The posterior vermis of the cerebellum selectively inhibits 10-Hz sympathetic nerve discharge in anesthetized cats

Susan M. Barman and Gerard L. Gebber†
Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan
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Barman SM, Gebber GL. The posterior vermis of the cerebellum selectively inhibits 10-Hz sympathetic nerve discharge in anesthetized cats. Am J Physiol Regul Integr Comp Physiol 297: R210–R217, 2009. First published May 20, 2009; doi:10.1152/ajpregu.90989.2008.—We studied the changes in inferior cardiac sympathetic nerve discharge (SND) and mean arterial pressure (MAP) produced by aspiration or chemical inactivation (muscimol microinjection) of lobule IX (uvula) of the posterior vermis of the cerebellum in baroreceptor-denervated and baroreceptor-innervated cats anesthetized with urethane. Autospectral analysis was used to decompose SND into its frequency components. Special attention was paid to the question of whether the experimental procedures affected the frequency composition of SND. Despite the absence of a statistically significant increase in MAP, the current investigation was initiated to determine whether the basal level of SND and resting blood pressure would be acutely increased following aspiration or chemical inactivation of the posterior vermis of urethane-anesthetized cats. The effects of such manipulations on rhythmic and aperiodic components of SND were characterized in baroreceptor-denervated cats, in which SND often has a 10-Hz rhythmic component and in baroreceptor-innervated cats, in which the cardiac-related rhythm is dominant.

METHODS

General procedures. The protocols used in these studies on 19 adult cats (3.27 ± 0.14 kg) were approved by Michigan State University’s Institutional Animal Care and Use Committee. Cats were initially anesthetized with 2.5% isoflurane mixed with 100% O2. A femoral artery and the femoral veins were cannulated to measure arterial pressure and to inject drugs, respectively. Urethane (1.08 ± 0.04 g/kg iv) was then administered, and isoflurane inhalation was stopped. This dose of urethane has been shown to maintain a surgical level of anesthesia in cats for a period [8–10 h; (9)] exceeding the duration of our experiments.

Each cat was placed in a stereotaxic apparatus, paralyzed (gal- lamine triethiodide, 4 mg/kg iv, initial dose), pneumothoracotomized, and artificially resired with room air. Normocapnia (end-tidal CO2, 4.32 ± 0.13%) was maintained with the parameters of artificial ventilation set at 37.20 ± 0.7 cc and 20.1 ± 0.8 cycles/min. Rectal temperature was kept near 38°C with a heat lamp. Before neuromuscular blockade, the adequacy of anesthesia was indicated by the absence of a palpebral reflex. When cats were paralyzed, an adequate level of anesthesia was indicated by the inability of noxious stimuli (pinch, heat, surgery) to increase arterial pressure or change the pattern of SND.

Baroreceptor denervation. The carotid sinus, aortic depressor, and cervical vagus nerves were sectioned bilaterally in 16 cats. Two observations verified the completeness of baroreceptor denervation in these experiments. First, there was not a sharp peak in the autospectrum of SND at the frequency of the heart beat, and the coherence value relating SND to the arterial pulse wave was <0.1 at this frequency. Second, SND was not reflexly inhibited during the pressor response produced by a bolus injection of norepinephrine bitartrate (1–2 μg/kg iv).

Neural recordings. As described in other reports from this laboratory (4, 10–12), the inferior cardiac postganglionic branch of the left stellate ganglion was exposed retropleurally by removing the head of the stellate ganglion...
the first rib. Potentials were recorded monophasically from the central ends of the cut nerves placed on platinum bipolar electrodes. The capacity-coupled preamplifier bandpass was set at 1–1,000 Hz, so that the synchronized discharges of sympathetic fibers appeared as slow waves (2, 8).

Aspiration of the cerebellar vermis. The posterior vermis of the cerebellum was exposed by removing the nuchal muscles and a portion of the occipital bone. Portions of lobule IX, and in some cases lobule X, of the cerebellar vermis were aspirated by gentle suction using a pipette with an O.D. of 1 mm attached to a vacuum pump. The pipette was manually guided into the posterior vermis with reference to surface landmarks in the maps of Ito (15) and Larsell (16, 17).

Muscimol microinjection into the cerebellar vermis. Muscimol was diluted in PBS to make a 2 mM-solution and adjusted to a pH of 6–8 (litmus paper test). Muscimol was placed in a glass micropipette (~40-μm tip diameter) that was glued (cyanoacrylate) to the needle of a 5-μl Hamilton syringe and mounted on a microinjection unit (David Kopf Instruments, model 5000). As described by Bradley et al. (7), the micropipette was placed into lobule IX by entering the cerebellum at an angle through lobule VIII. A total of four injections were made in two tracks that were separated by 1 mm in the rostral-caudal plane. The injections in each track were made in lobule IX at depths between 2.0 and 3.5 mm below the point of entry. A 100-nl injection was made slowly (~15 s) at each site by turning the calibrated micrometer on the microinjection unit. Muscimol was purchased from RBI Sigma (St. Louis, MO). As a control, vehicle (PBS) was injected into the cerebellum of three baroreceptor-denervated cats; neither SND nor arterial pressure were changed by this injection.

Data analysis. Data were acquired continuously with a Digitata 1322A digitizer (Axon Instruments, Union City, CA) using a sampling frequency of 200 Hz. Fast Fourier transform of SND was performed on 2-min data blocks (47 5-s windows of data with 50% overlap) that were collected just before, 3–5 min after removal of the cerebellar vermis or microinjection of muscimol into the cerebellar vermis, and later at ~30-min intervals until recovery of SND from the effects of muscimol microinjection. Normalized autospectra of SND were displayed on a scale of 0 to 20 Hz with a bin resolution of 0.2 Hz. Essentially all of the power in SND is contained in the 0- to 20-Hz band when recordings are made with a preamplifier bandpass of 1–1,000 Hz (2).

ASCII files of the autospectra of SND were saved for transfer to spreadsheet, graphics, and statistical programs (GraphPad Prism ver. 5.00 for Windows and GraphPad Instat, GraphPad Software, San Diego CA). The autospectra of SND constructed from data collected before and after each procedure described above were displayed on the same power scale. A macro written in Microsoft Excel version 7.0 was used to measure 10 Hz and cardiac-related power in SND. Briefly, 10-Hz power was calculated as the area above a line that connected the left and right limits of the 10-Hz band (i.e., the range of frequencies comprising the sharp peak in the autospectrum of SND in the 8- to 12-Hz band). Likewise, cardiac-related power was calculated as the area above a line that connected the left and right limits of the peak in the autospectrum of SND at the frequency of the heart beat. Low frequency (~6-Hz) power was calculated by arithmetically summing the values for the bins in the 0- to 6-Hz band. Total power in SND refers to the arithmetic sum of the values for the bins in the 0- to 20-Hz frequency band. Both rhythmic and aperiodic components of inferior cardiac SND are eliminated by autonomic ganglionic blockade with hexamethonium chloride (5 mg/kg iv).

Fig. 1. Effects of aspiration of the posterior vermis of the cerebellum on arterial pressure (AP) and left inferior cardiac sympathetic nerve discharge (SND) in a baroreceptor-denervated cat. A: oscillographic records (top to bottom) show AP (mmHg), SND, end tidal CO2 (%), and time base (10 s/division). Aspiration was performed over a 12-s period (see bars above time base). Horizontal bars (1 and 2) above SND are presented on an expanded time base (B). Vertical calibration, 100 μV. C: autospectra of SND before (black trace) and 3–5 min (gray trace) after ablation of the posterior vermis. Autospectra in this and subsequent figures are based on 47 5-s windows with 50% overlap and have a frequency resolution of 0.2 Hz per bin.
Statistical analysis. Values in the text and figures are means ± SE. A paired t-test was used to evaluate the effects of ablation or chemical inactivation (muscimol microinjection) of the posterior vermis on mean arterial pressure (MAP), power in the 10-Hz, cardiac-related, and 0- to 6-Hz bands, and total power in SND. P ≤ 0.05 indicated statistical significance. An unpaired t-test was used to compare the effects produced by ablation and chemical inactivation of the posterior vermis on SND and MAP.

Reconstruction of cerebellar ablations. The cerebellum and brain stem were removed at the end of each experiment and fixed in 10% buffered formalin. After at least 10 days in formalin, the brain stem and cerebellum were hemisected in the midsagittal plane and then carefully inspected. Using the fissures separating cerebellar lobules V-X as reference landmarks, the portion of the posterior vermis removed was drawn on a standard map obtained from the classic work of Larsell (16, 17). Subsequently, sagittal sections of 30-μm thickness were cut on a cryostat and stained with cresyl violet. These sections showed that the tissue removed by aspiration extended no more than 0.5–1.0 mm lateral on either side of the midsagittal plane. The brain stem was not damaged in any case, and there was no evidence of micropipette tracks in the brain stem.

RESULTS

Aspiration of the posterior vermis in baroreceptor-denervated cats. As shown in earlier studies (2, 5, 8, 10–12, 25), SND in urethane-anesthetized, baroreceptor-denervated, and vagotomized cats is characterized by a variable mixture of 10-Hz rhythmic bursting and aperiodic, lower-frequency (≤6 Hz) activity. Aspiration of portions of lobule IX (uvula) and, in some cases, lobule X (nodulus) differentially affected these frequency components in SND. Specifically, 10-Hz power in SND was either selectively enhanced or unmasked in 11 of 13 cats. There was no change in SND in the other two cats.

The results from one of three experiments in which a 10-Hz rhythm was unmasked following aspiration of the posterior vermis are illustrated in Fig. 1. Aspiration of a portion of lobule IX led to a 20 mmHg rise in MAP and an increase in the amplitude of bursts of SND (Fig. 1A). Furthermore, removal of this portion of the posterior vermis led to a change in the pattern of ongoing SND as shown by the recordings on an expanded time base (Fig. 1B). Note that in these 5-s segments, the discharges of the inferior cardiac nerve appear more rhythmic after (trace 2) than before (trace 1) aspiration of the posterior vermis. The changes in SND were quantified by constructing autospectra of SND based on 2-min data blocks. Before ablation of the posterior vermis, the power in SND was distributed over a wide band with a small peak near 5 Hz (black trace in Fig. 1C). After aspiration of lobule IX, a sharp peak near 9 Hz appeared in the autospectrum of SND (gray trace in Fig. 1C). Thus, removal of lobule IX unmasked a strong rhythm in the 10-Hz band. In this case, there was little change in power at lower frequencies (≤6-Hz). The tissue aspirated in this experiment was confined to portions of sublobules IXb, IXc, and IXd (Fig. 2B).

Figure 3 shows the autospectra of SND from one of the eight experiments in which preexisting 10-Hz activity was enhanced after ablation of the posterior vermis. Note that the peak near 10 Hz in control (black trace) was more than doubled in size after posterior vermis ablation (gray trace). In contrast, there was little change in power at frequencies ≤6 Hz. In this cat, the tissue removed was confined to the superficial portions of sublobules IXa, IXb, and IXc (see Fig. 2C).

Figure 4A summarizes the results obtained in 13 baroreceptor-denervated cats, including the two in which ablation of the
posterior vermis did not change 10-Hz power in SND. On the average, power in the 10-Hz band of SND was increased approximately three-fold ($P = 0.0019$) after ablation of the posterior vermis. The change in aperiodic, low-frequency ($\leq 6$-Hz) power was more variable (see Table 1); nonetheless, on the average, there was a small but statistically significant decrease in power in this band ($P = 0.0451$). Total power was not significantly changed. Despite this, MAP was significantly increased by 15 mmHg ($P = 0.0001$) after ablation of the posterior vermis. In the eight cats with a preexisting 10-Hz rhythm, the peak frequency in this band was unchanged by aspiration of the posterior vermis ($9.5 \pm 0.3$ Hz vs. $9.6 \pm 0.3$ Hz).

Table 1 shows the region of the posterior vermis aspirated for each of the 13 cats. It also shows that the magnitude of the change in power in 10-Hz SND was not related to the number of lobules or sublobules aspirated. In all 13 cats, all or part of sublobules IXb and IXc was removed. The largest cerebellar ablation (including portions of lobule IX and most of lobule X) in this series of experiments is shown in Fig. 2D; 10-Hz power increased 8.8-fold in this cat, but 10-Hz power increased only 2.5-fold in another cat in which a similar portion of the cerebellum was aspirated.

### Chemical inactivation of the posterior vermis in baroreceptor-denervated cats

We characterized the changes in SND and blood pressure produced by microinjection of muscimol into lobule IX of five cats (see Methods). In each of these experiments, there was a 10-Hz rhythm in SND that was enhanced after chemical inactivation of lobule IX. Figure 5 shows the results from two of these experiments. The control autospectra of SND (Fig. 5A, top, black trace) contained a sharp peak between 8 and 9 Hz and additional wideband power at frequencies $\leq 6$-Hz. The peak in the 10-Hz band was almost doubled in size 3–5 min after completing the series of muscimol injections into lobule IX (Fig. 5A, top, gray trace). Wideband power at lower frequencies was little affected. In this and one other cat, 10-Hz power returned to near control level within 2 h after microinjection of muscimol into the posterior vermis (Fig. 5A, bottom, black trace). At this time, aspiration of the region of lobule IX (including the portion into which muscimol had been injected) led to an increase in 10-Hz power in SND (Fig. 5A, bottom, gray trace) equivalent to that produced previously by microinjection of muscimol. The changes in SND produced by posterior vermis ablation in these cases are included in the summary presented in Fig. 4A.

In the remaining three experiments in this series, lobule IX was aspirated soon (within 20 min) after muscimol had been microinjected. The changes in SND produced by chemical inactivation were still optimal at this time. Figure 5B shows representative results from one of these experiments. Note that 10-Hz power in SND was selectively increased 3–5 min after microinjection of muscimol into lobule IX (compare control solid black trace with gray trace). No further changes in SND occurred 15 min later when lobule IX was aspirated (dotted black trace). The effects of posterior vermis ablation in these cases are not included in the summary presented in Fig. 4A.

Following posterior vermis ablation in each of the five experiments, the micropipette was repositioned at the stereotaxic coordinates used earlier for microinjection of muscimol. The tip of the micropipette was not within brain tissue. This means that aspiration had removed the portion of the posterior cerebellum into which muscimol had been injected, and it verified that the micropipette did not reach the underlying brain stem in any case.

Figure 4B summarizes the changes in SND and MAP produced by microinjections of muscimol into the posterior vermis of five cats. Power in the 10-Hz band of SND was significantly increased approximately three-fold after chemical inactivation of lobule IX ($P = 0.0251$). Lower frequency ($\leq 6$-Hz) and total power were not significantly changed. MAP was increased by 11 mmHg ($P = 0.0385$) after muscimol microinjection.

The magnitudes of the changes in 10 Hz ($5.98 \pm 1.51 \times 10^{-1} \text{ V}^2/\text{Hz}$), low frequency ($-3.24 \pm 1.45 \times 10^{-1} \text{ V}^2/\text{Hz}$), and total power ($2.80 \pm 2.36 \times 10^{-1} \text{ V}^2/\text{Hz}$) in SND produced by aspiration of the posterior vermis were not significantly different than those produced by chemical inactivation of the area ($4.42 \pm 1.26 \times 10^{-1} \text{ V}^2/\text{Hz}$, $0.10 \pm 3.39 \times 10^{-1} \text{ V}^2/\text{Hz}$, and $6.43 \pm 4.82 \times 10^{-1} \text{ V}^2/\text{Hz}$, respectively). Also, the reductions in MAP were similar following the two procedures ($13 \pm 2$ vs. $11 \pm 5$ mmHg).

### Chemical inactivation and aspiration of the posterior vermis in baroreceptor-innervated cats

In urethane-anesthetized, baroreceptor-innervated cats, most of the power in the SND autospectrum is in a band surrounding a peak at the frequency of the heart beat (cardiac-related rhythm), particularly when MAP is above 140 mmHg (2, 3). At lower levels of MAP, the cardiac-related rhythm can coexist with the 10-Hz rhythm (2, 3). To determine whether the posterior cerebellum inhibits the cardiac-related rhythm in SND, we studied the effects of muscimol microinjection and cerebellar ablation in three cats under conditions, in which there was a prominent cardiac-related but not a 10-Hz rhythm in SND (Fig. 6). Although not shown, the coherence value relating SND to the arterial pulse at the frequency of the heart beat ($3.80 \pm 0.23$ Hz) was $0.91 \pm 0.03$ in these cats.

Figure 6A shows data from one of the three baroreceptor-innervated cats, in which muscimol was microinjected into lobule IX of the posterior cerebellar vermis. Despite making four injections (100 nl each) into the area (same protocol as used for baroreceptor-denervated cats), there was no change in SND. Figure 4D summarizes the effects of chemical inactiva-
tion of the posterior vermis on SND and MAP in these three cats. About 30 min after completing the microinjections of muscimol, the posterior cerebellum was aspirated in each of these cats. As shown by the example in Fig. 6B, this procedure also did not alter the pattern of SND. Figure 4C summarizes the effects of posterior cerebellar aspiration on SND and MAP in these three cats. Histological examination showed that portions of sublobules IXb, IXc, and IXd were removed in all cases; and in one cat the ablation extended into lobule X.

**DISCUSSION**

Several laboratories have reported decreases in blood pressure and renal SND during electrical or chemical stimulation of lobule IX of the posterior vermis in anesthetized cats and rabbits (6, 7, 13, 18–21). To our knowledge, the current study is the first to demonstrate a role of the posterior vermis in determining the pattern of naturally occurring SND. Importantly, the ability of the cerebellum to modulate SND was only revealed in baroreceptor-denervated cats. Specifically, we found that aspiration of lobule IX (uvula) and, in some cases, lobule X (nodulus) of the posterior vermis or chemical inactivation of the uvula with muscimol unmasked or enhanced the 10-Hz rhythmic component of the discharges of the inferior cardiac postganglionic nerve, while the wide-band, lower-frequency (≤ 6-Hz) discharges of the nerve were either reduced or unchanged. This selective increase in 10-Hz SND was accompanied by a significant increase in MAP. Thus, at least in baroreceptor-denervated cats anesthetized with urethane, the posterior vermis exerts a tonic inhibitory effect on those brain stem mechanisms responsible for generating the 10-Hz rhythm in SND (2, 4, 5, 25) and consequently MAP. The fact that both SND and MAP were changed similarly with either aspiration or chemical inactivation of the posterior vermis indicates that cerebellar cortical neurons rather than simply axons of passage mediated the sympathoinhibitory effect.

In contrast to the results in baroreceptor-denervated cats, in cats with an intact baroreceptor reflex, a tonically active cerebellar inhibitory influence on SND was not unmasked. In
these animals, neither chemical inactivation nor ablation of the posterior vermis changed the rhythmic (cardiac-related) or aperiodic components of SND. Apparently, in these animals, the powerful baroreceptor-mediated inhibitory influence on the 10-Hz rhythm generator (2, 3) overpowered the cerebellar sympathoinhibitory process. The failure to alter the cardiac-related rhythm further supports the contention that the cerebellar sympathoinhibitory pathway acts selectively on the 10-Hz rhythm-generating mechanism. Our data from baroreceptor-inervated cats lends support to the findings of Holmes et al. (14), who showed that resting levels of arterial pressure and heart rate were similar before and 1–4 wk after ablation of the posterior vermis in baroreceptor-intact, conscious cats.

In nine of 11 baroreceptor-denervated cats in which 10-Hz SND was enhanced or unmasked, the portion of the posterior vermis that was removed was restricted to lobule IX. Thus, it seems likely that cortical neurons in lobule IX played a role in modulating the 10-Hz component of SND. The tissues aspirated in each of these cats extended over at least two of the four sublobules (a–d) of the uvula. Bradley et al. (6, 7) have elicited decreases in blood pressure and SND in anesthetized cats by electrical and chemical stimulation of sublobules IXb, IXc, and IXd. Thus, it is likely that a wide portion of the uvula contains neurons that can inhibit SND. We were not able to look for the location of tracks in the cerebellum made with the pipette used for microinjection of muscimol because the cerebellum was subsequently aspirated. Nonetheless, since the volume of injectate (100 nl) would have spread to more than one sublobule, defining a precise sublobule as the target of the injection would not have been possible. Importantly, it was not the intent of the current study to identify the precise sublobule of the posterior cerebellum that contains neurons that inhibit SND. Rather, our goal was to determine whether cerebellar neurons that mediate sympathoinhibition are tonically active, thereby helping to regulate the resting level or pattern of SND and blood pressure. Regardless of the deficiencies in experimental design, the data support the view that the uvula of the posterior vermis contains neurons that suppress the 10-Hz sympathetic rhythm generating mechanism.

One might argue that the changes in SND seen with aspiration or chemical inactivation of the posterior vermis in baroreceptor-denervated cats resulted from inadvertent damage or injection into the underlying midline medullary structures. This is unlikely for several reasons. First, inspection of histological sections of the brain stem revealed no evidence for trauma or micropipette tracks as would be expected if the pipettes used...
for suction of the cerebellum or muscimol microinjection had penetrated the medulla. Second, aspiration or chemical inactivation of the midline of the medulla (raphe nuclei) in baroreceptor-denervated cats actually reduces rather than enhances the 10-Hz rhythm in SND (24, 25). Third, lesion of the nucleus of the tractus solitarius (NTS) in baroreceptor-denervated, anesthetized cats does not affect SND (1). Fourth, had the aspiration or chemical inactivation impinged on the NTS, one would have expected disruption of the cardiac-related rhythm in SND of baroreceptor-innervated cats; in contrast, there was no change in SND of these cats.

Additional studies are required to determine the pathway from the cerebellar cortex to the brain stem sympathetic 10-Hz rhythm generator. But assuming this is the same pathway that involves the rostral region of the lateral parabrachial nucleus (PBN). They showed that chemical lesion of this region abolished or significantly attenuated the depressor effect evoked by stimulation of sublobule IXb in anesthetized rabbits. Anatomical studies also show a projection from the uvula to the PBN (22). Work from our laboratory (3, 5) has shown a role of this pontine region in control of the 10-Hz rhythm in SND. Specifically, chemical inactivation of the rostral dorsolateral pons (including the PBN) eliminated the 10-Hz but not the cardiac-related rhythm in SND (5). Also, single neurons in this area have activity correlated to the 10-Hz rhythm in SND (3).

Perspectives and Significance

The end point in most studies in which SND is recorded is to determine whether a particular manipulation leads to an increase or decrease in total neural activity per unit time. This approach does not consider the possibility that changes in the frequency composition of SND alone may also be physiologically relevant. Indeed, linear coherence analysis has revealed that the 10-Hz discharges of sympathetic nerve pairs with different cardiovascular targets (e.g., heart, viscera, and skeletal muscle vasculature) are much more strongly correlated than their aperiodic, lower-frequency components (2, 4, 10). Thus, the degree to which the discharges of sympathetic nerves with different targets are correlated is dependent on the frequency composition of the signals. Moreover, the efficiency with which target organs respond to their sympathetic inputs may depend on the frequency composition of the inputs as well as changes in total activity per unit time. Some support for this possibility was obtained in the current investigation. We found that aspiration of the posterior vermis produced a statistically

Fig. 6. Effects of chemical inactivation (muscimol microinjection) and aspiration of the posterior vermis in a baroreceptor-innervated cat. A: autospectra of SND before (black trace) and after (gray trace) microinjection of muscimol into the posterior vermis. B: autospectra of SND before (black traces) and after (gray trace) aspiration of the posterior vermis 30 min later.
significant increase in MAP without significantly affecting total power in SND. Providing that the changes in inferior cardiac SND were representative of those in other postganglionic nerves with cardiovascular targets, this suggests that the increase in blood pressure was linked to the selective enhancement of the 10-Hz rhythmic component of SND. In summary, we have identified a cerebellar control mechanism acting selectively to dampen the naturally occurring 10-Hz rhythmic component in SND, at least in baroreceptor-denervated cats anesthetized with urethane.

REFERENCES

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