Classification of Caudal Ventrolateral Pontine Neurons With Sympathetic Nerve-Related Activity

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Barman, Susan M. and Gerard L. Gebber. Classification of caudal ventrolateral pontine neurons with sympathetic nerve-related activity. J. Neurophysiol. 80: 2433–2445, 1998. This study was designed to answer three questions concerning caudal ventrolateral pontine (CVLP) neurons whose naturally occurring discharges are correlated to sympathetic nerve discharge (SND). 1) What are the proportions of CVLP 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) Do CVLP neurons with activity correlated to the cardiac-related and/or 10-Hz rhythm in SND subserve a sympathoexcitatory or sympathoinhibitory function? 3) Do CVLP neurons with activity correlated to the cardiac-related and/or 10-Hz rhythm in SND project to the thoracic spinal cord? To address these issues we recorded from 476 CVLP neurons in 24 urethan-anesthetized cats. Spike-triggered averaging, arterial pulse-triggered analysis, and coherence analysis revealed that the discharges of 66 of these neurons were correlated to inferior cardiac postganglionic SND. For 39 of these neurons, we were able to determine whether their discharges were correlated to one or both rhythms. The results showed that the CVLP contained a heterogeneous population of neurons with sympathetic nerve-related activity. The discharges of 21 neurons were correlated to both the 10-Hz and cardiac-related rhythms in SND, 9 neurons had activity correlated to only the 10-Hz rhythm, and 9 neurons had activity correlated to only the cardiac-related rhythm. The firing rates of CVLP neurons with activity correlated to both rhythms or to only the 10-Hz rhythm were increased during the inhibition of SND induced by baroreceptor reflex activation (rapid obstruction of the abdominal aorta). These neurons are presumed to exert sympathoexcitatory actions. The time-controlled collision test verified that 11 of 12 CVLP neurons with activity correlated to both rhythms were antidromically activated by stimulation of the first thoracic segment of the spinal cord. Antidromic mapping at this level showed that the site requiring the least stimulus current to elicit the longest latency response (nearest the terminus) was in the vicinity of the intermediolateral nucleus (IML). In contrast, only 1 of 13 CVLP neurons with activity correlated to only one of the rhythms in SND could be antidromically activated by spinal stimulation. These data demonstrate for the first time that there is a direct pathway from the CVLP to the IML that is comprised almost exclusively of sympathoexcitatory neurons whose discharges are correlated to both the 10-Hz and cardiac-related rhythms in SND.

INTRODUCTION

Two centrally generated rhythms (the 10-Hz and cardiac-related) often coexist in the discharges of sympathetic nerves that control a variety of cardiovascular targets in urethan-anesthetized or decerebrate-unanesthetized cats (Barman and Gebber 1997; Barman et al. 1992, 1994; Cohen and Gootman 1970). Evidence is available to support the hypotheses that the two rhythms are generated by different pools of brain stem neurons (Barman and Gebber 1993; Barman et al. 1994) and that the outputs of the two generators converge on bulbospinal neurons in the rostral ventrolateral medulla (RVLM) and caudal medullary raphe (CMR) (Barman and Gebber 1997).

Whereas the studies cited previously dealt with medullary neurons, there is also evidence that caudal ventrolateral pontine (CVLP) neurons play a role in control of sympathetic nerve discharge (SND). First, l-glutamate-induced activation of neurons in this region produces an increase in splanchnic sympathetic nerve activity in anesthetized rats (Huangfu et al. 1992). Second, anatomic studies identified a direct projection from this region to the intermediolateral nucleus (IML) of the thoracic spinal cord of the cat (Miura et al. 1983) and rat (Strack et al. 1989). Third, Guyenet and colleagues (Byrum et al. 1984; Huangfu et al. 1991) recorded from neurons in the A5 region of the CVLP of the rat whose axons appeared to terminate in the vicinity of the IML and whose firing rates were inhibited during baroreceptor reflex activation. The naturally occurring discharges of a few of these neurons showed a cardiac-related rhythm. Fourth, Barman et al. (1997) reported that the CVLP contains neurons that are essential for the expression of the 10-Hz rhythm in SND of baroreceptor-denervated cats. Specifically, chemical inactivation (muscimol microinjections) of the CVLP at the level of the lateral nucleus of the superior olive blocked the 10-Hz rhythm in SND. Moreover, recordings of local field potentials or from individual neurons in this region showed a 10-Hz component correlated to that in SND. Because these recordings were made in baroreceptor-denervated cats, a decision could not be made as to whether the discharges of any of these CVLP neurons were also correlated to the cardiac-related rhythm in SND, nor could we determine whether CVLP neurons with activity correlated to the 10-Hz rhythm in SND exert sympathoexcitatory or sympathoinhibitory actions in the cat. Also, no attempt was made to antidromically activate these neurons by electrical stimulation of the spinal cord. The current study was designed to deal with these important unresolved issues. The data obtained indicate that ~14% of the CVLP neurons studied at the level of the lateral nucleus of the superior olive had activity correlated to SND. These neurons were heterogeneous in terms of their patterns of relationship to SND. Whereas the discharges of most of these CVLP neu-
rons were correlated to both the 10-Hz and cardiac-related rhythms in SND, other neurons had activity correlated to only one of the two rhythms. Most of these CVLP neurons appear to subserve a sympathoexcitatory function because their firing rates were decreased during the inhibition of SND induced by baroreceptor reflex activation. With one exception, only those neurons whose discharges were correlated to both rhythms could be antidromically activated by spinal cord stimulation.

**METHODS**

**General procedures**

The protocols used in these studies on 24 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 brachial artery and femoral vein were cannulated to measure arterial pressure and to administer drugs, respectively. 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 initial dose of urethan has been shown to maintain a surgical level of anesthesia for 8–10 h in cats (Fleckenell 1987). The frontal-parietal electroencephalogram (EEG) showed a mixture of 7- to 13-Hz spindles and δ-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, cautery) did not change the EEG pattern. As reported by Barman et al. (1995a, 1997), coherence analysis showed that there is no correlation between SND and either the EEG spindles or δ-slow waves in 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 sectioned bilaterally in all cats, but the carotid sinus nerves remained intact. Under these conditions, the pattern of SND is dependent on the level of mean arterial pressure (Barman and Gebber 1997; Barman et al. 1994). Brachial arterial pressure was increased above the resting level by slowly inflating the balloon-tipped end of a Fogarty embolectomy catheter (4F) that was placed in the abdominal aorta via the left femoral artery (partial aortic obstruction). In the current study when mean brachial arterial pressure was 80 ± 3 (mean ± SE) mmHg, the 10-Hz rhythm in SND coexisted with irregular oscillations primarily at frequencies ≤6 Hz; the cardiac-related rhythm was weak or absent under these conditions. When mean brachial arterial pressure was 96 ± 3 mmHg, SND contained a mixture of the 10-Hz and cardiac-related rhythms; when mean brachial arterial pressure was 132 ± 4 mmHg, essentially all of the power in SND was in a narrow band surrounding a peak at the frequency of the heartbeat.

To study the effect of baroreceptor reflex activation on CVLP neurons, the abdominal aorta was abruptly obstructed for 5–10 s by rapid inflation of the balloon-tipped end of the Fogarty embolectomy catheter. This procedure inhibited SND. The firing rates of CVLP neurons were compared before and during aortic obstruction. If the firing rate of a CVLP neuron was decreased in parallel to 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 (Barman et al. 1994).

**Neural recordings**

The methods used to make monophasic recordings of 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 surface of the brain stem was exposed by removing portions of the occipital and parietal bones and cerebellum. The obex and midline were used as landmarks for placement of the recording microelectrode. The CVLP was explored on the left side at the level of the lateral nucleus of the superior olive, 7.5–9 mm rostral to the obex, 3.5–4.5 mm lateral to the midline, and ≤3.0 mm of the ventral surface. This is the same region where muscimol microinjections blocked the 10-Hz rhythm in SND (Barman et al. 1997). We recorded extracellularly from single CVLP neurons by using a tungsten microelectrode (FHC; 1-μm tip diam, ~3-MΩ tip impedance) connected to a hydraulic microdrive (David Kopf Instruments, model 650). Capacity-coupled preamplification with a band-pass of 0.1–3 kHz was used. The reference electrode was a clip placed on crushed muscle overlying the skull. The duration of neuronal action potentials was ≈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 (Humphrey and Schmidt 1990).

**Electrical stimulation**

The upper thoracic spinal cord was exposed by laminectomy and resection of the dura. Either a bipolar stainless steel electrode (Rhodes model SNE-100) or a tungsten microelectrode (FHC; tip impedance, 10–30 MΩ) was positioned into the T1 spinal segment. In the latter case an indifferent electrode was placed on crushed muscle surrounding the vertebral column. A Grass S8800 stimulator and PSIU-6 constant current unit were used to deliver cathodal square-wave pulses (0.5–ms duration) through the electrode. The electrode was initially positioned ipsilateral to the nerve recording either in the dorsolateral funiculus (DLF) or in the vicinity of the IML. However, the gray and white matter of the T1 spinal segment were extensively searched bilaterally to identify sites from which a CVLP neuron could be activated with a constant onset latency.

**ANTIDROMIC ACTIVATION.** Time-controlled collision of spontaneous and stimulus-induced action potentials was used as a test for antidromic activation (Barman and Gebber 1985, 1997; 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 elicited a constant latency 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.

In some cases after determining the threshold current for eliciting an antidromic response of a neuron, the stimulus intensity was increased in steps to ≈1 mA. We recorded the onset latency of antidromic activation at each stimulus intensity. A current-dependent reduction in the onset latency of antidromic activation suggests the presence of an axonal branch in the vicinity of the stimulating electrode (Barker et al. 1971; Barman and Gebber 1985; Barman et al. 1995b; Morrison and Gebber 1985). The longer-latency response evoked by threshold current presumably reflects slowed
conduction velocity in the axonal branch (Jankowska and Roberts 1972; Lipski 1981).

DEPTH-THRESHOLD CURVES. In three cases, antidromic mapping of the T1 gray and white matter was used to identify sites requiring the least stimulus current to antidromically activate the CVLP neuron. The stimulating microelectrode was moved in 200-μm increments from the ventral to dorsal surface of the spinal cord in tracks separated by 0.3–0.5 mm. At each site stimulated we recorded the threshold current for antidromic activation, the antidromic response latency, and the depth of the electrode tip below the dorsal surface. Depth-threshold curves were constructed from these data. The site in the gray matter requiring the least stimulus current to elicit the longest latency antidromic response is considered to be a site nearest the axonal terminal (Barman and Gebber 1985; Morrison and Gebber 1985). The site in the white matter requiring the least stimulus current to elicit the shortest latency antidromic response is considered to be a site nearest the main axon.

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 (A. P. Circuit, model 260-5) has unity gain and a roll-off rate of 24 db/octave. The action potentials of individual CVLP neurons were isolated using window discrimination (FHC amplitude analyzer) and represented by 5-ms square-wave pulses (CWE, PX-934 Pulse Stretcher). Data were processed (5-ms sampling interval) with software and an A/D convertor board from R. C. Electronics (Santa Barbara, CA). Time-domain analyses used R. C. Electronics software. Frequency-domain analysis used a modified version (Barman et al. 1995b; Gebber et al. 1994) of the software of Cohen et al. (1987) and Kocsis et al. (1990).

SPIKE-TRIGGERED AVERAGING. Standardized square-wave pulses representing the action potentials of single pontine 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. Averages of the arterial pulse (AP) and SND and a histogram of pontine neuronal activity were constructed by using a point on the systolic phase of the AP as the reference signal. The ratio of peak-to-background counts in the histograms was measured; a ratio of 2:1 was considered to reflect cardiac-related activity in a CVLP 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 APs in the average.

FREQUENCY-DOMAIN ANALYSES. Fast Fourier transform was performed on 32 5-s windows (160-s data block) to construct autospectra of SND, AP, EEG, and CVLP single neuronal activity. Coherence functions relating pairs of these signals were also constructed. Digital low-pass filtering (cutoff at 250 Hz) of the standardized pulses representing the action potentials of single neurons was performed by convolving the trains with a sine 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 1 in the case of a perfect linear relationship and 0 if two signals are unrelated. A coherence value ≥0.1 was considered to reflect a statistically significant relationship when 32 windows were averaged (Benignus 1970). Spectral analysis was 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 ≤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 ± SE. A paired t-test was used to compare the firing rates of CVLP neurons before and during baroreceptor reflex activation. Other comparisons used the unpaired t-test. $P \leq 0.05$ indicated statistical significance.

Histology

The brain stem and upper thoracic spinal cord were removed and fixed in 10% buffered formalin. Frontal sections of 30-μm thickness were cut and stained with cresyl violet. CVLP neuronal recording sites were identified with reference to the tracks made with the recording microelectrode and the stereotaxic planes of Berman (1968). Sites in the T1 spinal segment from which CVLP neurons were antidromically activated were also identified from tracks made with the stimulating electrode.

RESULTS

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

We recorded from 476 neurons in the CVLP at the level of the lateral nucleus of the superior olive in 24 urethane-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 11.4 Hz) and/or a peak at the frequency of the heart beat (ranging from 2 to 3.6 Hz) in these cats. Spike-triggered averaging revealed that the discharges of 66 CVLP neurons were correlated to SND; none of these neurons had activity correlated to the EEG. While recording from 39 of these neurons we were able to assess whether their discharges were correlated to both or only one of the two rhythms in SND. The results of these experiments follow. Three classes of neurons with sympathetic nerve-related activity were identified in the CVLP, sometimes in the same experiment.

CVLP NEURONS WITH ACTIVITY CORRELATED TO BOTH THE 10-HZ AND CARDIAC-RELATED RHYTHMS IN SND. The discharges of 21 CVLP neurons were correlated to both rhythms in SND. In 13 cases, data were collected at two levels of arterial pressure (one at which the 10-Hz rhythm was predominant in SND and one at which the cardiac-related rhythm was predominant) to demonstrate the correlation of CVLP neuronal activity to both rhythms in SND. In the other eight cases, only one recording session was needed because both rhythms were prominent in SND.

The data in Fig. 1 are for one of the CVLP neurons studied at two levels of arterial pressure. During the first recording session, the mean arterial pressure was 82 mmHg, and the autospectra of SND and CVLP neuronal activity (Fig. 1A, top and middle) contained a sharp peak at 8.2 Hz (i.e., the 10-Hz rhythm) but not at the frequency of the heart beat (3.2 Hz). 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 CVLP neuronal activity contained peaks time-locked to the phases of the cardiac cycle. As demonstrated by using coherence analysis (Fig. 1A, bottom), the 10-Hz rhythms in SND and CVLP neuronal activity were strongly correlated; the coherence value at 8.2 Hz was 0.76. The correlation between SND and CVLP neuronal activity was also demonstrated by using spike-triggered averaging (Fig. 1B, top). This average shows inferior cardiac SND for 500 ms before and after CVLP neuronal spike occurrence at time 0. The peaks in the spike-triggered average were regularly spaced at ~120-ms intervals, and their amplitudes greatly exceeded those in the corresponding “dummy” average of SND (Fig. 1B, bottom). The interval between CVLP neuronal spike occurrence and the first peak to the right of time 0 in the average of SND was 50 ms.

Slowly raising mean blood pressure to 112 mmHg (partial aortic obstruction) produced a cardiac-related rhythm in SND (see the autospectrum of SND in Fig. 1D, top, and the AP-triggered average of SND in Fig. 1F). This procedure also induced a cardiac-related rhythm in the discharges of the CVLP neuron; this is most evident in the AP-triggered histogram in Fig. 1F. There is also a small peak at 3.2 Hz rising out of background power in the autospectrum of CVLP neuronal activity (Fig. 1D, middle). Because SND still contained a 10-Hz rhythm (although diminished in power), the spike-triggered average (Fig. 1E, top) and coherence function (Fig. 1D, bottom) showed evidence of a relationship of CVLP neuronal activity to both rhythms. The presence...
arterial pressure from 82 to 112 mmHg. started that the 10-Hz discharges of the CVLP neuron and slow wave in inferior cardiac SND. The firing rate of this neuronal activity (Fig. 4) unit spike occurrence and the peak of the cardiac-related was a weak cardiac-related rhythm in SND but not in CVLP coherence value relating the cardiac-related rhythm in SND was 0.86

{\text{range: 0.11 ± 0.75}}$. The peak coherence value (0.22 not signiﬁcantly different from the corresponding value for spikes / s, when mean arterial pressure was raised from 82 to 112 mmHg.

Figure 2A (●) shows the recording sites of CVLP neurons with activity correlated to both the 10-Hz and cardiac-related rhythms in SND. These neurons were located at the level of the lateral nucleus of the superior olive, ±2.7 mm of the ventral surface of the pons. This distribution is similar to that reported for single neuron and population recording sites with activity correlated to the 10-Hz rhythm in SND of baroreceptor-denervated cats (Barman et al. 1997).

Coherence analysis revealed a signiﬁcant correlation between the discharges of 19 of these CVLP neurons and the 10-Hz rhythm in SND (peak coherence value: 0.30 ± 0.05; range: 0.11–0.75). The peak coherence value (0.25 ± 0.05; range: 0.10–0.48) at the frequency of the heart beat in the CVLP–SND coherence function was ≥0.1 for only 10 of these neurons. Despite the lack of a signiﬁcant coherence value in many cases, these neurons were deﬁned as having cardiac-related activity based on AP-triggered histograms. The ratio of peak-to-background counts in the histograms for each of these 21 CVLP neurons was >3:1. The peak coherence value relating the cardiac-related rhythm in SND to the AP was 0.84 ± 0.03 at a time when this rhythm was predominant.

The change in ﬁring rate of nine CVLP neurons with activity correlated to both the 10-Hz and cardiac-related rhythms in SND was monitored during a short period of baroreceptor reﬂex-induced inhibition of SND produced by rapid aortic obstruction. The ﬁring rates of these neurons were signiﬁcantly decreased, from 2.8 ± 0.6 to 0.8 ± 0.2 spikes / s, when mean arterial pressure was raised from 82 ± 2 to 138 ± 4 mmHg. Figure 3 shows an example of baroreﬂex-induced inhibition of a CVLP neuron with activity correlated to both rhythms in SND.

CVLP NEURONS WITH ACTIVITY CORRELATED TO THE 10-HZ BUT NOT THE CARDIAC-RELATED RHYTHM IN SND. The data in Fig. 4 are for one of the nine CVLP neurons whose discharges were correlated to only the 10-Hz rhythm in inferior cardiac SND. When the mean arterial pressure was 80 mmHg, the autospectra of SND and CVLP neuronal activity (Fig. 4A, top and middle) contained a sharp peak at 8.0 Hz (the 10-Hz rhythm). AP-triggered analysis showed that there was a weak cardiac-related rhythm in SND but not in CVLP neuronal activity (Fig. 4C). Spike-triggered averaging (Fig. 4B, top) and coherence analysis (Fig. 4A, bottom) demonstrated that the 10-Hz discharges of the CVLP neuron and inferior cardiac nerve were signiﬁcantly correlated. When arterial pressure was slowly raised to 135 mmHg by partial aortic obstruction, a strong cardiac-related rhythm replaced the 10-Hz rhythm in SND but not in CVLP neuronal activity (Fig. 4, D and F).

The relationship between CVLP neuronal activity and SND was eliminated as shown by the essentially flat spike-triggered average of SND (Fig. 4E, top) and the absence of a signiﬁcant coherence between CVLP neuronal activity and SND (Fig. 4D, bottom).

Six of the nine CVLP neurons with activity correlated to only the 10-Hz rhythm in SND were studied at two levels of arterial pressure. The other three were studied at only one level of arterial pressure at which the amplitude of the peak at the frequency of the heart rate in the autospectra of SND was at least twice as large as that near 10 Hz. The ratio of peak-to-background counts in the AP-triggered histograms of the nine CVLP neurons with activity correlated to only the 10-Hz rhythm was <1.2:1 when the peak coherence value relating the cardiac-related rhythm in SND to the AP was 0.86 ± 0.04. This latter value was not signiﬁcantly different from that relating SND and the AP when recording from CVLP neurons with activity correlated to both rhythms in SND.

The peak coherence value (0.22 ± 0.06; range: 0.11–0.60) near 10 Hz in the CVLP-SND coherence function was statistically signiﬁcant for eight of the nine neurons with activity correlated to only the 10-Hz rhythm. This value was not signiﬁcantly different from the corresponding value for CVLP neurons with activity correlated to both rhythms in SND. The recording sites of CVLP neurons with activity correlated to the 10-Hz but not the cardiac-related rhythm in SND (Fig. 2B, ▲) were intermingled with those whose discharges were correlated to both rhythms in SND.

We recorded the effect of baroreceptor reﬂex activation produced by rapid aortic obstruction on the ﬁring rates of four CVLP neurons with activity correlated to only the 10-Hz rhythm in SND. Their ﬁring rates were signiﬁcantly decreased from 3.7 ± 1.1 to 0.9 ± 0.6 spikes / s during the
inhibition of SND produced by raising mean arterial pressure from 80 ± 2 to 134 ± 6 mmHg.

CVLP neurons with activity correlated to the cardiac-related but not the 10-Hz rhythm in SND. The data in Fig. 5 are for one of the nine CVLP neurons whose discharges were correlated to only the cardiac-related rhythm in SND. As demonstrated by autospectral (Fig. 5A, top and middle) and AP-triggered analyses (Fig. 5C), a cardiac-related rhythm was evident in both CVLP neuronal activity and SND at a mean arterial pressure of 140 mmHg. Spike-triggered averaging (Fig. 5B, top) and coherence analysis (Fig. 5A, bottom) showed a correlation between the cardiac-related rhythms in these two signals. The coherence value relating CVLP neuronal activity and SND at the frequency of the heartbeat (2.8 Hz) was 0.26. When mean arterial pressure was 98 mmHg, there was a sharp peak at 8.0 Hz (i.e., the 10-Hz rhythm) in the autospectrum of SND (Fig. 5D, top) but not in the autospectrum of CVLP neuronal activity (Fig. 5D, middle). The spike-triggered average of SND (Fig. 5E) failed to reveal a significant relationship between CVLP neuronal activity and the 10-Hz rhythm in SND, and the coherence value at 8.0 Hz was not statistically significant in the CVLP–SND coherence function (Fig. 5D, bottom). In this particular example, a cardiac-related rhythm persisted in the discharges of the CVLP neuron and inferior cardiac nerve at the lower level of arterial pressure as shown by AP-triggered analysis (Fig. 5E). Both spike-triggered averaging and coherence analysis showed a weaker relationship between the cardiac-related rhythms in these signals. The autospectrum of CVLP neuronal activity did not contain a peak at the frequency of the heartbeat when mean arterial pressure was 98 mmHg, probably because its mean firing rate was only 1.5 spikes/s compared with 2.5 spikes/s when mean arterial pressure was 140 mmHg.

Six of the nine neurons identified as having discharges correlated to only the cardiac-related rhythm were actually identified at a time when the 10-Hz rhythm in SND was more prominent than the cardiac-related rhythm, as demonstrated by autospectral analysis. The peak coherence value at the frequency of the heartbeat in the CVLP-SND coherence function was 0.27 ± 0.05 (range: 0.11–0.53) for these nine CVLP neurons, and the corresponding value in the AP-SND coherence function was 0.80 ± 0.09. These values were not significantly different from those obtained while recording from CVLP neurons with activity correlated to both rhythms in SND. The ratio of peak-to-background counts in the AP-triggered histogram was >4:1 for the CVLP neurons with activity correlated to only the cardiac-related rhythm. The recording sites of CVLP neurons with activity correlated to only the cardiac-related rhythm in SND (Fig. 2B, ▼) were generally intermingled with other CVLP neurons with sympathetic nerve-related activity. However, most of these neurons were located dorsal to those whose discharges were correlated to only the 10-Hz rhythm in SND.

We assessed the responses of three CVLP neurons whose discharges were correlated to the cardiac-related but not the 10-Hz rhythm in SND to abrupt increases in arterial pressure (from 81 ± 5 to 137 ± 7 mmHg) produced by aortic obstruction. One neuron showed an increased firing rate, one showed a decreased firing rate, and one did not change its firing rate when SND was completely inhibited.

**Firing times of CVLP neurons during the 10-Hz and cardiac-related slow waves in SND**

The histogram in Fig. 6A shows the distribution of firing times of 52 CVLP neurons relative to the peak of the 10-Hz slow wave in inferior cardiac SND. The interval between unit spike occurrence and the peak of the 10-Hz slow wave to the right of time 0 was measured from the spike-triggered average of SND. The distribution of intervals was similar for neurons whose discharges were correlated to both rhythms or to only the 10-Hz rhythm; thus the data from 52 CVLP neurons whose activity correlated to the 10-Hz rhythm in SND were pooled. This includes the data from 22 neurons that were not tested for cardiac-related activity. The mean interval for the 52 neurons was 69 ± 3 ms. This value is similar to that for CVLP neurons with activity correlated to the 10-Hz rhythm in SND of baroreceptor-denervated cats (Barman et al. 1997). The mean firing rate of these 52 neurons was 3.2 ± 0.3 spikes/s, a value that was significantly lower than the frequency (8.2 ± 0.1 Hz) of the population rhythm recorded from the inferior cardiac nerve.

The histogram in Fig. 6B shows the distribution of firing
times of CVLP neurons relative to the peak of the cardiac-related slow wave in inferior cardiac SND, as determined from spike-triggered averages. Although AP-triggered analysis showed evidence for a cardiac-related rhythm in the discharges of 35 CVLP neurons, the interval between unit spike occurrence and the peak of the cardiac-related slow wave in SND could be measured for only 27 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. The intervals for the 27 neurons were not normally distributed; thus a mean value was not calculated. The mean firing rate of CVLP neurons with activity correlated to the cardiac-related rhythm in SND was 2.5 ± 0.3 spikes/s, a value that was not significantly different from the frequency of the heartbeat (2.8 ± 0.1 Hz).

Spinal axonal projections of CVLP neurons with sympathetic nerve-related activity

The gray and/or white matter of the T1 spinal cord was electrically stimulated in an effort to antidromically activate 12 CVLP neurons whose discharges were correlated to both rhythms in SND, six neurons whose discharges were correlated to only the 10-Hz rhythm, and seven neurons whose discharges were correlated to only the cardiac-related rhythm.

As demonstrated by using the time-controlled collision test, the axons of 11 of 12 CVLP neurons with activity correlated to both rhythms projected to the thoracic spinal cord. In all cases the axonal trajectory was ipsilateral to the recording site. Despite an extensive search at the T1 spinal segment, none of the six CVLP neurons with activity corre-
related to only the 10-Hz rhythm, and only one of the seven CVLP neurons with activity correlated to only the cardiac-related rhythm could be antidromically activated. Figure 7A shows the antidromic responses of a CVLP neuron whose discharges were correlated to both the 10-Hz and cardiac-related rhythms in SND to stimuli applied to a site in the T1 DLF. This neuron was activated with a constant onset latency of 36.5 ms when single shocks were applied once per second to the DLF (Fig. 7, traces 1–3) and faithfully followed paired stimuli separated by a minimum of 5 ms (trace 6). Thus the critical delay for antidromic activation was 41.5 ms. This neuron was indeed antidromically activated because a response was recorded when the interval between the naturally occurring action potential and the stimulus was 41.5 ms (Fig. 7, trace 5) but not 40.5 ms (trace 4).

Eight CVLP neurons with sympathetic nerve-related activity were antidromically activated by stimuli applied in the vicinity of the IML. We identified current-dependent shifts in the onset latency of antidromic activation for six of these neurons. Onset latencies of antidromic activation decreased from 45 ± 4 to 37 ± 5 ms by raising the stimulus current to 600 µA shortened the latency to 45 ms. We mapped the T1 spinal segment to identify low-thresh-
Although we did not extensively map the white matter to identify the location of the main axons of CVLP neurons with sympathetic nerve-related activity, four neurons were antidromically activated by low-intensity (≤300 μA) stimulation of the DLF, and three other neurons were antidromically activated by low-intensity stimulation of the medial portion of the ventral funiculus. The CVLP neuron with activity correlated to only the cardiac-related rhythm in SND was one of the neurons whose axon appeared to traverse the ventral funiculus.

Spinal axonal conduction velocity was estimated by dividing the distance (85 ± 105 mm) between the stimulating electrode in the spinal cord and the recording microelectrode in the CVLP by the onset latency of antidromic activation of the neuron. The shortest latency antidromic response was used to make this measurement. Figure 9 shows the estimated spinal axonal conduction velocities of the 12 CVLP neurons that were antidromically activated by thoracic spinal cord stimulation. The mean axonal conduction velocity was 2.3 ± 0.2 m/s.

**DISCUSSION**

We identified a direct pathway from the CVLP to the thoracic spinal cord comprised of neurons whose discharges are correlated to both the 10-Hz and cardiac-related rhythms in SND. Whereas bulbospinal neurons with activity correlated to both rhythms in SND were identified in two regions (RVLM and CMR) of the medulla (Barman and Gebber 1997), the current study is the first to demonstrate the presence of such neurons in the pons. Like their counterparts in the RVLM (Barman and Gebber 1985, 1997), CVLP-spinal neurons appear to subserve a sympathoexcitatory function because their firing rates were decreased in parallel to SND during baroreceptor reflex activation. In contrast, the CMR-spinal pathway is comprised primarily of sympathoinhibitory neurons (Barman and Gebber 1997; Morrison and Gebber 1985).

The results of the antidromic mapping studies support the view that the axons of CVLP neurons with activity correlated to both the 10-Hz and cardiac-related rhythms in SND terminated in the IML of the thoracic spinal cord. First, depth-threshold curves for each of three CVLP neurons revealed that the longest latency antidromic response was elicited with the least stimulus current when the stimulating microelectrode was in the IML. Second, current-dependent shifts in the onset latency of antidromic activation were noted when the stimulating microelectrode was in the vicinity of the IML. Reductions in the onset latency of antidromic activation were noted when the stimulating microelectrode was in the vicinity of the IML. Reductions in the onset latency of antidromic activation were noted when the stimulating microelectrode was in the vicinity of the IML. Reductions in the onset latency of antidromic activation were noted when the stimulating microelectrode was in the vicinity of the IML. Reductions in the onset latency of antidromic activation were noted when the stimulating microelectrode was in the vicinity of the IML. Reductions in the onset latency of antidromic activation were noted when the stimulating microelectrode was in the vicinity of the IML.

The main axons of CVLP-spinal neurons appeared to be distributed ipsilaterally in the DLF and the ventral funiculus within the upper thoracic spinal cord.

The current study is also the first to show that the CVLP contains a heterogeneous pool of neurons with sympathetic nerve-related activity. The naturally occurring discharges of the largest group (54%) of neurons were correlated to both
the 10-Hz and cardiac-related rhythms in SND. The discharges of a second group (23%) of neurons were correlated to only the 10-Hz rhythm, and a third group (23%) of neurons had activity correlated to only the cardiac-related rhythm in SND. The existence of the first two groups of neurons may account for the fact that chemical inactivation of this region blocked the 10-Hz rhythm in SND (Barman et al. 1997). Some of the CVLP neurons with sympathetic nerve-related activity were in the vicinity of the A5 noradrenergic cell group (Jones and Friedman 1983; Lackner 1980; Poitras and Parent 1978), which was implicated in arterial chemoreflex-induced sympathoexcitation in the rat (Koshiya and Guyenet 1994). Whether one or more of the classes of CVLP neurons defined here are part of the A5 cell group and, if so, whether they participate in chemoreflex regulation of SND, remain to be determined.

The RVLM and CMR also contain the same three classes of neurons with sympathetic nerve-related activity (Barman and Gebber 1997). In contrast, the medullary lateral tegmental field (LTF) and caudal ventrolateral medulla (CVLM) do not contain neurons whose discharges are correlated to both rhythms in SND. The LTF contains neurons with activity correlated to the cardiac-related but not the 10-Hz rhythm (Barman and Gebber 1993; Gebber and Barman 1985), whereas the CVLM contains neurons whose discharges are correlated to only the 10-Hz rhythm (Barman et al. 1994). 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.

As was the case for RVLM and CMR neurons with activity correlated to both the 10-Hz and cardiac-related rhythms (Barman and Gebber 1997), virtually all (11/12) of such CVLP neurons were antidromically activated by thoracic spinal cord stimulation. Thus the data from the current study support the hypothesis (Barman and Gebber 1997) that the
outputs of the 10-Hz and cardiac-related rhythm generators converge onto bulbospinal neurons. If the outputs of the two generators converged at a level antecedent to bulbospinal neurons, one would have expected that a substantial proportion of brain stem neurons with activity correlated to both rhythms would not have been antidromically activated by spinal cord stimulation. To date, few such neurons were found in the CVLP, RVLM, and CMR (Barman and Gebber 1997). Nonetheless, if a substantial number of such neurons are eventually identified in an as-yet unexplored region of the brain stem, then we would have to reevaluate this hypothesis.

With only one exception, CVLP neurons whose discharges were correlated to only the 10-Hz rhythm or to only the cardiac-related rhythm in SND could not be antidromically activated by thoracic spinal cord stimulation. Similarly, with the exception of one RVLM neuron, none of the RVLM, CMR, LTF, and CVLM neurons whose discharges were correlated to only one of the two rhythms in SND could be antidromically activated by stimulation of the thoracic spinal cord (Barman and Gebber 1993, 1997; Barman et al. 1995b; Gebber and Barman 1985). Thus we have yet to identify a major bulbospinal pathway that selectively relays information from only one of the rhythm generators to pre-ganglionic sympathetic neurons.

Despite the fact that CVLP neurons with activity correlated to only the 10-Hz rhythm in SND did not project to the spinal cord, they likely were elements of a network that controlled SND. This suggestion is based on the proposal of Barman et al. (1995a, 1997) that the 10-Hz rhythm in SND reflects the organization of a brain stem network that specifically governs SND. They found that the 10-Hz rhythm in SND was not correlated to that in other systems, including the naturally occurring or harmaline-induced 10-Hz rhythm in inferior olivary activity and the 10-Hz spindles in the EEG. Because the firing rates of CVLP neurons whose discharges were correlated to only the 10-Hz rhythm were decreased during the inhibition of SND produced by rapid aortic obstruction, they are presumed to subserve a sympathoexcitatory function. The firing rates of RVLM, CMR, and CVLM neurons with activity correlated to only the 10-Hz rhythm are also affected (decreased or increased) by barore-
ceptor reflex activation. Like such neurons in the CVLP, their activity does not become pulse synchronous even at high levels of arterial pressure (Barman and Gebber 1997; Barman et al. 1994). Such neurons apparently respond to tonic rather than pulsatile baroreceptor afferent nerve activity.

The targets of CVLP neurons whose discharges are correlated to only the 10-Hz rhythm in SND remain to be defined. At least three possibilities should be considered in future studies. First, these neurons might be interposed in pathways from the 10-Hz rhythm generator to CVLP-spinal neurons, i.e., they may be short-axon intrinsic interneurons. Second, CVLP neurons with activity correlated to only the 10-Hz rhythm in SND may project to other neurons in other brain regions involved in the control of SND. For example, such CVLP neurons might project to a corresponding pool of neurons in the CVLM. This possibility is consistent with the fact that the average interval between CVLP unit spike occurrence and peak of the 10-Hz slow wave in inferior cardiac SND (69 ± 3 ms) was significantly longer than that for CVLM neurons (59 ± 1 ms; n = 135) (Barman et al. 1994, 1995b). Thus CVLP neurons fire earlier during the 10-Hz slow wave in SND than CVLM neurons. A third possibility is that CVLP neurons with activity correlated to only the 10-Hz rhythm in SND might project to the cervical spinal cord to innervate propriospinal neurons in pathways that influence SND (Poree and Schramm 1992).

The functions of CVLP neurons whose discharges were correlated to only the cardiac-related rhythm in SND are even more uncertain. First, some may be elements of the network responsible for this component of SND. Second, others may be interneurons in the afferent limb of the baroreceptor reflex arc. Third, they may be elements of a nonsympathetic network that receive inputs from the baroreceptors. None of these possibilities can be ruled out, especially in view of the variety of responses of these neurons to baroreceptor reflex activation. The firing times of CVLP neurons during the cardiac-related slow waves in SND were too widely distributed to allow a comparison with those of neurons in the RVLM, CVLM, LTF, and CMR (Barman and Gebber 1997; Barman et al. 1994, 1995b; Gebber and Barman 1985).

Only 11% of the CVLP neurons studied at the level of the lateral nucleus of the superior olive had activity correlated to the 10-Hz rhythm in SND. In contrast, ~25% of RVLM, CVLM, and CMR neurons studied were identified as having this pattern of activity (Barman and Gebber 1992; Barman et al. 1994). However, in several ways, the characteristics of CVLP neurons with activity correlated to the 10-Hz rhythm in SND were similar to those of their counterparts in the RVLM, CMR, and CVLM (Barman and Gebber 1992, 1997; Barman et al. 1994, 1995b). First, their firing rates were considerably less than the frequency of the 10-Hz rhythm in SND. This 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 pacemaker neurons (Barman and Gebber 1992). Second, the degree to which the discharges of CVLP neurons were correlated to the 10-Hz rhythm in inferior cardiac SND (as reflected by the range of peak coherence values) was similar to that for RVLM, CVLM, and CMR neurons (Barman and Gebber 1992, 1997; Barman et al. 1994, 1995b).

Third, the spinal axonal conduction velocities of CVLP neurons were similar to those of RVLM- and CMR-spinal neurons with activity correlated to both the cardiac-related and 10-Hz rhythms in SND (Barman and Gebber 1997). Fourth, there is considerable overlap in the firing times of these four groups of neurons during the 10-Hz slow wave in SND (Barman and Gebber 1992, 1997; Barman et al. 1994, 1995b).

As a first step in understanding how the 10-Hz rhythm in SND is generated, the connections among the aforementioned pools of neurons need to be identified. Anatomical studies show that the medullary and pontine regions containing these neurons are richly interconnected [see review by Dampney (1994)]. However, with the exception of one electrophysiological study (Barman et al. 1995b) which used antidromic mapping and synaptic activation to identify reciprocal connections between CVLM and CMR neurons with activity correlated to the 10-Hz rhythm in SND, there is no specific information on the interconnections of brain stem neurons with activity correlated to this rhythm.

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REFERENCES


