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Differential control of vasomotion by angiotensins in the rostral ventrolateral medulla of hypertensive rats

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ABSTRACT

The central and peripheral renin–angiotensin systems are known for playing a key role in cardiovascular control. In the present study, we evaluated the hemodynamic effects produced by nanoinjections of angiotensin II (Ang II) or angiotensin-(1–7) [Ang-(1–7)] into the rostral ventrolateral medulla (RVLM) of adult male normotensive (Wistar—WT) and spontaneously hypertensive rats (SHR). Animals were anesthetized (urethane 1.2 g/kg) and instrumented for recording blood pressure (BP), heart rate (HR) and blood flow (BF) in the femoral, renal or mesenteric arteries. Afterwards, rats were positioned in a stereotaxic and prepared for nanoinjections (100 nl) of saline (NaCl 0.9%), Ang-(1–7) (40 ng) or Ang II (40 ng) into the RVLM. The vascular resistance (VR) was calculated by ΔMAP/ΔBF ratio. In WT, Ang-(1–7) or Ang II caused equipotent pressor effects that were not accompanied by changes in vascular resistance. However, MAP changes were greater in SHR. This strain also showed a concomitant increase in relative vascular resistance (ΔVR/VRbaseline) of renal (0.31 ± 0.07 and 0.3 ± 0.07 vs. 0.02 ± 0.01; Ang-(1–7), Ang II and Saline, respectively) and mesenteric beds (0.3 ± 0.06 and 0.33 ± 0.04 vs. 0.05 ± 0.02; Ang-(1–7), Ang II and saline, respectively). We conclude that Ang II and Ang-(1–7) at the RVLM control the vascular resistance of renal and mesenteric beds during hypertension.

1. Introduction

The rostral ventrolateral medulla (RVLM) is a major vasomotor center in the brainstem, containing sympathetic premotor neurons responsible for generating and maintaining vasomotor tone and resting levels of arterial blood pressure. RVLM is under modulatory influence of the renin–angiotensin system (RAS). Several studies demonstrated that some medullary areas are activated by injections of Angiotensin II (Ang II) and Angiotensin (1–7) [Ang-(1–7)](Allen et al., 1988; Alzamora et al., 2002; Andreatta et al., 1988; Fontes et al., 1997, 1994; Li et al., 2012; Muratani et al., 1991, 1993; Silva et al., 1993). In normotensive rats, injections of Ang II or Ang-(1–7) into the RVLM increases arterial pressure and sympathetic nerve activity (Allen et al., 1988; Averill et al., 1994; Du et al., 2013; Fontes et al., 1994; Hirooka et al., 1997; Li et al., 2013, 2012; Oliveira et al., 2013; Potts et al., 2000; Silva et al., 1993; Zhou et al., 2010). While Ang II effects are mediated by its type 1 (AT_1) receptor (Averill et al., 1994; Hirooka et al., 1997), the G protein-coupled MAS receptor, mediates those evoked by Ang-(1–7) (Santos et al., 2003).

Spontaneously hypertensive rats (SHR) are a well-known model, widely used to investigate the contribution of brain RAS to the development and maintenance of primary hypertension. Several studies showed changes in the activity and in the levels of RAS components of SHR brains (Hirooka et al., 1997; Ito et al., 2000; Nakagaki et al., 2011; Phillips and de Oliveira, 2008). Increases in central angiotensinogen expression precede the development of hypertension in SHR (Tamura, 1996). These animals also show increases in the diencephalic angiotensinogen levels, likely evident from the fourth week of life (Shibata et al., 1993). In addition, renin-like activity in the anterior hypothalamus and in the nucleus tract solitarii (nTS) is higher during the development of hypertension in SHR, when compared to its control, Wistar rats (WT) (Ruiz et al., 1990). Ang II content as well as its turnover within the hypothalamus, and Ang II immunoreactivity within the paraventricular hypothalamus (PVN) and within the supraoptic nucleus (SFO) are substantially increased in adult SHR when compared to WT (Ganten et al., 1983; Phillips and Kimura, 1986; Weyhenmeyer and Phillips, 1982). SHR also exhibit increased density of Ang II binding sites within the median preoptic nucleus (MnPO), SFO, PVN and nTS,

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and AT1 receptor mRNA within the preoptic area (Gutkind et al., 1988; Komatus et al., 1996). Furthermore, it was demonstrated that the RVLM of SHR show an increase in the ACE and AT_1 receptor density, besides showing greater pressor responses to intracerebroventricular (i.c.v.) injection of Ang II and by nanoinjections of Ang II into the preoptic area, nTS, RVLM and caudal ventrolateral medulla (CVLM) (Agarwal et al., 2011; Du et al., 2013; Matsuda et al., 1987; Muratani et al., 1991; Nakagaki et al., 2011; Phillips and de Oliveira, 2008; Wright et al., 1987; Zhu et al., 1998). More recently, Agarwal et al. demonstrated that levels of ACE_2 and Mas receptor in the PVN and RVLM are reduced in SHR (Agarwal et al., 2011). Besides the involvement of central RAS in the pathophysiology of hypertension, Biancardi and colleagues suggested that changes in peripheral levels of Ang II modify the central permeability, which would facilitate its access to brain regions strongly involved in the control of blood pressure (Biancardi et al., 2014).

Although blood flow assessments show that different vascular beds are involved in arterial pressure changes evoked from both CVLM (Ferreira et al., 2008) and RVLM (Dampney, 1994; Dampney and McAllen, 1988; de Paula and Machado, 2001; Dean et al., 1992; Lovick, 1987; Willette et al., 1987), the question that still remains open is which peripheral vascular bed would be controlled by Ang II and Ang-(1–7) signaling in the RVLM. Since RVLM and the peptides of RAS are important players in cardiovascular regulation, the present study shall evaluate whether nanoinjections of Ang-(1–7) and Ang II into the RVLM are able to alter regional blood flow in hypertensive rats.

2. Materials and methods

2.1. General procedures

2.1.1. Surgeries

Experiments were performed in adult male Wistar and spontaneously hypertensive rats (260–300 g). All experiments conformed to the regulation set forth by the Institutional Animal Welfare Committee (CETEA, UFMG), which are in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH publication 80-23, revised in 1996). After anesthesia with urethane (1.2 g/kg i.p., Sigma Chemical Co) animals were submitted to surgical procedures as described previously (Ferreira et al., 2008). Briefly, all the animals underwent tracheotomy and catheterization of femoral artery for arterial pressure measurement. Afterwards, animals were placed in a stereotaxic head holder (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. A heating pad maintained a constant body temperature (35–37 °C).

2.1.2. Injections into RVLM

Injections into the RVLM were performed with a thin tip glass micropipette as previously described (Alzamora et al., 2006; Ferreira et al., 2008). Unilateral injections of Ang-(1–7) (40 ng; Bachem), Ang II (40 ng; Bachem) or sterile saline (vehicle — NaCl 0.9%) were made into the RVLM (2.1 mm anterior, 1.8 mm lateral to the obex, and just above pia mater in the ventral surface). For all experiments, only one site of the RVLM was tested per animal and peptides were injected in a random order.

Arterial pressure and heart rate changes produced by injections into RVLM were recorded continuously (MP150 — AcqKnowledge Software/ Biopac System). A minimum interval of 20 min was waited between the pipette positioning and first central injection. A period of 30 min was waited among injections into RVLM.

2.1.3. Cardiac output measurements

Cardiac output (CO) was measured by the thermodilution method using a CARDIOTHERM 500 apparatus (Columbus Instrument, Columbus, OH). A thermistor (Fr1.5 microprobe, outer diameter of 0.64 mm) was inserted into the aortic arch through the left carotid artery for blood temperature measurement in WT (n = 6) and SHR (n = 4). A polyethylene catheter (PE10) was placed into the right atrium via jugular vein for saline injection. CO was measured by rapidly injecting 0.1 ml of cold saline (18–20 °C) with a pump (HAMILTON, Microlab 500 series) into the right atria as a thermal tracer indicator. Three to four thermodilution curves were generated (minimum of 10 min interval) in the control period to assure the reproducibility of the measurement. The CO values obtained 5 min before and at the peak of the response elicited by RVLM microinjections were used to express the baseline and peptide-evoked responses, respectively. Total peripheral resistance (TPR) was calculated from MAP and CO values (TPR = MAP/CO, mm Hg × ml^{-1} × min^{-1}).

2.1.4. Blood flow measurements

In different groups of WT and SHR, femoral (n = 10 and 9), renal (n = 6 and 4) or mesenteric (n = 6 and 5) blood flow were determined according to the method of Welch and colleagues (Welch et al., 1995) using a transit-time blood flowmeter (model T206; Transonics, NY, USA). A midline laparotomy was performed and miniature ultrasonic transit-time flow probe (0.5 or 0.7 mm V-series) was carefully placed around the artery (mesenteric, renal or femoral). Blood flow was recorded in an acquisition system (MP150 — Acqknowledge Software/ Biopac System). After 20 min of stabilization period, injections into RVLM were performed as described above (see section — Injections into RVLM). Mean vascular resistance was calculated as the ratio between mean arterial pressure and mean blood flow (mm Hg/ml/min).

2.2. Histological verification of injection sites

At the end of the experiments, the animals were euthanized with an overdose of anesthetic and the brain stem was carefully removed and fixed in 10% phosphate-buffered formalin for histological examination. Serial coronal sections (40–50 μm) of the medulla oblongata were performed and stained with neutral red. The atlas of Paxinos and Watson (Paxinos and Watson, 1986) was used as reference. Only experiments with injections confined to the ventral part of the paragigantocellular nucleus were used in this study.

2.3. Analysis

Values at the peak of the responses were considered for each nanoinjection. All values were expressed as means ± SEM. Values obtained before and after injections into RVLM were evaluated by Student's t-test. Comparisons among different groups were made by one-way ANOVA followed by Newman–Keuls. Significance was set at $p < 0.05$.

3. Results

Tables 1 and 2 show the pre injection (baseline) values of cardiovascular parameters measured in normotensive (WT) and hypertensive (SHR) animals, respectively, used in current experiments.

3.1. Injections of Ang II and (1–7) into RVLM change heart rate and blood pressure of normotensive and hypertensive rats

Fig. 1 is an example of coronal sections depicting injection site into RVLM. Representative chart recordings (Fig. 2) show the typical responses caused by injections of vehicle, Ang II and Ang-(1–7) into RVLM. In this case, the traces are from an experiment with SHR, where the increases in MAP and BF evoked by injections of Ang II and Ang-(1–7) were greater. Injections of Ang-(1–7) and Ang II into the RVLM of SHR produced a higher pressor response when compared to those evoked by the same injections in WT (ΔMAP: 17 ± 2 vs. 12 ± 1 mm Hg and ΔMAP: 21 ± 2 vs. 13 ± 1 mm Hg; $p < 0.05$, respectively). Injections of saline into RVLM of SHR and WT induced small and