Effect of barodenervation on c-Fos expression in the medulla induced by static muscle contraction in cats

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Effect of barodenervation on c-Fos expression in the medulla induced by static muscle contraction in cats. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H901–H908, 1998.—A previous study has shown increased Fos-like immunoreactivity (FLI), a marker of neural activation, in the nucleus of the solitary tract (NTS) and the ventrolateral medulla (VLM) after static muscle contraction elicited by electrical stimulation of L7 and S1 ventral roots of the spinal cord in anesthetized, baroreceptor-intact cats. Because the electrically induced static muscle contraction reflexly increased arterial blood pressure, the concomitant activation of the arterial baroreceptor reflex during static muscle contraction may have resulted in some of the FLI labeling that was observed in the medulla. The purpose of this study was to determine regions in the medulla that are activated by muscle contraction in the absence of arterial baroreceptor input. The electrical stimulation of L7 and S1 ventral roots of the spinal cord was used to elicit static muscle contraction, and FLI in the medulla was determined in barointact and barodenervated cats. In barointact contraction cats, FLI was observed in the lateral reticular nucleus (LRN), NTS, lateral tegmental field (FTL), subretrofacial nucleus (SRF), and A1 region of the medulla. In barodenervated contraction cats, FLI increased in the same regions; however, the number of FLI-labeled cells in the NTS, FTL, and A1 region was significantly less than in barointact contraction animals. No significant difference in the number of FLI-labeled cells was found in the LRN and SRF between the two groups. These results clearly demonstrate that cardiovascular regions in the medulla are activated by input fromafferent activity originating in skeletal muscle independently of concomitant arterial baroreceptor reflex activation.

exercise pressor reflex; heart rate; nucleus of the solitary tract; ventrolateral medulla

STATIC MUSCLE CONTRACTION induced by stimulation of ventral roots increases c-Fos expression in the nucleus of the solitary tract (NTS) and in the ventrolateral medulla (VLM) (22). Also, Fos-labeled cells in the NTS and the VLM have been induced by electrical stimulation of the carotid sinus nerve and by elevation of arterial pressure (9, 32). Because induced static muscle contraction reflexly increases arterial blood pressure (24, 25, 27, 28), it is possible that the observed c-Fos expression was due to activation of the arterial baroreceptor reflex.

Neuroanatomic tracing studies using injections of horseradish peroxidase into the triceps surae of the cat have demonstrated that muscle afferents terminate in several laminae of the spinal cord as well as ascending to terminate in the NTS (17). In addition, it has been shown that excitatory neuronal responses were recorded from neurons in the NTS by electrical stimulation of the tibial nerve in the hindlimb of rats (37), and in the VLM by contraction of skeletal muscle (3, 4) and by electrical stimulation of the tibial nerve (34). These results indicate that afferent fibers from muscle terminate in the NTS and VLM (3, 4, 34, 37). Because induced static muscle contraction activates group III and group IV muscle afferents (18, 25), it is possible that c-Fos expression in the medulla resulted from direct activation by skeletal muscle afferent fibers.

In the present study, we examined Fos-like immunoreactivity (FLI) in the medulla induced by electrical stimulation of L7 and S1 ventral roots of the spinal cord, which elicited alternate static contraction of both hindlimb muscles in anesthetized cats. To determine whether specific regions of the medulla were activated by skeletal muscle receptors, a comparison was made between the number of FLI-labeled cells evoked by muscle contraction in barointact animals and the number of FLI-labeled cells evoked by muscle contraction in barodenervated animals. Thus the present study determined whether regions of the VLM and NTS were activated by muscle contraction in the absence of baroreceptor input. A preliminary report of these findings has been published (23).

METHODS

General surgical preparation. The experiments were performed on 12 anesthetized cats weighing 3.2–5.3 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 arterial blood pressure, 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 if 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; PO2 80–100 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 37.0 and 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).
A pressure transducer (model P23ID, Statham, Oxnard, CA) was connected to an arterial catheter for measurement of blood pressure. Mean arterial pressure (MAP) was obtained by integrating the arterial signal with a time constant of 4 s. Heart rate (HR) was derived from the arterial pressure pulse by a biotachometer (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. The pelvis was stabilized in a spinal unit (Kopf Instruments, Tujunga, CA), and both the knee joints were secured by attaching the patellar tendon to two steel posts. All measured variables were continuously recorded on an eight-channel chart recorder (Gould Instruments, model 2800Cs).

Experimental protocol. The cats were allowed to stabilize for 4 h after surgery. Arterial pressure (AP), HR, and muscle tension were measured during alternating static contractions of the left and the right triceps surae muscles. Contractions were 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 ensure that a significant increase in muscle tension occurred with the ventral root stimulation. Three groups of animals were studied: 1) barointact cats that received electrical stimulation of the L7 and S1 ventral roots (barointact contraction group, n = 5); 2) barodenervated cats that received electrical stimulation of the ventral roots (barodenervated contraction group, n = 4); and 3) barodenervated cats without electrical stimulation of the ventral roots (barodenervated control group, n = 3). In the barodenervated cats, the denervation was accomplished by bilateral transection of the vagus and the carotid sinus nerves. Denervation was evaluated by measuring the increase in MAP while common carotid arteries were briefly clamped (23 ± 4 mmHg before and 3 ± 1 mmHg after denervation). Ninety minutes after the end of L7 and S1 ventral root stimulation, the cats were perfused transcardially with 1 liter of saline followed by 1.5 liter of 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4). Expression of c-Fos gene is induced within 30 min after activation of the cell. The nuclei of activated cells showed the characteristic dark brown staining under a light microscope. The c-Fos reaction product appeared as dark brown staining in the cell nucleus. This specific staining was abolished by omission of the primary antibody.

Cell counts and statistical analysis. Tissue sections were examined under standard light microscope. The 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 (6) were selected for each of the brain structures in each animal. The structures were subdivided rostrocaudally by using known anatomic cytoarchitectural landmarks. 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 as described elsewhere (20, 30).

A one-way repeated-measures analysis of variance was used for statistical comparison of changes in MAP and HR (across time, and intact vs. barodenervation) and cell count labeling with c-Fos per slice (barointact contraction vs. barodenervated contraction, or barodenervated contraction vs. barodenervated control). 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 ± SE.

### RESULTS

Blood pressure and HR responses to static muscle contraction. Table 1 shows changes in MAP, HR, and tension after electrical stimulation of the L7 and S1 ventral roots of the spinal cord to induce static muscle contraction in barointact and barodenervated cats. The MAP and HR responses to induced muscle contraction were significantly increased above baseline over the

#### Table 1. Baseline and Changes in MAP and HR after Static Muscle Contraction

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Peak 20 min</th>
<th>40 min</th>
<th>60 min</th>
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<tbody>
<tr>
<td>Barointact MAP mmHg</td>
<td>98 ± 7</td>
<td>154 ± 8*</td>
<td>129 ± 9*</td>
<td>116 ± 13</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>177 ± 8</td>
<td>205 ± 12*</td>
<td>213 ± 11*</td>
<td>203 ± 12</td>
</tr>
<tr>
<td>Tension, kg</td>
<td>0.8</td>
<td>8 ± 0.7</td>
<td>6 ± 1.2</td>
<td>5 ± 0.5</td>
</tr>
<tr>
<td>Barodenervated MAP mmHg</td>
<td>96 ± 15</td>
<td>157 ± 22*</td>
<td>130 ± 14*</td>
<td>106 ± 12</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>179 ± 17</td>
<td>205 ± 15*</td>
<td>213 ± 21*</td>
<td>206 ± 27</td>
</tr>
<tr>
<td>Tension, kg</td>
<td>0.8</td>
<td>8 ± 0.8</td>
<td>6 ± 0.8</td>
<td>5 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 5 baroreceptor-intact and n = 4 baroreceptor-denervated cats. Peak changes were induced during 1st or 2nd muscle contraction. Muscle tension values are for left hindlimb. MAP, mean arterial pressure; HR, heart rate. *Significantly different from baseline (P < 0.05).
first 40 min of muscle contraction in barointact and barodenervated cats. No significant difference in increases of MAP and HR was observed over 60 min of muscle contraction between the two groups.

Distribution of FLI in the NTS. FLI was observed in the NTS rostrocaudally from −1.2 to +0.6 mm to the obex (slices caudal and rostral to the obex were designated as negative and positive, respectively). FLI was found at these rostrocaudal areas of the NTS after stimulation of the L7 and S1 ventral roots in barointact animals. In barodenervated contraction cats, FLI also was seen at these same areas of the NTS. The photomicrographs of sections for FLI staining in the NTS at −0.6 mm to the obex are shown in Fig. 1. Figure 1A illustrates that only a few Fos-positive neurons were scattered throughout the NTS in a barodenervated control cat. Figure 1B was taken from a barointact contraction cat. Fewer FLI-labeled cells are seen in Fig. 1C, which was taken from a barodenervated contraction animal.

The mapping of FLI-labeled cells in representative coronal sections of the NTS from animals after muscle contraction either in barointact or barodenervated animals is shown in Fig. 2. The number of FLI neurons in the NTS (at −1.2, −0.6, and +0.6 mm to the obex) was significantly less in barodenervated contraction animals compared with barointact contraction animals; however, it was significantly higher than the number in barodenervated control animals without electrical stimulation of the ventral roots (Fig. 3). The number of FLI-labeled cells in the NTS at −1.2, −0.6, and +0.6 mm to the obex in barodenervated contraction animals was 43, 33, and 62% of the number of FLI-labeled cells in the same areas of the NTS in barointact contraction animals, respectively.

Distribution of FLI in the VLM. FLI was observed in the VLM rostrocaudally from −0.6 to +4.1 mm to the obex. Distinct FLI was seen in the VLM after ventral root stimulation in barointact contraction cats. In a barodenervated control group, a few Fos-positive neurons were scattered throughout the VLM (Fig. 4A). Figure 4B and C, shows low-power photomicrographs of FLI staining in the subretrofacial nucleus (SRF) of the rostral VLM obtained from barointact contraction and barodenervated contraction animals, respectively. Figure 4D shows the same region illustrated in Fig. 4C at a greater magnification.

Figures 2 and 5 show the mapping of FLI-labeled cells in representative coronal sections of the VLM from barointact animals after muscle contraction. FLI was also observed in those same areas of the VLM in barodenervated contraction cats (Figs. 2 and 5). However, the number of FLI-labeled cells is significantly less in the A1 region, which is a part of the caudal VLM, consisting of noradrenergic cells at +1.4 and +2.5 mm to the obex, compared with barointact contraction animals, whereas it is significantly higher than in barodenervated control animals at +2.5 mm to the obex (Fig. 6). Also, the number of FLI-labeled cells in the lateral tegmental field (FTL) was significantly less at −0.6 and +0.6 mm to the obex in barodenervated contraction cats than in barointact contraction cats; however, it was significantly more than in barodenervated control animals (Fig. 6). Figure 6 also shows that no significant difference in the number of FLI-labeled cells in the lateral reticular nucleus (LRN) at −0.6 and +0.6 mm to the obex, and in the SRF at +3.3 and +4.1 mm to the obex, was found between barointact contraction and barodenervated contraction groups; however, a significant difference for the number of FLI-labeled cells was observed in the LRN at −0.6 and +0.6 mm to the obex and in the SRF at +3.3 and +4.1 mm to the obex between barodenervation contraction and barodenervated control cats (Fig. 6).

**DISCUSSION**

Previous studies have shown that the medulla is a crucial area for the expression of the cardiovascular response to static muscle contraction (3, 4, 13–15). Recently, the determination of c-Fos activity has been used for identifying activated neurons during static

Fig. 1. Fos-like immunoreactivity (FLI) cells shown in nucleus of solitary tract (NTS) at 0.6 mm caudal to obex. Photomicrographs are of histological sections of NTS stained immunohistochemically for FLI in barodenervated control (A), barointact contraction (B), and barodenervated contraction animals (C). ce, Central canal; bar, 250 μm.
muscle contraction in anesthetized cats and in conscious rats during treadmill exercise (16, 21, 22). FLI was found in the NTS and the LRN, FTL, SRF, and A1 region of the VLM after static muscle contraction induced by electrical stimulation of the L7 and S1 ventral roots of the spinal cord (22). The present study confirms the findings of FLI in the NTS and the LRN, FTL, SRF, and A1 region of the VLM being induced by static muscle contraction. In addition, studies performed in barodenervated cats suggest that the NTS and the LRN, FTL, SRF, and A1 region of the VLM are directly activated by input originating from static contraction-induced activation of skeletal muscle afferents.

The NTS is the site of primary synapse for afferent fibers projecting from arterial baroreceptors to the brain stem (19). It has been previously reported that FLI was induced in neurons within the NTS after electrical stimulation of the carotid sinus nerve in rats (9), and the number of FLI-labeled neurons induced by hypertension with the infusion of phenylephrine was reduced within the NTS in rabbits after sinoaortic denervation (32). In the present study, the number of FLI-labeled cells in the NTS was reduced in barodenervated contraction cats compared with barointact animals after electrical stimulation of the L7 and S1 ventral roots. This result suggests that the elevated arterial pressure elicited by electrically induced static muscle contraction in barointact contraction cats increased the afferent input to the NTS from the baroreceptors in the aortic arch and carotid sinuses. In a previous study (22), it was reported that the number of c-Fos-labeled cells in the NTS (~0.6 mm to the obex) induced by static muscle contraction significantly increased compared with barointact control animals (barointact cats without electrical stimulation of ventral roots). In this study, the number of c-Fos-labeled cells in the NTS at ~0.6 mm to the obex in barodenervated control cats (barodenervated cats without electrical stimulation of ventral roots) is 9 ± 1, which is significantly fewer than the number in barointact control cats (36 ± 5). This result also indicates that neural input from baroreceptor afferents activated neurons in the NTS.

In addition, the NTS has been considered a terminating site for afferent fibers from skeletal muscle. A
previous study using the horseradish peroxidase (HRP) tracing technique has demonstrated that afferent fibers from skeletal muscle have direct monosynaptic connections to the NTS, as well as forming second-order afferents in laminae I to laminae V that ascend to the NTS (17). These central projecting fibers from skeletal muscle may contribute to the cardiovascular adjustments during muscular exercise (17). Recently, an electrophysiological study has shown that electrical stimulation of the tibial nerve in the hindlimb resulted in excitatory neuronal responses in the NTS (37). In combination with our present finding that FLI in the NTS persisted after static contraction of skeletal muscle in the absence of arterial baroreceptor input, it appears that the neurons in the NTS may be activated by input from afferent fibers from skeletal muscle independently of concomitant activation of baroreceptor afferents.

Because baroreflex activity is altered by the elevation of arterial pressure that occurs during static contraction of skeletal muscle (26), cells in the NTS may be activated by inputs from arterial baroreceptors and by inputs from muscle afferents. Because the primary synapses of afferents projecting from arterial baroreceptors and from contracting skeletal muscle are located within the NTS (17, 19), this site may be one of the areas in the central nervous system that integrates cardiovascular responses during muscle contraction. From the present results, a large number of FLI-labeled cells (62%) still appeared in the rostral NTS (0.6 mm rostral to obex) in barodenervated contraction cats; however, only 33% FLI-labeled cells occurred in the caudal NTS (−0.6 mm to the obex) in barodenervated contraction cats after static muscle contraction. This finding indicates that the projections of inputs from muscle afferent fibers and of inputs from baroreceptors may have different distributions in the NTS. That is, relatively more inputs from muscle afferent activity may project to the rostral NTS, and the caudal NTS may receive more inputs from baroreceptor activity. It has been reported that the dense Fos labeling

Fig. 3. Histogram showing mean number of FLI cells per section in NTS. *P < 0.05: significant differences for number of FLI cells were seen within NTS at 1.2 and 0.6 mm caudal to obex (−1.2 and −0.6, respectively) and at 0.6 mm rostral to obex (+0.6) in barointact contraction animals (hatched bars) vs. barodenervated contraction animals (filled bars). †P < 0.05: significant differences for number of FLI cells within NTS were seen in barodenervated contraction animals vs. barodenervated control animals (open bars).

Fig. 4. FLI cells shown in rostral ventrolateral medulla (VLM) [subretrofacial nucleus (SRF)]. Low-power photomicrographs are of histological sections of rostral VLM stained immunohistochemically for FLI in barodenervated control (A), barointact contraction (B), and barodenervated contraction animals (C). D: boxed region in C presented at greater magnification. Bar for A–C, 500 μm; bar in D, 200 μm.
induced by an increase in blood pressure (intravenous infusion of phenylephrine) occurred principally in the caudal NTS (30). This finding is consistent with neuroanatomic studies showing that aortic, carotid, and vagal baroreceptor fibers terminate densely in the caudal NTS (5, 31). However, the possible mechanisms of this interaction for integrating cardiovascular responses during muscular exercise in the NTS is not known.

Electrolytic lesions, radioactive glucose labeling, and single-unit recording have shown that the LRN is involved in the expression of the pressor response to static muscle contraction (13–15). In the present study, the number of FLI-labeled cells in the LRN after electrical stimulation of the L2 and S1 ventral roots was similar between barointact and barodenervated cats. This finding indicates that the neurons in the LRN were likely activated by independent afferent input from contracting skeletal muscle.

It has been shown that electrical stimulation of the FTL increases sympathetic nerve discharge, and activity of neural cells in the FTL has been shown to be temporally correlated with sympathetic nerve discharge (10). Neurons in this region project to the intermediolateral columns of the spinal cord via connection with the neurons in the VLM (2, 10). Distinct FLI was expressed in this region after electrically induced muscle contraction, which indicated that this region may be involved in regulating the cardiovascular responses to static exercise. Furthermore, a fewer number of FLI-labeled cells was found in the FTL of barodenervated contraction cats than in barointact contraction cats. These results demonstrate that the activated neural cells in the FTL during static muscle contraction resulted partly from the input of baroreceptor activity because arterial blood pressure was elevated during static muscle contraction. An electrophysiological study has shown excitatory neural responses in

Fig. 5. Distribution of FLI cells in representative coronal sections of medulla from either barointact contraction or barodenervated contraction animals. A–C: muscle contraction-induced FLI expression in VLM at 2.5, 3.3, and 4.1 mm rostral to obex, respectively. Each dot represents 1 labeled cell nucleus. FTG, gigantocellular tegmental field; VN, vestibular nucleus; P, pyramidal tract; RFN, retrofacial nucleus.
eral columns (IML) of the spinal cord, where they form excitatory projection. Their neural cells in the A1 region in barodenervated cats originated from the NTS and send projections to the rostral VLM (11, 12, 38). However, some neural cells were still activated during static muscle contraction in barodenervated cats. This finding indicates that the A1 region is a part of the overall system that is associated with the cardiovascular response induced during static muscle contraction. After barodenervation, static muscle contraction induced a fewer number of FLI-labeled cells in the A1 region. This clearly demonstrates that stimulation of baroreceptor afferents during static muscle contraction activated neural cells in the caudal VLM by the projection from the NTS (11, 12, 38). However, some neural cells in the A1 region were still activated during static muscle contraction in barodenervated cats. This finding suggests that afferent input from the contracting skeletal muscle activates the A1 region independently of input from arterial baroreceptors. However, it is not known whether these activated neurons in the A1 region in barodenervated cats originated from the NTS excitatory projection.

The SRF of the cat is known to play a crucial role in the cardiovasculary reflex (10). Previous study has shown that the caudal VLM receives direct projections from regions of the NTS (1). Furthermore, functional evidence provides that the projection from the NTS to the caudal VLM is excitatory (11, 12, 38). In the present study, distinct FLI-labeled cells were found after static muscle contraction in the A1 region, which is a part of the caudal VLM. This finding indicates that the A1 region is a part of the overall system that is associated with the cardiovascular response induced during static muscle contraction. After barodenervation, static muscle contraction induced a fewer number of FLI-labeled cells in the A1 region. This clearly demonstrates that stimulation of baroreceptor afferents during static muscle contraction activated neural cells in the caudal VLM by the projection from the NTS (11, 12, 38). However, some neural cells in the A1 region were still activated during static muscle contraction in barodenervated cats. This finding suggests that afferent input from the contracting skeletal muscle activates the A1 region independently of input from arterial baroreceptors. However, it is not known whether these activated neurons in the A1 region in barodenervated cats originated from the NTS excitatory projection.

Fig. 6. Histogram showing mean number of FLI cells per section in the SRF (A), LRN (B), FTL (C), and A1 area (D) at rostrocaudal extents. Positive and negative numbers on x-axes represent distances (in mm) rostral and caudal to obex, respectively. *P < 0.05; significant differences for number of FLI cells were seen in barointact contraction animals (hatched bars) vs. barodenervated contraction animals (filled bars). †P < 0.05; significant differences for number of FLI cells were seen in barodenervated contraction animals vs. barodenervated control animals (open bars).

In summary, significant FLI was found in the NTS and in the regions of the VLM in barointact and barodenervated cats after static muscle contraction elicited by electrical stimulation of the L7 and S1 ventral roots of the spinal cord. These FLI-positive regions are known to be involved in cardiovascular regulation. However, fewer FLI-stained cells were found in the NTS, FTL, and A1 region in barodenervated contraction cats compared with barointact contraction animals. In the LRN and the rostral VLM (SRF) there were no differences in the number of FLI-labeled cells among these two groups. Because FLI still occurred in some areas of the NTS and the VLM after static contraction of the hindlimb muscles in the absence of baroreceptor afferent input, these cardiovascular control regions can be activated by input from afferent activity originating in skeletal muscle independently of concomitant activation of arterial baroreceptors.

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