Subgroups of Rostral Ventrolateral Medullary and Caudal Medullary Raphe Neurons Based on Patterns of Relationship to Sympathetic Nerve Discharge and Axonal Projections

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Barman, Susan M. and Gerard L. Gebber. Subgroups of rostral ventrolateral medullary and caudal medullary raphe neurons based on patterns of relationship to sympathetic nerve discharge and axonal projections. J. Neurophysiol. 77: 65–75, 1997. This study was designed to answer three questions concerning rostral ventrolateral medullary (RVLM) and caudal medullary raphe (CMR) neurons with activity correlated to sympathetic nerve discharge (SND). 1) What are the proportions of RVLM and CMR neurons that have activity correlated to both the cardiac-related and 10-Hz rhythms in SND, to only the 10-Hz rhythm, and to only the cardiac-related rhythm? 2) Which of these cell types project to the spinal cord? 3) Do the outputs of the cardiac-related and 10-Hz rhythm generators converge at the level of bulbospinal neurons or their antecedent interneurons? To address these issues we recorded from 44 RVLM and 48 CMR neurons with sympathetic nerve-related activity in urethan-anesthetized cats with intact carotid sinus nerves, but sectioned aortic depressor and vagus nerves. Spike-triggered averaging, arterial pulse-triggered analysis, and coherence analysis revealed that the naturally occurring discharges of 24 of these RVLM neurons and 41 of these CMR neurons were correlated to both the 10-Hz and cardiac-related rhythms in inferior cardiac postganglionic SND. The discharges of the other neurons were correlated to only the 10-Hz rhythm (15 RVLM and 6 CMR neurons) or to only the cardiac-related rhythm (5 RVLM neurons and 1 CMR neuron) in SND. The time-controlled collision test verified that 16 of 18 RVLM and 31 of 34 CMR neurons with activity correlated to both rhythms were antidromically activated by stimulation of the white matter of the first thoracic (T1) segment of the spinal cord. In contrast, only 1 of 10 RVLM neurons and 0 of 4 CMR neurons with activity correlated to only the 10-Hz rhythm could be antidromically activated by stimulation at T1. Also 0 of 3 RVLM neurons with activity correlated to only the cardiac-related rhythm in SND were antidromically activated by spinal stimulation. These data show for the first time that bulbospinal sympathetic pathways emanating from the RVLM and CMR are comprised almost exclusively of neurons whose discharges are correlated to both the cardiac-related and 10-Hz rhythms in SND. Moreover, the data support the hypothesis that the outputs of the cardiac-related and 10-Hz rhythm generators converge on RVLM and CMR bulbospinal neurons rather than on their antecedent interneurons. Finally, the data demonstrate that a substantial proportion of RVLM neurons and a small group of CMR neurons with activity correlated to SND do not project to the thoracic spinal cord. Their discharges were correlated to only one of the rhythms in SND. Their axonal trajectories and functions are unknown.

INTRODUCTION

The 10-Hz and cardiac-related rhythms can coexist in sympathetic nerve discharge (SND) of urethan-anesthetized or decerebrate-unanesthetized cats (Barman and Gebber 1992; Barman et al. 1992, 1994; Cohen and Gootman 1970). Although both rhythms originate in the brain stem (Barman and Gebber 1992; Gebber et al. 1994; Zhong et al. 1993), data support the view that they are generated by different pools of neurons. Specifically, spike-triggered averaging and coherence analysis showed that caudal ventrolateral medullary (CVLM) neurons whose discharges are correlated to the 10-Hz rhythm do not have activity correlated to the cardiac-related rhythm in SND (Barman et al. 1994). In contrast, the discharges of medullary lateral tegmental field (LTF) neurons are correlated to the cardiac-related rhythm (Barman and Gebber 1987, 1989; Gebber and Barman 1985) but not to the 10-Hz rhythm in SND (Barman and Gebber 1993). Several studies from this laboratory have focused on the central neural circuitry responsible for the cardiac-related rhythm in SND. Data from antidromic mapping studies, comparison of neuronal firing times, and synaptic activation studies support the contention that LTF neurons with activity correlated to this component of SND innervate rostral ventrolateral medullary (RVLM) and caudal medullary raphe (CMR) neurons, at least some of which project to the intermediolateral nucleus (IML) of the thoracic spinal cord (Barman and Gebber 1987, 1989; Gebber and Barman 1985). Much less is known about the interconnections of central neurons responsible for the 10-Hz rhythm in SND. Barman et al. (1995b) provided data supporting the view that interconnections of CVLM and CMR neurons may contribute to the expression of the 10-Hz rhythm in SND. Intramedullary connections of RVLM neurons whose discharges are correlated to the 10-Hz rhythm in SND are unknown. Interestingly, Barman and Gebber (1992) identified some RVLM and CMR neurons whose discharges were correlated to both the cardiac-related and 10-Hz rhythms in SND. These data imply that the outputs of the generators of the two rhythms converge at a supraspinal site. It remains to be determined whether the convergence is on bulbospinal neurons or their antecedent interneurons. Whether there are bulbospinal neurons that selectively relay information from only one of the two generators to the spinal cord is also unknown.

The current study was designed to provide new information on the networks responsible for the 10-Hz and cardiac-related rhythms in SND. The following questions were the focus of these experiments. 1) What are the proportions of RVLM and CMR neurons that have activity correlated to...
both the cardiac-related and 10-Hz rhythms in SND, to only the 10-Hz rhythm, and to only the cardiac-related rhythm? 2) Which of these cell types in the RVLm and CMR project to the spinal cord? 3) Do the outputs of the cardiac-related and 10-Hz rhythm generators converge at the level of RVLm-spinal and CMR-spinal neurons or their antecedent interneurons?

**METHODS**

**General procedures**

The protocols used in these studies on 29 cats were approved by the All-University Committee on Animal Use and Care of Michigan State University. Cats were initially anesthetized with 2.5% isoflurane mixed with 100% O2. The right femoral artery and vein were cannulated to measure arterial pressure and to administer drugs, respectively. A Fogarty embolectomy catheter (4F) was inserted into the aorta via the left femoral artery. Urethane (1.2–1.8 g/kg iv, initial dose) was then administered, and isoflurane inhalation was terminated. Supplemental doses (0.2 g/kg iv) of urethane were given every 4–6 h. The frontoparietal electroencephalogram (EEG) showed a mixture of 7- to 13-Hz spindles and delta slow waves, indicative of unconsciousness and blockade of information transfer through the thalamus (Steriade and Llinas 1988; Steriade and McCarley 1990). Noxious stimuli (e.g., pinch, cauterizing muscle) did not change the EEG pattern. As reported by Barman et al. (1995a), coherence analysis showed that there was no correlation between SND and either the EEG spindles or delta slow waves in these urethane-anesthetized cats.

Cats were paralyzed (gallamine triethiodide, 4 mg/kg iv, initial dose), pneumothoracotomized, and artificially resired with room air. End-tidal CO₂ was held near 4% (Traverse Medical Monitors Capnometer, model 2200), and rectal temperature was kept near 38°C with a heat lamp.

The aortic depressor and vagus nerves were cut, but the carotid sinus nerves were intact. Barman et al. (1994) showed that under these conditions the amplitudes of the peaks in the autospectra of SND at the frequency of the heart rate and near 10 Hz can be controlled by changing the level of mean arterial pressure with aortic obstruction (inflating the balloon-tipped end of the Fogarty embolectomy catheter). This facilitates testing whether the discharges of an individual neuron are correlated to both the cardiac-related and 10-Hz rhythms or to only one of these rhythms in SND.

In the current study when mean arterial pressure was 89 ± 2 (SE) mmHg, the autospectrum of SND had a peak near 10 Hz (8.8 ± 0.08 Hz) but little if any power at the frequency of the heart beat. When mean arterial pressure was 124 ± 5 mmHg, the autospectrum of SND showed prominent peaks near 10 Hz and at the frequency of the heart beat. When mean pressure was 157 ± 4 mmHg, the autospectrum of SND showed a peak at the frequency of the heart beat but little if any power near 10 Hz. In cats in which the 10-Hz rhythm was not prominent under basal conditions, we administered idazoxan HCL (100 μg/kg iv; Sigma, St. Louis, MO). Orer et al. (1996) showed that administration of this α₂-adrenoceptor antagonist can induce or enhance the 10-Hz rhythm in SND.

**Neural recordings**

The methods used to record left inferior cardiac postganglionic SND and the EEG can be found in earlier reports (Barman and Gebber 1985; Barman et al. 1995a). The preamplifier band pass was 1–1,000 Hz. The synchronized discharges of sympathetic fibers appear as slow waves (i.e., envelopes of spikes) when this band pass is used (Gebber and Barman 1985).

The dorsal aspect of the brain stem was exposed by removing portions of the occipital bone and cerebellum. The obex and midline were used as landmarks for placement of the recording tungsten microelectrode (FHC; 1-μm tip diam, ~3-MΩ tip impedance) with a hydraulic microdrive (David Kopf Instruments, model 650). The reference electrode was a gold-plated disk on the skull. Capacitively-coupled preamplification with a band pass of 0.1–3 kHz was used. Extracellular recordings were made from two medullary regions that have been shown to contain neurons with activity correlated to SND (Barman and Gebber 1985, 1992; Barman et al. 1994, 1995b; Morrison and Gebber 1985). RVLm neurons were located 3.5 mm to the left of the midline, 4.7–6 mm rostral to the obex, and within 2.5 mm of the ventral surface in 16 cats. CMR neurons were located on the midline, 2–3.5 mm rostral to the obex, and within 3.5 mm of the ventral surface in 13 cats. When the microelectrode approached the ventral surface of the brain stem, 60-Hz noise appeared in the recording. The duration of the action potentials was at least 1.5 ms for all neurons described in this report, and in many cases there was an inflection on the rising phase of the spike. These properties indicate that recordings were made from cell bodies rather than axons (Humphrey and Schmidt 1990).

**Electrical stimulation**

A Grass S8800 stimulator and PSIU-6 constant current unit were used to deliver cathodal square-wave pulses (1 mA; 0.5-ms duration) through a bipolar stainless steel electrode (Rhodes model SNE-100) positioned in the white matter of the first thoracic (T1) segment of the spinal cord. In most cases the electrode was initially positioned at a site in the dorsolateral funiculus, ipsilateral to the nerve recording.

**ANTIDROMIC ACTIVATION.** Time-controlled collision of spontaneous and stimulus-induced action potentials was used as a test for antidromic activation (Barman and Gebber 1985; Barman et al. 1995b; Lipski 1981; Morrison and Gebber 1985). Neuronal responses were considered antidromic when the minimum interval between a spontaneous action potential and the stimulus that always elicited a response was close to the sum of the onset latency of the stimulus-induced action potential and the axonal refractory period (i.e., critical delay for antidromic activation). A measure of the axonal refractory period was obtained by determining the minimum interval between paired stimuli producing two action potentials 100% of the time. This measure overestimates the axonal refractory period when the recording is somal in origin. Nonetheless, the error in critical delay is small because the antidromic response latency far exceeded the estimated value of axonal refractory period.

**Data analysis**

Before all analyses on a Zenith 486 Z-Station 500 computer, the action potentials of individual neurons were isolated by using window discrimination, and SND was low-pass filtered at 100 Hz. The Butterworth analog filter (A.P. Circuit, model 260-5) has unity gain and a roll-off rate of 24 dB/octave. Data were processed (5-ms sampling interval) with software and an A/D converter board from R.C. Electronics (Goleta, CA). Time-domain analyses used R.C. Electronics software. As described by Barman et al. (1995b) and Gebber et al. (1994), frequency-domain analyses used a modified version of the software of Cohen et al. (1987) and Kocsis et al. (1990).

**SPIKE-TRIGGERED AVERAGING.** Standardized pulses representing the action potentials of single medullary neurons were used as references to construct averages of SND. A series of randomly generated pulses with the same mean frequency as the neuronal spike train was used to construct a “dummy” average from the same data sample of SND. The discharges of a medullary neuron...
were considered to be correlated to SND if the amplitude of the first peak to the right of time 0 in the spike-triggered average was at least 4 times that of the largest deflection in the dummy average.

**ARterial PULSE (AP)±TRIGGERed ANALYsis.** The threshold for initiating this analysis was set at a point on the systolic phase of the AP. Averages of the AP and SND and a histogram of medullary neuronal activity were constructed.

**Autospectral and Coherence Analyses.** Fast Fourier transform was performed on 32 or 65 contiguous 5-s windows of data to construct autospectra of SND, the AP, and the discharges of single medullary neurons and corresponding coherence functions. Digital low-pass filtering (cutoff at 250 Hz) of the standardized pulses representing neuronal spikes was performed by convolving the trains with a sinc function having parameters so that the autospectrum reflected the interspike intervals rather than the shape of the pulses (Christakos et al. 1988). The autospectrum of a signal shows how much power (voltage squared) is present at each frequency. The coherence function (normalized cross-spectrum) is a measure of the strength of linear correlation of two signals at each frequency. The squared coherence value (referred to as coherence value) is one in the case of a perfect linear relationship and zero if two signals are unrelated. A coherence value of $\geq 0.1$ was considered to reflect a statistically significant relationship when 32 windows were averaged (Benignus 1970). Only coherence values that met this level of significance are considered in the data quantified in this report. Spectral analyses were done over a frequency band of $0-100$ Hz with a resolution of 0.2 Hz/bin. The figures in this report show only frequencies $\leq 20$ Hz because $>90\%$ of the total power in SND was within this band (Barman et al. 1992).

**Statistical analysis**

Data are expressed as means $\pm$ SE. Student’s $t$-test for unpaired data and calculation of the Pearson product-moment correlation coefficient ($r$) were used in this study; $P < 0.05$ indicated statistical significance.

**Histology**

The medulla was removed and fixed in 10% buffered Formalin. Frontal sections of 30-μm thickness were cut and stained with cresyl violet. Medullary neuronal recording sites were approximated with reference to the bottom of electrode tracks and the stereotoxic planes of Berman (1968).

**Results**

**Classification of RVLM and CMR neurons based on the relationship between their discharges and the 10-Hz and cardiac-related rhythms in SND**

Spike-triggered averaging has shown that the naturally occurring discharges of $\sim 25\%$ of the neurons in the regions of the RVLM and CMR explored in the current study (see METHODS) are correlated to SND (Barman and Gebber 1992; Barman et al. 1994). The purpose of this series of experiments was to determine the proportions of such neurons that receive convergent inputs from the generators responsible for the cardiac-related and 10-Hz rhythms in SND and those that receive input from only one of these generators. This assessment was made for 44 RVLM and 48 CMR neurons with sympathetic nerve-related activity. RVLM AND CMR NEURONS WITH ACTIVITY CORRELATED TO BOTH THE 10-Hz AND CARDIAC-RELATED RHYTHMS IN SND. The discharges of 24 RVLM and 41 CMR neurons were correlated to both rhythms in inferior cardiac postganglionic SND. The data in Figs. 1 and 2 are for one of these RVLM neurons. At a mean arterial pressure of 93 mmHg, the autospectra of SND and RVLM neuronal activity (Fig. 1A, middle and bottom) contained a sharp peak at 9.4 Hz but not at the frequency of the heart beat (3.6 Hz). The absence of cardiac-related discharges is also shown by the low coherence value at 3.6 Hz in the AP-SND and AP-RVLM coherence functions (Fig. 1B, middle and bottom) and the essentially flat AP-triggered average of SND and histogram of RVLM neuronal activity (Fig. 2A). The spike-triggered average in Fig. 2A, top, shows inferior cardiac SND for 500 ms before and after RVLM neuronal spike occurrence at time 0. The peaks in the spike-triggered average were regularly spaced at $\sim 105$-ms intervals, and their amplitudes greatly exceeded those in the corresponding dummy average of SND (Fig. 2A, bottom). The interval between RVLM neuronal spike occurrence and the first peak to the right of time 0 in the average of SND was 60 ms. The relationship between the 10-Hz discharges of this RVLM neuron and the inferior cardiac nerve was confirmed with coherence analysis (Fig. 1B, bottom). The coherence value at 9.4 Hz was 0.64.

When mean arterial pressure was raised to 150 mmHg by aortic obstruction, the autospectra of SND and RVLM neuronal activity (Fig. 1B, middle and bottom) contained peaks at the frequency of the heart beat and its second harmonic. The cardiac-related rhythm was also evident in the AP-triggered average of SND and histogram of RVLM neuronal activity (Fig. 2B). A relationship between the cardiac-related rhythms in these two signals was demonstrated by spike-triggered averaging (Fig. 2B, middle and bottom) and by coherence analysis (Fig. 2B, bottom). The interval between RVLM unit spike occurrence and peak of the first cardiac-related slow wave to the right of time 0 in the average of SND was $180$ ms. The coherence value relating the cardiac-related rhythms in these two signals was 0.62.

Figure 3 shows the firing times of neurons with activity correlated to both the cardiac-related and 10-Hz rhythms in SND. Panels IA and IIA show the distributions of the intervals between unit spike occurrence and the peak of the 10-Hz slow wave to the right of time 0 in the spike-triggered averages of inferior cardiac SND for these 24 RVLM and 41 CMR neurons, respectively. The distribution of firing times of these CMR neurons is similar to that reported by Barman and Gebber (1992) and Barman et al. (1994, 1995b). In contrast, the rather narrow distribution of firing times of these RVLM neurons contrasts with the wider range of intervals reported by Barman and Gebber (1992). This difference will be addressed in the DISCUSSION. The distributions of firing times of RVLM and CMR neurons during the 10-Hz slow-wave overlap, but Table 1 shows that the mean lag in the spike-triggered averages for CMR neurons was significantly shorter than that for RVLM neurons. That is, on the average, CMR neurons fired 8 ms later than RVLM neurons during the 10-Hz slow wave in inferior cardiac SND.

Figure 3, IB and IIB, show the distributions of the intervals between unit spike occurrence and the peak of the cardiac-related slow wave to the right of time 0 in the spike-triggered averages of inferior cardiac SND for 19 of the RVLM and 28 of the CMR neurons with activity correlated to both rhythms. This interval could not be measured for the other...
such neurons because they were studied only at a time when both rhythms were present in the autospectra of SND (e.g., Fig. 7). In these cases, the relationship to the 10-Hz rhythm dominated the spike-triggered average of SND. The ranges of intervals shown here are comparable with those reported by Gebber and Barman (1985) for RVLM and CMR neurons with cardiac-related activity in barbiturate-anesthetized cats. As shown in Table 1, the intervals between unit spike occurrence and peak of the cardiac-related slow waves were significantly longer than those during the 10-Hz slow waves for both RVLM and CMR neurons.

Table 1 summarizes other properties of RVLM and CMR neurons with activity correlated to both rhythms in SND. The peak coherence values relating their 10-Hz discharges to those of the inferior cardiac nerve were significantly higher than those relating their cardiac-related discharges to SND. These coherence values are similar to those relating RVLM and CMR neuronal activity to the 10-Hz rhythm in SND of decerebrate-unanesthetized cats (Barman and Gebber 1992) and to the cardiac-related rhythm in SND of barbiturate-anesthetized cats (Gebber et al. 1990). These values relating the cardiac-related rhythm in SND to the AP were 0.85 \pm 0.02 and 0.80 \pm 0.03 while recording from these RVLM and CMR neurons, respectively. As shown by Table 1, the steady-state firing rates of these RVLM and CMR neurons were not significantly changed when blood pressure was slowly elevated to produce a prominent cardiac-related rhythm in SND. The total power in SND (as reflected by the value of the root mean square voltage of the 0- to 100-Hz band of the autospectrum) was also not significantly changed (99.7 \pm 2.6% of control) by this manipulation. However, the firing rates of 7 RVLM and

![Figure 1](image1.png)

**FIG. 1.** Frequency-domain analyses showing a relationship between the discharges of a rostral ventrolateral medullary (RVLM) neuron and inferior cardiac sympathetic nerve discharge (SND) in a urethan-anesthetized cat with intact carotid sinus nerves at mean arterial pressures of 93 mmHg (I) and 150 mmHg (II). A: traces (top to bottom) are autospectra (AS) of the arterial pulse (AP), SND, and RVLM neuronal activity. B: corresponding coherence functions. Spectra are based on 32 5-s windows; frequency resolution is 0.2 Hz per bin here and in subsequent figures.

![Figure 2](image2.png)

**FIG. 2.** Time domain-analyses showing a relationship between the 10-Hz and cardiac-related discharges of an RVLM neuron and inferior cardiac SND at 2 levels of arterial pressure (I and II). Data are from the same experiment as Fig. 1. A: spike-triggered (top) and dummy-triggered (bottom) averages of SND (569 and 522 trials in I and II, respectively). Binwidth is 5 ms in this and all averages in subsequent figures. B: arterial-pulse triggered analyses (589 and 489 trials in I and II, respectively). Binwidth is 10 ms for this and all AP-triggered histograms in subsequent figures. Vertical calibrations are 43 \mu V in A and 170 \mu V in B.
The firing rate of six RVLM neurons was significantly decreased from 3.8 ± 0.7 to 1.5 ± 0.7 spikes/s, and the firing rate of one was increased from 0.6 to 1.4 spikes/s when mean arterial pressure was raised from 83 ± 5 to 162 ± 10 mmHg. The firing rate of nine CMR neurons significantly increased from 1.7 ± 0.4 to 4.6 ± 0.7, and the firing rate of two decreased (from 1.8 to 1.2 and from 3.2 to 0.4 spikes/s) when mean arterial pressure was increased from 112 ± 6 to 187 ± 6 mmHg.

Figure 4 (circles) shows the recording sites of RVLM and CMR neurons with activity correlated to both rhythms in SND were monitored during short periods of baroreceptor reflex-induced inhibition of SND produced by abrupt increases in blood pressure (rapid aortic obstruction). The firing rate of six RVLM neurons was significantly decreased when mean arterial pressure was raised from 112 to 187 mmHg. The firing rate of one was increased from 0.6 to 1.4 spikes/s when mean arterial pressure was raised from 83 ± 5 to 162 ± 10 mmHg.

The data in Fig. 5 are for an RVLM neuron with this pattern of relationship to SND. During the initial recording period, a 10-Hz rhythm but not a cardiac-related rhythm (heart rate was 3.0 Hz) was prominent in the autospectra of SND and RVLM neuronal activity (Fig. 5IA, top and middle). AP-triggered analyses verified the lack of a cardiac-related rhythm in these signals (Fig. 5IC). Spike-triggered averaging (Fig. 5IB, top) and coherence analysis (Fig. 5IA, bottom) demonstrated a relationship between the 10-Hz rhythms in RVLM neuronal activity and SND. When arterial pressure was raised to induce a strong cardiac-related rhythm in SND (Fig. 5IA, top, and Fig. 5IIC), the relationship between RVLM neuronal activity and SND was abolished.

FIG. 3. Distribution of intervals between unit spike occurrence and peak of the next slow wave in the spike-triggered average of inferior cardiac SND for RVLM (I) and caudal medullary raphe (CMR) neurons (II). A and C: firing times during the 10-Hz slow waves. B: firing times during the cardiac-related slow waves. A and B: for neurons with activity correlated to both rhythms. C: for neurons with activity correlated to only the 10-Hz rhythm.

11 CMR neurons with activity correlated to both rhythms in SND were monitored during short periods of baroreceptor reflex-induced inhibition of SND produced by abrupt increases in blood pressure (rapid aortic obstruction). The firing rate of six RVLM neurons was significantly decreased from 3.8 ± 0.7 to 1.5 ± 0.7 spikes/s, and the firing rate of one was increased from 0.6 to 1.4 spikes/s when mean arterial pressure was raised from 83 ± 5 to 162 ± 10 mmHg. The firing rate of nine CMR neurons significantly increased from 1.7 ± 0.4 to 4.6 ± 0.7, and the firing rate of two decreased (from 1.8 to 1.2 and from 3.2 to 0.4 spikes/s) when mean arterial pressure was increased from 112 ± 6 to 187 ± 6 mmHg.

The firing rate of 8 of 10 RVLM neurons with activity correlated to the 10-Hz but not the cardiac-related rhythm in SND was significantly decreased from 2.8 ± 0.7 to 0.8 ± 0.4 spikes/s, and the firing rate of the other two were unaffected when mean arterial pressure was rapidly increased from 92 ± 4 to 163 ± 6 mmHg. The firing rate of the one CMR neuron tested was increased from 0.4 to 1 spike/s during baroreceptor reflex activation.

The recording sites of RVLM and CMR neurons with activity correlated to the 10-Hz but not the cardiac-related rhythm in SND (Fig. 4; squares) were intermingled with those whose discharges were correlated to both rhythms in SND.

RVLM AND CMR NEURONS WITH ACTIVITY CORRELATED TO THE 10-Hz BUT NOT THE CARDIAC-RELATED RHYTHM IN SND. The discharges of 15 RVLM and 6 CMR neurons were correlated to only the 10-Hz rhythm in inferior cardiac SND. Raising blood pressure to a level that induced a marked cardiac-related rhythm in SND did not produce cardiac-related activity in these neurons. The peak coherence values relating SND to the AP were 0.86 ± 0.03 and 0.84 ± 0.06 while recording from these RVLM and CMR neurons, respectively.

The data in Fig. 5 are for an RVLM neuron with this pattern of relationship to SND. During the initial recording period, a 10-Hz rhythm but not a cardiac-related rhythm (heart rate was 3.0 Hz) was prominent in the autospectra of SND and RVLM neuronal activity (Fig. 5IA, top and middle). AP-triggered analyses verified the lack of a cardiac-related rhythm in these signals (Fig. 5IC). Spike-triggered averaging (Fig. 5IB, top) and coherence analysis (Fig. 5IA, bottom) demonstrated a relationship between the 10-Hz rhythms in RVLM neuronal activity and SND. When arterial pressure was raised to induce a strong cardiac-related rhythm in SND (Fig. 5IA, top, and Fig. 5IIC), the relationship between RVLM neuronal activity and SND was abolished.

Note the essentially flat spike-triggered average of SND (Fig. 5IB, top) and the absence of a significant coherence between RVLM neuronal activity and SND at the frequency of the heart beat (Fig. 5IA, bottom). The absence of a cardiac-related rhythm in RVLM neuronal activity is also shown by the flat AP-triggered histogram (Fig. 5IIC).

Figure 3, IC and IIIC, shows the distributions of intervals between unit spike occurrence and peak of the 10-Hz slow wave in inferior cardiac SND for these RVLM and CMR neurons, respectively. Table 1 summarizes the properties of RVLM and CMR neurons with activity correlated only to the 10-Hz rhythm in SND. The interval between unit spike occurrence and peak of the 10-Hz slow wave for these RVLM neurons was significantly longer than that for RVLM neurons with activity correlated to both rhythms in SND. That is, the former group of neurons fired, on the average, 12 ms earlier than the latter group of neurons during the 10-Hz slow wave in inferior cardiac SND. In contrast, both types of CMR neurons fired, on the average, at the same time during the 10-Hz slow wave in inferior cardiac SND. The peak coherence value relating the 10-Hz discharges of these CMR neurons and the inferior cardiac nerve was significantly lower than that for the corresponding group of RVLM neurons and for RVLM and CMR neurons with activity correlated to both rhythms in SND. The steady-state firing rate of CMR neurons with activity correlated to only the 10-Hz rhythm in SND was significantly lower than that of RVLM neurons in this group.

The firing rate of 8 of 10 RVLM neurons with activity correlated to the 10-Hz but not the cardiac-related rhythm in SND was significantly decreased from 2.8 ± 0.7 to 0.8 ± 0.4 spikes/s, and the firing rate of the other two were unaffected when mean arterial pressure was rapidly increased from 92 ± 4 to 163 ± 6 mmHg. The firing rate of the one CMR neuron tested was increased from 0.4 to 1 spike/s during baroreceptor reflex activation.
TABLE 1. Characteristics of RVLM and CMR neurons with activity correlated to sympathetic nerve discharge

<table>
<thead>
<tr>
<th>Group</th>
<th>STA Lag, ms</th>
<th>Peak Coherence</th>
<th>Firing Rate, spikes/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 Hz CR</td>
<td>10 Hz CR</td>
<td>10 Hz CR</td>
</tr>
<tr>
<td>RVLM</td>
<td>Related to both the 10-Hz and cardiac-related rhythms in SND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>55 ± 2</td>
<td>0.42 ± 0.05</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>CMR</td>
<td>41</td>
<td>47 ± 2†</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>100 ± 11*</td>
<td>0.25 ± 0.05*</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>112 ± 9*</td>
<td>0.25 ± 0.03*</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>CMR</td>
<td>Related to only the 10-Hz rhythm in SND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>67 ± 5‡</td>
<td>0.51 ± 0.08</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>CMR</td>
<td></td>
<td>47 ± 7†</td>
<td>0.16 ± 0.03†‡</td>
</tr>
<tr>
<td></td>
<td>Related to only the cardiac-related rhythm in SND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVLM</td>
<td>65 ± 16</td>
<td>0.14 ± 0.05</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>CMR</td>
<td></td>
<td>150</td>
<td>0.38</td>
</tr>
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</table>

Values are means ± SE; n is number of neurons. STA Lag refers to the interval between unit spike occurrence and peak of the 10-Hz or cardiac-related (CR) slow wave from the spike-triggered average of inferior cardiac sympathetic nerve discharge (SND). Peak Coherence refers to the maximum value near 10 Hz or the CR rhythm in the medullary neuron-SND coherence function. Firing rate refers to the steady state firing rate of the medullary neuron during the run used to establish a relationship with SND. RVLM, rostral ventrolateral medullary; CMR, caudal medullary raphe; STA, spike-triggered average. * Significantly different (P < 0.05) from corresponding value for the 10-Hz rhythm. † Significantly different from corresponding value for RVLM neurons. ‡ Significantly different from corresponding value for neurons with activity correlated to both rhythms in SND.

RVLM AND CMR NEURONS WITH ACTIVITY CORRELATED TO THE CARDIAC-RELATED BUT NOT THE 10-Hz RHYTHM IN SND. The discharges of five RVLM neurons and one CMR neuron were correlated to only the cardiac-related rhythm in SND. The data in Fig. 6 are for one of these RVLM neurons. The cardiac-related rhythms in the discharges of this neuron and SND are evident in the AP-triggered analyses (Fig. 6IC) and autospectra (Fig. 6IA, top and middle). The relationship between these two signals was apparent in the spike-triggered average (Fig. 6IB, top) and by the significant coherence value (0.18) at the frequency of the heart beat in the RVLM-SND coherence function (Fig. 6IA, bottom). When mean arterial pressure was lowered, a 10-Hz rhythm dominated the autospectrum of SND (Fig. 6IIA, top). This manipulation eliminated the relationship between the discharges of this RVLM neuron and the inferior cardiac nerve as indicated by the essentially flat spike-triggered average of SND (Fig. 6IIB) and lack of a statistically significant coherence value in the RVLM-SND coherence function (Fig. 6IIIA, bottom).

Table 1 summarizes the properties of RVLM and CMR neurons with activity correlated to the cardiac-related but not the 10-Hz rhythm in SND. The peak coherence value relating the cardiac-related rhythm in SND to the AP was 0.92 ± 0.02 and 0.93 when recording from these RVLM and CMR neurons, respectively. None of the values listed in Table 1 for RVLM neurons with activity correlated to only the cardiac-related rhythm in SND was significantly different from corresponding values for RVLM neurons with activity correlated to both rhythms. None of these neurons was studied during abrupt increases in blood pressure that produced sympathoinhibition. The recording sites of RVLM and CMR neurons with activity correlated to the cardiac-related but not the 10-Hz rhythm in SND (Fig. 4; triangles) were intermingled with neurons whose discharges were correlated to the 10-Hz rhythm in SND.

Classification of RVLM and CMR neurons based on spinal axonal trajectories

Because most of the RVLM and CMR neurons studied had activity correlated to both the cardiac-related and 10-Hz rhythms, a major site of convergence of the two rhythm generators must be at a supraspinal level. One aim of the next series of experiments was to test the hypothesis that the outputs of the 10-Hz and cardiac-related rhythm generators converge on RVLM and CMR neurons with spinally projecting axons. Ideally, all RVLM and CMR neurons with activity correlated to both rhythms in SND should project to the
in the white matter of the T1 level of the cord were stimulated. In the case of CMR neurons, both sides of the cord were searched. In the case of RVLM neurons, only the left side of the cord (ipsilateral to the neuronal recording site) was searched in most cases because neuroanatomic (see review by Dampney 1994) and electrophysiological (Barman and Gebber 1985) studies have demonstrated a largely ipsilateral projection of RVLM neurons to the IML.

RVLM-SPINAL AND CMR-SPINAL NEURONS WITH ACTIVITY CORRELATED TO BOTH THE 10-Hz AND CARDIAC-RELATED RHYTHMS IN SND. As demonstrated by using the time-controlled collision test, 16 of 18 RVLM and 31 of 34 CMR neurons with activity correlated to both the cardiac-related

spinal cord if this hypothesis is correct. There could also be RVLM and CMR neurons that selectively relay information from one of the two rhythm generators to preganglionic neurons. Thus a second aim of this series of experiments was to determine whether RVLM and CMR neurons with activity correlated to only one rhythm in SND have spinal axons.

The white matter of the T1 spinal cord was stimulated in an attempt to antidromically activate 31 RVLM and 38 CMR neurons with sympathetic nerve-related activity. The existence of a spinal axon was not ruled out until multiple sites

FIG. 5. Relationship between the 10-Hz discharges of an RVLM neuron and inferior cardiac SND at 2 levels of arterial pressure (I and II). A: traces (top to bottom) are AS of SND and RVLM neuronal activity and corresponding coherence function. B: spike-triggered (top) and dummy-triggered (bottom) averages of SND (885 and 534 trials in I and II, respectively). C: arterial-pulse triggered analyses (700 and 694 trials in I and II, respectively). Vertical calibrations are 70 μV in B and 160 in C.

FIG. 6. Relationship between the cardiac-related discharges of an RVLM neuron and inferior cardiac SND. Format same as Fig. 5. Analyses are based on 375, 315, 330, and 417 trials in IB, IC, IIB, and IIC, respectively. Vertical calibrations are 50 μV in B and 170 μV in C.
ing the distance (85 ± 110 mm) between the stimulating and recording microelectrodes by the onset latency of antidromic activation. Figure 8, A and B, shows the distributions of axonal conduction velocities for RVLM-spinal and CMR-spinal neurons, respectively. The mean axonal conduction velocity of RVLM-spinal and CMR-spinal neurons was 2.8 ± 0.6 and 1.9 ± 0.1 m/s, respectively. These values are similar to those for RVLM-spinal and CMR-spinal neurons with activity correlated to the cardiac-related rhythm in SND of barbiturate-anesthetized cats (Barman and Gebber 1985; Morrison and Gebber 1985).

RVLM AND CMR NEURONS WITH ACTIVITY CORRELATED TO ONLY ONE OF THE RHYTHMS IN SND. Only 1 of 10 RVLM neurons and 0 of 4 CMR neurons with activity correlated

and 10-Hz rhythms in SND were antidromically activated by stimulation of the T1 spinal cord. The data in Fig. 7 are for one of these CMR neurons studied at a time when both the cardiac-related (3.8 Hz) and 10-Hz rhythms appeared in the autospectra of SND and CMR neuronal activity (Fig. 7B, top and middle). The cardiac-related rhythms in these signals were also revealed by AP-triggered analyses (Fig. 7D). Both coherence analysis (Fig. 7B, bottom) and spike-triggered averaging (Fig. 7C, top) showed the relationship between CMR neuronal activity and each of the rhythms in SND. Figure 7A shows the antidromic response of this CMR neuron to T1 spinal cord stimulation. This neuron was activated with a constant onset latency of 67 ms when single shocks were applied once every 1.2 s to a site in the T1 dorsolateral funiculus (traces 1–3). A response was not recorded when the interval between the naturally occurring action potential and the stimulus was 69 ms (trace 4), but a stimulus-induced action potential was recorded when the interval was increased to 70 ms (trace 5). This neuron faithfully followed paired stimuli separated by a minimum of 3 ms (trace 6).

There was no significant relationship between the onset latency of antidromic activation and the interval between unit spike occurrence and peak of the 10-Hz slow wave in SND for either RVLM or CMR neurons (r = 0.439 and 0.163 for RVLM and CMR neurons, respectively). Likewise, there was no significant relationship between the onset latency of antidromic activation and the interval between unit spike occurrence and peak of the cardiac-related slow wave in SND for these neurons (r = 0.076 and 0.277 for RVLM and CMR neurons, respectively).

Spinal axonal conduction velocity was estimated by divid-
to only the 10-Hz rhythm in SND could be antidromically activated by stimulation of the white matter at T1. The onset latency of antidromic activation was 9 ms for this one RVLM neuron, and its axonal conduction velocity was 11.1 m/s. None of the three RVLM neurons with activity correlated to only the cardiac-related rhythm in SND was activated by T1 spinal stimulation.

DISCUSSION

The current study provides important new information on central networks responsible for the 10-Hz and cardiac-related rhythms in SND of the cat. First, the data show for the first time that bulbospinal sympathetic pathways emanating from the RVLM and CMR are comprised almost exclusively of neurons whose naturally occurring discharges are correlated to both the cardiac-related and 10-Hz rhythms in SND. Second, the data support the hypothesis that the outputs of the generators of the two rhythms converge on RVLM-spinal and CMR-spinal neurons rather than on antecedent interneurons in the same nuclei. Third, this study is the first to show that there are subgroups of RVLM and CMR neurons with sympathetic nerve–related activity whose axons do not project to the thoracic spinal cord.

Sixteen of 17 RVLM-spinal and all 31 CMR-spinal neurons identified in the current study had activity correlated to both the cardiac-related and 10-Hz rhythms in SND. These data indicate that the generators of these two rhythms use common rather than separate pools of RVLM and CMR neurons to relay information to the spinal cord. However, the data do not rule out the possibility that pathways emanating from other brain regions selectively relay information from only one of the rhythm generators to preganglionic sympathetic neurons. In addition to RVLM and CMR neurons, the thoracolumbar IML is also innervated by neurons in the rostral ventromedial medulla, the caudal pontine A5 noradrenergic cell group, and hypothalamic paraventricular neurons (Strack et al. 1989).

The data available before this study implied that the outputs of the generators of the 10-Hz and cardiac-related rhythms in SND converged at a supraspinal site because some RVLM neurons had activity correlated to both rhythms (Barman and Gebber 1992). The results of the current study are the first to support the hypothesis that the site of convergence of the cardiac-related and 10-Hz rhythm generators is on RVLM-spinal and CMR-spinal neurons rather than on antecedent interneurons in these nuclei. Essentially all of the RVLM (16 of 18) and CMR (31 of 34) neurons with activity correlated to both the cardiac-related and 10-Hz rhythms in SND could be antidromically activated by stimulation of the T1 spinal cord. The fact that a few (5 of 52) neurons with activity correlated to both rhythms were not antidromically activated by T1 spinal cord stimulation was not unexpected. Because it is impossible to stimulate every fiber in the spinal cord while recording from an individual neuron, one should expect to miss the axons of some bulbospinal neurons. Also, the axon of a bulbospinal neuron could have been inadvertently damaged while searching for the axon of another neuron identified earlier in the experiment. Indeed four of these five neurons were studied late in the experiment, after searching for the axonal trajectories of at least five other neurons. Nonetheless, there is one caveat to the claim that the outputs of the two generators converged on bulbospinal neurons. The outputs of the two generators might have actually converged on a group of nonbulbospinal neurons in another region of the brain stem. These neurons in turn might have relayed both cardiac-related and 10-Hz rhythmic discharges to RVLM-spinal and CMR-spinal neurons. Although both the LTF and CVLM contain neurons that project to the RVLM or CMR (Barman and Gebber 1987, 1989; Barman et al. 1995b), these neurons are not the site of convergence of the two generators because their discharges are not correlated to both the cardiac-related and 10-Hz rhythms in SND (Barman and Gebber 1993; Barman et al. 1994). It remains to be determined whether there is any group of nonbulbospinal neurons with activity correlated to both rhythms in SND.

There were marked differences in the proportions of RVLM and CMR neurons whose discharges were correlated to both or only one of the two rhythms in SND. Whereas the overwhelming majority (41 of 48) of CMR neurons with sympathetic nerve–related activity received inputs from both the cardiac-related and 10-Hz rhythm generators, a substantial proportion (20 of 44) of RVLM neurons with sympathetic nerve–related activity received input from only one of the two rhythm generators. It is likely that neurons with activity correlated only to the 10-Hz rhythm in SND were elements of a network involved in the control of SND. This suggestion is based on the findings of Barman et al. (1995a) that the 10-Hz rhythm in SND is not correlated to that in other systems, including the naturally occurring or h carboline-induced 10-Hz rhythm in inferior olivary activity and the 10-Hz spindles in the EEG.

The targets of RVLM and CMR neurons with activity correlated to only the 10-Hz rhythm in SND are unknown. Several possibilities should be entertained in future studies. First, these neurons might have been interposed in pathways from the 10-Hz rhythm generator to RVLM- or CMR-spinal noradrenergic cell group, and hypothalamic paraventricular neurons; i.e., they may have been short-axon intrinsic interneurons. This possibility is consistent with the observation that such RVLM neurons fired significantly earlier during the 10-Hz sympathetic nerve slow wave than RVLM-spinal neurons. Second, RVLM and CMR neurons with activity correlated to only the 10-Hz rhythm in SND may have projected to neurons in other brain regions involved in the control of SND. Barman et al. (1995b) showed that the axons of some CMR neurons with activity correlated to the 10-Hz rhythm in SND of baroreceptor-denervated cats projected to and likely synapsed on their counterparts in the CVLM. Because CVLM neurons with activity correlated to the 10-Hz rhythm never have cardiac-related activity (Barman et al. 1994), it is reasonable to speculate that the same would be true for the CMR neurons that projected to the CVLM. The axons of RVLM neurons with activity correlated to the 10-Hz rhythm in SND do not appear to terminate in the CMR or CVLM (Barman, unpublished observation). However, anatomic and electrophysiological studies indicate that the axons of some RVLM neurons ascend to supramedullary structures purported to have a role in control of cardiovascular function, including the pontine locus coeruleus, A5, and parabrachial regions (Dampney 1994; Haselton and Guyenet 1990). Data are available that suggest that one or more of...
these regions is involved in the control of the 10-Hz rhythm in SND. Specifically, Zhong et al. (1992) showed that pontomedullary border transection eliminated the 10-Hz rhythm in SND. Cohen et al. (1991) reported that <1% of the neurons in the medial portion of the parabrachial nucleus had activity correlated to the 10-Hz rhythm in SND, but whether neurons in any other supramedullary region have such activity has yet to be tested. A third possibility is that the axons of RVLM and CMR neurons with activity correlated to only one of the rhythms in SND might have projected to the cervical spinal cord to innervate propriospinal neurons in pathways that influence SND (Poree and Schramm 1992).

RVLM and CMR neurons with activity correlated to only the cardiac-related rhythm in SND may be elements of the network responsible for this component of SND or interneurons in the afferent limb of the baroreceptor reflex arc. Both types of neurons have been identified in these regions (Barman and Gebber 1985; Morrison and Gebber 1985).

Barman and Gebber (1992) showed that the firing times of RVLM neurons in decerebrate-unanesthetized, baroreceptor-denervated cats were evenly distributed over a range of 15–95 ms before the peak of the 10-Hz slow wave in inferior cardiac SND. Data from the current study suggest that the wide range of firing times of RVLM neurons during the 10-Hz slow wave reflected recordings from different populations of these neurons. Most RVLM neurons with activity correlated to both rhythms in SND (and whose axons projected to the spinal cord) discharged 45–60 ms before the peak of the 10-Hz slow wave in inferior cardiac SND (Fig. 3A). In contrast, the firing times of RVLM neurons with activity correlated to only the 10-Hz rhythm were rather evenly distributed over a wider range (45–105 ms). Thus, as a group, RVLM-spinal neurons fired more synchronously during the 10-Hz slow wave in SND than RVLM neurons whose axons did not project to the spinal cord.

There was not a significant relationship between the onset latency of antidromic activation from the T1 spinal cord and the interval between unit spike occurrence and peak of the 10-Hz or cardiac-related slow waves in inferior cardiac SND for RVLM- and CMR-spinal neurons. Moreover, the intervals between neuronal firings and the peak of the cardiac-related and 10-Hz slow waves for an individual neuron were significantly different (Table 1). Thus these intervals do not provide information on the conduction time in the pathway from the site of medullary recording to the sympathetic nerve. Rather, it shows where in the cycle of peripheral nerve activity the neuron is most apt to fire. Two neurons with the same firing times presumably are activated simultaneously by inputs from the sympathetic rhythm generator; but if their spinal axonal conduction velocities are not the same, they will influence preganglionic sympathetic neurons at different times.

Barman and Gebber (1985) classified RVLM neurons that innervated the IML as sympathoexcitatory because their firing rates decreased during abrupt increases in blood pressure that inhibited SND (i.e., baroreceptor reflex activation). In the current study, the majority of RVLM neurons tested also were inhibited during abrupt increases in blood pressure during aortic obstruction, independent of whether their discharges were correlated to both the cardiac-related and 10-Hz rhythms in SND or to only the 10-Hz rhythm. Morrison and Gebber (1985) classified CMR neurons that innervated the IML as sympathoinhibitory because their firing rates increased during baroreceptor reflex activation. Likewise, in the current study we found that most of the CMR neurons with activity correlated to the 10-Hz rhythm in SND were excited during the inhibition of SND produced by aortic obstruction.

In summary, data from this study have enhanced our understanding of the organization of central circuits responsible for basal SND. The results show for the first time that RVLM-spinal and CMR-spinal sympathetic pathways are comprised almost exclusively of neurons whose discharges are correlated to both the cardiac-related and 10-Hz rhythms in SND. It is likely that the two rhythm generators converge at the level of these bulbospinal neurons rather than on antecedent interneurons.

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