Pontine neurons are elements of the network responsible for the 10-Hz rhythm in sympathetic nerve discharge

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Barman, Susan M., Heather L. Kitchens, Aaron B. Leckow, and Gerard L. Gebber. Pontine neurons are elements of the network responsible for the 10-Hz rhythm in sympathetic nerve discharge. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1909–H1919, 1997.—The current study was designed to test the hypothesis that pontine neurons are elements of the network responsible for the 10-Hz rhythm in sympathetic nerve discharge (SND). The first series of experiments tested whether chemical inactivation of neurons in the rostral dorsolateral pons (RDLP) or caudal ventrolateral pons (CVLP) affected inferior cardiac postganglionic SND of urethan-anesthetized cats. Muscimol microinjections into either region eliminated the 10-Hz rhythm in SND, supporting the view that pontine neurons are involved in the expression of this rhythm. Additional experiments were designed to determine if pontine neurons have activity correlated to the 10-Hz rhythm in SND or whether they merely provide a tonic (nonrhythmic) driving input to the rhythm generator. Coherence analysis revealed that local field potentials recorded from the RDLP or CVLP had a 10-Hz component that was significantly correlated to SND. Also, spike-triggered averaging and coherence analysis showed that the naturally occurring discharges of individual RDLP or CVLP neurons were correlated to the 10-Hz rhythm in SND. Taken together, these data support the hypothesis that RDLP and CVLP neurons are essential for the expression of the 10-Hz rhythm in SND and that they are elements of or receive input from the rhythm generator.

blood pressure; caudal ventrolateral pons; frequency-domain analyses; muscimol microinjections; rostral dorsolateral pons; spike-triggered averaging

A 10-Hz rhythm often appears in the discharges of sympathetic nerves that control a variety of target organs in urethan-anesthetized or decerebrate unanesthetized cats (4, 14). The results of a study by Gebber et al. (19) support the hypothesis that the 10-Hz rhythm is generated in the brain stem rather than the spinal cord. Specifically, local field potentials recorded from selected sites in the medulla showed a 10-Hz rhythmic component strongly correlated (coherence values close to 1.0) to the 10-Hz rhythm in sympathetic nerve discharge (SND) of decerebrate cats. Importantly, the 10-Hz rhythm in the mediullary population activity persisted after cervical spinal cord transection had eliminated the rhythm in SND.

The results of several studies support the view that the integrity of the rostral ventrolateral medulla (RVLM), caudal ventrolateral medulla (CVLM), and caudal medullary raphe (CMR) are required for the expression of the 10-Hz rhythm in SND. First, this rhythm is eliminated by chemical inactivation (muscimol microinjections) or irreversible lesion of any one of these medullary regions (5, 37, 38). Second, microinjection of the α2-adrenoceptor agonist clonidine into either the RVLM or CVLM (29) or microinjection of the serotonergic (5-HT1A) agonist 8-hydroxy-2(di-n-propylamino)tetrinal (8-OH-DPAT) into the CMR (28) reversibly blocked this rhythm in SND. These drugs are thought to act within these regions to inhibit catecholaminergic or serotonergic neurons, respectively. Finally, each of these medullary regions contained neurons whose naturally occurring discharges are correlated to the 10-Hz rhythm in SND (1, 3, 5, 7).

Data from our laboratory imply that supramedullary regions are also involved in the control of the 10-Hz rhythm in SND (37). Specifically, this component of SND was eliminated by pontomedullary border transection or radiofrequency lesions of the parabrachial and Kölliker-Fuse complex (PB/KF) in the rostral dorsolateral pontine (RDLP) in decerebrate cats. There are at least three ways to explain the loss of the 10-Hz rhythm in SND in these experiments: 1) pontine neurons may be elements of or receive input from the 10-Hz rhythm generator, 2) pontine neurons may provide a tonic (nonrhythmic) excitatory drive to a medullary 10-Hz rhythm generator, and 3) these manipulations may have disrupted fibers of passage of a critical group of neurons located elsewhere in the brain stem. Distinguishing between these possibilities was the aim of the current study. We designed a series of experiments to test the hypothesis that RDLP or caudal ventrolateral pontine (CVLP) neurons are elements of the network responsible for the 10-Hz rhythm in SND. Neurons in these regions have been implicated in the control of SND and blood pressure, including A5 noradrenergic neurons in the CVLP that project to the intermediolateral nucleus (IML) of the thoracolumbar spinal cord (10, 21, 36) and PB/KF (20, 26) and locus ceruleus (LC; Ref, 32) neurons in the RDLP. To test whether RDLP or CVLP neurons play a role in expression of the 10-Hz rhythm in SND, we first studied the effects on SND produced by microinjection of muscimol into either of these regions. Second, we recorded local field potentials in the RDLP and CVLP to determine whether pontine population activity had a 10-Hz rhythmic component correlated to that in SND. Finally, we searched for individual RDLP and CVLP neurons whose naturally occurring discharges were correlated to the 10-Hz rhythm in SND.

METHODS

General Procedures

The protocols used in these studies on 34 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% O$_2$. The right femoral artery and vein were cannulated to measure arterial pressure and to administer drugs, respectively. Urethan (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 urethan were given every 4–6 h. 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 (34, 35). Noxious stimuli (e.g., pinch, cautering muscle) did not change the EEG pattern. As reported by Barman et al. (6), coherence analysis showed that there was no correlation between SND and either the EEG spindles or delta slow waves in these urethan-anesthetized cats.

Cats were paralyzed (gallamine triethiodide, 4 mg/kg iv, initial dose), pneumothoraxacotomized, and artificially respiring with room air. End-tidal CO$_2$ was held near 4% (Traverse Medical Monitors Capnometr, model 2200), and rectal temperature was kept near 38°C with a heat lamp. The aortic depressor and vagus nerves were sectioned in all cats, and in some cats, the carotid sinus nerves were also sectioned. In cats with intact carotid sinus nerves but sectioned aortic depressor and vagus nerves, the pattern of SND is dependent on the level of mean arterial pressure (3. 5). When mean arterial pressure is ~90 mmHg, the 10-Hz rhythm in SND coexists with irregular oscillations primarily at frequencies ≤6 Hz. The cardiac-related rhythm is weak or absent under these conditions. In contrast, when mean arterial pressure is ~150 mmHg, the cardiac-related rhythm predominates. At mean arterial pressures between these levels, SND contains a mixture of the cardiac-related and 10-Hz rhythms.

In preparation for muscimol microinjections or pontine recordings, the dorsal surface of the brain stem was exposed by removing portions of the occipital and parietal bones, bony tentorium, and cerebellum. The obex, midline, and caudal border of the inferior colliculi were used as landmarks for placement of the microinjection pipettes or recording electrodes.

Neural Recordings

The methods used to record left inferior cardiac postganglionic SND and the EEG can be found in earlier reports (4, 6). 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 (18).

Pontine field potentials (population activity) were recorded by using a monopolar electrode (Rhodes model NE-300; 0.5-mm exposed tip). The reference electrode was a clip placed on crushed muscle overlying the skull. The preamplifier band pass was set at 1–1,000 Hz. The RDLP was explored on the left side (ipsilateral to the nerve recording) at the level of the PB/KF, 1–2 mm caudal to the inferior colliculus, 2–4.5 mm lateral to the midline, and within 3 mm of the dorsal surface. The CVLP was explored on the left side at the level of the lateral nucleus of the superior olive, 8–9 mm rostral to the obex, 3.5–5 mm lateral to the midline, and within 3.5 mm of the ventral surface. This region contains the A5 norepinephrine-containing neurons that project to the IML of the thora-columbar spinal cord (10, 21, 36). We recorded extracellularly from single neurons in these pontine regions by using a tungsten microelectrode (FHC; 1-µm tip diameter, ~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 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 (22).

Muscimol Microinjections

The general procedures used for chemical inactivation of pontine neurons are the same as those used by us to chemically inactivate medullary neurons (5, 38). The γ-aminobutyric acid agonist muscimol was injected into the pons through a glass micropipette (~40-µm tip diameter) glued to the needle of a 5-μl Hamilton syringe. Muscimol acts on the soma-dendritic region but not the axons of neurons (16). The syringe and micropipette were filled with a 10 mM solution of muscimol (diluted in 0.9% saline and adjusted to pH 7.2–7.4). Muscimol (1 nmol/100 nl) was injected slowly (10–20 s) into the pons by advancing the plunger of the syringe (marked in 50-nl increments). In six experiments, the micropipette was positioned into the RDLP, 1–2 mm caudal to the inferior colliculus. Injections were made bilaterally in five cats and ipsilateral to the nerve recording in one cat. In tracks 2, 3, and 4, and mm lateral to the midline at depths of 1 and 2 mm below the dorsal surface. In three other cats, muscimol was injected bilaterally into the brain stem 1 mm rostral to or 4 mm caudal to the caudal border of the inferior colliculi. The depth and lateral positions of these injections were the same as described for RDLP injections. To inactivate neurons in the CVLP, the micropipette was positioned 8 and 9 mm rostral to the obex. Injections were made bilaterally in six cats in tracks 4 or 4.5 mm lateral to the midline at depths of 0.5 and 1.5 mm from the ventral surface.

Data Analysis

Before all analyses on a Zenith 486 Z-Station 510 computer, SND and EEG were low-pass filtered at 100 Hz, and the action potentials of individual pontine neurons were isolated by using window discrimination. Pontine field potentials were band-pass filtered at 4–100 Hz to eliminate the high-power, low-frequency components that often appear in these signals. This did not affect the coherence between SND and the pontine field potentials in the 10-Hz frequency range. 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 analog-to-digital convertor board from RC Electronics (Santa Barbara, CA).

Frequency-domain analyses. Frequency-domain analyses were made by using a modified version (24) of the software of Cohen et al. (15). Fast Fourier transform was performed on 32 5-s windows of data (160 s) to construct autospectra of SND, the arterial pulse, EEG, and either pontine population or single 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 autospectra reflected the interspike intervals rather than the shape of the pulses (11). 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 was considered to reflect a statistically
significant relationship when 32 windows were averaged (8). 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 ≤ 20 Hz, since at least 90% of the total power in SND was within this band (4).

Except when showing the effects of muscimol microinjections on SND, the autospectra were scaled so that the height of the largest peak was the same for all analyses, regardless of the actual power at that frequency. In experiments with muscimol microinjections, the autospectra of SND before and after injection were displayed on the same power scale. As described by Orer et al. (29), total power in SND refers to power at frequencies ≤ 20 Hz; it was calculated by arithmetically summing the values for the bins in this frequency range (Statmost for Windows, Datamost). Power at frequencies ≤ 6 Hz was calculated the same way. The 10-Hz band is defined as the range of frequencies surrounding the sharp peak in the autospectrum of SND. This peak occurred between 7.2 and 12.0 Hz in these experiments. Power of the 10-Hz rhythm refers to that in the 10-Hz band after subtracting background power. Background was determined by extrapolating the tail of the lower frequency band of power.

Spike-triggered averaging. Standardized 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 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.

Statistical Analysis

Data are expressed as means ± SE. Student’s t-test for paired data was used to quantify the effects of muscimol microinjections on the frequency components in SND and mean arterial pressure. An unpaired t-test was used to compare properties of CVLP and RDLP neurons with activity correlated to the 10-Hz rhythm in SND. 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. Pontine injection and recording sites were identified with reference to the tracks made with the pipettes or recording electrodes and the stereotaxic planes of Berman (9).

RESULTS

Chemical Inactivation of Pontine Neurons

Muscimol microinjections into the RDLP. Muscimol was microinjected into the RDLP 1–2 mm caudal to the inferior colliculi in six cats (see METHODS for dose and injection sites). In four of these cats, the control mean arterial pressure (85 ± 4 mmHg) was such that the autospectra of SND contained a peak near 10 Hz (9.2 ± 0.6 Hz) but not at the frequency of the heartbeat. Figure 1A shows a 5-s record of the discharges of left inferior cardiac SND from one of these cats. As shown

![Fig. 1. Oscilloscopic records of sympathetic nerve discharge (SND) in a urethan-anesthetized cat before (A) and after muscimol microinjections into rostral dorsolateral pons ipsilateral (B) and contralateral (C) to nerve recording and immediately after high-frequency stimulation (50 Hz, 250 μA for 15 s) of caudal medullary raphe (D). Raphe was stimulated 45 min after completing muscimol microinjections. Horizontal calibration is 500 ms; vertical calibration for SND is 250 µV.](http://ajpheart.physiology.org/ by 10.220.33.2 on April 16, 2017)
by autospectral analysis (Fig. 2, trace 1), SND contained a prominent peak centered at 9.6 Hz (the 10-Hz rhythm) before muscimol was microinjected into the RDLP. Within 7 min of chemical inactivation of the ipsilateral RDLP, the power in the 10-Hz band was substantially reduced (Fig. 2, trace 2); the 10-Hz rhythm was nearly eliminated after injecting muscimol into the contralateral RDLP (Fig. 2, trace 3). Power in SND at frequencies >6 Hz was increased to 120% of control, and total power was reduced to 43% of control by inactivation of RDLP neurons bilaterally in this experiment. The changes in SND produced by chemical inactivation of the RDLP are also apparent by examining the 5-s records of SND in Fig. 1, B and C. Mean arterial pressure fell from 95 to 80 mmHg after bilateral chemical inactivation of the RDLP in this cat.

Muscimol was microinjected into the RDLP in two cats in which the control mean arterial pressure (100 and 105 mmHg) was such that both cardiac-related and 10-Hz rhythms were evident in SND. The data in Fig. 3 are representative of what happened in both experiments. Before muscimol was microinjected into the RDLP, the autospectrum of SND (Fig. 3, trace 1) contained two prominent peaks, one at 3.4 Hz (the cardiac-related rhythm) and one at 9.0 Hz (the 10-Hz rhythm). Although not shown here, the 3.4-Hz rhythm in SND cohered to the arterial pulse (peak coherence value, 0.85). After bilateral microinjections of muscimol into the RDLP, both rhythms in SND were eliminated (Fig. 3, trace 2). This was accompanied by a fall in mean arterial pressure from 105 to 90 mmHg. When mean arterial pressure was restored to 105 mmHg by an
intravenous injection of dextran, the cardiac-related but not the 10-Hz rhythm immediately reappeared in the autospectrum of SND (Fig. 3, trace 3).

Table 1 summarizes the effects of chemical inactivation of the RDLP 1–2 mm caudal to the inferior colliculus on SND and blood pressure in six cats. In one cat, muscimol microinjections into the ipsilateral RDLP were sufficient to eliminate the 10-Hz rhythm in SND. In the other five cats, bilateral injections were required. The data from the six animals are pooled in Table 1. Chemical inactivation of the RDLP virtually eliminated the 10-Hz rhythm in SND without significantly affecting power at frequencies ≤6 Hz or total power in SND. Mean arterial pressure was significantly decreased in these experiments. In three cats, muscimol microinjections placed bilaterally into the dorsolateral brain stem rostral or caudal to these sites (see Methods) did not significantly change power in the 10-Hz band (107 ± 13% of control), power at frequencies ≤6 Hz (98 ± 4% of control), total power in SND (106 ± 12% of control), or mean arterial pressure (97 ± 9 vs. 99 ± 13 mmHg).

In urethan-anesthetized or decerebrate unanesthetized cats in which the 10-Hz rhythm is absent or very weak under basal conditions, the intravenous administration of the α2-adrenoceptor antagonist idazoxan (29), elevating end-tidal CO2 (14), or a brief period of high-frequency stimulation of the CMR (unpublished observations) readily induces a 10-Hz rhythm in SND. Because full recovery from the effects of muscimol microinjections can take up to 3–5 h (27), we reasoned that as the effects of muscimol began to wane, these procedures might be able to accelerate recovery of the 10-Hz rhythm in SND. We stimulated the CMR (50 Hz for 15 s) every 15–20 min beginning 30–60 min after completing the microinjections in five cats. In three of these cats, idazoxan was also administered once between 30 and 90 min after chemical inactivation of the RDLP; and in two of the cats, 30–60 min after muscimol microinjections, end-tidal CO2 was elevated to 7–8% for 90 s. For at least 40 min after chemical inactivation of the RDLP, these manipulations failed to produce a 10-Hz rhythm in SND. However, in each of the cats, one or more of these procedures led to the reappearance of the 10-Hz rhythm in SND between 45 and 90 min after chemical inactivation of the RDLP. Figure 1D shows recovery of the 10-Hz rhythm in SND immediately after CMR stimulation (50 Hz, 250 µA for 15 s) 45 min after muscimol was microinjected bilaterally into the RDLP. During the stimulation, SND was inhibited (data not shown). Recovery of the 10-Hz rhythm in SND in this experiment is also indicated by the reappearance of a peak centered at 9.8 Hz in the autospectrum of SND after CMR stimulation (Fig. 2, trace 4). The CMR stimulus-induced 10-Hz rhythm persisted for several minutes and could be readily reinstated by an additional episode of high-frequency stimulation.

Muscimol microinjections into the CVLP. Muscimol was microinjected bilaterally into the CVLP 8–9 mm rostral to the obex in six cats in which the control autospectrum of SND contained a sharp peak centered at 9.0 ± 0.9 Hz but not at the frequency of the heartbeat (4 of these cats were baroreceptor denervated). In the example shown in Fig. 4, the 10-Hz rhythm in SND (trace 1) was attenuated after muscimol was microinjected into the CVLP ipsilateral to the nerve recording (trace 2) and was then eliminated by chemical inactivation of neurons in the contralateral CVLP (trace 3). In this experiment, power at frequencies ≤6 Hz was 280% of control, and total power was 95% of control after microinjections of muscimol bilaterally into the CVLP; mean blood pressure was reduced from 98 to 75 mmHg.

Table 1 summarizes the effects of microinjections of muscimol bilaterally into the CVLP in six cats. Chemical inactivation of this region essentially eliminated the 10-Hz rhythm in SND. On a group basis, power at frequencies ≤6 Hz was not significantly affected, but

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<th>Injection Site</th>
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<td>RDLP</td>
<td>6</td>
<td>7 ± 3*</td>
<td>110 ± 13</td>
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<tr>
<td>CVLP</td>
<td>6</td>
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Values are means ± SE; n, no. of experiments. CVLP, caudal ventrolateral pons; MAP, mean arterial pressure; RDLP, rostral dorsolateral pons. *Significantly different from control (P = 0.05).

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Fig. 4. Effects of muscimol microinjections into caudal ventrolateral pons on sympathetic nerve discharge of a baroreceptor-denervated cat. Traces 1–3: autospectra before and after muscimol microinjections ipsilateral and contralateral to nerve recording, respectively.
total power and mean arterial pressure were significantly decreased.

We attempted to induce recovery of the 10-Hz rhythm in SND for up to 2 h after completing the bilateral injections of muscimol into the CVLP. In no case were we successful in producing a 10-Hz rhythm in SND by the intravenous administration of idazoxan, elevating end-tidal CO₂, or high-frequency stimulation of the CMR. We also returned blood pressure to control levels by intravenous infusion of dextran, but this did not alter the autospectrum of SND.

In two cats, saline (100 nl/injection) was microinjected bilaterally into the same sites in the CVLP and RDLP where muscimol microinjections led to blockade of the 10-Hz rhythm in SND. Saline injections did not lead to changes in the autospectra of SND in these cats.

Identification of Pontine Sites With Activity Correlated to SND

RDLP field potentials. We recorded population activity (field potentials) within the RDLP 1–2 mm caudal to the inferior colliculus in six cats in an attempt to identify sites with activity correlated to the 10-Hz rhythm in SND. In the example shown in Fig. 5, there was a sharp peak centered at 7.4 Hz in the autospectra of SND, RDLP activity, and the EEG (Fig. 5B, top to bottom). Coherence analysis (Fig. 5C, top) showed that RDLP activity was significantly correlated to SND with a peak coherence value of 0.28 at 7.4 Hz. RDLP activity was also correlated to the EEG with a peak coherence value of 0.27 at 7.2 Hz (Fig. 5C, bottom), but there was no significant coherence between the EEG and SND (Fig. 5C, middle).

We recorded from 253 sites in the RDLP ipsilateral to the nerve recording in these cats. In 45 cases, the peak coherence value (0.22 ± 0.01) relating the 10-Hz activity (9.1 ± 0.2 Hz) in the RDLP and sympathetic nerve was significantly different from zero. The distribution of these 45 recording sites is shown in Fig. 6A (solid circles). These sites were in the area of the PB/KF and LC and adjacent reticular formation where muscimol microinjections eliminated the 10-Hz rhythm in SND. Field potentials from 28 of these sites were also significantly correlated to the EEG, with a peak coherence value of 0.37 ± 0.03 at 7.1 ± 0.4 Hz. The 10-Hz rhythm in SND was not correlated to the EEG in any of these cats.

CVLP field potentials. We recorded population activity from the CVLP 8–9 mm rostral to the obex in seven cats in an attempt to identify sites with activity correlated to the 10-Hz rhythm in SND. In the example shown in Fig. 7B, there was a sharp peak at 7.6 Hz in the autospectrum of SND (top), while the autospectrum of CVLP activity had a small peak at this frequency and a larger peak at 10.2 Hz superposed on a high level of background power (middle). The peak at 6.0 Hz in the autospectrum of the EEG also rose out of substantial background power (Fig. 7B, bottom). Coherence analysis (Fig. 7C, top) showed that CVLP activity was significantly correlated to SND with a peak coherence value of 0.39 at 7.6 Hz. CVLP activity was also correlated to the EEG with a peak coherence value of 0.29 at 9.8 Hz (Fig. 7C, bottom), but SND did not significantly cohere to the EEG (Fig. 7C, middle).

We recorded from 217 sites in the CVLP ipsilateral to the nerve recording in these cats. In 32 cases, the peak coherence value (0.21 ± 0.01) relating the 10-Hz activity (9.6 ± 0.1 Hz) in the CVLP and inferior cardiac nerve was significantly different from zero. The distribution of these 32 recording sites is shown in Fig. 6B (solid circles). These sites were in the region of the...
CVLP where muscimol microinjections eliminated the 10-Hz rhythm in SND. The field potentials from 21 of these CVLP sites were also significantly correlated to the EEG with a peak coherence value of 0.34 ± 0.03 at 8.4 ± 0.5 Hz. The 10-Hz rhythms in SND and the EEG were not significantly correlated in any of these cats.

Pontine Neurons With Activity Correlated to the 10-Hz Rhythm in SND

We searched for individual CVLP and RDLP neurons whose naturally occurring discharges were correlated to the 10-Hz rhythm in SND in experiments in which the autospectra of SND contained a peak near 10 Hz (8.5 ± 0.2 Hz) but not at the frequency of the heart rate (mean blood pressure, 84 ± 2 mmHg). We recorded from 60 CVLP neurons at the level of the lateral nucleus of the superior olive in 3 cats and from 55 RDLP neurons at the level of the PB/KF in 3 cats. As demonstrated by using spike-triggered averaging and coherence analysis, 13 of these CVLP neurons and 14 of these RDLP neurons had activity correlated to the 10-Hz rhythm in SND. The spike-triggered average in Fig. 8B, top, is for a CVLP neuron. The 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 ~115-ms intervals, and their amplitudes greatly exceeded those in the corresponding dummy average of SND (Fig. 8B, bottom). The interval between neuronal spike occurrence and the first peak to the right of time 0 in the spike-triggered average of SND was 65 ms (Fig. 9B, top). There was a peak at 8.8 Hz in the autospectra of SND and RDLP neuronal activity (Fig. 9C, top and middle), and coherence analysis (Fig. 9C, bottom) demonstrated that these signals were significantly correlated at this frequency (coherence value was 0.31). This neuron had a mean firing rate of 5.9 spikes/s. The discharges of this neuron were not correlated to the EEG (data not shown).

Table 2 summarizes the data from the 13 CVLP and 14 RDLP neurons with activity correlated to the 10-Hz rhythm in SND. The firing times of the two groups of neurons during the 10-Hz slow wave in inferior cardiac SND were similar, as were their mean firing rates. However, the coherence value relating CVLP neuronal activity to SND was significantly greater than that relating RDLP neuronal activity to SND. The location of the recording sites of RDLP and CVLP neurons with activity correlated to the 10-Hz rhythm in SND are indicated by solid triangles in Fig. 6, A and B, respectively. None of the pontine neurons with activity correlated to SND had activity correlated to the EEG (as indicated by coherence analysis and spike-triggered averaging). We did not keep a record of pontine neurons with activity correlated to the EEG but not SND.

DISCUSSION

The results of the current study are the first to demonstrate that pontine neurons are essential for the expression of the 10-Hz rhythm in SND. First, chemical inactivation of neurons in either the RDLP or CVLP eliminated the 10-Hz rhythm in SND. Second, field potentials recorded from these regions had a 10-Hz component correlated to that in SND. Third, the naturally occurring discharges of individual RDLP and CVLP neurons were correlated to the 10-Hz rhythm in SND. Muscimol microinjections made at the level of the PB/KF and LC in the RDLP were effective in reversibly eliminating the 10-Hz rhythm in SND. In contrast,
injections placed 1–2 mm further rostral or caudal in the dorsolateral brain stem did not significantly affect SND. Thus a restricted portion of the dorsolateral pons appears to serve a vital role in the expression of the 10-Hz rhythm in SND. Because the 10-Hz rhythm is most pronounced during late inspiration (4, 13) and the PB/KF plays an important role in respiratory regulation (12), one might argue that the loss of the 10-Hz rhythm attendant to chemical inactivation of the RDLP was secondary to its effects on respiration. This was not the case in the current study. This could mean that different populations of RDLP neurons are involved in the control of respiration and SND. It is unclear why only 1 of 61 RDLP field potential recordings made by Zhong et al. (37) had a 10-Hz rhythmic component correlated to SND, whereas ~18% of the RDLP field potentials recorded in the current study showed this relationship to SND.

Chemical inactivation of CVLP neurons at the level of the lateral nucleus of the superior olive also blocked the 10-Hz rhythm in SND. We did not test the effects of muscimol microinjections at more rostral levels of the ventrolateral pons; thus we do not know whether other portions of the ventrolateral pons are involved in control of the 10-Hz rhythm. It might be argued that muscimol injected into the CVLP spread to the RVLM, thereby leading to blockade of the 10-Hz rhythm in SND. This seems unlikely because the RVLM is ~3 mm caudal to the CVLP, a distance that far exceeds the radius of spread of a 100-nl volume of injection (30). Moreover, both population activity and the discharges of individual neurons in the CVLP were significantly correlated to the 10-Hz rhythm in SND. Taken together, the results of muscimol microinjections and electrical recordings support the view that CVLP neurons are essential for the appearance of the 10-Hz rhythm in SND. Some of these CVLP recording sites were located medial and dorsal to the A5 noradrenergic neurons (23, 25, 31). Thus CVLP neurons in addition to or other than A5 neurons may be involved in the control of the 10-Hz rhythm in SND.

The results of the current study favor the view that rather than merely providing a tonic, nonrhythmic excitatory drive to a medullary rhythm generator, RDLP and CVLP neurons are either elements of or receive input from the 10-Hz rhythm generator. First, the autospectra of the discharges of individual pontine neurons and pontine field potentials often contained a sharp peak at the frequency of the 10-Hz rhythm in SND (see Fig. 1) as occurs when SND contains a respiratory-related component (37, 39). Second, lesions of PB/KF produce an apneustic pattern of respiration (12, 37). The increased inspiratory activity would be expected to enhance not diminish the 10-Hz rhythm in SND (4, 13). Third, RDLP neuronal activity showed a 10-Hz rhythmic component correlated to that in SND.

Cohen et al. (13) were generally unsuccessful in their attempt to identify RDLP neurons with activity correlated to the 10-Hz rhythm in SND. Only one of the over 100 PB/KF neurons they sampled had such activity. In contrast, in the current study, the naturally occurring discharges of ~25% of the individual RDLP neurons sampled were correlated to the 10-Hz rhythm in SND. The difference between our results and those of Cohen et al. (13) may reflect the fact that they were primarily interested in identifying neurons that might function to coordinate sympathetic and respiratory discharges; thus they restricted their analysis to neurons that had respiratory-modulated activity. This was not the case in the current study. This could mean that different populations of RDLP neurons are involved in the control of respiration and SND. It is unclear why only 1 of 61 RDLP field potential recordings made by Zhong et al. (37) had a 10-Hz rhythmic component correlated to SND, whereas ~18% of the RDLP field potentials recorded in the current study showed this relationship to SND.

Chemical inactivation of CVLP neurons at the level of the lateral nucleus of the superior olive also blocked the 10-Hz rhythm in SND. We did not test the effects of muscimol microinjections at more rostral levels of the ventrolateral pons; thus we do not know whether other portions of the ventrolateral pons are involved in control of the 10-Hz rhythm. It might be argued that muscimol injected into the CVLP spread to the RVLM, thereby leading to blockade of the 10-Hz rhythm in SND. This seems unlikely because the RVLM is ~3 mm caudal to the CVLP, a distance that far exceeds the radius of spread of a 100-nl volume of injection (30). Moreover, both population activity and the discharges of individual neurons in the CVLP were significantly correlated to the 10-Hz rhythm in SND. Taken together, the results of muscimol microinjections and electrical recordings support the view that CVLP neurons are essential for the appearance of the 10-Hz rhythm in SND. Some of these CVLP recording sites were located medial and dorsal to the A5 noradrenergic neurons (23, 25, 31). Thus CVLP neurons in addition to or other than A5 neurons may be involved in the control of the 10-Hz rhythm in SND.

The results of the current study favor the view that rather than merely providing a tonic, nonrhythmic excitatory drive to a medullary rhythm generator, RDLP and CVLP neurons are either elements of or receive input from the 10-Hz rhythm generator. First, the autospectra of the discharges of individual pontine neurons and pontine field potentials often contained a sharp peak at the frequency of the 10-Hz rhythm in SND. Second, coherence analysis showed that pontine activity was significantly correlated to SND only in the frequency range of the 10-Hz rhythm.
The effects of muscimol in the RDLP were reversible; 45–90 min after completing the microinjections, several manipulations (stimulation of the CMR, intravenous injections of idazoxan, increasing end-tidal CO₂) led to the reappearance of the 10-Hz rhythm in SND. However, the effects of chemical inactivation of CVLP neurons could not be reversed for the duration (2 h) of the experiments. The reason for this difference is unclear. One possibility is that the time course of recovery is different in these two regions. Because the actions of muscimol can persist for up to 3–5 h after microinjection (27), we may not have waited long enough to see recovery after CVLP microinjections. A second possibility is that the significant fall in blood pressure resulting from chemical inactivation of CVLP neurons might have led to nonspecific depression of the 10-Hz rhythm generator. However, this is not likely to be the explanation for our inability to reverse the effects of muscimol in the CVLP because returning blood pressure to control levels by the intravenous infusion of dextran did not change SND in these experiments. Also, it is unlikely that the microinjections physically damaged neural elements in the CVLP leading to permanent loss of the 10-Hz rhythm because saline injections (same volume and same sites) did not alter the pattern of SND.

To date, five brain stem regions have been shown to contain neurons that are critical for expression of the 10-Hz rhythm in SND. In addition to the RDLP and CVLP, chemical inactivation of the RVLM, CVLM, or CMR blocks the 10-Hz rhythm in SND (5, 38), and each of these regions contains neurons whose naturally occurring discharges are correlated to the 10-Hz rhythm in SND (1, 3, 5, 7). The fact that the 10-Hz rhythm is dependent on the functional integrity of so many anatomically separated brain stem regions favors the view that this rhythm is a property of a distributed network of neurons rather than of a local generator. It is unlikely that the neurons in these five regions form a single in-series pathway. Anatomic studies showed that these areas are interconnected via multiple, redundant routes (see review by Dampney, Ref. 17). Reciprocal connections between CVLM and CMR neurons with activity correlated to the 10-Hz rhythm in SND were identified by Barman et al. (7) using antidromic mapping and synaptic activation techniques. The interconnections of RDLP and CVLP neurons with activity correlated to the 10-Hz rhythm in SND and their counterparts in the medulla remain to be determined.

Data from earlier studies by this laboratory have demonstrated that the cardiac-related and 10-Hz rhythms are generated by different pools of brain stem neurons. Specifically, spike-triggered averaging and coherence analysis showed that CVLM neurons whose discharges are correlated to the 10-Hz rhythm do not have activity correlated to the cardiac-related rhythm.
in SND (5). In contrast, the discharges of medullary lateral tegmental field neurons are correlated to the cardiac-related rhythm (18) but not to the 10-Hz rhythm in SND (2). Because RVLM- and CMR-spinal neurons have activity correlated to both the cardiac-related and 10-Hz rhythms in SND (3), the outputs of the two generators converge at the level of bulbspinal neurons. Data from the current study offer additional support for the view that the cardiac-related and 10-Hz rhythm-generating networks are comprised, in part, of different pools of neurons. Specifically, in the few experiments in which both the cardiac-related and 10-Hz rhythms were evident in SND, chemical inactivation of the RDLP selectively eliminated the 10-Hz rhythm. Thus the RDLP neurons inactivated in these experiments were not essential for generation of the cardiac-related rhythm in SND, although PB/KF neurons with pulse-synchronous activity have been identified in the cat (33).

Many of the RDLP and CVLP field potentials that cohered to the 10-Hz rhythm in SND also cohered to the EEG; however, SND and the EEG were not coherent. Moreover, none of the individual RDLP or CVLP neurons identified had activity correlated to both signals. Thus it is reasonable to assume that the field potentials reflected the combined activity of neurons that subserved different functions. Barman et al. (6) identified RVLM, CVLM, and CMR neurons with 10-Hz rhythmic discharges uncorrelated to SND; they were intermingled with neurons whose discharges cohered to the 10-Hz rhythm in SND. This points to the necessity of using correlational procedures such as spike-triggered averaging and coherence analysis to identify elements of sympathetic networks. The mere existence of an activity pattern like that in SND is not an adequate criterion to identify these neurons.

Selective blockade of the 10-Hz rhythm in SND produced by chemical inactivation (38) or ablation (37) of the CMR or by intravenous administration of clonidine (29) or 8-OH-DPAT (28) is accompanied by a significant reduction in mean arterial pressure. These observations point to a role of the 10-Hz rhythm in cardiovascular regulation. The results of the current study lend additional support to this proposal. Blood pressure fell significantly when the 10-Hz rhythm was eliminated by chemical inactivation of the RDLP or CVLP.

<table>
<thead>
<tr>
<th>Recording Site</th>
<th>n</th>
<th>STA Lag, ms</th>
<th>Peak Coherence</th>
<th>Firing Rate, spikes/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVLP</td>
<td>13</td>
<td>72 ± 3</td>
<td>0.46 ± 0.08</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>RDLP</td>
<td>14</td>
<td>68 ± 8</td>
<td>0.24 ± 0.04*</td>
<td>2.7 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of neurons. CVLP, caudal ventrolateral pons; RDLP, rostral dorsolateral pons; STA, spike-triggered average. STA lag refers to interval between unit spike occurrence and peak inferior cardiac sympathetic nerve discharge in spike-triggered average. Peak coherence refers to value near 10 Hz in neuron-to-nerve coherence function. *Significantly different (P ≤ 0.05) from corresponding value relating CVLP neuronal activity to sympathetic nerve discharge.
REFERENCES


