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Role of serotonergic input to the ventrolateral medulla in expression of the 10-Hz sympathetic nerve rhythm

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Orer HS, Gebber GL, Barman SM. Role of serotonergic input to the ventrolateral medulla in expression of the 10-Hz sympathetic nerve rhythm. Am J Physiol Regul Integr Comp Physiol 294: R1435–R1444, 2008. First published March 12, 2008; doi:10.1152/ajpregu.00012.2008.—We studied the changes in inferior cardiac sympathetic nerve discharge (SND) produced by unilateral microinjections of 5-hydroxytryptamine (5-HT) receptor agonists and antagonists into the ventrolateral medulla (VLM) of urethane-anesthetized, baroreceptor-denervated cats. Microinjection of the 5-HT2 receptor agonist LY-53857 (10 mM) into either the rostral or caudal VLM significantly reduced (P ≤ 0.05) the 10-Hz rhythmic component of basal SND without affecting its lower-frequency, aperiodic component. The selective depression of 10-Hz power was accompanied by a statistically significant decrease in mean arterial pressure (MAP). Microinjection of LY-53857 into the VLM also attenuated the increase in 10-Hz power that followed tetanic stimulation of depressor sites in the caudal medullary raphe nuclei. Microinjection of the 5-HT2 receptor agonist 1-(2,5-dimethoxy-4-i odophenyl)-2-amino-propyl-amino-tetralin (8-OHDPAT; 10 mM), into either the rostral or caudal VLM also selectively attenuated 10-Hz SND and significantly reduced MAP. The reduction in 10-Hz SND produced by 8-OHDPAT was partially reversed by intravenous WAY-100635 (1 mg/kg), which selectively blocks 5-HT1A receptors. These results support the view that serotonergic inputs to the VLM play an important role in expression of the 10-Hz rhythm in SND.

5-HT1A receptors; 5-HT2 receptors; caudal medullary raphe; caudal ventrolateral medulla; mean arterial pressure; rostral ventrolateral medulla; sympathetic nerve discharge

SYMPATHETIC NERVES EXHIBIT 10-Hz rhythmic discharges in urethane-anesthetized or decerebrate cats (4–11, 14, 15, 20–24, 27, 33, 37–39). Linear coherence analysis has revealed that the 10-Hz discharges of sympathetic nerve pairs with different targets are much more strongly correlated than are their lower-frequency, aperiodic discharges (8, 9, 20–22, 38). Thus, the mechanism responsible for generation of the 10-Hz rhythm serves the purpose of coordinating the discharges of sympathetic nerves with targets such as the heart and the vasculature of the viscera and skeletal muscle. Moreover, the 10-Hz rhythm has been implicated in the formulation of differential patterns of spinal sympathetic outflow as occurs, for example, during the defense reaction (21, 22). The functional relevance of the 10-Hz rhythm is further indicated by the observation that its sudden appearance in sympathetic nerve discharge (SND) is accompanied by a rise in arterial pressure (6).

The view that the 10-Hz rhythm in SND of the cat is generated in the brain stem is supported by the following observations. First, the rhythm in SND is maintained after midcollicular decerebration, but not after high spinal transection (8, 14, 24, 38). Second, rhythmic medullary field potentials, correlated to 10-Hz SND in decerebrate cats, persist after 10-Hz SND is eliminated by spinal transection (20). Such field potentials have been recorded from the rostral ventrolateral medulla (VLM), caudal VLM, and caudal medullary raphe nuclei (10, 20). Moreover, single neurons with activity correlated to 10-Hz SND have been identified in each of these regions (4, 5, 10, 11, 15). Third, chemical inactivation of the rostral VLM, caudal VLM, or medullary raphe with microinjection of muscimol reduces or eliminates 10-Hz SND (10, 39).

Barman and Gebber (7) reported that unilateral microinjections of as little as 6.25 pmol of the selective GABA-A receptor antagonist SR-95531 into the VLM at any level between 1 and 5 mm rostral to the obex eliminated 10-Hz SND. This led Barman and Gebber (7) to propose that 1) GABAergic transmission in the VLM is critical for generation of the 10-Hz rhythm, and 2) neurons in caudal and rostral portions of the VLM act in combination to help generate the 10-Hz rhythm in SND.

The role of the medullary raphe in expression of 10-Hz SND is less clear. The present study was initiated with this in mind. Specifically, we tested the hypothesis that raphe serotonergic input to the VLM promotes the generation of the 10-Hz rhythm in SND. The rationale for this hypothesis is as follows. First, 20% of caudal medullary raphe neurons with activity correlated to 10-Hz SND have axons that project to and branch in the VLM (11). Second, 10-Hz SND is reduced by microinjection of 8-hydroxy-2-(di-n-propylamino)tetratin (8-OHDPAT) into the caudal medullary raphe (33). The action of this 5-hydroxytryptamine 1A (5-HT1A) receptor agonist in the
raphé may, in part, involve autoinhibition of the discharges of serotonergic neurons (1, 16, 17, 25, 36). Third, microinjection of N-methyl-d-aspartate into the caudal medullary raphé enhances the 10-Hz rhythm in SND (33). Thus, it is possible that raphé serotonergic input to the VLM may act to enhance 10-Hz SND. We tested this by recording changes in SND produced by microinjection of 5-HT2 and 5-HT1A receptor antagonists and agonists into the rostral and caudal portions of the VLM.

METHODS

General procedures. The protocols used in these studies on 25 adult cats (3.03 ± 0.48 kg) were approved by Michigan State University’s Institutional Animal Care and Use Committee. Cats were initially anesthetized with 2.5% isoflurane mixed with 100% O2. A femoral artery and the femoral veins were cannulated to measure arterial pressure and to administer drugs, respectively. Urethane (1.15 ± 0.16 g/kg iv) was then administered, and isoflurane inhalation was stopped. This dose of urethane has been shown to maintain a surgical level of anesthesia in cats for a period [8–10 h; (19)] exceeding the duration of our experiments.

Cats were placed in a stereotaxic apparatus, paralyzed (galamine triethiodide, 4 mg/kg iv, initial dose), pneumothoracotomized, and artificially respired with room air. Normocapnia (end-tidal CO2 near 4.6%) was maintained with the parameters of artificial ventilation at 40.2 ± 4.2 ml and 20.9 ± 3.2 cycles/min. Rectal temperature was kept near 38°C with a heat lamp. Before neuromuscular blockade, the adequacy of anesthesia was indicated by the absence of a palpebral reflex. When cats were paralyzed, an adequate level of anesthesia was indicated by the inability of noxious stimuli (pinch, heat, surgery) to increase arterial pressure or change the pattern of SND.

Baroreceptor denervation. The carotid sinus, aortic depressor, and cervical vagus nerves were sectioned bilaterally. Two observations verified the completeness of baroreceptor denervation in these experiments. First, there was not a sharp peak in the autospectrum of SND at the frequency of the heart beat, and the coherence value relating SND to the arterial pulse wave was <0.1 at this frequency. Second, SND was not reflexly inhibited during the pressor response produced by a bolus injection of norepinephrine bitartrate (1–2 µg/kg iv).

Neural recordings. As described in other reports from this laboratory (8, 20, 38), the inferior cardiac postganglionic branches of the left and/or right stellate ganglion were exposed retropleurally by removing the head of the first rib. Bilateral recordings were made in four cats. Potentials were recorded monophasically from the central ends of the cut nerves placed on platinum bipolar electrodes. The capacity-coupled preamplifier band pass was set at 1–1,000 Hz so that the synchronized discharges of sympathetic fibers appeared as slow waves (4, 5, 7, 24).

Microinjections. The dorsal surface of the brain stem was exposed by removing portions of the occipital bone and cerebellum. The midline, obex, and dorsal medullary surface were used as landmarks for placement of the micropipette into the VLM. A micropipette was positioned into either the caudal VLM (1.5 and 2.5 mm ahead of the obex) or the rostral VLM (4.5 and 5.5 mm ahead of the obex) in tracks located 3.8–4.2 mm lateral to the midline. Unilateral microinjections were made at two depths (~4.0 and 5.5 mm below the dorsal surface) in each track. Thus, a set of four injections was made in the caudal or rostral VLM on either the left or right side of the neuraxis. Injection sites were in the regions of VLM from which we have recorded from neurons with activity correlated to the 10-Hz rhythm in SND (4, 5, 10, 11). In 16 cats, only one set of injections was made into either the caudal or rostral VLM. In the other nine cats, a second set of injections was made after recovery of SND from the first set of injections.

All chemicals used for microinjection were diluted in phosphate-buffered saline (PBS). Solutions were adjusted to a pH of 6–8 (litmus paper test) and placed in a glass micropipette (~40-µm tip diameter) that was glued (cyanoacrylate) to the needle of a 5-µl Hamilton syringe and mounted on a microinjection unit (model 5000; David Kopf Instruments). A 50-nl injection was made slowly (~10 s) at each medullary site (see above) by turning the calibrated micrometer on the microinjection unit. The following drugs were injected: the 5-HT2 receptor antagonist LY-53857 (10 mM) or methysergide (10 mM), the 5-HT2 receptor agonist 1-(2,5-dim ethoxy-4-iodophenyl)-2-amino-propane (DOI; 10 µM), and the 5-HT1A receptor agonist 8-OHDPAT (10 mM). All drugs were purchased from RBI Sigma (St. Louis, MO). These drug concentrations are within the range used in past studies from this and other laboratories (16, 17, 32, 33). As a control, vehicle (PBS) was injected into the rostral or caudal VLM of three cats. Injections of PBS did not change SND or arterial pressure. Placement of the micropipette into the VLM itself did not change the power and frequency composition of SND in the 25 cats used in this study.

Intravenous injections. We administered DOI (1 mg/kg) or the 5-HT1A receptor antagonist WAY-100635 (1 mg/kg) intravenously in a total of seven experiments. DOI was dissolved in PBS, and WAY-100635 was dissolved in 5 mM sodium citrate.

Electrical stimulation of the medullary raphé. A bipolar stainless steel electrode (model SNE-100; Rhodes) with a 0.25-mm tip exposure separated by 0.75 mm was positioned into the caudal medullary raphé (on the midline, 0.5 to 3 mm ahead of the obex, and 2–3 mm below the dorsal surface). A Grass S8800 quartz-timed digital stimulator and PSI6 constant current unit were used to deliver 10-s trains of high-frequency (50 Hz), 1-ms square-wave pulses to depressor sites in this region. Stimulus intensity was 0.25–1 mA.

Data analysis. Data were acquired continuously with a Digidata digitizer (model 1322A; Axon Instruments; Union City, CA) using a sampling frequency of 200 Hz. Fast Fourier transform of basal SND was performed on 2-min data blocks (47 5-s windows of data with 50% overlap) that were collected just before, 3–5 min after drug injection, and later at ~30-min intervals until recovery of SND from the effects of the drug reached its maxima. Normalized autospectra of SND were displayed on a scale of 0 to 20 Hz with a bin resolution of 0.2 Hz. Essentially all of the power in SND is contained in the 0- to 20-Hz band when recordings are made with a preamplifier band pass centered at 1–1,000 Hz (8). Fast Fourier transform was also used to construct autospectra of SND from 30-s data blocks (26 5-s windows, 80% overlap) that were collected 5–35 s after a 10-s period of tetanic raphé stimulation. The frequency scale and bin resolution for these autospectra were the same as for the autospectra constructed using 2-min data blocks.

ASCII files of the autospectra of SND were saved for transfer to spreadsheet, graphics, and statistical programs (Prism version 5.00 for Windows and Instat, GraphPad Software, San Diego CA). The autospectra of SND constructed from data collected before and after microinjection or intravenous administration of a drug were displayed on the same power scale. The 10-Hz band of SND is defined as the range of frequencies surrounding the sharp peak in the autospectrum of SND near 10 Hz. A macro written in Microsoft Excel version 7.0 was used to measure 10-Hz power. Briefly, 10-Hz power was calculated as the area above a line that connected the left and right limits of the 10-Hz band in the autospectrum of SND. Low-frequency (~<5 Hz) power was calculated by arithmetically summing the values for the bins in the 0- to 5-Hz band. Both the 10-Hz rhythmic and lower-frequency, aperiodic components of inferior cardiac SND are eliminated by autonomic ganglionic blockade with hexamethonium chloride (5 mg/kg iv). Total power in SND refers to the arithmetic sum of the values for the bins in the 0- to 20-Hz frequency band.

Statistical analysis. Values in the text and figures are means ± SE. A paired t-test was used to evaluate the effects of microinjection of a drug on mean arterial pressure (MAP) and power in the 10-Hz and 0- to 5-Hz bands and total power in SND. An unpaired t-test was used to compare 1) the effects of microinjection of a drug on 10-Hz power in SND recorded ipsilateral and contralateral to the injection sites, and
2) the changes in 10-Hz activity produced by microinjection of a drug into the rostral and caudal VLM. \( P \leq 0.05 \) indicated statistical significance. Raw values of power were used for statistical analyses, but changes in SND are expressed as percent of control in the text and figures.

**Histology.** The brain stem was removed at the end of each experiment and fixed in 10% buffered Formalin. Frontal sections of 30-μm thickness were cut and stained with cresyl violet. Sites of microinjection were identified with reference to the bottom of the tracks made with the micropipette and the stereotaxic planes of Berman (13).

**RESULTS**

*Effects of LY-53857 in the VLM.* We studied the changes in basal (spontaneously occurring) SND produced by unilateral microinjections of the selective 5-HT2 receptor antagonist, LY-53857, into the rostral or caudal VLM of 13 cats. Figure 1 shows the results from an experiment in which we microinjected LY-53857 into the rostral VLM (left side), while recording the discharges of the left and right inferior cardiac nerves (ICN, rCN). The autospectra of the discharges of both nerves contained a large, sharp peak near 10 Hz (Fig. 1, A and B, solid black traces). The peak near 10 Hz in the autospectra of ICN activity (ipsilateral recording) was eliminated 3 min after microinjection of LY-53857 into the left rostral VLM (Fig. 1A, gray trace). In this case, the reduction in 10-Hz power in rCN activity (contralateral recording) was less pronounced (Fig. 1B, gray trace). In contrast to the dramatic reductions in 10-Hz power, the power in SND at frequencies \( \leq 5 \) Hz was increased in this experiment. Approximately 1 h after microinjection of the 5-HT2 receptor antagonist, the autospectra of SND again showed a prominent peak near 10 Hz (Fig. 1, A and B, dotted traces). In this case, the peak was at a slightly lower frequency (7.8 Hz) than during control (8.4 Hz).

Figure 2 summarizes the changes in basal SND and MAP produced by unilateral microinjections of LY-53857 into the rostral or caudal VLM. The effects of microinjection of LY-53857 into these two regions were similar. On the average, 10-Hz power in SND recorded ipsilateral to the injection sites was significantly reduced to \( \sim 25\% \) of control by microinjection of LY-53857 into either the rostral (Fig. 2A; \( P < 0.0001 \)) or caudal VLM (Fig. 2B; \( P = 0.0002 \)). In contrast, power at frequencies \( \leq 5 \) Hz in ipsilateral SND was not significantly changed. Thus, the statistically significant reduction (\( P < 0.0004 \)) in total power in ipsilateral SND reflected the decrease in 10-Hz power. Although there was a tendency for the reduction in 10-Hz SND recorded contralateral to the VLM injection sites to be less pronounced, the changes in 10-Hz activity recorded ipsilateral and contralateral to the injections were not significantly different. As shown in Fig. 2C, the selective decrease in 10-Hz SND was accompanied by a statistically significant fall in MAP when LY-53857 was microinjected into either the rostral (\( P = 0.0005 \)) or caudal (\( P = 0.0003 \)) VLM. MAP averaged \( 130 \pm 4 \) mmHg before microinjection of LY-53857 into the VLM.

Recovery of 10-Hz SND toward control level routinely began \( \sim 45 \) min after unilateral microinjection of LY-53857 into the VLM. From its nadir, 10-Hz power in SND significantly increased to \( 81 \pm 8\% \) of control (\( P < 0.0001 \)) within 60–120 min after microinjection of LY-53857. MAP recovered to a value (122 \( \pm 4 \) mmHg) not significantly different from control.

In 16 cases, a peak near 10 Hz was still evident in the autospectrum of SND after microinjection of LY-53857 into the rostral (\( n = 7 \)) or caudal VLM (\( n = 9 \)). The peak frequency (9.9 \( \pm 0.5 \) Hz) after microinjection of LY-53857 into the rostral VLM was not significantly different from that (9.7 \( \pm 0.4 \) Hz) before the injection. The peak frequency in the 10-Hz band of SND was also unchanged by microinjection of LY-53857 into the caudal VLM (10.7 \( \pm 0.3 \) Hz vs. 10.6 \( \pm 0.3 \) Hz).

The histological sections in Fig. 3 show the tracks made by a micropipette whose tip was in the caudal VLM (Fig. 3A) at \( \sim 1.5 \) and 2.5 mm ahead of the obex of one cat and in the rostral VLM (Fig. 3B) at \( \sim 4.5 \) and 5.5 mm ahead of the obex in another cat. The dots in the schematics on the right side of Fig. 3, A and B, show the most ventral of two injection sites of LY-53857 made in each track through the VLM of 13 cats. The second injection in each track was \( \sim 1.5 \) mm dorsal to the dots. Eight sets of injections were made in the rostral VLM, and nine sets of injections were made in the caudal VLM. A set of injections refers to two injections in each of two tracks through either the rostral or caudal medulla.
Intravenous administration of the selective 5-HT2 receptor agonist DOI (1 mg/kg) partially reversed the reduction in basal 10-Hz SND produced by unilateral microinjections of LY-53857 into either the rostral or caudal VLM. In these experiments, DOI was administered 10 min after microinjection of LY-53857. The results from one of these experiments is shown in Fig. 4. The autospectra are for SND recorded ipsilateral to the sites of microinjection of LY-53857. The solid black trace (Fig. 4A, gray trace) was partially reversed by the intravenous administration of DOI (dotted trace). At this time, the peak frequency in the 10-Hz band was increased from 9.0 to 9.6 Hz. The low-frequency, aperiodic component of SND was not affected by intravenous DOI. In these four cats, microinjection of LY-53857 into either the rostral or caudal VLM reduced 10-Hz power to 20 ± 3% of control, and intravenous DOI significantly increased 10-Hz power back to 84 ± 14% of control (P = 0.0331).

In three cats, microinjection of methysergide into the VLM produced changes in basal SND and MAP similar to those reported for LY-53857. Although less selective in its actions than LY-53857, methysergide does block 5-HT2 receptors (12). Microinjection of methysergide into the rostral VLM of two cats reduced 10-Hz power in SND (ipsilateral recording) to 15 and 42% of control without affecting power at frequencies ≤5 Hz. When injected into the caudal VLM in one cat, 10-Hz power was reduced to 24% of control ipsilateral to the injection sites, also without a change in low-frequency power. By combining the data from these three cats, we found that microinjection of methysergide into the VLM significantly reduced 10-Hz power in SND to 27 ± 8% of control (P = 0.0117) and significantly reduced MAP by 21 ± 7 mmHg (P = 0.0438). The time course for recovery of 10-Hz SND from the effects of methysergide was similar to that seen after microinjection of LY-53857 into the VLM.

Changes in SND following tetanic raphé stimulation. The 10-Hz component in SND was selectively enhanced for a period of 30–60 s following a 10-s train of high-frequency (50 Hz) stimuli applied to sympahtoinhibitory sites in the caudal medullary raphé nuclei. An example is shown in Fig. 5A and B. SND was completely inhibited during the 10-s period of tetanic raphé stimulation, and MAP was reduced from 143 to 95 mmHg (Fig. 5A). Within a few seconds after stimulation, SND increased to above control level and MAP began to recover. As shown in the autospectra of SND constructed from data collected before (Fig. 5B, black trace) and 5–35 s after raphé stimulation (Fig. 5B, gray trace), the increase in SND was attributable to enhanced power in the 10-Hz band.

Tetanic raphé stimulation significantly reduced MAP from 133 ± 2 to 96 ± 2 mmHg (P < 0.0001) and completely inhibited SND in 19 cats. Figure 5C summarizes the changes in SND that occurred 5–35 s after tetanic raphé stimulation in these cats. The 10-Hz power in SND was significantly increased to ~300% of control (P < 0.0001), whereas power at frequencies ≤5 Hz was unchanged. Thus, the significant increase (P = 0.0019) in total power reflected the dramatic enhancement of 10-Hz activity. The peak frequency in the 10-Hz band was also significantly increased from 10.5 ± 0.2 to 11.0 ± 0.2 Hz (P = 0.0001).

The level to which 10-Hz power increased following tetanic raphé stimulation was markedly reduced by microinjection of LY-53857 into either the rostral or caudal VLM. In the example shown in Fig. 6, SND was recorded ipsilateral to the sites of microinjection of LY-53857 into the caudal VLM. The solid black trace is the control autospectrum of SND recorded 5–35 s following a 10-s period of tetanic raphé stimulation. The gray trace is the corresponding autospectrum of SND recorded ~5 min after LY-53857 was microinjected into the caudal VLM. Note the selective reduction in postraphe tetanus 10-Hz power. As shown by the dotted trace in Fig. 6, intravenous adminis-
tration of DOI partially reversed the effects of LY-53857 on postraphé tetanus 10-Hz power in SND. Although not shown, microinjection of LY-53857 into either the rostral or caudal VLM did not prevent the inhibition of SND and reduction in arterial pressure during tetanic raphé stimulation.

Figure 7 summarizes the effects of microinjections of LY-53857 into the VLM on SND recorded 5–35 s following tetanic raphé stimulation in 12 cats. There was a significant reduction in postraphé tetanus 10-Hz activity recorded ipsilateral to the sites of microinjection of LY-53857 into the rostral VLM (Fig. 7A; \( P = 0.0416 \)) or caudal VLM (Fig. 7B; \( P = 0.0009 \)). Although there was a tendency for the reduction in 10-Hz SND recorded contralateral to the injection sites to be less pronounced, the changes in 10-Hz activity recorded ipsilateral and contralateral to the injections were not significantly different. Importantly, the difference between postraphé tetanus 10-Hz power in ipsilateral SND before and after microinjection of LY-53857 into the rostral or caudal VLM exceeded the 10-Hz power in SND under basal conditions (i.e., no raphé stimulation and before drug microinjection) by 109 ± 57% and 54 ± 9%, respectively. Thus, microinjection of LY-53857 into the VLM attenuated the enhancement of 10-Hz SND produced by tetanic raphé stimulation as well as basal 10-Hz activity. In contrast to the reductions in postraphé tetanus 10-Hz power, microinjection of LY-53857 into the VLM did not significantly change power in SND at frequencies ≤5 Hz.

Effects of DOI in the VLM. We studied the changes in basal SND produced by unilateral microinjection of the selective 5-HT2 receptor agonist DOI into the rostral (2 cats) or caudal (1 cat) VLM. Recordings were made only from the inferior cardiac nerve ipsilateral to the injection sites. Figure 8 shows the results from one of the experiments in which DOI was microinjected into the rostral VLM. In this case, 10-Hz power in SND was increased to 209% (gray trace) of control (solid black trace) 3 min after microinjection of DOI. Moreover, the peak frequency in the 10-Hz band increased from 10.2 to 11.0 Hz. These changes were similar to those observed 5–35 s following a 10-s period of tetanic raphé stimulation (dotted trace) performed before microinjection of DOI in the same cat. The low-frequency (≤5-Hz) component of SND was unchanged by DOI microinjections into the VLM. In the other two experiments, DOI microinjections increased 10-Hz power to 386% (rostral VLM) and 229% (caudal VLM) of control. By combining data from the three cats, we found that microinjection of DOI into the VLM significantly increased 10-Hz power in SND to 275 ± 56% of control \( (P = 0.0446) \) and significantly increased the peak frequency in the 10-Hz band from 10.5 ± 0.8 to 11.1 ± 0.8 Hz \( (P = 0.0286) \). These changes were similar to those produced by tetanic raphé stimulation before microinjection of DOI into the VLM in these cats. Specifically, raphé stimulation significantly increased 10-Hz power to 260 ± 30% of control \( (P = 0.0341) \) and increased the peak frequency in the 10-Hz band from 10.5 ± 0.8 to 11.2 ± 0.8 Hz \( (P = 0.0198) \). In addition, the increase in 10-Hz power produced by DOI microinjection was accompanied by a significant increase in MAP of 12 ± 2 mmHg \( (P = 0.0099) \) in these cats.
Effects of 8-OHDPAT in the VLM. We studied the changes in basal SND produced by unilateral microinjections of the 5-HT1A receptor agonist 8-OHDPAT into the rostral or caudal VLM of six cats. Recordings were made only from the inferior cardiac nerve ipsilateral to the injection sites. Figure 9A shows the results from an experiment in which 8-OHDPAT was microinjected into the caudal VLM. Power in the 10-Hz band of SND was reduced to 38% of control 3–5 min after microinjection of 8-OHDPAT (compare solid black and gray traces). There was also a reduction to 53% of control in low-frequency (≤5 Hz) SND in this experiment. The dotted trace in Fig. 9A is the autospectrum of SND constructed from data collected 45 min after microinjection of 8-OHDPAT into the caudal VLM. At this time, 10-Hz power actually exceeded that in control.

Fig. 5. Effects of a 10-s period of tetanic (50-Hz) raphe stimulation on SND and arterial pressure. A: oscillographic records (top and bottom) show arterial pressure, SND, and time base (1 s/division). Period of raphe tetanus is shown by bar above time base. B: autospectra of SND before (solid black trace) and 5–35 s after (gray trace) high-frequency raphe stimulation. C: summary of the changes in SND power that occurred 5–35 s after a 10-s period of tetanic raphe stimulation in 19 cats. The autospectra of SND after raphe stimulation were based on 26 5-s windows with 80% overlap. Values are means ± SE. *Statistically different from before raphe stimulation (P ≤ 0.05; paired t-test); n = number of cases.

Effects of 8-OHDPAT in the VLM. We studied the changes in basal SND produced by unilateral microinjections of the 5-HT1A receptor agonist 8-OHDPAT into the rostral or caudal VLM of six cats. Recordings were made only from the inferior cardiac nerve ipsilateral to the injection sites. Figure 9A shows the results from an experiment in which 8-OHDPAT was microinjected into the caudal VLM. Power in the 10-Hz band of SND was reduced to 38% of control 3–5 min after microinjection of 8-OHDPAT (compare solid black and gray traces). There was also a reduction to 53% of control in low-frequency (≤5 Hz) SND in this experiment. The dotted trace in Fig. 9A is the autospectrum of SND constructed from data collected 45 min after microinjection of 8-OHDPAT into the caudal VLM. At this time, 10-Hz power actually exceeded that in control.

Fig. 6. Effects of microinjection of LY-53857 in the VLM on SND recorded 5–35 s after a 10-s period of tetanic raphe stimulation. Autospectra of SND before (solid black trace) and after (gray trace) unilateral microinjection of LY-53857 into the CVLM. The dotted trace shows partial reversal of the effects of LY-53857 after intravenous DOI (1 mg/kg). SND was recorded ipsilateral to the injection sites in the VLM. Data are from the same cat as in Fig. 4. Absolute 10-Hz power in the control postraphe tetanus autospectrum was 185% of that in the control autospectrum of basal SND.

Fig. 7. Summary of the effects of unilateral microinjections of LY-52857 into the VLM on SND recorded 5–35 s after a 10-s period of tetanic (50 Hz) raphe stimulation. A: changes in 10-Hz, 0- to 5-Hz, and total power in SND recorded ipsilateral and contralateral to the injection sites in the RVLM, expressed as % of control. B: same as in A, except that injection sites are in the CVLM. Values are means ± SE. *Statistically different from before raphe stimulation (P ≤ 0.05; paired t-test); n = number of cases.
VLM (Fig. 10A after microinjections of 8-OHDPAT into either the rostral or caudal VLM of six cats. The effects produced by microinjection of 8-OHDPAT into each of these regions significantly and selectively reduced the level of postraphé tetanus 10-Hz power in SND. The difference between postraphé tetanus 10-Hz power before and after 8-OHDPAT microinjection into the rostral or caudal VLM exceeded the 10-Hz power in SND under basal conditions (i.e., no raphe stimulation, before drug injection) by 88 ± 39% and 96 ± 29%, respectively. Thus, microinjections of 8-OHDPAT into the VLM attenuated the enhancement of 10-Hz SND produced by tetanic raphe stimulation as well as basal 10-Hz activity. Although not shown, microinjection of 8-OHDPAT into the VLM did not prevent the sympathoinhibition or depressor response during high-frequency raphe stimulation.

Figure 10 summarizes the changes in basal SND and MAP observed 3–5 min after microinjections of 8-OHDPAT into the rostral or caudal VLM of six cats. The effects produced by microinjection of 8-OHDPAT into these two regions were similar. The 10-Hz power in SND was significantly reduced after microinjections of 8-OHDPAT into either the rostral VLM (Fig. 10A; \( P < 0.0001 \)) or caudal VLM (Fig. 10B; \( P = 0.0011 \)). In contrast, power at frequencies ≤5 Hz was not significantly changed. As shown in Fig. 10C, a statistically significant fall in MAP accompanied the selective decrease in 10-Hz SND produced by microinjection of 8-OHDPAT into either the rostral (\( P = 0.0141 \)) or caudal VLM (\( P = 0.0026 \)). MAP averaged 133 ± 9 mmHg before microinjection of 8-OHDPAT into the VLM.

Recovery of 10-Hz SND toward control level routinely began no less than 30 min after unilateral microinjections of 8-OHDPAT into VLM. From its nadir, 10-Hz power in SND was significantly increased to 96 ± 20% of control within 90 min (\( P = 0.0039 \)), MAP returned to a value (129 ± 9 mmHg) not significantly different from control.

In the three cats in which there was residual 10-Hz power in SND after 8-OHDPAT microinjection into the rostral VLM, the peak frequency in the 10-Hz band was not significantly different from control (10.6 ± 0.9 Hz vs. 10.2 ± 0.9 Hz). This was also the case for the three cats in which there was residual 10-Hz power in SND after 8-OHDPAT microinjection into the caudal VLM. The peak frequency in the 10-Hz band was 10.0 ± 0.7 Hz before and 9.6 ± 0.8 Hz after microinjection of 8-OHDPAT.

In three cats, we studied the effects of intravenous administration of a selective 5-HT1A receptor antagonist, WAY-100635 (1 mg/kg), on the changes in SND produced by microinjection of 8-OHDPAT into the rostral (\( n = 2 \)) or caudal (\( n = 1 \)) VLM. Figure 9B shows the results from an experiment in which microinjection of 8-OHDPAT into the rostral VLM essentially eliminated the peak near 10 Hz in the SND autospectrum (compare postinjection gray trace with control solid black trace). Intravenous injection of WAY-100635 10 min later partially reversed the reduction in 10-Hz SND. This is indicated by the reemergence of the sharp peak near 10 Hz in the autospectrum of SND (dotted trace) 1–3 min after the intravenous administration of WAY-100635. In these three cats, 8-OHDPAT microinjections into the VLM reduced 10-Hz power to 4 ± 3% of control, and intravenous WAY-100635 significantly increased 10-Hz power back to 81 ± 22% of control (\( P = 0.0435 \)).

We also quantified the effects of microinjections of 8-OHDPAT into the rostral (Fig. 11A) or caudal (Fig. 11B) VLM on SND recorded 5–35 s following tetanic raphe stimulation. Note that microinjections of 8-OHDPAT into each of these regions significantly and selectively reduced the level of postraphé tetanus 10-Hz power in SND. The difference between postraphé tetanus 10-Hz power before and after 8-OHDPAT microinjection into the rostral or caudal VLM exceeded the 10-Hz power in SND under basal conditions (i.e., no raphe stimulation, before drug injection) by 88 ± 39% and 96 ± 29%, respectively. Thus, microinjections of 8-OHDPAT into the VLM attenuated the enhancement of 10-Hz SND produced by tetanic raphe stimulation as well as basal 10-Hz activity. Although not shown, microinjection of 8-OHDPAT into the VLM did not prevent the sympathoinhibition or depressor response during high-frequency raphe stimulation.
DISCUSSION

The present study is the first to demonstrate dependency of the 10-Hz rhythm in SND on serotonergic input to the VLM. We found that unilateral microinjections of a 5-HT2 receptor antagonist, LY-53857, into either the rostral or caudal VLM significantly reduced 10 Hz but not the low-frequency (≤5 Hz) component of SND recorded ipsilateral to the injection sites. The selective decrease in 10-Hz SND was accompanied by a statistically significant fall in MAP. Similar results were seen when methysergide, another drug that acts as a 5-HT2 receptor antagonist, was microinjected into the VLM.

Unilateral microinjections of DOI into either the caudal or rostral VLM led to a selective enhancement of power in the 10-Hz band of SND as well as an increase in MAP. Thus, the direction of change in 10-Hz power and MAP presumably produced by 5-HT2 receptor activation in the VLM was opposite to that produced by microinjection of LY-53857 or methysergide into the VLM. These findings are consistent with the view that serotonergic inputs act on postsynaptic 5-HT2 receptors in the VLM to enhance selectively 10-Hz SND and, consequently, MAP. Indeed, 5-HT2 receptor activation in a variety of brain regions has been reported to lead to neuronal excitation (2, 12, 28, 31, 34). Immunohistochemical mapping has demonstrated the presence of postsynaptic 5-HT2 receptor-like protein on neurons distributed throughout the caudal and rostral VLM of the rat (18).

That neurons in the caudal medullary raphé nuclei are the source of excitatory serotonergic input to the VLM is supported by the following observations. First, microinjection of LY-53857 into the VLM significantly reduced the level to which 10-Hz power was increased following a 10-s period of tetanic raphé stimulation. Second, the selective increase in 10-Hz SND following tetanic raphé stimulation was mimicked by microinjection of DOI into the VLM. The enhancement of 10-Hz SND following tetanic raphé stimulation was accompanied by an increase in peak frequency. Increased peak frequency in the 10-Hz band strongly suggests that the rhythm generator rather than just its follower circuits was affected by stimulation of the caudal medullary raphé nuclei.

![Fig. 10. Summary of the changes in SND and MAP produced by unilateral microinjections of 8-OHDPAT into the VLM.](image-url)

**A:** changes in 10 Hz, 0 to 5 Hz, and total power in basal SND recorded ipsilateral to microinjection sites in the RVLM, expressed as a percent of control. **B:** same as in **A**, except that data are for microinjections in the CVLM. **C:** changes in MAP produced by injections into the RVLM or CVLM. Values are means ± SE. *Statistically different than before microinjection of 8-OHDPAT (P ≤ 0.05; paired t-test); n = number of cases.

**Fig. 11. Summary of the effects of unilateral microinjections of 8-OHDPAT into the VLM on SND recorded 5–35 s after a 10-s period of tetanic (50 Hz) raphé stimulation.**

**A:** changes in 10-Hz, 0- to 5-Hz, and total power in SND recorded ipsilateral to the injection sites in the RVLM, expressed as % of control. **B:** same as in **A**, except that data is for injection sites in the CVLM. Values are means ± SE. *Statistically different from before microinjection of 8-OHDPAT (P ≤ 0.05; paired t-test); n = number of cases.
The events responsible for the enhancement of the 10-Hz rhythm in SND following high-frequency raphé stimulation remain to be determined. There may have been an overflow of serotonin released in the VLM during raphé stimulation, the effects of which outlasted the stimulation. Initially, the effects of increased serotonin release in the VLM may have been masked by the powerful, but short-lasting, sympathoinhibition, which was not affected by microinjections of LY-53857 into the VLM. The sympathoinhibition coincident with the stimulation may have occurred in the spinal cord rather than the brain stem. Regarding this possibility, some cat raphé sympathoinhibitory neurons with activity correlated to SND send their axons to the thoracic spinal cord (5, 29). The enhancement of 10-Hz SND produced by tetanic raphé stimulation might also reflect a rebound increase in the excitability of serotonergic neurons projecting to the VLM subsequent to their inhibition during the tetanus. Future studies should deal with these alternatives.

It is unlikely that the effects of LY-53857, methysergide, and DOI on 10-Hz SND were due to nonspecific actions of the drugs in the VLM. First, microinjection of vehicle into the VLM did not affect 10-Hz SND. Second, with few exceptions, the changes in power produced by microinjection of these drugs into the VLM were restricted to the 10-Hz band of SND. Third, as might be expected, microinjection of a 5-HT2 receptor antagonist, LY-53857 or methysergide, into the VLM produced a change in 10-Hz power (reduction) opposite in direction to that produced by microinjection of the 5-HT2 receptor agonist DOI. Fourth, recovery of 10-Hz SND toward control levels was routinely observed within 45 min after microinjection of these drugs into the VLM.

Our data also support a role of 5-HT1A receptors in the VLM in the expression of the 10-Hz rhythm in SND. Specifically, unilateral microinjections of 8-OHDPAT into the caudal or rostral VLM caused a selective decrease in 10-Hz power in basal SND and a reduction in MAP. In addition, 8-OHDPAT was effective in attenuating the enhancement of 10-Hz power that followed tetanic raphé stimulation. Importantly, the reductions in 10-Hz SND and MAP produced by microinjection of 8-OHDPAT into the VLM were partially reversed by the intraventricular administration of a selective 5-HT1A receptor antagonist, WAY-100635. Decreases in arterial pressure and SND have been observed upon microinjection of 8-OHDPAT into the rat rostral VLM (32). In this species, however, the changes in arterial pressure and SND were attenuated by α1-adrenoceptor antagonists injected into the VLM.

The mechanism by which 8-OHDPAT acts in the VLM to selectively reduce 10-Hz SND remains unclear. One possibility is that 8-OHDPAT acts to reduce transmitter release from the terminals of raphé serotonergic neurons projecting to the VLM. However, it is not known whether 5-HT1A receptors exist on the terminals of such neurons, as they are known to exist on the soma and dendrites of raphé serotonergic neurons. It is well established that the activation of 5-HT1A soma-dendritic autoreceptors on raphé serotonergic neurons inhibits their firing (1, 16, 17, 25, 36). Regarding this point, microinjection of 8-OHDPAT into the caudal medullary raphé has been reported to reduce selectively 10-Hz SND (33). However, whether this effect of 8-OHDPAT in the medullary raphé is attributable solely to the inhibition of serotonergic neurons projecting to VLM neurons is uncertain (25). A second possibility is that 5-HT1A receptors are located postsynaptically on VLM neurons, as they are in other brain areas including the hippocampus and frontal cortex (3, 35, 36). If postsynaptic 5-HT1A receptors are located on VLM neurons, then their activation would lead to a change in 10-Hz activity diametrically opposed to those elicited by activation of postsynaptic 5-HT2 receptors in the VLM. The question would then arise whether postsynaptic 5-HT1A and 5-HT2 receptors are located on different populations of VLM neurons.

The discharges of individual rostral VLM neurons with axons projecting to the spinal intermediolateral nucleus are correlated to both the 10-Hz rhythmic and lower-frequency, aperiodic components of SND (5). Yet, microinjection of either LY-53857 or 8-OHDPAT into the VLM reduced only the 10-Hz rhythmic component of SND. Thus, it is doubtful that serotonergic inputs directly contact putative rostral VLM-spinal sympathoexcitatory neurons. It is more likely that serotonergic inputs are directed to VLM neurons with activity correlated to 10 Hz but not to the lower-frequency, aperiodic component of SND in baroreceptor-denervated cats or the cardiac-related rhythm in baroreceptor-innervated cats (4, 5, 11). Such neurons with activity correlated to only the 10-Hz rhythm, which are located in both the rostral and caudal VLM, do not send their axons to the spinal cord (5, 11). If, as suggested by Barman and colleagues (5, 6, 11), these neurons play a role in 10-Hz rhythm generation, then their reduced activity after microinjection of LY-53857 or 8-OHDPAT into either the rostral or caudal VLM would be expected to reduce blood pressure as was found to be the case in the present study.

Lipski et al. (26) and Nicholson (30) have estimated that a 50-nl injectate spreads to form a sphere with a radius <0.5 mm. On this basis, it is unlikely that the similarities in the changes in the 10-Hz rhythm produced by microinjection of 5-HT receptor agonists and antagonists at sites in the caudal and rostral VLM separated by as much as 2–3 mm can be explained by widespread diffusion of the drugs. Rather, we suggest that raphé inputs are widely distributed in the VLM and that caudal and rostral VLM neurons act together to express the 10-Hz rhythm.

Perspectives and Significance

The 10-Hz rhythm in SND has been implicated in setting the level of arterial pressure, coordinating the discharges of sympathetic nerves with targets, such as the heart and vasculature of the viscera and skeletal muscle, and in the formulation of differential patterns of spinal sympathetic outflow as occurs, for example, during the defense reaction (6, 8, 9, 21, 22). The present study has provided new information concerning the potential role played by 5-HT2 and 5-HT1A receptors in the VLM in the expression of this rhythm. The results support the view that 5-HT2 receptor activation in the VLM by serotonergic inputs from the caudal raphé nuclei leads to a selective enhancement of 10-Hz SND. 5-HT1A receptor activation in the VLM produced the opposite effect. Whether caudal medullary raphé inputs to the VLM play a permissive or a critical role in 10-Hz rhythm generation remains in question. If, as suggested by Barman and Gebber (7), the 10-Hz rhythm is generated locally by a network of VLM GABAergic interneurons, then raphé serotonergic input to the VLM might simply aid in the recruitment of a greater number of active GABAergic interneurons.
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