Rostral dorsolateral pontine neurons with sympathetic nerve-related activity

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Barman, Susan M., Gerard L. Gebber, and Heather Kitchens. Rostral dorsolateral pontine neurons with sympathetic nerve-related activity. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H401–H412, 1999.—Spike-triggered averaging, arterial pulse-triggered analysis, and coherence analysis were used to classify rostral dorsolateral pontine (RDLP) neurons into groups whose naturally occurring discharges were correlated to only the 10-Hz rhythm (n = 29), to only the cardiac-related rhythm (n = 15), and to both rhythms (n = 15) in inferior cardiac sympathetic nerve discharge (SND) of urethan-anesthetized cats. Most of the neurons with activity correlated to only the cardiac-related rhythm were located medial to the other two groups of neurons. The firing rates of most RDLP neurons with activity correlated to only the 10-Hz rhythm (9 of 12) or both rhythms (7 of 8) were decreased during baroreceptor reflex-induced inhibition of SND produced by aortic obstruction; thus, they are presumed to be sympathoexcitatory. The firing rates of four of seven RDLP neurons with activity correlated to only the cardiac-related rhythm increased during baroreceptor reflex activation; thus, they may be sympathoinhibitory. We conclude that the RDLP contains a functionally heterogeneous population of neurons with sympathetic nerve-related activity. These neurons could not be antidromically activated by stimulation of the thoracic spinal cord.

baroreceptor reflex; locus coeruleus; parabrachial and K ölliker-Fuse complex; sympathetic rhythms

SYMPATHETIC NERVE DISCHARGE (SND) to a variety of cardiovascular organs of urethan-anesthetized or decerebrate-unanesthetized cats often shows two centrally generated rhythmic (10-Hz and cardiac-related) components (1–9, 15). The rostral ventrolateral medulla (RVLM), caudal medullary raphe (CMR), and caudal ventrolateral pons (CVLP) each contain a heterogeneous population of neurons whose naturally occurring discharges are correlated to SND (1, 3, 4). The largest group of such neurons in each of these regions has activity correlated to both the 10-Hz and cardiac-related rhythms in SND, whereas other neurons have activity correlated to only one of the two rhythms. Importantly, chemical inactivation (muscimol microinjections) of any one of these regions eliminates the 10-Hz rhythm in SND (6, 43). In contrast to these three brain stem regions, the medullary lateral tegmental field (LTF) and caudal ventrolateral medulla (CVLM) do not contain neurons with activity correlated to both rhythms in SND. Rather, the LTF contains neurons with activity correlated to only the cardiac-related rhythm (2, 20), and the CVLM contains neurons whose discharges are correlated to only the 10-Hz rhythm or to only the cardiac-related rhythm (7). The existence of such cell groups supports the hypothesis that the networks responsible for the 10-Hz and cardiac-related rhythms are comprised in part of different pools of brain stem neurons.

There is also evidence that rostral dorsolateral pontine (RDLP) neurons in the vicinity of the parabrachial and K ölliker-Fuse (PB/KF) complex and the locus coeruleus (LC) regulate SND. First, chemical stimulation of this region either decreases or increases blood pressure and SND (13, 17, 23, 32, 33, 41, 42). Second, Barman et al. (6) reported that bilateral chemical inactivation (muscimol microinjections) of the RDLP reversibly blocked the 10-Hz rhythm. Moreover, recordings of local field potentials or from individual neurons in the RDLP showed a 10-Hz component correlated to that in SND (6). Because the single neuron recordings were made in baroreceptor-denervated cats, a decision could not be made as to whether the discharges of any of these RDLP neurons were also correlated to the cardiac-related rhythm in SND. Although muscimol microinjections in the RDLP failed to block the cardiac-related rhythm in SND (6), Sieck and Harper (37) reported that some RDLP neurons of unanesthetized, unrestrained cats have cardiac-related activity.

It is clear that much remains to be learned about RDLP neurons with sympathetic nerve-related activity. With this in mind, the current study was designed to answer the following questions. 1) Are the discharges of individual RDLP neurons correlated to both the 10-Hz and cardiac-related rhythms in SND or to only one of these rhythms? 2) Do RDLP neurons with activity correlated to the 10-Hz and cardiac-related rhythms in SND subserve a sympathoexcitatory or sympathoinhibitory function? 3) Do the axons of RDLP neurons with activity correlated to the 10-Hz and/or cardiac-related rhythm project to the spinal cord? The data obtained indicate that the RDLP contains a heterogeneous population of neurons in terms of their patterns of relationship to SND and their responses to baroreceptor reflex activation. None of the RDLP neurons with sympathetic nerve-related activity had axons that descended to the thoracic spinal cord.

METHODS

General Procedures

The protocols used in these studies on 24 mongrel cats of either sex 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 brachial artery and right femoral vein were cannulated to measure arterial pressure and to administer drugs, respectively. Urethan (1.1–1.8 g kg−1, initial dose) was then administered, and isoflurane inhalation was terminated. Supplemental doses (0.2 g kg−1 of urethan were given every 4–6 h for the duration of the experiment (up to 12 h). The initial dose of urethan has been shown to maintain a surgical level of anesthesia for 8–10 h in cats (19). The frontal-parietal 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 (38, 39). Noxious stimuli (e.g., pinch, cautery) did not change the EEG pattern. As reported by Barman et al. (6, 8), coherence analysis showed that there is no correlation between SND and either the EEG spindles or mean arterial pressure (3, 4, 7). Brachial arterial pressure was increased during the inhibition of SND, it was classified as a sympathoexcitatory neuron. If the firing rate of a neuron was increased during the inhibition of SND, it was classified as a sympathoinhibitory neuron. SND is unaffected by aortic obstruction after complete baroreceptor denervation produced by bilateral section of the carotid sinus, aortic depressor, and vagus nerves (7).

Neural Recordings

The methods used to make monophasic recordings of left inferior cardiac postganglionic SND and the EEG can be found in earlier reports (5, 8). 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 (20). The dorsal surface of the brain stem was exposed by removing portions of the occipital and parietal bones, bony tentorium, and cerebellum. The midline and caudal borders of the inferior colliculi were used as landmarks for placement of the recording microelectrode. The RDLP was explored on the left side (ipsilateral to the nerve recording) 1–2 mm caudal to the inferior colliculus, 2–4.5 mm lateral to the midline, and within 3.5 mm of the dorsal surface. This is the region where chemical inactivation of neurons (muscimol microinjections) reversibly and selectively eliminated the 10-Hz rhythm in SND (6). We recorded extracellularly from single RDLP neurons by using a tungsten microelectrode (FHC; 1-µm tip diameter, ~3-MΩ tip impedance) connected to a hydraulic microdrive (model 650; David Kopf Instruments). Capacity-coupled preamplification with a band pass of 0.1–3 kHz was used. The duration of neuronal action potentials was at least 1.5 ms, and in some 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 (25).

Electrical Stimulation

The upper thoracic spinal cord was exposed by laminectomy and resection of the dura. A bipolar stainless steel semimicroelectrode (Rhodes model SNE-100) was positioned in the T1 spinal segment. 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 the electrode. The electrode was initially positioned ipsilateral to the nerve recording either in the dorsolateral funiculus or in the vicinity of the intermediolateral nucleus (IML). However, the gray and white matter at this level were extensively searched bilaterally in an attempt to identify a site from which an RDLP neuron could be antidromically activated. The criterion for antidromic activation was a response with a constant onset latency that followed high-frequency (~100 Hz) stimulation and collided with spontaneously occurring action potentials (3, 4, 30). After the axon of an RDLP neuron with sympathetic nerve-related activity was located, the stimulating electrode was moved 0.25–0.5 mm further rostral in the T1 segment. Thus failure to antidromically activate a neuron encountered later in the experiment could not be due to damage of its axon during earlier episodes of spinal stimulation.

Data Analysis

Before all analyses on a Zenith 486 Z-Station 510 computer, SND and EEG were low-pass filtered at 100 Hz; the Butterworth analog filter (model 260–5; A.P. Circuit) has unity gain and a roll-off rate of 24 dB/octave. The action potentials of individual RDLP neurons were isolated by using window discrimination (FHC amplitude analyzer) and were converted to a standardized 5-ms square wave pulse (PX-934 Pulse Stretcher; CWE). Data were processed (5-ms sampling interval) with software and an analog-to-digital converter board from R. C. Electronics (Santa Barbara, CA). Time-domain analyses used R. C. Electronics software. Frequency-domain analysis used a modified version (21) of the software of Cohen et al. (16) and Kocsis et al. (29).

Spike-triggered averaging. Standardized pulses representing the action potentials of individual RDLP neurons were used as reference signals to construct averages of SND. A series of randomly generated pulses with the same number and 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 neuron were considered to be correlated to SND if the amplitude of the first peak to the right of time 0 (neuronal spike occurrence) in the spike-triggered average was at least four times that of the largest deflection in the dummy average.
Arterial pulse-triggered analysis. The analysis was triggered when the systolic phase of the arterial pulse (AP) reached a specified pressure level. Averages of the AP and SND and a histogram of RDLP neuronal activity were constructed. The ratio of peak-to-background counts in the histograms was measured; a value of 2:1 was considered to reflect cardiac-related activity in an RDLP neuron. Also, to be classified as a neuron with cardiac-related activity, the histogram had to contain a peak at the same phase of each of the averaged APs.

Frequency-domain analyses. Fast-Fourier transform was performed on 32 5-s windows of data (160 s) to construct autospectra of SND, AP, EEG, and RDLP neuronal activity. Coherence functions relating pairs of these signals were also constructed. Digital low-pass filtering (cut off at 250 Hz) of the standardized pulses representing the action potentials of single neurons 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 (14). 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 > 0.1 reflects a statistically significant relationship when 32 windows are averaged (10). Spectral analyses were done over a frequency band of 0–100 Hz with a resolution of 0.2 Hz/bin. Figures 1–3 in this report show only frequencies ≤20 Hz since at least 90% of the total power in SND was within this band (5).

Statistical Analysis

Data are expressed as means ± SE. A paired t-test was used to compare the firing rate of RDLP neurons before and during baroreceptor reflex activation. The unpaired t-test was used for other analyses. The chi-square test was used to compare the distribution of the three classes of neurons with sympathetic nerve-related activity in the vicinity of the PB/KF complex and LC, and the Fischer’s exact test was used to compare the proportion of neurons with sympathetic nerve-related activity at different rostral-caudal or medial-lateral portions of the RDLP. P < 0.05 indicated statistical significance.

Histology

The brain stem was removed and fixed in 10% buffered Formalin. Frontal sections of 30-µm thickness were cut and stained with cresyl violet. RDLP recording sites were identified with reference to the tracks made with the recording electrodes and the stereotaxic planes of Berman (11).

RESULTS

Classification of RDLP Neurons Based on the Relationship Between Their Discharges and the 10-Hz and Cardiac-Related Rhythms in SND

We recorded from 526 neurons in the RDLP of 24 urethan-anesthetized cats with intact carotid sinus nerves. The autospectra of inferior cardiac postganglionic SND contained a sharp peak near 10 Hz (ranging from 6.8 to 11.0 Hz) and/or a peak at the frequency of the heartbeat (ranging from 2.2 to 4.2 Hz) in these cats. Spike-triggered averaging revealed that the naturally occurring discharges of 89 RDLP neurons had sympathetic nerve-related activity. None of these neurons had activity correlated to the EEG. The Fisher’s exact test showed that the proportion of the total number of neurons sampled that had sympathetic nerve-related activity was significantly greater when the recording microelectrode was positioned 1.7–2.0 mm caudal to the inferior colliculus (82 of 419 neurons) than 1.0–1.6 mm caudal to the inferior colliculus (7 of 107 neurons). We also compared the proportion of RDLP neurons with sympathetic nerve-related activity in the LC and underlying pontine reticular formation (2–3 mm lateral to the midline) with that in the PB/KF complex and surrounding pontine reticular formation (3.2–4.5 mm lateral to the midline). The Fisher’s exact test showed that the proportions of such neurons were similar in the two regions, 35 of a total of 174 neurons in the vicinity of the LC and 54 of a total of 352 neurons in the vicinity of the PB/KF complex. The greater number of neurons with sympathetic nerve-related activity in the vicinity of the PB/KF complex reflects the fact that more tracks (n = 73) were made through this region than the LC region (n = 47).

While recording from 59 of the RDLP neurons with sympathetic nerve-related activity, we were able to determine whether their discharges were correlated to both the 10-Hz and cardiac-related rhythms in SND or to only one of these rhythms. Three types of neurons with sympathetic nerve-related activity were found in the RDLP, sometimes in the same animal. These data are described below.

RDLP neurons with activity correlated to the 10-Hz but not the cardiac-related rhythm in SND. The data in Fig. 1 are for 1 of the 29 RDLP neurons whose discharges were correlated to only the 10-Hz rhythm in SND. At a mean brachial arterial pressure of 92 mmHg, the autospectrum of SND (Fig. 1A, top) contained a sharp peak at 8.8 Hz (i.e., the 10-Hz rhythm) but not at the frequency of the heartbeat (3.4 Hz). There was also a small peak rising out of background at 8.8 Hz in the autospectrum of RDLP neuronal activity (Fig. 1A, middle). The absence of a cardiac-related rhythm in these signals was formally demonstrated by AP-triggered analysis (Fig. 1C); neither the average of SND nor the histogram of RDLP neuronal activity contained peaks time locked to the cardiac cycle. As demonstrated by using coherence analysis (Fig. 1A, bottom), the discharges of this RDLP neuron were significantly correlated to the 10-Hz rhythm in SND; the coherence value at 8.8 Hz was 0.36. The correlation between SND and RDLP neuronal activity was also demonstrated by using spike-triggered averaging (Fig. 1B, top). The average shows inferior cardiac SND for 500 ms before and after RDLP neuronal spike occurrence at time 0. The peaks in the spike-triggered average were regularly spaced at ~115-ms intervals, and their amplitudes greatly exceeded those of the deflections in the corresponding dummy average of SND (Fig. 1B, bottom). The interval between unit spike occurrence and the first peak to the right of time 0 in the average of SND was 40 ms.
When mean brachial arterial pressure was slowly increased to 140 mmHg by partial aortic obstruction, the autospectrum of SND but not RDLP neuronal activity contained a sharp peak at 3.2 Hz, the frequency of the heartbeat (Fig. 1D, top and middle). Although not shown here, the coherence value relating SND to the AP at this frequency was 0.83. The presence of a cardiac-related rhythm in SND is also shown by AP-triggered analysis (Fig. 1F). Note, however, that the AP-triggered histogram of RDLP neuronal activity was flat. Moreover, RDLP neuronal activity and SND were not significantly coherent at the frequency of the heartbeat (Fig. 1D, bottom). Thus this RDLP neuron did not develop cardiac-related activity when this component
was predominant in SND. Although diminished in power, the 10-Hz rhythm persisted in SND at the higher level of arterial pressure (Fig. 1D, top). As demonstrated with spike-triggered averaging (Fig. 1E, top) and coherence analysis (Fig. 1D, bottom), the relationship between RDLP neuronal activity and the 10-Hz rhythm in SND was maintained. The firing rate of this RDLP neuron was decreased from 5.3 to 3.3 spikes/s by slowly raising mean arterial pressure from 92 to 140 mmHg.

We studied 22 of the RDLP neurons with activity correlated to only the 10-Hz rhythm in SND at two levels of arterial pressure. The other seven neurons were studied at only one level of arterial pressure, at which the amplitude of the peak at the frequency of the heartbeat in the autospectra of SND was at least two times as large as that near 10 Hz. Coherence analysis revealed a significant correlation between the discharges of 21 of these RDLP neurons and the 10-Hz rhythm in SND (peak coherence value: 0.20 ± 0.02; range: 0.11–0.36). The relationship between the discharges of the other eight neurons and the 10-Hz rhythm in SND was demonstrated with spike-triggered averaging. The ratio of peak-to-background counts in the AP-triggered histograms of the 29 RDLP neurons with activity correlated to only the 10-Hz rhythm was <1:3:1 when the peak coherence value relating the cardiac-related rhythm in SND to the AP was 0.82 ± 0.01.

RDLP neurons with activity correlated to the cardiac-related but not the 10-Hz rhythm in SND. The data in Fig. 2 are for 1 of the 15 RDLP neurons whose discharges were correlated to only the cardiac-related rhythm in SND. The autospectrum of SND (Fig. 2A, top) contained a sharp peak at the frequency of the heartbeat (4.2 Hz) when mean brachial arterial pressure was 120 mmHg. The peak at this frequency in the autospectrum of RDLP neuronal activity (Fig. 2A, middle) was broader. The cardiac-related rhythm in these two signals was formally demonstrated by AP-triggered analysis (Fig. 2C). Both spike-triggered averaging (Fig. 2B, top) and coherence analysis (Fig. 2A, bottom) showed that the discharges of this RDLP neuron were correlated to the cardiac-related rhythm in SND. The interval between unit spike occurrence and the peak of the cardiac-related slow wave in inferior cardiac SND was 45 ms, and the peak coherence value at the frequency of the heartbeat in the RDLP-SND coherence function was 0.26.

When mean brachial arterial pressure was 85 mmHg, the cardiac-related rhythm in RDLP neuronal activity and SND was eliminated (Fig. 2F). There was now a sharp peak at 10.2 Hz in the autospectrum of SND but not RDLP neuronal activity (Fig. 2D, top and middle). The spike-triggered average (Fig. 2E) failed to reveal a relationship between RDLP neuronal activity and the 10-Hz rhythm in SND, and the coherence value at 10.2 Hz in the RDLP-SND coherence function (Fig. 2D, bottom) was not statistically significant. The firing rate of this RDLP neuron was 3.8 and 3.4 spikes/s when mean arterial pressure was 120 and 85 mmHg, respectively.

We studied 11 of the 15 RDLP neurons with activity correlated to only the cardiac-related rhythm in SND at two levels of arterial pressure. The other four neurons were identified when the 10-Hz rhythm in SND was more prominent than the cardiac-related rhythm, as demonstrated by autospectral analysis. Coherence analysis revealed a significant correlation between the discharges of 13 of these 15 RDLP neurons and the cardiac-related rhythm in SND (peak coherence value: 0.21 ± 0.04; range: 0.10–0.60). The relationship between the discharges of the other two neurons and the cardiac-related rhythm in SND was demonstrated with spike-triggered averaging and AP-triggered analysis. The ratio of peak-to-background counts in the AP-triggered histogram for each of these 15 CVLP neurons was >3:1 at a time when the peak coherence value at the frequency of the heartbeat in the AP-SND coherence function was 0.84 ± 0.04. This coherence value was not significantly different from that relating the AP and SND when recording from RDLP neurons with activity correlated to only the 10-Hz rhythm in SND.

RDLP neurons with activity correlated to both the 10-Hz and cardiac-related rhythms in SND. The data in Fig. 3 are for 1 of 15 RDLP neurons whose discharges were correlated to both rhythms in SND. When mean arterial pressure was 90 mmHg, the autospectra of SND and RDLP neuronal activity (Fig. 3A, top and middle) contained a sharp peak at 8.8 Hz (i.e., the 10-Hz rhythm) but not at the frequency of the heartbeat (3.6 Hz). The absence of a cardiac-related rhythm in these signals was also shown by AP-triggered analysis (Fig. 3C). Both coherence analysis (Fig. 3A, bottom) and spike-triggered averaging (Fig. 3B, top) showed that the discharges of this RDLP neuron were correlated to the 10-Hz rhythm in SND. The coherence value at 8.8 Hz was 0.48, and the interval between RDLP neuronal spike occurrence and the first peak to the right of time 0 in the average of SND was 25 ms.

When mean brachial arterial pressure was slowly raised to 140 mmHg by partial aortic obstruction, a peak at the frequency of the heartbeat replaced the peak at 8.8 Hz in the autospectra of SND and RDLP neuronal activity (Fig. 3D, top and middle). AP-triggered analysis confirmed the existence of a cardiac-related rhythm in both signals (Fig. 3F). The relationship between the cardiac-related discharges of the RDLP neuron and the inferior cardiac nerve was demonstrated with coherence analysis (Fig. 3D, bottom) and spike-triggered averaging (Fig. 3E). The coherence value at the frequency of the heartbeat in the RDLP-SND coherence function was 0.47, and the interval between RDLP neuronal spike occurrence and the first peak to the right of time 0 in the average of SND was 25 ms. The firing rate of this RDLP neuron was 7.8 and 3.6 spikes/s when mean arterial pressure was 90 and 140 mmHg, respectively.

Data were collected at two levels of arterial pressure to show that 13 RDLP neurons had activity correlated to both the 10-Hz and cardiac-related rhythms in SND,
and in two cases only one recording session was needed because both rhythms were prominent in SND. Coherence analysis revealed a significant correlation between the discharges of 14 of these RDLP neurons and the 10-Hz rhythm in SND (peak coherence value: 0.29 ± 0.04; range: 0.12–0.62). The peak coherence value (0.26 ± 0.04; range: 0.13–0.48) at the frequency of the heartbeat in the RDLP-SND coherence function was statistically significant for 12 of these neurons. The ratio of peak-to-background counts in the AP-triggered histograms for each of these 15 RDLP neurons was >3:1 when the peak coherence value relating the
cardiac-related rhythm in SND to the AP was $0.81 \pm 0.05$.

**Recording Sites of RDLP Neurons with Sympathetic Nerve-Related Activity**

Figure 4 shows the distribution of recording sites for the three groups of RDLP neurons with sympathetic nerve-related activity. Most (22 of 29) of the neurons with activity correlated to only the 10-Hz rhythm (Fig. 4A) were located 3.2–4.5 mm lateral to the midline within the PB/KF complex and surrounding pontine reticular formation. The other seven neurons were located 2–3 mm lateral to the midline, within the reticular formation surrounding the LC. Most (13 of 15) of the neurons with activity correlated to both the 10-Hz and cardiac-related rhythms in SND (Fig. 4C)
were also located 3.2–4.5 mm lateral to the midline, within or adjacent to the PB/KF complex. The other two neurons with activity correlated to both rhythms were near the LC. In contrast, most (10 of 15) of the neurons with activity correlated to only the cardiac-related rhythm (Fig. 4B) were in the LC or the surrounding pontine reticular formation 2–3 mm lateral to the midline; the other five neurons were in the vicinity of the PB/KF complex 3.2–4.5 mm lateral to the midline. The chi-square test indicated that the relative distributions of the three types of neurons in the vicinity of the PB/KF complex and in the vicinity of the LC were significantly different. Specifically, 55% of the neurons with sympathetic nerve-related activity in the former region had activity correlated to only the 10-Hz rhythm, 13% had activity correlated to only the cardiac-related rhythm, and 32% had activity correlated to both rhythms. In contrast, in the vicinity of the LC, the corresponding percentages were 37, 53, and 10.

Firing Times of RDLP Neurons During the 10-Hz and Cardiac-Related Slow Waves in SND

The histogram in Fig. 5A shows the distribution of firing times of RDLP neurons relative to the peak of the 10-Hz slow wave in inferior cardiac SND. For each neuron, we measured the interval between unit spike occurrence and the first peak to the right of time 0 in the spike-triggered average of SND. The distribution of intervals was similar for neurons whose discharges were correlated to only the 10-Hz rhythm or to both rhythms, as well as for neurons in the vicinity of the PB/KF complex or in the LC region. Thus the data from 60 RDLP neurons with activity correlated to the 10-Hz rhythm in SND were pooled. This included the data from 16 neurons that could not be tested for cardiac-related activity. The mean interval for the 60 neurons was 56 ± 4 ms. The mean firing rate of these 60 neurons was 3.3 ± 0.3 spikes/s, a value that was significantly lower than the frequency (8.3 ± 0.2 Hz) of the population rhythm recorded from the inferior cardiac nerve.

The histogram in Fig. 5B shows the distribution of firing times of RDLP neurons relative to the peak of the cardiac-related slow wave in inferior cardiac SND. Although 44 RDLP neurons had cardiac-related activity, the interval between unit spike occurrence and the first peak to the right of time 0 in the spike-triggered average of SND could be measured for only 40 neurons. The coexistence of the 10-Hz and cardiac-related components in the spike-triggered average precluded us from measuring the precise interval in the other cases (3, 4). The intervals for the 40 neurons were not normally distributed, and thus a mean value was not calculated. The range of intervals was similar for neurons in the vicinity of the PB/KF complex or in the LC region, as well as for neurons with activity correlated to only the cardiac-related rhythm or to both rhythms in SND. The mean firing rate of RDLP neurons with activity correlated to the cardiac-related rhythm in SND was 2.9 ± 0.3 spikes/s, a value that was not significantly different from the frequency of the heartbeat (3.1 ± 0.1 Hz).

Responses of RDLP Neurons to Baroreceptor Reflex Activation

The firing rates of 27 RDLP neurons with activity correlated to the 10-Hz and/or cardiac-related rhythms in SND were monitored before and during a short period of baroreceptor reflex-induced inhibition of SND produced by rapid aortic obstruction. This included neurons in the vicinity of the PB/KF complex (n = 19) and the LC (n = 8). There was no apparent relationship
between response type (decreased or increased firing rate) and anatomic location.

The firing rates of 9 of 12 neurons with activity correlated to only the 10-Hz rhythm were significantly decreased from $4.5 \pm 1.0$ to $1.6 \pm 0.5$ spikes/s when mean brachial arterial pressure was raised from $96 \pm 6$ to $147 \pm 7$ mmHg. Figure 6A shows an example of baroreceptor reflex-induced inhibition of SND and the discharges of one of these neurons. Note that the inhibition of SND and RDLP neuronal activity occurred in parallel. The firing rates of the other three RDLP neurons with activity correlated to only the 10-Hz rhythm were significantly increased from $5.1 \pm 2.0$ to $5.1 \pm 2.0$ spikes/s during baroreceptor reflex-induced inhibition of SND.

We monitored the responses of seven RDLP neurons whose discharges were correlated to only the cardiac-related rhythm in SND when mean brachial arterial pressure was abruptly increased from $94 \pm 6$ to $147 \pm 13$ mmHg by aortic obstruction. The firing rates of four of these neurons were significantly increased from $2.4 \pm 0.7$ to $2.8 \pm 1.1$ spikes/s, and the firing rates of the other three neurons were significantly decreased from $4.9 \pm 2.8$ to $2.3 \pm 1.3$ spikes/s during the inhibition of SND produced by aortic obstruction. Figure 6B shows an example of baroreflex-induced activation of an RDLP neuron with activity correlated to only the cardiac-related rhythm in SND.

The firing rates of seven of eight RDLP neurons with activity correlated to both the 10-Hz and cardiac-related rhythms in SND were significantly decreased from $3.5 \pm 0.6$ to $1.6 \pm 0.6$ spikes/s when mean brachial arterial pressure was raised from $87 \pm 4$ to $143 \pm 5$ mmHg.
mmHg. The firing rate of the other neuron with activity correlated to both rhythms was unchanged during rapid aortic obstruction.

Spinal Cord Stimulation

We attempted to antidromically activate 26 RDLP neurons with sympathetic nerve-related activity. This included neurons in the vicinity of the PB/KF complex (n = 20) and in the LC region (n = 6). Within the population studied, eight neurons had activity correlated to both the 10-Hz and cardiac-related rhythms in SND, nine neurons had activity correlated to only the 10-Hz rhythm, and four neurons had activity correlated to only the cardiac-related rhythm. The other five neurons were studied only at a time when one of the two rhythms was present in the autospectrum of SND. Despite an extensive bilateral search of both the gray and white matter at the T1 spinal segment, none of these RDLP neurons were antidromically activated by thoracic spinal cord stimulation. That is, none of these neurons responded with a constant onset latency to single shocks (up to 1 mA) applied to the spinal cord. However, during the course of these experiments, we noted responses with a constant onset latency and high following frequencies for other RDLP neurons that were quiescent or whose discharges were not correlated to SND.

**DISCUSSION**

The current study is the first to show that the RDLP contains a heterogeneous population of neurons whose naturally occurring discharges are correlated to inferior cardiac SND. The heterogeneity was expressed both in terms of the patterns of relationship between RDLP neuronal activity and the 10-Hz and cardiac-related rhythms in SND and neuronal responses to baroreceptor reflex activation. The most common neuronal type identified was one whose activity was correlated to only the 10-Hz rhythm and whose firing rate was decreased in response to baroreceptor reflex activation. The fact that such neurons did not develop cardiac-related activity when this component was predominant in SND implies that they represented a group distinct from those whose members had activity correlated to only the cardiac-related rhythm or to both rhythms in SND. This view is supported further by the fact that more than one of the three types of RDLP neurons with sympathetic nerve-related activity could be identified in the same experiment. None of the RDLP neurons with sympathetic nerve-related activity were elements of a pathway that directly influenced spinal preganglionic sympathetic neurons because they could not be antidromically activated by stimulation of the gray or white matter of the T1 spinal segment.

Interestingly, the different groups of neurons with sympathetic nerve-related activity were not uniformly distributed within the RDLP. Two-thirds of the RDLP neurons with activity correlated to only the cardiac-related rhythm were in the vicinity of the LC. In contrast, 76% of the RDLP neurons with activity correlated to only the 10-Hz rhythm and 87% of the neurons with activity correlated to both rhythms were in the PB/KF complex and surrounding pontine reticular formation.

In agreement with the results of other studies (12, 18, 22, 26, 34, 35), some RDLP neurons were inhibited, and others were excited, during baroreceptor reflex activation. However, this is the first study to describe baroreceptor-induced responses for neurons whose naturally occurring discharges were correlated to SND. Moreover, our results show some relationship between the neuronal type and the direction of change in its firing rate during baroreceptor reflex activation. The majority of RDLP neurons with activity correlated to only the 10-Hz rhythm (9 of 12) or to both rhythms (7 of 8) in SND responded with a decrease in firing rate during rapid aortic obstruction. Such neurons are presumed to subserve a sympathoexcitatory function. The reduction in firing rate of neurons with activity correlated to only the 10-Hz rhythm was not surprising because power in the 10-Hz band of SND is reduced during baroreceptor reflex activation. This may reflect a tonic rather than pulse-synchronous component of baroreceptor-induced sympathoinhibition. RDLP neurons with activity correlated to only the cardiac-related rhythm were almost equally divided into those that were excited or inhibited by baroreceptor reflex activation. RDLP neurons that were excited during rapid aortic obstruction are presumed to exert sympathoinhibitory actions because the increase in their firing rate occurred in parallel to the inhibition of SND.

The suggestion that most RDLP neurons with sympathetic nerve-related activity exert sympathoexcitatory actions may seem at odds with the observation that microinjection of L-glutamate in the RDLP most often induces a fall in arterial pressure (13, 17, 32, 33, 41). Perhaps this depressor response reflected the activation of a pool of neurons that were quiescent under basal conditions. Nevertheless, the fact that a fall in blood pressure was noted when the 10-Hz rhythm was eliminated by chemical inactivation of the RDLP (6) supports the view that this region contains a pool of spontaneously active sympathoexcitatory neurons.

Despite the fact that both retrograde and anterograde axonal transport studies have demonstrated a modest projection from the PB/KF complex to the IML (31, 36), none of the neurons with sympathetic nerve-related activity in this region could be antidromically activated by electrical stimulation of the thoracic spinal cord. Such was also the case for neurons with sympathetic nerve-related activity in the vicinity of the LC. Although neurons in the vicinity of the LC project to the spinal cord (27), there is no evidence for a direct projection from this pontine region to the thoracolumbar IML (27, 31, 40). It is unlikely that technical problems prevented us from stimulating the axons of RDLP neurons with sympathetic nerve-related activity because during the course of these experiments other RDLP neurons, including quiescent neurons and neurons whose discharges were not correlated to SND, responded with a constant onset latency and followed
high frequencies of spinal stimulation, features that are consistent with antidromic activation (30). Moreover, after the axon of an RDLP neuron with sympathetic nerve-related activity was located, the stimulating electrode was moved 0.25–0.5 mm further rostral in the T1 segment. Thus failure to antidromically activate a neuron encountered later in the experiment could not be due to damage of its axon during earlier episodes of spinal stimulation. Finally, we have routinely antidromically activated RVLM, CMR, and CVLP neurons with activity correlated to both the 10-Hz and cardiac-related rhythms in SND (3, 4).

The relatively low incidence of RDLP neurons with activity correlated to both the 10-Hz and cardiac-related rhythms in SND compared with those with activity correlated to only one rhythm is in contrast to our findings for RVLM, CMR, and CVLP neurons (3, 4). In each of these regions, >50% of the neurons with sympathetic nerve-related activity had discharges correlated to both rhythms. Moreover, we likely underestimated the proportion of RDLP neurons with activity correlated to only the cardiac-related rhythm in SND because these neurons tended to be located in the vicinity of the LC, and fewer electrode tracks were made through this region. Thus the proportion of RDLP neurons with sympathetic nerve-related activity whose discharges were correlated to only one of the two rhythms in SND is probably even higher than reported here.

The fact that RDLP neurons whose discharges were correlated to both the 10-Hz and cardiac-related rhythms in SND could not be antidromically activated by stimulation of the thoracic spinal cord is also in sharp contrast to the finding that the axons of almost all such RVLM, CMR, and CVLP neurons projected to the thoracic spinal cord (3, 4). The data obtained from these earlier studies support the hypothesis that the outputs of the 10-Hz and cardiac-related rhythm generators converge on bulbospinal neurons. It was reasoned that if the outputs of the generators converged on neurons antecedent to bulbospinal neurons, then one should have been able to identify a substantial pool of neurons whose discharges were correlated to both rhythms but whose axons did not project to the spinal cord. We have now identified such neurons, in the RDLP. These RDLP neurons may be a site of convergence of the outputs of the two independent generators. Alternatively, they may receive input from axon collaterals of bulbospinal neurons in the RVLM, CMR, and/or CVLP whose discharges are correlated to both rhythms in SND. The similarity in firing times of medullary and pontine neurons relative to the peak of the 10-Hz slow wave in inferior cardiac SND (1, 3, 4, 6, 7, 9) makes it difficult to distinguish between these possibilities. Indeed, there is anatomic evidence that RDLP neurons receive input from the RVLM, CMR, and CVLP (see Ref. 17) but whether these connections involve bulbospinal neurons with sympathetic nerve-related activity remains to be determined.

Although RDLP neurons with activity correlated to the 10-Hz rhythm (including those with activity correlated to both rhythms) do not project to the thoracic IML, there are at least two reasons to suggest that they are elements of a pathway that controls SND. First, the 10-Hz rhythm is blocked by chemical inactivation of the RDLP (6). Thus the integrity of this region is required for the expression of the 10-Hz rhythm in SND. Second, the 10-Hz rhythm in SND is not correlated to that in other systems, including the naturally occurring or harmaline-induced 10-Hz rhythm in inferior olivary activity (8) and the 10-Hz spindles in the EEG (6–8 and this study). Thus Barman et al. (8) suggested that the 10-Hz rhythm in SND reflects the organization of a brain stem network that specifically governs SND. Additional studies are needed to determine how RDLP neurons with activity correlated to the 10-Hz rhythm are linked with other such brain stem neurons. For example, neurons in the RDLP project to the region of the RVLM that innervates the IML (see Ref. 17). Such studies may help us to understand the precise role of RDLP neurons in the control of SND.

The functions of RDLP neurons with activity correlated to only the cardiac-related rhythm remain to be defined. This region is not critical for the expression of this component of SND because chemical inactivation of the RDLP does not disrupt the cardiac-related rhythm in SND (6). RDLP neurons with activity correlated to only the cardiac-related rhythm in SND may relay information from the baroreceptors to other brain regions. There are numerous studies that have proposed such a role for RDLP neurons (17, 24, 28).

As is the case for RVLM, CMR, CVLM, and CVLP neurons (1, 3, 4, 7, 9), the firing rates of RDLP neurons with activity correlated to the 10-Hz rhythm were considerably lower than the frequency of the population rhythm in SND. This further supports the view that the 10-Hz rhythm is an emergent property of a distributed network comprised of neurons of different types, none of which may be inherent pacemakers (1).

We thank P. Nick Regis and Shannon M. Sykes for technical assistance.

This study was supported by National Heart, Lung, and Blood Institute Grant HL-33266.

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Received 6 August 1998; accepted in final form 6 October 1998.

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