sub>) and time of expiration (T<sub>e</sub>) averaged over 10 breaths. There was also an apparent increase in baseline dEMG activity, but this was not analyzed. They also reported an increase in nucleus tractus solitarius inspiratory neuron activity. Although their results provide a foundation for the study of the respiratory behavior mediated by the dPAG, the ventilatory pattern response to dPAG stimulation remains unknown. In

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addition, it is unknown if the dPAG respiratory behavior is normally inhibited by GABAergic mechanisms.

In the present study, it was hypothesized that stimulation of the dPAG would increase diaphragm activity, increase respiratory frequency, and increase neural minute activity. It was further hypothesized that this respiratory response to dPAG stimulation is normally inhibited by GABA. These hypotheses were tested in anesthetized rats. The respiratory activity was measured from dEMG activity. The respiratory response to dPAG neural excitation was studied by electrical stimulation of the nucleus, and dPAG inhibition was studied by injection of bicuculline methobromide (BicM), a GABA receptor antagonist.

METHODS

All experiments were performed on adult male Sprague-Dawley rats (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 to 6 PM)-dark cycle (6 PM to 6 AM). All experimental procedures were approved by the University of Florida Institutional Animal Care and Use Committee.

General preparation. Animals were anesthetized with an intraperitoneal injection of urethane (1.2–1.4 g/kg) and instrumented with femoral arterial and venous catheters for recording of arterial pressure and administration of intravenous fluids, respectively. While the animal was in the supine position, a midline incision was made on the ventral surface of the neck. A tracheotomy was performed, and the animal was intubated. All animals were spontaneously breathing a mixture of room air and 100% oxygen. Two small (0.003-mm-diameter) Teflon-coated, stainless steel wires with bared 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 was then placed in the prone position in a stereotaxic head holder (Kopf Instruments, Tujunga, CA), and the brain region overlying the PAG was exposed by a limited craniotomy and removal of the dura.

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 (Cambridge Electronics Design (CED) 1401 computer interface, Cambridge, UK). The dEMG electrode wires were connected to a Grass preamplifier probe (H1P5, Grass Instruments, West Warwick, RI) in series with a signal amplifier (P511). The dEMG signal was amplified (5–50 K), bandpass 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 CED data-sampling system (Spike2, CED). Resting rectified and integrated dEMG burst amplitude was arbitrarily adjusted to a value of 1.0–2.0 (arbitrary units) at the beginning of the experiment. Rectified and integrated dEMG activity 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). Supplemental anesthesia was given as necessary throughout the experiment (0.1 g/kg iv), as evidenced by fluctuations in blood pressure, HR, or respiration during surgery or in response to a pinch of the hind paw.

Protocol. Each rat underwent only one type of PAG stimulation, either electrical or chemical, following stabilization of resting blood pressure and respiratory pattern. For electrical stimulation, an insulated, stainless steel wire monopolar electrode (1 MΩ) was connected to an isolated voltage stimulator (DS2A, Digitimer, Hertfordshire, UK), in series with a programmable stimulator (Master8, AMPI, Jerusalem, Israel). The monopolar electrode was then secured to a micropositioner (MP-600, Kopf Instruments, Tujunga, CA) for stereotaxic placement into the brain. Alternatively, for chemical stimulation, a single-barrel microinjection pipette, attached to a pressure injection system (PPS-2, Medical Systems, Greenvale, NY), was secured to the micropositioner. The pipette or stimulating electrode was positioned into the region of the dPAG, according to stereotaxic coordinates described by Paxinos and Watson’s The Rat Brain in Stereotaxic Coordinates (28), between 7.7 and 8.0 mm caudal from bregma, 0.2–0.3 mm lateral from midline, and 3.8 and 4.1 mm ventral to the surface of the brain.

Electrical stimulation parameters of the dPAG were set between 7 and 10 V, 0.2-ms pulse duration, and 25– to 40-Hz stimulation frequencies. Mean arterial pressure calibration was applied for 10–15 s followed by periods of 30–90-s rest. Chemical stimulation of the dPAG was induced by local microinjection of the GABA receptor antagonist BicM (0.2–1.0 mM; Sigma Chemical, St. Louis, MO). BicM mediates disinhibition of the dPAG, uncovering endogenous excitatory inputs (6, 31). BicM was diluted in artificial cerebrospinal fluid (aCSF) containing 122 mM NaCl, 3 mM KCl, 25.7 mM NaHCO3, and 1 mM CaCl2, with pH adjusted to 7.4. Small amounts of fluorescent latex microspheres (Lumafuor, Naples, FL) were mixed into the aCSF to facilitate later identification of the microinjection sites. After stereotaxic placement of the microinjection pipette into the left side of the dPAG, 20–65 nl of BicM were microinjected into the brain over 30 s. The ventilatory and cardiovascular responses were recorded. The volume of microinjecate was determined by carefully monitoring the movement of the meniscus in the microinjection pipette with a monocular microscope equipped with a calibrated eyepiece (Titan Tools, Buffalo, NY). To control for the effects of microinjection alone, some animals also received unilateral microinjections of 20–65 nl of aCSF. One minute after completion of a central injection, the pipette was retracted from the brain. Each animal received only two doses of BicM in a randomized order. A separate group of control animals received central injections of aCSF. All microinjections were made at intertrial intervals no less than 30 min.

At the end of the experiment, the animal was euthanized. For those animals that underwent electrical stimulation, a electrolytic lesion was made in the stimulation site (10 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 to −16°C, and the midbrain was sliced into 40-μm transverse sections with a cryostat (HM101, Carl Zeiss, Thornwood, NY). The tissue was mounted on slides and sealed with a coverslip (Antifade, Molecular Probes, Eugene, OR). Injections and stimulation sites were imaged with a microscope equipped with both bright field and epifluorescence.

Data analysis. All data were analyzed off-line by using Spike2 software (CED). 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. The HR and MAP were measured at the end of inspiration. Respiratory parameters were calculated from...
individual bursts in the integrated dEMG signal and then averaged. Tt 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 Tt to the onset of the following inspiration. Time of the respiratory cycle (Ttot) was determined by calculating the sum of Tt and TE. Baseline dEMG was defined as the minimum dEMG value measured between bursts. dEMG burst amplitude was measured as the peak amplitude during Tt. The change in integrated dEMG during inspiration (delta dEMG) was calculated as the difference between baseline and dEMG peak burst amplitude. Neural minute activity was calculated by multiplying the delta dEMG amplitude by the instantaneous frequency.

For both electrical and chemical stimulation trials, baseline respiratory and cardiovascular values were averaged over the five-breath period collected just before the onset of stimulation. After the onset of stimulation, cardiorespiratory parameters were then averaged over successive 10-breath periods. One 10-breath average was taken during stimulation, referred to as the “stimulation-on average.” Immediately after the offset of stimulation, three 10-breath averages were taken (referred to as stimulation-off averages 1, 2, and 3). During chemical stimulation, successive 10-breath averages were calculated every 30 s after completion of the microinjection period. A maximum of eighteen 10-breath averages over ~520-s post-microinjection were measured.

Absolute values for MAP, HR, Tt, TE, Ttot, and respiratory frequency were compared before and after dPAG stimulation. Peak changes during dPAG stimulation were calculated as the difference between prestimulus vs. poststimulus values. Baseline dEMG, delta dEMG amplitude, and neural minute ventilation were expressed as a percentage of control. A maximum of eighteen 10-breath averages over ~520-s post-microinjection were measured.

Absolute values for MAP, HR, Tt, TE, Ttot, and respiratory frequency were compared before and after dPAG stimulation. Peak changes during dPAG stimulation were calculated as the difference between prestimulus vs. poststimulus values. Baseline dEMG, delta dEMG amplitude, and neural minute ventilation were expressed as a percentage of control. A one-way ANOVA with repeated measures was used to identify significant changes in cardiorespiratory parameters after electrical stimulation of the dPAG. A two-way ANOVA (factors: time and dose) was used to test for significant dose effects of BicM. A one-way ANOVA was used to compare the effect of dose on changes in cardiorespiratory values. When differences were indicated, a Bonferroni/Dunn or Tukey post hoc comparison was used to identify significant effects. P < 0.05 was accepted as significant for all tests used. All data are reported as means ± SE.

RESULTS

In all animals, the position of the electrical stimulation electrode was in the dPAG (Fig. 1). The microinjection site for all animals exposed to BicM or aCSF injection was also in the dPAG (Fig. 2). The mean resting MAP, HR, and respiratory frequency of all animals that underwent electrical stimulation vs. chemical stimulation are shown in Table 1.

Electrical stimulation. Electrical stimulation of the dPAG elicited an immediate increase in respiratory activity in all animals (Fig. 1). The average change in respiratory parameters is shown in Figs. 3 and 4. During electrical stimulation of the dPAG, there was a significant reduction in both stimulus-on TE and Ttot relative to control (Fig. 3A). Ti did not change significantly from control during stimulus-on conditions. Associated with the decrease in TE and Ttot, there was a significant increase in respiratory frequency (Fig. 3B). At the offset of stimulation (stimulus-off 1), Tt, Ttot, and respiratory frequency were not significantly different from control. Within 30 breaths (stimulus-off 3), Tt, Ttot, and respiratory frequency had all returned to control and were significantly different from the stimulus-on condition. After the offset of stimulation, there was a small reduction in Ti, but this change was not significantly different from control or the stimulus-on condition.

Electrical stimulation also elicited an immediate increase in baseline dEMG activity and peak burst amplitude (Fig. 1). The average increase in baseline activity during electrical stimulation was significantly different from control in all animals (Fig. 4A). Immediately after the offset of stimulation, baseline activity remained high, but this increase was not significantly
different from control. Peak dEMG burst amplitude increased in the first breath and was sustained throughout stimulation. The average stimulus-on peak activity was 170 ± 10% above control ($P < 0.001$). The peak dEMG burst activity was the combination of baseline activity and the change in dEMG activity with each breath. After the subtraction of baseline dEMG levels, the average change in dEMG amplitude or delta dEMG during inspiration showed a significant increase during electrical stimulation relative to control (Fig. 4B). Immediately after the offset of stimulation, delta dEMG remained elevated and was not significantly different from either control or stimulation values. Delta dEMG returned to control levels and was significantly different from stimulus-on levels by stimulus-off 2. Associated with the significant increase in both delta dEMG and respiratory frequency, there was a

Table 1. Resting cardiorespiratory parameters of different experimental groups

<table>
<thead>
<tr>
<th>Stimulation Condition</th>
<th>Mean Arterial Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>Respiratory Frequency, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical stimulation (n = 5)</td>
<td>108 ± 16 vs.</td>
<td>396 ± 11 vs.</td>
<td>97 ± 9 vs.</td>
</tr>
<tr>
<td>Bicuculline microinjection</td>
<td>118 ± 16*</td>
<td>406 ± 18*</td>
<td>121 ± 15*</td>
</tr>
<tr>
<td>Artificial cerebrospinal fluid</td>
<td>107 ± 4 vs.</td>
<td>381 ± 15 vs.</td>
<td>114 ± 9 vs.</td>
</tr>
<tr>
<td>microinj (n = 4)</td>
<td>125 ± 9*</td>
<td>447 ± 14*</td>
<td>199 ± 11*</td>
</tr>
<tr>
<td>Artificial cerebrospinal fluid</td>
<td>99 ± 13 vs.</td>
<td>378 ± 13 vs.</td>
<td>107 ± 8 vs.</td>
</tr>
</tbody>
</table>

Values are means ± SE of control vs. average; n, no. of animals. *Significant difference from control, $P < 0.05$. #Significant difference between stimulus-off average and stimulus-on average, $P < 0.05$.  

Fig. 3. Effects of electrical stimulation on ventilation timing. A: mean duration of time between inspiratory bursts (Ttot; ⬤), time of expiration (TE; ●), and time of inspiration (TI; ○) averaged from 5 breaths before (control), 10 breaths during (Stim on), and 3 successive groups of 10 breath averages each (Off-1, Off-2, Off-3) after the offset of electrical stimulation of the dPAG. B: mean respiratory frequency (Resp. Freq.) measured at control, Stim on, and after electrical stimulation of the dPAG. All data were averaged from 5 animals, and values are means ± SE. *Significant difference from control, $P < 0.05$. #Significant difference between stimulus-off average and stimulus-on average, $P < 0.05$. 

Fig. 2. Composite of chemical (shaded ovals) and artificial cerebrospinal fluid (asterisks) microinjection sites and an original recording of AP, HR, and dEMG from 1 animal before, during, and after bicuculline methobromide (BicM) microinjection into the dPAG (right). Left: reconstructed microinjection sites (n = 9) are based on the locations of fluorescent microsphere deposits. Schematics of midbrain PAG were adapted from Paxinos and Watson’s The Rat Brain in Stereotaxic Coordinates (28). Right: completion of the microinjection of BicM (0.3 mM, 45 nl) into the dPAG (25 Hz, 8 V, 0.2 ms) is indicated by "v." Arrow from left panel indicates location of reconstructed microinjection site.
significant increase in neural minute activity during the stimulus-on condition compared with control (Fig. 4B). Immediately after the offset of stimulation, neural minute activity was not significantly different from the stimulus-on condition but by stimulus-off 3 had returned to control and was significantly different from stimulus-on.

Associated with the increase in respiration during electrical stimulation of the dPAG, there was a significant increase in both MAP and HR (Table 1). Immediately after the offset of stimulation, MAP and HR returned to control levels and were not significantly different from control. MAP remained similar to control during stimulus-off 2 and 3. In contrast, during the same time period, HR dropped significantly below control.

**Bicuculline disinhibition.** BicM induced an increase in respiratory activity that typically occurred within 20 s of completion of dPAG microinjection. Respiration continued to increase until it reached a steady-state 3–5 min postinjection (see Fig. 2). The onset of the respiratory response was slower than for electrical stimulation but was sustained for the entire 520-s postinjection measurement period. As illustrated in Fig. 5, disinhibition of the dPAG was associated with a dose-dependent decrease in both T₁ and Tₑ. T₁ and Tₑ after 0.3 mM BicM (n = 4) were significantly greater than after 0.5 mM BicM (n = 4), and that after 0.5 mM BicM was significantly greater than that after 1.0 mM BicM (n = 2) (Fig. 5). Associated with the combined changes in T₁ and Tₑ, Ttot decreased and breathing frequency increased in a dose-dependent manner (Fig. 6). Comparisons between control values and the 10-breath average taken 300 s after the offset of microinjection demonstrated significant changes across all doses for T₁, Tₑ, Ttot, and respiratory frequency relative to the preceding control (Table 2).

BicM microinjection into the dPAG also elicited a marked increase in baseline dEMG activity (Fig. 2). As shown in Fig. 7A, baseline activity increased significantly in a dose-dependent manner: 0.5 and 1.0 mM were significantly greater than 0.3 mM; 1.0 mM was significantly greater than 0.5 mM. The average increase in baseline activity measured 300 s after the offset of microinjection had increased above control level to a steady state of 611 ± 418% for 0.3 mM, over

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**Fig. 4.** Effects of electrical stimulation on baseline and inspiratory dEMG and neural minute activity. A: mean amplitude of baseline dEMG measured as the minimum amplitude between control, Stim on, and Off-1, Off-2, and Off-3 after the offset of electrical stimulation of the dPAG. Averages are represented as percentage of control amplitude within animals (control = 100%). B: mean change in dEMG inspiratory burst amplitude from baseline (ΔdEMG; ○) and neural minute activity (▲) measured at control, Stim on, and after electrical stimulation of the dPAG. Averages are represented as percentage of control within animals (control = 100%). All data represent averages from 5 animals. Values are means ± SE. *Significant difference from control, P < 0.05. # Significant difference between stimulus-off averages and stimulus-on average, P < 0.05. Statistical indicators below symbols correspond to analysis associated with ΔdEMG.

**Fig. 5.** Dose-related changes in T₁ (A) and Tₑ durations (B) after unilateral microinjection of BicM into the dPAG. Zero reflects time that microinjection was completed. Arrow indicates the position of the first average taken immediately after microinjection completion. Values are means ± SE. *Significant differences between groups, P < 0.05. Statistical comparisons between doses at single 10-breath average points after BicM administration were not indicated. Data from animals receiving 0.3 and 0.5 mM BicM reflect an average of 4 each. Data from animals receiving 1 mM BicM reflect an average of 2.
733 ± 191% for 0.5 mM, and over 1,478 ± 741% for 1.0 mM. The increase in baseline activity was sustained throughout the postinjection measurement period (Fig. 7A). Comparisons between the percent increase in baseline activity 300 s after the offset of microinjection vs. the percent increase in baseline activity during electrical stimulation demonstrated no significant difference between baseline dEMG activity after 0.5 mM and electrical stimulation. The increase in baseline activity induced by 1.0 mM BicM, however, was significantly greater, and 0.3 mM BicM was significantly less than electrical stimulation. There was a significant increase in the delta dEMG activity over control for all doses of BicM when measured 300 s after the offset of microinjection (133 ± 27% for 0.3 mM, 136 ± 26% for 0.5 mM, and 138 ± 4% for 1.0 mM). There was, however, no significant difference in the delta dEMG activity across doses (Fig. 7B). A comparison with the delta dEMG evoked during electrical stimulation demonstrated no significant difference across all three BicM doses.

Associated with the significant increase in both delta dEMG and respiratory frequency, there was a significant and dose-dependent increase in neural minute activity after BicM microinjection. The change in neural minute activity was significantly less after 0.3 mM than 0.5 and 1.0 mM BicM, whereas that after 0.5 mM was significantly less than that after 1.0 mM (Fig. 8). Ten-breath averages taken 300 s after the offset of microinjection demonstrated that neural minute activity increased significantly above control for all doses of

Table 2. Dose-related changes in respiratory timing after BicM microinjection into the dPAG

<table>
<thead>
<tr>
<th>BicM Dose</th>
<th>Ti, s</th>
<th>Te, s</th>
<th>Ttot, s</th>
<th>Respiratory Frequency, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 mM (n = 4)</td>
<td>0.23 ± 0.02 vs. 0.18 ± 0.02*</td>
<td>0.38 ± 0.05 vs. 0.27 ± 0.04*</td>
<td>0.61 ± 0.07 vs. 0.46 ± 0.06*</td>
<td>103 ± 11 vs. 139 ± 11*</td>
</tr>
<tr>
<td>0.5 mM (n = 4)</td>
<td>0.25 ± 0.02 vs. 0.16 ± 0.01*</td>
<td>0.28 ± 0.01 vs. 0.15 ± 0.02*</td>
<td>0.54 ± 0.05 vs. 0.28 ± 0.04*</td>
<td>114 ± 9 vs. 199 ± 11*</td>
</tr>
<tr>
<td>1.0 mM (n = 2)</td>
<td>0.28 ± 0.009 vs. 0.16 ± 0.01</td>
<td>0.37 ± 0.01 vs. 0.15 ± 0.01</td>
<td>0.65 ± 0.01 vs. 0.31 ± 0.02</td>
<td>97 ± 4 vs. 198 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SE of control vs. average; n, no. of animals. Ti, inspiration time; Te, expiration time; Ttot, respiratory cycle time. Control vs. average taken 300 s post-BicM microinjection; *Significantly different from control. Statistical comparisons for 1.0 mM were not made due to low number of subjects.
BicM (205 ± 67% for 0.3 mM, 253 ± 68% for 0.5 mM, and 268 ± 2% for 1.0 mM). The increases in neural minute activity above control associated with 0.5 mM BicM were not significantly different from increases associated with electrical stimulation. Increases in neural minute activity induced by 1.0 mM, however, were significantly greater, and those induced by 0.3 mM were significantly less, than electrical stimulation.

Associated with the increase in respiration during disinhibition of the dPAG, there was a simultaneous increase in both MAP and HR (Table 1). The increase in MAP was dose related (14 ± 3 mmHg for 0.3 mM, 19 ± 6 mmHg for 0.5 mM, and 26 ± 12 mmHg for 1.0 mM). The increase in HR induced by 1.0 mM BicM (34 ± 3 beats/min), however, was not significantly different from that induced by 0.5 mM (65 ± 3 beats/min) or 0.3 mM (44 ± 8 beats/min) BicM. Alternatively, the increase in HR induced by all three doses of BicM was significantly greater than that induced by electrical stimulation. Yet the increase in MAP induced by 0.3 mM BicM was not significantly different from that induced by electrical stimulation. The increase in MAP after 0.5 and 1.0 BicM was significantly greater than that induced by electrical stimulation.

DISCUSSION

The results of this study demonstrate for the first time that disinhibition of neurons in the dPAG induces a profound increase in respiratory muscle activity. The BicM-mediated disinhibition of dPAG neurons is presumably via inhibition of GABA_A receptors. This increase in respiratory muscle activity included changes in both the Ttot and dEMG baseline activity and burst amplitude. The increase in overall respiratory activity was accompanied by significant increases in both MAP and HR. Whereas our results clearly demonstrated that dPAG neurons involved in respiratory muscle activation receive tonic inhibitory inputs, the central integration and descending pathway(s) mediating this response remains unknown.

Breath-timing effects. The effect of dPAG activation on breath timing resulted in an increase in the rate of ventilation. Electrical stimulation elicited an immediate increase in breathing frequency, with the first breath of the stimulus-on condition exhibiting a decreased Ttot. The Ttot is divided into inspiratory and expiratory durations. The overall decrease in Ttot observed with electrical stimulation was the result of a decreased Te with little change in Ti. Bassal and Bianchi (4) electrically stimulated the PAG without determination of the specific portion of the PAG activated. They also reported a decrease in Te when single pulses were delivered during the expiratory phase.

The advantage of the electrical stimulation is the ability to observe the latency of the stimulus-on and stimulus-off effects. The stimulus-on response occurred within the first breath. The breathing rate also rapidly returned to normal when the electrical stimulation was removed. The first stimulus-off measurement period showed a decrease in respiratory frequency to a level that was not significantly different from the control period. In several animals, the first breath after the stimulator was turned off showed a large inspiratory activation indicative of a sigh or gasp. The Ti and Te increased, and the breathing frequency was similar to...
prestimulation levels. These results suggest that there is a short latency pathway between the dPAG and the neural centers mediating breath timing. Increasing dPAG neuronal activity stimulates this timing pathway, resulting in a change in the brain stem pattern generator.

The dPAG influence in breath timing is normally suppressed. Application of BicM at the lowest dose in the present study resulted in an increase in respiratory frequency greater than observed with electrical stimulation. There was a dose-dependent increase in respiratory frequency, with the highest dose eliciting more than a doubling in respiratory frequency above resting levels to a rate of ~200 breaths/min. The disinhibition of dPAG increased frequency by decreasing both TI and TE. The application of BicM in all concentrations produced a progressive decrease in Ti and Te over the initial 6–10 breaths and then remained at a steady-state level for hundreds of seconds. This response was not observed with saline injections. Huang et al. (18) also found an increased breathing frequency with chemical stimulation of dPAG. Their increase in frequency was measured as the 10-breath mean change in respiratory frequency during the stimulation period. They reported a 50–60% increase in respiratory frequency. The respiratory frequency change was a result of a decrease in both Ti and Te. The maximum respiratory frequency reported in their study was 122 breaths/min with 60-nl DLH (3). In the present study, activation of the dPAG with electrical stimulation increased respiratory frequency to approximately the same rate, 120 breaths/min. Thus the disinhibition effect has a greater effect on respiration than either electrical or chemical stimulation. Furthermore, the results of our study and those of Huang et al. (18) suggest that either endogenous or chemical activation of the dPAG elicits a change in the respiratory control center, resulting in an increase in cycle rate and a decrease in cycle time by an effect on both inspiratory and expiratory durations. In contrast, electrical stimulation appears to predominantly modulate expiratory duration.

Diaphragm EMG effects. The stimulation of dPAG neurons increased dEMG activity. The peak of the integrated diaphragm signal increased in the first breath during which electrical stimulation was applied. The peak dEMG was elevated by an increase in both the magnitude of the inspiratory burst activity and an increase in tonic baseline activity. Bassal and Bianchi (4) also mentioned an augmentation of phrenic burst activity with electrical stimulation of the PAG. Huang et al. (18) noted, but did not analyze, an increase in diaphragm activity when DLH was injected into the dPAG. Examination of their Fig. 1 shows an increase in inspiratory burst amplitude and an apparent increase in baseline activity with the higher dose of DLH. In the present study, we were able to partition the increase in diaphragm activity with dPAG stimulation into two components: change in activity and baseline activity. While the magnitude of the inspiratory burst (change in EMG) increased by ~50%, the baseline activity increased 4- to 10-fold. This suggests a dual effect on the magnitude of the drive to the diaphragm, a large increase in resting muscle tone, and an increased phasic activation. Although we did not measure respiratory mechanical ventilation, the increased baseline tone suggests that functional residual capacity (FRC) may have increased by less relaxation during expiration. Future studies recording the mechanical effect of this increased diaphragm activation are needed.

Disinhibition of the dPAG again had a greater effect on the dEMG activity than electrical stimulation. There was a dose-dependent increase in peak integrated dEMG activity primarily due to a dose-dependent progressive increase in baseline dEMG activity. The inspiratory burst amplitude (delta dEMG) increased for all three doses, but there was no significant difference in either the time course of the response or the strength of the response as a function of dose. The change in diaphragm activity reached a plateau at ~30–40% above normal. The baseline activity, however, increased in a dose-dependent manner. The increase ranged from 2- to 15-fold. This indicates that there was a profound increase in phrenic motor neuron activity during the expiratory phase, again suggesting reduced diaphragm relaxation during expiration and an increase in FRC. The specific neural pathways mediating this response are unknown. It is likely that the dPAG increases the cycle timing and inspiratory magnitude of the brain stem neural oscillator. It is also possible that the descending influence of the dPAG acts in parallel to the respiratory oscillator acting on phrenic motor neurons to increase their steady-state excitability, thus producing an increase in the baseline diaphragm EMG.

The combined ventilatory drive response to dPAG activation by direct stimulation or disinhibition is illustrated by the change in neural minute activity. Similar to previous reports using phrenic integrated electromyogram magnitude and cycle rate (14), we estimated the ventilatory drive by multiplying the respiratory rate times the magnitude of the inspiratory dEMG burst (change in activity). Although this is not a measure of minute ventilation, it does allow us to predict (14) the ventilatory effect of dPAG activation. Both direct activation by electrical stimulation and disinhibition elicited an increase in the neural minute activity. With disinhibition, the dose-dependent effect was due to the dose-dependent change in respiratory frequency because there was no dose-dependent effect on inspiratory burst magnitude. The results indicate that the greater the degree of activation of the dPAG, the greater the increase in ventilation. The increased neural minute activity occurs in the presence of an increased tonic EMG activation. Future studies are needed to determine whether the increase in neural ventilation results in an increase in mechanical ventilation. It is possible that mechanical minute ventilation is not increased because of the possible increase in FRC due to the increase in baseline dEMG activity.
Cardiovascular effects. Both chemical and electrical stimulation of the dPAG induced significant increases in MAP and HR. The cardiovascular changes that we observed were similar to those reported by other investigators in both conscious and anesthetized preparations (10, 13, 27, 29). The increases in MAP and HR were proportional to the level of dPAG activation. Furthermore, comparisons between electrical vs. chemical stimulation suggest that disinhibition of the dPAG, particularly after 0.5 and 1.0 mM BicM, had a significantly greater effect on sympathetic drive compared with electrical stimulation. Recruitment of sympathoexcitatory neurons in the rostral ventrolateral medulla has been shown to mediate dPAG-evoked pressor and tachycardia responses (20, 33). DPAG activation has also been documented to attenuate baroreflex function (27, 30). Although baroreflex function was not assessed in the present study, it is possible that a portion of dPAG-evoked change in respiration may have been secondary to altered baroreflex function; blockade of baroreceptor input to the brain stem increases respiratory drive through changes in both Ti and Tc (17).

Summary. Simultaneous increases in MAP, HR, and respiration, coupled with attenuation of baroreflex function, prepare the animal to meet the physiological demands of the body during “fight or flight” responses (1). The dPAG and nearby structures have been identified as one of the few regions of the brain to contain the neural components sufficient to coordinate these behaviors (2, 11, 29, 30, 34). The presence of tonic inhibitory input to the dPAG has been well documented in relation to both behavioral and cardiovascu lar components of the defense response (6, 8, 29, 31). Similar to other regions of the brain, including certain respiratory areas (24, 26), tonic suppression and the subsequent withdrawal of inhibition allow for rapid gain adjustment of excitatory inputs (36). Although the exact origin of this inhibitory input is unknown, descending projections from forebrain structures as well as local GABAergic interneurons may contribute to tonic suppression of dPAG neurons (1, 7, 16). In the present study, we used BicM to block this tonic inhibitory input. In addition to selectively blocking GABA\(_A\)-receptor function, BicM has been shown to block \(Ca^{2+}\)-activated \(K^+\) currents. Blockade of these \(K^+\) currents increases neuronal discharge rate, independent of GABA\(_A\)-receptor blockade. We did not test the effects of a second receptor antagonist; thus we cannot completely rule out confounding effects of altered \(K^+\) conductances. Yet results from other investigators suggest that our findings were primarily due to the withdrawal of tonic inhibitory inputs. For example, microinjection of the noncompetitive GABA\(_A\)-receptor blocker picotorxin produces a similar increase in MAP, HR, and respiratory frequency as that described after BicM microinjection (29). Furthermore, both in vitro and in vivo, GABA\(_A\)-receptor blockade increases synaptic activity and blocks the inhibitory effect of GABA (22, 29). In vitro, the influence of BicM on neuronal discharge is blocked after synaptic blockade (29). Thus we are reasonably confident that the changes in respiratory cycle activity that we report here are primarily the result of neuronal disinhibition and reflect the expression of another component of the behavioral defense response.

In conclusion, the results of the present study demonstrate that neurons of the dPAG are involved in modulating respiratory function and are normally suppressed by GABA\(_A\) inhibition. Furthermore, tonic excitatory inputs to these neurons can be uncovered by GABA\(_A\)-receptor blockade. Disinhibition of the dPAG produces a powerful increase in respiratory activity characterized by a decrease in Ttot, increased inspiratory burst amplitude, and an increase in diaphragmatic activity during expiration. The pathways mediating these changes remain to be determined.

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REFERENCES


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