Role of pressor mechanisms from the NTS and CVLM in control of arterial pressure

Thiago Santos Moreira, Monica Akemi Sato, Ana Carolina Thomaz Takakura, José Vanderlei Menani, and Eduardo Colombari

Department of Physiology, Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brazil; Department of Physiology and Pathology, Faculdade de Odontologia, Universidade Estadual Paulista-UNESP, Araraquara, Brazil; and Department of Physiology, Faculdade de Medicina do ABC, Santo André, Brazil.

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Moreira, Thiago Santos, Monica Akemi Sato, Ana Carolina Thomaz Takakura, José Vanderlei Menani, and Eduardo Colombari. Role of pressor mechanisms from the NTS and CVLM in control of arterial pressure. Am J Physiol Regul Integr Comp Physiol 289: R1416–R1425, 2005. First published July 28, 2005; doi:10.1152/ajpregu.00053.2005.—In the present study, we investigated the effects of inhibition of the caudal ventrolateral medulla (CVLM) with the GABA receptor agonist muscimol combined with the blockade of glutamatergic mechanism in the nucleus of the solitary tract (NTS) with kynurenic acid (kyn) on mean arterial pressure (MAP), heart rate (HR), and regional vascular resistances. In male Holzman rats anesthetized intravenously with urethane/chloralose, bilateral injections of muscimol (120 pmol) into the CVLM or bilateral injections of kyn (2.7 nmol) into the NTS alone increased MAP to 186 ± 11 and to 142 ± 6 mmHg, respectively, vs. control: 105 ± 4 mmHg; HR to 407 ± 15 and to 412 ± 18 beats per minute (bpm), respectively, vs. control: 352 ± 12 bpm; and renal, mesenteric and hindquarter vascular resistances. However, in rats with the CVLM bilaterally blocked by muscimol, additional injections of kyn into the NTS reduced MAP to 88 ± 5 mmHg and mesenteric and hindquarter vascular resistances below control baseline levels. Moreover, in rats with the glutamatergic mechanisms of the NTS blocked by bilateral injections of kyn, additional injections of muscimol into the CVLM also reduced MAP to 92 ± 2 mmHg and mesenteric and hindquarter vascular resistances below control baseline levels. Simultaneous blockade of NTS and CVLM did not modify the increase in HR but also abolished the increase in renal vascular resistance produced by each treatment alone. The results suggest that important pressor mechanisms arise from the NTS and CVLM to control vascular resistance and arterial pressure under the conditions of the present study.

The medullary circuit related to cardiovascular control involves the nucleus of the solitary tract (NTS), nucleus ambiguus, and ventrolateral medulla (rostral and caudal) (8, 15, 17). The NTS is the site of the first synapse of the viscerosensory afferents in the brain stem, including those related to cardiovascular baroreceptor and chemoreceptor afferents. The neurotransmitter released by these afferents in the NTS is suggested to be l-glutamate (8, 15, 16, 32, 48). From the NTS, the baroreceptor afferent signals project to the caudal ventrolateral medulla (CVLM) (45, 47). Through GABAergic mechanisms, the CVLM inhibits neurons in the rostral ventrolateral medulla (RVLM) that innervate the preganglionic sympathetic neurons involved in controlling the heart and vascular beds (5, 15, 29, 36, 37). Disinhibition of the RVLM via deactivation of CVLM by electrolytic lesions or injections of the GABA receptor agonist muscimol, or the blockade of GABAergic transmission in the RVLM, results in sustained sympathoexcitation and increase in arterial pressure and heart rate (4, 14, 46).

Parallel to the inhibitory mechanisms, the RVLM also receives important excitatory projections (17, 20, 24, 26). Anatomical and immunohistochemical studies have shown that the NTS sends monosynaptic connections to the RVLM (13, 21, 35, 39, 41) and these projections from the NTS to the RVLM may convey peripheral chemoreceptor signals (30, 31, 49). The existence of pressor mechanisms in the NTS is supported by the increase in arterial pressure produced by l-glutamate injections into the NTS in awake rats (9, 33). Although l-glutamate injected into the NTS in anesthetized rats usually reduces arterial pressure, similar to baroreflex activation, l-glutamate into the NTS induces pressor responses in anesthetized rats after the inhibition of the CVLM with muscimol (49). This pressor response to l-glutamate into the NTS in anesthetized rats is abolished by the blockade of excitatory amino acid (EAA) receptors in the RVLM, which suggests the existence of a pressor pathway from the NTS to the RVLM (50, 54). Besides the inhibitory projection, there are some studies that suggest that the CVLM by direct or indirect projections may activate RVLM neurons (26, 38). In spite of some controversies (25), Ito and Sved (26) have shown that the blockade of the EAA receptors by bilateral injections of kyn into the RVLM combined with the inhibition of the CVLM with bilateral injections of muscimol reduced arterial pressure to a level similar to that produced by complete autonomic blockade, suggesting that the RVLM receives important tonic excitatory drive. However, bilateral injections of kyn alone into the RVLM in rats do not significantly change resting arterial pressure, which indicate that these excitatory mechanisms are not active in resting conditions. According to Ito and Sved (26), under resting conditions the excitatory drive to the RVLM is counterbalanced by the activation of the CVLM.

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Address for reprint requests and other correspondence: E. Colombari, Dept. of Physiology, Universidade Federal de São Paulo/EPM, Rua Botucatu, São Paulo, SP, Brazil (E-mail: colombari@fcr.epm.br)

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inhibitory mechanisms that, in turn, is also activated by signals under control of the RVLM excitatory drive. When the inhibitory mechanism is blocked by injections of muscimol into the CVLM, the excitatory mechanisms that reach the RVLM are released and sympathetic activity and arterial pressure increase (26).

Therefore, according to the work of Ito and Sved (26), the deactivation of the inhibitory influences that reach the RVLM by blocking the CVLM with muscimol or the EAA receptors with kyn into NTS increases sympathetic activity, arterial pressure, and heart rate. Additional studies have suggested that the RVLM also receives excitatory projections from the NTS and from the CVLM. Considering that the relative importance of the excitatory and inhibitory signals from the CVLM and NTS to the RVLM for cardiovascular regulation is still not completely clear, in the present study, we investigated the effects produced by the inhibition of the CVLM with muscimol combined with the blockade of EAA receptors in the NTS with kyn on mean arterial pressure (MAP), heart rate (HR) and renal, mesenteric and hindquarter vascular resistances. To better compare the relative importance of the mechanisms present in each area for cardiovascular control, two sequences of treatments were tested in different rats: in one group of rats the first treatment was muscimol into the CVLM, and the second treatment, performed 10 min later, was kyn into the NTS; in another group of rats, the first treatment was kyn into the NTS followed 10 min later by muscimol into the CVLM.

MATERIALS AND METHODS

Surgical procedures. All experiments were performed in accordance with the Brazilian National Health and Medical Research Council code of practice for the care and use of animals for scientific purposes and were approved by the Animal Experimentation Ethics Committee of the Federal University of São Paulo, School of Medicine.

Male Holtzman rats weighing 300–350 g were used. One day before the experiment, the rats were anesthetized with ketamine (80 mg/kg body wt) combined with xylazine (7 mg/kg body wt), and the femoral artery and vein were cannulated for arterial pressure measurement and drug administration, respectively. The arterial and venous catheters (PE-10 connected to PE-50) were tunneled subcutaneously and fixed on the back of the rat with suture thread. On the next day, immediately before the experiments, the animals were anesthetized with urethane (1.0 g/kg iv) combined with α-chloralose (60 mg/kg iv). A midline laparotomy was performed and miniature pulsed Doppler flow probes were placed around the renal artery, superior mesenteric artery, and lower abdominal aorta for measurement of renal, mesenteric, and hindquarter blood flows, respectively. The probes were fixed to the surrounding tissues with suture thread, and the animals were immediately placed in a stereotaxic apparatus in a prone position with the incisor bar at 11 mm below the intra-aural line. A partial occipital craniotomy was performed to expose the dorsal surface of the caudal brain stem.

Arterial pressure, heart rate and regional blood flow recordings. The catheter inserted into the femoral artery was connected to a P23 Db pressure transducer (Statham Gould) coupled to a preamplifier (model ETH-200 Bridge Bio Amplifier, CB Sciences) connected to the Powerlab computer recording system (model Powerlab 16SP, ADInstruments) for measurement of pulsatile arterial pressure, MAP, and HR. The flow probes were connected to a Doppler flowmeter (Dept. of Bioengineering, University of Iowa, Iowa City, IA) also coupled to the Powerlab computer recording system. Details of the Doppler flow recording technique, including the reliability of the method for estimation of flow velocity, have been described previously by Haywood and colleagues (22). Relative renal, mesenteric, and hindquarter vascular resistance changes were calculated as the ratio of MAP and Doppler shifts. Data from animals in which the probes moved during the experiment were not considered for analysis.

The rectal temperature was maintained at 37°C with a thermostatically controlled heating pad. During the surgical procedure or recording period, if the animals were responsive to noxious toe pinch, a supplementary dose of urethane (0.1 g/kg) combined with α-chloralose (20 mg/kg) was administered intravenously.

Central injections. Injections (50 nl, delivered over 5 s) of muscimol (120 pmol) or saline into the CVLM and kyn (2.7 nmol) or vehicle into the intermediate regions of the NTS were performed using the same single-barrel glass pipette (20 μm tip diameter) coupled to a pressure injection apparatus (Picospirter II). An injection was first performed in one side; the pipette was withdrawn from the brain and the contralateral injection was made; thus the two injections were made ~1 min apart. The volume of each injection was estimated from the displacement of the fluid meniscus in the pipette using a calibrated reticle. Injections into the CVLM were made 0.5 mm rostral to the calamus scriptorius, 1.8 mm lateral to midline and...
1.9 to 2.2 mm below the dorsal surface of the brain stem. Injections into the NTS were made 0.5 mm rostral to the calamus scriptorius, 0.5 mm lateral to midline, and 0.5 mm below the dorsal surface of the brain stem.

**Drugs.** Muscimol and kyn were purchased from Sigma Chemical. Muscimol was dissolved in isotonic saline. Kyn was initially dissolved in 100 mM sodium bicarbonate (in a volume that corresponded to 10% of the final volume) and then diluted with isotonic saline until reaching the final volume. The pH of kynurenic acid solution was around 7.4.

**Histology.** At the end of the experiments, a 2% solution of Evans blue was injected into the CVLM and NTS (50 nl) using the same pipette that was previously used for drug injection. Saline followed by 10% buffered formalin was perfused through the heart. The brains were removed, fixed in 10% formalin for at least 2 days, frozen, cut coronally into 50-μm sections and stained with Giemsa. The sections were analyzed by light microscopy to confirm the injections bilaterally into the CVLM and NTS.

**Experimental Protocols**

Effects of kynurenic acid into the NTS on MAP, HR, and regional vascular resistances in rats pretreated with muscimol into the CVLM. Blood flows, MAP and HR were continuously recorded during 60 min and were analyzed at every 10 min starting the recording 10 min after the connection of the arterial line to the pressure transducer. Control (baseline) values were recorded for 10 min and were analyzed immediately before bilateral injections of muscimol or saline into the CVLM (first treatment). These values were used as reference to calculate the changes produced by the treatments. Ten minutes after muscimol or saline into the CVLM, kyn or vehicle was bilaterally injected into the NTS and the cardiovascular responses were evaluated during the next 40 min.

Four groups of animals (n = 8 in each group) were used to investigate the cardiovascular effects of the combination of injections of muscimol or saline into the CVLM followed by injections of kyn or vehicle into the NTS. In each rat, only one of the following combinations was tested: 1) saline into the CVLM followed by vehicle into the NTS (control); 2) saline into the CVLM followed by kyn into the NTS; 3) muscimol into the CVLM followed by vehicle into the NTS; and 4) muscimol into the CVLM followed by kyn into the NTS.

Effects of muscimol into the CVLM on MAP, HR, and regional vascular resistances in rats pretreated with kynurenic acid into the NTS. The protocol used was similar to that described above (item 1), except that the first treatment was kyn or vehicle into the NTS and the second treatment was muscimol or saline into the CVLM.

Four groups of animals (n = 8 each group) were also used, and in each rat only one of the following combinations was tested: 1) Vehicle into the NTS followed by saline into the CVLM (control); 2) Kyn into the NTS followed by kyn into the CVLM (control); 3) Vehicle into the NTS followed by muscimol into the CVLM (control); and 4) Kyn into the NTS followed by kyn into the CVLM.

**Statistical analysis.** Data are expressed as means ± SE. Statistical analysis of baseline MAP, HR, and changes in vascular resistances were performed using two-way ANOVA followed by the Student-Newman-Keuls post hoc test. Significance level was set at P < 0.05.
into the NTS followed by saline into the CVLM; 3) Vehicle into the NTS followed by muscimol into the CVLM; and 4) Kyn into the NTS followed by muscimol into the CVLM.

**Baroreflex test in rats treated with muscimol into the CVLM and kynurenic acid into the NTS.** In 6 rats that received muscimol into the CVLM followed by kyn into the NTS, and in 5 rats treated with injections of kyn into the NTS followed by injections of muscimol into the CVLM, the baroreflex was tested by intravenous injection of a pressor dose of phenylephrine (5 μg/kg body wt) and a depressor dose of sodium nitroprusside (30 μg/kg body wt). The injections of phenylephrine and sodium nitroprusside were performed from 4 to 8 min before the first central injection (control) and from 10 to 20 min after the second central injection.

**Effects of kynurenic acid and muscimol injected in sites outside the NTS and CVLM on MAP, HR, and regional vascular resistances.** To confirm the specificity of injection sites for the effects of muscimol and kynurenic acid on MAP, HR, and regional vascular resistances, results from rats in which the injections did not reach the NTS or the CVLM bilaterally (misplaced injections) were also analyzed and presented in the **RESULTS** section.

**RESULTS**

**Histological analysis.** Figure 1A is a photomicrograph showing the typical sites of the bilateral injections into the intermediate region of the NTS and into the CVLM in one rat representative of the rats used in the present study. Figure 1B is a diagrammatic composite showing the injection sites into the medulla in rats that received injections of muscimol and kyn. According to Paxinos and Watson (40), these coronal sections are located ~13.7 mm caudal to bregma. The injection sites in the NTS shown in Fig. 1 are the same that previous studies have already shown to produce depressor responses to L-glutamate injections and pressor responses to bilateral injections of kyn (18, 32). Hypothalamic neurons have been described in the CVLM in sites similar to those shown in Fig. 1 (1, 3, 28, 34), and previous studies have showed that bilateral injections of muscimol in these sites induced pressor responses (25, 26, 49).

**Changes in MAP, HR, and regional vascular resistances induced by kynurenic acid into the NTS in rats pretreated with muscimol into the CVLM.** The baseline levels of MAP and HR were similar in all four experimental groups tested (Table 1). Bilateral injections of muscimol (120 pmol/50 nl) into the CVLM followed by vehicle into the NTS resulted in sustained (for at least 30 min) hypertension (186 ± 11 mmHg vs. saline: 105 ± 4 mmHg), tachycardia [407 ± 15 beats per minute (bpm) vs. saline: 352 ± 12 bpm] and increase in renal (364 ± 38% vs. saline: 6 ± 12%), mesenteric (389 ± 53% vs. saline: 8 ± 4%) and hindquarter vascular resistances (403 ± 48% vs. saline: 7 ± 9%), whereas renal (−123 ± 51% vs. saline: 5 ± 5%), mesenteric (−184 ± 46% vs. saline: 11 ± 13%) and hindquarter (−196 ± 47% vs. saline: 8 ± 10%) blood flows were reduced (Figs. 2 and 3).

Similar to muscimol into the CVLM, bilateral injections of kyn (2.7 nmol/50 nl) into the NTS immediately reduced MAP to 88 ± 5 mmHg and mesenteric (−28 ± 19%) and hindquarter vascular resistances (−31 ± 11%) below control preinjection baseline levels and abolished the changes in renal vascular resistance but did not modify the effects of muscimol on HR (Figs. 2-4). Mesenteric (33 ± 12%) and hindquarter (52 ± 16%) blood flows increased to above control preinjection baseline levels, and renal blood flow was restored to control baseline level by the combination of muscimol into the CVLM and kyn into the NTS (Figs. 3 and 4). No significant changes in breathing occurred after injections of kyn into the NTS or muscimol into the CVLM.
Changes in MAP, HR, and regional vascular resistances induced by muscimol into the CVLM in rats pretreated with kynurenic acid into the NTS. The baseline levels of MAP and HR were similar in all four experimental groups tested (Table 1). Bilateral injections of kyn (2.7 nmol/50 nl) into the NTS followed by saline into the CVLM produced sustained (for at least 30 min) increases in MAP (142 ± 110 mmHg vs. vehicle: 101 ± 2 mmHg), HR (412 ± 18 bpm vs. vehicle: 334 ± 16 bpm) and renal (138 ± 13% vs. saline: 8 ± 9%), mesenteric (168 ± 26% vs. saline: 11 ± 7%) and hindquarter vascular resistances (154 ± 34% vs. saline: 6 ± 5%), while renal (−81 ± 19% vs. saline: −5 ± 15%), mesenteric (−94 ± 21% vs. saline: 4 ± 6%), and hindquarter (−72 ± 22% vs. saline: 9 ± 14%) blood flows were reduced (Figs. 5 and 6).

Bilateral injections of muscimol (120 pmol/50 nl) into the CVLM preceded by vehicle into the NTS also increased MAP to 183 ± 7 mmHg, HR to 443 ± 11 bpm, and renal, mesenteric and hindquarter vascular resistances, while the blood flows in the same beds were reduced for at least 30 min (Figs. 5 and 6).

However, bilateral injections of muscimol (120 pmol/50 nl) into the CVLM in rats pretreated with kyn (2.7 nmol/50 nl) into the NTS immediately reduced MAP to 92 ± 2 mmHg and mesenteric and hindquarter vascular resistances, while the blood flows in the same beds were reduced for at least 30 min (Figs. 5 and 6).
HR (Figs. 5-7). Mesenteric (51 ± 14%) and hindquarter (55 ± 7%) blood flows increased above control preinjection baseline levels, and renal blood flow was restored to control baseline level by the combination of kyn into the NTS and muscimol into the CVLM (Figs. 6 and 7).

Baroreflex test in rats treated with muscimol into the CVLM and kynurenic acid into the NTS. The combination of bilateral injections of muscimol into the CVLM and kyn into the NTS abolished the reflex bradycardia (−1 ± 2 bpm vs. control: −54 ± 6 bpm; n = 11) produced by an intravenous injection of phenylephrine (5 μg/kg body wt) and also abolished the reflex tachycardia (3 ± 4 bpm vs. control: 66 ± 7 bpm) to intravenous injection of sodium nitroprusside (30 μg/kg body wt). The pressor response to intravenous phenylephrine (36 ± 5 mmHg vs. control: 44 ± 9 mmHg) and the hypotension to intravenous sodium nitroprusside (−35 ± 3 mmHg vs. control: −37 ± 5 mmHg) were not modified by the simultaneous blockade of the CVLM with muscimol and the NTS with kyn.

Effects of muscimol and kynurenic acid injected outside the CVLM and NTS on MAP, HR, and regional vascular resistances. In the first series of experiments, bilateral injections of muscimol or kyn outside the CVLM or NTS, respectively (Fig. 1), produced no significant changes in the baseline MAP (8 ± 3 mmHg vs. vehicle: 9 ± 6 mmHg; n = 12 and 8, respectively), HR (13 ± 11 bpm vs. vehicle: 7 ± 7 bpm) or renal (11 ± 6% vs. vehicle: 9 ± 15%), mesenteric (7 ± 5% vs. vehicle: 12 ± 17%) and hindquarter (6 ± 8% vs. vehicle: 6 ± 11%) vascular resistances. Injections of kyn outside the NTS after muscimol into the CVLM produced no additional changes in MAP (158 ± 32% vs. vehicle: 85 ± 32%; n = 12 and 8, respectively), HR (40 ± 11 bpm vs. vehicle: 413 ± 7 bpm) or renal (345 ± 26% vs. vehicle: 363 ± 32%), mesenteric (352 ± 35% vs. vehicle: 366 ± 13%) and hindquarter (374 ± 51% vs. vehicle: 358 ± 33%) vascular resistances.

In a second series of experiments, the bilateral injections of kyn or muscimol outside the NTS or CVLM, respectively, produced no significant changes in the baseline MAP (4 ± 6 mmHg vs. vehicle: −2 ± 11 mmHg; n = 9 and 8, respectively), HR (7 ± 9 bpm vs. vehicle: 13 ± 6 bpm) or renal (21 ± 16% vs. vehicle: 23 ± 11%), mesenteric (12 ± 5% vs. vehicle: 11 ± 11%) and hindquarter (9 ± 12% vs. vehicle: 8 ± 9%) vascular resistances. Injections of muscimol outside the CVLM after kyn into the NTS produced no additional changes in MAP (115 ± 32% vs. vehicle: 158 ± 32%; n = 9 and 8, respectively), HR (417 ± 9 bpm vs. vehicle: 423 ± 16 bpm) or renal (142 ± 36% vs. vehicle: 153 ± 41%), mesenteric (162 ± 25% vs. vehicle: 161 ± 13%) and hindquarter (159 ± 32% vs. vehicle: 158 ± 19%) vascular resistances.

DISCUSSION

As previously demonstrated, the blockade of the glutamatergic receptors in the NTS by injections of kyn or the inhibition of the CVLM with muscimol increases MAP and HR (15, 18, 26, 53). The novelty in the present study is that the blockade of the glutamatergic receptors of the NTS simultaneously with the inhibition of the CVLM reduces MAP and mesenteric and hindquarter vascular resistances below control preinjection baseline levels. Misplaced injections of muscimol or kyn outside the CVLM or NTS produced no significant changes in the baseline vascular resistances, MAP, and HR. Therefore, the present results suggest that important pressor mechanisms arising from the NTS and the CVLM are involved.
Important connections among the NTS, CVLM, and RVLM in the control of vascular resistance and arterial pressure in the conditions used in the present study.

Important connections among the NTS, CVLM, and RVLM for cardiovascular regulation have been proposed (11, 30). Chemoreceptors probably activate an excitatory pathway that connects the NTS to sympathetic premotor neurons in the RVLM either directly (10, 31, 49) or through brain stem pathways that involve neurons also in the A5 region (19). Baroreceptor afferents provide excitatory inputs to second-order neurons in the NTS that project to the CVLM and activate inhibitory projections from CVLM to the RVLM (11, 16). Glutamate is suggested to be the neurotransmitter released by baroreceptor and chemoreceptor afferent fibers in the NTS (8, 15, 16, 32, 48). Blocking the baroreflex with injections of kyn into the NTS, the CVLM inhibitory mechanism is deactivated reducing the inhibition of the RVLM which in turn increases sympathetic activity, vascular resistance, MAP, and HR. Injections of muscimol into the CVLM also remove the inhibition of the RVLM producing effects qualitatively similar to kyn into the NTS (4, 12, 53). Although baroreceptor signals that arise through NTS are important to inhibit the RVLM (2, 12, 18, 28), it is necessary to consider that part of the inhibition that the RVLM receives from the CVLM is not baroreceptor dependent (12), which may explain why vascular resistance and MAP increases are almost double after CVLM blockade with muscimol than after only the blockade of the baroreflex influences with kyn into the NTS.

Although the treatment with muscimol into the CVLM or kyn into the NTS independently disinhibits the RVLM and increases sympathetic activity, vascular resistance, MAP, and HR, when both treatments are combined simultaneously, MAP and mesenteric and hindquarter vascular resistances fall below control preinjection levels without changing the HR increase. These results suggest that the increases in MAP and regional vascular resistances produced by the inhibition of the CVLM alone depend on pressor mechanisms arising from the NTS. Moreover, the effects on MAP and vascular resistances produced by the blockade of the glutamatergic mechanisms of the NTS alone depend on pressor mechanisms that arise from the CVLM. Excitatory mechanisms arising from different central areas are important to activate RVLM neurons in resting conditions or hypertensive states (26, 27, 43). Although the blockade of EAA receptors in the RVLM produces no significant effects on baseline arterial pressure in rats (18, 26), it reduces arterial pressure and sympathetic nerve activity in rabbits (23). Ito and Sved (26) also showed that arterial pressure was reduced below control resting levels by the blockade of EAA receptors with kyn in the RVLM combined with the blockade of the CVLM with muscimol, which suggests that the increase in arterial pressure produced by the blockade of the CVLM is dependent on RVLM excitation produced by EAA release. Similar to Ito and Sved (26), the present results also support the importance of facilitatory mechanisms to the RVLM for the pressor response, resulting from muscimol into the CVLM, with the difference that the present results suggest that the facilitatory projection to the RVLM arises and is activated by glutamatergic mechanisms in the NTS.

The existence of pressor mechanisms in the NTS is suggested by the pressor responses usually produced by L-glutamate injections into the NTS in awake rats or by the antihypertensive effects of commissural NTS lesions in spontaneously hypertensive rats (9, 33, 42, 44). Although L-glutamate injected into the NTS in anesthetized rats usually induces depressor responses, the same injection into the NTS after the blockade of the CVLM with muscimol produces pressor responses in anesthetized rats (49), which suggest that L-glutamate injections into the NTS may also activate pressor mechanisms in anesthetized rats. However, in anesthetized rats the pressor mechanism activated by L-glutamate injections into the NTS is completely masked by the larger depressor responses dependent on CVLM. Anatomical and immunohistochemical studies have suggested the existence of a direct excitatory projection from the NTS to the RVLM (49, 50), which may convey peripheral chemoreceptor signals (30, 31). Although kyn injections into the NTS might block chemoreflex pathways (6, 7, 51), it is necessary to consider that chemoreceptors are usually silent in the absence of a proper stimulus. Therefore,
during the blockade of the CVLM with muscimol, a mechanism probably not related to the chemoreflex seems to activate EAA receptors in the NTS causing sympathetic activation and pressor responses. A previous study suggested that aortic depressor nerve activity can stimulate pressor mechanisms and increase arterial pressure when the CVLM was blocked by muscimol (52). Although the involvement of mechanisms related to the aortic depressor nerve should be considered, the present results do not illuminate the mechanism that activates NTS glutamatergic pressor pathways after muscimol injections into the CVLM. However, it is clear that MAP and vascular resistance are strongly dependent on NTS glutamatergic mechanisms after the blockade of the CVLM. On the other hand, the pressor response produced by kyn injections into the NTS is abolished by muscimol injections into the CVLM, suggesting that excitatory mechanisms from the CVLM blocked by muscimol injections are involved in the pressor response to kyn injections into the NTS. Injections of kyn into the RVLM abolish the pressor response to muscimol injections into the NTS (26), which suggests that the excitatory projection to the RVLM arises from other sources in addition to the NTS. Similar to previous studies (26, 38), the present results showing
that the pressor response to kyn injections into the NTS is abolished by muscimol injections into the CVLM also suggest that the CVLM by direct or indirect projections may activate RVLM neurons. Other studies have already suggested that non-glutamatergic excitatory projections arise from the CVLM to the RVLM (26) and that the cardiovascular effects of the caudal pressor area (CPA) activation also depend on an excitatory projection from the CVLM to the RVLM (38).

It is important to note that independent of the sequence of the CVLM and NTS blockades, HR increased after the first blockade, and this increase was not affected by the second blockade. Therefore, the fact that dual blockade produced different effects on MAP and HR suggests that different brainstem mechanisms are activated in these responses and may also involve changes in parasympathetic activity for the tachycardic responses. It is also important to consider that the combination of muscimol into the CVLM and kyn into the NTS reduced mesenteric and hindquarter vascular resistances below control baseline levels, while renal vascular resistance was maintained at the baseline level, which suggests that the hypotension after the combination of the two treatments depends on vasodilation of specific vascular beds. Although NTS glutamatergic and CVLM excitatory mechanisms are important for the increase in renal, mesenteric and hindquarter vascular resistances after the blockade of each area individually, these excitatory mechanisms apparently are not important to maintain baseline renal vascular resistance when both areas are blocked simultaneously. This differs from hindquarter and mesenteric vascular resistances that are dependent on NTS and CVLM excitatory mechanisms to maintain resting levels.

In conclusion, the present results suggest that the pressor responses produced by the blockade of the inhibitory mechanism to the RVLM are strongly dependent on the excitatory projections that arise from the CVLM and from the NTS. In addition, these excitatory pressor mechanisms are also important to maintain baseline arterial pressure and vascular resistance because the blockade of both mechanisms simultaneously reduced MAP and vascular resistance to below control resting levels.

**Perspectives**

One important mechanism still not completely understood is how RVLM neurons are activated to maintain tonic baseline activity of sympathetic nervous system. Besides the importance of the inhibitory mechanisms to control RVLM and sympathetic activity, recent studies have suggested that excitatory projections to the RVLM seem to play an important role in cardiovascular regulation. The present results suggest that excitatory mechanisms that arise from the NTS and CVLM are important to activate RVLM and the sympathetic system. In addition, they suggest that a balance between NTS and CVLM excitatory and inhibitory mechanisms seems to be essential to maintain baseline arterial pressure. Studies using different methodologies are necessary to show more details and to establish the role of the excitatory mechanisms proposed in the present study in the control of sympathetic activity and arterial pressure. An important mechanism still not understood is how the excitatory projections from CVLM and NTS to RVLM are activated under physiological conditions. Other questions for further investigation include whether the pathway relaying excitatory signals from the NTS to RVLM is also involved in chemoreceptor signaling and whether baroreceptor signaling is involved in the cardiovascular responses evoked from NTS when the CVLM is blocked by muscimol.

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