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RVLM GLYCINE RECEPTORS MEDIATE GABA_A AND GABA_B INDEPENDENT SYMPATHOINHIBITION FROM CVLM IN RATS.

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Abstract

The caudal ventrolateral medulla (CVLM) provides tonic inhibitory and also excitatory inputs to the rostral ventrolateral medulla (RVLM). These experiments evaluated the role of RVLM γ -amino butyric acid (GABA) receptor subtypes and glycine receptors in mediating CVLM sympathoinhibition. In Inactin anesthetized female rats, the CVLM and RVLM were functionally defined by pressor and depressor responses to microinjected GABA (500 pmol, 50 nl). Although reduced, pressor and sympathoexcitatory responses due to inhibition of the CVLM with GABA persisted following ipsilateral RVLM GABA_A receptor blockade (bicuculline, BIC, 400 pmol, 100 nl; n=12) in rats with contralateral nucleus tractus solitarius (NTS) lesion. In the presence of either ipsilateral (+contralateral NTS lesion; n= 8) or bilateral (n=6) GABA_A and GABA_B receptor blockade of the RVLM (400 pmol BIC + 400 pmol CGP35348, 100 nl), inhibition of the CVLM still increased MAP and renal sympathetic nerve activity (RSNA). Thus neither GABA_B receptors nor a contralateral CVLM to RVLM GABAergic pathway explains residual responses CVLM blockade. The addition of strychnine (300 pmol, 100 nl) to the RVLM eliminated responses to CVLM inhibition, suggesting that a GABA_A and GABA_B independent sympathoinhibitory influence from CVLM to RVLM is mediated by glycine receptors. Decreases in MAP and RSNA due to activation of the CVLM with glutamate (500 pmol, 50 nl) were reversed to increases in the presence of RVLM GABA_A receptor blockade (n=7). Thus, a sympathoexcitatory pathway from the CVLM can be activated in the presence of RVLM GABA receptor blockade, but sympathoinhibitory influences from the CVLM predominate.

Keywords

caudal ventrolateral medulla; rostral ventrolateral medulla; CGP35348; Bicuculline; Strychnine; contralateral baroreflex pathway

1. INTRODUCTION

The rostral ventrolateral medulla (RVLM) is a critical brainstem site involved in the regulation of cardiovascular function. Excitation of the RVLM produces an increase in sympathetic outflow and arterial blood pressure while inhibition of neurons in the RVLM results in a decrease in sympathetic outflow and arterial pressure demonstrating that this region is tonically active (13,14,16). As a final common site for modulation of sympathoexcitatory drive to preganglionic sympathetic neurons in the intermediolateral cell column of the spinal cord (IML), the RVLM receives and integrates neural inputs from several areas in the central nervous system.

Tonic activity of RVLM neurons appears to be largely dependent on synaptic inputs (31). While stimulation of several central nervous system sites excites the RVLM, the majority of these areas have not been shown to provide tonic excitation to the RVLM under normal physiological conditions (13,14). The pontine reticular formation (18) and the caudal pressor area (21,35) are regions which have been identified as potential sources of tonic excitatory drive to the RVLM. Sympathoexcitation from the caudal pressor area has been reported to be mediated through an excitatory pathway including the CVLM (35) or through inhibition of a GABAergic pathway between the CVLM and RVLM (21). Others have suggested that the CVLM (23,33) and the NTS (33) may also be important sources of tonic excitatory drive to the RVLM.

In regard to inhibitory influences, one area in particular, the caudal ventrolateral medulla (CVLM), has repeatedly been shown to have an important role in the modulation of RVLM pre-sympathetic neurons which project to the IML (10,11,13,16,25,40). GABA is the primary neurotransmitter mediating inhibition of sympathetic premotor neurons in the RVLM (10–14,16,25,40) and CVLM neurons are antidromically activated from the RVLM (1,13,25). Retrograde labeling from the RVLM of GABAergic CVLM neurons, which also express Fos protein in response to an increase in arterial pressure, suggest a direct GABAergic projection from the CVLM to the RVLM (8). Using a combination of electrophysiological and anatomical techniques, Schreihöfer & Guyenet demonstrated that baro-activated, pulse-modulated CVLM neurons express the GABA synthetic enzyme, GAD₆₇, and project rostrally (41). Taken together these studies provide convincing evidence for a tonically active monosynaptic GABAergic projection from CVLM to RVLM in the medullary baroreflex pathway.

Inhibitory GABAergic projections from the CVLM to the RVLM that are tonically active and independent of the baroreflex also have been described (10–12,40,41). Even at low arterial pressures, when arterial baroreceptor input is minimal, iontophoretic application of bicuculline results in increased firing of spinally projecting RVLM neurons (45). Following acute NTS lesion in rabbits (12) and chronic NTS lesion or SAD in rats (42), microinjection of bicuculline into the RVLM results in large sympathoexcitatory responses. In acutely (11) and chronically (42) sino-aortic denervated (SAD) rats, neuronal blockade of the CVLM results in increased arterial pressure and sympathetic nerve activity. Thus, there is a major baroreflex independent GABAergic inhibitory influence from the CVLM to the RVLM.

There is strong evidence demonstrating that arterial baroreflex initiated inhibition of the RVLM by the CVLM is mediated through GABA_A receptors (13,14,16,46). However, GABA_B receptor-like immuno-reactivity has been demonstrated in the region of the RVLM of rats (32) and cats (36). Several studies suggest that GABA_B receptors participate in GABAergic inhibition of the RVLM (2,4,9,27,28,29) and may be tonically activated (2,4,9). Since arterial baroreflex responses appear to be mediated solely by GABA_A receptors in the RVLM (13, 14,16), we considered that a possible role for GABA_B receptors might include mediation of baroreflex independent inhibitory influences from the CVLM. In addition, although all reports do not agree (2), there is evidence for tonic glycinergic inhibition of sympathetic outflow in the RVLM (6,8) and a role for glycine in the RVLM was evaluated.

We observed that ipsilateral RVLM GABA_A receptor blockade in contralateral NTS lesioned rats did not eliminate pressor and sympathoexcitatory responses to inhibition of the CVLM. The goal of subsequent experiments was to evaluate potential mechanisms for the remaining response to CVLM inhibition. Neither RVLM GABA_B receptors, nor a contralateral CVLM to RVLM pathway accounted for the remaining inhibition from the CVLM. The major new finding is that, although the predominant inhibitory influence from the CVLM is mediated by GABA_A receptors in the RVLM, in the presence of both GABA_A and GABA_B receptor blockade, glycine receptors in the RVLM provide the remaining inhibitory influence originating from the CVLM.

2. RESULTS

Preliminary experiments were performed in right NTS lesioned rats, to determine a treatment regimen to provide effective block of GABA receptors in the left RVLM. Efficacy of receptor blockade was evaluated by testing for elimination of baroreflex sympathoinhibitory responses following injection of BIC in the left RVLM (Protocols 1 and 2) or loss of MAP and RSNA responses to microinjection of GABA (1mM) into the left RVLM following administration of BIC + CGP35348 (Protocol 2). The concentrations of BIC (4mM) and CGP35348 (4mM) used in this study were based on those previously reported in the literature (2,43,47). In the current experiments a single microinjection of either BIC or BIC + CGP35348 was inadequate for maintenance of a complete GABA receptor blockade over a ten minute period. However, a single injection (400 pmol, 100 nl) of the antagonist(s) followed by supplemental injection (200 pmol, 50nl) at 5 minute intervals was found to reliably establish and maintain GABA receptor blockade for one hour. Using this regimen, supplemental injections of the GABA antagonists did not produce any additional effects on MAP or RSNA.

2.1 Nucleus tractus solitarius (NTS) lesion

Right NTS lesion in Protocol 1 (n=12) and Protocol 2 (n=8), had no lasting effect on either baseline mean arterial pressure (MAP) or renal sympathetic nerve activity (RSNA) (Table 1). In 17 of 20 animals from Protocols 1 and 2, sympathoinhibitory responses following elevation of MAP with a bolus injection of PE were tested 15–25 minutes following right NTS lesion and again at the end of the experiment following interruption of baroreflex responses from the left side, using either a left NTS lesion (n=2) or muscimol in the left CVLM (n=15). A ratio of change in RSNA to change in MAP (Δ RSNA/ Δ MAP) was used as an estimate of baroreflex gain. Heart rate responses were minimal in these anesthetized animals and therefore baroreflex gain for HR was not determined. In the animals tested in Protocol 1 (n=11) and Protocol 2 (n=6), interruption of both left and right sides of the baroreflex pathway resulted in 99% (Protocol 1) and 96% (Protocol 2) reduction in the ratio, Δ RSNA/ Δ MAP (Table 2). Elimination of the baroreflex, through interruption of the pathway on the left side at the end of the experiment, indicated that the initial right NTS lesion had been complete.

2.2 Responses to microinjection of GABA in the CVLM and RVLM

At the beginning of each protocol proper pipette placement was verified by microinjection of GABA (500 pmol, 50 nl). Mean responses to GABA in the CVLM and RVLM for the three protocols are provided in Table 3. RSNA was processed as impulses/sec and values are expressed as a percent of baseline. Peak responses were observed within 20 seconds and returned to stable levels near baseline within two minutes of microinjection. The experimental preparations for Protocols 1 and 2 were similar and comparison of responses to GABA in CVLM and RVLM were not different between the two groups.

Protocol 1: Effect of BIC in RVLM—In rats with prior right NTS lesions, the effects of inhibiting the left CVLM were evaluated before and during GABA_A receptor blockade in the left RVLM. Figure 1 depicts responses from a representative animal. Mean data are shown in Figure 2. Microinjection of GABA (500 pmol, 50 nl) in the left CVLM prior to GABA_A receptor block in the left RVLM elicited significant increases in MAP and RSNA (G_1), both of which recovered to levels not different from control within two minutes (R). Following GABA in the left CVLM, at least ten minutes were allowed before microinjection of the GABA_A receptor antagonist, BIC (400 pmol, 100 nl), in the left RVLM. BIC in the RVLM produced an increase in MAP and RSNA (B). Similar to previous studies (46,50) peak MAP was achieved 1–3 minutes after BIC injection and stabilized at the new higher level within 5 minutes, at which time BIC in the RVLM was supplemented. Within two minutes, responses to inhibition of the CVLM were again tested. In the presence of GABA_A receptor blockade of

the left RVLM, microinjection of GABA in the left CVLM (G_2) produced a further increase in MAP and RSNA. However, compared to responses to GABA in the left CVLM prior to $GABA_A$ receptor blockade in the left RVLM ($\Delta MAP = +29 \pm 3.4$ mmHg; $\Delta RSNA = +49 \pm 9.4$ %), the changes in MAP and RSNA due to inhibition of the left CVLM with GABA were smaller in the presence of BIC in the left RVLM ($\Delta MAP = +20 \pm 3.7$ mmHg; $\Delta RSNA = +26 \pm 9.7$ %). Baroreflex sympathoinhibition due to elevated MAP (PE bolus) was eliminated by BIC in the left RVLM in the seven rats tested (Table 4), suggesting that $GABA_A$ receptor blockade in the left RVLM was adequate (12,16).

Prior to $GABA_A$ receptor blockade in the left RVLM, GABA in the left CVLM reversibly increased heart rate (HR) from 316 ± 6.8 to 339 ± 10.0 bpm. Within two minutes HR returned to values not different from control (322 ± 8.7 bpm). Following BIC in the left RVLM, HR did not change significantly in response to GABA in the left CVLM (Control = 296 ± 10.4 ; BIC = 292 ± 14.3 ; BIC+GABA = 304 ± 18.0 bpm).

Effect of l-glutamate (l-glu) in CVLM—In a subset of seven right NTS lesioned rats from Protocol 1, the responses to excitation of the left CVLM (l-glu; 500 pmol, 50 nl) were also tested before and after $GABA_A$ receptor blockade (BIC) in the left RVLM. Figure 3 shows responses to l-glu in the left CVLM before and after BIC in the left RVLM in a representative animal. In the seven rats tested, excitation of the left CVLM with l-glu prior to $GABA_A$ receptor block in the left RVLM resulted in significant depressor (-17 ± 4.1 mmHg) and sympathoinhibitory (-34 ± 6.1 %C) responses. Following blockade of left RVLM $GABA_A$ receptors (BIC), the responses to l-glu in the left CVLM were reversed to significant pressor ($+19 \pm 7.9$ mmHg) and sympathoexcitatory ($+18 \pm 8.1$ %C) responses, suggesting that a sympathoexcitatory pathway exists from the CVLM. However, in this same subset of seven rats, in the presence of $GABA_A$ blockade in the left RVLM, inhibition of tonic activity from the left CVLM with GABA tended to increase MAP ($+20 \pm 6.3$ mmHg; $P \leq 0.06$) and significantly increased RSNA ($+19 \pm 10.5$ %C; $P < 0.05$), similar to mean responses in the entire group of 12 rats from Protocol 1 (Fig. 2).

Protocol 2: Effect of Combined $GABA_A$ and $GABA_B$ receptor blockade in the RVLM—To determine if $GABA_B$ receptors in the RVLM contributed to inhibition from CVLM to RVLM in the presence of $GABA_A$ blockade, a combination of BIC and CGP35348, a specific $GABA_B$ receptor antagonist, was microinjected into the left RVLM of eight rats with right NTS lesions and an experimental protocol similar to Protocol 1 was performed. In order to verify that both $GABA_A$ and $GABA_B$ receptors in the left RVLM were blocked, responses to exogenously administered GABA (50 pmol, 50 nl) into the left RVLM were evaluated before and after microinjection of the combined antagonists. Since BIC is a competitive $GABA_A$ receptor antagonist, a dose of exogenously administered GABA that produced moderate yet reproducible responses was used. Prior to administration of the blockers, microinjection of GABA (50 pmol, 50 nl) into the left RVLM resulted in significant decreases in MAP and RSNA. In the presence of BIC (400 pmol)+CGP35348 (400 pmol) (100 nl total volume) in the RVLM, MAP and RSNA responses to microinjection of GABA (50 pmol, 50 nl) into the RVLM were eliminated, suggesting that both $GABA_A$ and $GABA_B$ receptors in the left RVLM were blocked (Fig. 4).

Microinjection of GABA (500 pmol, 50 nl) in the left CVLM prior to injection of BIC + CGP35348 in the left RVLM produced significant and reversible increases in MAP and RSNA (G_1 , Fig. 5). The initial increases in MAP and RSNA following microinjection of the combination of GABA antagonists in the left RVLM tended to decrease over several minutes before stabilizing, although MAP remained significantly elevated above baseline (GX). In order to verify that GABA receptors remained blocked despite partial recovery, the effect of microinjected GABA (50 pmol, 50 nl) was tested periodically and no response to GABA in

the left RVLM was observed during combined GABA_A and GABA_B receptor blockade (not shown). In addition, arterial baroreflex responses to a bolus injection of phenylephrine were attenuated by 89% following combined GABA_A and GABA_B receptor blockade in the left RVLM (Table 4).

In the presence of GABA_A and GABA_B receptor blockade in the left RVLM (GX), responses to inhibition of the left CVLM with GABA were evaluated. Similar to responses in Protocol 1, microinjection of GABA (500 pmol, 50 nl) in the left CVLM continued to elicit a further significant increase in MAP (G₂). In the presence of BIC+CGP35348 in the left RVLM, the RSNA response following GABA in the left CVLM ($+13 \pm 5.2\%$) was significantly less than the RSNA response prior to GABA_A and GABA_B receptor blockade ($+41 \pm 5.0\%$). The increases in MAP due to left CVLM GABA before ($+25 \pm 3.9$ mmHg) and after ($+17 \pm 3.2$ mmHg) left RVLM GABA_A + GABA_B receptor blockade were not significantly different.

Before GABA_A + GABA_B receptor blockade in the left RVLM, GABA in the left CVLM reversibly increased HR (not shown) ($C_1 = 317 \pm 7.7$; $G_1 = 350 \pm 12.6$; $R = 320 \pm 11.2$ bpm). Following BIC + CGP35348 in the left RVLM, HR did not change significantly in response to GABA in the left CVLM ($C_2 = 314 \pm 11.5$; $GX = 318 \pm 12.4$; $GX+G_2 = 315 \pm 12.6$ bpm).

The experimental preparations were similar for Protocols 1 and 2. The increases in MAP and RSNA due to inhibition of the left CVLM in the presence of GABA_A and GABA_B receptor blockade of the left RVLM (Protocol 2) were no different from responses during GABA_A receptor block of the left RVLM (Protocol 1).

Protocol 3: Effects of bilateral GABA_A and GABA_B + glycine receptor blockade in the RVLM—In Protocols 1 and 2 the right NTS was lesioned to eliminate downstream effects of baroreceptor input from the right side. Left RVLM BIC blocked arterial baroreflex responses in the rats tested, suggesting that the minor contralateral CVLM to RVLM pathway that has been described anatomically (8) was not providing baroreflex compensation in these experiments. However it is possible that residual responses to GABA in the left CVLM could be due to contralateral baroreflex independent GABAergic inhibition from the left CVLM to the right RVLM. Therefore experiments were performed in which inhibitory neurotransmitter receptors in the RVLM were blocked bilaterally ($n = 6$). Responses to activation of cardiopulmonary reflexes (i.v. PBG) and unilateral activation of central baroreflex pathways (NTS l-glu) were eliminated by the combination of bilateral GABA_A, GABA_B, and glycine receptor blockade in the RVLM (Table 5).

Figure 6 shows responses from a representative rat. Mean data are summarized in Figure 7. Microinjection of GABA (500 pmol, 50 nl) in the CVLM prior to bilateral injection of BIC + CGP35348 in the RVLM produced significant and reversible increases in MAP and RSNA (G₁, Fig. 7). Similar to results from Protocol 2, following the initial increase in MAP and RSNA due to bilateral GABA_A and GABA_B receptor blockade in the RVLM, values tended to decrease over several minutes. However, following bilateral injections both MAP and RSNA stabilized at levels significantly above baseline (GX₁). In the presence of bilateral BIC + CGP35348 in the RVLM, CVLM microinjection of GABA (500 pmol, 50 nl) continued to elicit further increases in MAP and integrated RSNA (G₂). In the continued presence of bilateral GABA_A and GABA_B receptor blockade (GX₂), microinjection of strychnine hydrochloride (S) in the RVLM did not significantly increase MAP and RSNA, but blocked both MAP and RSNA responses to GABA in the CVLM (G₃). Baseline HR was 303 ± 22.1 in this group of anesthetized rats and responses to brainstem microinjections were small and not statistically significant.

2.3 Histology

Post hoc estimation of unilateral microinjection sites from animals in Protocols 1 and 2 are summarized in Figure 8. Figure 9 contains original photomicrographs from one animal that received bilateral injections in the RVLM and CVLM. In the six rats from Protocol 3, injection sites within the RVLM were estimated to be between -11.6 to -12.3 caudal to bregma, and injection sites within the CVLM were estimated to be between -13.68 to -13.8 caudal to bregma.

3. DISCUSSION

3.1 Evaluation of Inhibitory Influences

In experiments in Protocol 1, blockade of RVLM GABA_A receptors with BIC resulted in the expected increases in MAP and RSNA (13,16). Although reduced, pressor and sympathoexcitatory responses to inhibition of the CVLM persisted following GABA_A receptor blockade in the RVLM. The continued increase in MAP and RSNA observed in these experiments suggests that there is a source of inhibition from the CVLM which is not mediated by GABA_A receptors in the RVLM. Natarajan and Morrison (34) reported similar findings, whereby bilateral inhibition of the CVLM with muscimol produced increases in splanchnic nerve activity above those produced by prior microinjection of bicuculline into the RVLM.

Based on these data with RVLM GABA_A receptor blockade, we considered that RVLM GABA_B receptors might contribute to tonic baroreflex independent sympathoinhibition and may account for the remaining CVLM sympathoinhibitory influence seen in the presence of RVLM GABA_A receptor blockade. Using a similar experimental preparation to Protocol 1, responses to inhibition of the CVLM were tested before and after GABA_A + GABA_B receptor blockade in the RVLM (Protocol 2). The increases in MAP, HR, and RSNA following BIC + CGP35348 in the RVLM partially recovered towards control levels before stabilizing, although MAP and HR remained significantly elevated. Despite the partial recovery, elimination of baroreflex responses and responses to exogenously administered GABA in the RVLM verified that receptor blockade was maintained. Similar observations were reported by Sved and Tsukamoto following microinjection of CGP35348 in the NTS (47). In their study, the depressor response elicited by microinjection of CGP35348 in the NTS was transient, but responses to the GABA_B agonist, baclofen, remained blocked.

Although the pressor response to inhibition of the CVLM was attenuated by combined GABA_A and GABA_B receptor blockade in the RVLM, MAP and RSNA responses persisted, suggesting that the CVLM continued to provide a sympathoinhibitory influence in the presence of combined GABA receptor blockade in the RVLM. Additionally, since there were no significant differences in the responses to inhibiting the CVLM in the presence of BIC versus BIC+CGP35348 in the RVLM, it appears that GABA_B receptors do not make a major contribution to GABAergic inhibition from the CVLM to the RVLM.

In Protocols 1 and 2, NTS lesion on the right side eliminated downstream effects of afferent baroreceptor input from the right side. Subsequent GABA_A receptor blockade in the left RVLM eliminated baroreflex responses mediated through the left RVLM. However, although predominantly unilateral, a minor contralateral projection from the CVLM to the RVLM has been described anatomically (8) and CVLM neurons can be antidromically activated by electrical stimulation of the contralateral RVLM (15). Although functional significance of this pathway has not been demonstrated, we considered that the combination of minor effects of arterial baroreceptor input from the left NTS to CVLM pathway and baroreflex independent inhibition from the left CVLM to the right RVLM could have accounted for residual responses to inhibition of the left CVLM (Protocols 1 and 2). In Protocol 3, bilateral blockade of

GABA_A and GABA_B receptors in the RVLM was performed to block effects of both ipsilateral and contralateral GABAergic inputs to the RVLM. However, despite the substantial increase in MAP due to bilateral blockade of RVLM GABA receptors, inhibition of the CVLM still resulted in a further pressor response.

Thus, pressor and sympathoexcitatory responses due to inhibition of the CVLM persisted following ipsilateral blockade of RVLM GABA_A (Protocol 1) and GABA_A + GABA_B receptors (Protocol 2) in rats with contralateral NTS lesion, and following bilateral blockade of RVLM GABA_A (34) and GABA_A + GABA_B receptors (Protocol 3). Taken together these data suggest that 1) a contralateral CVLM to RVLM pathway does not account for responses in these experiments, and 2) there is an inhibitory influence from the CVLM that is independent of GABA receptors in the RVLM.

Tonic inhibition from the CVLM to the RVLM is largely GABAergic (6,13,14,16,40), but receptors for the inhibitory transmitter, glycine, have been described in the RVLM of rats (30) and cats (36). Although found in less abundance than GABAergic neurons, barosensitive glycinergic neurons are present in the CVLM of the rat (8). In order to determine if RVLM glycine receptors contributed to the remaining inhibitory influence of the CVLM, we evaluated the effects of bilateral RVLM glycine receptor blockade in the presence of bilateral GABA_A and GABA_B receptor blockade. The addition of strychnine in the RVLM eliminated the increase in MAP and RSNA that had been observed when the CVLM was inhibited in the presence of RVLM GABA receptor blockade alone. Therefore it appears that RVLM glycine receptors were involved in the remaining response to inhibition of the CVLM.

Blessing et al (6) reported that in rabbits microinjection of strychnine in the RVLM resulted in small but reproducible increases in MAP consistent with removal of tonic glycinergic inhibition of RVLM neurons. In contrast, bilateral injection of strychnine in the RVLM of rats was without effect (2). In the current experiments, in the presence of GABA_A and GABA_B receptor blockade, microinjection of strychnine into the RVLM did not significantly increase MAP or RSNA. If the residual pressor response to inhibition of the CVLM is mediated by removal of glycinergic influences in the RVLM, we might have expected that strychnine in the RVLM would result in small increases in MAP and RSNA. It is possible that the lack of response to blockade of RVLM glycine receptors in these experiments involves effects on multiple pathways, both inhibitory and excitatory, such that the net effect of strychnine in the RVLM is minimal. A similar mechanism has been proposed to explain the lack of effects when the excitatory amino acid antagonist, kynureate, is microinjected into the RVLM of normal animals (23, see discussion below). In general, effects of microinjected glycine in the RVLM are inhibitory (5,6,39). In conscious rats, depending on the dose, microinjection of glycine into the RVLM can produce either depressor (low dose) or pressor (high dose) responses (3). Neuronal excitation by glycine has been attributed to either positive allosteric modulation of NMDA receptors (24) or to potentiated release of acetylcholine (48). Glycine's effect to release acetylcholine is blocked by strychnine (48), whereas effects on NMDA receptors are strychnine insensitive (24). Therefore if strychnine blocked a sympathoexcitatory effect, which balanced a sympathoinhibitory effect of endogenous glycine in the current experiments, it would most likely involve blockade of glycine mediated acetylcholine release.

Alternately, the pathway from the CVLM involving glycine receptors in the RVLM may not be tonically active. In this regard, all of the projections of CVLM neurons have not been carefully defined and it is conceivable that the CVLM normally silences a pathway which, when activated, results in glycine release in the RVLM. For example, one possibility could involve the pontine reticular formation (PRF): Glycinergic neurons are present in the PRF (38); the CVLM projects to the PRF (44); and depending on the site, activation of cell bodies within the PRF results in either sympathoexcitation or sympathoinhibition (17) presumably

through actions at the RVLM (18). Also, reciprocal connections exist between the CVLM and PVN (13,14) and may participate in regulation of the sympathetic nervous system through actions in the RVLM (51). Thus, although inhibition of CVLM neurons had effects on sympathetic outflow in the current experiments, the pathway involving glycine in the RVLM is not necessarily tonically active nor does it necessarily involve a direct projection from the CVLM to the RVLM.

3.2 Evaluation of Excitatory Influences

Tonic inhibitory influences from the CVLM to the RVLM act to restrain tonic activity in neurons in the RVLM. However, the origin of tonic excitatory influences in the RVLM is not as well understood. Ito and Sved (23) confirmed that blockade of excitatory amino acid receptors (EAA) in the RVLM was without effect in intact normotensive rats. However, in the presence of neuronal blockade of the CVLM, blockade of EAA receptors in the RVLM, elicited a profound decrease in MAP. These authors proposed a model whereby EAAs in the RVLM excite both presympathetic neurons and neurons projecting to the CVLM, which in turn project to, and inhibit, sympathoexcitatory neurons in the RVLM. Since RVLM neurons apparently remained tonically active after blockade of EAA receptors in the RVLM in rats with CVLM intact, a separate tonic non-EAA excitatory input from the CVLM to the RVLM was proposed. In contrast, Horiuchi et al. (22) found evidence for only a minor EAA excitatory input to the RVLM in CVLM blocked rats.

In the current study, we reasoned that if a substantial tonic excitatory influence from the CVLM to the RVLM existed, then blockade of the CVLM in the presence of inhibitory neurotransmitter receptor blockade in the RVLM, should produce a decrease in MAP and RSNA. MAP and RSNA still increased when the CVLM was inhibited during RVLM GABA_A and GABA_B receptor blockade. The addition of RVLM glycine receptor blockade eliminated the pressor response to CVLM inhibition, but did not unmask a tonic excitatory influence from the CVLM. Since depressor and sympathoinhibitory responses to microinjection of l-glutamate (l-glu) in the CVLM were reversed to pressor and sympathoexcitatory responses following GABA_A receptor blockade in the RVLM, it is clear that a sympathoexcitatory pathway exists between the CVLM and RVLM. However, under the conditions of the current experiments, it does not appear that this particular pathway is tonically active.

Recently Moreira et. al (33) proposed that both the CVLM and NTS provide tonic excitatory input to the RVLM, which is normally masked by inhibitory input from these same regions. The authors proposed that the excitatory response observed following inhibition of either the NTS or CVLM alone was dependent on excitatory input from the remaining intact region. In the current experiments, in the presence of RVLM GABA receptor blockade, inhibition of the CVLM resulted in sympathoexcitatory responses which were eliminated by the addition of glycine receptor blockade in the RVLM. However, MAP and RSNA remained elevated above initial control levels in the presence of the inhibitory receptor antagonists in the RVLM. Thus, a site other than the CVLM, such as the NTS, must have provided tonic excitation to the RVLM to maintain arterial pressure and sympathetic nerve activity under these conditions.

3.3 Special Considerations

These experiments were performed in female rats in the estrus stage of the cycle and provide basic physiological data using a female animal model. Since rats were studied at the same stage of the estrous cycle, any concern regarding use of female animals based on confounding effects of varying ovarian hormone levels is not an issue in the current experiments. It is important to note that our data have been interpreted based on the existing literature, and the overwhelming majority of published reports contain results in male animal models only. Responses to

glutamate in the CVLM and RVLM, and responses to GABA_A receptor blockade alone in the RVLM of females in the current study were similar to those reported in the literature in males. Therefore we would not predict that the results of these experiments are specific to female rats.

The CVLM is heterogeneous and lacks clear anatomical boundaries. Anatomical studies have shown that CVLM neurons retrogradely labeled from the RVLM are mingled with neurons of the A1 (CVLM region) and C1 (RVLM region) cell groups (8,40,49). In the current experiments, microinjection sites were chosen based on functional criteria of pressor (CVLM) and depressor (RVLM) responses to GABA. However, given the anatomical distribution, any experiments involving microinjection of drugs into the functionally defined CVLM could have effects on some sympathoexcitatory neurons and vice versa. Since GABA in the CVLM region consistently resulted in sympathoexcitation and GABA in the RVLM region consistently resulted in sympathoinhibition, our results are interpreted based on the predominant effects due to inactivation of sympathoinhibitory CVLM and sympathoexcitatory RVLM neurons respectively. Potential minor effects on the alternate cell group could not account for the results of the current experiments.

Multiunit nerve activity was recorded in these experiments and normalized as percent of control (baseline) values. Previously we have noted that interpretation of normalized mean nerve activity data is similar whether the raw nerve activity is processed as i/sec or as a rectified and integrated voltage (unpublished observations). In Protocol 3, the raw nerve activity signal was processed as both i/sec and as a rectified and integrated signal using the rate and root mean square (RMS) functions of Power Lab data acquisition software. Results using the two methods were qualitatively similar, although in this relatively small group of rats statistical significances similar to those seen with MAP were most evident using RMS to process the raw signal. Greater sensitivity of the RMS method to detect changes in multiunit nerve activity is likely due to the fact that the integrated signal (RMS function) incorporates changes in both amplitude and frequency of the multiunit signal (20).

3.4 Summary & Conclusions

The major findings of these experiments are: 1) Contrary to our original hypothesis, GABA_B receptors in the RVLM do not appear to significantly contribute to tonic baroreflex independent GABAergic inhibition from the CVLM to the RVLM; 2) Although a contralateral CVLM to RVLM pathway has been described, in these microinjection experiments we saw no evidence for influences from a contralateral CVLM to RVLM GABAergic pathway; 3) Glycine receptors in the RVLM mediate a GABA_A and GABA_B independent inhibition from the CVLM; and 4) Although a sympathoexcitatory pathway may be activated from the CVLM in the presence of GABA_A receptor blockade in the RVLM, it does not appear to be tonically active under the conditions of the current experiments.

4. EXPERIMENTAL PROCEDURE

All procedures were performed in accordance with the NIH guidelines in *Guide for the Care and Use of Laboratory Animals*. All protocols were approved by the Institutional Animal Care and Use Committees at University of Missouri and The Ohio State University.

4.1 Surgical Preparation

Experiments were performed in 26 virgin female Sprague Dawley rats (3–5 months of age; Harlan Sprague-Dawley, Indianapolis, IN) weighing 225–280g. Two complete estrous cycles were documented by daily vaginal smear cytology in all rats and experiments were performed on the day of estrus. The estrus stage of the cycle is easily identified and characterized by low and consistent levels of estrogen and progesterone (7). Rats were anesthetized with

intraperitoneal Inactin (100 mg/Kg) and supplemented (0.1 mg/Kg, i.v.) as needed. The trachea was cannulated and the rat artificially ventilated (CWE SAR830 Ventilator) with O₂ enriched room air. Body temperature was monitored (Yellow Springs Instrument Co) and maintained at 37°C. The rat was then instrumented with a left femoral arterial catheter (Microline tubing with 28 gauge Teflon tip) to monitor arterial blood pressure and a left femoral venous catheter (polyethylene tubing 50) for subsequent systemic drug administration. The left renal nerve was isolated retroperitoneally, placed on a bipolar platinum recording electrode, and secured in place with dental impression material (Coltene President's dental acrylic). All wounds were sutured closed.

4.2 Drugs and Solutions

Inactin was obtained from Research Biochemicals International (Natick, MA) and dissolved in sterile water. Tubocurarine chloride was obtained from Bristol Myers Squibb (Princeton, NJ). Phenylephrine was purchased from Sigma Chemical Co, (St. Louis, MO) and diluted in isotonic saline. L-glutamic acid, γ -amino-butyric acid, muscimol, bicuculline methiodide, and strychnine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in phosphate buffered saline. CGP35348, a GABA_B antagonist, was generously provided by Novartis (Basel, Switzerland). Phenylbiguanide and Chicago Sky Blue 6B (80%) were obtained from Aldrich Chemical Company (Milwaukee, WI) and neutral red was purchased from National Diagnostics (Highland Park, NJ).

4.3 Brainstem Microinjections

Protocols 1&2—After catheter and electrode placement, the rat was placed in a stereotaxic apparatus and an occipital craniotomy performed. The occipital parietal membrane and dura were cut and folded laterally to expose the brainstem. Tubocurarine (0.1 mg/Kg, i.v.) was administered to paralyze the rat. The rat's head was tilted forward until the calamus scriptorius was located 2.4 mm posterior to interaural zero (26). To eliminate compensatory effects from baroreceptor inputs from the right side, the right nucleus tractus solitarius (NTS) was electrolytically lesioned (1 mA anodal current, 10 s) using a Teflon insulated tungsten electrode. Relative to calamus scriptorius, medial-lateral (ML), anterior-posterior (AP) and dorsal-ventral (DV) coordinates for the NTS were: ML = 0.5, AP = 0.5, DV = 0.5 mm. Subsequent unilateral microinjections into the CVLM and RVLM were performed on the left side (n = 20). Initially, the left CVLM and RVLM were functionally mapped based on responses to microinjection of GABA (500 pmol, 50 nl). The CVLM was defined as the site producing an increase in mean arterial blood pressure (MAP) $\geq$ 15 mmHg and an increase in renal sympathetic nerve activity (RSNA) following microinjection of GABA (500 pmol, 50 nl). Coordinates for the CVLM were: ML = 1.9–2.0, AP = –0.2–0.4, DV = –2.4–2.6 mm, relative to calamus scriptorius. The site where microinjection of GABA (500 pmol, 50 nl) produced a decrease in MAP $\geq$ 15 mmHg and a decrease in RSNA was defined as the RVLM (ML = 1.9–2.0, AP = 0.5–0.7, DV = –3.5–3.8 mm relative to calamus scriptorius). An equivalent volume of vehicle (phosphate buffered saline) injected into the same site produced no changes in MAP or RSNA. For experimental protocols, microinjections into the left CVLM were performed using a triple barrel glass micropipette filled with γ -amino-butyric acid (GABA, 10 mM), l-glutamic acid (l-glu, 10 mM), and muscimol (2 mM), in separate barrels and mounted on a micromanipulator (David Kopf). A double barrel glass micropipette, filled with GABA (10 mM) in one barrel and either bicuculline methiodide (BIC, 4 mM; Protocol 1) or BIC (4 mM) + CGP35348 (4mM) (Protocol 2) in the other barrel was mounted on another micromanipulator, and used for microinjections into the left RVLM. Responses to GABA confirmed pipette location in the functionally defined CVLM or RVLM at the beginning of each protocol.

The RSNA signal was amplified 100,000–200,000 times using a Grass P511 amplifier with a band pass filter (high frequency cutoff = 10,000 Hz; low frequency cutoff = 30–100 Hz). The signal was monitored on a loudspeaker and on a dual beam storage oscilloscope (Tektronix, R5113). A rate meter/window discriminator (Winston Electronics, RAD-IIA) was set to count multiunit nerve activity exceeding a selected voltage just above noise level and ratemeter output was electronically filtered (19). Ratemeter output for renal sympathetic nerve activity (RSNA), heart rate (HR), and mean arterial blood pressure (MAP) were monitored on a polygraph (MFE Instruments Corp., MFE 1800) and stored on videotape (Neuro Data Instrument Corp., DR-886) for later analysis. Any signal remaining 30 minutes to one hour after the animal's death, was considered electrical noise and subtracted from experimental nerve activity values. Since the absolute value of multiunit nerve activity is dependent on recording conditions, nerve activity was standardized as a percent of the initial baseline value preceding recorded experimental values.

Protocol 3—Similar to procedures in Protocols 1 and 2, rats were prepared for brainstem microinjections, except that unilateral NTS lesions were not performed ($n = 6$). The CVLM and RVLM were mapped bilaterally with GABA (500 pmol, 50 nl), using stereotaxic coordinates and criteria as described above. Average number of penetrations per side for identifying the CVLM was 1.2 ± 0.16 (range = 1–2) and 2 ± 0.39 (range = 1–4) for the RVLM. A triple barrel pipette filled with GABA (10 mM), BIC (4 mM) + CGP35348 (4 mM), and strychnine hydrochloride (S; 3mM) in separate barrels was used for bilateral microinjections into the CVLM and RVLM. A single barrel micropipette filled with l-glutamate (l-glu, 10 mM) and mounted on a separate micromanipulator was used for microinjections into the NTS. MAP, HR and the raw RSNA signal were acquired and stored on a computer hard-drive using Power Lab Data Acquisition System (AD Instruments). RSNA was analyzed as impulses/second (i/sec) similar to Protocols 1&2, except that Power Lab software, rather than a Winston Electronics ratemeter, was used to determine rate. In addition, the raw nerve activity signal was rectified and integrated using Power Lab software (root mean square function, RMS; time constant = 28 msec). Output signals were smoothed using Power Lab software. Any signal remaining 30 minutes to one hour after the animal's death, was considered electrical noise and subtracted from experimental nerve activity values. Nerve activity was expressed as percent of the baseline value preceding recorded experimental values.

4.4 Experimental Protocols

Protocol 1—Following right NTS lesion, reflex inhibition of RSNA due to elevated arterial pressure (phenylephrine, PE, bolus, 5 μ g/Kg, i.v.) was measured before beginning the experimental protocol (baroreflex test). Changes in MAP, HR and RSNA due to microinjection of GABA (500 pmol, 50 nl) in the left CVLM then were obtained before (control) and after blockade of GABA_A receptors in the left RVLM with BIC (400 pmol, 100 nl; $n = 12$). As determined by preliminary experiments, supplementation of BIC (200 pmol, 50 nl) at 5 minute intervals maintained blockade of GABA_A receptors in the RVLM throughout the protocol. Blockade of GABA_A receptors in the RVLM was verified by elimination of reflex decreases in RSNA due to elevations of MAP with PE ($n = 7$). In five animals, MAP was greater than 175 mmHg following BIC in the RVLM and therefore reflex responses to further elevations of MAP were not tested. Responses in these five rats were similar to group responses and therefore data from these animals were included in analysis. In seven of the rats from Protocol 1, the effects of l-glu (500 pmol, 50 nl) in the CVLM were also determined before and after GABA_A receptor blockade in the RVLM. Following CVLM microinjection of short-acting amino acid transmitters (GABA or l-glu), all parameters recovered to levels near control values within two minutes and at least ten minutes were allowed before new control measurements were obtained and another portion of the protocol was performed.

Protocol 2—Similar to Protocol 1, the right NTS was lesioned and reflex inhibition of RSNA due to elevated arterial pressure (phenylephrine, PE, bolus, 5µg/Kg, i.v.) was measured before and after GABA_A and GABA_B receptor blockade in the left RVLM (baroreflex test). MAP, HR and RSNA responses to microinjection of GABA in the left CVLM were determined before and after microinjection of the combined GABA antagonists (400 pmol BIC + 400 pmol CGP-35348; 100 nl) in the left RVLM (n= 8). GABA receptor blockade in the left RVLM was maintained with supplements of the combined antagonist solution (50 nl) at 5 minute intervals and blockade of the baroreflex response to increased MAP was verified. Additionally, GABA receptor blockade was verified by testing the response to microinjection of GABA in the left RVLM.

In 17 of the 20 animals used in Protocols 1 and 2, completeness of the right NTS lesion was further verified at the end of the experiment by testing for elimination of the baroreflex response following either inhibition of left CVLM with muscimol (n=15) or left NTS lesion (n=2).

Protocol 3—In a third group of rats, the effects of bilateral blockade of GABA_A + GABA_B receptors, and additional glycine receptor blockade in the RVLM were evaluated. Before ventral medulla microinjections, cardiopulmonary reflex responses to phenylbiguanide (PBG, 5µ/kg, i.v.) and responses to activation of central baroreflex pathways with l-glu in the NTS (500 pmol, 50 nl) were determined. MAP, HR and RSNA responses to microinjection of GABA in the left and right CVLM were determined before RVLM injections, after bilateral microinjection of the combined GABA antagonists (400 pmol BIC + 400 pmol CGP-35348; 100 nl), and after the addition of glycine receptor blockade with strychnine hydrochloride (S; 300 pmol, 100 nl) in the RVLM. Bilateral RVLM injections were made by repositioning the pipette to predetermined coordinates and left and right side injections were accomplished within 60–90 seconds. At the end of the protocol, responses to PBG and NTS l-glu were again tested.

At the end of the experiments in all three protocols the RVLM and CVLM were marked by injection of Chicago Sky Blue dye (1%, 25–50 nl). Standard histological techniques were used to fix and section the brainstem (30–60µm sections, neutral red stain). The injection site was estimated by comparison with a rat brain atlas (33).

4.5 Data Analysis and Statistical Comparisons

Within each protocol data subsets were analyzed using a one way ANOVA for repeated measures followed by Student Newman Keuls post hoc test. Paired t-tests were used to compare baroreflex gain before and after blockade of the left RVLM or contralateral blockade of baroreflex pathways in right NTS lesioned rats (Protocols 1 & 2) and to compare responses to PBG and NTS l-glu microinjection before and after the protocol (Protocol 3). Paired t-tests (Protocols 1 & 2) or one way ANOVA (Protocol 3) were used to compare changes in MAP, HR and RSNA due to GABA in the CVLM before and after antagonist injections into the RVLM. Unpaired t-tests were used to compare responses between Protocols 1 and 2. $P \leq 0.05$ was considered significant. Values are expressed as mean $\pm$ SEM.

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Figure 1.

Inhibition of left CVLM before and during GABA_A receptor blockade in left RVLM in a representative animal (Protocol 1). In a right NTS lesioned rat, microinjection of GABA in the left CVLM resulted in reversible increases in heart rate (HR), arterial pressure (AP), mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) (Control, left panel). Bicuculline in the left RVLM increased HR, AP, MAP, and RSNA. Subsequent blockade of the left CVLM with GABA resulted in further small increases in all parameters (RVLM Bic, right panel).

Figure 2.

Protocol 1: Mean data of responses to inhibition of left CVLM during GABA_A receptor blockade in left RVLM. Grouped bars indicate data sets compared by one-way ANOVA. In right NTS lesioned rats, inhibition of the left CVLM with GABA (G₁) reversibly increased mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA). Blockade of GABA_A receptors in the left RVLM with bicuculline increased MAP and RSNA (B). During GABA_A receptor blockade in the left RVLM, inhibition of the left CVLM resulted in further increases in MAP and RSNA (G₂). C = control; G = GABA in CVLM; R = recovery; B = bicuculline in RVLM. * different from C; # different from B alone; $P \leq 0.05$.

Figure 3.

Activation of left CVLM with l-glutamate (l-glu) before (Control) and during GABA_A receptor blockade in left RVLM (RVLM Bic) in a representative animal. In a right NTS lesioned rat, microinjection of l-glu in the left CVLM resulted in a reversible decrease in arterial pressure (AP), mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) (Control, left panel). Bicuculline in the left RVLM increased AP, MAP, and RSNA. In the presence of GABA_A receptor blockade in left RVLM, activation of the left CVLM with l-glu increased AP, MAP, and RSNA (RVLM Bic, right panel).

Figure 4.

Blockade of GABA_A and GABA_B receptors in left RVLM (Protocol 2). In right NTS lesioned rats, microinjection of GABA (G, 50 pmol, 50 nl) into the left RVLM decreased MAP and RSNA. Following combined GABA_A and GABA_B receptor blockade (GX = Bicuculline + CGP35348), responses to GABA in the left RVLM were eliminated (GX + G). * different from change with G alone. $P \leq 0.05$.

Figure 5.

Protocol 2: Inhibition of left CVLM during blockade of GABA_A and GABA_B receptors in left RVLM. Grouped bars indicate data sets compared by one-way ANOVA. In right NTS lesioned rats, inhibition of the left CVLM with GABA reversibly increased MAP and RSNA (G₁). Following blockade of GABA_A and GABA_B receptors in the left RVLM, MAP stabilized (GX) at a level significantly above control (C₂). During combined GABA_A and GABA_B receptor blockade in the left RVLM, inhibition of the left CVLM resulted in further increases in MAP, and RSNA was significantly elevated above control (G₂). C = control; R = recovery; * different from C ; # different from GX; $P \leq 0.05$.

Figure 6.

Effects of bilateral GABA_A, GABA_B and glycine receptor blockade in the RVLM on responses to GABA in the left CVLM in a representative rat (Protocol 3). Microinjection of GABA in the left CVLM resulted in reversible increases in arterial pressure (AP), mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) (Control, left panel). Bilateral Bicuculline + CGP35348 in the RVLM (GX) increased AP, MAP and RSNA. Subsequent blockade of the left CVLM with GABA resulted in further increases in these parameters (RVLM GX, middle panel). In the presence of bilateral GABA_A, GABA_B, and glycine receptor blockade in the RVLM(GX + S), responses to microinjection of GABA in the left CVLM were blocked.

Figure 7.

Protocol 3: Unilateral inhibition of CVLM during bilateral blockade of GABA_A, GABA_B, and glycine receptors in RVLM. Responses to GABA in the left and right CVLM were not different and therefore values were averaged for each rat and are presented as mean responses to unilateral CVLM injections. RSNA was analyzed as both i/sec and RMS and expressed as % of control levels. Grouped bars indicate data sets compared by one-way ANOVA. Unilateral inhibition of the CVLM with GABA reversibly increased MAP and RSNA (G₁). Following bilateral blockade of GABA_A and GABA_B receptors in the RVLM, MAP and RSNA stabilized (GX₁) at levels significantly above control (C₂). During combined GABA_A and GABA_B receptor blockade in the RVLM, unilateral inhibition of the CVLM resulted in further increases in MAP and the further increase in RSNA reached statistical significance when analyzed as RMS (G₂). Bilateral injection of strychnine in the RVLM did not significantly increase arterial pressure or RSNA (S), but blocked responses to unilateral CVLM GABA (G₃). C = control; R = recovery; * different from C ; # different from GX; P ≤ 0.05.

Figure 8.

Protocols 1 & 2 microinjection sites. Left = RVLM: The site functionally defined as the RVLM was marked with Chicago sky blue dye (50 nl) and estimated histologically (closed circles). Right = CVLM: The site functionally defined as the CVLM was marked with Chicago sky blue dye (50 nl) and estimated histologically (closed squares). Brainstem sections were adapted from *The Rat Brain in Stereotaxic Coordinates* (Paxinos & Watson, 1998)

Figure 9.

Photomicrographs demonstrating bilateral injection sites for the RVLM (left) and CVLM (right) in one rat (Protocol 3). Top: Blue regions and black arrow (RVLM) indicate Chicago sky blue dye in unprocessed sections. Bottom: Same sections following histological staining with neutral red. 4V = 4th ventricle; py = pyramidal tract; AP = area postrema; CC = central canal; IO = inferior olive.

Table 1

Effect of right NTS lesion on baseline values

Protocol	Before NTS Lesion	After (15 min) NTS lesion
Protocol 1 (n=12)		
MAP (mm Hg)	123 ± 4.2	119 ± 2.9
RSNA (% Control)	100	100 ± 6.3
Protocol 2 (n=8)		
MAP (mm Hg)	125 ± 5.8	116 ± 4.7
RSNA (% Control)	100	143 ± 29.4

Electrolytic lesion of the right NTS had no effect of either baseline mean arterial pressure (MAP) or renal sympathetic nerve activity (RSNA). Values = mean ± SEM.

Table 2

Verification of right NTS lesion

Protocol	After right NTS lesion		After right NTS lesion + left BX Disruption	
	Baseline MAP (mm Hg)	Δ RSNA/ Δ MAP (%C/mm Hg)	Baseline MAP (mm Hg)	Δ RSNA/ Δ MAP (%C/mm Hg)
Protocol 1 (n=11)	123 $\pm$ 4.6	-2.1 $\pm$ 0.19	162 $\pm$ 3.8 [*]	-0.03 $\pm$ 0.04 [*]
Protocol 2 (n=6)	126 $\pm$ 7.6	-1.7 $\pm$ 0.11	152 $\pm$ 5.5 [*]	-0.07 $\pm$ 0.06 [*]

In 17 of 20 animals, response to baroreflex activation was obtained after lesion of only the right NTS and again following the additional disruption of the baroreflex pathway on the left side (left CVLM (n=15) or left NTS lesion (n=2)). Baroreflex (BX) responses were reduced by 99% (Protocol 1) or 96% (Protocol 2) following removal of the left side of the baroreflex, indicating that the initial right NTS lesion was complete. Values = mean $\pm$ SEM;

* Elevated MAP and reduced baroreflex gain following left BX disruption ($P \leq 0.05$).

Table 3

CVLM & RVLM responses to GABA

Group	Baseline MAP (mm Hg)	CVLM Δ MAP (mmHg)	Δ RSNA (%C)	Baseline MAP (mm Hg)	RVLM Δ MAP (mmHg)	Δ RSNA (%C)
Protocol 1 (n=12)	114 $\pm$ 3.5	28.9 $\pm$ 3.4 *	49.01 $\pm$ 9.4 *	135 $\pm$ 3.8	-20.5 $\pm$ 3.5 *	-25 $\pm$ 5.1 *
Protocol 2 (n=8)	117 $\pm$ 6.1	24.8 $\pm$ 3.9 *	40.5 $\pm$ 4.9 *	142 $\pm$ 5.0	-17.4 $\pm$ 2.9 *	-24.6 $\pm$ 2.5 *
Protocol 3 (n=6)	108.1 $\pm$ 5.3	43.6 $\pm$ 3.4 *	99.5 $\pm$ 26.5 *	100 $\pm$ 8.1	-13.0 $\pm$ 0.9 *	-16.2 $\pm$ 2.6 *

Responses to microinjection of GABA at the beginning of the each protocol are shown. Significant increases in both arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) were observed following injection of GABA in the CVLM. Microinjection of GABA in the RVLM produced significant decreases in MAP and RSNA. Rats in Protocols 1 and 2 had prior right NTS lesions and responses shown are to left side injections. The averaged data for left and right side injections is shown for Protocol 3. Values = mean $\pm$ SEM;

* Significant change, $P \leq 0.05$.

Table 4

Baroreflex response before and after GABA receptor blockade in the RVLM

	Δ MAP (mm Hg)	Control Δ RSNA (% C)	Δ RSNA/ Δ MAP	Δ MAP (mm Hg)	RVLM GABA Receptor Blockade Δ RSNA (% C)	Δ RSNA/ Δ MAP
Protocol 1 (n=7)	37.5 $\pm$ 5	-61 $\pm$ 8.1	-1.8 $\pm$ 0.25	34 $\pm$ 4.1	-1.3 $\pm$ 1.2 *	-0.04 $\pm$ 0.04 *
Protocol 2 (n=8)	40 $\pm$ 4.3	-55 $\pm$ 10.5	-1.4 $\pm$ 0.2	30 $\pm$ 2.2	-5 $\pm$ 2.4 *	-0.15 $\pm$.08 *

In right NTS lesioned rats, phenylephrine (PE, 5 μ g/kg, i.v.) increased MAP similarly before and after left RVLM Bicuculline (BIC) (Protocol 1) or BIC + CGP35348 (Protocol 2). Decreases in RSNA were reduced by left RVLM GABA receptor blockade. Estimated baroreflex gain (Δ RSNA/ Δ MAP) was decreased by 98% with RVLM GABA_A receptor block and by 89% with GABA_A + GABA_B receptor blockade. Values = mean $\pm$ SEM;

* $P \leq 0.05$ compared to corresponding control value.

Table 5

Responses to activation of cardiopulmonary reflexes (i.v. phenylbiguanide, PBG) and central baroreflex pathways (microinjection of NTS l-glu) (Protocol 3)

	Δ MAP (mm Hg)	Control Δ RSNA (% i/sec)	Δ RSNA (% RMS)	Δ MAP (mm Hg)	After RVLM GX+S Δ RSNA (% i/sec)	Δ RSNA (% RMS)
PBG	-33.9 ± 7.2	-59.3 ± 10.1	-82.8 ± 4.4	$-2.7 \pm 4.0^*$	$4.6 \pm 5.3^*$	$5.1 \pm 4.2^*$
NTS l-glu	-35 ± 4.0	-51.9 ± 10.9	-74.4 ± 6.9	$0.4 \pm 3.9^*$	$6.5 \pm 3.3^*$	$-6.9 \pm 3.5^*$

At the beginning of the protocol (Control), both PBG and unilateral microinjection of l-glu into the NTS resulted in significant decreases in MAP and RSNA. Bilateral blockade of GABA_A, GABA_B, and glycine receptors (GX + S) in the RVLM eliminated reflex responses. Values = mean $\pm$ SEM;

* $P \leq 0.05$ compared to corresponding control value.