Role of Endogenous Angiotensin II on Glutamatergic Actions in the Rostral Ventrolateral Medulla in Goldblatt Hypertensive Rats

Taís Helena F. Carvalho, Cássia T. Bergamaschi, Oswaldo U. Lopes, Ruy R. Campos

Abstract—In this study, we investigated the cardiovascular responses mediated by rostral ventrolateral medulla neurons (RVL) in the Goldblatt hypertension model (2K-1C) treated or not treated with captopril. The actions of glutamate into the RVLM were tested, injecting glutamate (0.1 mol/L, 100 nL) and its antagonist kynurenic acid (0.02 mol/L, 100 nL). Glycine (0.5 mol/L, 100 nL) was also microinjected. Experiments were performed in male Wistar rats (weight, 250 to 300 g); 5 groups were studied: (1) 2K-1C nontreated (H, n=6); (2) 2K-1C treated with captopril, 10 mg/kg per day (Ht10, n=10); (3) 2K-1C treated with captopril, 50 mg/kg per day (Ht50, n=7); (4) control normotensive rats (N, n=7); and (5) normotensive rats treated with captopril, 50 mg/kg per day (Nt50, n=8). All experiments in 2K-1C were performed 6 weeks after renal surgery; captopril treatment lasted for the last 2 weeks. In urethane-anesthetized rats (1.2 g/kg IV), bilateral microinjection of glycine into the RVLM caused a depressor response; there was no difference between groups in relation to the change of variation (N: 54±2; H: 46±12; Ht10: 50±3, and Ht50: 42±7 mm Hg). Only in the H group, kynurenic acid microinjection into the RVLM caused a depressor response (H: 158±8 to 132±8 mm Hg). Glutamate response was larger in hypertensive than in normotensive rats (N: 38±2.6 and H: 55±6); no difference was observed between hypertensive groups. The data suggest that glutamate acts tonically to drive the RVLM in 2K-1C rats, and this action is modulated by endogenous angiotensin II. The increase in the glutamate actions within the RVLM may contribute to the pathogenesis of renovascular hypertension. ([Hypertension. 2003;42(part 2):707-712.])

Key Words: brain ■ angiotensin ■ captopril ■ amino acid ■ sympathetic nervous system

The rostral ventrolateral medulla (RVL) plays a central role in neural control of the circulation.1 Ongoing activity of premotor RVLM neurons is responsible for the generation of sympathetic vasomotor tone. Inhibition of RVLM neurons causes a decrease in arterial blood pressure (BP) similar to that seen after total inhibition of the autonomic nervous system.1,2

Previous studies demonstrated that microinjection into the RVLM of excitatory amino acid (EAA) receptor antagonist has no effect on basal level of BP.3 This fact has been interpreted as suggesting that the ongoing RVLM activity is not dependent of EAA inputs to RVLM. However, we showed previously that in the Goldblatt 2-kidney, 1-clip (2K1C) model, injection of kynurenic acid (Kyn), a broad-spectrum EAA receptor antagonist, into the RVLM reduced BP to the same extent as autonomic blockade.4 A similar result was shown in SHR.5 On the basis of these results, it was proposed that tonic actions of glutamatergic inputs to the RVLM are involved in the pathogenesis of experimental hypertension.

Although there is general agreement on the importance of the renin-angiotensin system for increased BP in the early phase of renovascular hypertension, several findings suggest that the hypertensive response to angiotensin II (Ang II) is neurogenically mediated.6 In this case, a possible mechanism responsible for the glutamatergic hyperactivity in the RVLM in renovascular hypertension is the increase of circulating Ang II, which, for example, indirectly can drive glutamatergic inputs to RVLM through the area postrema.7 The purpose of the present study was to test the hypothesis that Ang II may be an important mediator determining an increase in glutamatergic drive to the RVLM in the Goldblatt model. In the first series of experiments, we tested in renovascular hypertensive rats the actions of glutamate into the RVLM, injecting glutamate and its antagonist kynurenic acid. Glycine was also microinjected. In the second series, we examined the effects of the same drugs in Goldblatt animals previously treated with captopril.

Methods

Experiments were performed in male Wistar rats (n=38; weight, 250 to 300 g). All animal procedures were conducted according to the Guidelines for Ethical Care of Experimental Animals and were approved by the Institutional Ethics Committee of the Federal University of São Paulo School of Medicine.
Five experimental groups were studied: (1) 2-kidney, 1-clip hypertensive rats (H/n=6); (2) 2-kidney, 1-clip hypertensive rats treated with captopril, 10 mg/kg orally (HnT50/n=10); (3) 2-kidney, 1-clip hypertensive rats treated with captopril, 50 mg/kg orally (H50/n=7); (4) control normotensive rats (N/n=7); and (5) normotensive rats treated with captopril, 50 mg/kg orally (NT50/n=8).

To obtain hypertensive animals, the left renal artery was partially obstructed with a silver clip of 0.2-mm width (Goldblatt hypertension, 2-kidney, 1-clip model); the animals were submitted to the experimental procedures after 6 weeks of the surgery. For antihypertensive treatment, the animals received captopril orally for 14 days (10 mg/kg per day or 50 mg/kg per day). The treatment started in the 4th week after renal clipping surgery and lasted for the last 2 weeks. In the 4th week, BP was measured by a tail-cuff method to verify the evolution of hypertension, and only animals with BP ≥140 mm Hg were submitted to captopril treatment. The efficacy of captopril after 2 weeks of treatment was tested by intravenous AI (from 0.01 to 1 pg/kg IV) administration. A partial blockade in the pressor response to AI was observed in rats treated with 10 mg/kg captopril, and only when 50 mg/kg was used, a total blockade was observed.

After the end of 6 weeks, rats were anesthetized with ketamine and xylazine (40 and 20 mg/kg IP, respectively) and instrumented with femoral venous and arterial catheters constructed from PE-50 and PE-10 tubing filled with heparinized saline for drug injection and arterial pressure recording, respectively. On the experimental day, rats were anesthetized with urethane (1.2 g/kg IV), given a tracheotomy, and ventilated artificially with oxygen-enriched air. Rectal temperature was maintained at 37°C by means of a servo-controlled electric blanket. An adequate depth of anesthesia was monitored by observing BP, heart rate, and the corneal and paw-pinch reflexes; if needed, an additional administration of anesthetic was performed (5% of initial dose).

Animals were placed in a stereotaxic frame, and an occipital cranietomy was performed to expose the dorsal surface of the brain stem and cerebellum. The dura mater was opened and retracted, exposing the Obex, whose vertex was taken as a landmark for the stereotaxic coordinates. The RVLM was located 3 mm rostral to the Obex, 1.7 to 1.8 mm lateral to midline, and 3 mm deep to the dorsal medullary surface.

Bilateral microinjections were made with the use of glass micropipettes with a tip diameter of 10 to 20 μm, connected to a Hamilton syringe, and the volume injected was measured by determining the displacement of the meniscus in the pipette with respect to a horizontal grid viewed through an operating microscope. Microinjections consisted of glutamate (0.5 mol/L), glycine (0.1 mol/L), and kynurenic acid (0.02 mol/L) in a volume of 100 nL; the pH of the solutions was adjusted to 7.4. At the end of the experiment, 100 nL of Evans blue dye was injected into the site. The animals were killed by an overdose of urethane, and the brain stem was then removed and fixed by immersion for at least 72 hours in 4% paraformaldehyde solution. Transverse 40-μm frozen sections were cut and mounted. Figure 1 is a representative histological coronal view showing the dye distribution within the RVLM region. When the dye was bilaterally deposited ventral to nucleus ambiguous and lateral to inferior olivary nucleus, this was considered a positive histology.

All values are expressed as mean±SEM. The significance of change in mean arterial pressure (MAP) or heart rate (HR) after microinjections was determined within each group by the Student paired t test. Differences between groups were assessed by 1-way ANOVA followed by the Student-Newman-Keuls method. A value of P<0.05 was considered significant.

**Results**

**Effects of Glutamate Microinjection Into the RVLM of Hypertensive rats, Treated or Not Treated With Captopril**

Six weeks after renal surgery, the rats that were submitted to partial left renal artery obstruction had a significant increase in MAP (182±6.7 mm Hg, n=6) compared with normotensive rats (103±4 mm Hg, n=7). There was no difference between groups in relation to heart rate or body weight.

Before we tested the effects of different microinjections into the RVLM, the functional pressor sites were identified by previous bilateral glutamate microinjection into the region (0.1 mol/L, 100 nL).

Bilateral microinjections of glutamate into the RVLM of nontreated hypertensive rats caused a significant increase in MAP (from 182±7 to 237±8 mm Hg, P<0.05, n=6), as shown in Figure 2B, accompanied by a no significant change in HR (from 454±13 to 436±17 bpm).

In normotensive rats, a significant increase in MAP was also observed (from 103±4 to 141±2 mm Hg, P<0.05, n=7), as shown in Figure 2A, without significant changes in HR (from 368±14 to 404±20 bpm). The response to glutamate in hypertensive rats was greater than in normotensive rats, with a significant difference between groups when compared with the absolute change of variation (H: 55±6, N: 38±2 mm Hg, P<0.05).

The treatment with captopril for 2 weeks reduced BP in the hypertensive animals in a dose-dependent manner. Treated animals (captopril, 10 mg/kg per day) showed MAP levels of 150±5.6 mm Hg. A further reduction was achieved in ani-

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**Figure 1.** Typical microinjection site in the RVLM evaluated by 100 nL of Evans blue diffusion (left); schematic representation on right. CST indicates corticospinal tract; IO, inferior olivary nucleus; NA, nucleus ambiguous; NTS, nucleus of the tractus solitarii; and STN, spinal trigeminal nucleus.

**Figure 2.** Values of MAP reached after bilateral microinjection of glutamate (0.1 mol/L, 100 nL) into the RVLM in normotensive group (N, n=7), hypertensive group (H, n=6), hypertensive group treated with 10 mg/kg per day captopril (H10, n=10), and hypertensive group treated with 50 mg/kg per day captopril (H50, n=7). *P<0.05 in relation to basal level; +P<0.05 in relation to basal level of H group.
mals treated with 50 mg/kg per day (119±7 mm Hg). Both groups showed a significant reduction in MAP in relation to nontreated animals (182±6.7 mm Hg).

In the hypertensive treated group (captopril, 10 mg/kg), glutamate microinjection into the RVLM produced a significant pressor response (from 150±6 to 198±9 mm Hg (n=10), as shown in Figure 2C. There was no significant change in HR (from 398±14 to 396±14 bpm). The response evoked in this group (absolute change of variation) did not differ in relation to the response observed in the nontreated hypertensive group (Ht10: 47±6 mm Hg, H: 55±6 mm Hg).

In the hypertensive group treated with 50 mg/kg of captopril, glutamate microinjection into the RVLM also produced a significant increase in MAP (from 119±7 to 162±12 mm Hg, n=7), as shown in Figure 2D, with no significant change in HR (from 427±19 to 401±35 bpm). Also, in this group, the absolute change of variation in MAP in response to glutamate was not significant different in relation to the nontreated group (Ht50: 43±6, H: 55±6 mm Hg). In addition, the response to glutamate did not differ between the two hypertensive treated groups (Ht10: 47.4±6, Ht50: 43±6 mm Hg).

Effects of Glycine Microinjection Into RVLM of Hypertensive Rats Treated or Not Treated With Captopril
Glycine microinjection (0.5 mol/L, 100 nL) into the RVLM provoked a significant decrease in BP in all groups.

In 6 hypertensive nontreated animals, glycine microinjection into the RVLM significantly decreased MAP (from 168±10 to 122±9 mm Hg, P<0.05), as shown in Figure 3B. There was no significant change in HR (from 436±25 to 440±24 bpm).

In normotensive rats, however, glycine microinjection produced a fall in MAP comparable to that seen after acute spinal cord transection (from 108±4 to 54±2 mm Hg; P<0.05, n=6), as shown in Figure 3A, without significant changes in HR (from 380±22 to 377±14 bpm). However, when compared with the absolute change of variation between groups in response to glycine, no significant difference was observed (H: 46±12 mm Hg, N: 54±2 mm Hg).

When glycine was injected into the RVLM of hypertensive captopril-treated (10 mg/kg per day) animals, MAP fell from 139±8 to 89±7 mm Hg (P<0.05, n=7), as shown in Figure 3C, followed by a nonsignificant change in HR (from 398±16 to 378±19 bpm). Despite the fact that MAP reached 89 mm Hg after glycine in the treated group, a significantly different level in relation to the nontreated hypertensive group (125±8.2 mm Hg), the change of variation was not different between groups (Ht10: 50±3.7, H: 46±12 mm Hg).

A similar result was observed in animals treated with 50 mg/kg per day captopril. Glycine microinjection into the RVLM evoked a significant decrease in MAP (from 133±10 to 91±8 mm Hg, n=5), as shown in Figure 3D, with no significant change in HR (from 417±26 to 369±32 bpm). The change of variation in MAP in response to glycine microinjection in this group was not different in relation to nontreated hypertensive rats (Ht50=42±7, H=46±12 mm Hg). In addition, there was no difference in the glycine response between the two treated hypertensive groups (Ht10=50±3.7, Ht50=42±7 mm Hg) and between treated groups against the normotensive group (N=54±2 mm Hg).

Effects of Kynurenic Acid Microinjection Into RVLM of Hypertensive Rats Treated or Not Treated With Captopril
Bilateral microinjections of kynurenic acid (0.02 mol/L, 100 nL)—a broad-spectrum ionotropic glutamate receptor antagonist—into the RVLM of hypertensive nontreated animals induced a significant reduction in MAP (from 158±8 to 132±8, n=5) without significant changes in HR (from 435±14 to 436±15 bpm), as shown in Figure 4B. However, in normotensive rats, the same microinjection did not promote significant changes in MAP (from 110±4 to 122±8 mm Hg, n=5) or HR (from 379±13 to 407±18 bpm), as demonstrated in Figure 4A.

When kynurenic acid was microinjected into the RVLM of captopril-treated (10 mg/kg per day) hypertensive rats, the depressor response previously seen was null. There were no significant changes in MAP (from 144±9 to 145±10 mm Hg, n=7) or HR (from 405±15 to 405±12 bpm) after kynurenic acid microinjection. The same result was observed in the hypertensive animals treated with the
higher dose of captopril (50 mg/kg per day); kynurenic acid microinjection did not promote significant changes in MAP (from 127±14 to 136±13 mm Hg, n=6) or HR (from 399±11 to 406±13 bpm). Figures 4C and 4D, shows the MAP responses to kynurenic acid into the RVLM in the low- and high-dose captopril-treated groups, respectively. Figure 5 shows typical BP and HR responses to kynurenic acid microinjection into the RVLM in a hypertensive rat and in a hypertensive rat treated with captopril (50 mg/kg per day).

Effects of Glutamate, Glycine, or Kynurenic Acid Microinjection Into RVLM of Normotensive Rats Treated With Captopril

In a control series of experiments, to test the effects of chronic captopril treatment on RVLM responses of normotensive rats, glutamate, glycine, and kynurenic acid were microinjected into the RVLM in normotensive rats treated for 2 weeks with captopril (50 mg/kg per day, n=8). The magnitude of changes in MAP evoked by glutamate (from 105±4 to 152±8 mm Hg), kynurenic acid (from 97±5 to 113±11 mm Hg), or glycine (from 104±4 to 63±6 mm Hg) into the RVLM were not different when compared with those observed in normotensive nontreated rats.

Discussion

The RVLM is considered a major center for regulation of sympathetic and cardiovascular activities. An increase in RVLM activity may be an important mechanism in the pathogenesis of experimental hypertension. A previous study from our laboratory demonstrated that in renovascular hypertensive rats, the glutamatergic synapses within the RVLM are tonically active, since injection of the broad-spectrum ionotropic glutamate receptor antagonist kynurenic acid (Kyn) into the RVLM causes a long-lasting fall in blood pressure.4 The present study demonstrated that the depressor response to microinjection of Kyn into the RVLM in renovascular hypertensive rats is dependent of endogenous Ang II, since chronic treatment with captopril blocked the response to Kyn. However, exogenous glutamate actions induced by glutamate microinjection into the region elicited a similar response in BP in renovascular hypertensive rats, treated or not treated with captopril.

Apparently, the inefficacy of Kyn in decreasing BP in the captopril-treated groups could be attributed to the fact that these animals had a lower BP than nontreated rats. However, this possibility appears to be remote, because in the treated group (10 mg/kg per day), the level of BP was ≈150 mm Hg. A similar level was observed in hypertensive nontreated animals, in which Kyn microinjection into the RVLM was able to decrease BP. The fact that Kyn was unable to decrease BP in the hypertensive low-dose treated group, in which there was only a partial blockade of circulating Ang II, suggests that angiotensin may play a modulatory role in the RVLM acting on synaptic transmission and that this action is in part dependent of the endogenous level of the peptide.

The role of tonic glutamatergic synapses within the RVLM in the maintenance of hypertension was demonstrated not only in the Goldblatt model4 but also in spontaneous hypertensive rats (SHR) and Dahl salt-sensitive rats.5,8 These results suggest that an increase in the glutamatergic inputs to RVLM may be a common mechanism causing an increase in sympathetic drive in experimental hypertension, at least in these models.

In normotensive rats, in contrast to the ability of Kyn to block the excitation of RVLM in response to activation of certain central pathways, Kyn administration into the RVLM of anesthetized or conscious rats did not change basal level of arterial pressure.3,9–11 Based on these observations, it has been proposed that in normotensive rats, the RVLM is not tonically driven by EAA inputs. However, other alternative have been proposed. Ito and Sved12 have suggested that Kyn into the RVLM blocks excitatory inputs to tonically active local inhibitory interneurons as well as excitatory inputs to RVLM, causing no change in the basal sympathetic tone and BP. Another possible explanation is that under conditions in which all tonic glutamatergic synaptic inputs have been blocked, an intrinsic pacemaker-like mechanism may become operational.13 In this case, the contribution of tonic glutama-
ergic actions on RVLM activity may be greater than estimated only by the effects of blockade of ionotropic glutamatergic receptors within the RVLM.

The question that arises is which are the sources of the tonic glutamatergic inputs to the RVLM in hypertensive rats? One possible candidate is the hypothalamic paraventricular nucleus (PVN). The excitatory effects on vasomotor RVLM neurons of PVN stimulation was prevented by simultaneous microiontophoretic application of Kyn into the RVLM; however, the antagonist did not change the ongoing activity of RVLM neurons. Another possible candidate is the area postrema. This hypothesis is supported by studies showing that lesion of the area postrema prevents the chronic hypertension induced by intravenous infusion of Ang II. In addition, microinjection of Ang II into the area postrema increases arterial pressure acutely. Finally, the pontine reticular formation (PRF) is also a candidate, since it is a source of glutamatergic inputs to the RVLM. Inhibition of the PRF in normotensive anesthetized rats causes a decrease in BP that appears to be mediated by Kyn-sensitive input to the RVLM. Therefore, Ang II can act through these pathways, causing an increase in the glutamatergic drive to the RVLM and an increase in sympathetic vasomotor tone and BP. There are several different possible mechanisms by which Ang II can reach these pathways. The RVLM contains fibers that are immunoreactive for Ang II; however, the origin of such fibers is unknown. Possible candidates are the nucleus of the tractus solitarii and hypothalamus, both of which project to the RVLM and contain angiotensin-immunoreactive neuronal cell bodies. Alternatively, it is possible that Ang II acts in the brain from a nonneuronal origin; the peptide may be formed from angiotensinogen in the extracellular fluid and then reaches its receptors through diffusion.

The importance of EAA drive to the RVLM in hypertension was also reported in acute experiments, in a model of mechanical compression of the RVLM that causes an increase in BP and sympathetic outflow in rats. The pressor response to compression was inhibited after microinjection of Kyn into the RVLM. Taken together, the results suggest that an increase in glutamatergic activity within the RVLM is probably a major mechanism to increase arterial pressure, acutely or chronically, in different experimental models of hypertension.

A substantial body of evidence suggests that elevation in the sympathetic drive participates in the pathogenesis of hypertension. Sympathectomy and antihypertensive agents that work by blocking sympathetic transmission can interrupt the development and maintenance of experimental hypertension. In the present series of experiments, inhibition of the RVLM by glycine caused a substantial fall in arterial pressure in hypertensive rats. The MAP level reached by glycine into the RVLM in captopril-treated rats was significantly lower in relation to the nontreated hypertensive group, suggesting that both sympathetic drive and the Ang II system work together to maintain the high BP in the Goldblatt model. This result is in agreement with a previous study in which the level of BP reached after glycine into the RVLM was amplified by intravenous captopril administration. In addition, either glycine into the RVLM or acute sympathetic ganglionic blockade caused the same effect on BP, indicating that all sympathetic vasomotor tone is dependent of RVLM activity in the Goldblatt (2K-1C) model.

A most interesting feature of the study, in which glutamate was microinjected into the RVLM in hypertensive rats treated or not treated with captopril, was that the exogenous action of EAA was not changed by treatment. A similar result was obtained by Tsuchihashi et al in another model of experimental hypertension. They showed that SHR treated with enalapril had the same response to glutamate into the RVLM as nontreated hypertensive rats.

In summary, the present data show that blockade of EAA receptors in the RVLM of renovascular hypertensive rats but not in normotensive animals decreases BP. This mechanism is dependent of endogenous level of Ang II. On the other hand, the exogenous action of EAA was not affected by captopril treatment. The difference in the role of RVLM in the control of arterial pressure in the Goldblatt model and normotensive rats supports the hypothesis that there is an increase in the glutamatergic drive to RVLM premotor neurons mediated by Ang II in Goldblatt (2K-1C) hypertension.

Perspectives

The RVLM contains neurons involved in the tonic and reflex regulation of cardiovascular system and sympathetic drive. The present data suggest that EAA act tonically to drive RVLM neurons only in hypertensive rats, and the tonic action is modulated by endogenous Ang II. The increase in the EAA actions within the RVLM may contribute to the pathogenesis of renovascular hypertension.

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