Sympathoinhibition from ventrolateral periaqueductal gray mediated by the caudal midline medulla

C. Dean

Departments of Anesthesiology and Physiology, The Medical College of Wisconsin and the Department of Veterans Affairs Medical Center, Milwaukee, Wisconsin

Submitted 6 May 2005; accepted in final form 15 July 2005

Dean, C. Sympathoinhibition from ventrolateral periaqueductal gray mediated by the caudal midline medulla. Am J Physiol Regul Integr Comp Physiol 289: R1477–R1481, 2005; doi:10.1152/ajpregu.00326.2005.—Activation of neurons in the ventrolateral region of the periaqueductal gray (vIPAG) can elicit a decrease in renal sympathetic nerve activity and blood pressure. The present study investigated whether the vIPAG-evoked sympathoinhibitory response depends on neurons in the caudal midline medulla (CMM). In pentobarbital-anesthetized rats, activation of neurons in the vIPAG evoked a decrease in renal sympathetic nerve activity to 29.4 ± 4.8% below baseline levels and arterial blood pressure fell 8.9 ± 1.6 mmHg (n = 20). Microinjection of the GABA agonist muscimol into sympathoinhibitory regions of the CMM significantly attenuated the vIPAG-evoked sympathoinhibition to 17.9 ± 4.1% below baseline and the depressor response to 4.3 ± 1.2 mmHg. At 65% (13/20) of the sites examined, the vIPAG-evoked sympathoinhibition was responsive to CMM muscimol microinjection and attenuated from 34.2% to 11.5%, with the depressor response reduced from 14.8 to 3 mmHg. Microinjection of muscimol at the remaining 35% of the CMM sympathoinhibitory sites was ineffective on the vIPAG-evoked sympathoinhibition and depressor response. These data indicate that sympathoinhibitory and hypotensive responses elicited by activation of neurons in the vIPAG can be mediated by neurons in the sympathoinhibitory region of the CMM. The finding that the vIPAG-evoked response is not affected by muscimol at all CMM sympathoinhibitory sites also suggests that sympathoinhibitory sites in the CMM are not homogeneous and can mediate functionally different responses.

SYMPATHOINHIBITION ELICITED by activation of neurons in the ventrolateral region of the periaqueductal gray matter (vIPAG) is mediated through 5-HT1A (serotonin; 5-hydroxytryptamine) receptors in the sympathoexcitatory region of the rostroventrolateral medulla (RVLM) (1). However, whether the vIPAG-RVLM sympathoinhibitory pathway is direct or indirect remains unknown, with evidence available to support both. Evidence for a direct projection comes from anatomical studies in which a retrograde tracer microinjected into sympathoexcitatory sites in the RVLM is transported to the vIPAG (2, 20), and anterograde tracers microinjected into the vIPAG are transported to the RVLM (19). Some vIPAG projections to the RVLM have been shown to be serotonergic (2), which could provide a source of serotonin at 5-HT1A receptors in the RVLM.

An indirect vIPAG-RVLM pathway could include the caudal midline medulla (CMM). The CMM region runs in the midline from 1.5 mm caudal to 1.5 mm rostral to obex and includes a vasodepressor region located 0.5–1.5 mm rostral to obex (rostro CMM). The rostral CMM encompasses caudal raphe nuclei, including the rostral part of the raphe pallidus, the raphe obscurus, and the caudal raphe magnus. Anatomical evidence demonstrates that neurons in the vIPAG project to the CMM (10), and there are serotonergic and nonserotonergic projections from the caudal raphe to the sympathoexcitatory region of the RVLM (2). Sympathoinhibition evoked by activation of neurons in the caudal raphe and CMM could be mediated through the sympathoexcitatory region of the RVLM (6, 17, 21) or via raphe-spinal projections (15).

Sympathoinhibition has been shown to be an integral component of the cardiovascular response to severe hemorrhage (7). The response to hemorrhage includes an initial compensatory sympathoexcitation followed by an intense sympathoinhibition and hypotension, with increasing blood loss. In the anesthetized rat, the descending pathway mediating the sympathoinhibition has been shown to include vIPAG neurons (7) and 5-HT1A receptors in the sympathoexcitatory region of the RVLM (8). In support of a role for the CMM in mediating vIPAG-evoked sympathoinhibition, the onset of the hypotensive component of the response to severe hemorrhage, which is associated with a sympathoinhibition, is attenuated by inactivation of neurons in the CMM (11). The present study was designed to determine whether vIPAG-evoked sympathoinhibition is mediated through neurons in the CMM.

MATERIALS AND METHODS

The protocol for this study was approved by the Animal Care and Use Committees at the Medical College of Wisconsin and at the Zablocki Department of Veterans Affairs Center. The experiments were performed in Sprague-Dawley rats (280–390 g) anesthetized with pentobarbital sodium (50 mg/kg ip) with a catheter inserted into a femoral vein for supplemental administration of anesthetic. Arterial blood pressure was monitored continuously from a femoral arterial cannula via a pressure transducer (Statham). Blood samples were taken at intervals, and arterial blood gases were maintained within physiological limits by infusion of bicarbonate. A heating pad was used to maintain body temperature at 37°C.

The head of the animal was fixed in a stereotoxic frame (Kopf), and sympathetic nerve activity was recorded using flexible silver wire electrodes positioned on a renal nerve via a retroperitoneal approach. The electrodes were fixed in position with silastic gel allowing adjustment of the body of the animal without disturbing neural recordings. The electrophysiological signals were directed to high-impedance differential amplifiers (gain = 1,000; 0.1–10-kHz passband), followed by filter/amplifiers (gain up to 400; high- and low-bandpass filtering 10 Hz–3 kHz). The amplifier output was displayed.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
online and also directed to precision full-wave rectifiers and averaged using Bessel linear averaging filters (averaging interval = 100 ms) to obtain an online moving time average. Arterial blood pressure, renal sympathetic nerve activity, and a moving time average of renal sympathetic nerve activity were monitored and recorded on a computer-based data acquisition and storage system (Apple Macintosh G4 computer, ADInstruments PowerLab/8SP with chart software). All data were also recorded on tape (Vetter PCM recording adaptor Model 3000A) for subsequent data analysis.

For central microinjections, dorsal craniotomies were performed, and the dura was reflected to allow the insertion of micropipettes. Initial coordinates (in millimeters) for targeting the vIPAG with respect to bregma were 7.6 caudal, 0.6 lateral, 5.5 depth and for the CMM with respect to calamus scriptorius, 3.0 rostral, 0.5 lateral, 2.0 depth, with target sites identified functionally as described below. A single-barrel glass micropipette was used for microinjections into the vIPAG, and a multibarreled glass micropipette (20–30-μm total tip diameter) was used for microinjections into the CMM. The micropipettes were attached to a four-channel pressure ejection system developed in the laboratory. The volume of ejection was measured by observing the level of the fluid meniscus through a graduated monocular microscope eyepiece (7 nl/division). The volume of each drug administered was controlled by changing driving pressure, duration, or frequency.

The drugs for microinjection were diluted in a vehicle of artificial cerebrospinal fluid (NaCl, 124 mM; KCl, 2.0 mM; MgCl2, 2.0 mM; KH2PO4, 1.3 mM; CaCl2, 2.0 mM; NaHCO3, 24 mM; and glucose, 11.0 mM). The agents used were the synaptic excitant, D,L-homocysteic acid (DLH; 20 nl; 0.1 M; Sigma-Aldrich) and the GABA agonist muscimol (50 nl, 1.75 mM; Sigma-RBI). Pontamine sky blue dye (50 nl; 1%; BDH Laboratories) was used to mark CMM injection sites.

A single-barreled micropipette was inserted at coordinates targeting the vIPAG and 20 nl DLH was microinjected, and the effects on sympathetic nerve activity and arterial blood pressure were monitored. Minor adjustments of electrode position were made until a site was located at which DLH evoked a decrease in renal sympathetic nerve activity and blood pressure. This protocol and target coordinates have been used successfully in previous studies in this lab to indicate tip placement in the sym. The location of the vIPAG (1, 7). The micropipette remained at the sympathoinhibitory site in the vIPAG, and a multibarreled micropipette was inserted at coordinates targeting the CMM. D,L-homocysteic acid (20 nl) was microinjected, with fine adjustment of coordinates as necessary, to locate a CMM site at which a renal sympathoinhibitory and depressor response were evoked. Time was allowed for recovery of baseline nerve activity and blood pressure. The microinjection of DLH into the vIPAG was then repeated to provide control data for vIPAG-evoked sympathoinhibitory and depressor responses. After recovery of baseline parameters, the GABA agonist muscimol (50 nl) was then microinjected from the multibarreled pipette at the CMM depressor site to inhibit local neurons (7, 14), and after 1 min, the microinjection of DLH in the vIPAG was repeated, while the effects on sympathetic nerve activity and blood pressure were monitored. The microinjection of DLH at the vIPAG site was subsequently repeated at 20 min after the CMM administration of muscimol to examine recovery of the vIPAG-evoked response.

At the conclusion of the protocol pontamine sky blue dye (50 nl) was microinjected from the multibarreled micropipette to mark the location of the sympathoinhibitory site in the CMM.

Data analysis. Analysis of taped data was performed by sampling averaged renal sympathetic nerve activity and arterial blood pressure at a rate of 20 Hz using a Hewlett-Packard 310 computer equipped with an Infotek 16-channel, 12-bit A/D converter. An analysis program was used to display averaged nerve activity and blood pressure on a CRT along with a movable cursor. The cursor was set at the onset of a microinjection and acted as a zero time marker for the analysis. Nerve activity and blood pressure were averaged over sequential 30-s periods, relative to the cursor, before, during, and after a microinjection. Nerve activity was subsequently expressed as a percentage change from baseline determined as three averaged 30-s premicroinjection periods. To eliminate noise, zero nerve activity was obtained at the end of the experiment in response to intravenous administration of phenylephrine (6 mg/kg) and was subtracted from the averaged nerve activity. The percent change in sympathetic nerve activity and change in blood pressure from baseline levels evoked by microinjection of DLH into the vIPAG was compared before (control) and after muscimol administration into the CMM using one-way ANOVA. Duncan’s post hoc was used with the level of significance set at P < 0.05, and group data are presented as means ± SE. After analysis of the group data, a threshold of 20% attenuation of the peak renal sympathoinhibitory response was set for a CMM muscimol injection to be considered effective in attenuating the vIPAG response. Responses meeting this criterion were placed in a responsive group, whereas those below it were placed in a nonresponsive group, and the vIPAG-evoked responses in each group are presented before and after CMM muscimol microinjection.

For identification of the location of CMM microinjection sites, brains were removed post mortem and frozen. Sequential 30-μm transverse sections of tissue through the medulla were cut, stained with neutral red, and examined microscopically to identify and locate the microinjection sites histologically.

RESULTS

In 20 animals, activation of neurons in the vIPAG elicited a decrease in sympathetic nerve activity and blood pressure (Fig. 1). Peak inhibition of sympathetic nerve activity and decrease in blood pressure occurred at 1–1.5 min, and baseline values were recovered at 2–2.5 min after microinjection of DLH. Arterial blood pressure decreased 12.9 ± 4.4 mmHg from baseline levels of 103.6 ± 4.3 mmHg, and renal sympathetic nerve activity was decreased by 30.8 ± 4.1% of its baseline value (n = 20).

In the same animals, sites were also located in the CMM at which microinjection of DLH elicited a decrease in sympathetic nerve activity and blood pressure (Fig. 1). At these sites, peak inhibition of sympathetic nerve activity and blood pressure occurred at 1 min, and baseline values recovered by 2.5 min after microinjection of DLH. Renal sympathetic nerve activity decreased to 29.4 ± 4.8% below baseline levels, and arterial blood pressure fell 8.9 ± 1.6 mmHg (n = 20) (Fig. 1).

The sympathoinhibitory and depressor responses to activation of vIPAG neurons were attenuated following microinjection of muscimol into sympathoinhibitory sites in the CMM, and an example is shown in Fig. 2. For the group (n = 20), the peak renal sympathoinhibition was significantly attenuated to 17.9 ± 4.1% below baseline, and the decrease in arterial blood pressure was significantly reduced to 4.3 ± 1.2 mmHg (Fig. 1).

The peak vIPAG-evoked sympathoinhibitory response was attenuated by at least 25% of the control response at 13 of the 20 sites, and less than 12% at the remaining 7 sites. In the group in which the vIPAG-evoked sympathoinhibitory response responded to muscimol microinjection into the CMM, with an attenuation greater than 20%, the control inhibition of the renal nerve of 34.2% below baseline levels was attenuated to 11.5% (Fig. 3, Responsive). The control depressor response in this group was 14.8 ± 6.6 mmHg, which was attenuated to 3.0 ± 0.8 mmHg (n = 13) after muscimol treatment. The renal sympathoinhibition in the group in which the vIPAG-evoked response was not responsive was 29.9 ± 8.5% below baseline levels before microinjection of muscimol and 24.7 ± 6.6% after (Fig. 3, Nonresponsive). The magnitude of the control depressor response was 9.2 ± 3.8 mmHg and was unchanged.
at 6.6 ± 2.9 after microinjection of muscimol in the CMM (n = 7). At 20 min after microinjection of muscimol, there was some recovery of the sympathoinhibitory and depressor responses evoked by activation of neurons in the vlPAG (Fig. 3).

Neither the vlPAG-evoked control responses nor the responses evoked by microinjection of DLH into the CMM were significantly different between the group in which the vlPAG-evoked response was attenuated vs. that in which it was unchanged after microinjection of muscimol into the CMM. Baseline blood pressure was not significantly different in the two groups, 103.2 ± 5.9 mmHg in the group in which the vlPAG-evoked response was attenuated vs. 104.3 ± 6.3 mmHg in the group in which the response was not changed. Muscimol had no effect on baseline nerve activity (4.0 ± 1.7% of baseline levels at 1 min after microinjection) or blood pressure (−0.6 ± 0.5 mmHg).

Histological analysis showed that the sympathoinhibitory sites in the CMM at which microinjection of muscimol attenuated the vlPAG-evoked sympathoinhibition were located from 0.6 to 1.8 mm rostral to the obex (Fig. 4). These sites were located in the ventral half of the medulla extending from the midline ventrolaterally over the medial portion of the pyramids. Caudal midline medullary sympathoinhibitory sites, at which muscimol had no effect on the vlPAG-evoked sympathoinhibition, were intermingled in this region and were also identified at more lateral and rostral locations.

**DISCUSSION**

The present study demonstrates that sympathoinhibition elicited by activation of neurons in the vlPAG is mediated at least in part through neurons in the CMM. These data enhance...
our understanding of the central organization of sympathoinhibitory pathways that play an integral role in diverse functional responses mediated through the vlPAG (7, 13). Previous studies have shown that vlPAG-evoked sympathoinhibition is mediated through 5-HT1A receptors in the sympathoexcitatory region of the RVLM, but the central pathway from vlPAG to RVLM remained unknown. Determining the involvement of the midline medulla in mediating sympathoinhibition from vlPAG has been difficult due to the heterogeneity of both the vlPAG and CMM. This study provides evidence for an indirect descending pathway via the CMM but does not preclude the existence of a direct component.

The CMM region targeted in this study was selected based on data from several studies. Sympathoinhibition can be elicited by activation of neurons in this region (5, 17, 21), and the sympathoinhibition can be mediated through the RVLM (6, 17, 21). In the present study the CMM region, which contained neurons mediating the vlPAG-evoked sympathoinhibitory response was located from 0.5 to 1.5 mm rostral to obex, extending from the midline laterally over the medial pyramidal tract. This region is within the rostral CMM as defined by Heslop et al. (12), which includes rostral raphe pallidus, raphe obscurus, and caudal raphe magnus, although none of the effective sites in the present study were located in the raphe pallidus.

The framework for a caudal medullary relay in the vlPAG-RVLM sympathoinhibitory pathway has been demonstrated anatomically as projections to midline medullary regions from vlPAG (3) and from midline medullary regions to RVLM (2, 12) have been identified. Electrophysiological evidence suggests that midline medullary neurons can mediate sympathoinhibitory responses. Activation of midline raphe regions has been shown to elicit sympathoinhibition and inhibit the discharges of RVLM sympathoexcitatory neurons (21). In a study by Wang and Lovick (22), inhibition of RVLM neuronal discharges evoked by electrical stimulation of the vlPAG could be attenuated by GABA microinjection in the raphe magnus and obscurus. Interpretation of the data is limited as electrical stimulation would have activated both cell bodies and fibers of passage and may not have been limited to vlPAG neurons. In addition, 18 RVLM units were tested with 9 responding to CMM treatment, and it is not clear how this translates into changes in regional efferent sympathetic nerve activity. In the present study, local neurons were activated, and efferent sympathetic outflow was recorded. The data clearly demonstrate that neurons in the CMM are involved in the mediation of a vlPAG-evoked sympathoinhibition.
Sympathoinhibition from the vIPAG mediated through the midline medulla may play a functional role in the cardiovascular response to severe hemorrhage. The sympathoinhibitory component of severe hemorrhage has been shown to be mediated by neurons in the vIPAG (7) and through 5-HT1A receptors in the sympathoexcitatory region of the RVLM (8) in anesthetized rats. The central vIPAG-RVLM pathway mediating hemorrhagic sympathoinhibition has not been defined, but an indirect pathway through the CMM has been suggested. The vasodepressor response to severe hemorrhage, which is associated with sympathoinhibition, was delayed and reduced (11) by inactivation of the CMM region, from which the vIPAG-evoked sympathoinhibitory response was attenuated in the present study. Serotonergic and nonserotonergic neurons in the region of the raphe pallidus and raphe magnus are activated in response to severe hemorrhage (9, 16), whereas neurons in the raphe obscurus are not (4, 9). Raphe pallidus and raphe magnus provide inputs to the RVLM, some of which are serotonergic, which could mediate the sympathoinhibitory component of severe hemorrhage (2).

One-third of the CMM sympathoinhibitory sites that have been examined, including some located within the same region as effective sites, were not involved in the mediation of the vIPAG-evoked renal sympathoinhibition. The caudal medulla and midline raphe region contain mixed populations of sympathoexcitatory and sympathoinhibitory neurons, and these data indicate that CMM sympathoinhibitory neurons show specialization, relaying sympathoinhibitory responses from sources not confined to the vIPAG. Neuronal activation at some vIPAG sites may have engaged a direct pathway mediating sympathoinhibition from vIPAG-RVLM, which was not affected by microinjection of muscimol in the CMM.

**Perspectives**

Sympathoinhibition is an essential component of an array of physiological responses, the mechanisms underlying which are equally diverse. Raphe-spinal projections can mediate sympathoinhibition (15), perhaps as a component of antinociception (13). In the baroreflex, sympathoinhibition can be elicited by a GABA-mediated decrease in the activity of RVLM sympathoexcitatory neurons (18). The response to severe hemorrhage, including sympathoinhibition, is dependent upon the integrity of neurons in the vIPAG (7) and 5-HT1A receptor activation in the RVLM (1) in anesthetized rats. Data from the present study indicate that a pathway mediating sympathoinhibition from the vIPAG involves a relay in the CMM. The ventrolateral column of the PAG relays sympathoinhibitory and depressor components, which could be integrated into various responses, including antinociception, hemorrhage, and the defense response (7, 13). Whether the vIPAG sympathoinhibitory neurons use separate descending pathways to control individual functions or whether there is a common output pathway mediating vIPAG-evoked sympathoinhibition remains to be determined.

**ACKNOWLEDGMENTS**

The assistance of Claudia Hermes and Vicki Woyach is gratefully acknowledged.

**GRANTS**

This work was supported by NIH Neurological Disorders and Stroke Grant NS-43189.

**REFERENCES**