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Activation of angiotensin-(1–7)/Mas axis in the brain lowers blood pressure and attenuates cardiac remodeling in hypertensive transgenic (mRen2)27 rats

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ABSTRACT

Activation of the peripheral angiotensin-(1–7)/Mas axis of the renin–angiotensin system produces important cardioprotective actions, counterbalancing the deleterious actions of an overactivity of Ang II/AT_1 axis. In the present study we evaluated whether the chronic increase in Ang-(1–7) levels in the brain could ameliorate cardiac disorders observed in transgenic (mRen2)27 hypertensive rats through actions on Mas receptors. Sprague Dawley (SD) and transgenic (mRen2)27 hypertensive rats, instrumented with telemetry probe for arterial pressure (AP) measurement were subjected to 14 days of ICV infusion of Ang-(1–7) (200 ng/h) or Ang-(1–7) associated with Mas receptor antagonist (A779, 1 μg/h) or 0.9% sterile saline (0.5 μl/h) through osmotic mini-pumps. Ang-(1–7) infusion in (mRen2)27 rats reduced blood pressure, normalized the baroreflex control of HR, restored cardiac autonomic balance, reduced cardiac hypertrophy and pre-fibrotic alterations and decreased the altered imbalance of Ang II/Ang-(1–7) in the heart. Further, there was an attenuation of the increased levels of atrial natriuretic peptide, brain natriuretic peptide, collagen I, fibronectin and TGF-β in the heart of (mRen2)27 rats. Furthermore, most of these effects were mediated in the brain by Mas receptor, since were blocked by its selective antagonist, A779. These data indicate that increasing Ang-(1–7) levels in the brain can attenuate cardiovascular disorders observed in (mRen2)27 hypertensive rats, probably by improving the autonomic balance to the heart due to centrally-mediated actions on Mas receptor.

1. Introduction

The renin–angiotensin system (RAS) plays a key role in the control of arterial pressure (AP) and hydroelectrolytic balance (Hall, 1991). However, the inappropriate overactivity of the angiotensin-converting enzyme (ACE)/angiotensin (Ang) II/AT_1 receptor axis, is critically involved in the pathogenesis of cardiovascular diseases. On the other hand, the ACE2/Ang-(1–7)/Mas receptor axis can counterbalance and attenuate the deleterious actions of Ang II (Ferrario et al., 2010; Santos, 2014). It is well known that increase in circulating levels of Ang-(1–7) induces important cardioprotective actions mediated by Mas receptors (Ferrario et al., 2010; Santos, 2014). Ang-(1–7) is capable to induce antiarrhythmogenic effect against ischemia/reperfusion injuries in rats (Ferreira et al., 2001; Santos, 2014), as well as, to prevent atrial tachycardia and fibrillation in rats and dogs (Ferreira et al., 2011; Liu et al., 2011), cardiac hypertrophy and Ang II-induced pathological cardiac remodeling (Santos, 2014).

In the brain, Ang-(1–7) is a powerful facilitator of the bradycardic component of the baroreflex control of heart rate (HR) in normotensive (Campagnole-Santos et al., 1992; Santos, 2014) or hypertensive animals (Benter et al., 1995; Britto et al., 1997; Heringer-Walther et al., 2001; Oliveira et al., 1996). ICV infusion of Ang-(1–7) inhibits sympathetic outflow and increases vagal outflow in rabbits with chronic heart failure, thus contributing to enhanced baroreflex gain in this condition (Kar et al., 2011). In addition, ICV infusion of Ang-(1–7) for 4 weeks significantly reduces the expression of Ang II and AT_1 receptors in the brain of spontaneous hypertensive rats (Jiang et al., 2013).

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We have previously shown that chronic ICV infusion of Ang-(1–7) attenuated hypertension and prevented the increase in collagen type I mRNA expression, normalized the baroreflex control of the AP and the autonomic tone to the heart in DOCA-salt rats (Guimaraes et al., 2012). Similarly, selective overexpression of ACE2 throughout the brain attenuated neurogenic hypertension (Feng et al., 2010). Moreover, ACE2 gene therapy in the paraventricular nucleus of the hypothalamus in mice prevented Ang II mediated oxidative stress in the brain and restored autonomic dysfunction (Xia et al., 2011). The actions of Ang-(1–7) in the brain seems to be mostly mediated by Mas receptor (Santos et al., 2005) which was shown to be expressed in cardiovascular-related areas in the central nervous system (Becker et al., 2007; Freund et al., 2012).

More recently, Xue et al. (2013) showed that ICV infusion of A779, a selective Mas receptor antagonist (Santos et al., 1994) significantly augmented the blood pressure of DOCA-salt induced hypertension in female rats. Moreover these authors showed that ICV Ang-(1–7) infusion attenuated the increased blood pressure observed after ovariectomy in female DOCA-salt rats (Xue et al., 2013).

Transgenic model of renin-dependent hypertension created by insertion of the mouse Ren-2 gene into the rat genome, the transgenic (mRen2)27 hypertensive rats (Mullins et al., 1990), exhibit high levels of Ang II in the brain and develop hypertension at early age (Mullins et al., 1990). Elevated Ang II/Ang-(1–7) ratio in the medulla of transgenic (mRen2)27 hypertensive rats (Senanayake et al., 1994) is accompanied by impaired baroreflex function (Diz et al., 2008). Furthermore, these rats exhibit cardiac fibrosis, remodeling, hypertrophy and cardiac dysfunction (Langheinrich et al., 1996). Previous studies showed that ICV administration (Dobruch et al., 2003) of Ang-(1–7) or the delivery of an Ang-(1–7) fusion protein in the cisterna magna (Garcia-Espinosa et al., 2012) attenuated the hypertension of (mRen2)27 hypertensive rats.

Considering that Ang-(1–7) lowers blood pressure, we hypothesized that chronic increase in Ang-(1–7) levels in the brain could ameliorate cardiac disorders observed in transgenic (mRen2)27 hypertensive rats through actions on Mas receptor. To address this hypothesis, we evaluated the cardiovascular parameters in (mRen2)27 rats subjected to chronic intracerebroventricular (ICV) infusion of Ang-(1–7) or Ang-(1–7) with the selective Mas receptor antagonist.

2. Material and methods

The procedures used for: blood pressure monitoring, ICV infusion, baroreflex test, cardiac autonomic tone evaluation, echocardiography, histological analysis, measurement of ACE and ACE2 activity, angiotensin levels, AT_1 and Mas receptor protein expression, ANP, BNP, TGF-β levels and mRNA expression of components of the extracellular matrix are described only in the online Data Supplement.

2.1. Animals

Male heterozygous (mRen2)27 rats (10–12 weeks old) were separated in different groups: hypertensive control [(mRen-2)27; *n*: 18], hypertensive treated whit Ang-(1–7) [(mRen-2)27 A7; *n*: 18], hypertensive treated with Ang-(1–7) plus A779 [(mRen-2)27 A7 + A779; *n*: 18] and age-matched normotensive control Sprague–Dawley (SD; *n*: 16). Animals were obtained from the animal facility of the Laboratory of Hypertension, Institute of Biological Sciences, Federal University of Minas Gerais, Brazil. The rats were housed in the animal facility and kept at controlled room temperature (22–24 °C) and 12/12 h light/dark cycle. All experimental protocols were approved by the institutional committee that regulates the use of laboratory animals – Comitê de Ética no Uso de Animais (CEUA/UFMG, protocol 49/2013) and were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

2.2. Statistical analysis

Data are expressed as mean ± SEM. Differences among groups were assessed by one-way ANOVA followed by Newman–Keuls *post-hoc* test and were performed with GraphPad Prism software (version 5.0). The criterion for statistical significance was set at $p < 0.05$.

3. Results

3.1. Baseline systolic, diastolic, mean arterial pressure and heart rate

As shown in Fig. 1, conscious freely moving (mRen-2)27rats presented higher baseline SBP, DBP and HR compared to SD rats ($n = 5$). As expected, (mRen2)27-A7 showed a significant attenuation of hypertension [SBP = 168 ± 10 mmHg *vs.* 201 ± 3 mmHg in (mRen-2)27; or ΔMAP = −26 ± 4 mmHg in comparison to baseline, $n = 6$ each, Fig. 1A–C] and normalized baseline HR (372 ± 6 beats/min *vs.* 392 ± 6 beats/min, mmHg in (mRen-2)27; Fig. 1D). Furthermore, the co-infusion with A779 reversed the beneficial effects of Ang-(1–7) ICV infusion on BP and HR, suggesting that these effects were mediated by Mas receptor (Fig. 1). No alteration in the circadian rhythm of AP or HR was observed by any treatment.

3.2. Cardiac function

High blood pressure, activation of the tissue RAS and increase in the sympathetic drive altogether contribute to cardiac hypertrophy and dysfunction. Therefore, we have next assessed cardiac function by echocardiography. As it can be seen in Table S2, there was an increase in the left ventricle (LV) mass (990 ± 15.4 mg), the interventricular septum in systole (2.67 ± 0.16 mm), in LV posterior wall thickness in systole (3.21 ± 0.10 mm) and diastole (2.10 ± 0.07 mm) in (mRen-2)27 in comparison to SD normotensive rats (559 ± 36 mg, 2.30 ± 0.11 mm, 2.66 ± 0.07 mm, 1.57 ± 0.04, respectively). These changes show significant cardiac remodeling in hypertensive (mRen-2)27 rats. Ang-(1–7) infusion significantly altered LV mass and LV internal diameter in systole and diastole (778 ± 88 mg, 4.25 ± 0.09 mm, 7.86 ± 0.29, respectively), in hypertensive rats. A779 infusion prevented LV mass attenuating effect of Ang-(1–7). (mRen-2)27 and (mRen-2)27 A7 + A779 rats also showed an increased stroke volume and cardiac output compared to SD, characterizing a hyperdynamic state. The alteration in these cardiac parameters was significantly reduced by chronic infusion with Ang-(1–7).

3.3. Cardiac hypertrophy

In addition to the evaluation made with echocardiography, cardiac hypertrophy was evaluated by the morphometric measurement of the cardiomyocytes. As can be seen in Fig. 2 (mRen-2)27 rats had increased diameter of the cardiomyocytes in the free wall of the LV and (mRen2)27 + A7 rats had noticeable attenuation of cardiomyocyte hypertrophy (13 ± 0.11 μm *vs.* 14.4 ± 0.12 μm in (mRen2)27; $n = 5$ each; Fig. 2B, C and E). Moreover, this effect was blocked by the co-infusion of A779 (14 ± 0.13 μm, $n = 4$, Fig. 2D and E).

3.4. Baroreflex control of HR

Considering the effects of ICV infusion of Ang-(1–7) on the cardiac function and remodeling, we next evaluated its effect on the sensitivity of the baroreflex control of HR. (mRen-2)27 hypertensive rats presented ~50% reduction in the sensitivity of the baroreflex bradycardia (0.39 ± 0.06 ms/mmHg, $n = 5$) compared to SD rats (0.78 ± 0.08 ms/mmHg, $n = 6$; Fig. 3). In contrast, chronic ICV infusion of Ang-(1–7) normalized baroreflex sensitivity index in (mRen2)27 rats (0.64 ± 0.05 ms/mmHg, $n = 6$; Fig. 3), which was completely abolished by the co-infusion with A779 (0.31 ± 0.05 ms/mmHg, $n = 6$; Fig. 3). The improvement in baroreflex bradycardia in

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