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Glutamate release in midbrain periaqueductal gray by activation of skeletal muscle receptors and arterial baroreceptors

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Li, Jianhua, and Jere H. Mitchell. Glutamate release in midbrain periaqueductal gray by activation of skeletal muscle receptors and arterial baroreceptors. *Am J Physiol Heart Circ Physiol* 285: H137–H144, 2003. First published March 20, 2003; 10.1152/ajpheart.00904.2002.—We have previously reported that both skeletal muscle receptor and arterial baroreceptor afferent inputs activate neurons in the dorso-lateral (DL) and lateral regions of the midbrain periaqueductal gray (PAG). In this study, we determined whether the excitatory amino acid glutamate (Glu) is released to mediate the increased activity in these regions. Static contraction of the triceps surae muscle for 4 min was evoked by electrical stimulation of the L7 and S1 ventral roots in cats. Activation of arterial baroreceptor was induced by intravenous injection of phenylephrine. The endogenous release of Glu from the PAG was recovered with the use of a microdialysis probe. Glu concentration was measured by the HPLC method. Muscle contraction increased mean arterial pressure (MAP) from 98 ± 10 to 149 ± 12 mmHg ($P < 0.05$) and increased Glu release in the DL and lateral regions of the middle PAG from 0.39 ± 0.10 to 0.73 ± 0.12 μM (87%, $P < 0.05$) in intact cats. After sinoaortic denervation and vagotomy were performed, contraction increased MAP from 95 ± 12 to 158 ± 15 mmHg, and Glu from 0.34 ± 0.08 to 0.54 ± 0.10 μM (59%, $P < 0.05$). The increases in arterial pressure and Glu were abolished by muscle paralysis. Phenylephrine increased MAP from 100 ± 13 to 162 ± 22 mmHg and increased Glu from 0.36 ± 0.10 to 0.59 ± 0.18 μM (64%, $P < 0.05$) in intact animals. Denervation abolished this Glu increase. Summation of the changes in Glu evoked by muscle receptor and arterial baroreceptor afferent inputs was greater than the increase in Glu produced when both reflexes were activated simultaneously in intact state (123% vs. 87%). These data demonstrate that activation of skeletal muscle receptors evokes release of Glu in the DL and lateral regions of the middle PAG, and convergence of afferent inputs from muscle receptors and arterial baroreceptors in these regions inhibits the release of Glu. These results suggest that the PAG is a neural integrating site for the interaction between the exercise pressor reflex and the arterial baroreceptor reflex.

static muscle contraction; arterial baroreflex; exercise pressor reflex; microdialysis

THREE MECHANISMS ARE THOUGHT to contribute to the cardiovascular responses evoked during exercise. First, afferent input arising from contracting skeletal

muscle relayed to the brain stem causes increases in sympathetic nerve activities, arterial blood pressure, and heart rate (32, 35, 36, 39). Neural signals from contracting skeletal muscle are generated by activating mechanically and metabolically sensitive nerve ending (receptors) located in the muscle (22, 23, 39). These neural signals are subsequently carried to the central nervous system by group III and group IV afferent fibers (36, 39). Together, activation of these receptors by muscle contraction along with the reflex cardiovascular responses is termed the exercise pressor reflex (EPR) (36, 39). A second source of input to the brain stem that elicits cardiovascular responses during exercise originates in higher centers of the brain, such as the motor cortex and/or subcortical motor areas (13, 51), and has been termed “central command” (50). The third source of brain stem inputs arises from peripheral baroreceptor populations. While neural signals from the exercise pressor reflex and central command are activated during exercise, inputs from arterial baroreceptors are constantly being transmitted to the central nervous system and the arterial baroreflex (ABR) can modulate the cardiovascular responses produced by the EPR and central command (45, 46).

The midbrain periaqueductal gray (PAG) is an important neural substrate for autonomic regulation (4, 33, 48, 49) and plays a role in regulating ABR (18, 43, 49). The PAG is also linked to the EPR (20, 25, 53, 54). For example, muscle contraction increases PAG neuropeptide Y and enkephalin release in cats (53, 54). In addition, treadmill exercise in rats activates PAG neurons (20) and muscle contraction increases PAG neuron discharge (25). However, the effect of the activated ABR during muscle contraction or dynamic exercise was not considered in these previous studies (20, 53, 54). This is important because the PAG is activated when the ABR is engaged (41), and blood pressure rises with muscle contraction or dynamic exercise. We (28) recently found that both muscle receptor and baroreceptor afferent inputs activate neuronal cells in regions of the PAG during static muscle contraction by identifying c-Fos expression. Furthermore, the dorso-lateral (DL) and lateral regions of the middle PAG are independently activated by skeletal muscle afferents (28).

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On the basis of these data, we focused on the DL and lateral regions of the PAG in the present study.

Finally, the ABR and the EPR activate the same PAG regions via excitatory neuronal projections (28, 41). The excitatory amino acid glutamate (Glu) and its receptors also appear to play a role in cardiovascular regulation in the PAG (1, 3, 14, 34). Thus the first purpose of this study was to determine whether afferent inputs from both skeletal muscle receptors and arterial baroreceptors increase Glu release in these regions. The PAG neurons are excited by the ABR (41) and involved in modulating the ABR (18, 43, 49). An increase in Glu release in the rostral ventrolateral medulla (rVLM) is independent of activation of the ABR (29). However, this Glu increase is dependent on the ABR in the caudal ventrolateral medulla (cVLM) (15). Accordingly, the second purpose of this study was to examine whether denervation of arterial baroreceptors alters this Glu release in the PAG. Our hypothesis was that there is an inhibitory mechanism by which Glu release is decreased in the PAG when the two reflexes are activated simultaneously.

EXPERIMENTAL METHODS

General Methods

Animal surgical preparation. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center and complied with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Experiments were performed on eight anesthetized cats of either gender (3.8–5.8 kg wt). The animals were anesthetized by inhalation of a halothane-oxygen mixture (2–5% halothane in 100% oxygen). A tube was inserted into the trachea via a tracheotomy to maintain an open airway, and a femoral vein and artery were cannulated for drug administration and measurement of arterial blood pressure (ABP), respectively. Anesthesia was then maintained with α -chloralose (80 mg/kg) and urethane (200 mg/kg) injected intravenously. Throughout the experiment, supplemental injections of α -chloralose (15 mg/kg) and urethane (40 mg/kg) were given if the cats exhibited a corneal reflex or withdrew a limb in response to a noxious stimulus. Arterial blood gases and pH were periodically checked (model ABL-3, Radiometer; Copenhagen, Denmark) and maintained within normal limits (pH: 7.30–7.40; P_{CO_2} : 32–36 mmHg; P_{O_2} > 80 mmHg) by adjusting the ventilator (model 661, Harvard Apparatus; South Natick, MA) or by injecting 1 M 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.

Additional surgery was performed to allow an acute sino-aortic and cardiopulmonary baroreceptor deafferentation (44). The ventral surface of the neck was exposed by a midline incision. Superficial tissues were cauterized to expose the common carotid arteries and the carotid sinus bifurcation bilaterally. Silk sutures were then placed around the vagosympathetic nerves bilaterally to isolate input from aortic and cardiopulmonary baroreceptors. Sutures were also placed around the carotid sinus nerve, the internal carotid artery, and the occipital artery distal to the carotid sinus bifurcation bilaterally.

Laminectomy. The lower lumbar and upper sacral portions of the spinal cord were exposed. The dura was then opened. The L7 and S1 spinal ventral roots were carefully separated and cut close to the spinal cord. The peripheral ends of the transected L7 and S1 ventral roots were then placed on platinum bipolar stimulating electrodes, and the exposed spinal cord region was immersed in a pool of warm mineral oil (37°C). The calcaneal bone of one hindlimb was cut, allowing the Achilles tendon to be connected to a force transducer for measurement of developed tension during electrically stimulated muscle contraction. The pelvis was stabilized in a spinal unit (Kopf Instruments; Tujunga, CA) and the knee joints secured by attaching the patellar tendon to a steel post.

Craniotomy and microdialysis. The cat's head was fixed in a stereotaxic apparatus (Kopf Instruments), and a craniotomy was performed to expose the brain stem and cerebellum. For placement of probes into the PAG, the superior colliculi were exposed by gently reflecting back the cortex, by removing a portion of the cerebellum by suction, and by removing a portion of the tentorium. Gel foam was used minimize any bleeding during this procedure, and warmed (37°C) mineral oil was applied to the dorsal surface of the superior colliculi.

After surgery, the microdialysis probes (1 mm membrane and 0.5 mm outer diameter, Bioanalytical System; W. Lafayette, IN) were placed into the middle PAG (A0.6) (7) ipsilateral to the contracted muscle with the use of a stereotaxic carrier (Kopf Instruments). Each of the probes was inserted ~2.0 mm below the dorsal surface of the brain stem, and 1.2 mm lateral to midline for reaching the DL and lateral regions. Stimulation of skeletal muscle afferents and the ABR activate the DL and lateral regions of the PAG (28). Thus Glu releases from these regions of the PAG were collected with the use of microdialysis probes. The probes were continuously perfused at a rate of 5 μ l/min with artificial extracellular fluid (ECF; pH 7.4) that was made fresh for each experiment. The ECF contained 0.2% bovine serum albumin, 0.1% bacitracin, and the following ions (in mM): 6.2 K⁺, 134 Cl⁻, 2.4 Ca²⁺, 150 Na⁺, 1.3 P⁻, 13 HCO₃⁻, and 1.3 Mg²⁺ (29). Functional confirmation of the accurate placements of the microdialysis probes into these regions of the PAG was verified by perfusing the probes with 10 nM L-glutamate. Perfusion of L-glutamate into these regions produced an immediate increase in blood pressure.

Measurement of cardiovascular activities and muscle tension. ABP was measured with a pressure transducer (model P23ID, Statham; Oxnard, CA) connected to an arterial catheter. 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 with a biotach (Gould Instruments; Cleveland, OH). The developed tension during electrically stimulated muscle contraction was measured using a force transducer (model FT10, Grass Instruments) clamped to the cut end of the Achilles tendon. All measured variables were continuously recorded on an eight-channel chart recorder (model 2800s, Gould Instruments). These variables were also sampled with the use of a Pentium III-based Dell computer that was equipped with analog-to-digital conversion (model 1401, CED) and data-acquisition software (CED, Spike 2, version 3).

Experimental Protocols

After the microdialysis probe was inserted, 2 h were allowed for stabilization of the preparation. During this period, ECF was continuously dialyzed and timed collections were performed. Four-minute collection periods were performed

immediately postinsertion and at each of the 40-min recovery period for basal levels of glutamate. The extracellular Glu concentration is stabilized 120 min after insertion (29). Four minutes of static muscle contraction was then elicited by electrically stimulating the L7 and S1 ventral roots ($3\times$ motor threshold, 0.1 ms duration, 40 Hz). A sample was collected during the contraction. We chose a 4-min contraction and a 5 $\mu\text{l}/\text{min}$ perfusion rate to obtain dual 10 μl of sample, which was the amount needed for the HPLC analysis (15, 29). Control data were obtained 4 min before the contraction, and recovery data were collected for 4 min after each contraction. Activation of the ABR was then induced by continuous infusion of PE hydrochloride (Sigma, 0.5 mg/ml in saline) by connecting the femoral vein cannula to a syringe pump. The 1- to 5-ml/h infusion rate was adjusted to maintain the increase in the ABP (28). These protocols were repeated after the cats were denervated. There was a 40-min rest period between each of the interventions. A period of 1 h was allowed for ABP to stabilize after denervation. Afferent activity from aortic and cardiopulmonary baroreceptors was eliminated after bilateral transection of the vagosympathetic nerve bundles at the level the carotid sinus bifurcation. Afferent inputs from carotid sinus baroreceptors were eliminated by bilateral ligation of sutures around the carotid sinus nerve, the internal carotid artery, and the occipital artery complex. The ligation effectively crushed the carotid sinus nerve and eliminated afferent input from carotid baroreceptors (44). The efficacy of denervation was confirmed by the absence of a pressor response to brief bilateral occlusion of the common carotid arteries (MAP: 23 ± 4 mmHg before and 3 ± 2 mmHg after denervation). Finally, the ventral roots were stimulated after the muscle was paralyzed by an intravenous injection of pancuronium bromide (200 $\mu\text{g}/\text{kg}$ wt). This protocol allowed us to determine whether muscle contraction was necessary for Glu release. At the termination of the protocol, the microdialysis probe was perfused with a solution of 150 mM KCl for 4 min to obtain Glu concentration by neuronal depolarization (44).

Measurement of Glu by HPLC. Glu concentrations were measured using HPLC with *o*-phthaldialdehyde derivative based on the methods as described elsewhere (21). The *o*-phthaldialdehyde solution (3 μl) was mixed with 20 μl of perfusate and permitted to react for 2 min. Ten microliters of the reaction product were then injected into the HPLC system (Dupont). Separation was achieved by reverse-phase chromatography with the use of a 3 μm C16 column (BAS) over a 15-min wash period with two buffers: *buffer A* (0.1 M Na acetate, pH 6.4, 14% acetonitrile, and 2% tetrahydrofuran) was run for 5 min, followed by a 10-min ramping, and then 5 min with *buffer B* (same as *buffer A* with the addition of 75% acetonitrile). Peak areas were determined by integration and excitatory amino acid concentrations were quantified on the basis of linear calibration with 5 μM amino acid standards every seventh sample. Previous studies (15, 29) have used this method to measure Glu concentrations from dialysates sampled from the medullary region. All assays were performed in a blinded fashion.

Histology

At the end of each experiment, Evans blue dye was microdialyzed into the PAG for 40 min. The brain stem was then removed and placed in a solution of 10% phosphate-buffered formalin and then stored at 4°C. After the tissue was adequately fixed, the brain stem was blocked and subsequently sliced into 50- μm -thick sections on a cryostat (model 2800 Frigocut-E, Cambridge Instruments). The sections were

placed on coated slides. The tracks in the PAG produced by the microdialysis probes were examined. In addition, the extent of dye diffusion was examined with the use of a light microscope.

Statistical Analysis

A one-way repeated-measure analysis of variance was used for statistical comparison of changes in MAP, HR, peak muscle tension, and Glu concentration. Tukey's post hoc test was utilized as appropriate. All values were expressed as means \pm SE. For all analysis, differences were considered significant if $P < 0.05$. All statistical analyses were performed using SigmaStat for Windows version 2.03 (SPSS; Chicago, IL).

RESULTS

Basal Glu Concentration

Basal levels of Glu concentrations in the PAG were 4.1 ± 0.7 , 2.5 ± 0.6 , 0.85 ± 0.3 , and 0.44 ± 0.1 μM immediately after insertion and at 40-, 80-, and 120-min recovery periods, respectively. Glu concentration during the first precontraction period (0.39 ± 0.10 μM) was not different when compared with that at 120 min postinsertion ($P > 0.05$). This indicates that the extracellular concentration of Glu had stabilized after 120 min postinsertion, as previously reported (29).

Glu and Cardiovascular Activities During Control Studies

The original tracings in Fig. 1 show that 4-min static muscle contraction-induced increases in MAP and HR and that intravenous injection of PE induced an increase in MAP and a decrease in HR. Before baroreceptor denervation, static muscle contraction (at peak tension of 5.6 ± 0.6 kg) increased the extracellular concentration of Glu in the PAG from 0.39 ± 0.10 to 0.73 ± 0.12 μM ($P < 0.05$, 87%), MAP from 98 ± 10 to 149 ± 12 mmHg ($P < 0.05$), and HR from 189 ± 15 to 210 ± 20 beats/min ($P < 0.05$). The recovery levels for Glu, MAP, and HR after contraction were 0.51 ± 0.11 μM , 101 ± 12 mmHg, and 188 ± 16 beats/min, respectively. Intravenous injection of PE increased Glu concentration from 0.36 ± 0.10 to 0.59 ± 0.18 μM ($P < 0.05$, 64%), increased MAP from 100 ± 13 to 162 ± 22 mmHg ($P < 0.05$) and decreased HR from 185 ± 18 to 155 ± 25 beats/min. The recovery levels for Glu, MAP, and HR after injection of PE were 0.43 ± 0.12 μM , 103 ± 12 mmHg, and 180 ± 18 beats/min, respectively. These results are shown in Fig. 2. Figure 3 further shows a relationship between the percent change of Glu concentration and MAP response during muscle contraction ($r = 0.923$, $P < 0.001$).

Glu and Cardiovascular Activities after Baroreceptor Denervation

After denervation, muscle contraction (at peak tension of 5.4 ± 0.4 kg) increased Glu concentration in the PAG from 0.34 ± 0.08 to 0.54 ± 0.10 μM ($P < 0.05$, 59%) (see Fig. 2). The increased Glu concentration in the PAG during contraction was significantly reduced

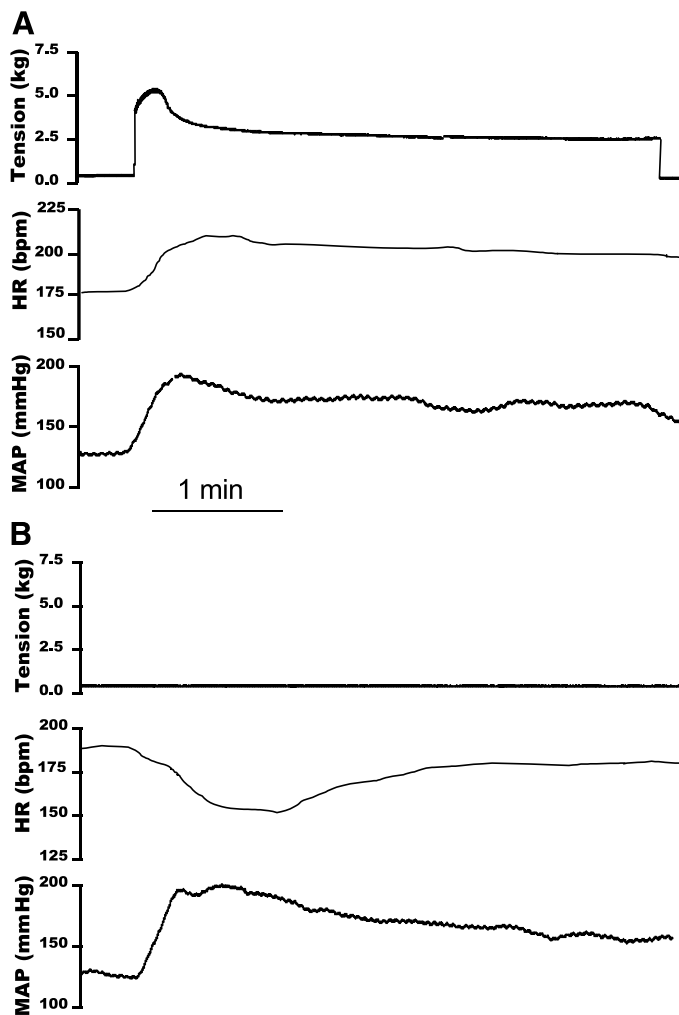


Fig. 1. Representative traces from one cat show typical cardiovascular responses to 4-min static muscle contraction (A) and to intravenous infusion of phenylephrine (PE) (B). MAP, mean arterial pressure; HR, heart rate; bpm, beats/min.

compared with that before denervation (87% vs. 59%) as shown in Fig. 4. Furthermore, summation of the changes in Glu (123%) evoked by muscle receptor (59%, muscle contraction in denervated cats) and arterial baroreceptor afferent inputs (64%, PE injection in intact cats) was greater than the increase in Glu produced when both reflex were activated simultaneously in intact state (87%, contraction in intact cats). This result is also shown in Fig. 4. This demonstrates that the release of Glu in the PAG was inhibited during convergence of afferent inputs from muscle receptors and arterial baroreceptors. Perfusion of the dialysis probe with KCl produced a robust increase in PAG Glu concentration ($0.37 \pm 0.11 \mu\text{M}$ at baseline to $1.78 \pm 0.38 \mu\text{M}$, $P < 0.05$). This increase (380%) is significantly greater than summation of the increases by activation of muscle afferent and by PE infusion (123%), and that by simultaneous activation of both the ABR and the EPR (87%). This demonstrates that Glu concentrations observed during the experimental interventions were below the maximal concentration of

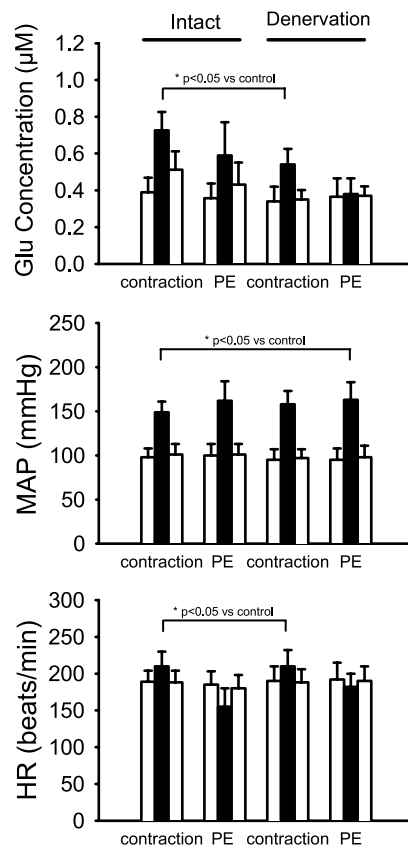


Fig. 2. The changes in glutamate (Glu) (top) concentration, MAP (middle), and HR (bottom) during 4-min static muscle contraction or during 4 min of intravenous infusion of PE in intact and barodennervated cats ($n = 8$). 1) Muscle contraction significantly increased Glu, MAP, and HR in intact and denervated cats; 2) PE infusion significantly increased Glu and MAP, and decreased HR in intact cats; 3) PE infusion also significantly increased MAP in denervated cats; and 4) denervation significantly reduced Glu increases during contraction, and during PE infusion. Open bars, data for baseline and recovery, respectively. Solid bars, data during contraction or during PE infusion.

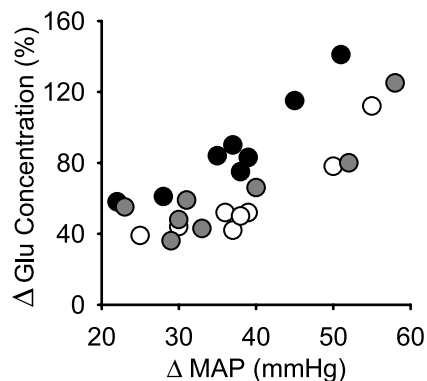


Fig. 3. Relationships between the average change of MAP and percentage change of Glu concentration during 4 min interventions. Solid circles ($r = 0.923$) and open circles ($r = 0.912$), muscle contraction in intact animals and denervated animals, respectively ($n = 8$). Gray circles ($r = 0.876$), activation of arterial baroreceptors by intravenous infusion of PE in intact animals.

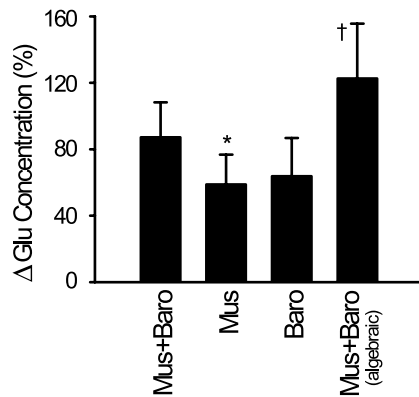


Fig. 4. The percent changes in Glu concentration during muscle contraction in intact animals (Mus+Baro), muscle contraction in denervated animals (Mus), PE infusion in intact animals (Baro), and summation of muscle contraction and PR infusion (Mus+Baro/algebraic). Isolated skeletal muscle afferents were activated by muscle contraction in denervated animals. PE activated only the arterial baroreceptors in intact animals. Muscle contraction activated both muscle afferents and the arterial baroreceptors in intact animals ($n = 8$). * $P < 0.05$ vs. intact (Mus+Baro); † $P < 0.05$ vs. intact (Mus+Baro).

Glu released in the regions of the PAG. The increases in MAP and HR induced by muscle contraction were 95 ± 12 to 158 ± 15 mmHg ($P < 0.05$) and 190 ± 20 to 210 ± 22 beats/min ($P < 0.05$), respectively. Table 1 shows the peak MAP response and the average MAP response during 4 min produced by these interventions. The recovery levels for Glu, MAP, and HR after contraction were 0.35 ± 0.05 μ M, 97 ± 10 mmHg, and 188 ± 18 beats/min, respectively. These results are also shown in Fig. 2. Figure 3 further shows that there is a correlation between increases of Glu and MAP ($r = 0.912$, $P < 0.001$) and that the percent increase of Glu concentration was smaller during the similar MAP response after denervation. An intravenous injection of PE did not cause a significant increase in Glu concentration. PE increased Glu concentration from 0.37 ± 0.10 to 0.38 ± 0.09 μ M ($P > 0.05$). The increase in MAP induced by PE was 95 ± 13 to 163 ± 20 mmHg ($P < 0.05$), and the decrease in HR by PE was 192 ± 23 to 182 ± 18 beats/min. The recovery levels for Glu, MAP, and HR after injection of PE were 0.37 ± 0.05 μ M, 98 ± 13 mmHg, and 190 ± 20 beats/min, respectively. These results are also shown in Fig. 2.

Glu and Cardiovascular Activities after Muscle Paralysis

The increases in Glu, MAP, and HR by electrical stimulation of the ventral roots were abolished by muscle paralysis with an intravenous injection of pancuronium bromide. The electrical stimulation altered Glu concentration from 0.38 ± 0.12 to 0.39 ± 0.13 μ M ($P > 0.05$), MAP from 96 ± 10 to 98 ± 12 mmHg ($P > 0.05$), and HR from 192 ± 20 to 192 ± 15 beats/min ($P > 0.05$), respectively. The recovery levels for Glu, MAP, and HR after stimulation of the ventral roots were 0.36 ± 0.10 μ M, 98 ± 14 mmHg, and 190 ± 16

beats/min, respectively. This demonstrates that the increases in Glu concentration, MAP, and HR were due to activation of skeletal muscle afferents but not due to direct stimulation of the ventral roots.

Histological Examination

Histological examination showed that microdialysis probes were located in the DL or lateral region of the PAG in all eight cats used in this study (Fig. 5). In addition, the extent of dye diffusion was examined using a light microscope. The regions that were stained by dialyzing Evans blue dye through the probes were restricted within the PAG in all eight cats.

DISCUSSION

The purpose of this study was to determine whether afferent inputs from contracting skeletal muscle are responsible for Glu release in the PAG and whether activation of arterial baroreceptors alters this release. Our results have shown that activation of muscle receptors and arterial baroreceptors increases 59% and 64% Glu in the PAG, respectively (Fig. 4). Summation of the changes in Glu evoked by muscle receptor and arterial baroreceptor afferent inputs was greater than the increase in Glu produced when both reflexes were activated simultaneously (123% vs. 87%, Fig. 4). These data demonstrate that activation of skeletal muscle receptors evokes release of Glu in the PAG, and convergence of afferent input from muscle receptors and arterial baroreceptors in this region inhibits the release of Glu. These results suggest that the PAG may act as one of the central integrating sites for an interaction between the EPR and the ABR and that the neurotransmitter glutamate is involved.

Role of PAG in EPR

The PAG is an important neural site for autonomic regulation (4, 33, 48, 49). Electrical or chemical stimulation of the PAG elicits a variety of autonomic responses (4, 8–10, 33). Furthermore, two distinct cardiovascular effector columns, pressor and depressor regions have been defined rostrocaudally in the PAG

Table 1. Changes in MAP

| | Peak MAP Response, mmHg | Average MAP Response, mmHg |
|----------------------|-------------------------|----------------------------|
| Mus+Baro | 51 ± 4 | 36 ± 3 |
| Mus | $63 \pm 5^*$ | 39 ± 4 |
| Baro | 62 ± 5 | 37 ± 4 |
| Mus+Baro (algebraic) | $125 \pm 4^*$ | 76 ± 5 |

Values are means \pm SE; $n = 8$ animals. Mus+Baro and Mus indicate muscle contraction in intact and denervated animals, respectively. Baro indicates phenylephrine perfusion in intact animals. Mus+Baro (algebraic) indicates summation of mean arterial pressure (MAP) changes by Mus and Baro. Peak MAP response was observed 5–60 s after start of the interventions. Average MAP response: MAP responses during 4-min interventions are averaged. * $P < 0.05$ vs. Mus+Baro.

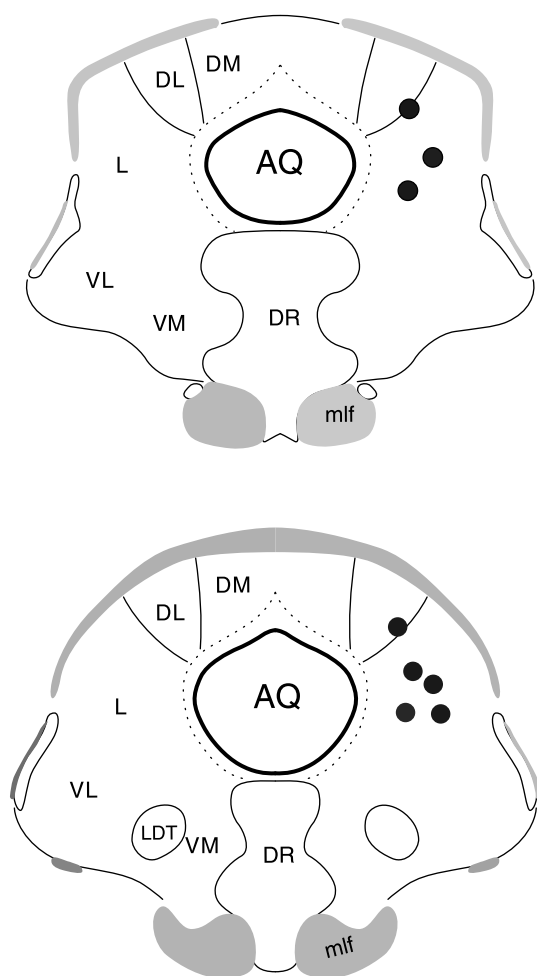


Fig. 5. The locations of the microdialysis probes (●) in the midbrain periaqueductal gray (PAG) from eight animals. The probes were inserted 1.2–3.0 mm below the dorsal surface of the brain stem and 0.8–2.0 mm lateral to midline. DL, dorsolateral; L, lateral; DM, dorsomedial; VL, ventrolateral; VM, ventromedial; AQ, cerebral aqueduct; mlf, medial longitudinal fascicle; LDT, laterodorsal tegmental nucleus; and DR, dorsal nucleus raphe.

(4). In both the cat and the rat, stimulation of the DL and lateral regions of the PAG elicits potent increases in ABP, HR, and lumbar and splanchnic sympathetic nerve discharge, whereas stimulation of the ventrolateral region of the PAG causes a decrease in ABP and HR, and increases blood flow through the hindlimb vascular beds (8–10). Furthermore, the PAG is involved in regulating the EPR and ABR (18, 20, 25, 28, 41, 43, 48, 49, 53, 54).

The reflex cardiovascular responses to static muscle contraction are mediated by both group III (thinly myelinated A δ) and group IV (unmyelinated C) fibers (36, 39). The majority of group III and group IV skeletal muscle afferent fibers make their first synapse in the dorsal horn of the spinal cord (31, 38). There is a direct neuronal projection from the dorsal horn of the lumbosacral cord terminating in the DL and lateral regions of the PAG (11, 24). In these same regions, distinct c-Fos expression was induced by activation of both the EPR and ABR (28). Furthermore, there are

descending projections from the DL and lateral regions of the PAG to the nucleus of the solitary tract and the cVLM and rVLM (16) that are involved in the EPR and ABR (15, 17, 20, 27, 29, 30, 42, 49).

It has been reported that the ABR activates the lateral region of the PAG (41). However, Fos-labeled cells in the lateral region of the PAG induced by muscle contraction are independent of the activation of the ABR because the number of the activated neurons is still distinct after denervation (28). Chemical or electrical stimulation of the column of neurons within the lateral PAG evokes an increase in blood pressure (8, 10). This effect is mediated by neurons in the rVLM (48). In previous reports, we have shown that distinct Fos-labeled cells were induced by static muscle contraction in the rVLM (27) and that there was no difference in the number of Fos-labeling cells in this region after barodenervation (30). Together, these results suggest that there are descending excitatory pathways between the lateral PAG and the rVLM during the EPR. Thus the excitatory amino acid in the rVLM is increased by activation of the EPR (2, 29). In addition, there is also a descending pathway arising from the PAG to the cVLM (16) that is involved in both the ABR and the EPR (2, 15, 27, 30). Evidence has shown that the role played by cVLM in the EPR is dependent on the ABR. This statement is based on the finding in barodenervated animals that the increase in Glu concentration during the EPR is significantly attenuated (15) and the number of Fos-labeled cells in the cVLM during muscle contraction is reduced (30). Thus it seems likely that neurons in the lateral PAG are activated by muscle contraction and by arterial baroreceptors for the integration of cardiovascular control during the EPR.

It is unknown whether PAG plays a role in modulating the cardiovascular responses during the EPR because little difference was observed between the EPR of anesthetized cats with intact brain and midcollicular decerebration (19). First, Waldrop and Stremel (52) reported that static muscle contraction increases the discharge of cells in the posterior hypothalamus. Muscle contraction also activates hypothalamic vasopressin and oxytocin neurons (26). Second, it has recently been reported that electrical stimulation of the mesencephalic locomotor region (central command) inhibits the discharge of dorsal horn neurons at lumbar spinal cord receiving muscle afferents (12) and also resets carotid sinus baroreflex (37). When those results are viewed together, we believe that regions of the higher brain are important for the cardiovascular responses during the EPR and for integration of this reflex. The PAG is located in a key area between the higher brain and the medullary cardiovascular regulating center and appears to be involved in the EPR and ABR (17, 18, 20, 28, 33, 34, 41, 43, 53, 54). Thus the PAG may serve as one of the important sites for integration of cardiovascular responses during exercise. Our data support this hypothesis.

Role of PAG Glu in EPR

Glu and its receptors appear to play a role in cardiovascular regulation in the PAG (1, 3, 14, 34). Studies (34) have shown that an increase in ABP induced by L-glutamic acid in the PAG is significantly reduced by a pretreatment with AP-5, a selective antagonist of Glu NMDA receptor. Glu receptor antagonists block an increase of excitatory synaptic events related to cardiovascular activity by perfusion of sodium nitroprusside into the PAG (14). In addition, glutamate and glutamate-positive terminals were found throughout the PAG in the cat (5). Furthermore, Glu is accumulated in synaptic terminals ascending from the spinal cord to the PAG (3). In the present study, we provide evidence showing that the PAG Glu plays a role in integrating the EPR and ABR.

Inhibition of Glu Release

GABAergic neurotransmission consisting of short interneurons (47) has a decisive action in the negative modulation of all functions including cardiovascular control by the PAG (4). GABAergic modulation may lower the excitation of neurons in the PAG to influence firing of the rVLM (6, 40). An increase in arterial blood pressure during muscle contraction activates the ABR, which excites GABAergic neurons in the cVLM projecting to the rVLM. Furthermore, it has been reported that muscle contraction increases GABA concentration in the rVLM (2, 42). Similarly, it is assumed that GABA release in the PAG is caused by activation of arterial baroreceptors. Furthermore, GABA may inhibit the activity of neurons that evoke the pressor response during muscle contraction. Also, GABA released by the ABR during the EPR may inhibit either PAG Glu interneurons or Glu presynaptic release activated by muscle contraction to reduce Glu level in the PAG. This may be one of the mechanisms by which Glu increase is inhibited in the PAG during simultaneous activation of the EPR and the ABR. Our preliminary study (unpublished data) shows that activation of PAG GABA receptors by microinjection of the GABA agonist muscimol into the lateral PAG attenuates the pressor response to muscle contraction. However, to make a definitive conclusion, it must be determined whether or not GABA release is increased by activation of the ABR.

Study Limitations

Finally, two concerns must be addressed. First, the convergence of afferent inputs from skeletal muscle receptors and arterial baroreceptors inhibits the Glu release in the PAG. This finding may be explained by a nonlinear summation of inputs, which is represented as nonlinear saturation, occlusion, and/or redundancy because the data cannot exclude a possibility of nonlinear summation between the afferent inputs.

Second, it must be determined that an increase in Glu concentration was neuronal in origin. In the recent report (2), Glu level in dialysate samples were de-

creased significantly and could not be detected by HPLC method after 10 μ M tetrodotoxin was added to the perfusate solution and microdialyzed into the medullary regions. This suggests that the measured Glu by microdialysis method was a neuronal release.

In conclusion, activation of skeletal muscle receptors and arterial baroreceptors evokes Glu release in the PAG, and convergence of afferent input from both receptors inhibits this release. The data suggest that the PAG is a neural integrating site in the interaction between the EPR and ABR and that Glu plays a role in this interaction.

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