Sympathetic nerve and cardiovascular responses to chemical activation of the midbrain defense region

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Larson, Peter D., Sheng Zhong, Gerard L. Gebber, and Susan M. Barman. Sympathetic nerve and cardiovascular responses to chemical activation of the midbrain defense region. Am J Physiol Regulatory Integrative Comp Physiol 280: R1704–R1712, 2001—The changes in mean arterial pressure (MAP), renal (RBF) and femoral (FBF) blood flows, and inferior cardiac (CN) and vertebral nerve (VN) sympathetic nerve discharges (SND) produced by chemical activation (0.1-homocysteic acid) of the midbrain periaqueductal gray (PAG) were compared in baroreceptor-denervated and -innervated cats anesthetized with urethan. Defenselike cardiovascular responses in both states were similar in magnitude and consisted of increased MAP and FBF and decreased RBF; however, the nerve responses differed. In baroreceptor-denervated cats, PAG activation increased CN 10-Hz activity, decreased VN 10-Hz activity, and lengthened the CN-VN phase angle. In baroreceptor-innervated cats in which the rhythm in SND was cardiac related, PAG activation increased CN activity, but VN activity and the CN-VN phase angle were unchanged. These results demonstrate that chemical activation of PAG neurons induces differential patterns of sympathetic outflow generally consistent with accompanying defenselike cardiovascular responses. However, the mechanisms responsible for the changes in 10-Hz and cardiac-related SND appear to be different.

Periaqueductal gray; regional blood flows; sympathetic nerve discharge

TWO STUDIES FROM OUR LABORATORY (10, 11) described the patterns of sympathetic outflow evoked by electrical stimulation of the defense region of the subventricular midbrain periaqueductal gray (PAG). In baroreceptor-denervated cats, we observed immediate and sustained increases in the 10-Hz discharges of the inferior cardiac (CN) and renal (RN) postganglionic sympathetic nerves, but a decrease in vertebral sympathetic nerve (VN) 10-Hz activity. The CN, RN, and VN provide sympathetic outflow to the heart, kidney, and forelimb vasculature, respectively. The reciprocal changes in VN vs. CN and RN discharges were accompanied by significant lengthening of the CN-VN phase angle and shortening of the VN-RN phase angle in the 10-Hz band. Using the work of Haken (12) and Kelso (16) as a guide, we proposed that the changes in phase angle reflected alterations in the coupling of multiple brain stem 10-Hz oscillators, which, in turn, led to the differential responses of the three nerves (10, 11). This proposal was supported by the temporal correlation of the changes in phase angle and nerve activities and the fact that the magnitude of changes in phase angle was directly related to the extent to which PAG stimulation reciprocally affected the 10-Hz discharges of the CN and VN. Moreover, the response pattern to PAG stimulation was switched to one of increased activity in all three nerves when the frequency of stimulation was reduced to just below that of the free-running 10-Hz rhythm in sympathetic nerve discharge (SND). Importantly, phase angles were unchanged when CN, VN, and RN 10-Hz discharges were uniformly increased. This observation and the results obtained with partial phase spectral analysis (10) virtually ruled out the possibility that the phase angle between the discharges of nerve pairs simply reflected the difference in conduction times to the nerves from the site of PAG stimulation.

The pattern of changes in CN, RN, and VN discharges was different in baroreceptor-innervated cats in which the predominant rhythm in SND was at the frequency of the heart beat (near 3 Hz) rather than near 10 Hz. Under this condition, CN and RN discharges were dramatically increased during the first 20 s of PAG stimulation, but VN activity was either unchanged or modestly increased. Later, as blood pressure rose, VN activity usually was reduced, whereas the increases in CN and RN discharges were somewhat blunted. At no time during PAG stimulation was there a change in the CN-VN or VN-RN phase angles at the cardiac frequency. It was proposed that the late decrease in VN cardiac-related activity reflected increased baroreceptor nerve discharge attendant to the rise in blood pressure (11). Thus we attributed the differential changes in the 10-Hz vs. cardiac-related discharges of the CN, RN, and VN to different central mechanisms.

The experiments with electrical stimulation of the PAG left at least two important questions unanswered. First, did the sympathetic nerve responses involve the...
activation of neuronal cell bodies in the PAG or fibers of passage? Second, were the differential sympathetic nerve responses accompanied by regional changes in blood flows characteristic of the defense reaction? These include decreased renal blood flow and increased skeletal muscle blood flow (1, 5, 13, 20). In the current study, we addressed these two issues by recording renal and femoral blood flows together with CN and VN discharges during chemical activation of the defense region of the PAG with the excitatory amino acid D,L-homocysteic acid (DLH).

METHODS

Experimental subjects and general procedures. The protocols used on 10 cats of either sex (2.75–4.25 kg) were approved by the All-University Committee on Animal Use and Care of Michigan State University. The cats were initially anesthetized with 2.5–3.5% isoflurane in oxygen. Urethane (1.2–1.8 g/kg iv) was administered via a catheter inserted into the right femoral vein, and isoflurane inhalation was terminated. This dose range of urethan has been reported (8) to maintain a surgical level of anesthesia for a period (8–10 h) that exceeded the duration of our experiments. Noxious stimuli (pinch, cauterization of muscle, surgery) failed to desynchronize the electroencephalogram (EEG) at any time during the experiment. The EEG was recorded with an electrode placed on the skull overlying the frontal-parietal cortex (3). Blood pressure was measured from a catheter inserted into the right femoral artery. The cats were paralyzed (galantine triethiodide, 4 mg/kg iv, initial dose) and artificially ventilated with room air; a bilateral pneumothoracotomy was performed. End-tidal CO₂ was maintained near 4.0% by adjusting the parameters of ventilation. Rectal temperature was maintained near 38°C with a heat lamp.

Baroreceptor denervation was performed in six of the cats by bilateral section of the carotid sinus, aortic depressor, and vagus nerves (3). Sympathetic nerve recordings were begun before baroreceptor denervation in two of these cats. In these preparations, section of the baroreceptor and vagus nerves eliminated the cardiac-related rhythm in SND and the inhibition of SND produced by raising blood pressure with a bolus intravenous injection of norepinephrine bitartrate (2 μg/kg). Intravenous norepinephrine also failed to inhibit SND in the four cats in which baroreceptor denervation was performed before sympathetic nerve recordings were begun. SND contained a variable mixture of the 10-Hz rhythm and irregular low-frequency (<6 Hz) oscillations after baroreceptor denervation.

Blood flow was measured ultrasonically with a Transonic T206 small animal flowmeter and perivascular 28 flow probes (Transonic Systems). The blood flow probes were placed around the left renal and the left femoral artery. Zero flow was determined by occlusion of the arteries just distal to the probes. The left hindlimb was skinned, and the paw was tied so that femoral blood flow was largely directed to skeletal muscle. As previously described (3), bipolar platinum electrodes were used to record monophasically from the central ends of the cut postganglionic sympathetic CN and VN near their exits from the left stellate ganglion. The CN and VN provide sympathetic outflow to the heart and forelimb, respectively. Nerve recordings were made with the band-pass filter set at 1–1,000 Hz (Grass Instruments 7P3 preamplifier), so that envelopes of multiunit spikes appeared as slow waves (3, 6). Data were stored on magnetic tape.

Portions of the occipital and parietal bones and medial cerebellum were removed. The cerebral aqueduct and inferior colliculus were used as landmarks for placement of microinjection needles or stimulating electrodes. The brain stem was removed and fixed in 10% buffered formalin. Paraffin-embedded frontal sections of 30-μm thickness were cut and stained with cresyl violet, and sites of microinjection and electrical stimulation were identified with reference to electrode tracts. Relative to the stereotoxic coordinates of Snider and Niemer (21), placements in the PAG were beneath the bony tentorium at posterior (P) 0 to P1, lateral (L) 0.5 to L2.0, and height (H) +3 to H+0. This region of the PAG is referred to as the subtentorial PAG from which cardiovascular and behavioral components of the flight component of the defense response are elicited (1, 2, 4, 5, 20).

Chemical activation was performed by injecting 200 nl of a 500-mM solution of DLH over 2–5 s through the needle (outside diameter of 0.5 mm) of a 1-μl Hamilton syringe mounted on a David Kopf Instruments (DKI) stereotoxic instrument. We made one or two injections per track, with two to four tracks per animal. At least 20 min elapsed between injections that were made on either the left or right side of the PAG. Electrical stimulation was performed by using a Grass Instruments S8800 quartz-timed digital stimulator and PSIU6 constant-current unit to deliver 1-ms square-wave pulses of variable intensity at 25 Hz through concentric bipolar stainless steel electrodes (Rhodes model SNE-100, with 0.25-mm tip exposures separated by 0.75 mm) mounted on a DKI stereotoxic instrument.

Data analysis. We measured mean arterial pressure (MAP, mmHg) and mean blood flows (ml/min) through the femoral and renal arteries. Changes in mean blood flow are expressed as a percentage of control. Mean vascular conductance was calculated as mean blood flow divided by MAP. We measured the time from drug injection to the onset and peak of the rise in MAP.

Time series analysis was performed by using software written in our laboratory by Lewis (see Refs. 10 and 18). We extracted 10-Hz and cardiac-related activity in SND from the original recordings using a digital band-pass filter (symmetric, nonrecursive type with a Lanczos smoothing function; RC Electronics, Santa Barbara, CA) with a width of 4 Hz centered at the frequency of the sharp peak in the autospectrum of SND. This filtering caused minimal phase distortion of the signal (10). The roll-off slope of the filter was 39%/Hz outside of the band pass. The slow waves in SND extracted by digital filtering are smoother and more sinusoidal-like than the originals, thus aiding in the accurate detection of peaks and troughs. We made cycle-by-cycle measurements of the peak-to-trough amplitudes of the filtered 10-Hz or cardiac-related slow waves (normalized on a scale of 0 to 1.0). The phase lag (in degrees) of VN activity relative to CN activity was calculated as a three-point (cardiac-related SND) or ten-point (10-Hz SND) moving average. Cycle-by-cycle values of the CN-VN phase angle were derived from intervals between the peaks of corresponding slow waves in the two nerves (10).

The method of autospectral analysis used in this study has been described in detail (3, 10). Briefly, autospectral analysis was performed by using fast Fourier transform (FFT) after SND had been low-pass filtered at 100 Hz (original recordings). The sampling rate of 200 Hz gave a resolution of 0.2 Hz/bin. The power spectra (autospectra) derived from 40-s data segments were averages of 29 5-s data windows with 75% overlap. The autospectrum of a signal shows how much power is present at each frequency. Although FFT was performed over a bandwidth of 0–100 Hz, the spectra are displayed on a scale of 0–15 Hz, which contained >90% of the
power in SND (3). Total power in SND was defined as the sum of the absolute values in the bins between 0 and 15 Hz. A macro written in Microsoft Excel 7.0 was used to measure the power above background activity in the 10-Hz and cardiac-related bands of SND. A line was fitted to connect the left and right limits of the sharp peak near 10 Hz or at the cardiac frequency in the autospectrum of SND, and power in these bands was calculated as the area above this line. In baroreceptor-denervated cats, low-frequency power was calculated as the sum of the values in the bins from 0 to 6 Hz.

**Statistical analysis.** Comparisons before and after microinjection of DLH into the PAG were made by using the Wilcoxon-signed rank test; comparisons between baroreceptor- innervated and -denervated conditions were made by using the Mann-Whitney U test. Statistical tests were performed by using Statmost 3.2 (DataMost).

**RESULTS**

We made a total of 45 microinjections of DLH into the PAG; 22 of the injections were in baroreceptor-denervated cats and 23 in baroreceptor-innervated cats. The responses to 18 of the injections were classified as defenselike on the basis of an increase in MAP, an increase in femoral blood flow (FBF), and a decrease in renal blood flow (RBF). Eight defenselike responses were noted in five baroreceptor-denervated cats, and ten defenselike responses were observed in six baroreceptor-innervated cats. No more than two defenselike responses were seen in a given experiment. There was only one cat in which a defenselike response was observed both before and after baroreceptor denervation. The sites in the PAG from which the defenselike responses were chemically induced are illustrated in Fig. 1. These sites were in the same vicinity as those (not shown) from which decreases in both FBF and RBF \( (n = 6) \) or no cardiovascular responses \( (n = 21) \) were observed on microinjection of DLH.

**Responses to chemical activation of the PAG in baroreceptor-denervated cats.** A defenselike response elicited by microinjection of DLH into the PAG of a baroreceptor-denervated cat is shown in Fig. 2. Within 10 s of completing the microinjection of DLH, MAP began to rise from 114 mmHg, reaching a peak of 125 mmHg \(-25\) s later. Subsequently, MAP slowly decreased toward the control level. RBF decreased to a minimum of 66% of control, and FBF increased to a maximum of 328% of control. As reported by others (1, 5), the maximal changes in RBF and FBF occurred after MAP reached its peak. Renal arterial conductance was reduced to a minimum of 60% of control, and femoral arterial conductance was increased to a maximum of 298% of control. DLH microinjection also increased the amplitude of CN 10-Hz slow waves and decreased the amplitude of VN 10-Hz slow waves. The reciprocal changes in nerve activity were accompanied by an increase in the phase lag of VN 10-Hz activity relative to CN 10-Hz activity from near 50 to near 80°.

Spectral analysis of CN and VN discharges before and after injection of DLH into the PAG is shown in Fig. 3. In this experiment, the frequency of the free-running rhythm in SND was 9.4 Hz. Chemical activation of the PAG produced an increase in CN 10-Hz power (248% of control) and a decrease in VN 10-Hz power (72% of control). Power is the square of voltage (10), thus the changes in 10-Hz power are larger than the changes in slow-wave amplitudes in the corresponding time series (Fig. 2). CN total power increased to 210% of control, and VN total power decreased to 75% of control. There was no change in low-frequency power for either the CN or VN.

Summary data for the eight defenselike responses elicited by DLH microinjection in the PAG of five baroreceptor-denervated cats are presented in Tables 1 and 2. The responses elicited by microinjections into the left and right sides of the PAG were indistinguishable; thus the data were pooled. Table 1 shows that the increase in MAP, decrease in RBF and increase in FBF, and corresponding changes in conductances were sta-

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**Fig. 1.** Sites from which microinjection of d,L-homocysteic acid (DLH) into the midbrain periaqueductal gray (PAG) elicited a defenselike cardiovascular response as defined by increases in mean arterial pressure (MAP) and femoral blood flow (FBF) and a decrease in renal blood flow (RBF). IC, inferior colliculus; •, microinjection in baroreceptor-denervated cats; ■, microinjection in baroreceptor-innervated cats; scale bar is 1 mm. P0.2 and P0.9 correspond to stereotaxic planes of Snider and Niemer (21).
tistically significant. Table 2 shows that DLH microinjection into the PAG produced a statistically significant increase in CN 10-Hz power and CN total power, but VN 10-Hz and VN total power were decreased significantly. There was no change in low-frequency power for either nerve. The reciprocal changes in 10-Hz power for the two nerves were accompanied by a statistically significant increase in the CN-VN phase angle in this band.

We electrically activated the PAG in three baroreceptor-denervated cats. Figure 4 shows the defenselike response to electrical stimulation of the PAG for the same baroreceptor-denervated cat as is shown in Figs. 2 and 3. Electrical stimuli (60 μA, 25 Hz) were applied for 40 s (between the 2 arrows). There was an increase in MAP of 15 mmHg that was not maintained during the period of stimulation. Peak MAP was reached 20 s after PAG stimulation was begun. The maximal decrease in RBF (71% of control) and peak increase in FBF (270% of control) were reached later than the maximal increase in MAP. Electrical stimulation of the PAG produced a sustained increase in CN 10-Hz slow-wave amplitude and a sustained decrease in VN 10-Hz power.

Table 1. Defenselike cardiovascular responses to microinjection of DLH into the midbrain PAG of baroreceptor-denervated and baroreceptor-innervated cats

<table>
<thead>
<tr>
<th></th>
<th>Baroreceptor Denervated</th>
<th>Baroreceptor Innervated</th>
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<tbody>
<tr>
<td></td>
<td>( (n = 8) )</td>
<td>( (n = 10) )</td>
</tr>
<tr>
<td>Change in MAP, mmHg</td>
<td>13 ± 8*</td>
<td>19 ± 6*†</td>
</tr>
<tr>
<td>RBF, % of control</td>
<td>85 ± 10*</td>
<td>86 ± 12*</td>
</tr>
<tr>
<td>R cond, % of control</td>
<td>75 ± 11*</td>
<td>77 ± 11*</td>
</tr>
<tr>
<td>FBF, % of control</td>
<td>156 ± 72*</td>
<td>214 ± 76*</td>
</tr>
<tr>
<td>F cond, % of control</td>
<td>139 ± 66*</td>
<td>191 ± 65*</td>
</tr>
<tr>
<td>Time to onset, s</td>
<td>10 ± 5</td>
<td>17 ± 19†</td>
</tr>
<tr>
<td>Time to peak, s</td>
<td>40 ± 13</td>
<td>73 ± 26†</td>
</tr>
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Values are means ± SD. Time to onset and time to peak refer to mean arterial pressure (MAP) response. RBF, renal blood flow; R cond, renal conductance; FBF, femoral blood flow; F cond, femoral conductance; DLH, D,L-homocysteic acid; PAG, periaqueductal gray. *Significantly different from control \( (P < 0.05, \) Wilcoxon-signed rank test); †significantly different from baroreceptor-denervated state \( (P < 0.05, \) Mann-Whitney U test).
slow-wave amplitude whose onsets preceded the increase in MAP. These changes were associated with an increase in the phase lag of VN 10-Hz activity relative to CN 10-Hz activity from near 90° to near 160°.

Responses to chemical activation of the PAG in baroreceptor-innervated cats. A defenselike response produced by microinjection of DLH in the PAG of a baroreceptor-innervated cat is shown in Fig. 5. After completion of the microinjection of DLH (indicated by the arrow), there was an increase in MAP and FBF and a decrease in RBF. Peak MAP was not reached until 55 s after the microinjection of DLH and represented a rise of 20 mmHg. RBF decreased maximally to 84% of control (a decrease in conductance to 76% of control), and FBF increased to 239% of control (an increase in conductance to 215% of control). Whereas CN cardiac-related slow-wave amplitude was increased after microinjection of DLH, the amplitude of VN cardiac-related slow waves and the CN-VN phase angle were essentially unchanged.

Spectral analysis of CN and VN discharges before and after microinjection of DLH is shown in Fig. 6 (same experiment as Fig. 5). The autospectra of CN and VN discharges show a peak at the frequency of the heart beat (3.6 Hz), indicating that the predominant rhythm in SND was cardiac related. There was a significant increase in CN cardiac-related power (173% of control) and total power (156% of control) after microinjection of DLH. VN cardiac-related power (93% of control) and total power (98% of control) were little affected.

Summary data for the 10 defenselike responses elicited by DLH microinjection in the PAG of six baroreceptor-innervated cats are presented in Tables 1 and 2. There was a significant rise in CN-VN phase angle within the cardiac-related band of activity in response to microinjection of DLH in the caudal PAG.

Comparison of the responses elicited by DLH microinjection in baroreceptor-innervated and -denervated cats shows that the increase in MAP was significantly greater in baroreceptor-innervated preparations (Table 1). On the average, the onset and peak of the increase in MAP occurred later in baroreceptor-innervated cats. There were no significant differences in the maximal changes in regional blood flow or conductance for either the renal or femoral arteries. The increase in total power for CN was not different in the two states, but VN total power was significantly decreased only in the baroreceptor-denervated state (Table 2).

We electrically activated the PAG in three baroreceptor-innervated cats. One of these cases is shown in

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**Table 2. Sympathetic nerve responses to microinjection of DLH into the midbrain PAG of baroreceptor-denervated and baroreceptor-innervated cats**

<table>
<thead>
<tr>
<th></th>
<th>10-Hz Power, % control</th>
<th>Low-Frequency Power, % control</th>
<th>Total Power, % control</th>
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<tbody>
<tr>
<td><strong>Baroreceptor denervated (n = 8)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>128 ± 42*</td>
<td>96 ± 22</td>
<td>122 ± 22*</td>
</tr>
<tr>
<td>VN</td>
<td>73 ± 12*</td>
<td>96 ± 17</td>
<td>77 ± 9*</td>
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<tr>
<th></th>
<th>Cardiac-Related Power, % control</th>
<th>Total Power, % control</th>
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<tr>
<td><strong>Baroreceptor innervated (n = 10)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>150 ± 53*</td>
<td>140 ± 45*</td>
</tr>
<tr>
<td>VN</td>
<td>100 ± 29</td>
<td>97 ± 23†</td>
</tr>
</tbody>
</table>

Values are means ± SD. CN, inferior cardiac nerve; VN, vertebral nerve. Change in CN-VN phase angle in the 10-Hz band was 14 ± 6°. Change in CN-VN phase angle in the cardiac-related band was 3 ± 11°. *Significantly different from control ($P < 0.05$, Wilcoxon-signed rank test); †significantly different from baroreceptor-denervated state ($P < 0.05$, Mann-Whitney $U$ test).
Stimuli (100 μA, 25 Hz) were applied for 40 s (between the 2 arrows). MAP in this experiment showed large fluctuations on the time scale of respiratory activity. Soon after the onset of stimulation, MAP increased, reaching a peak of 120 mmHg 20 s after stimulation began. RBF decreased to a minimum of 85% of control (71% of control conductance), and FBF increased to a maximum of 298% of control (250% of control conductance). Maximal blood flow changes lagged that of MAP. CN cardiac-related slow-wave amplitude barely increased in this case, whereas VN slow-wave amplitude clearly decreased. CN-VN phase angle was unchanged. Note that the decrease in VN cardiac-related slow-wave amplitude did not occur until MAP began to rise.

**DISCUSSION**

To our knowledge, this is the first study in which regional blood flows were recorded together with the discharges of sympathetic nerves with different targets during chemical activation of the defense region of the midbrain PAG. In both baroreceptor-denervated and -innervated cats, microinjection of DLH into the PAG produced a defenselike cardiovascular response, as defined by increases in MAP and FBF and a decrease in RBF. The changes in regional blood flows were attributable to alterations in vascular resistance as reflected by a significant increase in femoral arterial conductance and a decrease in renal arterial conductance. Although CN activity was increased in both baroreceptor-denervated and -innervated cats, there were notable differences in the responses of the VN and changes in CN-VN phase angle. VN 10-Hz activity was decreased by chemical activation of the PAG in baroreceptor-denervated cats, whereas VN cardiac-related activity was unchanged in baroreceptor-innervated cats. CN-VN phase angle in the 10-Hz band was lengthened, whereas the phase angle in the cardiac-related band was unaffected. The sympathetic nerve responses in baroreceptor-denervated and -innervated cats...
cats were similar to those observed during electrical stimulation of the PAG (10, 11). Thus the changes in SND elicited by electrical stimulation likely reflected the excitation of neuronal cell bodies in the PAG rather than fibers of passage.

It is reasonable to propose that the changes in SND in response to chemical activation of the subtentorial PAG contributed to the defenselike cardiovascular pattern. The increase in CN 10-Hz and cardiac-related discharges is consistent with tachycardia occurring during the defense reaction (1, 4, 13). Although we did not record RN discharges in the current study, it is likely that an increase in the 10-Hz and cardiac-related discharges of this nerve contributed to the decrease in RBF produced by chemical activation of the PAG. Regarding this point, our laboratory (11) has reported parallel increases in CN and RN discharges during electrical stimulation of the PAG defense region in both baroreceptor-denervated and -innervated cats.

Activation of the sympathetic cholinergic vasodilator system and circulating epinephrine released from the adrenal gland are also involved in increasing skeletal muscle blood flow during the defense reaction in the cat (13, 22). These factors may have been primarily responsible for the increase in FBF produced in baroreceptor-innervated cats, because neither total VN power nor VN cardiac-related power was changed by DLH microinjection into the PAG. Thus reduced vasoconstrictor outflow to skeletal muscle may not have contributed to the increase in FBF in baroreceptor-innervated cats. This interpretation is subject to the provisos that VN activity is generally representative of sympathetic outflow to all skeletal muscle and increases in vasoconstrictor outflow to forelimb skin, and sympathetic cholinergic vasodilator outflow to forelimb skeletal muscle did not obscure a decrease in vasoconstrictor outflow to skeletal muscle. The latter possibility seems remote, because the cardiac-related rhythm is only weakly apparent in skin vasoconstrictor discharge (15). Moreover, skeletal muscle sympathetic vasodilator fibers neither have cardiac-related activity (14) nor respond to baroreceptor reflex activation (19, 22).

Regarding this issue, almost all of the power in VN activity was cardiac related in baroreceptor-innervated cats.

The fact that VN cardiac-related activity was not reduced by DLH microinjection in the PAG does not eliminate reduced vasoconstrictor outflow as a potential mechanism of skeletal muscle vasodilation in the baroreceptor-innervated cat. We have reported a delayed reduction in VN cardiac-related activity occurring in parallel with an increase in MAP during high-frequency electrical stimulation of the PAG defense region (11). The delayed decrease in VN cardiac-related activity was attributed to increased baroreceptor nerve discharge accompanying the pressor response. The increase in MAP (19 mmHg) produced by chemical activation of the PAG in the current study was only about one-third of the magnitude reported during electrical stimulation (11). Thus the enhancement of baroreceptor nerve discharge in response to chemical activation of the PAG may have been insufficient to reduce VN cardiac-related activity under the conditions of our experiments.
The reciprocal changes in CN and VN 10-Hz discharges observed in response to both chemical or electrical stimulation of the defense region of the PAG in baroreceptor-denervated cats were accompanied by significant lengthening of the CN-VN phase angle. The increase in the phase lag of VN 10-Hz activity relative to CN 10-Hz activity produced by microinjection of DLH into the PAG was smaller than that observed during electrical stimulation (10, 11). However, our laboratory previously demonstrated that the magnitude of the change in CN-VN phase angle is directly proportional to the extent to which CN and VN 10-Hz discharges are differentially affected by PAG stimulation (10). The increase in CN and decrease in VN 10-Hz discharges produced by chemical activation of the PAG were not as large as those seen during electrical stimulation (10), and therefore the smaller changes in CN-VN phase angle were expected.

In baroreceptor-innervated cats, the increase in MAP produced by chemical activation of the PAG was significantly greater than that in baroreceptor-denervated cats, and peak pressure was reached later (see Table 1). These findings are counterintuitive given that the buffering provided by the baroreceptor reflex would be expected to decrease the magnitude of the change in MAP and possibly shorten its time course. The basis for the differences in the magnitude and duration of the blood pressure responses in baroreceptor-innervated and -denervated cats remains to be determined.

Hilton (13) proposed that the sympathetic component of the baroreceptor reflex is suppressed during the defense response. If so, cardiac-related power in SND should have decreased during activation of the PAG defense region, because the cardiac-related rhythm is the consequence of entrainment of centrally generated oscillations by pulse-synchronous baroreceptor afferent nerve activity (9, 18). This was not found to be the case for either the CN or VN. Thus Hilton’s contention is not supported by the results of our experiments.

**Perspectives**

Our laboratory has offered a new model to explain the reciprocal changes in CN and VN 10-Hz discharges induced by electrical stimulation of the PAG defense region (10, 11). We have proposed that this pattern arises from reorganization of the coupling of multiple brain stem oscillators with different targets, as reflected by the changes in the phase angles between the 10-Hz discharges of sympathetic nerve pairs. The results of the current study point to the physiological relevance of this model in explaining the defense reaction in baroreceptor-denervated cats. First, because reciprocal changes in CN and VN 10-Hz discharges were induced by chemical activation of the PAG, this pattern can be attributed to excitation of neurons in this region rather than fibers of passage. Second, defenselike changes in regional blood flows accompanied the reciprocal changes in CN and VN 10-Hz discharges. Third, the reciprocal changes in the 10-Hz discharges of the two nerves induced by chemical activation were accompanied by an increase in the CN-VN phase angle. On the basis of arguments presented in our previous studies (10, 11), we interpret the lengthening of phase angle as indicating that the state of coupling of 10-Hz oscillators with different targets is changed during the defense reaction in baroreceptor-denervated cats. In contrast, the phase angle between the cardiac-related discharges of the CN and VN was unchanged by PAG activation in baroreceptor-innervated cats. This supports the contention that different mechanisms account for the defenselike changes in 10-Hz and cardiac-related SND.

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