Extracellular serotonin changes in VLM during muscle contraction: effects of 5-HT$_{1A}$-receptor activation

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Extracellular serotonin changes in VLM during muscle contraction: effects of 5-HT$_{1A}$-receptor activation. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2899–H2909, 1997.—This study determined whether muscle contraction causes an increase in extracellular levels of serotonin (5-HT) in the rostral (rVLM) or caudal ventrolateral medulla (cVLM) in anesthetized rats. Muscle contraction, evoked by tibial nerve stimulation, increased mean arterial blood pressure (MAP) by 27 ± 4 mmHg (n = 8). In addition, 5-HT levels in the rVLM were elevated by 65 ± 9% during the contraction (n = 8). Results were similar over two repeated contractions. In contrast, muscle contraction increased MAP, but not 5-HT, levels in the cVLM (n = 6). Tibial nerve stimulation after muscle paralysis had no effect on either MAP or 5-HT levels in both rVLM and cVLM. Microdialysis of a 5-HT$_{1A}$ agonist, 8-OH-DPAT (10 mM), into the rVLM for 30 min (n = 6) blunted the MAP change and reduced 5-HT release during contraction. Administration of NAN-190, a 5-HT$_{1A}$ antagonist, into the rVLM had no effect on 5-HT release and cardiovascular responses during muscle contraction and blocked the changes in 5-HT, MAP, and heart rate to static contraction after subsequent microdialysis of 8-OH-DPAT. Results demonstrate that 5-HT levels in the rVLM increase during muscle contraction and that 5-HT$_{1A}$-receptor activation in the rVLM blunt MAP response to muscle contraction via a decrease in the extracellular concentration of 5-HT.

arterial blood pressure; heart rate; exercise pressor reflex; rostral ventrolateral medulla; caudal ventrolateral medulla; rat

THE VENTROLATERAL MEDULLA (VLM) is divided into caudal (cVLM) and rostral (rVLM) portions, and these regions have been suggested to be opposing in nature with regard to regulation and/or integration of cardiovascular responses (8). For example, the rVLM elicits pressor effects in response to electrical and chemical stimulation, whereas the cVLM evokes hypotension (8, 34, 43). The VLM has also been implicated in mediating increases in mean arterial blood pressure (MAP) and heart rate (HR) during static muscle contraction, commonly known as the exercise pressor reflex, in anesthetized cats (4, 11, 21, 22, 24, 29) and rats (2). The pressor response during muscle contraction was abolished after bilateral electrolytic lesioning of an area within the VLM (21). Also, c-Fos expression (24) and radioactive glucose (22) studies have highlighted regions within the medulla, including the rVLM and cVLM, that are active during static muscle contraction.

Serotonin (5-HT) within the rVLM and adjacent reticular formation contributes to descending control of autonomic functions and regulates sympathetic outflow (26, 27, 40). Release of 5-HT has been implicated in the interaction between antinociceptive/cardiovascular control and specific descending medullary neurons projecting into sympathetic or somatomotor regulatory regions in the spinal cord (28, 44). Studies using 5-HT$_{1A}$ agonists demonstrate 5-HT$_{1A}$-mediated cardiovascular effects through the rVLM, including the raphe pallidus and the C1 region of the rVLM (14, 15). Stimulation of 5-HT$_{1A}$ receptors in the rVLM evokes a decrease in sympathetic activity, resulting in hypotension and bradycardia (15, 19, 40). Recently, Ally et al. (2) found evidence that activation of 5-HT$_{1A}$ receptors within the rVLM, but not the cVLM, inhibits cardiovascular responses elicited during static muscle contraction and suggested that this attenuation is possibly mediated through changes in 5-HT release.

Therefore, the purpose of the present study was to determine whether rVLM or cVLM changes in extracellular 5-HT concentration are associated with cardiovascular responses during static muscle contraction and whether 5-HT$_{1A}$-receptor activation within the rVLM attenuates increases in MAP and HR during muscle contraction via a change in 5-HT release. We quantified the rVLM and cVLM extracellular fluid 5-HT in response to muscle contraction using microdialysis techniques. Also, we examined the effects of 8-hydroxy-2-(di-n-propylamino)tetrinal (8-OH-DPAT; RBI, Natick, MA), a 5-HT$_{1A}$-receptor agonist administered into the rVLM, on the exercise pressor reflex with concomitant measurement of 5-HT release. Receptor specificity was further confirmed by prior administration of 1-[2-methoxyphenyl]-4-[4-(2-phthalimido)butyl]piperazine (NAN-190; RBI), a 5-HT$_{1A}$-receptor antagonist, followed by subsequent microdialysis of 8-OH-DPAT into the rVLM.

METHODS

Surgery

Male Sprague-Dawley rats (300–350 g) were initially anesthetized with 25 mg/kg pentobarbital sodium (Sigma...
Chemical, St. Louis, MO) and 75 mg/kg chloral hydrate (Sigma Chemical). The rats were maintained at 37–38°C with the use of a heating pad and an infrared heat lamp. Additional doses of chloral hydrate were given based on appearance of a corneal reflex, changes in blood pressure during surgical manipulation, and/or a response to a noxious stimulus, i.e., paw or tail pinch. One common carotid artery was catheterized and coupled with a pressure transducer (model P23 ID; Statham, Oxnard, CA) to allow measurement of arterial pressure using a physiological chart recorder (model 79D; Grass Instruments, Quincy, MA). MAP and HR were obtained by integrating the arterial pressure signal with a time constant of 2 s. The animal was allowed to breathe spontaneously after cannulation of the trachea. However, during experiments involving neuromuscular blockade with intravenous administration of pancuronium bromide through a cannula inserted into a jugular vein, a respirator (model 681; Harvard Apparatus, South Natick, MA) was used for artificial ventilation (room air, 60 strokes/min, 1 ml/100 g body wt). Arterial blood gases and pH were periodically checked (ABL-3, Radiometer, Copenhagen, Denmark) and were maintained within normal limits by providing supplemental oxygen, infusing the lungs using a ventilator and/or injecting sodium bicarbonate intravenously.

After the left tibial nerve was isolated, the nerve was placed on a bipolar platinum hook electrode connected to a stimulator (model S88, Grass) via a stimulus isolation unit (model SI USC, Grass). The hip and left knee were secured to prevent movement during contractions. The triceps sura muscle was exposed and kept moist with mineral oil over wet gauze. Muscle tensions generated by tibial nerve stimulation were measured by a force transducer (model FT03, Grass) attached to the corresponding Achilles tendon.

Microdialysis

The head of the rat was fixed in a stereotaxic frame (Kopf Instruments, Tujunga, CA), and a static muscle contraction was induced by stimulating the tibial nerve (3 × motor threshold, 40 Hz, 0.1 ms) while monitoring arterial pressure, MAP, HR, and developed tension. The dorsal medulla was exposed after retraction of the dorsal neck muscles, the caudal half of the cerebellum, and the dura, thereby revealing the floor of the fourth ventricle rostral to the caudal aspect of the inferior cerebellar peduncle. Two microdialysis probes (model CMA-11; CMA, Acton, MA) with a 1-mm membrane tip (0.24 mm outer diameter) were placed bilaterally into either the rVLM (2.0 mm rostral to the caudal tip of the area postrema, 1.9 mm lateral to midline, and 2.4 mm ventral to the floor of the fourth ventricle) or the cVLM (0.5 mm rostral to the caudal tip of area postrema) based on the rat atlas (33). With the use of a microdialysis pump (CMA/100, CMA), the probes were continuously perfused at 1 µl/min with artificial cerebrospinal fluid (CSF: 125 mM NaCl, 1.26 mM CaCl₂, 2.5 mM KCl, 1.18 mM MgCl₂) at pH 7.4 and osmolality of ~309 mOsm/kg. This artificial fluid served as the delivery system for the drug used in the experiments.

After setup, verification of proper placement of microdialysis probes was performed before each experiment by perfusing 1 nM L-glutamate (RBI, Natick, MA) for 5 min into either the rVLM or the cVLM. If the probes were in the rVLM, an increase in MAP was noted after subsequent L-glutamate administration. Conversely, L-glutamate dialysis into the cVLM exhibited a decrease in MAP. After correct placement of probes was functionally assessed, a static muscle contraction was evoked by stimulating the tibial nerve at parameters similar to those described above. Arterial pressure, MAP, HR, and developed tension were recorded and compared with those recorded before insertion of the probes. This step was performed to determine whether insertion of the probes disrupted the functional integrity of the rVLM or the cVLM. New probes were used for each experiment.

Protocols

Release of 5-HT in rVLM and cVLM. Perfusion of artificial CSF continued at 1 µl/min, and nine 10-min collection periods were performed so that a stable baseline 5-HT release (control) was achieved over a 90-min period (Table 1). A 10-min collection was necessary in this protocol for the dialysate volume to be 20 µl (bilateral collection) to run the chemical assays for 5-HT. Subsequently, a 2-min static contraction was evoked by stimulating the tibial nerve (3 × motor threshold, 40 Hz, 0.1 ms) during a 10-min collection period. The animal was then allowed to recover for 60 min with six 10-min collections, followed by another 2-min stimulation-evoked muscle contraction (10-min collection). This was performed to determine whether repeated muscle contractions elicited similar 5-HT release patterns. Artificial CSF was then dialyzed for another 60 min (six 10-min collections) to establish a recovery of 5-HT. Lastly, the animal was paralyzed with 200 µg pancuronium bromide intravenously (Elkins-Sinn, Cherry Hill, NJ) to allow observation of whether identical stimulation of the tibial nerve using prior parameters produced a change in 5-HT or cardiovascular variables following neuromuscular blockade. This protocol was implemented in separate rats, and samples were collected from both the rVLM (n = 8) and the cVLM (n = 6). Muscle tension, HR, and MAP were continuously monitored and documented throughout the protocol.

Samples were immediately stored at −80°C. The 5-HT concentrations of the perfusates were measured using high-performance liquid chromatography with electrochemical detection (HPLC-EC; see Biochemical Assay of 5-HT). Analyses were done without bias, because samples were coded and the person performing the assays was unaware of the code or the protocol.

Effect of a 5-HT3A-receptor agonist (8-OH-DPAT) microdialyzed into rVLM. Six rats were used to determine the effects of the 5-HT3A-receptor agonist 8-OH-DPAT on cardiovascular responses and changes in extracellular 5-HT concentration during muscle contraction by dialyzing the drug into the rVLM. The cVLM did not warrant further investigation because 5-HT concentrations in this area did not change significantly after muscle contractions despite increases in MAP and HR (see Results). Furthermore, 8-OH-DPAT was not microdialyzed into the cVLM, because a recent study (2) demonstrated that such administration had no effect on cardiovascular responses during muscle contraction.

After the surgical setup, nine 10-min control collections were performed for 90 min (Table 2). A 2-min muscle contrac-

Table 1. Protocol for release of 5-HT in rVLM and cVLM

<table>
<thead>
<tr>
<th>Collection Time</th>
<th>Protocol</th>
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<tbody>
<tr>
<td>10-min collection for 90 min</td>
<td>After insertion of probes</td>
</tr>
<tr>
<td>10-min collection</td>
<td>First contraction</td>
</tr>
<tr>
<td>10-min collection for 60 min</td>
<td>Postcontraction</td>
</tr>
<tr>
<td>10-min collection</td>
<td>Second contraction</td>
</tr>
<tr>
<td>10-min collection for 60 min</td>
<td>Postcontraction</td>
</tr>
<tr>
<td>Paralysis</td>
<td></td>
</tr>
<tr>
<td>10-min collection</td>
<td>Third stimulation</td>
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</tbody>
</table>

5-HT, serotonin; rVLM and cVLM, rostral and caudal ventrolateral medulla, respectively.
tion was then elicited during another 10-min collection period (Table 2). The rat was allowed to recover for 60 min (six 10-min collections). After control cardiovascular responses were measured and the dialysates were collected, 8-OH-DPAT (10 mM) was microdialized for 30 min during three 10-min collection periods. The muscle contraction was then repeated as described, and the dialysate was collected for 10 min. Artificial CSF was dialyzed through the probes for an additional 60 min with six 10-min collection periods to determine whether 5-HT recovers from precontraction levels. The dialysate samples were stored at −80°C, and 5-HT levels were measured by HPLC-EC (see Biochemical Assay of 5-HT). Tibilal nerve stimulation was conducted using prior stimulation parameters after muscle paralysis with pancuronium, and the dialysate was collected for 10 min. The dose of 8-OH-DPAT used in this study was 10 mM, because a recent dose-response experiment (2) determined this to be the effective dose.

Effect of a 5-HT1A-receptor antagonist (NAN-190) microdialized into rVLM. The effects of the 5-HT1A-receptor antagonist NAN-190 on cardiovascular responses and changes in extracellular 5-HT concentration during muscle contraction were investigated by dialyzing the drug into the rVLM (n = 5). The effects on 5-HT, MAP, and HR during a static muscle contraction after a subsequent administration of 8-OH-DPAT into the rVLM were then determined.

In this protocol, nine 10-min control collections were performed for 90 min. A 2-min muscle contraction was then elicited during another 10-min collection period. The rat was allowed to recover for 60 min (six 10-min collections). After control cardiovascular responses were measured and the dialysates were collected, NAN-190 (10 mM) was microdialized for 30 min during three 10-min collection periods. The muscle contraction was then repeated as described, and the dialysate was collected for 10 min. Thereafter, 8-OH-DPAT (10 mM) was administered into the rVLM for 30 additional min and a contraction was repeated. The dialysate samples were stored at −80°C, and 5-HT levels were measured by HPLC-EC (see Biochemical Assay of 5-HT). Tibilal nerve stimulation was conducted using prior stimulation parameters after muscle paralysis with pancuronium, and the dialysate was collected for 10 min. The dose of NAN-190 was 10 mM, because a previous study (2) determined this dose to be effective in blocking the attenuating effects on cardiovascular responses during muscle contraction after a subsequent administration of 8-OH-DPAT into the rVLM.

Biochemical Assay of 5-HT

Analysis of 5-HT was done using HPLC-EC. A reversed phase C18 3-µm column (Rainin Instruments, Woburn, MA) was used with a mobile phase consisting of 75 mM monobasic sodium phosphate, 1.4 mM sodium octanyl sulfonate, 100 µM EDTA, and 12% acetonitrile brought to pH 5.6 with NaOH. Analysis of the eluent was by electrochemical detection (ESA Coulochem II, Bedford, MA). The area under the curve was compared with standards of 5-HT (Sigma Chemical) injected onto the column at the beginning of each run. Standards were prepared daily from a 10−6 M stock standard that was stored at −80°C. Serial dilutions of 10−6, 5 × 10−7, 10−7, and 10−8 M were analyzed to establish a standard curve. The use of Justice Innovations ChromPerfect (Palo Alto, CA) software allowed determination of regression for the standard. A minimum correlation coefficient of 0.95 was used for all standard curves. Routinely, voltammograms were generated to verify retention times of standards and to maximize the sensitivity of the system for 5-HT. Voltammograms of samples were also done to compare the retention time and oxidation-reduction ratios with authentic standards.

Histology

Methylene blue (10 mM) was dialyzed for 30 min at 1 µl/min through the microdialysis probes at the completion of every experiment. The animal was perfused transcardinally with 0.9% saline and then with 10% phosphate-buffered Formalin. The medulla was removed, fixed in 10% phosphate-buffered Formalin, and then stored at −4°C. The locations of microdialysis probes were later determined by mounting the medulla on the stage of a model Pelco 101 vibratome (Ted Pella, Redding, CA), taking 50-µm transverse sections, and examining under a microscope (Zeiss). The spread of the dye rostrocaudally as well as laterally was measured to compare from one animal to another. Only animals in which the probes were centered at the target sites (cVLM or rVLM) were included in the final data analyses.

Statistical Analyses

All data are expressed as means ± SE. Normality of the data was tested so that appropriate parametric and/or nonparametric statistics could be performed. Baseline and peak values of MAP, HR, tension, and percent 5-HT release elicited by two repeated muscle contractions were analyzed using a one-way analysis of variance (ANOVA) with repeated measures. Baseline value was the average of the 2-min period before a manipulation. Peak changes in MAP, HR, developed tension, and 5-HT were defined as the maximum values obtained during the contraction periods. The one-way repeated-measures ANOVA was also used to compare the hemodynamic, tension, and 5-HT data before and after administration of 8-OH-DPAT. Also, the one-way repeated-measures ANOVA was used to compare MAP, HR, tension, and 5-HT data before and after administration of NAN-190 and after administration of 8-OH-DPAT. Post hoc analyses for the ANOVAs were performed using Student-Newman-Keuls tests, and for all statistical evaluations P < 0.05 was considered significant.

RESULTS

Confirmation of Proper Probe Placements and Functional Integrity

Bilateral insertion of the microdialysis probes into either the rVLM or the cVLM had no effects on changes in arterial pressure, MAP, HR, and developed tension generated by contraction of the triceps surae muscle in response to tibial nerve stimulation. In all rats (n = 20), MAP, HR, and tension rose by 28 ± 3 mmHg, 28 ± 4 beats/min, and 410 ± 23 g, respectively, before insertion of the probes. After the probes were placed, a

Table 2. Protocol for microdialysis of 5-HT1A-receptor agonist 8-OH-DPAT into rVLM

<table>
<thead>
<tr>
<th>Collection Time</th>
<th>Protocol</th>
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<tbody>
<tr>
<td>10-min collection for 90 min</td>
<td>After insertion of probes</td>
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<tr>
<td>10-min collection</td>
<td>First contraction</td>
</tr>
<tr>
<td>10-min collection for 60 min</td>
<td>Postcontraction</td>
</tr>
<tr>
<td>10-min collection for 30 min</td>
<td>Administration of 8-OH-DPAT</td>
</tr>
<tr>
<td>10-min collection</td>
<td>Second contraction</td>
</tr>
<tr>
<td>10-min collection for 60 min</td>
<td>Postcontraction</td>
</tr>
<tr>
<td>Paralysis</td>
<td>Third stimulation</td>
</tr>
</tbody>
</table>

Acknowledgment: This research was supported by a training grant from the National Institute of Health (HL07327).
similar contraction increased MAP, HR, and tension by $27 \pm 3$ mmHg, $26 \pm 4$ beats/min, and $423 \pm 29$ g, respectively. If the probes were within the rVLM or the cVLM, L-glutamate (1 nM) administration elicited pressor or depressor responses, respectively. Microdialysis of L-glutamate into the rVLM revealed a significant increase in MAP of $40 \pm 4$ mmHg within 1 min, and such administration into the cVLM produced a significant depressor response of $-43 \pm 5$ mmHg. Postexperimental methylene blue dialysis followed by histological analysis confirmed successful implantation of probes in targeted areas.

Release of 5-HT During Muscle Contraction

For the experiments with the rVLM, after a 90-min collection period to achieve stable basal values of extracellular 5-HT, a tibial nerve stimulation-evoked static muscle contraction was performed. Basal levels of 5-HT were achieved at the second collection period, i.e., after a 20-min stabilization period. The novel finding was that extracellular fluid concentrations of 5-HT in the rVLM significantly increased from $0.7 \pm 0.3$ to $1.2 \pm 0.5$ fmol/20 µl dialysate (an increase of $65 \pm 9\%$, $P < 0.05$) following muscle contraction and paralleling a pressor response, as shown in Fig. 1. MAP and HR also increased by $27 \pm 4$ mmHg and $29 \pm 4$ beats/min, respectively, in response to a developed tension of $433 \pm 30$ g during the contraction ($n = 8$; Fig. 2). Percent change of extracellular 5-HT in the rVLM and cardiovascular changes during a second contraction were consistent with the first contraction in all rats (Figs. 1 and 2). During the second contraction, MAP, HR, and developed tension increased by $25 \pm 5$ mmHg, $27 \pm 5$ beats/min, and $423 \pm 32$ g, respectively. The 5-HT level returned to baseline value within 20 min after each contraction. Stimulation of the tibial nerve after neuromuscular blockade resulted in constant HR and MAP and a baseline extracellular level of 5-HT (Fig. 1). Hemodynamic, tension, and 5-HT-level data are summarized in Table 3.

For the protocol in which dialysate samples were collected from the cVLM, two repeated muscle contractions revealed no significant changes among baseline, contraction, and postcontraction values in extracellular fluid concentration of 5-HT (Fig. 3). However, during
the two contractions, MAP, HR, and tension increased similarly (Fig. 4). Basal and peak hemodynamic, tension, and 5-HT data are summarized in Table 3.

Effect of 5-HT<sub>1A</sub>-Receptor Activation on Extracellular 5-HT Levels in rVLM and Concomitant Cardiovascular Responses During Muscle Contraction

The effects of a 30-min perfusion with 10 mM 8-OH-DPAT on the rVLM concentrations of 5-HT before and after muscle contractions and after neuromuscular blockade are shown in Fig. 5. The dose (10 mM) and site of administration of this drug were selected on the basis of a recent study (2) that used a similar dose and site to affect cardiovascular responses during muscle contraction. Before 8-OH-DPAT was administered, MAP and HR increased by 26 ± 3 mmHg and 28 ± 4 beats/min, respectively, with a developed tension of 422 ± 19 g (n = 6; Fig. 6). After the drug was administered, increases were noted in MAP and HR during the contraction, but the elevations were significantly diminished (∆MAP = 13 ± 2 mmHg and ∆HR = 15 ± 3 beats/min; P < 0.05) (Fig. 6). Muscle tension developed to 429 ± 25 g and was similar to that before the drug.

Of novel importance was the percent change in extracellular fluid levels of 5-HT in the rVLM, which was significantly inhibited (−42 ± 6%; P < 0.05) during the muscle contraction after the 30-min perfusion of 8-OH-DPAT (Fig. 5). In addition, there was an ~10-fold increase in 5-HT baseline level after the 30-min 8-OH-DPAT microdialysis. Lastly, by paralyzing the animal, the percent change in extracellular 5-HT concentration, MAP, and HR did not deviate from baseline after a tibial nerve stimulation.

Effect of 5-HT<sub>1A</sub>-Receptor Antagonism on Extracellular 5-HT Levels in rVLM and Subsequent Cardiovascular Responses During Muscle Contraction Before and After 8-OH-DPAT Administration

The effects of a 30-min perfusion with 10 mM NAN-190 on the rVLM concentrations of 5-HT and on MAP and HR before and after muscle contractions and after neuromuscular blockade are shown in Table 4. Before NAN-190 was administered, muscle contraction increased MAP and HR by 24 ± 3 mmHg and 25 ± 4 beats/min, respectively, with a developed tension of 434 ± 25 g (n = 5). The extracellular concentration of 5-HT increased from 0.7 ± 0.2 to 1.5 ± 0.3 fmol/20 µl fluid (Δ5-HT = 0.8 ± 0.3 fmol/20 µl fluid) during a muscle contraction. After NAN-190 was administered for 30 min, MAP and HR increased by 22 ± 4 mmHg....

### Table 3. Hemodynamic, tension, and 5-HT levels during two 2-min tibial nerve stimulation-evoked muscle contractions before and after neuromuscular blockade in experiments in which microdialysis probes were placed into either rVLM or cVLM

<table>
<thead>
<tr>
<th></th>
<th>1st Stimulation</th>
<th>2nd Stimulation</th>
<th>3rd Stimulation (After Paralysis)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Peak</td>
<td>Control</td>
</tr>
<tr>
<td>rVLM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>114 ± 5</td>
<td>141 ± 4*</td>
<td>110 ± 4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>375 ± 7</td>
<td>399 ± 7*</td>
<td>367 ± 8</td>
</tr>
<tr>
<td>Tension, g</td>
<td>10 ± 4</td>
<td>443 ± 30*</td>
<td>9 ± 4</td>
</tr>
<tr>
<td>5-HT, fmol/20 µl</td>
<td>0.7 ± 0.3</td>
<td>1.6 ± 0.5*</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>cVLM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>108 ± 5</td>
<td>139 ± 4*</td>
<td>113 ± 4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>360 ± 9</td>
<td>390 ± 6*</td>
<td>369 ± 5</td>
</tr>
<tr>
<td>Tension, g</td>
<td>10 ± 4</td>
<td>410 ± 24*</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>5-HT, fmol/20 µl</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.7 ± 0.2</td>
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</table>

Values are means ± SE. MAP, mean arterial blood pressure; HR, heart rate. *Significantly different from corresponding control (P < 0.05).

Fig. 3. Percent extracellular fluid serotonin concentration sampled from caudal ventrolateral medulla (cVLM) during two 2-min tibial nerve stimulation-evoked static muscle contractions repeated after 60 min and during a tibial nerve stimulation following muscle paralysis. Values are means ± SE (n = 6). Control values are levels before respective contractions.
and 28 ± 5 beats/min, respectively, in response to another muscle contraction. In addition, microdialysis of NAN-190 into the rVLM resulted in no significant change in extracellular 5-HT concentration during the muscle contraction (Table 4). Developed muscle tension was 440 ± 29 g and was similar to that before the drug.

Furthermore, prior administration of NAN-190 blocked the attenuating effects on extracellular 5-HT level and cardiovascular responses after subsequent 8-OH-DPAT perfusion into the rVLM ($\Delta$MAP = 25 ± 5 mmHg, $\Delta$HR = 26 ± 5 beats/min, $\Delta$5-HT = 0.8 ± 0.3 fmoles/20 µl fluid, and developed tension = 430 ± 30 g) (Table 4).
information to the rVLM 5-HT1A-mediated system in-
finding adds significant conceptual and mechanistic
decrease in extracellular concentrations of 5-HT. This
HR in response to muscle contraction mediated via a
nist into the rVLM attenuates increases in MAP and
contractions before and after administration of 8-OH-DPAT into rVLM followed by neuromuscular

Histology and Diffusion of Methylene Blue

Histological sections of the medullary region showed that the membranes of the probes were within the
rVLM and the cVLM as described by the rat brain atlas
of Paxinos and Watson (33). Also, after histological
sections were visualized, the spread of methylene blue
dye for 30 min in each region was measured to be ~600
µm (Fig. 7), both rostrocaudally and laterally. This
diffusion was similar in all sections, and the distribu-
tion pattern corresponds to a recent study (2) that used
similar techniques.

DISCUSSION

This study is the first to demonstrate that an in-
crease in extracellular 5-HT concentration within the
rVLM is associated with cardiovascular responses during
static muscle contraction. Furthermore, our results have determined that administration of a 5-HT1A ago-
nist into the rVLM attenuates increases in MAP and
HR in response to muscle contraction mediated via a
decline in extracellular concentrations of 5-HT. This
finding adds significant conceptual and mechanistic
information to the rVLM 5-HT1A-mediated system in-
volved in static exercise. This research also showed a
lack of 5-HT change within the cVLM during muscle
contraction, implying that changes in 5-HT in the
cVLM have no possible role in the exercise pressor
reflex. The cardiovascular and neurotransmitter re-
sponses were due to contraction-evoked activation of
muscle afferents, because stimulation of the tibial
nerve after neuromuscular blockade evoked no changes
in MAP, HR, or 5-HT concentration.

The VLM is a functionally identified area within the
reticular formation of the medulla oblongata and has
been implicated in various autonomic regulatory roles
(8, 12). Furthermore, the rostral and caudal portions of
the VLM, i.e., the rVLM and the cVLM, respectively,
have been shown to play opposing roles in regulating
and integrating cardiovascular adjustments resulting
from local or peripheral stimulation (17, 42). The rVLM
is critical in central regulation of sympathetic nerve
discharge because it projects directly to preganglionic
neurons in the intermediolateral cell column of the
spinal cord (3, 17). In contrast, the cVLM neurons send
inhibitory projections to the rVLM neurons that appear
to tonically inhibit rVLM-neuronal activity (17, 43).
Both the rVLM and cVLM have been demonstrated to
be involved in integration of blood pressure and HR
responses during static muscle contraction. For ex-
ample, lesioning the rVLM (5) or locally administering
kynurenic acid, an excitatory amino acid antagonist (4),
abolishes the pressor response during muscle contrac-
tion. In addition, radioactive glucose (22) and c-Fos
expression (24) studies have identified both the rVLM
and cVLM as important areas active during the exer-
cise pressor reflex. Thus the role of the rVLM and
cVLM in integrating cardiovascular responses during
static exercise has become a focus of sustained interest.

Histofluorescence and neuroanatomic studies have
described the existence of several neurotransmitters in
the VLM, including catecholamines and other neuropep-
tides, contributing to regulation of cardiovascular func-
tions (for reviews, see Refs. 8 and 35). In addition,
clustered groups of 5-HT cell bodies/neurons have been
identified in the VLM of rats (6, 20, 36). The term “B1/3
cell group” for the 5-HT-containing neurons located
within the rVLM was introduced by Jacobs et al. (23).
Furthermore, the role of 5-HT in the VLM in evoking
centrally mediated cardiovascular effects has been well
documented by numerous investigators (10, 15, 19, 27,
28). However, a recent review of the involvement of
5-HT in the central regulation of cardiovascular homeo-
stasis notes that "findings regarding release of endog-
enous 5-HT itself in the VLM are scarce" (35). Also,
there is no literature with respect to release of 5-HT in
the extracellular space within either the rVLM or the
cVLM. The present study is the first determination of
such release of 5-HT from both the rVLM and cVLM using microdialysis techniques that allow a site-specific approach to the measurement of release of neurotransmitter(s). We have demonstrated that extracellular 5-HT in the rVLM increases during static muscle contraction, whereas 5-HT levels in the cVLM do not change during muscle manipulation. These results suggest that release of 5-HT in the rVLM appears to play a role in mediating cardiovascular responses during static exercise. It is noteworthy that plasma levels of 5-HT have been shown to increase during exercise in humans (31). Static muscle contraction increased 5-HT by ~75% (0.9 fmol). Although this femtomolar concentration seems quite small, it may represent a functionally important increase. A change of 0.9 fmol represents $15 \times 10^6$ molecules of 5-HT. Depending on the number of synapses that the probe sampled, this change may have a physiological significance on the neurochemistry of rVLM regulation during cardiovascular responses elicited by muscle contraction. The relation between this release of 5-HT and a cardiovascular response is shown in the present study. However, the potential functional implication of the $15 \times 10^6$ molecules of 5-HT increase cannot be fully explained by our study.

The VLM neurons contain a high density of 5-HT binding sites, particularly the 5-HT$_{1A}$ subtype (38). These 5-HT$_{1A}$ receptors are present in the C1 area of the rVLM, the raphe pallidus, the parapyramidal region, the ventromedial medulla, and scattered locations throughout the VLM (38, 39). These receptors play a role in mediating cardiovascular effects, because local or iontophoretic application of the 5-HT$_{1A}$-receptor agonist 8-OH-DPAT in the rVLM inhibits neuronal activity of the rVLM and elicits a decrease in MAP and HR by reducing efferent sympathetic outflow (19, 41). Furthermore, activation of 5-HT$_{1A}$ receptors in the rVLM, but not in the cVLM, results in attenuation of cardiovascular responses evoked during static muscle contraction in anesthetized rats (2), which is confirmed by the present study. In addition, the present study measures changes in extracellular fluid 5-HT levels during muscle contraction before and after administration of 8-OH-DPAT and supports the concept put forward by Ally and colleagues (2) that 5-HT$_{1A}$-receptor activation attenuates MAP and HR changes during muscle contraction via an inhibition of presynaptic 5-HT release. Presumably, 8-OH-DPAT is selective for 5-HT$_{1A}$ receptors, because previous studies have shown that prior administration of the 5-HT$_{1A}$-receptor antagonist NAN-190 blocked cardiovascular effects of the drug (2, 14, 15). Furthermore, because prior administration of NAN-190 blocked the attenuating effects of 8-OH-DPAT on 5-HT, MAP, and HR observed in the experiments in which 8-OH-DPAT was microdialyzed alone, it can be suggested that the selectivity for 5-HT$_{1A}$ receptors was functionally specific. However, the 2 mM dose of 8-OH-DPAT (final extracellular concentration of the drug as determined by in vitro recovery experiments; see below) may affect other receptors besides 5-HT$_{1A}$, including 5-HT$_{1B}$, 5-HT$_{2}$, and $\alpha_2$-adrenergic receptors (28, 30). Therefore, we cannot exclude the possibility that 8-OH-DPAT attenuated the cardiovascular responses and 5-HT concentration during muscle contraction via these other receptors. Along the same line, Adell et al. (1) suggested that 10 mM 8-OH-DPAT, administered into the raphe nucleus, may produce nonspecific effects. Similarly, it has been shown that a 2 mM dose of NAN-190 binds with 5-HT$_{1B}$, 5-HT$_{2}$, and $\alpha_2$-adrenergic receptors in addition to 5-HT$_{1A}$ receptors (16). However, Nóbrega et al. (32) demonstrated that the specific 5-HT$_{1B}$-receptor agonist 1-[3-(trifluoromethyl)phenyl]piperazine failed to modify cardiovascular responses to muscle contraction, suggesting that 5-HT$_{1B}$ receptors have no possible role in regulating blood pressure and HR responses during static contraction. In addition, it has been shown that the 5-HT$_{2}$-receptor antagonist ketanserin and the $\alpha_2$-adrenoceptor blocker idazoxan failed to inhibit cardiovascular effects of 8-OH-DPAT, thereby suggesting that 8-OH-DPAT effects are not

Fig. 7. Schematic diagram of a transverse section of medulla showing tract made by a microdialysis probe (MP) inserted into rVLM. An estimate of approximate lateral diffusion of methylene blue (MB; ~600 µm) is shown by transverse lines. A similar spread was seen in rostrocaudal plane and experiments with cVLM. MLF, medial longitudinal fasciculus; NTS, nucleus tractus solitarii; STN, spinal trigeminal nucleus.
mediated via 5-HT\textsubscript{2} or \alpha\textsubscript{2}-adrenergic receptors (14, 28). Nevertheless, the present study using the 2 mM concentration of 8-OH-DPAT and NAN-190 cannot clearly rule out the possibility of an effect of the drugs on other receptors besides 5-HT\textsubscript{1A}. Our findings of increased 5-HT release and successful pharmacological manipulation with a 5-HT\textsubscript{1A} agonist add to the scheme of central cardiovascular regulation via neurotransmitters and provide additional insights into the neural control of blood pressure during static exercise.

Microdialysis of 8-OH-DPAT into the rVLM resulted in a 10-fold increase in basal extracellular 5-HT levels. During the contraction period, 8-OH-DPAT was continuously dialyzed for 10 min. This procedure strengthens our finding that, despite a 10-fold increase in 5-HT while 8-OH-DPAT was administered during rest, muscle contraction indeed attenuated 5-HT release in the rVLM, further suggesting that 8-OH-DPAT attenuates cardiovascular responses during muscle contraction via a decrease in extracellular 5-HT concentration. However, because prior administration of NAN-190 blocked the 10-fold increase in 5-HT after administration of 8-OH-DPAT during rest and inhibited the increase in 5-HT during muscle contraction, it can be assumed that 8-OH-DPAT mediated its effects via 5-HT release. Using in vivo brain microdialysis, studies have measured the somatodendritic release of 5-HT in the raphé nuclei of the rat (1, 14). Perfusion of 1 µM 8-OH-DPAT into the raphe did not produce any significant effect on 5-HT release; however, at doses of 10 mM and higher, 8-OH-DPAT dose-dependently increased dialysate 5-HT within the raphe (1). That study (1) suggests that in vivo 5-HT release does not depend on nerve impulses of serotonergic neurons and that somatodendritic release of 5-HT is independent of local autoreceptor activation. Furthermore, Adell and co-workers (1) have also postulated that the increase in extracellular 5-HT concentration after local dialysis of 8-OH-DPAT is possibly due to a blockade of the 5-HT transport, because the drug inhibits the 5-HT reuptake process. Because our results revealed an increased baseline level of 5-HT in the rVLM after microdialysis of 10 mM 8-OH-DPAT, it is possible that a serotonergic mechanism similar to the raphe is present in the rVLM. However, this study cannot clearly explain potential mechanisms by which 8-OH-DPAT decreases 5-HT levels to less than control levels during muscle contraction but not during rest. To the best of our knowledge, this study is the first to present this data on the basis of measurement of 5-HT in the rVLM. However, it has been shown that 8-OH-DPAT administration into the ventromedial medulla (a region \textsim 1 mm medial to the rVLM) decreases extracellular concentrations of 5-HT in the ventromedial medulla, and it has been suggested that this decrease in 5-HT may play a role in modulation of nociception via 5-HT\textsubscript{1A}-receptor activation (37). It may be possible that a mechanism similar to that in the ventromedial medulla exists in the rVLM. This may also explain the decrease in 5-HT during muscle contraction after perfusion of 8-OH-DPAT. However, other potential mechanisms for the action of 8-OH-DPAT exist in addition to the effects on 5-HT release. For example, a recent study has shown that 8-OH-DPAT induces release of norepinephrine in the hippocampal formation using in vivo methods, suggesting a noradrenergic-serotonergic mechanism (18). Furthermore, 8-OH-DPAT has also been shown to facilitate acetylcholine release in the rat frontal cortex (9). These effects of 8-OH-DPAT raise the possibility of other potential mechanisms for its action. However, the exact mechanism of the increased 5-HT level in the rVLM after administration of 8-OH-DPAT cannot be explained by the present study. Doses of 8-OH-DPAT <10 mM have shown no attenuating effects on cardiovascular responses during muscle contraction (2). Nevertheless, it is possible that the increased baseline 5-HT level after microdialysis of 8-OH-DPAT might have contributed to the attenuation of 5-HT levels following muscle contraction. However, this seems unlikely given the fact that a negative percent change in 5-HT concentration occurred during the contraction following the drug, strongly suggesting that the higher baseline may not have been the causative factor.

The 10-fold increase in basal 5-HT following 8-OH-DPAT (10 mM) administration did not elicit a shift in baseline blood pressure or HR. The concentration of 5-HT in the rVLM after 8-OH-DPAT perfusion was increased to 7 fmol/20 µl of extracellular fluid. Thus far, no microdialysis studies have been done in which 5-HT is administered into the rVLM along with the recording of concomitant changes in blood pressure. However, intracerebroventricular administration or local application of 5-HT into the nucleus tractus solitarii has been performed in previous studies. For example, in one study (7) in which 5-HT was locally injected into the nucleus tractus solitarii, the dose of 5-HT was in the picomolar range to elicit a change in blood pressure. That picomolar dose (7) is higher than the femtomolar concentration of 5-HT measured following 8-OH-DPAT administration in our experiments, and hence it appears that the concentration of 5-HT in the femtomolar range is not sufficient to evoke a change in blood pressure. In another study (13) using intracerebroventricular administration of 5-HT, a dose of 100 nmol was shown to evoke an increase in blood pressure, a concentration much higher than that measured in the rVLM after 8-OH-DPAT perfusion in our study. Furthermore, the prolonged administration and the low rate of perfusion of 8-OH-DPAT also attribute to the lack of effect on baseline cardiovascular parameters. In addition, on the basis of in vitro recovery studies, it has been determined that \textsim 20% of 8-OH-DPAT crossed the microdialysis membrane, a concentration much less than that required to evoke a change in blood pressure as shown in a previous study (25). In our experiments, 5-HT was sampled site-specifically by the microdialysis probes from the rVLM or cVLM. We determined correct placement of the probes within the rVLM or cVLM by perfusing L-glutamate and observing a pressor or depressor response, respectively. Furthermore, analyses of the diffusion of methylene blue dye, microdialyzed for the same duration and at the same rate as the drug,
showed a spread of ~600 μm, suggesting that the dialysate was collected from within the respective area. Obviously, the possibility of collecting 5-HT from distant areas and a potentially wider diffusion of 8-OH-DPAT to structures other than the rVLM exists. However, our results demonstrated an increase in 5-HT during muscle contraction measured from the rVLM and no change when collected from the cVLM. The rVLM and cVLM in the rat are separated by ~1.5 mm. If 5-HT was sampled from an area that was 1.5 mm away or farther, then analyses of 5-HT collected from the cVLM region would have shown an increase during muscle contraction. Therefore, our experimental method proves that the sampling site was specific. Furthermore, Ally and co-workers (2) have shown that administration of 10 mM 8-OH-DPAT into the cVLM did not attenuate the cardiovascular responses during muscle contraction. In our study, the drug and dose used were the same as those used by Ally et al. (2), and dye diffusion studies indicate that 8-OH-DPAT did not spread to an area that is 1.5 mm away. This suggests that the drug possibly diffused similar to the dye and that 5-HT was sampled from within the rVLM or the cVLM.

In conclusion, the present study demonstrated that an increase in extracellular fluid concentration of 5-HT in the rVLM, but not in the cVLM, is associated with cardiovascular responses elicited during static muscle contraction. Furthermore, activation of 5-HT1A receptors within the rVLM inhibits increases in MAP and HR during muscle contraction mediated via a reduction in extracellular concentration of 5-HT. The present study provides conceptual information regarding the mechanism of 5-HT1A modulation of the exercise pressor reflex and sheds further light on the neurochemical basis of circulatory adjustments during static exercise.

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