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Hypertension. 2000;35:413-417

doi: 10.1161/01.HYP.35.1.413

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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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Excitatory Amino Acids in the Rostral Ventrolateral Medulla Support Blood Pressure in Spontaneously Hypertensive Rats

Satoru Ito, Kazutoshi Komatsu, Kazuyoshi Tsukamoto, Alan F. Sved

Abstract—Injection of the excitatory amino acid (EAA) antagonist kynurenic acid (KYN) into the rostral ventrolateral medulla (RVLM) of anesthetized rats has no effect on arterial pressure. However, we recently reported that after inhibition of the caudal ventrolateral medulla, injection of KYN into the RVLM decreased arterial pressure to the same level as produced by complete inhibition of the RVLM. We have suggested that these results reflect tonically active EAA-mediated inputs to the RVLM producing both direct excitation of RVLM vasomotor neurons and indirect inhibition of these neurons. On the basis of this model, we hypothesize that the balance between these EAA-driven direct excitatory and indirect inhibitory influences on the RVLM may be altered in models of experimental hypertension. To begin to test this hypothesis, the effects of injecting KYN into the RVLM of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) were compared. In chloralose-anesthetized WKY, bilateral injection of KYN into the RVLM did not alter arterial pressure, whereas similar injections in SHR reduced mean arterial pressure by ≈ 40 mm Hg. After inhibition of the caudal ventrolateral medulla, which similarly increased arterial pressure in both strains, injection of KYN into the RVLM reduced mean arterial pressure to the same level as produced by autonomic blockade. These results suggest that the balance of excitatory and inhibitory influences on RVLM vasomotor neurons driven by tonically active EAA-mediated inputs to the RVLM is disrupted in SHR and may contribute to the hypertension in SHR. (*Hypertension*. 2000;35[part 2]:413-417.)

Key Words: brain ■ hypertension, experimental ■ glutamate ■ neurotransmitter ■ amino acid ■ rats, inbred SHR

The rostral ventrolateral medulla (RVLM) is critical to the tonic and reflexive regulation of arterial blood pressure (AP). Ongoing activity of RVLM-spinal neurons is responsible for the generation of baseline sympathetic vasomotor tone, and acute inhibition of this region causes a marked decrease in AP, similar to that seen in response to cervical spinal cord transection or inhibition of the autonomic nervous system.^{1,2} Previous studies demonstrated that local injection into the RVLM of excitatory amino acid (EAA) receptor antagonists has no effect on resting AP,³⁻⁵ and this has been interpreted as indicating that RVLM neuronal activity at rest is not produced by EAA-mediated inputs to the RVLM. However, we have recently reported that although injection of kynurenic acid (KYN) into the RVLM of anesthetized rats had no effect on baseline AP, after inhibition of the caudal ventrolateral medulla (CVLM), a region that tonically inhibits the RVLM, injection of KYN into the RVLM reduced AP to the same extent as total autonomic blockade.⁶ On the basis of these results, it was proposed that tonically active EAA-mediated inputs to the RVLM excite RVLM vasomotor neurons and also indirectly inhibit these neurons through

excitation of an inhibitory input from the CVLM.⁶ Thus, we suggested that blockade of EAA receptors in the RVLM results in little change in AP because it simultaneously withdraws excitation and inhibition of RVLM vasomotor neurons. Furthermore, the lack of change in AP in response to injection of KYN into the RVLM implies that the direct excitatory influences of EAA and the indirect inhibitory influences of EAA are normally in perfect balance at resting AP in anesthetized rats.

The balance between the tonically active excitatory and inhibitory inputs to RVLM vasomotor neurons affects resting AP. If the excitatory input to these neurons was high relative to the inhibitory input, AP should be elevated. Indeed, there are suggestions in the literature that such an imbalance is responsible for the elevated AP in spontaneously hypertensive rats (SHR). For example, Smith and Barron^{7,8} reported that inhibition of the CVLM or blockade of its GABAergic inhibitory input to the RVLM by injection of a γ -aminobutyric acid (GABA) receptor antagonist into the RVLM caused a smaller increase in AP in SHR than in normotensive Wistar-Kyoto rats (WKY). These results sug-

Received September 14, 1999; first decision October 11, 1999; revision accepted October 22, 1999.

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gest that AP in SHR may be elevated because of a disinhibition of the RVLM, leading to a relative excess of excitatory drive of RVLM vasomotor neurons. Therefore, in SHR, in contrast to normotensive rats, blockade of EAA receptors in the RVLM may produce a decrease in AP. The present studies were conducted to test this hypothesis.

Methods

Adult male SHR and WKY (Charles River, Japan), 16 to 20 weeks of age and weighing between 300 and 450 g, were used in these experiments. Animals were housed in groups of 2 to 3 in hanging wire mesh cages in temperature-controlled rooms with a fixed 12-hour light/dark cycle for at least 2 weeks before experiments. Food (MF, Oriental Yeast Co) and tap water were available ad libitum.

For measuring AP, mean AP (MAP), and heart rate (HR) during injections of substances into the ventrolateral medulla, rats were prepared as previously described.⁶ Briefly, rats were initially anesthetized with halothane, and cannulas were inserted into a femoral artery and a femoral vein. The trachea was cannulated, and the rat was connected to a ventilator. The rat was placed in a stereotaxic frame with the incisor bar set 11 mm below the interaural point, and the dorsal surface of the medulla was exposed. After completion of all surgery, the rat was injected with α -chloralose (60 mg/kg IV), and the halothane was terminated. Additional chloralose (20 mg/kg IV) was administered hourly. Rats were injected with tubocurarine (0.5 mg/kg, supplemented hourly with 0.2 mg/kg) and ventilated with 100% oxygen for the remainder of the experiment. Injections of solutions into the brain stem were made with single-barrel glass micropipettes; all injections were in a volume of 100 nL artificial cerebrospinal fluid vehicle administered during several seconds with a PicoPump (WPI). KYN was initially dissolved in 100 mmol/L sodium bicarbonate and then diluted in artificial cerebrospinal fluid. Bilateral injections were made 1 side at a time, with \approx 30 seconds separating the 2 injections. Coordinates for injections into the CVLM were 1.2 mm rostral to the caudal tip of the area postrema, 2.0 mm lateral to the midline, and 2.4 mm below the dorsal surface of the brain stem. Coordinates for injections into the RVLM were, with the pipette tip angled 20° rostrally, 1.8 mm rostral to the caudal tip of the area postrema, 1.8 mm lateral to the midline, and 2.9 mm below the dorsal surface of the brain stem. In each case, test injections of 1 nmol of glutamate in 100 nL were made into the CVLM and/or RVLM on each side of the brain stem to verify that the coordinates were within the functional depressor and pressor regions of the CVLM and RVLM, respectively. If an injection did not alter MAP by \geq 25 mm Hg in the appropriate direction, then the coordinates were changed by 0.3 mm in 1 direction at a time until an acceptable physiological response was obtained. In most rats of each strain, the coordinates did not need to be adjusted, and there was no significant difference in the average coordinates between SHR and WKY.

At the conclusion of the experiment, \approx 20 nL of 1% Fast Green dye was injected into the RVLM for histological verification of the center of the microinjection site. All RVLM injection sites were located in the rostral medulla, just ventral to the compact portion of nucleus ambiguus, similar to the injection sites that we have published previously.⁹ Injections into the CVLM were located in the ventrolateral medulla at the approximate rostral-caudal level of the obex.

In other SHR and WKY, the effects of injection of the ganglionic blocking drug hexamethonium (20 mg/kg IV) on MAP and HR were determined.

KYN, muscimol, and hexamethonium were purchased from Sigma Chemical Co; all other drugs and chemicals were obtained from standard commercial suppliers.

Data are expressed as mean \pm SEM. Responses in SHR and WKY were compared by *t* test or ANOVA with the use of Statistica software.

Effect of Injection of Glutamate into RVLM and CVLM on AP and HR in SHR and WKY

	MAP, mm Hg		HR, bpm	
	Baseline	Change	Baseline	Change
RVLM				
WKY	106 \pm 2	+42 \pm 5	336 \pm 6	-43 \pm 21
SHR	161 \pm 4*	+44 \pm 3	370 \pm 8	-76 \pm 18
CVLM				
WKY	110 \pm 4	-35 \pm 3	325 \pm 10	-56 \pm 9
SHR	162 \pm 6*	-69 \pm 4*	362 \pm 11	-111 \pm 18

Values represent baseline MAP and HR and the maximal change in MAP and HR produced by unilateral injection of 1 nmol glutamate into the RVLM or CVLM. These values are from the groups of SHR (n=9) and WKY (n=7) that later received injections of muscimol into the CVLM and KYN into the RVLM.

*Significant difference ($P<0.01$) from WKY.

Results

Before we tested the effects of injecting KYN into the RVLM, functional depressor sites in the CVLM and/or functional pressor sites in the RVLM were identified by the local injection of glutamate (1 nmol in 100 nL). Injection of glutamate into the RVLM increased MAP in both strains of rats (Table), with the magnitude of the increase being similar between groups. The increase in MAP was associated with variable decreases in HR, which also did not differ between SHR and WKY. Injection of glutamate into the CVLM decreased MAP in all rats, although the response was substantially larger in SHR (Table). The decrease in MAP was associated with a decrease in HR that did not differ between the rat strains.

Bilateral injection of KYN into the RVLM of chloralose-anesthetized WKY had no effect on baseline AP or HR (Figures 1 and 2), as previously noted in Sprague-Dawley rats.⁶ In contrast, bilateral injection of 2.7 nmol KYN into the RVLM of SHR produced a decrease in AP of \approx 40 mm Hg, with no significant change in HR (Figures 1 and 2). The decrease in AP occurred rapidly and lasted for \approx 20 minutes. Even unilateral injection of KYN into the RVLM of SHR resulted in a significant decrease in AP (-18 ± 3 mm Hg; n=6). Bilateral injection of a smaller dose of KYN (1.35 nmol) into the RVLM of SHR still evoked a decrease in AP, although the response was significantly smaller than with the larger dose of KYN (Figure 2).

Bilateral injection of muscimol into the CVLM in WKY increased AP by 82 ± 3 mm Hg (n=7) (Figures 3 and 4). When AP had reached its peak, \approx 3 minutes after injection of muscimol, injection of KYN into the RVLM markedly decreased AP to \approx 65 mm Hg (Figures 3 and 4), a level similar to that produced by autonomic blockade with intravenous injection of hexamethonium (Figure 4). Similarly, in SHR, injection of muscimol into the CVLM increased AP by 85 ± 3 mm Hg (n=9), and the subsequent injection of KYN into RVLM reduced AP to the same extent as autonomic blockade (Figures 3 and 4).

Discussion

The major finding of these studies is that injection of KYN into the RVLM of SHR decreases AP. This observation is in

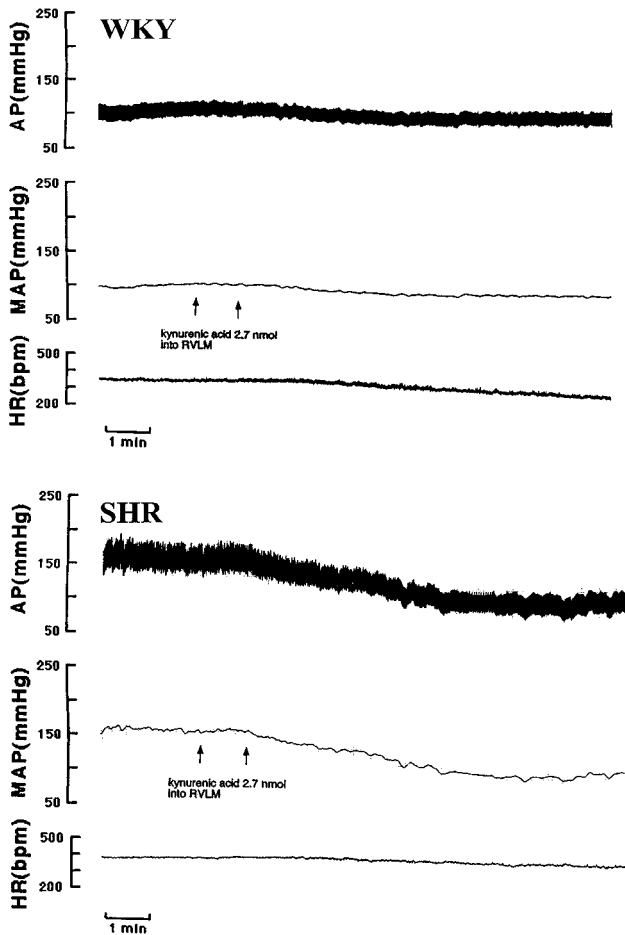


Figure 1. Representative polygraph recordings from an SHR (bottom) and a WKY (top) in which 2.7 nmol of KYN was injected bilaterally into the RVLM. The arrows indicate the points at which KYN was injected into the RVLM, first on the left side and then on the right side. These recordings are typical of the data included in Figure 2.

marked contrast to the lack of change in AP in response to injection of KYN into the RVLM of WKY, similar to observations reported previously for normotensive Sprague-Dawley rats.^{3,4,6} The dose of KYN used in these experiments (2.7 nmol) was demonstrated previously to effectively and selectively block ionotropic EAA receptors in the RVLM.³ Despite not changing resting MAP in normotensive rats, this dose of KYN does block evoked responses that are thought to be mediated via EAA transmission in the RVLM, such as the pressor response evoked by electric stimulation of the sciatic nerve.³ Thus, the most straightforward interpretation of these data is that blockade of ionotropic EAA receptors in the RVLM decreases AP in SHR but not in normotensive control rats.

After inhibition of the CVLM, with consequent withdrawal of prominent inhibitory control of RVLM sympathoexcitatory neurons,^{1,2} injection of KYN into the RVLM reduced AP to the same extent as total autonomic blockade in both SHR and WKY. We have previously reported a similar response in Sprague-Dawley rats.⁶ Indeed, it was largely this observation in Sprague-Dawley rats that prompted us to propose a model in which the balance between tonic EAA-mediated excitation

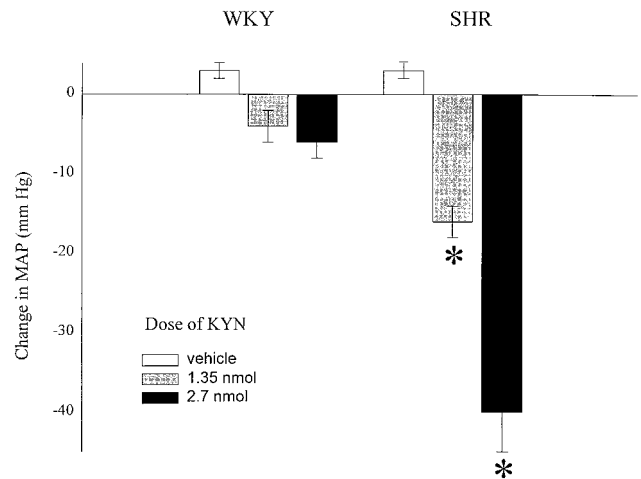


Figure 2. Effect of KYN injected into the RVLM on MAP in SHR and WKY. Groups of chloralose-anesthetized SHR and WKY ($n=5$ to 7 per group) received bilateral injections of KYN or vehicle into the RVLM. Values shown are maximal change in MAP from baseline. Baseline MAP in rats receiving injections of 2.7 nmol KYN were 102 ± 4 mm Hg for WKY and 155 ± 2 mm Hg for SHR. Bilateral injection of 2.7 nmol KYN into the RVLM decreased HR by 16 ± 6 bpm from a baseline of 340 ± 14 in WKY and by 35 ± 4 bpm from a baseline of 360 ± 11 in SHR. *Significant difference ($P < 0.01$) from response in WKY and from effect of vehicle injection in SHR.

of RVLM vasomotor neurons and indirect inhibition of RVLM vasomotor neurons driven by an EAA-mediated input to the RVLM plays a critical role in determining resting AP.⁶ This model predicts that chronically elevated sympathetic vasomotor tone would result from either increased EAA-mediated excitation of RVLM vasomotor neurons or from decreased inhibition of RVLM vasomotor neurons. In either case, this shift of balance toward excitation of RVLM vasomotor neurons would be reversed by injection of an EAA antagonist. Thus, in hypertensive rats injection of KYN into the RVLM should reduce AP. The present observations made in SHR are totally consistent with this model, as is a previous report that KYN injected into the RVLM decreased AP in rats made hypertensive by constriction of the renal artery (Goldblatt hypertensive rats).¹⁰

In Sprague-Dawley rats, after bilateral injection of muscimol into the CVLM, injection of KYN into the RVLM reduces AP to the same level as produced by total autonomic blockade.⁶ Because under these conditions KYN reduced AP not just to baseline levels before inhibition of the CVLM but to much lower levels, we suggested that the CVLM must normally provide a non-EAA-mediated excitatory input to RVLM vasomotor neurons⁶ in addition to the well-known inhibitory input.^{1,2} In the present study, similar data were obtained in SHR and WKY; after injection of muscimol into the CVLM, injection of KYN into the RVLM reduced AP to the same extent as total autonomic blockade in each strain. These data are consistent with the model presented above in which the balance between direct EAA-mediated excitation of RVLM vasomotor neurons and indirect EAA-mediated inhibition of these neurons is shifted toward excitation in SHR.

This model of RVLM function in SHR would predict that the activity of RVLM sympathoexcitatory neurons is elevated

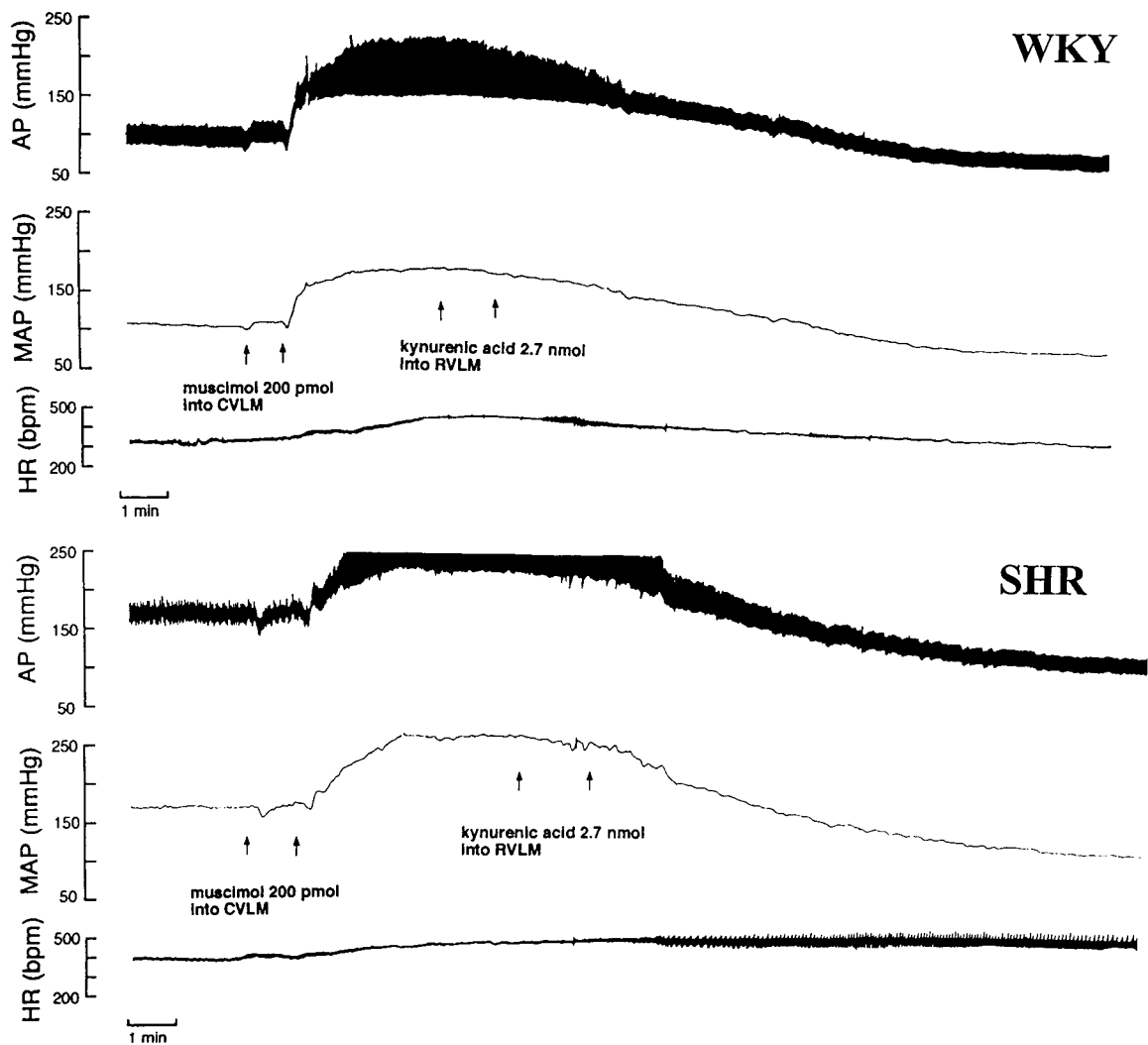


Figure 3. Representative polygraph recordings from an SHR (bottom) and a WKY (top) in which 2.7 nmol of KYN was injected bilaterally into the RVLM after bilateral injection of muscimol into the RVLM. These recordings are typical of the data included in Figure 4.

under baseline conditions in anesthetized rats. Indeed, this has been observed in some studies¹¹ but not others¹²; these different results might relate to the anesthetic agent used or the specific population of cells that were recorded.

On the basis of these data, it appears that the balance between excitatory and inhibitory inputs to RVLM vasomotor neurons that are influenced by EAA-mediated transmission in the RVLM are shifted toward excitatory influences in the SHR. However, the issue of whether this results from excitation or disinhibition is not completely clear. Several studies have reported differences in cardiovascular responses to pharmacological alteration of either the CVLM or RVLM in SHR compared with WKY, although in most cases the studies are difficult to interpret and the literature is often contradictory. For example, many studies, including the present one, report that the decrease in AP caused by injection of EAA agonists into the CVLM is larger in SHR than in WKY,^{7,8,13,14} and some authors^{7,8,13} have suggested that this reflects a decrease in basal inhibitory drive of the RVLM from the CVLM. However, because baseline AP is different

between these 2 strains of rats, it is difficult to interpret these data; AP might fall more in SHR because it starts out higher. If, instead, these data are compared on the basis of the decrease in MAP as a percentage of the decrease in MAP produced by autonomic blockade, then the values become quite similar ($\approx 85\%$ to 90%).

Some investigators have reported that disruption of the inhibitory input to the RVLM from the CVLM, either by injection of the GABA antagonist bicuculline into the RVLM or by injection of the neuroinhibitory agent tetrodotoxin into the CVLM, produces a much larger increase in AP in WKY than in SHR.^{7,8,13} This observation has been taken as evidence that the inhibitory input to the RVLM from the CVLM is much less active in SHR than it is in WKY. Again, these data are difficult to interpret because of the difference in baseline AP values. Furthermore, Muratani et al¹⁴ observed that bicuculline injected into the RVLM produced a similar large increase in MAP in both SHR and WKY. The present data showing similar large increases in MAP after injection of muscimol into the CVLM are consistent with the results of

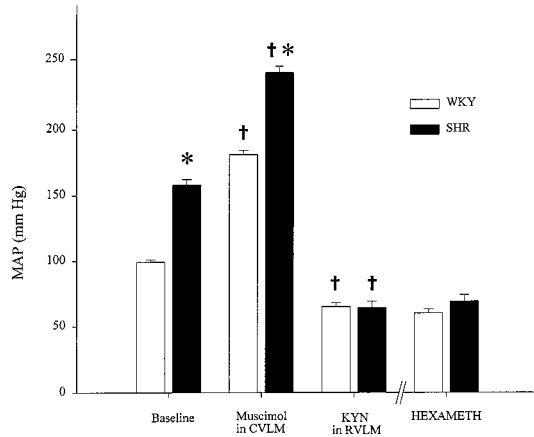


Figure 4. Effect of KYN injected into the RVLM after injection of muscimol into the CVLM on MAP in SHR and WKY. Chloralose-anesthetized SHR ($n=9$) and WKY ($n=7$) received bilateral injections of muscimol (200 pmol) into the CVLM. Approximately 3 minutes later, KYN was injected bilaterally into the RVLM. Values shown represent the MAP attained after each treatment. MAP values after intravenous injection of hexamethonium (20 mg/kg) in a separate group of SHR and WKY ($n=5$) are shown for comparison. *Significant difference ($P<0.01$) from response in WKY. †Significant change ($P<0.01$) in MAP from the previous treatment.

Muratani et al.¹⁴ The reason for the large difference between these 2 groups of data is not clear at present. However, large doses of glutamate or other EAA injected into the RVLM produce increases in MAP of similar magnitude in SHR and WKY,^{7,8,14,15} suggesting that the tonic inhibitory control of RVLM sympathoexcitatory neurons is not grossly abnormal since we have previously noted that disruption of this inhibitory input enhances the pressor response to injection of glutamate into the RVLM.⁶

In summary, the present data show that blockade of EAA receptors in the RVLM of SHR, but not WKY, decreases AP. This qualitative difference in the central neural control of AP between SHR and WKY is consistent with the hypothesis that there is a relative increase in EAA-mediated drive of RVLM vasomotor neurons in this model of hypertension.

Acknowledgments

This study was supported by grants from Nihon University School of Medicine and the National Institutes of Health (HL-55687 to Dr Sved).

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