Sympathoexcitatory CVLM neurons mediate responses to caudal pressor area stimulation

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Natarajan, Madhusudan, and Shaun F. Morrison. Sympathoexcitatory CVLM neurons mediate responses to caudal pressor area stimulation. Am J Physiol Regulatory Integrative Comp Physiol 279: R364–R374, 2000.—Neurons in the caudal pressor area (CPA) are a source of tonic sympathoexcitation that is dependent on activation of cardiovascular sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM). In the present study, we sought to clarify the mechanism through which CPA neurons elicit increases in RVLM neuronal discharge, vasoconstrictor sympathetic tone, and arterial pressure. In urethan-chloralose-anesthetized, paralyzed, and artificially ventilated rats, bilateral disinhibition of CPA with bicuculline (Bic) after bilateral disinhibition of caudal ventrolateral medulla (CVLM) caused increases in splanchnic sympathetic nerve activity (+277% control) and arterial pressure (+54 mmHg). Inhibition of CVLM neurons with muscimol abolished the pressor response to activation of CPA neurons, suggesting that neurons within CVLM mediate the excitatory responses from CPA. Disinhibition of CPA and CVLM with Bic enhanced the sympathoexcitatory responses to stimulation of CPA with dl-homocysteic acid, which were blocked by micro-injections of kynurenic acid into CVLM. We conclude that the pathway from CPA to RVLM involves an obligatory glutamatergic activation of sympathoexcitatory neurons in the vicinity of CVLM.

sympathetic nerve activity; arterial pressure; bicuculline; sympathetic premotor neuron; rostral ventrolateral medulla

NEURONS IN THE ROSTRAL VENTROLATERAL medulla (RVLM) provide the majority of maintained excitatory input to sympathetic preganglionic neurons controlling cardiovascular function. However, the basis for the tonic discharge of RVLM premotor neurons is not yet understood; the relative importance of self-sustaining pacemakers, interneuronal networks, and integration of multiple inputs remains to be determined in assessing the excitatory drive to spinal sympathetic neurons. The caudal pressor area (CPA), located in the caudal end of the ventrolateral medulla, is one of the few sites besides the RVLM that provides a tonic excitatory influence on cardiovascular function (9, 13, 15, 20, 34) in the rat and the cat (6, 13, 20). Thus stimulation of CPA neurons with excitatory amino acids produces pressor responses (6, 8, 20, 34), whereas inhibition of CPA with inhibitory amino acids results in vasodepressor responses (9, 13, 34).

Recent evidence indicates that the responses to stimulation of CPA are sympathoexcitatory, i.e., not entirely neurohumoral, and are mediated through increases in the discharge of RVLM sympathetic premotor neurons (8). Varying the activity of neurons in CPA with excitatory or inhibitory stimuli results in excitation or inhibition of RVLM neurons, respectively, although the magnitudes of the changes in RVLM unit activity were not reflected in the cardiovascular changes produced by CPA stimulation (8). Stimulation of CPA after inhibition of neurons in RVLM did not yield any change in arterial pressure (AP), indicating that RVLM activity is essential for responses to CPA stimulation (34).

In the present study, we sought to clarify the mechanism by which activation of CPA neurons increases the activity of vasomotor neurons in the RVLM. One mechanism through which CPA neurons might increase sympathetic nerve activity (SNA) is the inhibition of the inhibitory input from neurons in the caudal ventrolateral medulla (CVLM) to the sympathetic premotor neurons in the RVLM (9). Neurons in the CVLM project monosynaptically to RVLM neurons (22) and inhibit them by activation of GABA receptors on RVLM motor neurons (9). Neurons in the CVLM comprise both 1) a phasic baroreceptor-driven glutamatergic input to CVLM neurons from the nucleus of the solitary tract (14, 25) and 2) a tonic inhibition of RVLM premotor neurons that can be demonstrated in the absence of baroreceptor input (10, 11, 21). By regulating the level of inhibition exerted by CVLM neurons, CPA could affect the activity of RVLM sympathetic premotor neurons and thus modulate SNA, AP, and heart rate (HR). We tested this hypothesis by examining the responses to CPA stimulation after blockade of GABA_A receptors in CVLM with bicuculline (Bic). The results suggest that rather than inhibiting sympathoinhibitory CVLM neurons, CPA neurons excite sympathetic premotor neurons in the RVLM through glutamatergic activation of a population of sympathoexcitatory neurons in

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CVLM. Portions of this work have been presented in abstract form (32).

MATERIALS AND METHODS

Experiments were performed on Sprague-Dawley rats (36 males, 3 females, 289–650 g). Anesthesia was induced with isoflurane (2% in 100% O₂). The right femoral artery and left femoral vein were cannulated for arterial blood pressure measurement and drug administration. Anesthesia was maintained with urethane (0.8 g/kg iv) and α-chloralose (0.07 g/kg iv). The trachea was intubated for artificial respiration. Animals were placed in a stereotaxic apparatus with the incisor bar at 17 mm below the interaural line and a spinal clamp on the T9-T10 vertebral processes. Rectal temperature was maintained at 37°C with the use of a thermostatically controlled heating table and lamp. Animals were artificially respired with 100% O₂ (125–175 ml minute vol), pneumothoracotomized, and paralyzed with d-tubocurarine (0.8 g/kg iv).

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GABA<sub>A</sub> receptors in the RVLM on the increases in SNA. We examined the effect of blockade of GABA<sub>A</sub> receptors in RVLM on the increases in SNA. In seven animals, concomitant disinhibition of CPA and RVLM resulted in greater mean peak increases in SNA (207 ± 13% of control), AP (73 ± 7 mmHg, Fig. 2C), and HR (47 ± 29 beats/min). The efficacy of the blockade of GABA<sub>A</sub> receptors in RVLM was assessed by elimination of 1) the sympathoinhibitory response to electrical stimulation of baroreceptor afferents in the ADN and 2) the cardiac frequency-related component in the autospectrum of control SNA (data not shown). The finding that disinhibition of CPA neurons elicited a significant (P < 0.05) increase in SNA, AP, and HR above that resulting from disinhibition of RVLM alone indicates that the sympatoexcitatory responses to activation of CPA neurons persist in the absence of a GABA<sub>A</sub> receptor-mediated inhibition of sympathetic premotor neurons in RVLM, including that from CVLM sympathoinhibitory neurons.

Responses to CPA stimulation are abolished by inhibition of neurons in the CVLM. To determine if activation of CVLM neurons is required for the elaboration of the pressor responses to stimulation of CPA neurons, microinjections of DLH were made into CPA, first ipsilateral and then contralateral to a unilateral microinjection of muscimol into CVLM. In the example in Fig. 3, microinjection of DLH into the left CPA evoked a pressor response of 11 mmHg and a tachycardia of 24 beats/min. After inhibition of neurons in the left CVLM, subsequent microinjection of DLH into the ipsilateral CPA produced no change in AP and an increase of 2 beats/min in HR (Fig. 3). In contrast, microinjection of DLH into the right CPA increased AP by 20 mmHg and HR by 15 beats/min (Fig. 3), responses comparable to those under control conditions. These findings suggest 1) that activation of a population of neurons within the CVLM is essential for eliciting sympatoexcitation from stimulation of CPA and 2) that the sympatoexcitatory pathway from CPA to CVLM is primarily ipsilateral.

Disinhibition of CPA yields sympatoexcitation after blockade of GABA<sub>A</sub> receptors in CVLM. To determine whether CPA facilitates sympatoexcitation through a GABA<sub>A</sub> receptor-mediated inhibition of neurons in CVLM, we examined the sympatoexcitatory and pressor responses to disinhibition of CPA after disinhibition of neurons in CVLM. As illustrated in Fig. 4A, bilateral microinjection of Bic in CVLM produced decreases in splanchnic SNA (−63% of control), mean AP (−64 mmHg), and HR (−62 beats/min). In six animals, disinhibition of CVLM neurons decreased SNA by −54 ± 8.8% of control, AP by −45 ± 6 mmHg, and HR by −97 ± 16.7 beats/min. In the absence of further manipulations, these sympathoinhibitory and depressor responses persisted for at least 10 min. Microinjection of Bic into CPA subsequent to disinhibition of neurons in CVLM elicited consistent increases in SNA and AP. In the example in Fig. 4B, bilateral microinjections of Bic into CPA after those into CVLM increased SNA by 83% of the level after disinhibition of

![Figure 1](http://ajpregu.physiology.org/.../ Downloaded from http://ajpregu.physiology.org/.../ by .../7, 2017)
CVLM neurons. This was accompanied by a pressor response of +52 mmHg but little change (+4 beats/min) in HR. In six animals, disinhibition of CPA neurons evoked a mean sympathoexcitatory response of +277 ± 98% of the “control” level of SNA after disinhibition of CVLM neurons, a pressor response of +54 ± 7 mmHg above the level of AP resulting from disinhibition of CVLM neurons, and no significant change in HR.

Fig. 2. CPA neurons receive a tonic GABAergic inhibition. A: bicuculline (Bic) in CPA causes sympathoexcitation. Figure depicts 6 interrupted data segments from a single experiment shown as 6 vertical panels (from left to right: panels 1–6, respectively) with 4 traces (AP, SNA, I-SNA, and HR, as in Fig. 1) in each panel. Panel 1: control response to DLH (40 nl, 5 mM, ▼) microinjection in CPA. Panels 3 and 4: sympathoexcitatory responses to microinjection of Bic (50 nl, 4 mM, ▼) in CPA on the left (panel 3) and right (panel 4) side of the medulla. Panel 6: subsequent DLH microinjection in CPA (▼) yields smaller sympathoexcitatory responses than under control conditions. Panels 2 and 5: traces at ×30 scale bar indicate improved synchrony of SNA bursts to the cardiac cycle. Panels 1, 3, 4, and 6: horizontal scale bar represents 30 s. The vertical scale represents 350 μV for SNA trace. B: disinhibition of CPA with the use of Bic does not eliminate the cardiac frequency-locked component of SNA. SNA autospectrum before (heavy trace) and after (thin trace) Bic microinjections in CPA indicate a prominent spike at the respective cardiac frequencies (▼ and ▼). HR was increased by disinhibition of CPA neurons. C: disinhibition of rostral ventrolateral medulla (RVLM) neurons does not block sympathoexcitation after disinhibition of CPA neurons. Mean increases in AP and SNA after bilateral Bic microinjections (50 nl, 4 mM) in CPA only (open bars), after bilateral Bic microinjections in RVLM only (shaded bars), and after bilateral Bic microinjections both in CPA and RVLM (filled bars). *Significant group differences at P < 0.05.

Fig. 3. Unilateral inhibition of caudal ventrolateral medulla (CVLM) eliminates the sympathoexcitatory responses from ipsilateral CPA but not those from contralateral CPA. Figure depicts 4 interrupted data segments from a single experiment shown as 4 vertical panels (from left to right: panels 1–4, respectively) with 4 traces (AP, SNA, I-SNA, and HR, as in Fig. 1) in each panel. Panel 1: control sympathoexcitatory responses to microinjection of DLH (40 nl, 20 mM, ▼) into CPA. Panel 2: unilateral microinjection of muscimol (50 nl, 2 mM, ▼) into CVLM causes sympathoexcitation. Panel 3: pressor response to subsequent microinjection of DLH in ipsilateral CPA is eliminated. Panel 4: pressor response to DLH activation of contralateral CPA neurons remains intact. Horizontal scale bar represents 30 s. Vertical scale represents 100 μV for the SNA trace.
HR (−5 ± 22 beats/min) after the bradycardia evoked by disinhibition of CPA. These results indicate that the sympathoexcitatory and pressor responses to increased activity of CPA neurons are not dependent on activation of GABA<sub>A</sub> receptors in the CVLM.

The sympathoexcitatory response to CPA stimulation involves a glutamatergic synapse in CVLM. Having established that activation of neurons in CVLM is required for the sympathoexcitatory responses to CPA stimulation, but that these responses can be achieved without decreased activation of GABA<sub>A</sub> receptors in RVLM, we reasoned that the requisite CVLM neurons are likely to be sympathoexcitatory and that they might be activated by glutamate during stimulation of CPA. To determine if ionotropic, glutamatergic neurotransmission in CVLM is involved in mediating the sympathoexcitatory and pressor responses to stimulation of CPA neurons, unilateral microinjections of DLH were made into the CPA ipsilateral and then contralateral to a unilateral microinjection of the glutamate receptor antagonist kynurenic acid in the CVLM. These experiments were performed immediately after bilateral microinjection of Bic into the CVLM and CPA to eliminate the tonic GABAergic inhibition to their neurons and to maximize the responses to subsequent application of DLH.

After bilateral microinjections of Bic into CVLM and CPA, microinjection of DLH into CPA evoked sympathoexcitatory and pressor responses (Fig. 5A) that were significantly (P < 0.005) larger than those evoked under control conditions (Figs. 1A, 2A, 3A, 5A). In the example shown in Fig. 5A, unilateral microinjection of DLH into the left CPA after sequential microinjections of Bic into CVLM and CPA increased SNA by 72% of control and AP by +26 mmHg, which were markedly greater than the DLH-evoked response before the Bic microinjections (SNA +11% of control and AP +9 mmHg). The responses to DLH stimulation of the right CPA (Fig. 5A) were of similar magnitude. In 24 trials in five animals, unilateral microinjection of DLH into CPA after sequential microinjections of Bic into CVLM and CPA evoked a mean peak increase in SNA of 44 ± 6.5% of control that was accompanied by a mean pressor response of 18 ± 2 mmHg.

Unilateral blockade of glutamate receptors in CVLM with kynurenic acid abolished the large sympathoexcitatory responses to microinjections of DLH into CPA occurring after sequential microinjections of Bic into CVLM and CPA. In the example shown in Fig. 5B, kynurenic acid microinjection into the left CPA caused an increase of 53% in SNA of control and 18 mmHg in AP. The increase in SNA presumably arose from blockade of the excitatory input to baroreceptor reflex interneurons in CVLM (21, 25). Subsequent microinjection of DLH into the left (ipsilateral) CPA failed to evoke a change in SNA or AP (Fig. 5B); however, the robust responses to DLH stimulation of CPA neurons were preserved on the right (contralateral) side (SNA +42% of control, AP 15 mmHg). In four animals that had received microinjections of Bic into both the CVLM and CPA, unilateral microinjection of kynurenic acid into the CVLM increased SNA by +53 ± 26.8% of control, AP by +23 ± 7.2 mmHg, and HR by +17 ± 3.9 beats/min. Microinjection of DLH into the CPA on the same side as the treated CVLM elicited no response in SNA, AP, or HR. The mean changes immediately after DLH microinjection were +2 ± 1.6% in SNA of control, +2 ± 1.6 mmHg in AP, and +7 ± 6.9 beats/min in HR. In contrast, DLH microinjection into the CPA on the side contralateral to the treated CVLM produced increases of +18 ± 5.2% in SNA of control, +13 ± 1.5 mmHg in AP, and +12 ± 7 beats/min in HR, which were significantly (P < 0.05) larger than the responses to stimulation of the CPA ipsilateral to the CVLM treated with kynurenic acid.
Histological localization of injection sites in the medulla. The recovered sites of microinjections, marked by iontophoresis of 2% Fast Green in the CPA, CVLM, and RVLM of most animals are plotted in Fig. 6. Figure 6A illustrates a thionin-stained section containing a Fast Green dye deposit in the CPA and a camera lucida drawing of the brain stem at the level of bregma \(-15.00\), on which 12 microinjection sites in CPA are plotted at a level \(0.8\) mm caudal to calamus scriptorius. These sites overlap the region of the CPA described in previous investigations of the cardiovascular responses to activation of CPA (8, 9, 15, 34). Figure 6B provides an example of a Fast Green dye deposit marking a microinjection site in the CVLM and an atlas drawing at bregma \(-13.68\) mm (33) with the locations of eight microinjection sites in CVLM. Similarly, Fig. 6C shows a Fast Green dye deposit marking a microinjection site in the RVLM and an atlas drawing at bregma \(-11.96\) mm (33) with the locations of nine microinjection sites in RVLM. These sites correspond to published locations of sympathoinhibitory neurons in CVLM (2, 3, 21, 26) and sympathetic premotor neurons in RVLM (7, 29, 36), respectively.

DISCUSSION

This study provides the first direct demonstration of the sympathetic effects of manipulation of the activity of neurons in the CPA. The principal novel findings are 1) that the sympathoexcitatory and pressor responses to stimulation of CPA are mediated through activation of a glutamatergic input to (presumably) sympathoexcitatory neurons in the CVLM and 2) that both the sympathoexcitatory neurons in the CPA and those in the CVLM receive a tonically active GABA\(_A\) receptor-mediated inhibitory input, removal of which enhances the responses to CPA stimulation. These findings allow us to propose a pathway (Fig. 7) mediating the increases in SNA and AP arising from activation of CPA neurons. This pathway is comprised of a series of sympathoexcitatory neurons in CPA, CVLM, and RVLM, with at least the excitation of CVLM neurons arising from glutamate receptor activation.

The CPA was initially characterized in cats (6, 13, 20) and rats (15) as a medullary site capable of influencing vasomotor tone, at least partly through alterations in the activity of sympathetic premotor neurons in the RVLM (8). Although several excitatory projec-
tions to RVLM have been identified, including those from the pontine reticular formation (16, 24), hypothal-amus (18, 44), and somatic and visceral afferents (12, 30, 37), few are tonically active. The present observa-
tions of alterations in splanchnic SNA, AP, and HR in response to excitatory and inhibitory stimuli applied to 
CPA are in agreement with previous conclusions that 
CPA neurons play a role in regulating the tonic sym-
pathetic outflow to cardiovascular targets, although 
the nature of this influence beyond the splanchnic 
outflow remains to be determined.

Our data also suggest that the pathway from sympa-thoexcitatory neurons in the CPA to those in the 
CVLM is mediated by glutamate and that it has a 
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The CVLM contains neurons that provide an impor-
tant regulation of sympathetic outflow through their 
inhibitory inputs to sympathetic premotor neurons in the RVLM. This inhibition has been attributed to a 
GABA_A receptor-mediated monosynaptic projection 
from the CVLM (1, 2, 5, 21, 23, 38, 40), with both a 
tonic and phasic (baroreceptor mediated) component 
(10, 11). Recently, the existence of a population of 
sympathoexcitatory neurons in the region of the CVLM 
has been proposed on theoretical and experimental 
grounds (17, 19). The results of the present study 
support this hypothesis in that CPA stimulus-evoked 
responses were eliminated by microinjection of 
kynurenic acid in CVLM, indicating that the sympa-thoexcitatory effects of neurons in the CPA are medi-
ated via excitatory synapses, presumably on a popula-tion of sympathoexcitatory neurons, in the region of 
CVLM. By extension, our finding that CPA neurons 
contribute to the tonic excitation of RVLM sympathetic 
premotor neurons provide strong support for the view
that sympathoexcitatory neurons in the CVLM region also play a role in determining basal sympathetic tone to cardiovascular targets. Although our results do not entirely preclude the possibility that the sympathoexcitatory neurons present in the CVLM are part of a population of RVLM neurons that extends caudal to the obex and intermingles with sympathoinhibitory CVLM neurons there, this would be contrary to anatomic and functional data. Spinally projecting sympathethetic premotor neurons in RVLM are located within 800 μm of the caudal pole of the facial nucleus (29, 35, 38, 41), and kynurenic acid microinjection into this vasomotor region of RVLM has no effect on CPA-evoked responses (9).

Each of the synaptic sites in the proposed pathway mediating the sympathoexcitatory effects of CPA stimulation is under a tonic GABA<sub>A</sub> receptor-mediated inhibitory regulation. This has been demonstrated for sympathethetic premotor neurons in the RVLM (5, 23, 38, 42, 43) and was also observed in this study (Fig. 4). Neurons in the CVLM are considered to be a primary source of the tonic and baroreceptor-related inhibition of vasomotor neurons in the RVLM (2, 3, 10, 21). Similarly, the increases in SNA and AP after Bac administration into the CPA indicate an active inhibition of the sympathoexcitatory neurons there. Our finding that blockade of GABA<sub>A</sub> receptors in both CPA and CVLM results in significantly larger responses to stimulation of CPA neurons than the same stimulation applied after only CPA neurons were disinhibited is consistent with the existence of a tonically active inhibitory regulation of sympathoexcitatory neurons in the CVLM. The source(s) of the GABAergic regulation of CPA and CVLM neurons remains unknown.

It is noteworthy that although disinhibition of CPA and RVLM both result in sympathoexcitation, disinhibition of CPA did not affect the baroreceptor reflex, unlike the elimination of baroreceptor reflex-mediated sympathoinhibition when RVLM is disinhibited (27, 31, 39, 42, 43). Although this result does not rule out the possibility that neurons in both CVLM and CPA receive baroreceptor-related input from the nucleus of the solitary tract, it would also be consistent with a tonic inhibition of CPA sympathoexcitatory neurons that arise from a nonbaroreceptor source. In this case, one would expect that the discharge of CPA neurons would not be modulated over the time course of the cardiac cycle by the phasic activity on baroreceptor afferent nerves. Additionally, the levels of sympathoexcitation induced by disinhibition of CPA are lower than those resulting from disinhibition of RVLM, consistent with the view that CPA neurons are responsible for only a portion of the excitatory drive to RVLM sympathetic premotor neurons. Third, disinhibition of CPA did not enhance the sensitivity of CPA neurons to excitatory stimulation with DLH, unlike the potentiated responses of RVLM neurons when CVLM is inhibited (19). There alsoexists a relatively large discrepancy between the size of the sympathoexcitatory responses to DLH stimulation of CPA neurons and the falls in SNA after inhibition of CPA neurons as noted previously (8, 9) and confirmed in this study. Together, these two observations suggest that neurons in the CPA normally receive a high level of tonic excitation, limiting the potential for an increased responsiveness to DLH stimulation.

A maintained GABA<sub>A</sub> receptor-mediated inhibition of the population of sympathoinhibitory neurons in the CVLM has been previously described (9, 23, 42) and formed a basis for the hypothesized role of CVLM sympathoinhibitory neurons in mediating the pressor responses to CPA activation; if stimulation of CPA neurons increased the inhibition of sympathoinhibitory neurons in the CVLM, a pressor response could arise from a reduced inhibition of sympathetic premotor neurons in the RVLM (9). Although our results do not rule out the possibility that neurons in the CPA may contribute to an inhibition of the sympathoinhibitory neurons in the CVLM, they do indicate that this cannot be the principal mechanism responsible for the increases in SNA and AP evoked by activation of CPA neurons. Increasing the activity of CPA neurons by removing their tonic GABAergic inhibition resulted in a marked increase in SNA and AP even after blockade of GABA<sub>A</sub> receptors either 1) in the CVLM, indicating that the CPA stimulus-evoked responses could not have been accomplished by activation of these GABA<sub>A</sub> receptors in CVLM, or 2) in the RVLM, indicating that the pressor responses to activation of CPA could not
arise from removal of a GABA receptor-mediated inhibition of RVLM neurons.

The differences between the results of the current study and the observations of Campos et al. (9) may be resolved by considering that the pressor response to excitation of CPA neurons is dependent on increasing the activity of sympathetic premotor neurons in the RVLM (8) and thus would be modulated by the level of excitability of vasomotor neurons in RVLM. As the inhibitory input to sympathetic premotor neurons in RVLM is markedly increased after bilateral disinhibition of CVLM neurons (42) (Fig. 4), the reduced excitability of RVLM neurons may have been sufficient to prevent the microinjection of glutamate into CPA from producing a sufficient depolarization of RVLM neurons to evoke a pressor response (9). One interpretation of this result would be that the CPA stimulus-evoked pressor responses were abolished because Bic had prevented the inhibition of CVLM sympathoinhibitory neurons.

This conjecture is supported by our finding that disinhibition of CPA neurons with Bic, likely providing a more sustained stimulus than glutamate or DLH microinjections, was needed to evoke increases in sympathetic activity after disinhibition of CVLM neurons. Indeed, once Bic was applied to CVLM, it was only after subsequent disinhibition of CPA neurons that microinjection of DLH into CPA produced sympathoexcitatory responses, and these were markedly enhanced over control responses. Also, it should be noted that the concentration of DLH used in our experiments is far lower than that used by others (8, 9, 34). Despite the lower concentrations of the drug and the correspondingly smaller sympathoexcitatory responses to control microinjections of DLH into CPA subsequent to disinhibition of both CVLM and CPA, these microinjections of DLH into CPA produced robust sympathoexcitatory responses not seen after disinhibition of either CPA or CVLM alone.

Thus the model that we suggest (Fig. 7) is the simplest pathway accounting for the effects that our drug microinjections had on SNA and AP. These experimental results must, however, be interpreted within the framework of the limitations of the microinjection technique. We cannot, for instance, assess the potential involvement of local interneurons in or among the CPA, CVLM, and RVLM. Thus administration of a glutamate receptor antagonist may disfacilitate GABA neurons, and the effects of disinhibition with Bic may include an increase in glutamate transmission. Additionally, we have no accurate measure of the diffusion sphere of the drugs, the variation of drug concentration within the diffusion sphere, or the efficacy of antagonists used in attenuation of synaptic transmission. Further characterization of the pathway by which CPA neurons influence the activity of RVLM premotor neurons will benefit from single-unit recordings and anatomic studies.

In summary, our data provide strong evidence that, in addition to the neurons that exert a tonic inhibition of sympathetic premotor neurons in the RVLM, the CVLM region also contains neurons that provide a tonic sympathoexcitatory input to RVLM premotor neurons. Through a glutamatergic synapse, neurons in the CPA comprise at least one of the sources of tonic excitation of these CVLM sympathoexcitatory neurons and thus contribute to the maintenance of basal levels of sympathetic tone and AP.

**Perspectives**

The model in Fig. 7 summarizes the results derived from the current study, in which sympathoexcitatory neurons in the CVLM are tonically excited by neurons in the CPA via a glutamate receptor-mediated projection, and from several previous investigations on neuronal function and transmitters in sympathetic regulatory pathways involving neurons in the CVLM and RVLM. These data emphasize two aspects of the organization of central autonomic control networks that affect the interpretation of experimental results.

Although the sympathetic and cardiovascular responses elicited from a region, such as the strong sympathoinhibition evoked from the CVLM, may suggest that the local neurons involved in sympathetic regulation comprise a functionally homogeneous population, additional populations of neurons may be present whose effects are masked or overshadowed under a particular experimental condition. The existence of a sympathoexcitatory projection from CVLM to RVLM was first proposed by Ito and Sved (19) as part of a conceptual model of interconnected populations of brain stem neurons providing tonic excitation to RVLM neurons. This model was significant as it accounted for the puzzling observation that the level of AP is refractory to glutamate receptor blockade in RVLM, and yet subsequent to inhibition of CVLM, kynurenic acid application to RVLM reduces AP to spinal levels, suggesting elimination of sympathetic tone. The current study suggests that neurons in the CPA are one of the sources of excitatory drive regulating the activity of sympathoexcitatory CVLM neurons. Networks of brain stem neurons have also been hypothesized to underlie the generation and regulation of SNA in the cat (4). In this model, the lateral tegmental field (LTF) comprises an important rostrally projecting excitatory input to sympathetic premotor neurons in the RVLM. It may be that such LTF neurons in the cat have a counterpart in the sympathoexcitatory neurons in the CVLM of the rat, which may only be distinguished with more precise recording techniques.

Second, the finding that many of the neuronal populations involved in the brain stem networks regulating sympathetic outflow are subject to a tonic inhibition, often mediated by GABA receptor activation, is significant. This finding suggests that the effects evoked by stimulation of a particular site could be strongly modulated by the level of inhibition at subsequent synaptic sites in that pathway. In the current study, it was only after removing the inhibition from both CVLM and CPA neurons that a robust sympathetic response could be elicited from DLH activation of CPA neurons. Similarly, the presence of tonic inhibitory inputs to both CPA and RVLM neurons would be...
a significant factor in determining the excitatory responses to disinhibition of either of these two sites. This was apparent from our finding that disinhibition of either CPA neurons or those in RVLM produced significant increases in SNA, but that simultaneous blockade of GABA<sub>A</sub> receptors in both sites resulted in a greater sympathetic response (Fig. 2C) than disinhibition of each site individually. Thus the net sympathetic response inducible by stimulation (or disinhibition) of either RVLM or CPA is intimately related to the level of activity of the other site which, in turn, is heavily influenced by its tonic inhibitory input. The potential for such nonlinearities in the responses evoked from pathways involving cascading populations of neurons will be important when assessing the contributions of multiple sources of excitation to sympathetic premotor neurons in the RVLM and it points to the difficulty in estimating such contributions based solely on changes induced by excitation or inhibition of a single source.

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