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Chronic infusion of angiotensin-(1-7) into the lateral ventricle of the brain attenuates hypertension in DOCA-salt rats

Priscila S. Guimaraes, Nivia M. Santiago, Carlos H. Xavier, Elizabeth P. P. Velloso, Marco A. P. Fontes, Robson A. S. Santos, and Maria Jose Campagnole-Santos

National Institute of Science and Technology-Nanobiofar, Department of Physiology and Biophysics, Biological Science Institute, Federal University of Minas Gerais, Belo Horizonte, MG

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Guimaraes PS, Santiago NM, Xavier CH, Velloso EP, Fontes MA, Santos RA, Campagnole-Santos MJ. Chronic infusion of angiotensin-(1-7) into the lateral ventricle of the brain attenuates hypertension in DOCA-salt rats. *Am J Physiol Heart Circ Physiol* 303: H393–H400, 2012. First published June 1, 2012; doi:10.1152/ajpheart.00075.2012.—Angiotensin-(ANG)-(1-7) is known by its central and peripheral actions, which mainly oppose the deleterious effects induced by accumulation of ANG II during pathophysiological conditions. In the present study we evaluated whether a chronic increase in ANG-(1-7) levels in the brain would modify the progression of hypertension. After DOCA-salt hypertension was induced for seven days, Sprague-Dawley rats were subjected to 14 days of intracerebroventricular (ICV) infusion of ANG-(1-7) (200 ng/h, DOCA-A7) or 0.9% sterile saline. As expected, on the 21st day, DOCA rats presented increased mean arterial pressure (MAP) (≈40%), and impaired baroreflex control of heart rate (HR) and baroreflex renal sympathetic nerve activity (RSNA) in comparison with that in normotensive control rats (CTL). These changes were followed by an overactivity of the cardiac sympathetic tone and reduction of the cardiac parasympathetic tone, and exaggerated mRNA expression of collagen type I (≈9-fold) in the left ventricle. In contrast, DOCA rats treated with ANG-(1-7) ICV had an improvement of baroreflex control of HR, which was even higher than that in CTL, and a restoration of the baroreflex control of RSNA, the balance of cardiac autonomic tone, and normalized mRNA expression of collagen type I in the left ventricle. Furthermore, DOCA-A7 had MAP lowered significantly. These effects were not accompanied by significant circulating or cardiac changes in angiotensin levels. Taken together, our data show that chronic increase in ANG-(1-7) in the brain attenuates the development of DOCA-salt hypertension, highlighting the importance of this peptide in the brain for the treatment of cardiovascular diseases.

renin-angiotensin system; deoxycorticosterone acetate-salt; baroreflex; collagen

THE RENIN-ANGIOTENSIN SYSTEM (RAS) is a key humoral mechanism to control arterial pressure and the hydroelectrolytic balance. However, the overactivity of this system toward the accumulation of tissue ANG II is importantly involved in the development of cardiovascular diseases (18, 42). Angiotensin-(1-7) is now recognized as an important modulator of cardiovascular function, and its actions mainly counterbalance the effects of ANG II in different tissues in several pathophysiological conditions (36).

In the brain, ANG-(1-7) is a powerful facilitator of the bradycardic component of the baroreflex control of heart rate (HR) in normotensive (6) or hypertensive animals (4, 10, 20, 32). The nucleus tractus solitarii (NTS) seems to be a site in the brain involved in the baroreflex facilitatory effect of ANG-(1-7) (8, 10). Intracerebroventricular (ICV) infusion of the specific ANG-(1-7) antagonist, A779, has highlighted the importance of endogenous ANG-(1-7) in the brain in mediating the benefits observed after central or peripheral treatment with angiotensin I-converting enzyme (ACE) inhibitors (4, 20). Moreover, chronic shift of brain RAS toward ANG-(1-7) synthesis by overexpression of ACE2 in different cardiovascular-related areas attenuates hypertension (11, 45). Such benefits appear to be mediated by a combination of a direct effect of ANG-(1-7) on receptor Mas (37), which is expressed in cardiovascular-related areas in the central nervous system (1), with counterbalancing effect of the ANG II effects on ANG II type 1 receptor (AT_1). These and other data from the literature support the beneficial role of ANG-(1-7) in the brain when the treatment of cardiovascular diseases is concerned.

In DOCA-salt model, a neurohumoral model of hypertension, in which tissues RAS are involved with the development and maintenance of hypertension, subcutaneous infusion of ANG-(1-7) prevents mid-myocardial and perivascular collagen deposition without affecting the level of blood pressure and cardiac hypertrophy (17). In addition, we have recently shown that a life-time increase in circulating ANG-(1-7) levels attenuates the development of DOCA-salt hypertension, as well as reduces left ventricle remodeling and cardiac dysfunction (35). These effects were accompanied by an important increase in ANG-(1-7) in the left ventricle (LV) that might have contributed to the attenuation of cardiac dysfunction and prefibrotic lesions (35).

Because hyperactivity of the brain RAS is an important factor in different models of hypertension (18, 42), such as DOCA-salt (21, 44), and ANG-(1-7) is implicated with several benefits of hypertension treatment (17, 35, 36), in this study we sought to evaluate whether a chronic increase in ANG-(1-7) levels in the brain would attenuate the progression of hypertension. To achieve this goal, DOCA-salt hypertensive rats were concomitantly subjected to a chronic lateral ventricle infusion of ANG-(1-7). As a first step, measurements of mean arterial pressure (MAP) and HR, baroreflex control of HR and efferent sympathetic nerve activity, and cardiac autonomic tone were performed. Furthermore, the level of angiotensin peptides and mRNA expression of collagen type I and III in the LV were analyzed as well.

Address for reprint requests and other correspondence: M. J. Campagnole-Santos, Depto de Fisiologia e Biofísica, Universidade Federal de Minas Gerais, Av. Antonio Carlos, 6627-ICB, 31270-901; Belo Horizonte, MG (e-mail: mjcs@icb.ufmg.br).

METHODS

Animals

Experiments were performed in male Sprague-Dawley rats (12–14 weeks of age, 300–400 grams of body wt) from the animal facilities of the Laboratory of Hypertension, ICB, Federal University of Minas Gerais, Brazil. The project was approved by the institutional committee that regulates the use of laboratory animals (Comitê de Ética em Experimentação Animal-CETEA/Federal University of Minas Gerais; protocols 137/2006 and 67/2007).

DOCA-salt Hypertension

Under ketamine (80 mg/kg) and xylazine (6 mg/kg) anesthesia, all rats were subjected to unilateral nephrectomy. A set of rats also received a silicon pellet (Silicone rubber encapsulant; Down Corning) containing DOCA (200 mg/kg) implanted subcutaneously between the scapulae. Two days after surgery, DOCA rats received a 0.9% NaCl + 0.2% KCl solution to drink. Normotensive control rats (CTL) received tap water to drink.

Chronic ICV Infusion of ANG-(1-7)

Seven days after inducing DOCA-salt hypertension, the lateral ventricle of the brain [dorsoventral: −4.5 mm, mediolateral: −1.5 mm, anteroposterior: −1.0 mm from Paxinos and Watson Atlas (33)] was cannulated to infuse ANG-(1-7) (200 ng/h; Bachem; DOCA-A7 group) or 0.9% sterile saline (0.5 μl/h; DOCA and CTL groups) for 14 days. Infusion was performed using an osmotic minipump (Alzet, model 2002) attached to the cannula through a silicon tube (3.0 cm) and placed subcutaneously between the scapulae. The site of infusion was verified postmortem by the presence of Alcian blue dye (2%/5 μl), injected through the ICV cannula, only in the ventricular system.

Experimental Designs

Study 1: evaluation of the baroreflex control of HR and the autonomic control of HR in conscious freely moving rats. On the 19th day after DOCA surgery, rats were anesthetized with the mixture of ketamine and xylazine for implanting polyethylene catheters into the femoral artery and vein for arterial pressure recording and intravenous injections, respectively. On the 20th day, pulsatile arterial pressure, MAP, and HR were continuously monitored by a data acquisition system (*Acqknowledge* software 4.1; Biopac System). The baroreflex control of HR was determined by the reflex changes in HR [converted into pulse interval (PI)] in response to transient increases in MAP (in mmHg) induced by bolus injection (0.1 ml) of phenylephrine (PE; 2.5 to 50 μg/ml iv), as described previously (4, 5, 8, 20, 32). The ratio between maximum changes in HR, expressed as PI (ΔPI; in ms) and maximum increases in MAP (ΔMAP; in mmHg) was used as an index of baroreflex sensitivity (baroreflex bradycardia) for each animal. The baroreflex sensitivity index for each group was calculated by the average of the indices (ΔPI/ΔMAP) of each rat. For illustrative purposes only, the best fit line that correlates mean changes in PI and mean changes in MAP for the entire group was plotted as well.

The cardiac autonomic tone was determined 30 min after the baroreflex analysis. Rats were subjected to methylatropine, a muscarinic blocker (3 mg/kg iv), and the maximum HR was obtained; 15 min later, propranolol, a β-adrenergic blocker (4 mg/kg iv), was injected. On the next day, blockers were injected in the reverse order to obtain the minimal HR. The intrinsic HR was obtained at the end of the protocol (iHR, arithmetic mean from the 2 days). The sympathetic tone was calculated by the difference between the maximum HR (first day) and iHR, and the parasympathetic tone by the difference between iHR and the minimum HR (second day).

Study 2: evaluation of the baroreflex control of renal sympathetic nerve activity in anesthetized rats. Another group of rats, 21 days after DOCA implant, were anesthetized with urethane (1.2 to 1.4 g/ kg ip), tracheostomized, and cannulated into the femoral artery and vein. The adequacy of urethane anesthesia was verified by the absence of a withdrawal response to nociceptive stimulation of a hindpaw. Body temperature was monitored with a rectal thermometer and kept in the range of 36.5° to 37.0°C with a heating lamp. With the use of a retroperitoneal approach, the left renal nerve was isolated, covered with mineral oil, and placed on a silver bipolar electrode. The activity of the renal sympathetic nerve activity (RSNA) was amplified by 10K, filtered (100–1,000 Hz), and subsequently rectified, integrated (reset every second), and displayed online. RSNA, MAP, and HR were acquired using Powerlab 2/4, LabChart 7.2 software (ADInstruments, Australia). All data were digitized at 1 kHz. The noise level of the RSNA recording system was determined postmortem and subtracted from RSNA values (19). Baseline RSNA (in spikes/s) was measured as previously described (39a).

Baroreflex control of RSNA was determined by the reflex RSNA changes in response to transient increases in MAP produced by bolus injections (0.1 ml) of PE (2.5 to 50 μg/ml). Changes in RSNA were converted into percentage, the RSNA before each PE injection was considered 100%, and the ratio between changes in RSNA (in percentages) and changes in MAP (in mmHg) (ΔRSNA/ΔMAP) was used as an index of baroreflex sensitivity for each animal. The sensitivity index of the baroreflex control of sympathetic activity for each group was calculated by the average of the indices (ΔRSNA/ΔMAP) of each rat. For illustrative purpose, the best fit line that correlates mean changes in RSNA and mean changes in MAP for the entire group was plotted.

Study 3: measurement of angiotensin peptides levels and collagen type I and III mRNA expression in the left ventricle DOCA-salt rats. A third set of rats was used in this protocol for radioimmunoassay (RIA) and quantitative RT-PCR analysis (30). On the 21st day after DOCA implant, rats were euthanized, and the trunk blood and the LV were collected. Fresh LV was weighted and quickly sectioned and frozen on dry ice. The LV weight was normalized by tibia length to estimate LV hypertrophy (46). The trunk blood was collected into chilled tubes containing an enzymatic inhibitors cocktail (1 mM *p*-hydroxymercuribenzoate, 30 mM of 1-10-phenanthroline, 1 mM pepstatin A, 1 mM PMSF, and 7.5% EDTA; 50 μl/ml of blood). For RIA, samples of LV were homogenized in 2.5 ml of 4 M guanidine thiocyanate/1% trifluoroacetic acid in water, modified from Campbell DJ and cols, 2004 (7). Total protein was determined by the Bradford method (3). Peptides were extracted onto BondElut C18 phenylsilane cartridge (Varian) and the levels of ANG II and ANG-(1-7) in plasma or LV were measured by RIA, as previously described (2). For quantitative RT-PCR, samples of LV were homogenized using the TRIzol reagent (Invitrogen, San Diego, CA). RNA samples (2 μg) were treated with DNAse, and reverse transcribed using MML-V (Moloney murine leukemia virus; Invitrogen) to obtain cDNA. The endogenous S26 ribosomal, collagen type I or III cDNA were amplified using specific primers (BioteZ Berlin-Buch) and SYBR green reagent (Applied Biosystems) in ABI Prism 7900 platform (Applied Biosystem). Analysis was performed following Livak KJ & Schmittgen TD method (30). Primers sequence of collagen type I are as follows: 5′-TGT TCA GTC TTG TGG ACC TC-3′ (forward) and CCT TAG GC ATT GTG TAT GC-3′ (reverse); collagen type III: 5′-CTT ATT TTG GCA CAG TCC-3′ (forward) and 5′-TTT GAC ATG GTT CTG GCT TCC-3′ (reverse); endogenous control S26: CGA TTC CTG ACA ACC TTG CTA TG-3′ (forward) and CGT GCT TCC CAA GCT CTA TGT (reverse).

Statistical Analysis

Data were expressed as means ± SE. Differences between groups were analyzed by parametric one-way ANOVA followed by Student-Newman-Keuls post hoc test. The criterion for statistical significance was set at $P < 0.05$.

RESULTS

Study 1: Conscious Rats

Baseline MAP and HR. Figure 1 summarizes cardiovascular parameters analyzed in conscious freely moving rats. As expected, baseline MAP was significantly higher (≈45 mmHg) in rats subjected to 20 days of DOCA-salt hypertension compared with CTL. Interestingly, DOCA rats receiving chronic ICV infusion of ANG-(1-7) had a noticeable attenuation of hypertension. However, baseline MAP in DOCA-A7 rats was still significantly higher than in CTL ($P < 0.01$). There were no differences in baseline HR among the experimental groups (Fig. 1).

Baroreflex control of HR. Figure 2 shows that the baroreflex sensitivity index of DOCA rats was reduced by ~50% compared with CTL. In contrast, chronic ICV infusion of ANG-(1-7) not only prevented this attenuation but also produced a clear improvement in baroreflex bradycardia index, which was even higher when compared with CTL. The improvement in baroreflex can also be visualized in the best fit line that correlates the changes in PI and changes in MAP (Fig. 2), in which the line that represents DOCA-A7 rats was greatly shifted to the left, whereas the representative line of DOCA rats was shifted to the right compared with the CTL line.

Cardiac autonomic tone and intrinsic HR. Pharmacological blockade of sympathetic and parasympathetic activity showed that DOCA rats had increased cardiac sympathetic tone (Fig. 3*A*) and reduced cardiac parasympathetic tone (Fig. 3*B*).

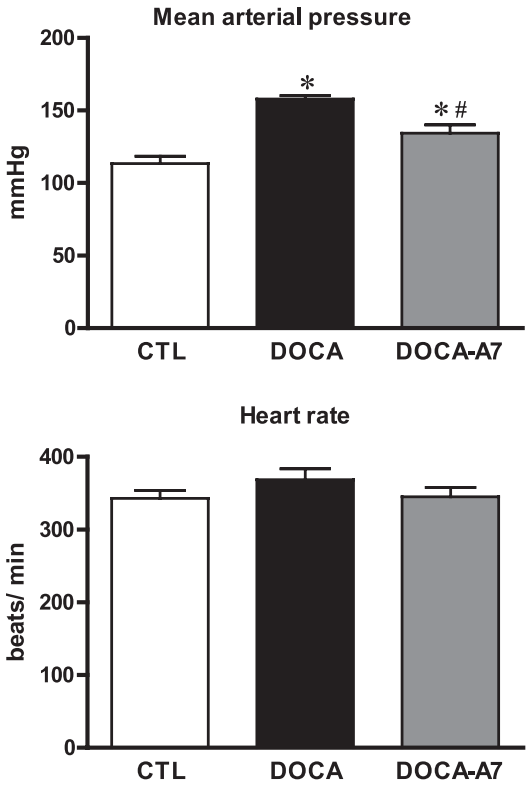

Fig. 1. Baseline mean arterial pressure (in mmHg) and heart rate (in beats/min) in conscious rats after 20 days of DOCA-salt hypertension. Control (CTL), $n = 8$; DOCA, $n = 6$; DOCA-A7, $n = 8$. Data are means ± SE. *$P < 0.01$ vs. CTL; #$P < 0.01$ vs. DOCA; 1-way ANOVA followed by Student-Newman-Keuls post hoc test.

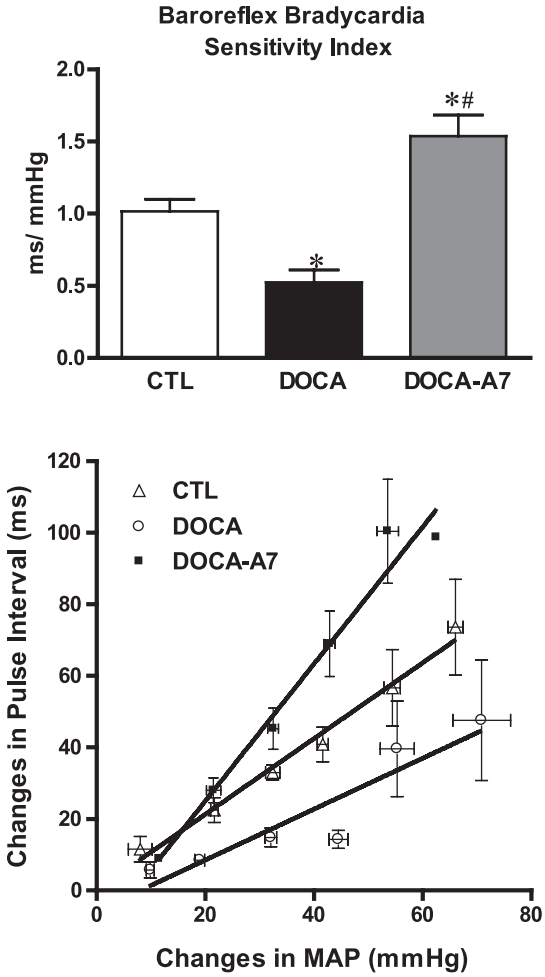

Fig. 2. Baroreflex control of heart rate in conscious rats after 20 days of DOCA-salt hypertension. Bradycardic baroreflex index (in ms/mmHg) and the best-fit regression line between the changes in mean arterial pressure (ΔMAP; in mmHg) produced by bolus intravenous injections of phenylephrine and the baroreflex-mediated changes in heart rate (Δpulse interval; in ms) are shown. CTL, $n = 7$; DOCA, $n = 6$; DOCA-A7, $n = 5$. Data are means ± SE. *$P < 0.01$ vs. CTL; #$P < 0.01$ vs. DOCA; 1-way ANOVA followed by Student-Newman-Keuls post hoc test.

Interestingly, chronic ICV infusion of ANG-(1-7) prevented this cardiac autonomic imbalance. In fact, in DOCA-A7 rats both cardiac sympathetic and parasympathetic tones were comparable with CTL, as shown in Fig. 3, *A* and *B*, and by the re-establishment of the ratio between autonomic tone to the heart (Fig. 3*D*). It is intriguing that intrinsic HR (Fig. 3*C*) was reduced in both DOCA and DOCA-A7 groups compared with CTL.

Study 2: Anesthetized Rats

Baseline mean arterial pressure and heart rate. As observed in Table 1, baseline MAP was significantly attenuated under anesthesia, and there was no difference between MAP levels measured in DOCA and DOCA-A7. However, these baseline values were different from those recorded in CTL rats. There was no statistical difference in baseline HR or baseline RSNA among the groups (Table 1).

Baroreflex control of sympathetic activity. The RSNA baroreflex sensitivity index of DOCA rats was reduced compared

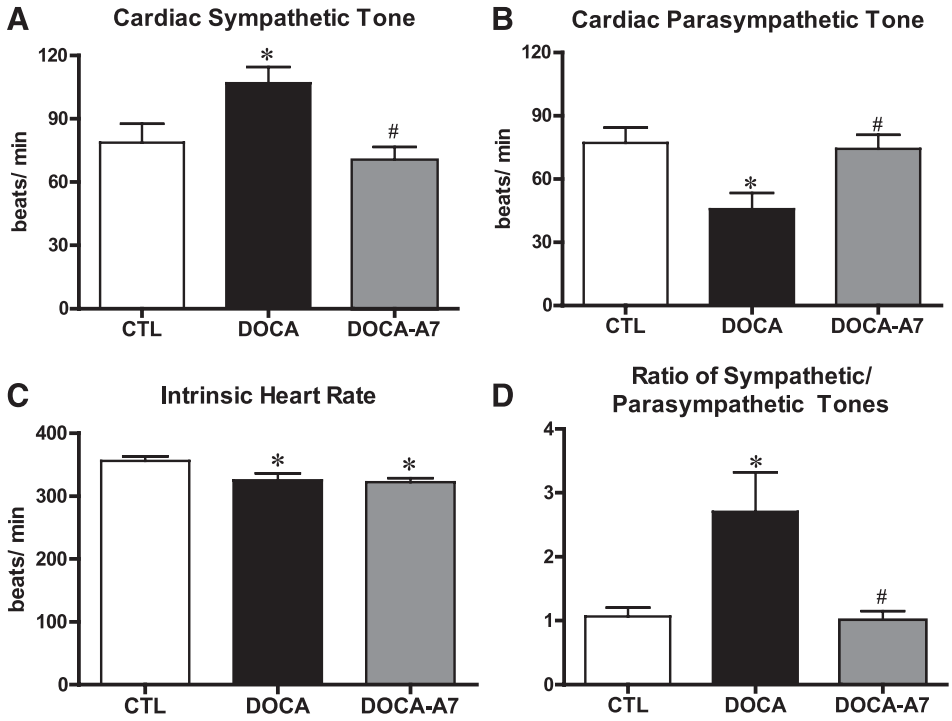

Fig. 3. Cardiac sympathetic tonus (in beats/min; *A*), cardiac parasympathetic tonus (in beats/min; *B*), intrinsic heart rate (in beats/min; *C*), and ratio of cardiac sympathetic and parasympathetic tones (*D*) of conscious rats after 21 days of DOCA-salt hypertension. CTL, $n = 7$; DOCA, $n = 5$; DOCA-A7, $n = 6$. Data are means ± SE. *$P < 0.05$ vs. CTL; #$P < 0.05$ vs. DOCA; 1-way ANOVA followed by Student-Newman-Keuls post hoc test.

with CTL; however, rats that received chronic ICV infusion of ANG-(1-7) presented a significant improvement, being this index comparable with CTL (Fig. 4). The best fit regression line that correlates changes in RSNA and changes in MAP for the entire group, plotted for illustrative purpose, shows that there was no displacement of DOCA-A7 line in relation to the CTL one, whereas there was a great shift to the right of the line that represents DOCA rats (Fig. 4).

Study 3: Plasma and Tissue Analysis

LV relative weight. The ratio between LV weight and tibia length was increased in DOCA rats (0.230 ± 0.006 mg/cm; $n = 16$) and DOCA-A7 rats (0.229 ± 0.005 mg/cm; $n = 19$) compared with CTL (0.203 ± 0.005 mg/cm; $n = 14$; $P < 0.05$), suggesting that DOCA-A7 presented similar LV hypertrophy as DOCA.

Angiotensin peptides levels. As seen in Table 2, DOCA-salt hypertension induced a moderate decrease in plasma and LV levels of ANG II in DOCA and DOCA-A7 rats compared with CTL. However, there was no statistically significant difference among the groups. A slight increase in ANG-(1-7) plasma levels was observed in DOCA and DOCA-A7 groups; likewise, no significant difference among the groups was observed.

Table 1. *Baseline MAP, HR, and RSNA in anesthetized rats*

Parameter	Experimental Groups		
	CTL	DOCA	DOCA-A7
MAP, mmHg	93 ± 1.4	120 ± 3.3*	115 ± 4.1*
HR, beats/min	333 ± 23	302 ± 15	300 ± 25
RSNA, spikes/s	127 ± 13	132 ± 16	126 ± 15

Values are means ± SE; $n = 5$ animals for all groups. MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity. *$P < 0.05$ vs. control (CTL).

mRNA expression of collagen type I and III in the LV. DOCA rats presented an approximately ninefold increase in mRNA expression of collagen type I compared with CTL (Fig. 5). In contrast, the increase in the expression of collagen type I observed in DOCA was abolished in DOCA-A7 rats, which presented values comparable with that of CTL (Fig. 5). There was no difference in the collagen type III mRNA expression among the experimental groups (Fig. 5).

DISCUSSION

The major findings of the present study were that ICV infusion of ANG-(1-7) in DOCA-salt hypertensive rats lowered baseline mean arterial pressure, and normalized the baroreflex control of arterial pressure and the cardiac autonomic tone. Furthermore, such improvements were followed by a blunted increase in the mRNA expression of collagen type I in the LV. Taken together, these data indicate that increased availability of ANG-(1-7) in the brain may have an important impact to attenuating the development of DOCA-salt hypertension.

The overactivity of the ANG II/AT_1 axis in the brain is a key component for the development and maintenance of different types of hypertension (18, 42), such as the DOCA-salt experimental model (21, 44). On the other hand, accumulating evidence shows that ANG-(1-7) may function as a counteracting peptide for ANG II hypertensive effects both acting at the brain (6, 10, 11, 20, 45) or more convincingly at peripheral tissues, such as the heart (17, 35, 36).

In the present study we addressed some of the effects of a chronic and direct increase in ANG-(1-7) levels in the central nervous system of DOCA-salt hypertensive rats. It is interesting that ICV infusion of ANG-(1-7) strongly attenuated the increase in arterial pressure in these animals. Although baseline MAP in DOCA rats increased 40% in comparison with

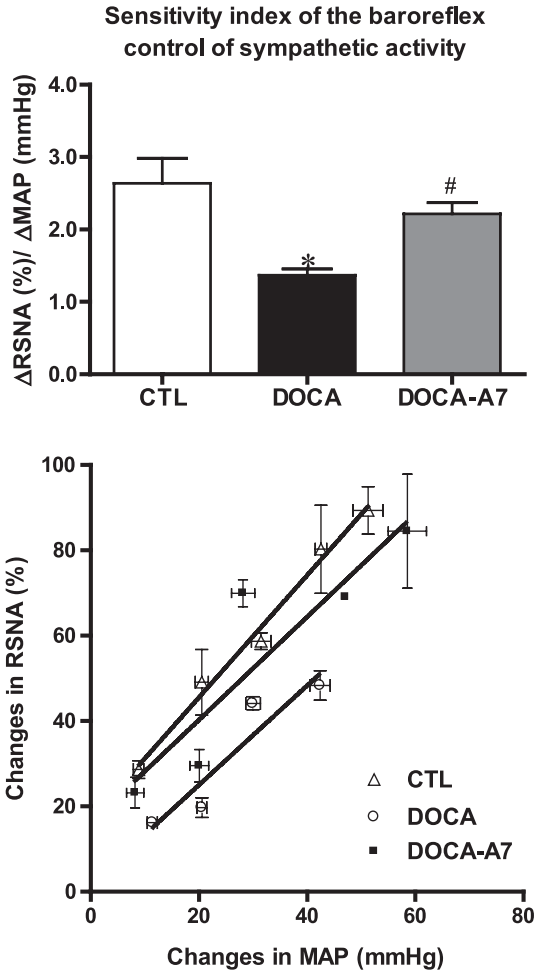

Fig. 4. Baroreflex control of renal sympathetic nerve activity (RSNA) of anesthetized rats after 21 days of DOCA-salt hypertension. Sensitivity index of the baroreflex control of RSNA (Δ%RSNA/mmHg) and the best-fit regression line between ΔMAP (in mmHg) produced by bolus intravenous injections of phenylephrine and the baroreflex-mediated changes in RSNA (Δ%RSNA) are shown. CTL, $n = 5$; DOCA, $n = 5$; DOCA-A7, $n = 5$. Data are means ± SE. *$P < 0.01$ vs. CTL; #$P < 0.05$ vs. DOCA; 1-way ANOVA followed by Student-Newman-Keuls post hoc test.

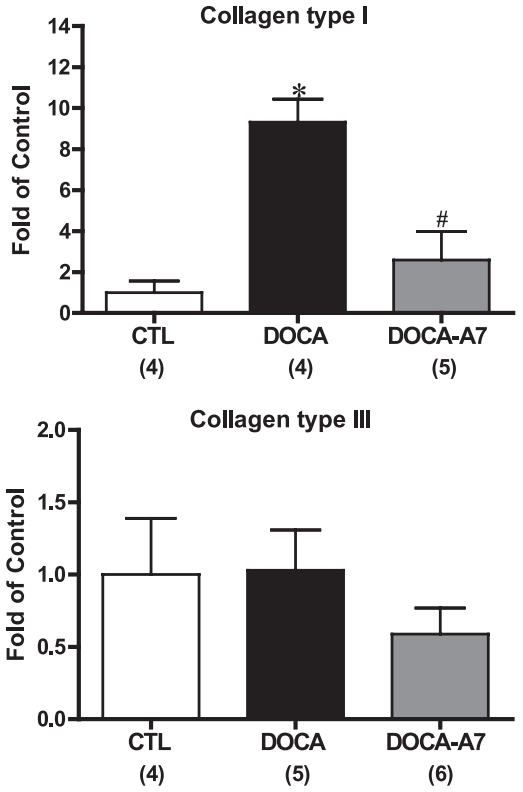

Fig. 5. Expression of mRNA for collagen type I and collagen type III (fold of control) performed by quantitative RT-PCR in the left ventricle of rats after 21 days of DOCA-salt hypertension. *$P < 0.05$ vs. CTL; #$P < 0.05$ vs. DOCA; (n), numbers of rats; 1-way ANOVA followed by Student-Newman-Keuls post hoc test.

CTL rats, MAP in DOCA rats treated with ANG-(1-7) increased ~20%. Several mechanisms may have accounted for the beneficial effects of ANG-(1-7) in the brain during hypertension. It has been shown that endogenous ANG-(1-7) in the brain is involved with the improvement of baroreflex sensitivity (32) after peripheral infusion (4) or after ICV infusion in ACE inhibitor treatment in hypertensive rats (20). More recently, Yamazato and colleagues (45) showed that the overexpression of ACE2 in the rostral ventrolateral medulla (RVLM) decreases MAP and HR in spontaneously hypertensive rats but not in normotensive Wistar-Kyoto rats. In addition, transgenic mice overexpressing ACE2 in different cardiovascular-related regions in the brain, such as NTS and RVLM, developed attenuated neurogenic hypertension partially due to an improvement of spontaneous baroreflex activity and the parasympathetic tone, through activation of the ANG-(1-7)/ Mas axis (11). Our present data are in keeping with these findings and further show that the possible mechanism of the antihypertensive effect triggered by ANG-(1-7) in the brain is related to improvement of baroreflex control and the re-establishment of autonomic control of the circulation.

Table 2. *ANG II-ir and ANG-(1-7)-ir in plasma and left ventricle of rats after 21 days of DOCA-salt hypertension*

Parameter	Experimental Groups		
	CTL	DOCA	DOCA-A7
Plasma, pg/ml			
ANG-(1-7)	39.0 ± 3.2 (5)	42.9 ± 4.2 (6)	47.1 ± 7.6 (4)
ANG II	27.7 ± 7.0 (5)	19.0 ± 4.8 (6)	16.7 ± 1.1 (4)
ANG-(1-7)/ANG II	1.8 ± 0.4 (5)	3.1 ± 0.8 (6)	2.9 ± 0.7 (4)
Left ventricle, pg/mg protein			
ANG-(1-7)	10.8 ± 1.5 (5)	10.6 ± 1.5 (5)	7.8 ± 2.0 (5)
ANG II	9.0 ± 3.2 (5)	4.7 ± 0.5 (5)	4.1 ± 1.0 (5)
ANG-(1-7)/ANG II	1.8 ± 0.5 (5)	2.3 ± 0.4 (5)	1.9 ± 0.1 (5)

Values are means ± SE; (n), number of animals.

Our data showed that 3 wk of DOCA-salt hypertension attenuated the baroreflex control of HR and of RSNA, whereas ICV infusion of ANG-(1-7) restored baroreflex sensitivity to levels seen in the normotensive rats. Previous data from our laboratory have shown that ANG-(1-7) strongly affects the gain of the baroreflex control of HR in other models of hypertension (4, 8, 20). The present study extended this observation, showing that increased brain ANG-(1-7) levels can also improve baroreflex control of sympathetic activity. Recently, Kar et al. (26) have shown that four days of ICV infusion of

ANG-(1-7) induced an enhancement of the baroreflex control of the RSNA in rabbits subjected to chronic heart failure.

It is well recognized that a wide range of cardiovascular diseases are associated with elevated sympathetic outflow (18, 27, 31), which may also precede the increase in blood pressure (23). Additionally, several studies have shown that overactivity of brain RAS and high levels of mineralocorticoids are important factors contributing to imbalance the autonomic nervous system to the periphery toward the sympathetic overactivity (47). In our study, we particularly evaluated the cardiac autonomic tone at rest. It is interesting that we found that DOCA rats presented an imbalanced cardiac autonomic activity, in which the sympathetic branch dominates the parasympathetic one. In contrast, the chronic ICV infusion of ANG-(1-7) in DOCA rats restored this imbalance, since sympathetic and parasympathetic tones were comparable with CTL. It is interesting that despite the high levels of mineralocorticoid continuously delivered in the body, the increase in ANG-(1-7) levels in the brain had a powerful effect against the cardiac autonomic imbalance observed in DOCA-salt hypertension. It is intriguing that the intrinsic HR was reduced in DOCA and DOCA-A7 rats, which suggests a direct effect of the mineralocorticoid on the pacemaker cells. Additional studies will be necessary to address the mechanisms underlying the reduced intrinsic HR observed in DOCA-salt rats.

The improvement of the baroreflex control of arterial pressure and the cardiac autonomic tone may be mainly due to the effect of ANG-(1-7) in hypothalamic and medullary areas related to cardiovascular control. It is well known that ANG-(1-7) improves the bradycardic component of baroreflex when microinjected in the NTS of hypertensive rats (8), or after infusion in the cerebral lateral ventricle of normotensive rats (6). In contrast, ANG-(1-7) is also known to mediate sympathoexcitatory effects in RVLM (12) and paraventricular nucleus of the hypothalamus (39). However, recent data have shown that ANG-(1-7) inhibits norepinephrine release in hypothalamus through nitric oxide pathway in different models of hypertension and prevents the ANG II stimulatory effect in this region (13–15). Even though some acute studies have shown an excitatory effect of ANG-(1-7), it is important to consider that chronic studies clearly show that ANG-(1-7) mediates antihypertensive effects in the brain.

In the present study, we did not observe differences in baseline MAP between anesthetized DOCA and DOCA-A7 rats, neither in baseline RSNA among all groups. Anesthesia could have buffered any pronounced difference that might arise from DOCA-salt or DOCA-salt subjected to ANG-(1-7) infusion, mainly concerning MAP levels. Studies in the literature, and more recently those from Kandlikar and Fink (24, 25), have shown no contribution of renal nerves to mild DOCA-salt hypertension, but these authors have shown a role of the splanchnic sympathetic nerves. Our present data are in agreement with these findings and show that in addition to the splanchnic nerve (25) there is an overactivation of sympathetic efferent activity to the heart. Thus physiopathological mechanism in DOCA-salt hypertension may include peripheral activation of sympathetic tonus to different vascular/organs, other than the kidney.

It is well established that in DOCA-salt model plasma renin activity is inhibited, and consequently the plasma angiotensin levels are also reduced (21, 38), but not suppressed. There is also evidence that myocardial renin and angiotensinogen levels are decreased in this model (28). In line with these observations, plasma and LV levels of ANG II in both DOCA and DOCA-A7 rats were reduced. No significant changes in plasma and LV ANG-(1-7) levels were observed, which rule out the possibility that the cardiovascular effects of chronic infusion with ANG-(1-7) in the lateral ventricle are due to a leakage of the peptide to the periphery.

The DOCA-salt hypertension was accompanied by an increase in LV/tibia length and collagen deposition, suggesting the development of hypertrophy and prefibrotic lesions. Although hypertrophy was not changed by ANG-(1-7) treatment, DOCA-A7 rats had a blunted mRNA expression of collagen type I in the LV. It is known that collagen type I is the most abundant collagen in the myocardium, ~80% of the total (9), which could explain the magnitude of such increase. In contrast, neither DOCA nor DOCA-A7 rats showed an increase in mRNA expression for collagen type III. The increase in the mRNA expression of collagen type III usually happens in the initial phase of this disease, whereas in collagen type I occurs more lately (43). Thus the lack of change in collagen III may be due to the time frame LV was analyzed in our study.

Taken together, our data suggest that the hypertrophy in the DOCA-A7 group might have happened more as a consequence of the increase in blood pressure (afterload) combined with increased cardiac levels of ANG II and effects of the mineralcorticoid. In contrast, the restoration of cardiac autonomic balance in these animals likely had a positive impact on collagen deposition, and this effect was present despite the moderate change in ANG II levels in the heart.

In vitro studies have shown that ANG-(1-7) mediates antifibrotic and antitrophic effects (22, 36, 40). However, in in vivo studies, different results on cardiac hypertrophy versus fibrosis versus high blood pressure have been obtained. The different results among these in vivo studies may be related to levels of ANG-(1-7) achieved locally in the heart. Previous studies from Grobe et al. (16, 17) showed that subcutaneous treatment with ANG-(1-7) reduced cardiac fibrosis without altering hypertrophy. Despite the fact that in such studies the cardiac angiotensin peptides were not measured, it is possible that ANG-(1-7) did not reach reasonable levels to induce a significant change in cardiac hypertrophy and to attenuate hypertension. Similar to this hypothesis, a recent finding of our laboratory (35) showed that transgenic rats with systemic overexpression of ANG-(1-7) presented attenuated hypertension, cardiac hypertrophy, and fibrosis when subjected to DOCA-salt hypertension model. Furthermore, we observed that these effects were accompanied by a remarkable (~4 times) increase in ANG-(1-7) in the LV. On the other hand, in the present study, ANG-(1-7) levels were not changed in the heart; therefore, the lowered blood pressure and the reduced cardiac fibrosis after ICV infusion were likely to be due to a reduction in sympathetic activity to the heart. Hence, in our view, ANG-(1-7), acting directly in the heart or indirectly by modulating the sympathetic outflow, may be sufficient to oppose collagen deposition, whereas the attenuation of cardiac hypertrophy would depend on the combination of reduction in high blood pressure, decrease in sympathetic activity, and a considerable increase in ANG-(1-7) locally in the heart.

It is well established that the autonomic activity affects myocardium remodeling (27, 29, 31, 34, 41). We believe that

both the increase in cardiac parasympathetic activity and the reduction in the sympathetic branch contributed to the blunted mRNA expression of collagen type I in DOCA-A7 rats. Similar to our hypothesis, it is known that the chronic stimulation of the vagus efferent nerve has a markedly beneficial impact in survival in rats with heart failure, at least by improving the cardiac output, decreasing the LV end-diastolic pressure and plasma norepinephrine levels (29). Moreover, chronic stimulation of the carotid sinus in dogs with heart failure decreases the LV end-diastolic pressure and plasma norepinephrine levels; normalizes the expression of cardiac β_1-adrenergic receptors, β-adrenergic receptor kinase, and nitric oxide synthase; and reduces interstitial fibrosis and cardiomyocyte hypertrophy (34). Otherwise, the sustained increase in sympathetic activity in different beds, the elevated levels of plasma norepinephrine, and the reduced HR variability are associated with hypertrophy, LV remodeling, more incidences of arrhythmias, and elevated index of mortality (27, 31).

In the present study we showed that chronic ICV infusion of ANG-(1-7) in DOCA-salt hypertensive rats, prevented changes in *1*) the baroreflex control of HR, *2*) the baroreflex control of RSNA activity, and *3*) the autonomic tone to the heart, which together had a dramatic effect to attenuating hypertension in these animals. Moreover, the combined effects of ANG-(1-7) on the cardiac autonomic tone and the reduced MAP are likely important factors blunting the increase in mRNA expression of collagen type I in the LV observed in DOCA-A7 rats. These results indicate a key role of ANG-(1-7) in the brain to the baroreflex control of arterial pressure and to attenuate hypertension and cardiac fibrosis in animal models. Furthermore, our study supports pharmacologic approaches that consider the increase in ANG-(1-7) levels in the brain to treat arterial hypertension.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: P.S.G., N.M.S., C.H.X., and E.P.P.V. performed experiments; P.S.G., N.M.S., R.A.S.S., and M.J.C.-S. analyzed data; P.S.G., N.M.S., M.A.P.F., R.A.S.S., and M.J.C.-S. interpreted results of experiments; P.S.G. and M.J.C.-S. prepared figures; P.S.G. and M.J.C.-S. drafted manuscript; P.S.G., M.A.P.F., R.A.S.S., and M.J.C.-S. edited and revised manuscript; P.S.G., N.M.S., C.H.X., E.P.P.V., M.A.P.F., R.A.S.S., and M.J.C.-S. approved final version of manuscript; M.J.C.-S. conception and design of research.

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