c-Fos expression in the midbrain periaqueductal gray during static muscle contraction

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Li, Jianhua, and Jere H. Mitchell. c-Fos expression in the midbrain periaqueductal gray during static muscle contraction. Am J Physiol Heart Circ Physiol 279: H2986–H2993, 2000.—The periaqueductal gray (PAG) of the midbrain is involved in the autonomic regulation of the cardiovascular system. The purpose of this study was to determine if static contraction of the skeletal muscle, which increases arterial blood pressure and heart rate, activates neuronal cells in the PAG by examining Fos-like immunoreactivity (FLI). Muscle contraction was induced by electrical stimulation of the L7 and S1 ventral roots of the spinal cord in anesthetized cats. An intravenous infusion of phenylephrine (PE) was used to selectively activate arterial baroreceptors. Extensive FLI was observed within the ventromedial region (VM) of the rostral PAG, the dorsolateral (DL), lateral (L), and ventrolateral (VL) regions of the middle and caudal PAG in barointact animals with muscle contractions, and in barointact animals with PE infusion. However, muscle contraction caused a lesser number of FLI in the VM region of the rostral PAG, the DL, L, and VL regions of the middle PAG and the L and VL regions of the caudal PAG after barodenervation compared with barointact animals. Additionally, the number of FLI in the DL and L regions of the middle PAG was greater in barodenervated animals with muscle contraction than in barodenervated control animals. Thus these results indicated that both muscle receptor and baroreceptor afferent inputs activate neuronal cells in regions of the PAG during muscle contraction. Furthermore, afferents from skeletal muscle activate neurons in specific regions of the PAG independent of arterial baroreceptor input. Therefore, neuronal cells in the PAG may play a role in determining the cardiovascular responses during the exercise pressor reflex.

Fos-like immunoreactivity, blood pressure; heart rate; exercise pressor reflex; barodenervation

STATIC CONTRACTION of the hindlimb muscles has been shown to increase arterial blood pressure (ABP), heart rate (HR), left ventricular contractility, and renal sympathetic nerve activity in anesthetized animals (26, 27, 30, 31). Studies have suggested that the superficial dorsal horn of lumbosacral cord (11, 42) and the ventrolateral medulla (VLM) (3, 4, 20, 22) are involved in the expression of the cardiovascular responses to static muscle contraction. Also, regions of the hypothalamus are responsive to static muscle contraction and are involved in the exercise pressor reflex (19, 37). In addition, the midbrain periaqueductal gray (PAG) has been shown to be linked to the cardiovascular responses during static exercise in anesthetized cats (18, 39, 40) and during dynamic treadmill exercise in rats (15).

The PAG is an important neural substrate for autonomic regulation, because it receives projections from the forebrain and the VLM and sends projections to the VLM (2, 12, 25, 34), all of which are known to be involved in cardiovascular regulation (10, 13, 23, 25, 33, 35). Previously, Fos mapping methods have been used to identify activated neurons in the medulla and the hypothalamus during muscle contraction (19, 20, 22). This method could also be used to determine if the PAG is involved in the exercise pressor reflex.

In the present study, we examined Fos-like immunoreactivity (FLI) in the regions of the PAG during alternating contractions of both hindlimb muscles in barointact and barodenervated cats. Also, FLI in these same regions was examined after intravenous infusion of phenylephrine (PE) to induce hypertension in barointact and barodenervated animals. Induced static contractions in barointact animals were used to determine the number of FLI in the regions of the PAG during activation of muscle afferents plus baroreceptor afferents and in barodenervated animals during activation of only muscle afferents. Furthermore, the number of FLI in barodenervated cats during muscle contraction was also compared with barodenervated control animals to determine activated regions of the PAG only by muscle afferent inputs. Intravenous infusion of PE was used in barointact cats to determine the number of FLI in the regions of the PAG induced by activation of baroreceptor reflex. Intravenous infusion of PE was used in barodenervated cats to determine the number of FLI in regions of the PAG induced by hypertension without baroreceptor reflex activation, and the number of FLI in barodenervated cats was also compared with that in barodenervated control animals.
A preliminary report of this study has been published (21).

METHODS

General Surgical Preparation

The experiments were performed on 20 anesthetized cats weighing 3.5–5.4 kg. The animals were anesthetized by inhalation of a halothane-nitrous oxide-oxygen mixture. An endotracheal tube was inserted into the trachea via a tracheotomy to maintain an open airway, and a jugular vein and carotid artery were catheterized for drug administration and measurement of ABP, respectively. Anesthesia was then maintained with α-chloralose (80 mg/kg) injected intravenously. Throughout the experiment, supplemental α-chloralose (15 mg/kg iv) was given if the cats exhibited a corneal reflex or they withdrew a limb in response to a noxious stimulus. Arterial blood gases and pH were periodically determined (Radiometer, ABL-3, Copenhagen, Denmark) and were maintained within normal limits (pH: 7.30–7.40; PCO₂: 32–36 mmHg; PO₂ > 80 mmHg) by adjusting the ventilator (model 661, Harvard Apparatus, South Natick, MA) or injecting a 1 M solution of sodium bicarbonate intravenously. Body temperature was continuously monitored with a rectal probe and was maintained between the range of 37.0–38.5°C with a water-perfused heating pad and an external heat lamp. A laminectomy was performed, exposing the lower lumbar and upper sacral portions of the spinal cord. The L7 and S1 ventral roots of the spinal cord were carefully separated and cut bilaterally close to the spinal cord. The peripheral ends of the transected L7 and S1 ventral roots were placed on platinum bipolar stimulating electrodes. The exposed spinal cord region was immersed in a pool of warm mineral oil (37°C). In the barodenervated animals, the common carotid artery, vagus nerve, and carotid sinus bifurcation were exposed bilaterally. Transection of the vagus nerve and carotid sinus nerve on each side eliminated afferent activity from cardio-pulmonary and arterial baroreceptors. The efficacy of barodenervation was confirmed by measuring the increase in mean arterial pressure (MAP) while bilateral common carotid arteries were briefly clamped (25 ± 3 mmHg before and 4 ± 2 mmHg after denervation).

ABP was measured with a pressure transducer (model P23 ID, Statham, Oxnard, CA) connected to an arterial catheter. MAP was obtained by integrating the arterial signal with a time constant of 4 s. HR was derived from the arterial pressure pulse by a Biotach (Gould Instruments, Cleveland, OH). The calcaneal bone of each hindlimb was cut, allowing the Achilles tendons to be connected to force transducers (FT10, Grass Instruments) for measurement of induced tension by the triceps surae muscle. The pelvis was stabilized in a spinal unit (Kopf Instruments, Tujunga, CA), and the knee joints were secured by attaching the patellar tendon to a steel post. All measured variables were continuously recorded on an eight-channel chart recorder (model 2800s, Gould Instruments).

Experimental Protocol

The cats were allowed to stabilize for 4 h after surgery. ABP and HR were monitored during static muscular contraction of the triceps surae muscle. The contraction was induced by electrical stimulation of the L7 and S1 ventral roots for 2 min at three times motor threshold, 30 Hz, and with 17-ms delay between L7 and S1 activation. Stimulus was alternately administered to contralateral roots such that while one leg was in a contracted state the other leg was resting. These 2-min alternating contractions were performed for a total of 60 min. The motor threshold was readjusted over the 60-min period of muscle contraction to attempt maintaining a consistent increase in muscle tension during the ventral root stimulation. To obtain similar level of the increase of ABP induced by electrical stimulation of the ventral roots, the jugular vein cannula was connected to a syringe pump for continuous infusion of PE hydrochloride (Sigma, 0.5 mg/ml in saline) for 60 min. A rate of infusion at 1–2.5 ml/h was adjusted to maintain the hypertension.

Five groups of animals were studied: 1) barointact cats that received electrical stimulation of the L7 and S1 ventral roots (barointact group, n = 6), 2) barodenervated cats that received electrical stimulation of the L7 and S1 ventral roots (barodenervated group, n = 4), 3) barodenervated cats without electrical stimulation of the ventral roots and without intravenous infusion of PE (barodenervated control group, n = 3), 4) barointact cats with intravenous infusion of PE (hypertension group, n = 4), 5) barodenervated cats with intravenous infusion of PE (barodenervated-hypertensive group, n = 3).

Ninety minutes after the end of L7 and S1 ventral roots stimulation or the end of intravenous infusion, the cats were perfused transcardially with 1 liter of saline followed by 1.5 liter 4% paraformaldehyde in PBS (pH 7.4). Robust c-Fos expression in the medulla induced by static muscle contraction has been found at 90 min after the end of the electrical stimulation of the ventral roots (22). Therefore, 90 min was used as the time point to perfuse the cat brain in present experiments. After perfusion, the brain was removed and stored in the same fixative solution for 2 h. Finally, it was transferred to a 30% sucrose solution overnight to prevent ice crystal formation. Coronal sections (25 μm) were cut on a cryostat (model 2800 Frigocut-E, Cambridge Instruments) and placed serially into four wells containing cryoprotectant, then kept at −20°C in a freezer.

Immunocytochemistry

Tissue was removed from cryoprotectant and then rinsed in PBS for 30 min. The sections were washed in PBS for 15 min followed by 0.5% hydrogen peroxide for 10 min to quench endogenous peroxidase activity. Sections were placed in PBS containing 1% normal goat serum and 0.1% Triton X-100 for 30 min followed by 0.5% hydrogen peroxide for 10 min to quench endogenous peroxidase activity. Sections were then incubated in a primary antibody to c-Fos (1:10,000 dilution, catalog no. sc-52, Santa Cruz Biotechnology) for 48 h at 4°C. At the end of this incubation period, sections were rinsed in PBS and then in PNT for 15 min. The sections were incubated in biotinylated goat anti-rabbit IgG (1:200, Vector Kit) for 30 min, washed in PBS for 30 min, and incubated with ABC solution (1:50, Vector Kit) for 30 min. After a serial rinse in PBS and Tris buffer, the c-Fos reaction product was made visible by incubating sections with hydrogen peroxide and diaminobenzidine. The sections were then washed in distilled water, mounted in PBS, and air-dried overnight. Subsequently, sections were cleared in ascending alcohol and xylene baths. Permount medium was used for a coverslip. Sections were examined under a light microscope. c-Fos reaction product appeared as dark brown staining in the cell nucleus.

Cell Counts and Statistical Analysis

Tissue sections were examined under standard light microscope. The cell nuclei of activated cells showed the characteristic dark brown staining of oxidized DAB as c-Fos labeling. Two to three sections that most closely matched the standard stereotaxic planes of Berman’s atlas (5) were se-
lected for three rostrocaudal levels of the PAG of each animal. The rostral, middle, and caudal PAG was stereotaxically anterior A2.5 to A3.3, A0.6-P0.2, and P0.9-P1.2, respectively. Furthermore, the PAG was delineated into the dorsomedial (DM), dorsolateral (DL), lateral (L), ventrolateral (VL), and ventromedial (VM) subdivisions (Fig. 1) as described elsewhere (16, 32). The total number of labeled cells was counted in each region for each animal. This number was then divided by the total number of sections counted to provide a mean cell count per slice for each region.

A one-way repeated-measure ANOVA was used for statistical comparison of changes in MAP, HR, and tension (across time and among barointact, barodenervated animals with muscle contraction, and barointact and barodenervated animals with PE) with a Student-Newman-Keuls post hoc analysis. A one-way ANOVA was used for statistical comparison of cell count labeling with c-Fos per slice (barointact contraction versus barodenervated contraction, barointact hypertension versus barodenervated hypertension, barodenervated contraction versus barodenervated control, barodenervated hypertension versus barodenervated control, and barointact contraction versus barointact hypertension). A Student-Newman-Keuls post hoc analysis was used to determine differences between groups. \( P < 0.05 \) was considered significant. All values are expressed as means \( \pm SE \).

RESULTS

Changes in MAP and in Muscle Tension

The changes in MAP after electrical stimulation of the L7 and S1 ventral roots of the spinal cord to induce static muscle contraction in barointact and barodenervated cats and during intravenous infusion of PE in barointact and barodenervated cats are shown in Table 1. The maximal increase in MAP was attained during the first or second muscle contraction. The MAP response to induced muscle contraction was significantly increased above baseline over the first 40 min of muscle contraction in barointact and barodenervated animals. Intravenous infusion of PE hydrochloride increased ABP to similar levels to that caused by static muscle contraction. The rate of infusion was adjusted to maintain hypertension during the 60-min period. There was an increase in MAP above baseline over the first 40 min in barointact and barodenervated cats with intravenous infusion of PE (\( P < 0.05 \)). No significant difference for the changes of MAP was seen over 60 min among barointact and barodenervated animals by induced muscle contraction or by intravenous infusion of PE.

The maximal peak tension developed by the triceps surae muscle was 8.4 \( \pm 0.5 \) and 8.5 \( \pm 0.7 \) kg in barointact and barodenervated cats, respectively. The peak tension produced by contraction at 20, 40, and 60 min after the start of the ventral roots stimulation was 7.3 \( \pm 0.4 \), 5.7 \( \pm 0.4 \), and 4.2 \( \pm 0.5 \) kg in barointact cats and 7.7 \( \pm 0.6 \), 6.2 \( \pm 0.5 \), and 4.5 \( \pm 0.4 \) kg in barodenervated cats.

Distribution of FLI in the PAG

Barointact with contraction versus barodenervated with contraction versus barodenervated control. Distinct FLI was found in the VM of the rostral PAG, the DL, L, and VL regions of the middle and caudal PAG after ventral root stimulation in barointact contraction cats. Compared with barodenervated cats with contraction, muscle contraction caused higher number of FLI in the VM region of the rostral PAG, the DL, L, and VL regions of the middle PAG, and the L and VL regions of the caudal PAG in the barointact animals (Fig. 2). Photomicrographs of FLI staining in DL, L, and VL of the middle PAG in a barointact animal with muscle contraction are shown in Fig. 3. Also, photomicrographs of FLI staining in DL, L, and VL of the middle PAG in a barodenervated animal with contraction are shown in Fig. 3. The number of FLI in bar-
including changes in peripheral blood flow, ABP, HR, and respiration, as well as pupil dilation and increased plasma catecholamine levels (1, 13, 25, 28, 36). Furthermore, two distinct cardiovascular effector columns, pressor and depressor regions, have been defined rostrocaudally in the PAG. In both the cat and the rat, stimulation of the PAG dorsolateral and lateral to the cerebral aqueduct elicits potent increases in ABP, HR, and lumbar and splanchic sympathetic nerve discharge, whereas stimulation of the PAG ventrolateral to the aqueduct causes a decrease in ABP and HR, and increases blood flow through the hindlimb vascular beds (6–9).

In addition, the PAG is linked to the pressor response during muscle contraction. Static muscle contraction increased the release of neuropeptide Y and enkephalin in the PAG (39, 40). Neurons excited by static muscle contraction have been recorded in the PAG (18), and c-Fos expression was increased in the PAG during treadmill exercise in rat (15). In the present study, static muscle contraction caused extensive Fos labeling within the VM region of the rostral PAG and the DL, L, and VL regions of the middle and caudal PAG in barointact cats. This further demonstrates that the PAG may be involved in the expression of the exercise pressor reflex. Because neuronal cells in the PAG respond to activation of the baroreceptor reflex (23, 32, 33), the increase in ABP during static muscle contraction may be responsible for the observed changes (27, 30, 31). The effect of activation of the baroreceptor reflex during muscle contraction or exercise was not considered in the previous studies (15, 39, 40). Therefore, Fos-labeled cells in these subdivisions of the PAG were observed in barodenervated cats dur-

### Table 1. Baseline and changes in MAP and HR after static muscle contraction

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Peak</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
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<tr>
<td><strong>BD Control</strong></td>
<td></td>
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<tr>
<td>MAP, mmHg</td>
<td>98 ± 10</td>
<td>0</td>
<td>5 ± 1</td>
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<td>HR, beats/min</td>
<td>182 ± 12</td>
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<td>0</td>
<td>2 ± 1</td>
<td>5 ± 1</td>
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<tr>
<td>Tension, kg</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>Barointact + Con</strong></td>
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<tr>
<td>MAP, mmHg</td>
<td>110 ± 8</td>
<td>54 ± 6*</td>
<td>36 ± 6*</td>
<td>27 ± 6*</td>
<td>15 ± 7</td>
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<tr>
<td>HR, beats/min</td>
<td>185 ± 15</td>
<td>22 ± 6*</td>
<td>18 ± 6*</td>
<td>18 ± 6*</td>
<td>10 ± 2</td>
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<tr>
<td>Tension, kg</td>
<td>0.8</td>
<td>8.4 ± 0.5*</td>
<td>7.3 ± 0.4*</td>
<td>5.7 ± 0.4*</td>
<td>4.2 ± 0.5*</td>
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<tr>
<td><strong>BD + Con</strong></td>
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<tr>
<td>MAP, mmHg</td>
<td>96 ± 15</td>
<td>60 ± 13*</td>
<td>44 ± 11*</td>
<td>34 ± 8*</td>
<td>20 ± 6</td>
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<tr>
<td>HR, beats/min</td>
<td>186 ± 12</td>
<td>25 ± 7*</td>
<td>19 ± 9*</td>
<td>19 ± 9*</td>
<td>12 ± 3</td>
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<tr>
<td>Tension, kg</td>
<td>0.8</td>
<td>8.5 ± 0.7*</td>
<td>7.7 ± 0.6*</td>
<td>6.2 ± 0.5*</td>
<td>4.5 ± 0.4*</td>
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<tr>
<td><strong>Barointact + Phen</strong></td>
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<tr>
<td>MAP, mmHg</td>
<td>108 ± 9</td>
<td>59 ± 11*</td>
<td>39 ± 7*</td>
<td>22 ± 9*</td>
<td>15 ± 8</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>192 ± 16</td>
<td>−21 ± 12*</td>
<td>−4 ± 3</td>
<td>−2 ± 1</td>
<td>0</td>
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<tr>
<td>Tension, kg</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>BD + Phen</strong></td>
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<tr>
<td>MAP, mmHg</td>
<td>100 ± 4</td>
<td>62 ± 15*</td>
<td>37 ± 12*</td>
<td>31 ± 15*</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>190 ± 18</td>
<td>−5 ± 2</td>
<td>0</td>
<td>3 ± 2</td>
<td>2 ± 1</td>
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<tr>
<td>Tension, kg</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
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Values are means ± SE for barodenervated control animals (BD control, n = 3), barointact (barointact + muscle contraction (Con), n = 6), and barodenervated (BD + Con, n = 4) animals with muscle contraction, and barointact (barointact + phenylephrine (Phen), n = 4), and barodenervated (BD + Phen, n = 3) animals with intravenous infusion of phenylephrine. MAP, mean arterial pressure; HR, heart rate. Muscle tension values are for left hindlimb. *Significantly different from baseline (P < 0.05). No muscle contraction or infusion of Phen was treated in BD control animals. One-way repeated measure ANOVA was not used for comparison in BD control group.

The PAG is an important neural substrate for autonomic regulation. Electrical or chemical stimulation of the PAG elicits a variety of autonomic responses, including changes in peripheral blood flow, ABP, HR,
ing static muscle contraction. In the present study, the major finding is that specifically organized regions of the PAG (the dorsolateral and lateral regions of the middle PAG) were activated by muscle contraction, which is independent of activation of the arterial baroreceptor reflex.

It has been reported that FLI was induced in neurons within the PAG by hypertension produced by an infusion of PE (23, 32) and that the number of induced FLI-labeled neurons was reduced in the PAG after sinoaortic denervation (33). This finding indicates that the responding neurons induced by hypertension are related to activation of baroreceptor reflex. In the present experiment, we also observed a fewer number of FLI in the VM of the rostral PAG and in the DL, L, and VL of the caudal and middle PAG in barodenervated cats with infusion of PE compared with barointact cats with infusion of PE. Furthermore, the number of Fos-labeled cells were compared between barodenervated cats with infusion of PE and barodenervated cats without infusion of PE. A few of FLI-labeled neurons were observed in those subdivision regions of the PAG in both groups of animal and there was no difference for the number of FLI between both groups. This indicates that induced hypertension caused no distinct c-Fos expression in those regions of the PAG in barodenervated cats in this study. Thus we may exclude the possibility that activation of baroreceptor reflex caused increased Fos expression in those regions of the PAG in barodenervated contraction cats in this study.

Furthermore, static muscle contraction induced extensive FLI within the VM region of the rostral PAG and the DL, L, and VL regions of the middle and caudal PAG. After barodenervation, fewer FLI-labeled cells were found in the VM region of the rostral PAG, the DL, L, and VL region of the middle PAG, and the L and VL regions of the caudal PAG compared with barointact cats. However, muscle contraction still caused a significantly higher number of FLI-labeled neurons in the DL and L regions of the middle PAG of barodenervated animals compared with barodenervated control animals. There was no difference in the number of Fos-labeled cells in other regions of the PAG between barodenervated animals and barodenervated control animals. These results suggest that activation of muscle afferent input activates the DL and L regions of the middle PAG independently of activation of arterial baroreceptor reflex, and the activated neurons in the VM of the rostral PAG, the VL region of the middle PAG, and the L and VL regions of the caudal PAG are induced by activation of baroreceptor reflex during static muscle contraction.

The majority of group III and IV afferent fibers forms synapses in the superficial dorsal horn of spinal cord (24, 29). Also, it has been previously demonstrated that this region may be a site of the first synapse for the exercise pressor reflex (41). Furthermore, it has been reported that there is a direct projection from the dorsal horn of the lumbosacral cord to the DL and L regions of the PAG (17, 38). In the present study, these
same regions demonstrated distinct Fos labeling during muscle contraction. This result suggests that muscle contraction activates the specific regions of the PAG by direct spinal-PAG pathways during the exercise pressor reflex.

The rostral VLM projects selectively to the lateral division of the PAG (12). In previous reports, we showed that distinct Fos expression was induced by static muscle contraction in the rostral VLM (22) and that there was no difference in the number of Fos-labeled cells in this region after barodenervation (20). In addition, the excited neurons by static muscle contraction were recorded in the medulla using electrophysiological methods (3, 4). Fos expression both in the rostral VLM (20) and in the lateral region of the PAG shown in this study were induced by muscle contraction independently of activation of the arterial baroreceptor reflex. These results clearly indicate that the
Fos-labeled cells in the lateral PAG may be caused by the rostral VLM ascending excitatory pathway. It has been reported that chemical or electrical stimulation of a column of neurons within the lateral PAG evokes a hypertensive response (8, 9). In addition, it has been shown that there is a descending pathway arising from the PAG (the lateral and ventrolateral regions) to the rostral VLM (12). Other regions of the medulla related to cardiovascular regulation such as the nucleus of the solitary tract (NTS) and caudal VLM also receive projections from those regions of the PAG (12). Thus the activated neurons in the lateral PAG by muscle contraction is likely to be involved in the expression of pressor response to muscle contraction by those descending pathways.

It has been demonstrated that static muscle contraction increased the discharge of cells in the posterior hypothalamus (37). We found that the activated neurons in the L and DL regions of the middle PAG were induced by muscle contraction independently of activation of baroreceptor reflex. In addition, vasopressin and oxytocin neurons of the paraventricular nucleus and the supraoptic nucleus of hypothalamus were activated by muscle contraction (19). Those brain structures may not be requisite to produce cardiovascular changes evoked by muscle contraction in anesthetized animals, because little difference was observed between the exercise pressor responses of anesthetized cats with intact brain and midcollicular decerebration (14). But we think that those structures may be important sites for integration of cardiovascular responses during exercise, such as the pressor response by muscle contraction may be modulated by descending projections from those structures to the medulla.

The ventrolateral PAG receives projections from medullary sites, which are involved in cardiovascular regulation. It has been reported that injection of the anterograde tracer into the NTS produced labeling concentrated within the ventrolateral PAG (12). In this study, muscle contraction caused the distinct number of c-Fos in the DL, L, and VL regions of the PAG in barointact animals, compared with that in barodenervated animals. However, there was no difference for number of c-Fos in the VL region of the PAG between barodenervated and barodenervated control animals. This suggests the VL area of the PAG was activated by the arterial baroreceptor reflex during static muscle contraction. Chemical or electrical stimulation of a column of neurons within the VL region of PAG resulted in a decrease in ABP (7, 8). Because the VL region of the PAG as the depressor area was activated by arterial baroreceptors during muscle contraction, activation of ventrolateral PAG may be part of a negative feedback reflex that opposes the increase in ABP that occurs during muscle contraction (27, 30, 31), such that the increase in ABP will not be overdone. The L and DL regions of the middle PAG as the pressor areas were activated by muscle afferents during muscle contraction. This may be one of resources to drive the pressor response during muscle contraction (27, 30, 31).

In summary, static muscle contraction caused extensive Fos expression within the VM region of the rostral PAG and the DL, L, and VL regions of the middle and caudal PAG in anesthetized cats. This result suggests that static muscle contraction activates neuronal cells in regions of the PAG. Neuronal cells in the PAG were responding to activation of the baroreceptor reflex, which may be caused by elevation of ABP during static muscle contraction. However, muscle contraction caused a significantly higher number of FLI-labeled neurons in the DL and L regions of the middle PAG in barodenervated animals compared with barodenervated control animals. This finding indicates that specifically organized regions of the PAG (the dorsolateral and lateral regions of the middle PAG) were activated by afferent activity from skeletal muscle independently of activation of arterial baroreceptor during static muscle contraction. Therefore, neuronal cells in the PAG may play a role in determining the cardiovascular responses during the exercise pressor reflex.

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