Midline medullary depressor responses are mediated by inhibition of RVLM sympathoexcitatory neurons in rats

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Verberne, Anthony J. M., Daniela M. Sartor, and Ayse Berke. Midline medullary depressor responses are mediated by inhibition of RVLM sympathoexcitatory neurons in rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1054–R1062, 1999.—Mechanisms underlying the depressor and sympathoinhibitory responses evoked from the caudal medullary raphe (MR) region were investigated in pentobarbital sodium-anesthetized, paralyzed rats. Intermittent electrical stimulation (0.5 Hz, 0.5-ms pulses, 200 µA) of the MR elicited a mixed sympathetic response that consisted of a long-latency sympathoexcitatory (SE) peak (onset = 146 ± 7 ms) superimposed on an inhibitory phase (onset = 59 ± 10 ms). Chemical stimulation of the MR (glutamate; Glu) most frequently elicited depressor responses accompanied by inhibition of sympathetic nerve discharge. Occasionally, these responses were preceded by transient pressor and SE responses. We examined the influence of intermittent electrical stimulation (0.5 Hz, 0.5-ms pulses, 25–200 µA) and Glu stimulation of the MR on the discharge of rostral ventrolateral medulla (RVLM) premotor SE neurons. Peri-stimulus-time histograms of RVLM unit discharge featured a prominent inhibitory phase in response to MR stimulation (onset = 20 ± 2 ms; duration = 42 ± 4 ms; n = 12 units). Glu stimulation of the MR reduced blood pressure (−37 ± 2 mmHg, n = 19) and inhibited the discharge of RVLM SE neurons (15 of 19 neurons). Depressor and sympathoinhibitory responses elicited by chemical and electrical stimulation of the MR region are mediated by inhibition of RVLM premotor SE neurons and withdrawal of sympathetic vasomotor discharge.

Lesions of the midline medullary area did not alter resting arterial blood pressure but elevated the discharge of the inferior cardiac nerve (17). Furthermore, Zhong and colleagues (35) reported that chemical inactivation of neurons within the cat MR uniformly reduced the 10-Hz rhythmic discharges of the left and right inferior cardiac nerves (35). These observations suggest that neurons within the midline medulla are part of a neuronal network associated with sympathetic rhythm generation.

Studies that have used intermittent electrical stimulation paradigms have identified short- and long-latency sympathoexcitatory responses associated with MR stimulation (18, 36). The contrasting effects reported in these studies may depend largely on the stimulation paradigm used. Thus the use of chemical exipients avoids activation of fibers of passage, but the observed effect may be the net result of stimulation of mixed populations of neurons. On the other hand, intermittent electrical stimulation techniques have the disadvantage of indiscriminately stimulating all neuronal elements, including axons of passage, but allow temporal resolution of the various components of an evoked response.

In this study, we have focused on the mechanism of the sympathoinhibitory vasomotor responses evoked by stimulation of the MR by examining the effects on lumbar sympathetic vasomotor discharge and on the discharge of premotor sympathoexcitatory neurons of the rostral ventrolateral medulla (RVLM). By comparing the effects of electrical and chemical stimulation of these sites, we have emphasized the heterogeneity of caudal midline medullary neurons with regard to sympathetic vasomotor function. In addition, we demonstrate that in the rat, depressor and sympathoinhibitory responses elicited by MR stimulation are mediated by inhibition of the discharge of sympathoexcitatory neurons of the RVLM (11, 14, 19).

MATERIALS AND METHODS

General procedures. All experiments were performed on 27 male Sprague-Dawley rats (290–380 g), were approved by the ethical review committee of the Austin and Repatriation Medical Centre, and complied with the guidelines of the National Health and Medical Research Council of Australia. Animals were anesthetized initially by placement into a container saturated with enflurane vapor (Ethrane; Abbott Australasia, Kurnell, Australia), and when a surgical level of anesthesia was attained, the trachea was cannulated. All animals were ventilated artificially with 100% O2 (1 ml/100 g body wt, 60–70 breaths/min) containing ~1.4% halothane (Fluothane; Zeneca, Macclesfield, UK). This concentration of halothane produced a deep surgical level of anesthesia and was maintained throughout the entire surgical procedure.
The depth of anesthesia was verified by noting the absence of responses to firm paw pinch and corneal probing. Core temperature was maintained at 36.5–37.5°C with a servo-controlled heating pad. Arterial blood pressure was measured from the right carotid artery, and drugs were injected intravenously via the right jugular vein.

After all surgery was completed, the halothane was discontinued and pentobarbital sodium (45 mg/kg iv Nembutal; Boehringer Ingelheim, Artarmon, Australia) was administered slowly. After ensuring that this dose of pentobarbital sodium produced an appropriate level of anesthesia, we administered the paralyzing agent pancuronium buse (David Bull Laboratories) intravenously (2 mg/kg, with 0.2-mg/kg supplements when required). Thereafter, the adequacy of the anesthesia was judged by the stability of the blood pressure and absence of a pressor response to firm toe pinch. Before administration of supplementary doses of pancuronium (0.2–0.5 mg/kg), the adequacy of the anesthesia was confirmed. If a pressor response was noted in response to firm toe pinch, additional pentobarbital sodium (9–10 mg/kg) was administered.

Single-unit recordings. Single-unit recordings of RVLM premotor sympathoexcitatory neurons were made as described previously (2, 10, 29). In the unit recording experiments, either a monopolar or a bipolar stimulating electrode was placed into the dorsolateral quadrant of the spinal cord ipsilateral to the recording electrode after a laminectomy at the T3-T4 level for antidromic activation of medullospinal neurons, either a monopolar or a bipolar stimulating electrode was described previously (2, 10, 29). In the unit recording experiments, either a monopolar or a bipolar stimulating electrode was placed into the dorsolateral quadrant of the spinal cord ipsilateral to the recording electrode after a laminectomy at the T3-T4 level for antidromic activation of medullospinal units. An indifferent electrode was connected to a silver wire implanted in a muscle adjacent to the stimulation site (monopolar electrode only). The antidromic nature of spikes elicited by spinal stimulation (0.5 Hz, 0.5-ms duration, and 0.3–2.5-mA intensity) was established by demonstration of invariant latency and the collision test. Barosensitivity of RVLM neurons was tested by inflation of a subdiaphragmatic abdominal aortic snares to increase systemic arterial blood pressure. These neurons were inhibited by elevation of arterial blood pressure in a reproducible and proportional manner.

The effect of MR electrical stimulation was tested on both RVLM-spinal and RVLM-nonspinal neurons. The activity of RVLM-nonspinal cells was clearly inversely related to the level of arterial blood pressure, and they were otherwise considered identical to, and colocalized with, the medullospinal neurons. Indeed, it has been shown that ~90% of the barosensitive neurons recorded in the area of the RVLM immediately caudal to the facial motor nucleus are bulbospinal (23). Failure to activate a spinal axon of these cells was most probably due to the use of a monopolar stimulation electrode that permits less current spread throughout the surrounding spinal cord tissue. In the study of chemical stimulation of the MR on RVLM sympathoexcitatory neurons (see Chemical stimulation of caudal MR region and single-unit recording in RVLM), a bipolar stimulation electrode was used, and all units recorded in this part of the study were barosensitive and bulbospinal.

Electrical stimulation of caudal MR region. Before insertion of a stimulating electrode into the MR, the location of the caudal pole of the facial motor nucleus was determined using antidromic field potential recording. This procedure localized both the caudal tip and the ventral boundary of the facial motor nucleus. Thereafter, the glass recording microelectrode was replaced with a monopolar stimulating electrode (2–3 MΩ impedance at 1 kHz, Frederick Haer) that was lowered stereotaxically through the cerebellum to the midline medulla oblongata to a depth ~2 mm dorsal to the ventral boundary of the facial motor nucleus. Stimuli were applied to the electrode as intermittent single pulses (0.4–0.5 Hz, 0.5-ms pulse duration, 25–200 µA). The selection of these stimulation parameters was based on the results of preliminary experiments conducted by the authors as well as those reported earlier by other investigators (13, 36). The electrode was lowered through the medulla incrementally (3,000-µm steps), and electrical stimulation (25–200 µA, 0.5 Hz, 0.5-ms pulses) was applied at each site. When electrical stimulation of the midline medulla was combined with extracellular single-unit recording of RVLM premotor sympathoexcitatory neurons, the RVLM was initially located as described above, and then the stimulation electrode was lowered into the brain using an additional micropositioner.

Chemical stimulation of caudal MR region. Chemical stimulation of the MR area was achieved by microinjection of sodium glutamate (Glu; 0.2 M, pH 7.4, 30–40 nl, in artificial cerebrospinal fluid) through a glass micropipette (tip diameter 20–40 µm) cemented to the tip of a 1-µl glass microsyringe fixed to a micropositioner. Rhodamine-tagged microbeads (LumaFluor, New York, NY, or Molecular Probes, Eugene, OR) were incorporated into the solutions of Glu to facilitate histological localization of the injection sites using fluorescence microscopy.

Histological analysis of stimulation, injection, and recording sites. Electrical stimulation sites within the MR area were marked by electrolytic deposition of iron that was identified using the Prussian blue reaction as described previously (24, 26, 27). At the end of each experiment, clonidine (200 µg/kg iv) was administered. The signal remaining after clonidine administration was regarded as electrical noise, systematically subtracted from the rectified signal on computer analysis, and considered as the zero level of the sympathetic discharge. The resting level of sympathetic nerve discharge measured at the beginning of the experiment was used as the 100% level. Thus the sympathetic discharge was quantified as arbitrary "units" of activity (24, 26, 27).

Data analysis and statistics. Arterial blood pressure, sympathetic nerve discharge, stimulation pulses, and single-unit activities were averaged and analyzed with the Statview program.
activity were recorded on a videocassette recorder in pulse code modulation format (Vetter Instruments). A microcomputer-based data acquisition system was then used to construct peristimulus time histograms and histograms of evoked sympathetic activity as described previously (28, 29). The magnitude of the inhibition of RVLM unit firing produced by electrical stimulation of the MR area was calculated by dividing the total number of spikes observed during the period of inhibition by the number of spikes observed during an equivalent period of prestimulus firing. This figure was subtracted from unity to yield an index of inhibition. The mean of the differences between the number of spikes observed in the prestimulus period and the poststimulus period was subjected to a Student’s paired t-test in which the null hypothesis was that the mean of the differences does not differ from zero. The level of significance was taken as 0.05. All data are expressed as means ± SE.

RESULTS

Electrical stimulation of caudal MR region and lumbar sympathetic nerve discharge. Intermittent electrical stimulation (0.5 Hz, single 0.5-ms rectangular pulses, 100–200 µA) of the MR area produced an evoked response that consisted of sympathoexcitatory and sympathoinhibitory components. In most cases (n = 5 experiments), this pattern consisted of a long-latency sympathoexcitatory response superimposed on a sympathoinhibitory phase. The onset latency of the long-latency sympathoexcitatory response was 146 ± 7 ms, and the peak latency was 176 ± 51 ms. Occasionally (2 of 5 experiments), a short-latency sympathoexcitatory response was also observed (onset latency = 40 ms). The inhibitory phase (onset latency = 59 ± 10 ms; duration = 268 ± 39 ms) always preceded the long-latency sympathoexcitatory response. Figure 1 depicts a representative series of responses observed on stimulation at various depths and rostrocaudal locations (Fig. 2) within the midline medullary region in a single experiment. The computer-averaged evoked sympathetic responses consisted of a prominent inhibitory trough whose nadir occurred −250 ms after the onset of stimulation (Fig. 1, F, H, and I). The trough is most prominent at ventral and caudal locations within the region of the medulla investigated. A large sympathoexcitatory peak is also apparent at ~175 ms (Fig. 1, C, F, and I). It should be noted that, due to the brevity of the

![Fig. 1. Effect of intermittent electrical stimulation (0.5 Hz, 0.5-ms pulse duration, 200 µA) of midline medulla on averaged evoked sympathetic response recorded from lumbar sympathetic nerve in a typical experiment. Each histogram is an average of 150 repetitions of response to stimulus presented at t = 0. Sympathetic discharge is expressed as units, where resting level of rectified and filtered signal is equivalent to 100 units and signal remaining after clonidine (200 µg/kg iv) is regarded as zero. Sharp peak at t = 0 appearing in some histograms is a stimulation artifact. A–I represent responses obtained by stimulation at sites identified in Fig. 2.](http://apregu.physiology.org/)
bar sympathetic nerve discharge. Microinjection of Glu due to activation of the arterial baroreflex. The response was not influenced by secondary effects on arterial blood pressure were observed. Consequently, evoked sympathetic response, no obvious changes in arterial blood pressure were observed. Consequently, the response was not influenced by secondary effects due to activation of the arterial baroreflex.

Chemical stimulation of caudal MR region and lumbar sympathetic nerve discharge. Microinjection of Glu typically elicited depressor and sympathoinhibitory responses whose magnitude was dependent on the location within the midline medulla area. Figure 3 shows blood pressure and sympathetic nerve responses to Glu microinjection obtained from two medullary sites depicted in the inset. In this example, the depressor and sympathoinhibitory responses were preceded by a transient (3–4 s) pressor and sympathoexcitatory response. This pattern of response was observed in three of seven cases. Pressor and sympathoexcitatory responses unaccompanied by depressor and sympathoinhibitory responses were never observed in this study. The lumbar sympathetic nerve responses always preceded the arterial blood pressure changes. Heart rate was either unchanged during the depressor responses, or bradycardic (10–30 beats/min) responses were observed (data not shown). Depressor responses to Glu ranged from 10 to 60 mmHg and lasted up to 30 s. These were always accompanied by prominent inhibition of lumbar sympathetic nerve discharge that returned to baseline levels just before return of blood pressure to preinjection levels. Results from several separate experiments (n = 7 rats) are depicted in Fig. 4. These data indicate that Glu-induced depressor responses may be elicited from a number of locations within the midline medulla oblongata and that the most effective sites are found within the ventral medulla at a level rostral to the area postrema and at the middle third of the inferior olive.

Caudal MR stimulation and RVLM premotor sympathoexcitatory neuronal discharge. Electrical stimulation (25–200 µA) of the MR region inhibited the discharge of 12 of 15 (80%) neurons classified as RVLM premotor sympathoexcitatory cells (2, 9, 26). All of these neurons were found within 300 µm of the caudal pole of the facial motor nucleus and were barosensitive, their discharge was pulse modulated, and they had a mean basal discharge rate of 13 ± 3 spikes/s (n = 15, range = 1–38 spikes/s). Approximately 53% (8 of 15) of these neurons were activated antidromically by spinal cord stimulation (mean antidromic latency = 30 ± 10 ms, range = 12–82 ms), indicating a spinally projecting axon. The onset latency of the inhibition induced by midline medullary stimulation was 20 ± 2 ms, and the duration of the inhibition was 42 ± 4 ms. Figure 5, A–C, depicts peristimulus time histograms of the discharge of an RVLM neuron produced by intermittent electrical stimulation (25–100 µA) of the midline medulla. At a low current intensity (25 µA), inhibition of this unit was still detectable. An oscilloscope view of the inhibition of RVLM unit discharge (50-µA stimulation intensity) induced by MR stimulation is also depicted (Fig. 5D). The mean inhibition index for these neurons was 0.85 ± 0.06 (0.40–1.00, n = 12, P < 0.05). Two neurons displayed evidence of excitation that preceded the inhibition produced by midline medullary stimulation (onset = 10 ms) at the highest current tested only (200 µA). In the remaining 10 cells, no evidence of an excitatory response was detected. RVLM premotor sympathoexcitatory neurons were not antidromically activated by MR stimulation.

Chemical stimulation of the MR with Glu was tested only with RVLM neurons with a spinal projection identified by antidromic activation using spinal stimulation (resting firing rate = 9.4 ± 1.6 spikes/s; conduction velocity = 3.7 ± 0.6 m/s; n = 19). Glu microinjection resulted in a depressor response (−37 ± 2 mmHg, n = 19) accompanied by bradycardia (−30 ± 3 beats/min, n = 19) and an inhibition of the discharge of 15 of 19 neurons tested (Fig. 6). The maximal change in neuronal discharge rate from resting was −86 ± 5%, and the duration of the inhibition was 38 ± 5 s. Three cells were unaffected by Glu microinjection, and one cell was activated. Most of these neurons (18 of 19) were also tested for their response to baroreceptor unloading produced by administration of the vasodilator agent sodium nitroprusside, and the majority (14 of 19) was activated. The cells that were unaffected by Glu
microinjection were activated by baroreceptor unloading (2 of 3 cells).

Localization of stimulation and recording sites. The locations of the stimulation electrode tips and recording sites in the single-unit recording experiments are shown in Fig. 7. These were based on the histological identification of the Prussian blue-stained spots in the midline and the locations of the Pontamine sky blue deposits made within the RVLM. All RVLM units were recorded within 300 µm of these sites. Locations of the Glu injection sites and RVLM recording sites are depicted in Fig. 8.

DISCUSSION

This study has provided evidence that the rat MR region contains neurons that exert a powerful inhibitory influence on sympathetic vasomotor discharge via an inhibitory action on RVLM premotor sympathoexcitatory neurons. A number of other studies have reported similar findings in studies in which more rostral midline structures were stimulated (16, 30). We have extended these investigations by studying the effects of electrical and chemical stimulation of the midline depressor area on sympathetic nerve discharge and RVLM premotor sympathoexcitatory neuronal discharge. The powerful influence of the MR on sympathetic vasomotor activity was evident after both chemical and intermittent electrical stimulation of the MR.

The findings of the present study suggest that the MR area contains mixed populations of neurons capable of influencing sympathetic vasomotor function. Intermittent electrical stimulation of the MR allowed discrimination of sympathoexcitatory and sympathoinhibitory components largely because these occurred at different latencies. On the other hand, responses to chemical excitants reflect the net effect of stimulation of both excitatory and inhibitory elements that may not be discriminated unless these have differing thresholds or are separated spatially. Despite this, chemical stimulation of the midline medullary region occasionally produced transient pressor and sympathoexcitatory responses that preceded the profound depressor and sympathoinhibitory responses. Although these observations support the notion of intermingled sympathoinhibitory and sympathoexcitatory elements within the midline medulla, it is also possible that the sympathoexcitatory effects were mediated in part by activation of passing fibers.

Short-latency sympathoexcitatory responses have been recorded from the splanchnic nerve in anesthetized rats on stimulation of rostral raphe obscurus (36). In contrast, Huangfu and colleagues (13) reported only
a long-latency sympathoexcitatory response that was predominantly, but not exclusively, mediated by stimulation of spinal serotonin receptors. These investigators also reported the presence of a distinct sympathoinhibitory phase on stimulation of the midline medulla, but this was not pursued further. In the present study, long-latency sympathoexcitatory responses predominated, but occasionally indications of a short-latency component were present. These discrepancies may be associated with differences in the specific sympathetic outflow studied in each investigation (e.g., lumbar sympathetic nerve trunk and splanchnic sympathetic nerve).

Because RVLM premotor sympathoexcitatory neurons were inhibited by MR stimulation, it is most probable that the depressor and sympathoinhibitory response is mediated by a propriomedullary pathway, which terminates onto RVLM neurons. In addition, the sympathoexcitatory response may be mediated by spinally projecting serotonergic neurons of the raphe obscurus/pallidus (13, 36). The net result of stimulation of these diverse elements may depend on experimental conditions (e.g., type of anesthetic and depth of anesthesia), location within the medulla, and also the nature of the excitatory amino acid receptor activated (6).

The most effective location for Glu-induced depressor responses coincided approximately with the location of the sites at which intermittent electrical stimulation produced an evoked sympathetetic response containing a profound sympathoinhibitory component. This area, which was within the rostrocaudal level of the middle third of the inferior olive, is similar to that described by Henderson and colleagues (12) in the rat but more rostral to that identified in the rabbit (4).

A major question arising from these studies is whether the input from the midline medullary depressor area to RVLM premotor sympathoexcitatory neurons is mono- or polysynaptic. Evidence from experiments performed in the cat would suggest that the latter alternative is most probable (16). This study found that neurons with the characteristics of premotor sympathoexcitatory vasomotor neurons were inhibited by rostral midline medullary stimulation. However, rostral midline medullary neurons were not antidromically activated by stimulation within the RVLM pressor area. In addition, some cells were excited by depressor area stimulation...
at a shorter latency than those cells inhibited by microstimulation. The inhibition of RVLM neuronal discharge induced by rostral midline medullary stimulation was blocked by iontophoretic application of the GABAA receptor antagonist bicuculline. Taken together, these observations suggest that midline medullary stimulation activates GABAergic interneurons that synapse onto RVLM premotor sympathoexcitatory neurons. Recent investigations performed in the rabbit have also supported the notion of an inhibitory GABAergic link (5). The location of this GABAergic pathway is presently unknown.

Chemical stimulation of the MR also inhibited the discharge of the majority of the RVLM medullospinal sympathoexcitatory neurons tested. Under pentobarbital sodium anesthesia, these neurons are under the influence of a powerful baroreceptor-mediated inhibition. This was reflected in the large increase in neuronal discharge observed on unloading the baroreceptors with sodium nitroprusside. Indeed, this effect is likely to result in underestimation of the magnitude of the inhibitory effect of MR stimulation with Glu, because the resultant depressor response would serve to unload the baroreceptors in a manner similar to the actions of

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**Fig. 5.** Peristimulus-time histograms of an RVLM sympathoexcitatory vaso-motor neuron obtained during stimulation of vasodepressor sites within midline medulla oblongata (0.5 Hz, 0.5-ms pulses, 150 sweeps). A: 25 µA, B: 50 µA, C: 100 µA. Electrical stimulation was presented at t = 0 (note stimulation artifact at t = 0), D: oscilloscope record of RVLM unit showing inhibition of firing produced by midline medullary stimulation (50 µA, 250 sweeps).

**Fig. 6.** Inhibition of an RVLM sympathoexcitatory neuron by activation of midline medullary depressor area with Glu (40 nl, 0.2 M). Elevation of MAP by gradual aortic occlusion (AOc) reproducibly inhibited discharge of neuron. Reduction of MAP with sodium nitroprusside (SNP; 5 µg/kg iv) resulted in baroreceptor unloading and an increase in neuronal discharge rate. Glu-induced depressor response is accompanied by a reduction in discharge rate of cell.
the vasodilator agent. Although this confounding factor could have been avoided by using baroreceptor-dener- 
vated animals, it would also make positive identifica-
tion of RVLM barosensitive cells difficult. Neurons that 
were not affected by Glu microinjection were neverthe-
less activated by reduction of arterial blood pressure. 
This suggests that these cells were also inhibited by 
Glu microinjection, but less powerfully. Importantly, 
the results of the chemical stimulation experiments 
support those obtained on intermittent electrical stimu-
lation of the MR.

Evidence obtained from anterograde tracing experi-
ments suggests that the midline medullary region 
projects directly to the RVLM premotor sympathoexcit-
atory neurons (20, 34). On the other hand, studies 
using retrograde tracers injected into the RVLM have 
not identified a major projection from the MR (3, 25). 
Anterograde tracing experiments using biotin-dextran 
(BD) conducted in our laboratory (unpublished observa-
tions) have indicated that direct contacts between 
BD-labeled efferents from the MR and tyrosine hydroxy-
lase (TH)-labeled cells of the RVLM were infrequent, 
although BD-labeled varicose fibers were intermingled 
with the TH-positive neurons. The discrepancies be-
tween these studies may be explained by the fact that 
the midline medullary injection sites were not charac-
terized functionally. Thus it is possible that direct 
projections to RVLM TH-positive neurons from the 
midline depressor sites may be much less prominent 
than from midline regions more rostral to the depressor 
area.

Perspectives

The present study characterized a midline medullary 
depressor and sympathoinhibitory region in the anes-
thesized, paralyzed rat. Sympathoinhibition elicited 
from this region is probably mediated in large part by 
inhibition of the discharge of RVLM premotor sympa-
thoexcitatory neurons rather than by direct inhibition of 
sympathetic preganglionic neurons. The pathway is 
likely to be an indirect one and may involve a GABAer-
gic inhibitory link. The MR region probably contains 
intermingled sympathoexcitatory and sympathoinhibi-
tory neurons. The depressor region of the MR is un-
likely to participate in baroreflex sympathoinhibition, 
because lesions of this area do not alter baroreflex 
function (17). However, the MR may relay inputs from 
supramedullary vasodepressor regions such as the
anterior hypothalamus (17) and/or the ventrolateral periaqueductal gray (12).

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