Differential Patterns of Spinal Sympathetic Outflow Involving a 10-Hz Rhythm

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Gebber, Gerard L., Sheng Zhong, Craig Lewis, and Susan M. Barman. Differential patterns of spinal sympathetic outflow involving a 10-Hz rhythm. J. Neurophysiol. 82: 841–854, 1999. Time and frequency domain analyses were used to examine the changes in the relationships between the discharges of the inferior cardiac (CN) and vertebral (VN) postganglionic sympathetic nerves produced by electrical activation of the midbrain periaqueductal gray (PAG) in urethane-anesthetized, baroreceptor-denervated cats. CN-VN coherence and phase angle in the 10-Hz band served as measures of the coupling of the central oscillators controlling these nerves. The 10-Hz rhythm in CN and VN discharges was entrained 1:1 to electrical stimuli applied to the PAG at frequencies between 7 and 12 Hz. CN 10-Hz discharges were increased, and VN 10-Hz discharges were decreased when the frequency of PAG stimulation was equal to or above that of the free-running rhythm. In contrast, stimulation of the same PAG sites at lower frequencies increased, albeit disproportionately, the 10-Hz discharges of both nerves. In either case, PAG stimulation significantly increased the phase angle between the two signals (VN 10-Hz activity lagged CN activity); coherence values relating their discharges were little affected. However, the increase in phase angle was significantly more pronounced when the 10-Hz discharges of the two nerves were reciprocally affected. Importantly, partialization of the phase spectrum using the PAG stimuli did not reverse the change in CN-VN phase angle. This observation suggests that the increase in the CN-VN phase angle reflected changes in the phase relations between coupled oscillators in the brain stem rather than the difference in conduction times to the two nerves from the site of PAG stimulation. In contrast to the effects elicited by PAG stimulation, stimulation of the medullary lateral tegmental field induced uniform increases in the 10-Hz discharges of the two nerves and no change in the CN-VN phase angle. Our results demonstrate that changes in the phase relations among coupled brain stem 10-Hz oscillators are accompanied by differential patterns of spinal sympathetic outflow. The reciprocal changes in CN and VN discharges produced by PAG stimulation are consistent with the pattern of spinal sympathetic outflow expected during the defense reaction.

INTRODUCTION

In addition to exerting uniform actions on the discharges of sympathetic nerves, the brain can formulate differential patterns of spinal sympathetic outflow. For example, the defense reaction is characterized, in part, by increased sympathetic drive to the heart, vasoconstriction in the viscera, and skeletal muscle vasodilation due to the withdrawal of sympathetic vasoconstrictor discharge and activation of sympathetic vasodilator fibers (Coote et al. 1973; Folkow et al. 1964; Hilton 1982). The underlying mechanisms responsible for increased sympathetic drive to the heart and viscera in the face of decreased vasoconstrictor outflow to skeletal muscle remain obscure. Two models have attracted the most attention in recent years. The first model is based on the observation that chemical activation of the rostral ventrolateral medulla elicits site-dependent differential changes in regional blood flows (Dampney and McAllen 1988; McAllen and Dampney 1990). This has led to the proposal that presynaptic neurons in the rostral ventrolateral medulla are topographically organized according to their peripheral targets. A logical extension of this proposal is that the discharges of such neuronal groups are coordinated by inputs from the defense regions of the midbrain periaqueductal gray (PAG) and hypothalamus (Carrive 1991; Dampney 1994). In the second model, the discharges of selected groups of preganglionic sympathetic nerves with different targets are coordinated by inputs from brain stem and hypothalamic neurons with diffusely branching spinal axons. Jansen et al. (1995) have proposed that such inputs, some of which arise in the defense regions of the PAG and hypothalamus, act as ‘‘command’’ neurons that, when activated, lead to differential patterns of spinal sympathetic outflow.

The models described above differ in terms of the level of the neuraxis at which cell groups with different targets are coordinated. Nevertheless, both models presuppose coordination as the result of inputs shared by groups of neurons that are not necessarily directly interconnected. The implication is that such inputs excite some cell groups and inhibit others. The current study deals with an alternative to these ‘‘hard-wired’’ models. In the alternative model, differential patterns of spinal sympathetic outflow elicited in the absence of baroreceptor reflex feedback control are emergent properties of a system of dynamically coupled brain stem oscillators that generate the 10-Hz rhythm in sympathetic nerve discharge (SND).

The background for this model is as follows. First, a 10-Hz rhythm uncorrelated to rhythms of similar frequency in the electroencephalogram (EEG) and motor systems (inferior olivary activity) is ubiquitous to the discharges of sympathetic nerves with cardiovascular targets in decerebrate or urethane-anesthetized cats (Barman et al. 1992, 1995; Gebber et al. 1994b). The 10-Hz rhythm in SND is most prominent after baroreceptor denervation or unloading (Barman and Gebber 1997; Barman et al. 1992, 1994). Second, the 10-Hz rhythm is generated in the brain stem by multiple oscillators, each of which preferentially or selectively controls a different portion of the spinal sympathetic outflow (Gebber et al. 1994b; Huang et al. 1992). Third, whereas the 10-Hz rhythmic discharges of sympathetic nerves with different targets normally are strongly correlated, the coupling of the brain stem oscillators control-
ling the nerves is both dynamic and nonuniform (Barman et al. 1992; Gebber et al. 1994b).

In the current study, we tested whether electrical activation of selected sites in the brain stem of baroreceptor-denervated cats reciprocally affects the 10-Hz discharges of the inferior cardiac (CN) and vertebral (VN) postganglionic sympathetic nerves, and if so, whether such changes are accompanied by alterations in coherence and phase angle in the 10-Hz band. Coherence and phase angle were used as measures of the relationships among the coupled oscillators controlling these nerves. The CN and VN innervate the heart and forelimb vasculature, respectively. The results demonstrate that differential patterns of spinal sympathetic outflow emerge when the phase relations among coupled 10-Hz oscillators are altered by stimulation of the defense region of the midbrain PAG.

METHODS

Experimental subjects and anesthesia

The protocols used on 15 cats were approved by the All-University Committee on Animal Use and Care of Michigan State University. The cats were initially anesthetized with 2.5% isoflurane mixed with 100% oxygen. Urethane (1.2–1.8 g/kg iv) was then administered, and isoflurane inhalation was terminated. This dose range of urethane has been reported to maintain a surgical level of anesthesia for a period (8–10 h), which exceeded the duration of our experiments (Flecknell 1987). Nevertheless, supplemental doses (0.2 g/kg iv) of urethane were given every 4–6 h. The frontal-parietal EEG showed a mixture of 7-to-13-Hz spindles and delta–slow waves, indicative of unconsciousness and blockade of information transfer through the thalamus (Steriade and Llinas 1988). The EEG was not changed by noxious stimuli (e.g., pinch) applied to the head or body and, as previously reported, was not correlated to 10-Hz or lower frequency SND (Barman et al. 1995).

General procedures

Blood pressure was measured from a catheter inserted into the abdominal aorta via a femoral artery. Spontaneous respiration during anesthesia was eupneic with end-tidal CO2 (Traverse Medical Monitors Capnometer, model 2200) in the normocapnic range. Subsequently, the animal was paralyzed (gallamine triethiodide; 4 mg/kg iv, initial dose), artificial ventilation with room air was begun, and bilateral pneumothoracotomy was performed. End-tidal CO2 was kept between 4.0 and 4.5% by adjusting the parameters of artificial ventilation. Rectal temperature was kept near 38°C with a heat lamp. Baroreceptor denervation was performed by bilateral section of the carotid sinus, aortic depressor, and vagus nerves (Barman et al. 1992). Section of these nerves eliminated the cardiac-related rhythm in SND and the inhibition of SND induced by raising blood pressure with a bolus iv injection of norepinephrine bitartrate (2 μg/kg).

Neural recordings and central stimulation

By using methods described by Barman et al. (1992), potentials were recorded monophasically with bipolar platinum electrodes from the central ends of the cut CN and VN near their exits from the left stellate ganglion. The CN and VN provide sympathetic outflows to the heart and forelimb vasculature, respectively. Nerve recordings initially were made with the band-pass of the Grass Instruments 7P3 preamplifier set at 1–1,000 Hz, so that envelopes of multiunit spikes appeared as slow waves (Barman et al. 1992; Cohen and Gootman 1970). The frontal-parietal EEG was recorded with a gold-plated disk electrode placed on the skull and the indifferent electrode on crushed muscle; the amplifier band-pass was 1–1,000 Hz. These data were stored on magnetic tape.

A Grass S8800 quartz-timed digital stimulator and PSIU6 constant-current unit were used to deliver 1-ms square-wave pulses of variable intensity and frequency through concentric bipolar stainless steel electrodes (Rhodes model SNE-100 with 0.25-mm tip exposures separated by 0.75 mm) to selected sites in two regions of the brain stem. Electrode placements into the midbrain PAG and medullary lateral tegmental field (LTF) were made visually after removal of portions of the occipital and parietal bones and medial cerebellum. Relative to the stereotactic coordinates of Snider and Niemer (1961), electrode placements in the midbrain PAG were caudal to the bony tentorium at P1 to P2, L1 to L2 (left), and H + 3 to H + 0. We refer to this portion of the midbrain PAG as the caudal PAG. The sites of stimulation in the medullary LTF were located 2–4 mm rostral to the obex, 2–3 mm lateral to the midline (left), and 2–4 mm below the dorsal surface. Sites of stimulation were identified with references to electrode tracks. The brain stem was removed and fixed in 10% buffered Formalin. Frontal sections of 30-μm thickness were cut on a cryostat, stained with cresyl violet and examined microscopically.

Data analysis

A flow chart depicting the methods and sequence of data analysis is presented in Fig. 1. The recordings of SND on magnetic tape were initially low-pass filtered at 50 Hz to obtain the “original” records shown in Fig. 1A. The analog filter had an attenuation slope of 24 dB/octave. In preparation for time series analysis, the original records were then digitally filtered without phase distortion to extract the 10-Hz band of SND. This was performed by using software obtained from R. C. Electronics, Santa Barbara, CA. The width of the bandpass for digital filtering usually was 4 Hz with the center frequency matched to that of the primary peak in the autospectrum of SND. The digitally filtered signals (Fig. 1B) are smoother and more sinusoidal-like than the originals with power reduced by no more than 10% in the designated band-pass. The roll-off slope of the digital filter was such that power outside of the band-pass setting was reduced by 39%/Hz. By using software written by one of us (Lewis), voltages and times of occurrence of the peaks and troughs of the digitally smoothed signals were detected and saved as an ASCII file. These data were used to construct time series of the amplitudes (peak to trough) of the slow waves in the two nerves, and the relative phase (i.e., phase angle in degrees) between the peaks of the CN and VN slow waves on a cycle-by-cycle basis (Fig. 1C). Slow-wave amplitudes were normalized (scale 0–1.0) relative to the largest slow wave in the time series. The phase angle between the peaks of corresponding CN and VN slow waves was plotted on a scale of 0–360° with the peak of the CN slow wave in each cycle serving as the reference. Thus the phase angle is the number of degrees that the peak of the VN slow wave lagged that of the CN slow wave. Phase angle (φ) was calculated on a cycle-by-cycle basis by using the formula

\[ \phi = \frac{t}{T} \cdot 360^\circ \]

where t is the interval (ms) between the peaks of the slow waves in the two nerves and T is the period of the rhythm in the reference signal (i.e., interval between the peaks of the CN slow waves that immediately preceded and followed each VN slow wave). The sampling rate for data collection was 500 Hz in the case of time series analysis. Thus, for a 100-ms slow wave, the resolution of measurement of phase angle was 7.2°/bin.

Spectral analysis was used to characterize CN and VN discharges (original recordings) in the frequency domain. This provided information not available in the time series such as the values of power in different frequency bands and coherence values relating signal pairs. Fast Fourier transform (FFT) was performed by using a modified
version (Gebber et al. 1994b) of the programs of Cohen et al. (1987) and Kocsis et al. (1990). The sampling rate was 200 Hz, and the resolution of measurement was 0.2 Hz/bin. Thirty-two 5-s windows were averaged with 50% overlap for 80-s data blocks and 75% overlap for 40-s data blocks. The analysis yielded autospectra of the discharges of the CN and VN (Fig. 1D, top 2 panels) and corresponding coherence functions (Fig. 1D, 3rd panel) and phase spectra (Fig. 1D, bottom) relating the discharges of the two nerves. In each panel, the spectra for data collected before (control; trace 1 or ○ for phase angle) and during (test; trace 2 or □) brain stem stimulation are superposed. The autospectrum of a signal shows how much power (voltage squared) is present at each frequency. Powers in the superposed autospectra are normalized on a scale of 0–1.0, with 1.0 representing the highest absolute power reading found in one of the bins of either the control or test spectrum. The coherence function (normalized cross-spectrum) measures the strength of linear correlation (scale 0–1.0) of pairs of signals as a function of frequency, whereas the phase spectrum measures the lag (scale 0–360°) of the second signal (VN) in a pair relative to the first (CN) at each frequency. Although FFT was performed for the 0- to 100-Hz band, the spectra are displayed on a frequency scale of 0–15 Hz. Less than 10% of the total power in SND was contained above 15 Hz.

In this study, the total power in SND is defined as the sum of the absolute values in the bins between 0 and 15 Hz. A macro written in Microsoft Excel version 7.0 was used to measure the power above background in the 10-Hz band. A line was fitted to connect the left and right limits of the sharp peak near 10 Hz in the autospectrum of SND. The power in the 10-Hz band was calculated as the area above the line. The power at frequencies between 0 and 6 Hz is the sum of the values in the bins comprising this band. Changes in power are expressed as a percent of control. Because the range over which power could change was not restricted to values between 0 and 100%, paired and unpaired comparisons were made with the Student’s t-test using raw percentages (Sokal and Rohlf 1969). However, coherence values

FIG. 1. Data analysis A: original recordings (low-pass filtered at 50 Hz) of the discharges of inferior cardiac (CN) and vertebral (VN) sympathetic nerves. Stimulation (500 μA, 9.4 Hz) of a site in the caudal periaqueductal gray (PAG) of the midbrain was begun at the vertical line. Vertical calibration is 100 μV; horizontal calibration is 500 ms. B: CN and VN discharges after digital filtering using a band-pass of 7.4 to 11.4 Hz. Horizontal calibration is 500 ms. C: amplitude- and relative phase-time series. Top panel: amplitude-time series for CN; PAG stimulation started at vertical line. Data points are cycle-by-cycle measurements of peak-to-trough amplitude (normalized on scale of 0–1.0 relative to largest slow wave) of digitally filtered 10-Hz slow waves. Middle panel: same for VN. Bottom panel: a relative phase-time series showing cycle-by-cycle measurements of peak-to-trough amplitude (normalized on scale of 0–1.0) of the VN slow wave relative to the peak of the CN slow wave (see methods). D: panels (top to bottom) show autospectra (AS) of CN activity; AS of VN activity; coherence functions (scale, 0–1.0) and phase spectra relating CN and VN discharges. In each panel, the control (trace 1 or ○ for phase angle) and test (during PAG stimulation; trace 2 or □) spectra are superposed on the same scale. Each spectrum is the average of 32 5-s windows with either 50% (control) or 75% (test) overlap. Frequency resolution is 0.2 Hz/bin.
were z-transformed (Rohlf and Sokal 1969) before paired comparisons were made. Values in the text are means ± SE with P ≤ 0.05 used to signify statistically significant differences. The Pearson product-moment correlation coefficient (r value) (Rohlf and Sokal 1969) was used to test for a significant relationship between the changes in sympathetic nerve powers and CN-VN lag time produced by brain stem stimulation.

Partialization of the CN-VN coherence function and phase spectrum was performed by using standardized 5-V square-wave pulses (5-ms duration) representing electrical stimuli applied to the PAG. This procedure allowed us to determine whether the responses of the two nerves (locked 1:1 to the stimuli) were attributable to factors in addition to the direct influences of their inputs from the PAG. As described in earlier reports from our laboratory (Gebber et al. 1994a,b), partialization involves, first, mathematical removal of the portion of two signals (CN and VN discharges) that is attributable to a third signal (PAG stimulus), and second, computation of the relationships between the residual components of CN and VN discharges. The mathematical formulas used to calculate the partial coherence and partial phase spectrum, and the algorithms on which our software is based can be found in Jenkins and Watts (1968) and Bendat and Piersol (1986). If the coherence of CN and VN discharges is attributable solely to the direct influences of inputs from the PAG, partialization will reduce the CN-VN coherence value at the frequency of stimulation to zero. If, on the other hand, the central circuits governing these nerves are tightly coupled, partialization will not necessarily reduce the coherence value to zero (Gebber et al. 1994a,b; Kalitzin et al. 1997; Lopes da Silva et al. 1980). In such cases, we also partialized the CN-VN phase spectrum using the PAG stimuli. This allowed us to test whether the changes in CN-VN phase angle (10-Hz band) produced by PAG activation reflected alterations in the phase relations among coupled oscillators rather than the difference in conduction times to the two nerves from the site of stimulation. In the former case, the phase angle during PAG stimulation should be the same before and after partialization (Jenkins and Watts 1968). In the latter case, the change in phase angle produced by PAG stimulation would be reversed by partialization if the residual coherence value in the 10-Hz band remained significantly different from zero. The value of phase angle would be random and, thus, meaningless if coherence is reduced to zero.

RESULTS

Effects of caudal PAG stimulation on 10-Hz rhythm in SND

of baroreceptor-denervated cats

SINGLE SHOCKS. The biphasic primary response elicited in both the CN and VN by single shocks (100–500 μA) applied once every 2 s to sites in the caudal PAG was an increase in activity (upward negative potential) followed by a decrease in activity (downward positive wave). A typical example is shown in Fig. 2, where the traces are peri-stimulus averages of 122 CN and VN responses with the PAG stimulus applied at time 0. In 9 cases, the onset latency of the negative potential was 64 ± 4 (SE) ms for the CN and 74 ± 6 ms for the VN. The ratio of the negative to positive wave (peak amplitudes measured from baseline) was 1.7 ± 0.2 for the CN and 0.9 ± 0.1 for the VN. The durations of the positive waves in the CN (200 ± 25 ms) and VN (192 ± 21 ms) were not significantly different. As shown in Fig. 2, the biphasic primary responses of the CN and VN were followed by damped oscillations whose periods corresponded to that of the free-running (no stimulation) 10-Hz rhythm. The damped oscillations indicate that PAG stimulation reset the 10-Hz rhythm in SND (Huang et al. 1992; Pavlidis 1973). The portions of the averages of CN and VN activities preceding the PAG stimuli are flat because the stimuli were delivered randomly with respect to the phases of the free-running 10-Hz rhythm.

FREQUENCIES OF STIMULATION (7–12 Hz) NEAR THAT OF THE FREE-RUNNING RHYTHM. The responses of the CN and VN were related to the frequency of PAG stimulation in a remarkable way. Frequencies of PAG stimulation at or slightly above that of the free-running rhythm produced reciprocal changes in the 10-Hz discharges of the two nerves, whereas lower frequencies of stimulation of the same sites did not. In the experiment illustrated in Fig. 3, the frequency of caudal PAG stimulation was the same as that (10.0 Hz) of the free-running rhythm in SND. The amplitude-time series show that PAG stimulation (begun at the 1st vertical line) almost immediately increased 10-Hz slow-wave amplitude in the CN (Fig. 3A, top) but decreased that in the VN (Fig. 3A, middle). The changes in slow-wave amplitudes were accompanied by an increase in the phase lag of VN 10-Hz activity relative to CN 10-Hz activity from between 40 and 90° to between 110 and 180°. The change in phase angle is shown in both the relative phase-time series (Fig. 3A, bottom) and the short strips of digitally filtered records of CN and VN discharges (Fig. 3, B and C) that formed part of the data block from which the time series were derived. Note that the effects of PAG activation were reversed soon after PAG stimulation was stopped at the second vertical line in Fig. 3A.

The spectra in Fig. 4A were derived from the same data block used to construct the time series in Fig. 3A. The control autospectra of CN and VN activities (Fig. 4A, trace 1 in top 2 panels) show a sharp peak at 10 Hz. Caudal PAG stimulation increased the power in the 10-Hz band of CN activity (Fig. 4A, trace 2 in top panel) and decreased VN 10-Hz power (Fig. 4A, trace 2 in 2nd panel). The changes in 10-Hz band power are disproportionate relative to the changes in slow-wave ampli-
tude in the corresponding time series (Fig. 3A). This is explained in large part by the fact that power is the square of the voltage. The changes in power at frequencies \( \leq 6 \) Hz will be described later. PAG stimulation had little effect on coherence in the 10-Hz band. Note that the peak coherence value relating the 10-Hz discharges of the two nerves was \( >0.9 \) both before and during PAG stimulation (Fig. 4A, traces 1 and 2 in 3rd panel). A coherence value \( \geq 0.1 \) reflects a statistically significant correlation between CN and VN activities when 32 windows are averaged (Benignus 1970). Figure 4A, bottom panel, shows the phase spectra relating CN and VN discharges. The phase angle (VN activity lags CN activity) at the frequency of the peak coherence in the 10-Hz band was lengthened from near 70° in control (\( F \)) to near 140° (\( E \)) during PAG stimulation. This change was statistically significant as determined by using the 95% confidence limits for the phase angle provided by Jenkins and Watts (1968). The 95% confidence limits are \( \pm 2^\circ \) for a coherence value of 0.9 when 32 windows are averaged. The values of phase angle at frequencies \( \leq 6 \) Hz before and during PAG stimulation were not significantly different. Nevertheless, because the low power components of CN and VN discharges at frequencies \( \leq 6 \) Hz were weakly correlated (coherence values generally \(<0.5\)), the values of phase angle at these frequencies are less reliable for comparative purposes. Regarding this point, the 95% confidence limits for the phase angle are \( \pm 20^\circ, \pm 30^\circ, \) and \( \pm 40^\circ \) for coherence values of 0.4, 0.2, and 0.1, respectively, when 32 windows are averaged.

The data in Fig. 1 are from another experiment in which the frequency of PAG stimulation was the same as that (9.4 Hz) of the free-running rhythm in SND. The time series in this case only shows the first several seconds of PAG stimulation (begun at vertical line). Such short time series demonstrated that the reciprocal changes in CN and VN 10-Hz slow-wave amplitudes were temporally correlated to the changes in CN-VN phase angle in this band.

The spectra in Fig. 4B are from an experiment in which the frequency of PAG stimulation (10.0 Hz) was slightly higher than that (8.8 Hz) of the free-running rhythm. As was the case when the stimulus frequency was the same as that of the free-running rhythm, CN 10-Hz power was increased (Fig. 4B, top panel), whereas VN 10-Hz power was decreased (Fig. 4B, 2nd panel). The reciprocal changes in 10-Hz power were accompanied by an increase in the CN-VN phase angle in the 10-Hz band from near 60° to near 160° (Fig. 4B, bottom panel). Although somewhat reduced, the peak coherence value in the 10-Hz band remained \( >0.8 \) during PAG stimulation (Fig. 4C, 3rd panel). Importantly, the superposed autospectra of CN and VN discharges show that power in the 10-Hz band was moved to the frequency of PAG stimulation at the expense of power at the frequency of the free-running rhythm (Fig. 4B, top 2 panels). This observation suggests that the 10-Hz rhythm was entrained in a 1:1 relation to the PAG stimuli.

In contrast to the responses elicited by frequencies of PAG stimulation equal to or somewhat higher than that of the free-running rhythm, stimulation of the same PAG sites at lower frequencies increased power in the 10-Hz band of VN as well as CN activity. Figure 5 shows a case in which the frequency of PAG stimulation was 8.0 Hz while that of the free-running rhythm was 9.0 Hz. In this case, the increases in CN and VN 10-Hz discharges were nearly proportional, and CN-VN phase angle and coherence in the 10-Hz band were not
The frequency of PAG stimulation was the same as that (10.0 Hz) of the free-running rhythm. CN 10-Hz band power was unchanged, whereas VN 10-Hz band power was significantly decreased. As a consequence, CN 10-Hz band power was increased more than total power. VN 10-Hz band power was significantly reduced. As a consequence, CN 10-Hz band power and stimulus intensity were inversely related.

The effects produced by low-frequency (7–12 Hz) stimulation of 18 sites in the caudal PAG of 15 baroreceptor-denervated cats are summarized below. The data include 34 cases of PAG stimulation at frequencies equal to or above that of the free-running rhythm and 26 cases at stimulus frequencies lower than that of the free-running rhythm. For seven of these PAG sites, we used frequencies of stimulation below, equal to, and above that of the free-running rhythm. For the remaining sites, the frequencies of stimulation were either below and above or below and at that of the free-running rhythm. For any given site, stimulus intensity (200–1,000 μA) was kept the same for each of the stimulus frequencies used. The data reported are for stimulus intensities that produced maximum changes in CN and VN discharges. The frequency of the free-running rhythm in these experiments ranged from 8.0 to 11.8 Hz.

Table 1A shows the changes in power in selected bands of SND produced by PAG stimulation at frequencies equal to or above that of the free-running rhythm. CN 10-Hz band power was significantly increased, whereas 0- to 6-Hz band power was significantly reduced. As a consequence, CN 10-Hz band power was increased more than total power. VN 10-Hz band power was significantly reduced, whereas 0- to 6-Hz band power was unchanged. As a consequence, VN 10-Hz power was reduced more than total power.
accounted for 31 ± 3% of total power in control and 58 ± 3% of total power during PAG stimulation. The corresponding values for the VN were 35 ± 2% (control) and 20 ± 2% (during PAG stimulation). Mean blood pressure was significantly increased from 98 ± 4 to 115 ± 5 mmHg during PAG stimulation at frequencies equal to or above that of the free-running rhythm. Table 2A1 shows the changes in CN-VN coherence, phase angle, and lag time in the 10-Hz band for the same cases. The reduction in peak coherence produced by PAG stimulation was small but statistically significant. Phase angle and lag time were significantly increased.

Table 1A2 shows the changes in power for cases in which the frequency of PAG stimulation was below that of the free-running rhythm. VN and CN 10-Hz band powers were significantly increased in these cases, but the change was significantly more pronounced for the CN. There were no significant changes in 0- to 6-Hz band power. CN 10-Hz band power accounted for 23 ± 2% of total power in control and 62 ± 3% of total power during PAG stimulation. The corresponding values for the VN were 32 ± 2% (control) and 53 ± 3% (during PAG stimulation). Frequencies of PAG stimulation below that of the free-running rhythm significantly increased mean blood pressure from 90 ± 2 to 109 ± 6 mmHg. Table 2A2 shows the changes in CN-VN coherence, phase angle, and lag time in the 10-Hz band for the same cases. Coherence in the 10-Hz band was not significantly affected by PAG stimulation, whereas phase angle and lag time were significantly increased. However, the changes in phase angle and lag time were not as large as those produced by stimulus frequencies at or above that of the free-running rhythm. These differences were statistically significant.

Figure 8 shows that the change in CN-VN lag time (10-Hz band) produced by PAG stimulation was directly correlated to the extent to which CN and VN 10-Hz band powers were differentially affected. We used the ratio of CN to VN 10-Hz band powers as a measure of the differential effects produced by PAG stimulation. The ratio was calculated from values of power expressed as a percent of control. The ratio in the absence of PAG stimulation was arbitrarily set at 1:1. Thus, if PAG stimulation increased CN 10-Hz band power to 400% of control and reduced VN 10-Hz band power to 50% of control, the ratio was 8:1 during PAG stimulation. In cases when PAG stimulation increased 10-Hz power in both nerves, the increase always was at least somewhat greater for the CN than VN. Therefore ratios >1:1 were the rule. The increase in CN-VN lag time in the 10-Hz band (derived from phase angle) was the difference between the values before and during PAG stimulation. The filled circles in Fig. 8 are for cases when low-frequency PAG stimulation led to reciprocal changes in CN and VN 10-Hz discharges. The open triangles are for cases when low-frequency PAG stimulation increased the 10-Hz discharges of both nerves. Nine additional data points (open circles) are for cases of high-frequency (25 Hz) PAG stimulation that will be described later. Pooling of the data points (n = 69) revealed that the ratio of CN to VN 10-Hz band powers during PAG stimulation and the increase in CN-VN lag time in this band were directly related. The Pearson product-moment correlation coefficient was highly significant.
A correlation coefficient (\(r\) value = 0.61) was statistically significant (\(P = 2 \times 10^{-6}\)).

Partialization of the CN-VN coherence function and phase spectrum was performed by using the standardized pulses representing the stimuli applied to the PAG. The purpose of this analysis was to determine whether the responses of the CN and VN locked 1:1 to PAG stimuli are attributable to factors in addition to the direct influences of their inputs from the site of stimulation. For example, if the oscillators governing the discharges of the two nerves are tightly coupled, partialization will not necessarily reduce the coherence value at the frequency of stimulation to zero (Gebber et al. 1994a,b; Kalitzin et al. 1997; Lopes da Silva et al. 1980). In 8 of 12 cases, the residual coherence value (after partialization) relating CN and VN activities at the frequency of PAG stimulation exceeded 0.5. A typical example is shown in Fig. 9. In this case, the frequency of PAG stimulation was the same as that (9.4 Hz) of the free-running rhythm. Partialization reduced the coherence value at the frequency of stimulation from 0.95 (Fig. 9B, top) to 0.67 (Fig. 9C, top). Importantly, the CN-VN phase angle at the frequency of PAG stimulation was not changed by partialization of the phase spectrum. The phase angle before PAG stimulation was near 50° (Fig. 9A, bottom). This value was increased to near 110° during PAG stimulation (Fig. 9B, bottom), and partialization using the standardized pulses representing the stimuli did not reverse this change (Fig. 9C, bottom). As determined with the 95% confidence limits of Jenkins and Watts (1968), the values of phase angle during PAG stimulation were not significantly affected by partialization in
and during (test) PAG stimulation in baroreceptor-denervated cats produced by PAG stimulation in baroreceptor-denervated cats.

TABLE 1. Changes in sympathetic nerve powers (% of control) produced by PAG stimulation in baroreceptor-denervated cats

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<th>CN</th>
<th>VN</th>
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<tr>
<td><strong>A. Low-frequency (7–12 Hz) PAG stimulation</strong></td>
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<tr>
<td>10-Hz band power</td>
<td>618 ± 98*†</td>
<td>33 ± 4*†</td>
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<tr>
<td>0- to 6-Hz power</td>
<td>74 ± 9*</td>
<td>112 ± 10</td>
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<tr>
<td>Total power</td>
<td>276 ± 33*</td>
<td>64 ± 6*</td>
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<td><strong>B. High-frequency (25 Hz) PAG stimulation</strong></td>
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<tr>
<td>10-Hz band power</td>
<td>803 ± 93*†</td>
<td>224 ± 22*†</td>
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<tr>
<td>0- to 6-Hz power</td>
<td>73 ± 22</td>
<td>112 ± 12</td>
</tr>
<tr>
<td>Total power</td>
<td>279 ± 32*</td>
<td>129 ± 9*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n is number of cases. PAG, periaqueductal gray; CN, inferior cardiac sympathetic nerve; VN, vertebral sympathetic nerve. Arrows point in the direction of the change of power in the 10-Hz band. Total power is for 0- to 15-Hz band. * Significantly different from control by paired comparison (P ≤ 0.05). † Significantly different from changes in 0- to 6-Hz band. Power is for 0- to 15-Hz band. * Significantly different from control by paired comparison (P ≤ 0.05).

TABLE 2. Peak coherence values, phase angles, and lag times relating the 10-Hz discharges of the CN and VN before (control) and during (test) PAG stimulation in baroreceptor-denervated cats

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>Coherence</th>
<th>Phase Angle, deg</th>
<th>Lag Time, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Low-frequency (7–12 Hz) PAG stimulation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.2 ± 0.1</td>
<td>0.96 ± 0.01</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>Test</td>
<td>9.6 ± 0.1</td>
<td>0.87 ± 0.02*</td>
<td>166 ± 9*</td>
</tr>
<tr>
<td><strong>B. High-frequency (25 Hz) PAG stimulation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.1 ± 0.1</td>
<td>0.96 ± 0.01</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>Test</td>
<td>8.2 ± 0.2</td>
<td>0.92 ± 0.02</td>
<td>91 ± 8*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n is number of cases. Phase angle and lag time refer to the delay of VN 10-Hz activity relative to that in the CN. For abbreviations, see Table 1. Arrows point in the direction of the change of power in the 10-Hz band. Frequency refers to the frequency of the 10-Hz rhythm in CN and VN activities, measured in Hz. Coherence (scale, 0–1.0), phase angle (in degrees), and lag time (ms) were measured at the frequency of the 10-Hz rhythm. * Significantly different from control by paired comparison (P ≤ 0.05).

FIG. 8. Relationship between ratio of powers in the 10-Hz band of CN and VN activities during PAG stimulation and the change in CN-VN lag time from control. The ratio was calculated from values of power expressed as a % of control (see text). The CN/VN powers ratio in the absence of PAG stimulation (control) was arbitrarily set at 1:1. The CN/VN powers ratio (log scale) is plotted against the change in CN-VN lag time at the frequency of peak coherence in the 10-Hz band. The data points are for 69 cases in 15 baroreceptor-denervated cats. Filled circles are for cases when low-frequency PAG stimulation increased CN 10-Hz band power and decreased VN 10-Hz band power. Open triangles are for cases when low-frequency PAG stimulation increased both CN and VN 10-Hz band powers. Open circles are for cases when high-frequency (25 Hz) PAG stimulation reciprocally affected CN and 10-Hz band powers. The r value for the pooled data was 0.61 (P = 2 × 10⁻⁴).

FIG. 9. Partialization. A: control coherence function (top) and phase spectrum (bottom) relating the discharges of the CN and VN in the absence of PAG stimulation. B: same, but during PAG stimulation at a frequency equal to that (9.4 Hz) of the free-running rhythm in SND. C: partial coherence function (top) and partial phase spectrum (bottom). Partialization of the spectra in B yielding the spectra in C was performed by using standardized pulses representing the PAG stimuli. Dotted lines can be used as guides in comparing phase angles in the 10-Hz band before and during PAG stimulation, and after partialization. The spectra are averages of 32 5-s windows and the frequency resolution is 0.2 Hz/bin.
CN 10-Hz band power and decreased VN 10-Hz band power (Table 1B). Powers in the 0- to 6-Hz band of CN and VN discharges were not changed significantly. CN 10-Hz band power accounted for $31 \pm 3\%$ of total power before and $36 \pm 4\%$ of total power during high-frequency PAG stimulation. The corresponding values for the VN were $37 \pm 3\%$ (control) and $20 \pm 4\%$ (during PAG stimulation). Mean blood pressure was significantly increased from $92 \pm 7$ to $123 \pm 12$ mmHg during high-frequency PAG stimulation. Table 2 shows that high-frequency PAG stimulation produced a small but statistically significant decrease in the peak coherence value in the 10-Hz band. Phase angle and lag time in the 10-Hz band were significantly increased but not to the same extent as during stimulation of the same PAG sites at frequencies equal to or just above that of the free-running rhythm. Regarding this point, the ratio of CN to VN 10-Hz band powers typically was higher when the frequency of stimulation was the same as that of the free-running rhythm (Fig. 8).

Stimulation of the medullary LTF

The effects on CN and VN discharges produced by electrical activation of eight sites in the medullary LTF (see METHODS) of six baroreceptor-denervated cats were strikingly different from those produced by PAG stimulation. Independent of whether the LTF stimulus frequency (7–14 Hz range) was below, equal to, or higher than that of the free-running rhythm, CN and VN 10-Hz band powers were increased to nearly the same extent (Fig. 11, A–C, top 2 panels). Moreover, phase angle in the 10-Hz band was essentially unchanged (Fig. 11, A–C, bottom panel). Peak coherence in this band also was little affected by LTF stimulation (Fig. 11, A–C, 3rd panel). As previously described for the PAG, peak power in the 10-Hz band was moved to frequencies of LTF stimulation that were below (Fig. 11A) or above (Fig. 11C) that of the free-running rhythm. Thus LTF as well as PAG stimulation entrained the 10-Hz rhythm in SND. Although not shown, high-frequency (25 Hz) LTF stimulation increased CN and VN discharges uniformly and raised mean blood pressure by $40$ mmHg.

Our summary of the effects of medullary LTF stimulation is limited to eight episodes when the stimulus frequency was the same as that of the free-running rhythm (e.g., Fig. 11B). Stimulus intensity ranged from 150 to 500 $\mu$A. Power in the 10-Hz band of CN activity was increased to $36 \pm 3\%$ of control, whereas VN 10-Hz power was increased to $39 \pm 6\%$ of control. CN-VN phase angle at the frequency of peak coherence in the 10-Hz band was not significantly affected. Values of phase angle were $40 \pm 23\degree$ before and $54 \pm 26\degree$ during medullary LTF stimulation. Peak coherence values in the 10-Hz band were $0.93 \pm 0.02$ before and $0.96 \pm 0.01$ during medullary LTF stimulation.
DISCUSSION

Reciprocal changes in sympathetic outflows to the heart (or kidney) and skeletal muscle in response to midbrain PAG or hypothalamic stimulation were first reported by Kollai and Koizumi (1980) and Dean and Coote (1986). These studies, however, were performed without reference to the frequency composition of SND or the phase relations between the discharges of nerve pairs. Moreover, no consideration was given to the question of whether the pattern of changes produced by central activation was dependent on the frequency of stimulation. The current study focused on these issues.

As did Kollai and Koizumi (1980) and Dean and Coote (1986), we used electrical stimuli to activate sites in the brain. It is obvious that electrical stimulation is an artificial means of eliciting sympathetic nerve responses. Nevertheless, this approach allowed us to uncover the dependency of pattern on stimulus frequency, thus providing a window through which underlying mechanisms for the reciprocal changes in CN and VN activities could be examined. Although we cannot attribute the changes in SND produced by electrical stimulation specifically to the activation of neuronal somata, enhanced cardiac function and reciprocal changes in blood flows to the viscera and skeletal muscle have been reported on chemical activation of neurons in the caudal PAG (Carrive 1991; Dampney 1994; Hilton and Redfern 1986).

Previous work from our laboratory demonstrated that the 10-Hz rhythm in SND is generated in the brain stem by a system of coupled oscillators, each of which preferentially or selectively controls a different portion of the spinal sympathetic outflow (Gebber et al. 1994b; Huang et al. 1992). The current study extends these findings in new directions. First, we found that this system of coupled oscillators is influenced by inputs from the defense region of the caudal PAG. Regarding this point, single shocks applied to the PAG reset the 10-Hz rhythm in SND. Furthermore, peak power in the 10-Hz band of SND was moved to the frequency of PAG stimulation (range, 7–12 Hz) at the expense of power at the frequency of the free-running rhythm. We interpret this finding to indicate that inputs from the PAG entrained the 10-Hz rhythm (Glass and Mackey 1988; Huang et al. 1992; Pavlidis 1973). Second, we found that the differential changes in CN and VN discharges elicited by PAG stimulation were largely restricted to the 10-Hz band of activity. Regarding this point, PAG stimulation either failed to significantly affect the power in the 0- to 6-Hz band of CN and VN discharges or, in one experimental series, changed 0- to 6-Hz power in CN discharges in a direction opposite that of the change in 10-Hz band power. Thus the differential changes in CN and VN discharges were attributable primarily to perturbation of the system responsible for the 10-Hz rhythm.

Concerning the mechanisms responsible for the reciprocal changes in CN and VN 10-Hz discharges, several observations argue against the possibility that PAG stimulation simply engaged hard-wired pathways that independently excited some brain stem oscillators or spinal cell groups while inhibiting others. First, single shocks applied to the caudal PAG elicited short-latency excitatory responses in both the CN and VN. Second, whereas VN 10-Hz activity was significantly reduced by PAG stimulation at frequencies equal to or above that of the free-running rhythm, stimulus frequencies just below that of the free-running rhythm significantly increased the 10-Hz discharges of this nerve. The critical stimulus frequency at which the reversal occurred always was that of the free-running rhythm. This observation is remarkable considering the fact that the frequency of the free-running rhythm ranged from 8.0 to 11.8 Hz. Third, the coherence value relating CN and VN discharges at the frequency of PAG activation (7–12 Hz range) remained significantly different from zero in most cases following partialization using the PAG stimuli. The residual coherence indicates that the 10-Hz oscillators controlling the two nerves remained tightly coupled during their entrainment in a 1:1 relation to the PAG stimuli (Gebber et al. 1994a,b; Kalitzin

FIG. 11. Spectral analysis of the effects of medullary lateral tegmental field (LTF) stimulation on the discharges of the CN and VN. The frequency of LTF stimulation was 9.2 Hz in A, 11.6 Hz in B, and 13.4 Hz in C. The frequency of the free-running rhythm was 11.6 Hz. Stimulus intensity was 500 μA. Sequence and format of panels are as in Fig. 1D, except that the phase spectra are scaled between 0 and 180°. The spectra are averages of 32 5-s windows, and the frequency resolution is 0.2 Hz/bin. Control (trace 1 or ●) and test (LTF stimulation; trace 2 or ○) spectra are superposed in each panel.
et al. 1997; Lopes da Silva et al. 1980). Importantly, the phase angle relating CN and VN discharges at the frequency of PAG stimulation was not changed after partialization. Thus lengthening of the CN-VN phase angle during PAG stimulation could not be attributed simply to the difference in conduction times to the two nerves from the site of stimulation. Rather, lengthening of the phase angle suggests that PAG stimulation altered the phase relations between the coupled 10-Hz oscillators governing the CN and VN. This suggestion is further supported by the results obtained with high-frequency PAG stimulation. As did frequencies of stimulation at or slightly above that of the free-running rhythm, high-frequency (25 Hz) stimulation reciprocally affected CN and VN discharges and lengthened the phase lag of VN 10-Hz activity relative to CN activity. In the case of high-frequency activation, it is unlikely that lengthening of the CN-VN phase angle can be explained by differences in conduction times to the two nerves from the site of stimulation because the 10-Hz rhythm was not entrained 1:1 to the PAG stimuli.

It is also unlikely that PAG stimulus-induced decreases in VN 10-Hz activity reflected postexcitatory depression that was more pronounced in the VN than CN. If postexcitatory depression was an important factor in explaining the reduction of VN 10-Hz activity, we would not have expected the frequency of PAG stimulation at which the reversal from increased to decreased VN 10-Hz activity occurred to be strictly tied to the frequency of the free-running rhythm. Moreover, we would not have expected LTF stimulation to increase CN and VN 10-Hz discharges proportionately if postexcitatory depression was more pronounced in the VN than CN. Finally, cycle-by-cycle measurements of slow-wave amplitude demonstrated that the inhibition of VN 10-Hz discharges produced by PAG stimulation was not preceded by an initial excitation (see Figs. 1A and 3A). Although Kollai and Koizumi (1980) reported that the duration of postexcitatory depression following hypothalamic stimulation was longer in the VN than CN of the cat, we did not find this to be the case when single shocks were applied to the PAG once every 2 s. Nevertheless, we did find that the ratio of amplitudes of the initial negative wave and succeeding positive wave elicited by single shocks was greater for the CN than VN.

Coherence and phase angle were used to characterize the coupling of the 10-Hz discharges of the CN and VN. The former measures the strength of coupling, whereas the latter measures the relative timing of the bursts in the two nerves. Whereas the strength of coupling was not much affected by PAG stimulation, the reciprocal changes in CN and VN 10-Hz discharges were accompanied by a dramatic increase in the phase lag of VN activity relative to CN activity. Under the assumption that the state of coupling of brain stem circuits is reflected by the CN-VN phase angle, the results of the current study are consistent with the hypothesis that dynamic changes in the phase relations between coupled 10-Hz oscillators lead to differential patterns of spinal sympathetic outflow. First, the differential changes in the 10-Hz discharges of the two nerves produced by PAG stimulation were temporally correlated to the increase in the phase lag of VN activity relative to CN activity. Second, the magnitude of change in phase angle was directly related to the extent to which PAG stimulation differentially affected the 10-Hz discharges of the two nerves. Thus the phase angle was increased more when CN and VN 10-Hz discharges were reciprocally affected than when the 10-Hz discharges of both nerves were increased, albeit disproportionately. Third, uniform increases in the 10-Hz discharges of the CN and VN produced by medullary LTF stimulation, and occasionally by frequencies of PAG stimulation below that of the free-running rhythm, were not accompanied by a significant change in phase angle in the 10-Hz band.

The question can be raised whether the changes in CN-VN phase angle produced by PAG stimulation reflected changes in the shape of the 10-Hz sympathetic nerve slow waves (e.g., from sinusoidal to asymmetric spikelike waves) rather than alterations in the phase relations between coupled brain stem oscillators. This possibility seems unlikely for two reasons. First, time series analysis was performed only after digital filtering was used to extract the 10-Hz slow waves from the total signal. The filtered slow waves were sinusoidal in shape both before and during PAG stimulation (see Figs. 1B and 3, B and C). Second, in the case of spectral analysis, FFT determines the phase angle by fitting series of harmonically related sine and cosine waves to the data (Bendat and Piersol 1986; Jenkins and Watts 1968).

Whereas our hypothesis is that changes in the phase relations among coupled 10-Hz oscillators reflect the events leading to differential patterns of spinal sympathetic outflow, it remains to be determined whether phase angle itself is the parameter responsible for the patterns that emerge. Discussion of this matter could be useful in guiding the direction of future studies. In general terms, coupled nonlinear oscillators are dynamical systems in that changes in the levels of control parameters (inputs to system) lead to abrupt changes in the relative timing (i.e., phase relations) of their outputs during each cycle of activity (Haken 1996; Kelso 1995). The phase relations characterizing the state of coupling in which the system exists at any given time is determined by the internal self-organizing properties of the system and the values of the control parameters. The repertoire of possible states is dependent, in addition, on such factors as the number of oscillators in the system and their interconnections (e.g., near vs. far neighbors). These factors remain to be defined for the brain stem system responsible for the 10-Hz rhythm in SND. Models of such systems have been used to explain how the pattern of locomotion might be changed from one characterized by in-phase movements of the limbs to another characterized by out-of-phase movements (Beek et al. 1992; Grillner 1981; Haken 1996; Jeka et al. 1993; Kelso 1995). Here, we invoke the same general principles to explain how a shift in phase angle might lead to reciprocal changes in sympathetic outflows to different targets.

Whereas skeletal muscle contractions generally follow the frequency of rhythmic motor nerve activity, this is not the case for vascular smooth muscle. Rather, vascular smooth muscle acts as a low-pass filter with a cutoff frequency well below that of the 10-Hz rhythm. How then might changes in the phase relations among coupled 10-Hz oscillators in the brain stem lead to differential patterns of spinal sympathetic outflow and, thus, changes in regional blood flow occurring in opposite directions? Because elimination of the 10-Hz rhythm in SND by chemical inactivation or ablation of selected brain stem regions significantly reduces blood pressure (Zhong et al. 1993), it seems reasonable to assume that 10-Hz SND is transduced into a “steady-state” level of vascular smooth muscle contraction. Under this condition, differential patterns of spinal sympathetic outflow would result if alter-
ations in the phase relations among coupled oscillators induced nonuniform changes in the amplitudes of the 10-Hz slow waves in nerves with different targets. Regarding this possibility, in-phase bidirectional coupling of two oscillators by excitatory connections can lead to mutual reinforcement of their outputs during each cycle of rhythmic activity (König and Schillen 1991). This situation can be changed by incorporating a lag into the system. For example, Reddy et al. (1998) have demonstrated that coupled limit cycle oscillators can drive one another to a state of zero amplitude output when their mutual interactions are suitably delayed. Perhaps most relevant to the current study is a model of oscillators coupled by mutual inhibitory connections (Glass and Mackey 1988). One of the possible functional states in such a system is characterized by a high rate of firing of the cells of one of the oscillators and a low rate of firing of cells in the second oscillator. The applicability of such models to the data reported in the current study remains to be determined.

Chemical or electrical activation of the caudal PAG elicits changes in heart rate and regional blood flows characteristic of the naturally occurring defense reaction (Carrive 1993; Damney 1994; Hilton 1982; Hilton and Redfern 1986). In cats, full expression of the increase in blood flow to skeletal muscle during the defense reaction requires not only the activation of sympathetic cholinergic vasodilator fibers and the release of epinephrine from the adrenal medulla, but also the selective inhibition of vasoconstrictor outflow to these vascular beds (Coote et al. 1973; Folkow et al. 1964). Thus the reciprocal changes in power in the 10- to 6-Hz band of SND was little affected by frequencies of stimulation of the same sites. This apparent nonuniform changes in the 10-Hz discharges produced by caudal PAG stimulation.


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