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# Angiotensin-(1-7) antagonist, A-779, microinjection into the caudal ventrolateral medulla of renovascular hypertensive rats restores baroreflex bradycardia

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#### ABSTRACT

In the present study we evaluated the effect of caudal ventrolateral medulla (CVLM) microinjection of the main angiotensin (Ang) peptides, Ang II and Ang-(1-7), and their selective antagonists on baseline arterial pressure (AP) and on baroreceptor-mediated bradycardia in renovascular hypertensive rats (2K1C). Microinjection of Ang II and Ang-(1-7) into the CVLM of 2K1C rats produced similar decrease in AP as observed in Sham rats. In both Sham and 2K1C, the hypotensive effect of Ang II and Ang-(1-7) at the CVLM was blocked, for up to 30 min, by previous CVLM microinjection of the Ang II AT<sub>1</sub> receptor antagonist, Losartan, and Ang-(1-7) Mas antagonist, A-779, respectively. As expected, the baroreflex bradycardia was lower in 2K1C in comparison to Sham rats. CVLM microinjection of A-779 improved the sensitivity of baroreflex bradycardia in either 2K1C or Sham rats. These results suggest that Ang-(1-7) at the CVLM may contribute to the low sensitivity of the baroreflex control of heart rate in renovascular hypertensive rats.

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#### 1. Introduction

The neural control of arterial pressure (AP) is mainly based on changes in sympathetic and parasympathetic outflow which, in turn, influences cardiac output and the total peripheral resistance. Increases in peripheral sympathetic activity and alterations in arterial baroreflex function appear to contribute to the pathogenesis of hypertension. The baroreceptor reflex acts basically through independent pathways that control sympathetic and parasympathetic outflow. The primary medullary circuitry of the baroreflex includes inhibition of sympathoexcitatory neurons of the rostral ventrolateral medulla (RVLM) by gabaergic neurons of the caudal

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ventrolateral medulla (CVLM) [1,25,51,54]. In addition, CVLM neurons appear to play a role in the regulation of sympathetic vasomotor tone and AP through gabaergic neurons that are tonically activated by inputs independent of arterial baroreceptors or the nucleus tractus solitarii (NTS), providing a gabaergicmediated inhibition of the presympathetic RVLM neurons that is autonomous of baroreceptor inputs [45]. The CVLM barodependent and baro-independent inhibitory mechanisms of presympathetic RVLM neurons may play an important role in determining the long-term level of sympathetic vasomotor tone and AP. Inhibition or destruction of the CVLM produces severe acute hypertension, consistent with blockade of baroreceptor reflexes and withdrawal of inhibition of RVLM sympathoexcitatory neurons. Further, other studies have shown that in spontaneously hypertensive rats (SHR), CVLM produced inhibition of the RVLM neurons seems to be attenuated, which might explain the increased sympathetic drive to the periphery that is observed in this model of hypertension [34,48].

CVLM is under the modulatory influence of the reninangiotensin system (RAS). Angiotensin-(1-7) is now recognized as an important mediator of the RAS in different tissues, especially in the heart and vasculature [9,18,44]. In the brain, Ang-(1-7) and its selective receptor Mas [43] were described to be present in

Abbreviations: 2K1C, Goldblatt renovascular hypertension 2-kidney, 1-clip; Ang II, angiotensin II; Ang-(1-7), angiotensin-(1-7); AP, arterial pressure; AT<sub>1</sub>, angiotensin II type 1 receptor; AT<sub>2</sub>, angiotensin II type 2 receptor; CVLM, caudal ventrolateral medulla; HR, heart rate; MAP, mean arterial pressure; ms, millisecond; NA, nucleus ambiguus; NTS, nucleus tractus solitarii; PI, pulse interval; RAS, renin–angiotensin system; RVLM, rostral ventrolateral medulla; Sham, normotensive rats.

different areas related to cardiovascular control [8]. Previous studies have shown that microinjection of Ang II and Ang-(1-7) into areas of the ventrolateral medulla (VLM) induces significant changes in AP [2–5,7,20,29,47]. These peptides also influence baroreflex control at the VLM in normotensive animals [6,34,42,47]. In addition, the use of selective angiotensin antagonists has produced evidence for the role of Ang II and Ang-(1-7) endogenous effects at the CVLM in rabbits and rats [16,20,30,53] and in hypertensive or in normotensive rats [7,32,34].

It is well established that in renovascular hypertensive rats the baroreflex bradycardia is attenuated [13,26]. However, the pathophysiological relevance of the CVLM, a key area for the medullary baroreceptor reflex pathway, for the genesis of hypertension has only been addressed in SHR [39,51]. It has been reported that in these animals, the CVLM exerts a lower tonic sympato-inhibition on the RVLM neurons [34,48,51]. On the other hand, there is considerable evidence on the overactivity of the brain RAS in the Goldblatt renovascular hypertension 2-kidney-1clip (2K1C) model. It is possible that the participation of brain RAS occurs in either the early phase of 2K1C hypertension, when plasma Ang II is elevated, or in the late phase, when plasma Ang II levels are back to normal [27,28].

The objective of the present study was to test the hypothesis that alterations of RAS components in the CVLM would contribute to tonic and reflex AP changes observed after hypertension. Therefore, we investigate the cardiovascular effects of CVLM microinjection of Ang II and Ang-(1-7) and the effect of selective  $AT_1$  and Mas receptor antagonists on baroreceptor-mediated bradycardia in renovascular, 2K1C, hypertensive rats.

#### 2. Materials and methods

#### 2.1. Drugs

Ang II, Losartan, Ang-(1-7) and A-779 were purchased from the Bachem Chemical Company (St. Louis, MO, USA) or Peninsula Laboratories (Belmont, CA, USA). Phenylephrine was obtained from Sigma Chemical Company (St. Louis, MO, USA).

Ang II (2 mg/ml), Losartan (2 mg/ml), Ang-(1-7) (2 mg/ml) and A-779 (2 mg/ml) were dissolved in sterile isotonic saline (NaCl, 0.9%), aliquoted (10  $\mu$ l) and stored at -20 °C. Phenylephrine was dissolved in sterile saline at 1 mg/ml concentration and 100  $\mu$ l aliquots were stored at -20 °C. At the moment of the experiment, the aliquots were diluted to the desired concentrations and used only once.

#### 2.2. Animals

Experiments were performed in male Fisher rats (n = 49) (ENUT, UFOP, Brazil). All animal procedures were according to the Guidelines for Ethical Care of Experimental Animals and were approved by the Institutional Ethics Committee of the Federal University of Ouro Preto, MG, Brazil.

#### 2.3. Induction of renal hypertension

Renovascular hypertension was induced according to a previously described method [41]. Briefly, the rats (150–200 g) were anesthetized with the mixture of ketamine and xylazine (50 mg/kg and 10 mg/kg, *i.p.*, respectively) and a silver clip (0.20 mm ID) was placed around the left renal artery through a midline incision (Goldblatt renovascular hypertension, 2-kidney, 1-clip model, 2K1C). Other rats were submitted to similar procedures but without the renal artery clip placement (Sham group or normotensive rats). Experiment was carried out after 30 days after the surgery (2K1C or Sham).

#### 2.4. Arterial pressure measurements

Pulsatile arterial pressure was monitored by a Gould pressure transducer (PM-1000, CWE) coupled to a blood pressure signal amplifier (UIM100A, Powerlab System). Mean arterial pressure (MAP) and heart rate (HR) were determined by the arterial pressure wave. All variables were continuously recorded and saved to a PowerLab digital acquisition system (Powerlab 4/20, ADInstruments) with an 800 Hz sampling rate.

#### 2.5. CVLM microinjections

After 30 days of surgery, 2K1C and Sham rats (260–300 g) were anesthetized with urethane (1.2 g/Kg, *i.p.*) and underwent a tracheostomy. Next, a polyethylene catheter was inserted into the abdominal aorta, through the femoral artery for arterial pressure measurement, and another catheter was inserted into the inferior cava vein, through the femoral vein for drugs injection. The animals were placed in a stereotaxic frame (David Kopf instruments, CA) as previously described by Rodrigues et al. [41]

Unilateral microinjections of Ang II, Ang-(1-7), angiotensin receptor antagonists (Losartan or A-779) or sterile saline (vehicle–NaCl 0.9%) in a volume of 100 nl were made over a 20–30 s period into the CVLM (0.7 mm anterior, 1.8 mm lateral to the obex, and just above pia mater in the ventral surface), as previously described by Alzamora et al. [5,6]. The dose of the peptides and antagonists was based in previous studies [5,6,20,41].

#### 2.6. Evaluation of the sensitivity of the baroreflex bradycardia

The baroreflex bradycadia was tested in different groups of animals, before and 5–10 min after CVLM microinjections of the



**Fig. 1.** Averaged changes in mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min) produced by the microinjection of saline (100 nl, n = 13-14), Ang-(1-7)(40 pmol, n = 8) and Ang II (40 pmol, n = 5-6) into the CVLM of Sham and 2K1C rats. \*p < 0.05 in comparison to saline (ANOVA followed by Dunnett's test).

angiotensin antagonists,  $AT_1$  Ang II receptor antagonist, Losartan (n = 5-6) or Ang-(1-7) antagonist, A-779 (n = 5-8). Baroreflex bradycardia was determined as previously described by Rodrigues et al. [41].

#### 2.7. Experimental procedures

The arterial pressure and HR of urethane anesthetized Sham (n = 26) and 2K1C (n = 23) rats were continuously recorded. After a 10 min stabilization period, the baroreflex bradycardia was evaluated. The micropipette was then positioned into the CVLM, Ang II (40 pmol) or saline (NaCl, 0.9%–100 nl) in a random order in one group, or, Ang-(1-7) (40 pmol) or saline (NaCl, 0.9%–100 nl) in a random order in another group were microinjected. Fifteen min after the microinjection of the peptides, Losartan (86 pmol), A-779 (50 pmol) or saline (100 nl) was microinjected and after 5, 15 and 30 min Ang II or Ang-(1-7) was repeated in each group, respectively. The baroreflex test was evaluated in different groups of animals before and 5–10 min after CVLM microinjection of the angiotensin antagonists, Losartan or A-779.

#### 2.8. Histological verification of injection sites

The histological verification of injection sites was done accordingly as previously described by Alzamora et al. [5,6] and by Rodrigues et al. [41].

#### 2.9. Statistical analysis

The results are expressed as means  $\pm$  SEM. Comparisons between two groups were assessed by Student's *t*-test. Comparisons of three or more groups were made by one-way ANOVA followed by Newman–Keuls or Dunnett's test, as appropriate. Statistical analyses were performed with the software *Graphpad Prism* (version 4.00). The criterion for statistical significance was set at p < 0.05.

#### 3. Results

#### 3.1. Baseline values of MAP and HR in 2K1C and in Sham rats

The baseline values of MAP of anesthetized 2K1C (148  $\pm$  4 mmHg, *n* = 23) were significantly higher than the baseline values of MAP of anesthetized Sham rats (106  $\pm$  2 mmHg, *n* = 26). The baseline values of HR were not significantly different between the Sham group (367  $\pm$  10 beats/min, *n* = 26) and the 2K1C group (379  $\pm$  9 beats/min, *n* = 23). The relative weight (kidney weight/body weight) of the left kidney (clipped) in 2K1C rats was significantly smaller (0.068  $\pm$  0.003, *n* = 23) in comparison to the relative weight of the left kidney (non-clipped) in Sham (0.086  $\pm$  0.002, *n* = 26). In contrast, the relative weight of the right kidney (non-clipped) in 2K1C rats was significantly greater (0.104  $\pm$  0.002, *n* = 23) in comparison to the weight of the right kidney (non-clipped) in 2K1C rats was significantly greater (0.104  $\pm$  0.002, *n* = 23) in comparison to the weight of the right kidney (non-clipped) in 2K1C rats was significantly greater (0.104  $\pm$  0.002, *n* = 23) in comparison to the weight of the right kidney (non-clipped) in 2K1C rats was significantly greater (0.104  $\pm$  0.002, *n* = 23) in comparison to the weight of the right kidney (non-clipped) in 2K1C rats was significantly greater (0.104  $\pm$  0.002, *n* = 23) in comparison to the weight of the right kidney (non-clipped) in 2K1C rats was significantly greater (0.104  $\pm$  0.002, *n* = 23) in comparison to the weight of the right kidney (non-clipped) in 2K1C rats was significantly greater (0.104  $\pm$  0.002, *n* = 23) in comparison to the weight of the right kidney (non-clipped) in 2K1C rats was significantly greater (0.104  $\pm$  0.002, *n* = 23) in comparison to the weight of the right kidney (non-clipped) in 2K1C rats was significantly greater (0.104  $\pm$  0.002, *n* = 23) in comparison to the weight of the right kidney (non-clipped) in 2K1C rats was significantly greater (0.104  $\pm$  0.002, *n* = 23) in comparison to the weight of the right kidney (non-clipped) in 2K1C rats was significanter (0.104  $\pm$  0.005  $\pm$  0.001, *n* = 26).



**Fig. 2.** Mean arterial pressure changes ( $\Delta$ MAP, mmHg) produced by CVLM microinjections of Ang II (40 pmol; panels A and C) or Ang-(1-7) (40 pmol; panels B and D) before and 5, 15 and 30 min after microinjection of Losartan (86 pmol, panel A) or A-779 (50 pmol, panel B), or saline (100 nl, panels C and D) in normotensive (Sham, *n* = 4–8) or hypertensive (2K1C, *n* = 4–8) rats. \**p* < 0.05 in comparison to before (ANOVA followed by Dunnett's test).



**Fig. 3.** Baroreflex mediated changes in heart rate (HR, bpm) expressed as pulse interval (ms) in response to changes in mean arterial pressure (MAP, mmHg) produced by graded doses of phenylephrine in normotensive (Sham, *n* = 7–8) or hypertensive (2K1C, *n* = 7–8) rats. In A, pulsatile (PAP, mmHg), mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min) recordings illustrating the typical effect produced by injection of phenylephrine (2.5 μg, i.v.) before and after CVLM microinjection of A-779 (50 pmol) in 2K1C rats. Panels B and C present the averaged baroreflex bradycardia indexes obtained before and after CVLM microinjection of A-779. Panels D and E present the averaged baroreflex bradycardia indexes obtained. Lines represent the least-square regression equation fitted through



**Fig. 4.** Image of a histological section of the medulla (A) illustrating disruption of the tissue caused by microinjection and diagrammatic representations of frontal sections of the medulla (13.30–13.80 mm caudal to the bregma); (B) showing the center of the microinjection into the CVLM (shaded area). The diagrams are from the atlas of Paxinos and Watson [37]. AP: area postrema; Amb: nucleus ambiguus; LR: lateral reticular nucleus; py: pyramidal tract; sol: nucleus of solitary tract; XII: hypoglossal nucleus.

#### 3.2. Effect of CVLM microinjection of Ang II, Losartan, Ang-(1-7) or A-779 in 2K1C and Sham rats

Unilateral microinjection of Ang-(1-7) into the CVLM produced significant decrease in MAP in 2K1C ( $-14 \pm 2 \text{ mmHg}$ ,  $n = 8 \text{ vs.} -2 \pm 0.6 \text{ mmHg}$ , n = 13; saline; Fig. 1), which was similar to that produced by the microinjection of Ang-(1-7) into the CVLM in Sham rats ( $-14 \pm 2 \text{ mmHg}$ ,  $n = 8 \text{ vs.} -2 \pm 0.4 \text{ mmHg}$ , saline; n = 14; Fig. 1). In addition, Ang II also induced significant fall in blood pressure in 2K1C ( $-10 \pm 0.9 \text{ mmHg}$ ,  $n = 5 \text{ vs.} -2 \pm 0.6 \text{ mmHg}$ , n = 13; saline; Fig. 1) similar to that observed in Sham rats ( $-11 \pm 1.3 \text{ mmHg}$ ,  $n = 6 \text{ vs.} -2 \pm 0.4 \text{ mmHg}$ , saline; n = 14; Fig. 1). The hypotensive effect induced by angiotensin peptides was not accompanied by significant changes in HR in both group of animals, Sham or 2K1C (Fig. 1).

The microinjection of the AT<sub>1</sub> Ang II antagonist, Losartan and saline produced similar hypotensive effects in 2K1C and Sham groups. In contrast, the Ang-(1-7) antagonist, A-779, produced a significant decrease in AP as compared to saline in 2K1C rats  $(-12 \pm 4 \text{ mmHg}, n = 6 \text{ vs. } 2 \pm 0.6 \text{ mmHg}, n = 13)$ . In Sham rats, the effect of A-779 was similar to saline  $(-5 \pm 2 \text{ mmHg}, n = 8 \text{ vs.} -2 \pm 0.4 \text{ mmHg}$ , saline; n = 14). HR did not significantly change in any group after the microinjection of these antagonists (data not shown).

As shown in Fig. 2, the microinjection of Losartan into the CVLM abolished Ang II hypotensive effect up to 30 min in Sham and 2K1C rats (Fig. 2A). Similarly, the microinjection of A-779 into the CVLM abolished the Ang-(1-7) hypotensive effect up to 30 min in Sham and 2K1C rats (Fig. 2B). In addition, the Ang II and Ang-(1-7) depressor effects were not different 5, 15 and 30 min after the

CVLM microinjection of saline, respectively (Fig. 2C and D). No significant changes in HR were observed in all groups at any of the different time points (data not shown).

## 3.3. Evaluation of the sensitivity of baroreflex bradycardia in 2K1C and in Sham rats

As expected, the reflex bradycardia of 2K1C rats  $(0.19 \pm 0.03 \text{ ms/mmHg}, n = 15)$  was significantly lower in comparison to that of Sham rats  $(0.42 \pm 0.02 \text{ ms/mmHg}, n = 15)$ . Fig. 3A illustrates the baroreflex bradycardia induced before and after the CVLM microinjection of A-779. As shown in Fig. 3B and C, the Ang-(1-7) antagonist significantly increased baroreflex bradycardia in 2K1C rats  $(0.44 \pm 0.05 \text{ ms/mmHg}, n = 7 \text{ vs}. 0.24 \pm 0.03 \text{ ms/mmHg}, n = 7$ ; before A-779). In contrast, the microinjection of Losartan into the CVLM did not change the baroreflex bradycardia of the 2K1C rats  $(0.2 \pm 0.04 \text{ ms/mmHg}, n = 7 \text{ vs}. 0.14 \pm 0.02 \text{ ms/mmHg}, n = 8$ , before Losartan; Fig. 3D and E). In Sham rats, baroreflex bradycardia was not significantly altered by the CVLM microinjection of A-779 (0.35  $\pm$  0.04 ms/mmHg,  $n = 7 \text{ vs}. 0.40 \pm 0.02 \text{ ms/mmHg}, n = 7$ , before A-779; Fig. 3B and C) or Losartan  $(0.52 \pm 0.06 \text{ ms/mmHg}, n = 8 \text{ vs}. 0.43 \pm 0.03 \text{ ms/mmHg}, n = 8$ , before Losartan; Fig. 3D and E).

#### 3.4. Histological examination

Fig. 4A shows an image of a histological section of the medulla illustrating the site of the microinjection in one representative animal. In Fig. 4B, diagrams of frontal sections of the medulla from the atlas of Paxinos and Watson [37] show the location (shaded

the average points (panels B and D) and the slope of these regression lines, baroreflex sensitivity index (ms/mmHg) are presented in panels C and E. Arrows mark the injections of phenylephrine (i.v., in bolus). \*p < 0.05 in comparison to the respective Sham group (ANOVA followed by Newman–Keuls test). \*p < 0.05 in comparison to before (ANOVA followed by Newman–Keuls test).

area) of the center of the microinjections in all animals of this study. The microinjections into the CVLM were located in the ventral portion of the lateral reticular nucleus, at the level of -13.3 to -13.8 mm, posterior to the bregma.

#### 4. Discussion

The main finding of the present study was to show that CVLM blockade of the Ang-(1-7) receptor with A-779 restored the sensitivity of the baroreflex bradycardia in 2K1C hypertensive rats to the level of normotensive rats. In addition, no change in baroreflex bradycardia was observed after administration of the AT<sub>1</sub> antagonist, Losartan. These data suggest that endogenous CVLM Ang-(1-7), and not Ang II, is contributing to the lower baroreflex bradycardia of 2K1C rats.

Regardless of the relevance of CVLM neurons for the tonic and reflex control of the cardiovascular system, the contribution of this medullary center for the genesis or maintenance of hypertension has not yet been completely established [7,51]. Brain RAS is activated in several hypertension models [21,22,27,28]. Muratani et al. [33] have shown that Ang II microinjection into the CVLM produces depressor responses that are significantly greater in SHR than in WKY rats. In contrast, we have recently shown that microinjection of Ang II into the CVLM had similar depressor responses in 2K1C rats [41] and in SHR [19], as compared to their normotensive controls. Similarly, the results of the present study showed that exogenously administrated Ang II and Ang-(1-7) into the CVLM produced significant decreases in MAP in 2K1C similar to that observed in Sham rats. However, studies in literature suggest that hypertensive rats may have decreased tonic GABAergic inputs to the RVLM, originated from the CVLM [34,48]. Thus, it is possible that the similarity of Ang peptides responses in the CVLM of Sham and 2K1C rats may be related to alterations in the angiotensin endogenous levels or angiotensin receptor densities among excitatory and inhibitory neurons or a combination of both, which could compensate for the decrease in CVLM neuronal activity.

In the present study, we have shown that the Ang II AT<sub>1</sub> receptor antagonist, Losartan significantly blocked Ang II AP effect at the CVLM for up to 30 min in both Sham and 2K1C rats, suggesting that AT<sub>1</sub> receptors mediate the hypotensive action of exogenously Ang II in the CVLM. However, in a previous study [41] we have shown in Sham and 2K1C rats that the Ang II AT<sub>2</sub> receptor antagonist, PD123319, also significantly attenuated Ang II effect at the CVLM, but for up to 15 min. Taken together, these studies indicate that the Ang II depressor effect at the CVLM may be mediated by both AT<sub>1</sub> and AT<sub>2</sub> receptor. With the data available, however, it is difficult to quantify the relative contribution of each receptor subtype for the Ang II response at the CVLM of normotensive and hypertensive animals. Future studies will be necessary to identify the distribution of the angiotensin receptors, AT<sub>1</sub> and AT<sub>2</sub>, in different subareas and neuronal cell types of the VLM.

It is well established that Ang-(1-7) acts as a counterregulatory modulator of Ang II in the baroreflex control in normotensive [11,18,40] and hypertensive rats [10,14,24,36]. While Ang II reduces the baroreflex sensitivity [12,23,46], Ang-(1-7) induces facilitation of the baroreflex both after peripheral [10] and central microinjection, ICV [10,11,36] and at the NTS [14,15,17]. However, distinct effects were observed after VLM microinjection of Ang peptides. We have recently shown [6] that the microinjection of Ang II at the CVLM induces a facilitatory effect in the reflex bradycardia while the microinjection of Ang-(1-7) produces a decrease in baroreflex bradycardia. In addition, the modulatory effect of Ang-(1-7) on baroreflex control was blocked by peripheral treatment with methyl-atropine [6], suggesting that these effects are mediated through modulation of the parasympathetic drive to the heart. The results of the present study corroborate these data

showing that CVLM microinjection of A-779 improved the sensitivity of reflex bradycardia, which suggests a tonic inhibitory effect induced by endogenous Ang-(1-7) at the CVLM, at least in 2K1C hypertensive rats. Most of the CVLM barosensitive neurons are GABAergic [13,38], however some neurons are catecholaminergic [50,52] and some are cholinergic, yet, some of the cholinergic neurons also express GABA [45]. Although these neurons are often depicted as simple relays to the RVLM, GABAergic cells, and possible the others, are likely to innervate multiple sites to provide a more widespread baroreceptormediated inhibition of other regions of the CNS. A possible reciprocal pathway between the CVLM and the nucleus ambiguous (NA) was suggested in previous studies [31,49] and could be the basis to explain a possible medullary interaction between sites that control the parasympathetic and sympathetic nervous systems. It is our hypothesis [6] that Ang-(1-7) facilitates the activity of inhibitory barosensitive neurons in the CVLM that project to the NA, while Ang II would induce opposite effect. As the baroreflex circuit is activated by the pressor response produced by phenylephrine, there was the expected increase in the activity of the parasympathetic pre-ganglionic neurons in the NA (through the NTS-NA pathway), which could be modulated by an inhibitory barosensitive neurons projection from the CVLM. The alteration of the parasympathetic drive to the heart would depend upon the balance between the activities of both pathways (NTS-NA and CVLM-NA). Facilitation of the inhibitory barosensitive cells induced by Ang-(1-7) microinjection into the CVLM would attenuate the HR changes (bradycardia). Future studies will be necessary to clearly demonstrate the existence of such pathway and to show whether angiotensin peptides may modulate parasympathetic activity through this putative CVLM/NA pathway.

Losartan, on the other hand, had no effect on reflex bradycardia either in 2K1C or in Sham rats. Similar results were also observed by Sesoko et al. [47] that showed that in normotensive rats the Ang II antagonist (Sar<sup>1</sup>, Thr<sup>8</sup>)-Ang II into the CVLM did not alter baroreflex bradycardia. The data in normotensive rats suggest that Ang-(1-7) and Ang II have no tonic effect on baroreflex modulation. However, an important role for local activation of the brain RAS for the maintenance of hypertension is suggested by our current data. In addition, our data are consistent with other reports showing that renovascular hypertension induces increases in angiotensinogen mRNA or Ang II levels in different brainstem regions [28,35]. Thus, taken together, these data suggest a selective alteration of the brain RAS with a possible increase in Ang-(1-7) or Mas receptor levels in the CVLM of renovascular hypertensive rats. In fact, Lazartigues et al. [28] showed that maintenance of hypertension is associated with greater activation of the brainstem RAS in 2K1C mice as compared to normotensive control animals. It is possible that in 2K1C rats the increased level of RAS can also induce an increase in Mas and/or AT<sub>2</sub> receptors expression in the CVLM.

It is interesting to notice that the CVLM microinjection of A-779 induced a significant hypotensive effect, but only in 2K1C hypertensive rats. It is possible that hypertension may lead to alteration in the activity of a specific pathway, increase in an excitatory pathway or attenuation of an inhibitory pathway or both. In addition, alteration in the expression of the Ang-(1-7) receptor, Mas, or yet in the enzymatic pathways involved in angiotensin peptide production may explain the hypotensive effect observed after A-779 injection in the CVLM of 2K1C.

In summary, our results suggest that Ang-(1-7) at the CVLM may contribute to the low sensitivity of the baroreflex control of heart rate in renovascular hypertensive rats. Further, our results also showed that blockade of the  $AT_1$  receptor at the CVLM do not change the baroreflex bradycardia in renovascular hypertension. These data suggest that differential activation of the RAS components in the VLM is induced by renovascular hypertension.

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