Extracellular serotonin changes in VLM during muscle contraction: effects of 5-HT1A-receptor activation

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Asmundsson, Gudbjorn, Daryl Caringi, David J. Mokler, Toshio Kobayashi, Takeshi Ishide, and Ahmmed Ally. Extracellular serotonin changes in VLM during muscle contraction: effects of 5-HT1A-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 $\pm 4$ mmHg ($n = 8$). In addition, 5-HT levels in the rVLM were elevated by $65 \pm 9\%$ 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-HT1A 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-HT1A 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-HT1A-receptor activation in the rVLM blunts 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; rats

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, 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-HT1A agonists demonstrate 5-HT1A-mediated cardiovascular effects through the rVLM, including the raphe pallidus and the C1 region of the rVLM (14, 15). Stimulation of 5-HT1A 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-HT1A 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-HT1A-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)tetratin (8-OH-DPAT; RBI, Natick, MA), a 5-HT1A-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-HT1A-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 P231D; 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, inflating 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 SIU5C, 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 mosmol/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 mM 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 biochemical 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-HT1A-receptor agonist (8-OH-DPAT) microdialyzed into rVLM. Six rats were used to determine the effects of the 5-HT1A-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>Third stimulation</td>
</tr>
<tr>
<td>10-min collection</td>
<td>5-HT, serotonin; rVLM and cVLM, rostral and caudal ventrolateral medulla, respectively.</td>
</tr>
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</table>
agonist 8-OH-DPAT into rVLM.

Effective dose. The 8-OH-DPAT used in this study was 10 mM, because a recent study 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. The dose of 8-OH-DPAT used in this study 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 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 −20°C. Dilutions of 10^−6, 5 × 10^−6, 10^−5, and 10^−4 M were analyzed to establish a standard curve. The use of Justice Innovations ChromPerfect (Calo 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 transcardially 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 diffusion of the drug. 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 Placement 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 mm Hg, 28 ± 4 beats/min, and 410 ± 23 g, respectively, before insertion of the probes. After the probes were placed, a

<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 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>

Table 2. Protocol for microdialysis of 5-HT1A-receptor antagonist 8-OH-DPAT into rVLM.
similar contraction increased MAP, HR, and tension by
27 ± 3 mmHg, 26 ± 4 beats/min, and 423 ± 29 g,
respectively. If the probes were within the rVLM or the
cVLM, L-glutamate (1 nM) administration elicited pres-
sor or depressor responses, respectively. Microdialysis
of L-glutamate into the rVLM revealed a significant
increase in MAP of 40 ± 4 mmHg within 1 min, and
such administration into the cVLM produced a signifi-
cant depressor response of −43 ± 5 mmHg. Postexperi-
mental 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 ±
0.3 to 1.2 ± 0.5 fmol/20 µl dialysate (an increase of 65 ±
9%; \( P < 0.05 \)) following muscle contraction and parallel-
ing a pressor response, as shown in Fig. 1. MAP and HR
also increased by 27 ± 4 mmHg and 29 ± 4 beats/min,
respectively, in response to a developed tension of 433 ±
30 g during the contraction (\( n = 8; \) Fig. 2). Percent
change of extracellular 5-HT in the rVLM and cardiovas-
cular changes during a second contraction were consis-
tent with the first contraction in all rats (Figs. 1 and 2).
During the second contraction, MAP, HR, and devel-
oped tension increased by 25 ± 5 mmHg, 27 ± 5
beats/min, and 423 ± 32 g, respectively. The 5-HT level
returned to baseline value within 20 min after each
contraction. Stimulation of the tibial nerve after neuro-
muscular blockade resulted in constant HR and MAP
and a baseline extracellular level of 5-HT (Fig. 1).

For the protocol in which dialysate samples were
collected from the cVLM, two repeated muscle contrac-
tions revealed no significant changes among baseline,
contraction, and postcontraction values in extracellular
fluid concentration of 5-HT (Fig. 3). However, during

Fig. 1. Percent extracellular fluid serotonin concen-
tration sampled from rostral ventrolateral
medulla (rVLM) during two 2-min tibial nerve
stimulation-evoked static muscle contractions re-
peated at an interval of 60 min and during a
nerve stimulation following neuromuscu-
lar blockade (muscle paralysis arrow). Values are
means ± SE (\( n = 8 \)). * \( P < 0.05 \) compared with
respective controls (values before each contrac-
tion).

Fig. 2. Average peak changes in mean arterial pressure (MAP), heart
rate (HR), and developed tension during two 2-min stimulations of
tibial nerve repeated at an interval of 60 min in experiments in which
microdialysis probes were placed within rVLM. Values are means ±
SE (\( n = 8 \)).
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>
</tr>
</thead>
<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>
</tr>
</tbody>
</table>

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

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 inhibit 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 muscle 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.

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 (∆5-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...
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 (ΔMAP = 25 ± 5 mmHg, ΔHR = 26 ± 5 beats/min, Δ5-HT = 0.8 ± 0.3 fmol/20 µl fluid, and developed tension = 430 ± 30 g) (Table 4).

Fig. 4. Average peak changes in MAP, HR, and developed tension during two 2-min stimulations of tibial nerve at an interval of 60 min in experiments in which microdialysis probes were inserted into cVLM. Values are means ± SE (n = 6).

Fig. 5. Percent extracellular fluid serotonin concentration sampled from rVLM during 2-min tibial nerve stimulation-evoked static muscle contractions before and 30 min after microdialysis of 10 mM 8-OH-DPAT and during a tibial nerve stimulation following muscle paralysis. Values are means ± SE (n = 6). *P < 0.05 compared with respective controls (values before each contraction).

Fig. 6. Average peak changes in MAP, HR, and developed tension during a 2-min tibial nerve stimulation-evoked static muscle contraction before (control) and after 30 min of microdialysis of 10 mM 8-OH-DPAT into rVLM. Values are means ± SE (n = 6). *P < 0.05 compared with control.
Table 4. Hemodynamic, tension, and 5-HT levels during 2-min tibial nerve stimulation-evoked muscle contractions before and after administration of 8-OH-DPAT into rVLM followed by neuromuscular blockade and during muscle contractions before and after microdialysis of NAN-190 and after perfusion of 8-OH-DPAT into rVLM

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>After 8-OH-DPAT</th>
<th>After Paralysis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Baseline Peak</td>
<td>Baseline Peak</td>
<td>Baseline Peak</td>
</tr>
<tr>
<td>rVLM</td>
<td>n</td>
<td>After Nan-190</td>
<td>After 8-OH-DPAT</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>6</td>
<td>110 ± 4</td>
<td>136 ± 4*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>6</td>
<td>365 ± 6</td>
<td>393 ± 7*</td>
</tr>
<tr>
<td>Tension, g</td>
<td>6</td>
<td>10 ± 4</td>
<td>432 ± 20*</td>
</tr>
<tr>
<td>5-HT, fmo/20 µl</td>
<td>6</td>
<td>0.7 ± 0.3</td>
<td>1.5 ± 0.3*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Baseline Peak</th>
<th>After 8-OH-DPAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>rVLM</td>
<td>5</td>
<td>112 ± 4</td>
<td>136 ± 5*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>5</td>
<td>370 ± 6</td>
<td>395 ± 7*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>5</td>
<td>10 ± 4</td>
<td>444 ± 25*</td>
</tr>
<tr>
<td>Tension, g</td>
<td>5</td>
<td>0.7 ± 0.2</td>
<td>1.5 ± 0.3*</td>
</tr>
<tr>
<td>5-HT, fmo/20 µl</td>
<td>5</td>
<td>0.3*</td>
<td>0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. NAN-190, 1-[2-methoxyphenyl]-4-[4-(2-phthalimido)-butyl]piperazine. *Significantly different from corresponding baseline (P < 0.05).

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 distribution pattern corresponds to a recent study (2) that used similar techniques.

DISCUSSION

This study is the first to demonstrate that an increase 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 agonist into the rVLM attenuates increases in MAP and HR in response to muscle contraction mediated via a decrease in extracellular concentrations of 5-HT. This finding adds significant conceptual and mechanistic information to the rVLM 5-HT1A-mediated system involved 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 responses 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 example, lesioning the rVLM (5) or locally administering kynurenic acid, an excitatory amino acid antagonist (4), abolishes the pressor response during muscle contraction. In addition, radioactive glucose (22) and c-Fos expression (24) studies have identified both the rVLM and cVLM as important areas active during the exercise 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 various neurotransmitters in the VLM, including catecholamines and other neuropeptides, contributing to regulation of cardiovascular functions (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 homeostasis notes that “findings regarding release of endogenous 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 × 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 × 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_{1A}-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_{2A}-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...
mediated via 5-HT$_2$ or $\alpha_2$-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$_{1A}$. Our findings of increased 5-HT release and successful pharmacological manipulation with a 5-HT$_{1A}$ 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 contractions 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 raphé 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 raphé (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 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 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 was site specific. Furthermore, Ally and co-workers (2) have shown that administration of 10 mM 8-OH-DPAT into the cVLM did not attenuate the cardiovasculary 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 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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