Respiratory muscle responses elicited by dorsal periaqueductal gray stimulation in rats

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The periaqueductal gray matter (PAG) refers to the midbrain region that surrounds the mesencephalic aqueduct. This region is an important neural structure in defense behavior, analgesia, vocalization, and autonomic regulation. Different behavior patterns have been elicited by activation of the longitudinal neuronal columns of the PAG (1, 3, 6, 30). The dorsal subdivision (dPAG) has been demonstrated to play a crucial role in fight/flight behavior and associated autonomic responses. Furthermore, the activation of the dPAG is closely related to the emotional responses of anxiety, panic, and fear (12, 22, 29). These emotional responses often have a respiratory component that may be mediated by the dPAG.

Electrical stimulation in the cat PAG elicited increased respiratory rate (fR) that was mainly due to shortening of inspiratory time (Ti) and Te was reported with microinjection of the excitatory amino acid N-methyl-D-aspartic acid (DLH) into the dPAG (17, 19). Similar respiratory responses could also be evoked by applying the GABA receptor antagonist bicuculline, activating this area by disinhibiting neurons in the dPAG (14). Inspiratory and expiratory tracheal airflow has also been reported to increase after activation of dPAG (19), suggesting the possible recruitment of expiratory muscle activity. Previous studies, however, only measured increased respiratory activity in an inspiratory muscle, the diaphragm. The present study was undertaken to test the hypothesis that dPAG activation involves the simultaneous recruitment of both inspiratory and expiratory muscles. Stimulation of the dPAG may also elicit a sustained change in the poststimulus state of the dPAG (14, 15). If this occurs, then the change of cardiorespiratory response would be sustained after the cessation of dPAG stimulation. Electrical stimulation is the technique of choice since the on- and off-stimulation timing can be reliably determined. Although electrical stimulation activates both neurons and fibers of passage, it has been demonstrated that controlled electrical stimulation in the dPAG elicits cardiorespiratory responses similar to chemical stimulation (3, 14, 25). Intensity- or frequency-dependent physiological responses have been described with electrical stimulation in the PAG (24, 28). In the present project, stimulating intensity and frequency were systematically investigated in dPAG-elicited respiratory responses. It was hypothesized that electrical stimulation of the dPAG would elicit an immediate (within the first respiratory cycle) increase in ventilation and that the increased ventilatory state would persist after the stimulation ceased. Thus this project studied the effect of dPAG activation by electrical stimulation with systematic variation of stimulus intensities and frequencies. Both inspiratory and expiratory muscle activities were analyzed. The cardiorespiratory responses were analyzed during and after the electrical stimulation of the dPAG.

MATERIALS AND METHODS

The experiments were performed on adult male Sprague-Dawley rats (250–400 g) housed in the University of Florida animal care facility. The rats (n = 13) were exposed to a normal 12:12-h light-dark cycle. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Florida.

General preparation. Rat were anesthetized with urethane (1.4 g/kg ip). Additional urethane (20 mg/ml) was administrated intrave-
nously as necessary. The adequacy of anesthesia was regularly verified by the absence of a withdrawal reflex or blood pressure and heart rate (HR) responses to a paw pinch. A tracheostomy was performed. The femoral artery and vein were catheterized. The body temperature was monitored with a rectal probe and maintained between 37 and 39°C with the periodic use of a heating pad. The rats respired spontaneously with room air. End-tidal \( \text{PCO}_2 \) (\( \text{PETCO}_2 \)) was measured with flow-through capnography (Capnogard, Novametris Medical System).

Inspiratory and expiratory EMG activities were recorded with bipolar Teflon-coated wire electrodes. The bare tips of the electrodes were inserted into the diaphragm through a small incision in the abdominal skin. A third wire served as an electrical ground inserted in the skin beside the ear. Another pair of electrodes was inserted into the external abdominal oblique muscle, ipsilateral to the diaphragm electrodes through a second incision in the abdominal skin. For three animals, the phrenic nerve was isolated via a dorsal approach in the cervical region ipsilateral to the diaphragm electrodes. The intact nerve was placed en passage on bipolar platinum electrodes for recording phrenic electroneurogram (ENG) and covered with warm mineral oil.

The recording electrodes for muscle EMGs or phrenic ENG were connected to high-impedance probes connected to an AC preamplifier (P511, Grass Instruments), amplified and band-pass filtered (0.3–3.0 kHz). The analog outputs were then connected to a computer data sampling system (CED model 1401, Cambridge Electronics Design) and processed by a signal analysis program (Spike 2, Cambridge Electronics Design). The arterial catheter and tracheal tube were attached to two calibrated pressure transducers connected to a polygraph system (model 7400, Grass Instruments). The analog outputs of the polygraph were sent to the computer data sampling system. All signals were recorded simultaneously and stored for subsequent offline analysis.

The animal was then placed prone in a small animal stereotaxic head holder (Kopf Instruments). The cortex overlying the PAG was exposed by removal of small portions of the skull with a high-speed drill. The dura was reflected, and warm mineral oil was applied on the surface. A monopolar stainless steel stimulating electrode, insulated to within 30–50 µm of the tip, was advanced into the dPAG based on a stereotaxic atlas of the rat brain (23). The coordinates for the caudal dPAG were 7.64–8.72 mm caudal to the bregma, 0.1–0.6 mm lateral to the midline, and depths of 3.8–4.5 mm below the dorsal surface of the brain. The dPAG was stimulated (S48 stimulator, Grass Instruments) with a 10-s train of electrical pulses (0.2-ms pulse width). The electrode tip was placed 1.5–2.0 mm lateral or dorsal to the dPAG and stimulated with 100-µA amplitude at 100-Hz frequency.

In the second set of experiments (\( n = 3 \)), electrical stimulation was delivered into the dPAG with a single stimulus paradigm: pulse trains of 10 s, 100-Hz frequency, 0.2-ms pulse width, and 50-µA current magnitude. The dEMG, ipsilateral phrenic ENG, HR, and blood pressure were recorded. The objective of this group of animals was to confirm that the dEMG response correlated with phrenic nerve activity during stimulation of the dPAG.

Data analyses. All data were analyzed off-line using Spike2 software (Cambridge Electronics Design). The dEMG, \( \text{aEMG} \), and ENG were rectified and integrated (time constant = 50 ms). The Ti, Te, and \( f_R \) were calculated from the tracheal pressure. Baseline dEMG, \( \text{aEMG} \), and ENG were defined as the minimum value measured between bursts at end of expiration. The amplitudes of integrated dEMG (\( \Delta \text{dEMG} \)), \( \text{aEMG} \) (\( \Delta \text{aEMG} \)), or ENG were calculated as the difference between baseline and peak burst amplitudes (Fig. 2). The mean arterial blood pressure (MAP) was calculated as the diastolic pressure plus one-third of the pulse pressure. HR was derived from the average interval between peak systolic pressure pulses in the arterial pressure trace.

The control respiratory and cardiovascular parameters were averaged over the 5 s before the onset of stimulation. The on- and off-stimulus respiratory effects were measured from the complete respiratory cycle or breath taken immediately before and after the onset of stimulation and the first complete respiratory cycle after cessation of stimulation. During electrical stimulation, Ti, Te, \( f_R \), baseline dEMG, baseline \( \text{aEMG} \), \( \Delta \text{dEMG} \) amplitude, MAP, and HR were averaged every 2.5 s. After the cessation of stimulation, these values were averaged for every 2.5 s during the first 10 s. Then, the parameters were averaged for 5 s of each 10-s period for the next 50 s
Fig. 2. Cardiorespiratory response elicited by dPAG stimulation with 75-μA intensity, 100-Hz frequency, 10-s duration, 0.2-ms pulse width from a single animal. A: integrated abdominal muscle EMG (aEMG) and diaphragm EMG (dEMG) with electrical stimulation in the dPAG. Top trace: arterial blood pressure (AP). Second trace: heart rate (HR) response. Third trace: tracheal pressure. Fourth trace: integrated aEMG from the external abdominal oblique muscle. Bottom trace: integrated dEMG. The horizontal bar represents the 10-s stimulation duration. The second horizontal bar represents total time duration for data analysis (70 s). The third horizontal broken line represents each time period for data analysis, with the long bars representing 5 s and the short bars representing 2.5 s. B: schematic representation of analysis on dEMG response. C: schematic representation of analysis of aEMG response.

R1340 RESPIRATORY RESPONSES EVOKED FROM dPAG

AJP-Regul Integr Comp Physiol • VOL 289 • NOVEMBER 2005 • www.ajpregu.org

(Fig. 2A). MAP, HR, Ti, Te, and fR were compared before, during, and after dPAG stimulation. The peak value for each analyzed parameter was defined as the highest average value that occurred during electrical stimulation. For diaphragm activity, baseline dEMG and ΔdEMG were expressed as a percentage of control (Fig. 2B). For the aEMG signal, the activity under the control condition was treated as zero because there was no control activity. The peak aEMG baseline activity or ΔaEMG was considered as arbitrary unit one. All aEMG measurements were calculated as a ratio to peak values (Fig. 2C).

A two-way ANOVA with repeated measures (factors of frequency and time or factors of intensity and time) was performed for comparisons of respiratory and cardiovascular responses due to the different stimulating conditions in the dPAG. A one-way ANOVA with repeated measures (factor: treatment) was performed for comparisons on respiratory parameter changes in two single breaths immediately before and after electrical stimulation or at the cessation of stimulation. When differences were indicated, a Tukey’s post hoc multiple comparison analysis was used to identify significant effects. A Pearson’s correlation test was performed to measure the correlation between dEMG and phrenic ENG activity. P < 0.05 was considered significant. All data are reported as means ± SE.

RESULTS

In all animals, the tips of the electrical stimulation electrodes were in the dPAG (Fig. 1). Before the stimulation, average fR was 102 ± 2 breaths/min, HR was 462 ± 3 beats/min, and MAP was 80 ± 3 mmHg. A typical response observed during and immediately after electrical stimulation (75 μA, 100 Hz, 10 s) of the dPAG is shown in Fig. 2. At these stimulation parameters, the maximal tracheal pressure increased immediately in both negative and positive directions, indicating increased inspiratory and expiratory efforts. Associated with these changes in tracheal pressure was a rapid increase in fR, peak tracheal pressure, dEMG activity, and recruitment of aEMG activity. The aEMG was silent during eupenic breathing, but aEMG activity was recruited after the onset of stimulation and persisted after the cessation of stimulation (Fig. 2). Parallel to the immediate change in respiratory function, there was a slower rate of change in both blood pressure and HR. At maximal stimulation intensity of 100 μA and 100 Hz, no significant ventilatory response was observed when stimulation sites were 1.5–2 mm dorsal or lateral to the dPAG.

Effect of stimulation intensity. To identify the dPAG stimulation intensity sufficient to increase respiratory activity, animals were stimulated with a 10-s electrical stimulus train of 100 Hz with various intensities of 10, 50, 75, or 100 μA (Figs. 3 and 4). Stimulation with 10 μA did not elicit significant changes in cardiorespiratory pattern. For those stimuli >10 μA, baseline activity of dEMG during stimulation increased significantly compared with control. In the first 2.5-s measurement period, both 75 and 100 μA evoked a greater increase in
baseline activity than 50 μA (P < 0.05). Ti and Te significantly decreased and fR significantly increased for stimulus intensities of 50, 75, and 100 μA. No significant changes in dEMG were observed for all stimulus intensities. MAP and HR significantly increased with stimulus intensities of 50, 75, and 100 μA, and no significant group differences were observed among these three stimulation intensities. The relationships between peak cardiorespiratory responses and stimulus intensity are presented in Table 1 and Fig. 4. The respiratory timing parameters and MAP reached their peaks during the second 2.5-s measurement period. Baseline dEMG peaked during the first 2.5-s measurement period with stimulation intensities of 75 and 100 μA. HR increased to peak at the fourth 2.5-s measurement period during stimulation. Stimulation with 10 μA did not significantly change peak cardiorespiratory parameters compared with control. No significant difference in peak values was found among stimulus intensities of 50, 75, and 100 μA. There was no significant difference in the ventilatory responses for animals tested with 10 and 25 μA; thus 10 μA results were used for subthreshold response analyses.

Table 1. Peak cardiorespiratory response to electrical stimulation of the dPAG

<table>
<thead>
<tr>
<th>100 Hz</th>
<th>Control</th>
<th>+10 μA</th>
<th>+50 μA</th>
<th>+75 μA</th>
<th>+100 μA</th>
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<tr>
<td>Ti, ms</td>
<td>218±16</td>
<td>211±19</td>
<td>147±12</td>
<td>127±7§</td>
<td>134±13§</td>
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<tr>
<td>Te, ms</td>
<td>377±39</td>
<td>325±33</td>
<td>168±10*§</td>
<td>147±8§</td>
<td>152±6*§</td>
</tr>
<tr>
<td>fR, breaths/min</td>
<td>104±7</td>
<td>116±9</td>
<td>195±15*§</td>
<td>222±14*§</td>
<td>215±13*§</td>
</tr>
<tr>
<td>Baseline dEMG activity, %</td>
<td>1.00±0.00</td>
<td>1.24±0.14</td>
<td>11.96±2.02*§</td>
<td>16.28±3.39×§</td>
<td>15.11±3.22×§</td>
</tr>
<tr>
<td>dEMG amplitude, %</td>
<td>1.00±0.00</td>
<td>1.04±0.05</td>
<td>1.30±0.28</td>
<td>2.08±1.02</td>
<td>1.48±0.50</td>
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<td>MAP, mmHg</td>
<td>80±9</td>
<td>90±7</td>
<td>141±13*§</td>
<td>151±11*§</td>
<td>152±17*§</td>
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<tr>
<td>HR, beats/min</td>
<td>462±9</td>
<td>476±8</td>
<td>511±13*§</td>
<td>535±16*§</td>
<td>527±16*§</td>
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<table>
<thead>
<tr>
<th>75 μA</th>
<th>Control</th>
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<th>+30 Hz</th>
<th>+100 Hz</th>
</tr>
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<tbody>
<tr>
<td>Ti, ms</td>
<td>219±17</td>
<td>223±22</td>
<td>171±15§</td>
<td>127±7§</td>
</tr>
<tr>
<td>Te, ms</td>
<td>382±39</td>
<td>375±34</td>
<td>202±16§</td>
<td>147±8§</td>
</tr>
<tr>
<td>fR, breaths/min</td>
<td>100±4</td>
<td>102±7</td>
<td>166±17§</td>
<td>222±14§</td>
</tr>
<tr>
<td>Baseline dEMG activity, %</td>
<td>1.00±0.00</td>
<td>1.01±0.06</td>
<td>5.85±3.39×§</td>
<td>16.28±3.39×§</td>
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<tr>
<td>dEMG amplitude, %</td>
<td>1.00±0.00</td>
<td>1.03±0.03</td>
<td>1.09±0.09</td>
<td>2.08±1.02</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>80±7</td>
<td>80±6</td>
<td>125±12*§</td>
<td>151±11*§</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>463±9</td>
<td>461±8</td>
<td>500±13§</td>
<td>535±16§</td>
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</table>

Values are means ± SE. dPAG, dorsal half of the periaqueductal gray matter; Ti and Te, inspiratory and expiratory time, respectively; fR, respiratory frequency; dEMG, diaphragm EMG; MAP, mean arterial pressure; HR, heart rate. *P < 0.05; †P < 0.001, compared with control. §P < 0.05; ¶P < 0.001, compared with peak values from 10 μA-100 Hz or 75 μA-10 Hz stimulation. *P < 0.05, †P < 0.05, §P < 0.05, ¶P < 0.001, compared with peak values from 75 μA-30 Hz stimulation.
**Effect of stimulation frequency.** To identify the dPAG stimulation frequency sufficient to increase respiratory activity, the animals were stimulated with a 10-s electrical stimulus train of 75 μA with 10, 30, and 100 Hz. Stimulation at 10 Hz did not elicit significant changes in cardiorespiratory pattern (Fig. 5). Baseline dEMG significantly increased at the fourth measurement period during stimulation with 30 Hz (P < 0.05), whereas 100-Hz stimulation elicited a significant increase in the first 2.5-s measurement period (P < 0.001). Stimulation with 100 Hz elicited a significantly greater increase in baseline dEMG compared with 10 and 30 Hz (P < 0.001). There was no significant change in ΔdEMG across all frequencies of stimulation. Ti and Te significantly decreased with 100-Hz stimulation; thus there was a significant increase in fR (Fig. 5). Stimulation with 30 Hz significantly increased Ti and Te and increased fR from the second 2.5-s measurement period. There was a significant difference in the Ti, Te, and fR between 30 and 100 Hz (P < 0.05).

Stimulation with 30 Hz increased both MAP and HR significantly at the second 2.5-s measurement period (Fig. 5). Stimulation at 100 Hz significantly increased MAP and HR at the first 2.5-s measurement period (P < 0.001). A significant difference in HR was observed with 30- and 100-Hz stimulation frequencies. There was no significant difference in the MAP change between 30 and 100 Hz.

The peak cardiorespiratory response relationships as a function of stimulus frequency are presented in Table 1 and Fig. 6. Ti, Te, and fR reached their peaks during the second 2.5-s measurement period with 100-Hz stimulation and reached peak at the fourth 2.5-s measurement period with 30 Hz. Baseline dEMG peaked during the first 2.5-s period with 100-Hz stimulation. Baseline dEMG peaked at the fourth 2.5-s measurement period with 30-Hz stimulation. HR peak was at the fourth 2.5-s measurement period for 100 Hz stimulation. Stimulation at 30 and 100 Hz elicited significant changes in peak Ti, Te, fR, MAP, and HR compared with 10-Hz stimulation (P < 0.05).

**Onset effect of dPAG stimulation.** The specific changes in respiration that occurred within the first breath after the onset of dPAG stimulation were analyzed in more detail. The respiratory timing and dEMG activity were compared in breaths immediately before and after the onset of electrical stimulation with 100 A and 100 Hz (Table 2). Within this first breath, Ti significantly decreased from 217 ± 7 to 143 ± 13 ms (P < 0.001), and Te significantly decreased from 404 ± 54 to 212 ± 9 ms (P < 0.05). Respiratory frequency significantly increased from 100 ± 8 to 170 ± 5 breaths/min (P < 0.001). There were significant increases in baseline dEMG activity (226 ± 67%).

**Off-stimulation and poststimulation effect.** After the offset of stimulation, dPAG-induced changes in cardiorespiratory activity persisted for a minimum of 60 s (Fig. 2). After the cessation of stimulation at 100 Hz, there were sustained and significant increases in baseline dEMG and fR relative to control, until the 7.5-s time period with 50 μA, the 20-s time period with 75 μA, and the 10-s time period with 100 μA (P > 0.05). Ti returned
to the control level at the 5-s time period after cessation of stimulation with 50 and 75 μA and the 10-s time period with 100 μA ($P > 0.05$). Te was significantly decreased after the cessation of stimulation until the 20-s time period with 50 μA, the 40-s time period with 75 μA ($P < 0.05$), and the 30-s time period with 100 μA ($P < 0.05$). With 50, 75, and 100 μA, HR remained significantly greater than control during the entire 1-min poststimulation measurement period ($P < 0.001$). MAP returned to the control level after cessation of stimulation by the 20-s time period with 50 μA, the 50-s time period with 75 μA, and the 30-s time period with 100 μA.

The first breath pattern after the offset of dPAG stimulation with 100 μA and 100 Hz (Table 2) was determined. The Ti, Te, fR, and dEMG activity were compared between the breaths immediately before and after the cessation of electrical stimulation. Ti significantly increased from 136 ± 2 to 144 ± 1 ms ($P < 0.001$). Te was not significantly different (169 ± 1 to 179 ± 2 ms). The fR significantly decreased from 200 ± 12 to 178 ± 8 breaths/min ($P > 0.05$). There were no significant changes of baseline dEMG activity (813 ± 133 to 754 ± 192%) and ΔdEMG amplitude (134 ± 36 to 123 ± 37%).

dPAG stimulation effect on phrenic ENG, aEMG, and $\text{PETCO}_2$. In the three animals tested, the phrenic ENG increased in parallel with the ipsilateral dEMG during the electrical stimulation of the dPAG. Baseline dEMG and phrenic ENG activities increased in the first breath after the onset of stimulation. The pattern of the phrenic ENG activity was significantly correlated with the dEMG activity ($r = 0.825, P < 0.001$). Peak phrenic ENG baseline increased 492 ± 212%, and peak amplitude increased 146 ± 101% from the control level.

The aEMG was silent during control breathing (Figs. 2 and 7). aEMG activity was recruited later and recovered earlier during dPAG stimulation than dEMG. dPAG stimulation increased ΔaEMG amplitude and aEMG baseline activity. There was increased aEMG baseline discharge during the entire inspiratory phase. The ΔaEMG was modulated with a respiratory rhythm in phase with expiration in three of seven animals. aEMG activity persisted after the cessation of stimulation with stimulus intensities of 50, 75, and 100 μA and stimulus frequencies of 30 and 100 Hz. With stimulation at 75 μA and 10 Hz, there was no change in aEMG (Fig. 7). Stimulation with 75 μA at 30 Hz elicited an aEMG increase; the peak response was 512 ± 141% from the baseline level (Fig. 7). The aEMG activity was further increased with 75 μA at 100-Hz stimulation to 3,099 ± 1,472% above control (Fig. 7).

$\text{PETCO}_2$ was recorded during electrical stimulation in the dPAG with 75 μA at 100 Hz. $\text{PETCO}_2$ decreased from 39.5 ± 0.6 to 27.8 ± 2.3 Torr on the first breath after the onset of stimulation. The $\text{PETCO}_2$ remained decreased throughout the stimulation. After the cessation of stimulation, $\text{PETCO}_2$ returned to control by the first poststimulus measurement period.

DISCUSSION

The results of this investigation demonstrated that electrical stimulation in the dPAG elicited enhanced respiratory activity...
that included both inspiratory and expiratory muscle recruitment. The fR increased significantly after dPAG activation, which included shortening of both Ti and Te. The changes in breath phase timing were the result of increased active inspiratory and expiratory motor output. The increase in respiratory activities was accompanied by significant increases in both HR and MAP. There were stimulus intensity and frequency thresholds for eliciting the dPAG-mediated respiratory response. Electrical stimulation in the dPAG also produced an immediate elevated respiratory dEMG and aEMG baseline activity, which was sustained after the cessation of electrical stimulation in dPAG. This sustained poststimulation effect may represent a sustained change of the poststimulus state of the dPAG and/or changes in descending respiratory pathways.

Table 2. On- and off-stimulus respiratory response to electrical stimulation with 100 μA and 100 Hz of the dPAG

<table>
<thead>
<tr>
<th></th>
<th>On-Stimulus Effect</th>
<th>Off-Stimulus Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Stimulus on</td>
</tr>
<tr>
<td>Ti, ms</td>
<td>217 ± 7</td>
<td>143 ± 13†</td>
</tr>
<tr>
<td>Te, ms</td>
<td>404 ± 54</td>
<td>212 ± 9*</td>
</tr>
<tr>
<td>fR, breaths/min</td>
<td>100 ± 8</td>
<td>170 ± 5†</td>
</tr>
<tr>
<td>Baseline dEMG, %</td>
<td>100 ± 0</td>
<td>226 ± 67</td>
</tr>
<tr>
<td>dEMG amplitude, %</td>
<td>100 ± 0</td>
<td>135 ± 14*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05; †P < 0.001, compared with control. ‡P < 0.001, compared with stimulus-on.

Methodological considerations. Electrical stimulation to activate neural structures in the PAG has inherent strengths and limitations as an electrophysiological research tool. The advantage of the electrical stimulation is the ability to observe the timing of on- and off-stimulus effects. This is especially important when studying time-related changes in neural structures. In the present study, the use of electrical stimulation allowed for the observation of a first breath onset effect and persistent changes after the cessation of stimulation. It is clear that there is a short-latency response to dPAG activation. Current spread with monopolar electrodes has been suggested (26) to range from 0.3 to 1.0 mm when the stimulus intensity was 50–200 μA in the central nervous system. In the present study, the highest intensity was 100 μA, so the current spread range would be >1.0 mm. In the present study, electrical stimulation outside the dPAG but in close proximity did not elicit a change in respiratory pattern or in cardiovascular parameters. This suggests that cardiorespiratory responses reported with electrical stimulation within the dPAG are mediated by neural elements within the dPAG. Although electrical stimulation can activate both neurons and fibers of passage, the thresholds of these neuronal elements are different. Indeed, electrical stimulation can still be used to specifically activate different components with specific stimulating parameters (4, 25). As previously suggested (14), low-intensity and high-frequency electrical stimulations elicited similar cardiovascular and respiratory responses from the dPAG as chemical disinhi-
bition. Thus, although electrical stimulation reduces the specificity of the structures activated, it has the advantage of allowing the observation of the timing of the onset of the respiratory response and sustained respiratory activity after the stimulation has ceased.

Although electrical stimulation can activate both neurons and fibers of passage, the thresholds of these neuronal elements are different. Indeed, electrical stimulation can still be used to specifically activate different components with specific stimulating parameters (3, 25). As previously suggested (14), low-intensity and high-frequency electrical stimulations elicited similar cardiovascular and respiratory responses from the dPAG as chemical disinhibition. In the present investigation, control studies were performed with electrical stimulation outside the dPAG. With the electrode tip <2 mm outside the dPAG, maximal electrical stimulation did not elicit an increase in respiratory activity or in cardiovascular parameters. This suggests that cardiorespiratory responses reported with electrical stimulation within the dPAG are mediated by neural elements within the dPAG. Thus, although electrical stimulation reduces the specificity of the structures activated, it has the advantage of allowing the observation of the timing of the onset of the respiratory response and sustained respiratory activity after the stimulation has ceased.

Respiratory response to dPAG stimulation. dPAG electrical stimulation elicited a significant increase in respiratory frequency with no significant change in ΔaEMG amplitude. This resulted in a frequency-dependent increase in neural minute ventilation. The increased respiratory frequency was the result of shortening of both Ti and Te. The results also showed that activation of the dPAG has a greater effect on Te than Ti. In addition, the reduction in Te was sustained after cessation of stimulation. Electrical stimulation frequencies at 25 and 40 Hz were previously reported to reduce Te with minimal effect on Ti (14). This effect on Te is consistent with the report in cats that electrical stimulation in the PAG decreased Te, but the specific region within the PAG that was stimulated was not identified (2, 8, 16). Thus there is a dPAG modulation of respiratory timing that appears to be greatest on modulation of expiration.

Stimulation of dPAG neurons by excitation with microinjection of DLH or disinhibition with bicuculline significantly reduced both Ti and Te in a dose-dependent manner (14, 17). In the present study, the magnitude of the respiratory responses was increased with increased current intensity and stimulation frequency in a dose-dependent manner, consistent with chemical stimulation (14, 17). There was a threshold for eliciting the response evidenced by the observation that low-stimulation intensity or low frequency did not elicit significant changes of cardiorespiratory pattern. As the intensity or frequency increased, the cardiorespiratory responses were recruited and increased to a plateau. The modulation of respiratory timing could therefore be attributed to dPAG elicited modulation of brain stem respiratory center activities by yet to be determined pathways.

Anatomical studies have reported direct and indirect connections between the PAG and brain stem respiratory network. A retrograde labeling study reported a connection between rostral ventral respiratory group and the PAG (11). Neuronal inhibition with GABA receptor agonist muscimol in the lateral parabrachial nucleus (LPBN) almost completely blocked the respiratory response elicited from the dPAG (13). Anatomical connections between the PAG and LPBN have been confirmed in various studies (4, 5, 18). The LPBN has been demonstrated as a critical region in neural control of breathing (7, 27). Thus it is likely that the respiratory response elicited by electrical stimulation in the present study is mediated by a LPBN pathway.

Fig. 7. External abdominal oblique muscle EMG activity following the electrical stimulation of the dPAG. A: data from 1 animal. Note that there was no aEMG activity under the prestimulation control condition and with the lowest frequency of stimulation (10 Hz). There was a delayed aEMG response with 30-Hz stimulation. aEMG activity was recruited on the first breath after the onset of 100-Hz stimulation. B: mean aEMG response to 100-Hz, 75-μA stimulation of the dPAG (n = 3). The horizontal bar represents stimulation duration of 10 s.
Electrical stimulation in the dPAG also elicited a significant change of dEMG that was evident in the first breath after the onset of electrical stimulation. The change in dEMG was due to an increase in the baseline dEMG activity with no significant change in ΔdEMG. The increase in inspiratory muscle activity is consistent with previous reports of electrical and chemical stimulation of the dPAG (14, 17). However, although it has been reported that dPAG activation decreases Te, there are no previous reports of active expiration and recruitment of expiratory muscle activity. dPAG activation recruited aEMG activity in this normally silent expiratory muscle. The dPAG-mediated activation of the abdominal muscle was sustained after the cessation of stimulation. The activation of both inspiratory and expiratory muscles was further associated with an increase of tracheal pressure changes in both inspiratory and expiratory directions. Thus the respiratory response elicited from the dPAG included recruitment of active expiration.

Elevated baseline activity in dEMG and phrenic ENG was observed in the present study. In a report by Huang et al. (17), DLH was microinjected into dPAG and there was an increased fę and the baseline dEMG activity (their Fig. 1). This increase in dEMG baseline was also reported with dPAG activation by electrical stimulation and GABA disinhibition (14). The increase in phrenic ENG activity parallels the change in dEMG, demonstrating that the change in dEMG was due to dPAG-mediated changes in respiratory neural mechanisms. Baseline dEMG and phrenic ENG elevation is not due to the stimulation artifact because the elevation continued after the completion of stimulation. The change in respiratory drive was also not an artifact of the enhanced intrinsic contractions of the diaphragm because this tonic activity was also observed in the phrenic neurogram. The tonic activity appears to be the result of increased neural output to respiratory muscles from spinal motor respiratory drive, although the exact source is not yet known. This tonic activity would represent an increase in resting muscle tone and may change functional residual capacity as previously suggested (14). The results of the present study extend these observations by showing that increased respiratory muscle tone occurs in both inspiratory and expiratory muscles. Stimulation of the hypothalamic locomotion region, another suprapontine structure involved in defense behaviors, with both electrical stimulation and GABA disinhibition elicited enhanced cardiorespiratory responses and elevation of baseline activity in the phrenic ENG in anesthetized and decorticated cats (9, 10). This elevation was evident without chemoreceptor or vagal inputs. Thus this enhancement and recruitment of respiratory muscles in response to stimulation of central neural defense regions may be a common characteristic of these elicited behaviors.

dPAG stimulation on cardiovascular responses. Both chemical and electrical stimulation in the dPAG evoked significant increases in MAP and HR. The response pattern in the present study was similar to previous studies with both conscious and anesthetized animals (3). The increase in MAP and HR was related to the intensity of stimulation of the dPAG, which were similar to dose-dependent responses of disinhibition (14) or DLH stimulation (17) of the dPAG. The rostral ventrolateral medulla has been demonstrated to mediate the pressor and tachycardia responses elicited from the dPAG (20). Huang et al. (17) suggested that dPAG-elicited cardiovascular and respiratory responses could be separated at the brain stem level.

Microinjection of propranolol into the NTS attenuated the respiratory response elicited from the dPAG but not the cardiovascular response. Blocking the LPBN eliminated 90% of the respiratory response evoked from the dPAG, whereas the cardiovascular response was only partially attenuated (13). These data suggest that cardiovascular and respiratory responses elicited from dPAG may descend by different pathways to the brain stem.

In summary, the results of the present study demonstrated that the respiratory response elicited with stimulation of the dPAG was characterized by increased active ventilation for both inspiration and expiration. The activity of the diaphragm was increased, and expiratory muscle activity was recruited. The cardiorespiratory response pattern is stimulus intensity and frequency dependent. Electrical dPAG stimulation that exceeded the threshold elicited a change in respiratory timing in the first breath after the onset of stimulation. Respiratory timing changes were sustained after the cessation of stimulation and may represent short-term respiratory neuroplasticity elicited from the dPAG. The increase in ventilation persisted despite a decreased Pco2. The neural mechanisms of enhanced respiratory muscle EMG activities and breathing pattern changes remain to be determined but may involve brain stem and spinal control systems.

REFERENCES


