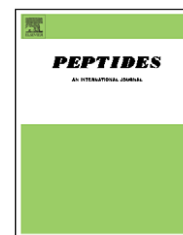


available at www.sciencedirect.comjournal homepage: www.elsevier.com/locate/peptides

Evidence for a role of AT₂ receptors at the CVLM in the cardiovascular changes induced by low-intensity physical activity in renovascular hypertensive rats

M.C. Rodrigues^{a,b}, M.J. Campagnole-Santos^c, R.P. Machado^{a,b}, M.E. Silva^b,
J.L.M. Rocha^a, P.M. Ferreira^d, R.A.S. Santos^c, A.C. Alzamora^{a,b,*}

^aDepartamento de Ciências Biológicas, Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil

^bNúcleo de Pesquisa em Ciências Biológicas, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil

^cDepartamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

^dDepartamento de Ciências Fisiológicas, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Go, Brazil

ARTICLE INFO

Article history:

Received 5 April 2007

Received in revised form

4 June 2007

Accepted 4 June 2007

Published on line 8 June 2007

Keywords:

Caudal ventrolateral medulla

Baroreflex control of heart rate

Angiotensin II

Low-intensity physical activity

Renovascular hypertension (2K1C)

ABSTRACT

In the present study, we evaluated the involvement of the rennin–angiotensin system (RAS) in the control of the blood pressure (BP), baroreceptor-mediated bradycardia and the reactivity of caudal ventrolateral medulla (CVLM) neurons to Ang II and to AT₂ receptor antagonist in sedentary or trained renovascular hypertensive rats. Physical activity did not significantly change the baseline mean arterial pressure (MAP), heart rate (HR) or the sensitivity of the baroreflex bradycardia in normotensive Sham rats. However, in 2K1C hypertensive rats, physical activity induced a significant fall in baseline MAP and HR and produced an improvement of the baroreflex function (bradycardic component). The microinjections of Ang II into the CVLM produced similar decreases in MAP in all groups, Sham and 2K1C, sedentary and trained rats. The hypotensive effect of Ang II at the CVLM was blocked by previous microinjection of the AT₂ receptors antagonist, PD123319, in all groups of rats. Unexpectedly, microinjection of PD123319 at the CVLM produced a depressor effect in 2K1C sedentary that was attenuated in 2K1C trained rats. No significant changes in MAP were observed after PD123319 in Sham rats, sedentary or trained. These data showed that low-intensity physical activity is effective in lowering blood pressure and restoring the sensitivity of the baroreflex bradycardia, however these cardiovascular effects are not accompanied by changes in the responsiveness to Ang II at CVLM in normotensive or hypertensive, 2K1C rats. In addition, the blood pressure changes observed after AT₂ blockade in 2K1C rats suggest that hypertension may trigger an imbalance of AT₁/AT₂ receptors at the CVLM that may be restored, at least in part, by low-intensity physical activity.

© 2007 Elsevier Inc. All rights reserved.

* Corresponding author at: Departamento de Ciências Biológicas, Universidade Federal de Ouro Preto, Morro do Cruzeiro, 35 400 000 Ouro Preto, MG, Brazil. Tel.: +55 31 3559 1693; fax: +55 31 3559 1633.

E-mail address: andreaalzamora@iceb.ufop.br (A.C. Alzamora).

0196-9781/\$ – see front matter © 2007 Elsevier Inc. All rights reserved.

doi:10.1016/j.peptides.2007.06.001

1. Introduction

It is well accepted that physical activity of low intensity induces resting bradycardia and a decrease in blood pressure (BP) in hypertensive animals and humans [36,48,51]. Previous studies [26,36] have shown that the lowering in blood pressure induced by exercise training may be related to fall in sympathetic drive and improvement of baroreflex function.

The sympathetic drive and baroreflex is primarily modulated by medullary neurons. The ventrolateral medulla (VLM) influences the intrinsic activity of spinal preganglionic sympathetic nerve and has a critical role in the control of blood pressure [7,14]. The rostral ventrolateral medulla (RVLM) contains excitatory neurons that directly project to the sympathetic preganglionic motor region involved in the spinal cord. Baroreflex inputs from nucleus tractus solitarii (NTS) stimulates the caudal ventrolateral medulla (CVLM) that elicits dramatic effects on blood pressure and sympathetic nerve activity, and it is highly likely the existence of direct connections from these regions to the RVLM [7,14,46].

The VLM neurons are modulated by peptides of the rennin-angiotensin system (RAS). Studies by us [1,2,6] and others [5,7,14] have shown that there is an important interaction between VLM neurons and angiotensin II (Ang II). Ang II microinjected into the RVLM and the CVLM acts as an excitatory agent [5,35,40]. The development of nonpeptide angiotensin antagonists has produced definitive evidence for the presence of Ang II receptor subtypes. The receptor subtype that is associated with losartan and other "sartans" and appears to mediate essentially all of the known effects of Ang II is designated AT₁. Further studies have shown that Ang II endogenous to the RVLM and CVLM modulates the tonic activity of cardiovascular neurons in rabbit [14,32] and hypertensive and normotensive rats [5,35,40]. In addition to AT₁, other receptor subtype, which is associated with PD123319 and its structural analogs, is designated AT₂. Recent evidence indicates that AT₂ receptor subtype does participate in multiple physiologic functions, including pressure-natriuresis [30], pressor response [19,49] and autoregulation of cerebral blood flow [45]. Whereas the physiologic role of AT₂ receptors has been a matter of debate, emerging information is presently available which suggests their participation in functions previously ascribed to the AT₁ subtype [19,30,31,45,49]. The engagement of AT₂ receptor subtype in the cardiovascular actions of RAS is ascertained in mutant mice targeted for disruption of the gene encoding this angiotensin receptor subtype where it was observed elevated blood pressure [16,20]. Studies also assigned a role of central AT₂ receptors subtype in the suppression of baroreflex sensitivity by showing an increased baroreceptor sensitivity and decreased blood pressure variability in AT₂ receptor-deficient mice [16,23]. In rats, Ang II was also shown to suppress the baroreflex, at least in part, through AT₂ receptors at the RVLM or NTS [23,29,31].

The Goldblatt renovascular hypertension model is characterized by high levels of tissue and circulating Ang II, and an increased sympathetic nerve discharge, which may be related to a central action of Ang II on the sympathetic nervous system. We hypothesized that the changes in sympathetic activity produced by physical training are also related to an

alteration of the responsiveness of CVLM neurons to Ang II. To test this hypothesis, in the present study, we evaluated the RAS involvement in the control of the BP, baroreceptor-mediated bradycardia and the reactivity of CVLM to Ang II and to AT₂ receptor antagonist in sedentary or trained renovascular hypertensive rats.

2. Methods

2.1. Animals

Experiments were performed in male Fisher rats ($n = 26$) (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.

2.2. Production of renal hypertension

To obtain hypertensive animals, the rats (150–200 g) were anesthetized with the mixture of ketamine and xylazine (50 and 10 mg/kg, i.p., respectively) and a silver clip (0.20 mm i.d.) 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). One week after the surgery, systolic blood pressure (SBP) was measured by tail-cuff method for 4 weeks. The 2K1C and Sham rats were separated in two experimental groups, sedentary and physical training. The training protocol started 4 days after the surgery.

2.3. Physical training protocol

Training was performed without workload by 20 sessions of swimming of 1 h duration daily, 5 days a week. At the first day the rats swam for 20 min, at the second day for 40 min and from third day until the end of training period, they swam for 1 h. The exercise was performed in group of four or five rats in a 38 cm × 60 cm × 50 cm tank. Water temperature was maintained at 30 ± 2 °C, controlled by a thermostat.

2.4. Arterial pressure measurements

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

2.5. Evaluation of baroreflex sensitivity

Baroreflex control of HR was determined by recording reflex heart rate changes in response to transient increases in MAP produced by repeated bolus injections of graded doses of phenylephrine (0.25–5 µg, i.v.) (baroreflex bradycardia) in urethane anesthetized rats. Phenylephrine doses were injected

1–2 min apart into a femoral vein in 0.1 mL of isotonic sodium chloride. Blood pressure and heart rate were allowed to return to basal levels before the next dose was given. Peak changes in HR occurring during the initial 5–10 s of the corresponding maximum change in MAP produced with phenylephrine. The HR was converted to pulse interval (PI, ms) by the formula: $60,000/\text{HR}$. Best-fit regression line was drawn from the mean \pm S.E.M. of pressure and HR changes for each dose of phenylephrine for each animal. The slope of the regression line was used as an index of baroreflex sensitivity (baroreflex gain).

2.6. CVLM microinjections

2K1C and Sham rats (260–300 g) after 32 days of surgery 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 injection of drugs. The animals were placed in a stereotaxic frame (David Kopf instruments, CA) with the tooth bar 11 mm below the level of the interaural line. The dorsal surface of the brainstem was exposed by a limited occipital craniotomy and an incision of the atlanto-occipital membrane and meninges was performed, as previously described [1].

Unilateral microinjections of Ang II, PD123319 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 [1]. Microinjections were made with a triple barreled glass micropipette (outside diameter = 90–130 μm), fixed to the stereotaxic manipulator that was inserted in the brain tissue through the dorsal surface. Experiments were made only at sites where the positioning of the micropipette produced a transitory depressor response (usually 10–20 mmHg).

2.7. Experimental protocol

The arterial pressure and HR of urethane anesthetized Sham and 2K1C sedentary ($n = 5$ – 6) and trained ($n = 7$) rats were continuously recorded. After 10 min of stabilization period, baroreflex control of heart rate was evaluated. Next, the micropipette was positioned in the CVLM and Ang II (40 pmol) or saline (NaCl, 0.9%, 100 nL) was microinjected in random order. After, a period of 15 min, PD123319 (50 pmol) was microinjected into the CVLM and after 5, 15 and 30 min Ang II microinjection was repeated in order to verify the duration of AT_2 receptor blockade.

2.8. Drugs

Ang II and PD123319 were purchased from Sigma Chemical Company (St. Louis, MO, USA) or Peninsula Laboratories (Belmont, CA, USA). Phenylephrine was from Sigma Chemical Company (St. Louis, MO, USA).

Ang II (2 mg/mL) and PD123319 (3.2 mg/mL) were dissolved in sterile isotonic saline (NaCl, 0.9%), aliquoted (10 μL) and stored at -20°C . Phenylephrine was dissolved in sterile saline at 1 mg/mL concentration and 100 μL aliquots were stored at

-20°C . At the moment of the experiment, the aliquots were diluted in the desired concentrations and used only once.

2.9. Histological verification of injection sites

At the end of each experiment, the animals were then killed with excess of anesthetic and the brain was carefully removed and fixed in 10% phosphate-buffered formalin. Serial coronal sections (40–50 μm) of the medulla oblongata were made and stained with neutral red for histological examination. Microinjections sites were identified by tissue rupture produced by the volume of microinjections under a light microscopy and referred to standard anatomical structures of the brain stem according to the atlas of Paxinos and Watson [39].

2.10. Statistical analysis

The results are expressed as means \pm S.E.M. Comparisons among different groups were assessed by one-way or two-way ANOVA followed by Bonferroni or Dunnett test where appropriated. The analysis was 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 sedentary and trained rats

The baseline MAP of 2K1C (161 ± 14 mmHg, $n = 5$) was significantly higher than the baseline MAP of Sham rats (105 ± 4 mmHg, $n = 6$) in sedentary groups. Physical training significantly lowered MAP of 2K1C (127 ± 6 mmHg, $n = 7$), however, the MAP of 2K1C trained rats was significantly higher than the baseline MAP of Sham training group (105 ± 4 mmHg, $n = 7$; Fig. 1A).

The baseline values of HR were not significantly different in Sham trained group (373 ± 10 beats/min, $n = 7$) in comparison to sedentary Sham rats (400 ± 13 beats/min, $n = 6$). However, 2K1C training group the baseline HR (375 ± 8 mmHg, $n = 8$) was significantly smaller than the baseline HR of sedentary 2K1C group (407 ± 12 mmHg, $n = 5$; Fig. 1B).

The reduction in the weight of the clipped kidney was similar in sedentary 2K1C ($-27 \pm 7\%$, $n = 5$) and 2K1C trainer group ($-39 \pm 9\%$, $n = 8$; Fig. 1C).

3.2. Evaluation of the sensitivity of reflex bradycardia in sedentary and trained rats

As expected, the reflex bradycardia in the sedentary 2K1C rats (0.09 ± 0.03 ms/mmHg, $n = 5$; Fig. 2) was significantly smaller in comparison to Sham sedentary rats (0.36 ± 0.07 ms/mmHg, $n = 6$). There was not significantly difference in the baroreflex bradycardia in the sedentary or trained Sham groups. Conversely, in 2K1C rats physical training significantly increased baroreflex bradycardia (0.2 ± 0.03 ms/mmHg, $n = 7$ versus 0.09 ± 0.03 ms/mmHg, $n = 5$, 2K1C sedentary; Fig. 2). The baroreflex sensitivity in 2K1C trained rats was not different from Sham trained rats (0.33 ± 0.03 ms/mmHg, $n = 7$; Fig. 2).

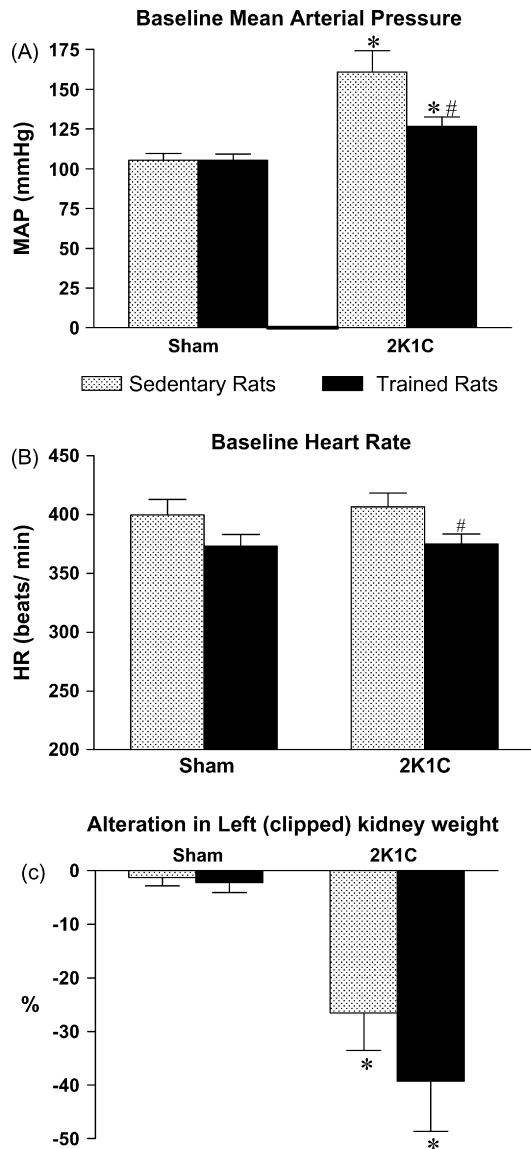


Fig. 1 – Baselines values of mean arterial pressure (MAP, mmHg; A) and heart rate (HR, beats/min; B) in Sham ($n = 6$) and 2K1C ($n = 5$) sedentary and Sham ($n = 7$) and 2K1C ($n = 7$) trained rats. Percentage of reduction in the weight of the left (clipped) kidney in Sham and 2K1C sedentary and trained rats (C). $p < 0.05$ in comparison to the respective Sham group (ANOVA followed by Bonferroni test). $^{\#}p < 0.05$ in comparison to sedentary 2K1C group (ANOVA followed by Bonferroni test).

3.3. Cardiovascular effects produced by microinjection of Ang II and PD123319 into the CVLM in sedentary and trained rats

In order to verify whether the changes involved by physical activity were related to the reactivity to Ang II at the CVLM we determined the cardiovascular responses to Ang II in normotensives and renovascular hypertensive rats submitted low-intensity physical activity. Microinjection of Ang II into the CVLM produced a significant decrease in MAP in 2K1C trained

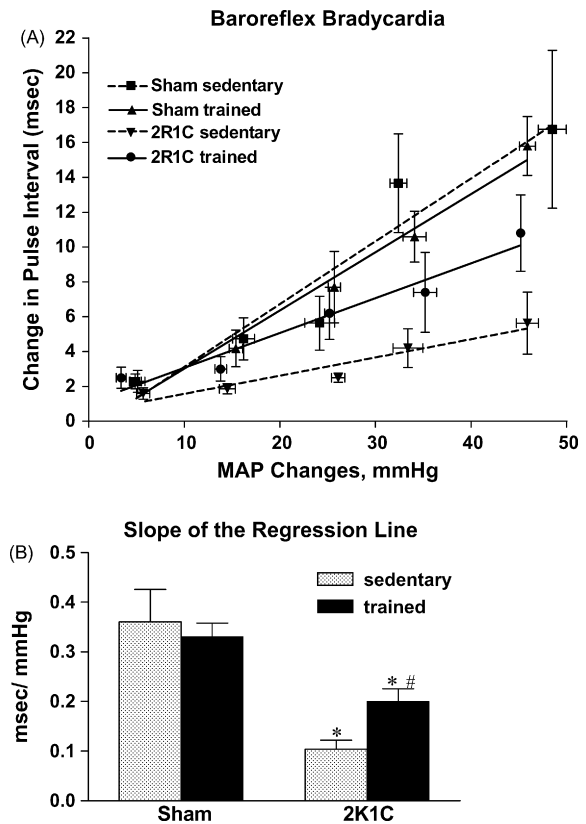


Fig. 2 – Baroreflex mediated changes in HR expressed as pulse interval (ms) in response to changes in mean arterial pressure (MAP, mmHg; A) produced by graded doses of phenylephrine in Sham ($n = 6$) and 2K1C ($n = 5$) sedentary and Sham ($n = 7$) and 2K1C ($n = 7$) trained rats. Lines represent the least-square regression equation fitted through the average points and the baroreflex sensitivity index (ms/mmHg) for bradycardia is expressed by the slope of these regression line (B). $^{\#}p < 0.05$ in comparison to the respective Sham group (ANOVA followed by Bonferroni test). $^{\#}p < 0.05$ in comparison to sedentary 2K1C group (ANOVA followed by Bonferroni test).

rats (-14 ± 3 mmHg, $n = 7$) was similar to that observed in sedentary 2K1C rats (-12 ± 1 mmHg, $n = 5$). Similar fall in blood pressure after CVLM microinjection of Ang II was observed in Sham rats (-13 ± 1 mmHg, $n = 6$ in sedentary Sham rats and -13 ± 2 mmHg, $n = 7$ in trained rats; Fig. 3A). All these hypotensive effects were significantly different from the hypotensive effect produced by microinjection of saline into the CVLM. Microinjection of Ang II into CVLM of Sham or 2K1C groups (sedentary or trained rats) did not significantly alter HR (Fig. 3B). The AT_2 Ang II antagonist, PD123319, produced a significant hypotensive effect compared to saline in 2K1C sedentary (-10 ± 1 mmHg versus -5 ± 1 mmHg; saline, $n = 5$), however in 2K1C trained rats these depressor effect is similar to saline (-6 ± 2 mmHg versus -5 ± 1 mmHg; saline, $n = 7$; Fig. 3A). In contrast, PD123319 has a similar hypotensive effect to saline in Sham sedentary or trained groups (Fig. 3A).

As shown in Fig. 4A, the microinjection of PD123319 into the CVLM abolished Ang II effect up to 5 min in Sham sedentary, Sham trained, 2K1C sedentary and 2K1C trained

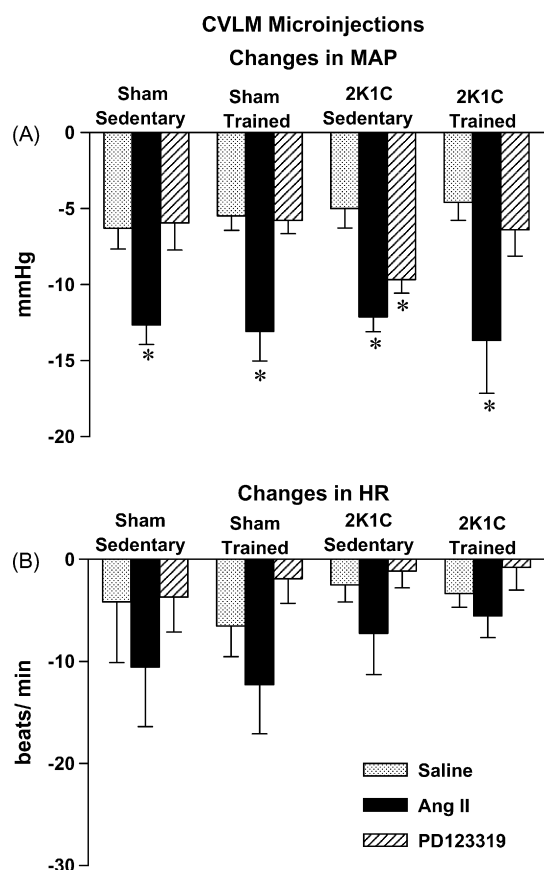


Fig. 3 – Averaged changes in mean arterial pressure (MAP, mmHg; A) and heart rate (HR, beats/min; B) produced by microinjection of saline (100 nL, $n = 5-7$), Ang II (40 pmol, $n = 5-7$) or PD123319 (50 pmol, $n = 5-7$) into the CVLM in Sham and 2K1C sedentary and trained rats. $p < 0.05$ in comparison to saline (ANOVA followed by Dunnett's test).

rats. In addition, Ang II was significantly reduced up to 15 min after PD123319 microinjection in all groups of animals (Fig. 4A). Thirty minutes after PD123319 microinjection into the CVLM, Ang II effect was not different from control (before PD123319, Fig. 4A). No significant changes in HR were observed in all groups at any of the different time points (data not shown). In addition, Ang II depressor effect were not different 5, 15 and 30 min after the CVLM microinjection of saline (time control, data not shown).

3.4. Histological examination

Fig. 4 presents diagrams of frontal sections of the medulla according to the atlas of Paxinos and Watson [39], showing the localization of the microinjections in the CVLM. The microinjections into the CVLM were located in the ventral portion of the lateral reticular nucleus.

4. Discussion

The results of the present study showed that the low-intensity physical activity is effective in reducing high BP, HR and in

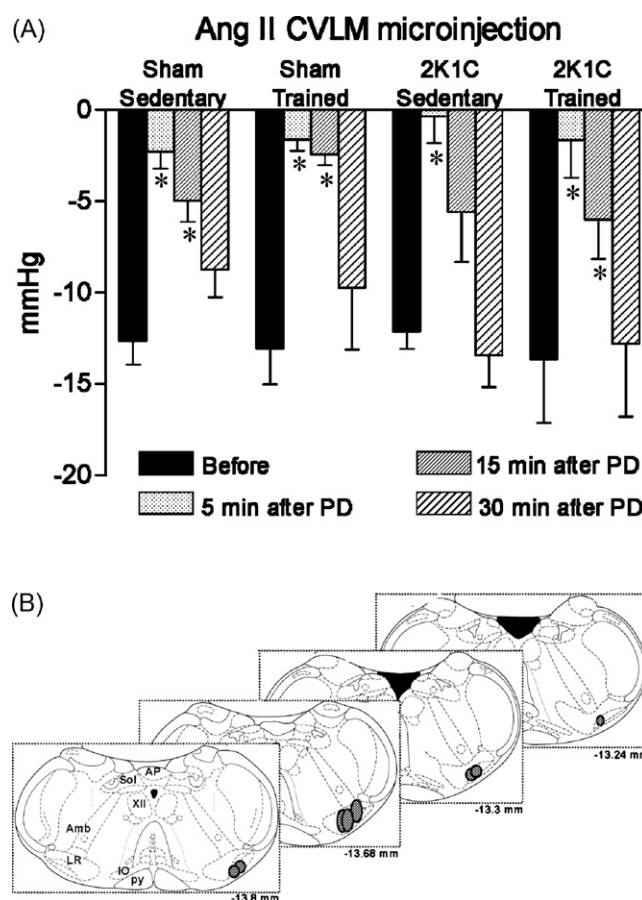


Fig. 4 – (A) Mean arterial pressure changes (MAP, mmHg) produced by CVLM microinjections of Ang II (40 pmol, $n = 5-7$) before and 5, 15 and 30 min after microinjection of PD123319 (PD, 50 pmol, $n = 5-7$) in Sham and 2K1C sedentary ($n = 5-6$) and trained ($n = 7$) rats. (B) Diagrammatic representation of frontal sections of the lower brain stem showing the center of the microinjections in the CVLM (shaded area) as determined by tissue rupture produced by the volume of microinjections in each experiment. Maps and coordinates (in mm, right margin) are from the atlas of Paxinos and Watson [39]. Amb, ambiguus nucleus; AP, area postrema; LRT, lateral reticular nucleus; LRTPC, lateral reticular nucleus, parvocellular; py, pyramidal tract; XII, hypoglossal nucleus. $p < 0.05$ in comparison to saline (two-way ANOVA followed by Bonferroni test).

restoring the sensitivity of the reflex bradycardia in renovascular hypertensive rats. Ang II microinjected into the CVLM had similar hypotensive effects that were significantly blocked by Ang II AT₂ receptor antagonist in sedentary or trained, 2K1C and Sham rats. In addition, the Ang II AT₂ receptor antagonist induced a fall in arterial pressure after CVLM microinjection in 2K1C sedentary rats that was attenuated in 2K1C trained rats. Overall, the present study showed that the low-intensity exercise provokes a significant improvement of the cardiovascular parameters in the renovascular 2K1C hypertension accompanied by an alteration of AT₂ mediated Ang II responses at neurons of the CVLM in hypertensive 2K1C rats.

In renovascular hypertensive rats the reflex bradycardia of the sedentary 2K1C rats was significantly smaller in comparison to Sham sedentary rats as extensively shown in previous studies [9,27,33]. In addition, low-intensity training was effective in reducing high BP and in restoring the sensitivity of the reflex bradycardia in renovascular hypertensive rats. These findings are in agreement with previous studies [48,51] that showed that exercise training can induce important changes in the cardiovascular system in animals and humans with hypertension. Studies in SHR have shown that low-intensity exercise training decreases HR and cardiac output, consequently, attenuates hypertension in SHR [48,51]. To address the mechanisms by which low-intensity exercise causes bradycardia, a variety of studies [15,26] have shown an attenuated sympathetic tone in SHR, but not the vagal tone, which remained unchanged. The low-intensity exercise training brings the sympathetic tone to the normal level. An increased arterial BRS after exercise training has been observed in hypertensive subjects [26,38]. In SHR during the postexercise, the BRS for bradycardiac responses was increased, while the BRS for tachycardiac responses remained depressed [26,43]. In addition, an increase in vascular compliance was also observed in humans after exercise training [25]. Increased shear stress during exercise may also enhance the release of endothelial factors [25]. All of these mechanisms may increase the sensitivity of the arterial baroreceptor afferents, thus increasing the BRS.

There are evidences suggesting that RAS and neurogenic mechanisms interact, central and peripherally, in the development and maintenance of hypertension. Despite of the number of studies [8,11] it is still not yet clear the involvement and dependence on activation of angiotensin AT₁ or AT₂ receptor subtype in 2K1C hypertensive model. Cervenka et al. [11] showed that the target disruption of AT_{1A} receptor gene prevents the development of 2K1C Goldblatt hypertension in mice. AT_{1A} receptors play an essential role in BP control [11,22,37] and moreover suggest that for the development of 2K1C hypertension, the presence of AT_{1A} receptor is critical. However, acute AT₂ receptors blockade increased BP in 2K1C AT₁ receptors knockout mice indicating that, at least to some extent and under specific conditions, AT₂ receptors participate in acute BP regulation [11].

Considering that low-intensity training is effective in reducing high BP and in restoring the sensitivity of the baroreflex bradycardia in 2K1C hypertensive rats, we postulated that low-intensity training may induce changes in the neuronal activity of the CVLM of 2K1C hypertensive model mediated by Ang II. Most forms of hypertension, in which the renovascular 2K1C is included, are related to an imbalance of the depressor and pressor sympathetic pathways in the medulla. Part of this imbalance appears to be due to the inability of CVLM neurons to counterbalance the apparent greater intrinsic pressor activity of RVLM neurons of hypertension animals [10].

Other studies have shown that the brain RAS may be involved in the early stages of 2K1C hypertension, inasmuch as intracerebroventricular injection of Ang II antagonists such as salarasin can reduce BP [24,47]. However, the participation of the RAS at the CVLM in the hypertension is still controversial. Muratani et al. [35] showed that microinjection

of Ang II in the CVLM produced depressor responses that were significantly greater in SHR than in WKY rats. In contrast, other studies have shown that SHR and WKY rats had similar pressor and depressor responses to microinjection of Ang II into the RVLM or into the CVLM, respectively [35,44]. In these studies tonic sympathoinhibitory activity of the CVLM seems smaller in young SHR than in age-matched WKY controls, suggesting that in SHR, an abnormal inhibitory activity of the CVLM would produce an increase in sympathetic and neurogenic hypertension that characterizes the initial phase of hypertension in these animals. Our results showed that Ang II into the CVLM produced a significant decrease in MAP in 2K1C groups similar to that observed in Sham groups, suggesting that there were no significant alteration of the RAS in the CVLM of 2K1C.

Several studies [4,21] have evaluated the role of the VLM areas on the adjustments that follows static skeletal contraction, however, few studies have assessed the interaction of the RAS and VLM in exercise trained animals. We have previously shown that exercise training induces changes in the Ang II responsiveness at the RVLM of normotensive rats [6]. In contrast, in present study, we have observed that the low-intensity physical activity did not change the hypotensive effects of Ang II microinjected into the CVLM in Sham or 2K1C rats.

The AT₂ receptor is moderately expressed in certain nuclei involved in cardiovascular regulation, such as, locus coeruleus, paragigantocellular nucleus, medullary reticular nucleus, lateral reticular nucleus, NTS and ambiguous nucleus [28,50], however, its contribution to BP regulation in these areas remains elusive. Few studies have described a direct role of AT₂ receptors in the cardiovascular effects elicited by brain microinjection of Ang II [12,17,18,41,42,52-54]. In the present study, we have shown that the AT₂ Ang II antagonist, PD123319, significantly attenuated Ang II effect at the CVLM for up to 15 min in Sham sedentary, Sham trained, 2K1C sedentary and 2K1C trained rats. These data suggest that AT₂ receptor, at least in part, mediates the hypotensive action of exogenously Ang II in the CVLM. Ambuhl et al. [3] showed that Ang II microinjection into the inferior olivary nucleus (ION) has an excitatory effect on a considerable number of ION neurons and that this effect is mediated by AT₂ receptors. Although unlikely, we cannot completely rule out the possibility that in our study, Ang II had also reached neurons in ION.

Unexpectedly, microinjection of PD123319 at the CVLM produced a depressor effect in 2K1C sedentary that was attenuated in 2K1C trained rats. No significant changes in MAP were observed after PD123319 in Sham rats, sedentary or trained. One possibility for the unexpected effect of PD123319 could be an increase in the expression of AT₁ receptor on the CVLM of hypertensive rats as reported for the RVLM [19]. Therefore, the blockade of AT₂ receptors by PD123319 would increase the availability of Ang II for AT₁ receptors at the CVLM thus resulting in a greater hypotensive effect. However, ongoing experiments in our laboratory do not support this hypothesis since microinjection of the AT₁ receptor antagonist, losartan, into the CVLM did not change MAP in 2K1C hypertensive rats. Considering that in some instances PD123319 can interfere with the response of other peptides

or can present an agonistic effect [13,34,53]. Further studies are necessary to clarify our present findings.

In summary, our results showed that low-intensity physical activity that is effective in reducing high BP and in restoring the sensitivity of the baroreflex bradycardia, does not induce changes in the responsiveness to Ang II at CVLM of normotensive or hypertensive, 2K1C rats. These results, on the other hand, changes in the responsiveness to AT₂ related stimuli at the CVLM appears to be involved in the cardiovascular effect of low-intensity physical activity and add new significant insights into RAS mechanism involved in cardiovascular homeostasis and its adaptation to exercise in renovascular hypertension.

Acknowledgements

This study was supported by FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) Pronex Project Grant (FAPEMIG/CNPq) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). M.C. Rodrigues was a recipient of CAPES fellowship (Master Degree) at the "Programa de Pós-graduação Ciências Biológicas", NUPEB, UFOP. We are thankful to Dr. Cláudia Martins Carneiro Associate Professor at the "Departamento de Análises Clínicas", UFOP, for histological analysis.

REFERENCES

- [1] Alzamora AC, Santos RAS, Campagnole-Santos MJ. Hypotensive effect of Ang II and Ang-(1-7) at the caudal ventrolateral medulla involves different mechanisms. *Am J Physiol (Regul Integr Comp Physiol)* 2002;283:R1187-95.
- [2] Alzamora AC, Santos RAS, Campagnole-Santos MJ. Baroreflex modulation by angiotensins at the rat rostral and caudal ventrolateral medulla. *Am J Physiol (Regul Integr Comp Physiol)* 2006;290:R1027-34.
- [3] Ambuhl P, Felix D, Imboden H, Khosla MC, Ferrario CM. Effects of angiotensin analogues and angiotensin receptor antagonists on paraventricular neurones. *Regul Pept* 1992;38(2):111-20.
- [4] Ally A, Phattananarudee S, Kabadi S, Patel M, Maher TJ. Cardiovascular responses and neurotransmitter changes during static muscle contraction following blockade of inducible nitric oxide synthase (iNOS) within the ventrolateral medulla. *Brain Res* 2006;1090(1):123-33.
- [5] Averill DB, Diz D. Angiotensin peptides and baroreflex control of sympathetic outflow: pathways and mechanisms of the medulla oblongata. *Brain Res Bull* 2000;51(2):119-28.
- [6] Becker LK, Santos RAS, Campagnole-Santos MJ. Cardiovascular effects of angiotensin II and angiotensin-(1-7) at the RVLM of trained normotensive rats. *Brain Res* 2005;1040(1-2):121-8.
- [7] Blessing WW, Reis DJ. Inhibitory cardiovascular function of neurons in the caudal ventrolateral medulla of the rabbit: relationship to the area containing A₁ noradrenergic cells. *Brain Res* 1982;253:161-71.
- [8] Blume A, Kaschina E, Unger T. Angiotensin II type 2 receptors: signaling and pathophysiological role. *Curr Opin Nephrol Hypertens* 2001;10:239-46.
- [9] Britto RR, Santos RAS, Fagundes-Moura CR, Khosla MC, Campagnole-Santos MJ. Role of angiotensin-(1-7) in the modulation of the baroreflex in renovascular hypertensive rats. *Hypertension* 1997;30:549-56.
- [10] Carvalho THF, Bergamaschi CT, Lopes OU, Campos RR. Role of endogenous angiotensin II on glutamatergic actions in the rostral ventrolateral medulla in spontaneously hypertensive rats. *Hypertension* 2003;42(pt 2):707-12.
- [11] Cervenka L, Horáček V, Vanecková I, Hubáček JA, Oliverio MI, Coffman TM, Navar LG. Essential role of AT1A receptor in the development of 2k1c hypertension. *Hypertension* 2002;40:735-41.
- [12] D'Amico M, Filippo C, Rossi F. Role of AT2 receptors in the cardiovascular events following microinjection of angiotensin II into the superior colliculus of anaesthetized rats. *Naunyn-Schmiedeberg Arch Pharmacol* 1998;357:121-5.
- [13] Gironacci MM, Vata M, Rodriguez-Fermepín M, Fernández BE, Pena C. Angiotensin-(1-7) reduces norepinephrine release through a nitric oxide mechanism in rat hypothalamus. *Hypertension* 2000;35:1248-52.
- [14] Dampney RAL. Functional organization of central pathways regulating the cardiovascular system. *Physiol Rev* 1994;74(2):323-64.
- [15] Gava NS, Vêras-Silva AS, Negrão CE, Krieger EM. Low-intensity exercise training attenuates cardiac β -adrenergic tone during exercise in spontaneously hypertensive rats. *Hypertension* 1995;26(pt 2):1129-33.
- [16] Gross V, Plehm R, Tank J, Jordan J, Diedrich A, Obst M, et al. Heart rate variability and baroreflex function in AT2 receptor-disrupted mice. *Hypertension* 2002;40:207-13.
- [17] Hein LGS, Barsh RE, Pratt VJ, Dzau BK, Kodilka. Behavioral and cardiovascular effects of disrupting the angiotensin II type-2 receptor gene in mice. *Nature* 1995;377:744-7.
- [18] Hogarty DC, Speakman EA, Puig V, Phillips MI. The role of angiotensin AT1 and AT2 receptors in the pressor, drinking and vasopressin responses to the central angiotensin. *Brain Res* 1992;586:289-94.
- [19] Hu L, Zhu D, Yu Z, Wang JQ. Expression of angiotensin II type 1 (AT1) receptor in the rostral ventrolateral medulla in rats. *J Appl Physiol* 2002;92:2153-61.
- [20] Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A, et al. Effects on blood pressure and exploratory behavior of mice lacking angiotensin II type-2 receptor. *Nature* 1995;377:748-50.
- [21] Ishide T, Preuss CV, Maher TJ, Ally A. Neurochemistry within ventrolateral medulla and cardiovascular effects during static exercise following eNOS antagonism. *Neurosci Res* 2005;52:21-30.
- [22] Ito S, Komatsu K, Tsukamoto K, Kanmatsuse K, Sved AF. Ventrolateral medulla AT1 receptors support blood pressure in hypertensive rats. *Hypertension* 2002;40:552.
- [23] Jöhren O, Dendorfer A, Dominiak P. Cardiovascular and renal function of angiotensin II type-2 receptors. *Cardiovasc Res* 2004;62(3):460-7.
- [24] Kagiya S, Varela A, Phillips MI, Galli SM. Antisense inhibition of brain renin-angiotensin system decreased blood pressure in chronic 2-kidney, 1 clip hypertensive rats. *Hypertension* 2001;37:371-5.
- [25] Katz SD. The role of endothelium-derived vasoactive substances in the pathophysiology of exercise intolerance in patients with congestive heart failure. *Prog Cardiovasc Dis* 1995;38:23-50.
- [26] Krieger EM, Farah VMA, Moreira ED, Pires MD, Irigoyen MCC. Comparison of three methods for the determination of baroreflex sensitivity in conscious rats. *Braz J Med Biol Res* 1999;32(3):361-9.
- [27] Kumagai K, Suzuki H, Ryuzaki M, Matsukawa S, Saruta T. Baroreflex control of renal sympathetic nerve activity is

- potenciado at early phase of two-kidney, one-clip Goldblatt hypertension in conscious rabbits. *Circ Res* 1990;67:1309-22.
- [28] Lenkei Z, Palkovits M, Corvol P, Llorens-Cortes C. Expression of angiotensin type-1 (AT1) and type-2 (AT2) receptor mRNAs in the adult rat brain: a functional neuroanatomical review. *Front Neuroendocrinol* 1997;18(14):383-439.
- [29] Lin KS, Chan JYH, Chan SHH. Involvement of AT2 receptors at NRVL in tonic baroreflex suppression by endogenous angiotensin. *Am J Physiol* 1997;272:2204-10.
- [30] Lo M, Liu KL, Lantelme P, Sassard J. Subtype 2 of angiotensin II receptors controls pressure-natriuresis in rats. *J Clin Invest* 1995;95:1394-7.
- [31] Luoh HF, Chan SHH. Participation of AT1 and AT2 receptor subtypes in the tonic inhibitory modulation of baroreceptor reflex response by endogenous angiotensins at the nucleus tractus solitarius in the rat. *Brain Res* 1998;782(1-2):73-82.
- [32] Matsumura Y, Hasser EM, Bishop VS. Central effect of angiotensin II on baroreflex regulation in conscious rabbits. *Am J Physiol (Regul Integr Comp Physiol)* 1989;256:R694-700.
- [33] Moreira ED, Oliveira M, Krieger EM. Impaired baroreflex control of heart rate in high-renin renal hypertension. *J Hypertens* 1988;6(8):619-25.
- [34] Moreira TH, Rodrigues AL, Beirao PS, Santos RAS, Cruz JS. Angiotensin II inhibition of Ca²⁺ currents is independent of ATR1 angiotensin II receptor activation in rat adult vagal afferent neurons. *Auton Neurosci* 2005;117(2):79-86.
- [35] Muratani H, Ferrario CM, Averill DB. Ventrolateral medulla of spontaneously hypertensive rats: role of angiotensin II. *Am J Physiol (Regul Integr Comp Physiol)* 1993;264:R388-95.
- [36] Negrão CE, Krieger EM, Brum PC. Influence of exercise training on neurogenic control of blood pressure in spontaneously hypertensive rats. *Hypertension* 1999;34:720-3.
- [37] Oliverio MI, Best CF, Smithies O, Coffman TM. Regulation of sodium balance and blood pressure by the AT_{1A} receptor for angiotensin II. *Hypertension* 2000;35:550-4.
- [38] Pagani M, Somers V, Furlan R, Dell OS, Conway J, Baselli G, et al. Changes in autonomic regulation induced by physical training in mild hypertension. *Hypertension* 1988;12:600-10.
- [39] Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. New York: Academic Press; 1986.
- [40] Sesoko S, Muratani H, Takeshita S, Teruya H, Kawazoe N, Fukuyama K. Modulation of baroreflex function by angiotensin II endogenous to the caudal ventrolateral medulla. *Brain Res* 1995;671:38-44.
- [41] Shi L, Yao J, Koos BJ, Xu Z. Induced fetal depressor or pressor responses associated with c-fos by intravenous or intracerebroventricular losartan. *Dev Brain Res* 2004;153:53-60.
- [42] Shi L, Mao C, Thornton SN, Sun W, Wu J, Yao J, et al. Effects of intracerebroventricular losartan on angiotensin II-mediated pressor responses and c-fos expression in near-term ovine fetus. *J Comp Neurol* 2005;493(4):571-9.
- [43] Silva GJJ, Brum PC, Negrão CE, Krieger EM. Acute and chronic effects of exercise on baroreflexes in spontaneously hypertensive rats. *Hypertension* 1997;30(2):714-9.
- [44] Smith JK, Barron KW. The rostral and caudal ventrolateral medulla in young spontaneously hypertensive rats. *Brain Res* 1989;506(1):153-8.
- [45] Stromberg C, Naveri L, Saavedra JM. Nonpeptide angiotensin AT1 and AT2 receptors ligands modulate the upper limit of cerebral blood flow autoregulation in the rat. *J Cereb Blood Flow Metab* 1993;13:293-303.
- [46] Sved AF, Ito S, Madden CJ. Baroreflex dependent and independent roles of the caudal ventrolateral medulla in cardiovascular regulation. *Brain Res Bul* 2000;51(2):120-33.
- [47] Sweet CS, Columbo JM, Gaul SL. Central antihypertensive effects of inhibitors of the rennin-angiotensin system in rats. *Am J Physiol* 1976;231:1794-9.
- [48] Tipton CM. Exercise training for the treatment of hypertension: a review. *Clin J Sports Med* 1999;9(2):104.
- [49] Tony GM, Porter JP. Functional role of brain AT1 and AT2 receptors in the central angiotensin II pressor response. *Brain Res* 1993;603:57-63.
- [50] Veerasingham SJ, Raizada MK. Brain renin-angiotensin system dysfunction in hypertension: recent advances and perspectives. *Br Pharmacol* 2003;139:191-202.
- [51] Véras-Silva AS, Mattos KC, Gava NS, Brum PC, Negrão CE, Krieger EM. Low-intensity exercise training decreases cardiac output and hypertension in spontaneously hypertensive rats. *Am J Physiol (Heart Circ Physiol)* 1997;273:H2627-31.
- [52] Von Bohlen U, Halbach O, Albrecht D. Angiotensin II inhibits long-term potentiation with the lateral nucleus of the amygdala through AT1 receptors. *Peptides* 1998;19(6):1031-6.
- [53] Walthers PE, Gaspari TA, Widdop RE. Angiotensin-(1-7) acts as a vasodepressor agent via angiotensin II type 2 receptors in conscious rats. *Hypertension* 2005;45:960-6.
- [54] Widdop RE, Gardiner SM, Kemp PA, Bennett T. Differential blockade of central effects of angiotensin II by AT2 receptor antagonists. *Am J Physiol (Heart Circ Physiol)* 1993;265:H226-31.