The nonpeptide ANG-(1–7) mimic AVE 0991 attenuates cardiac remodeling and improves baroreflex sensitivity in renovascular hypertensive rats

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ABSTRACT

Aims: The nonpeptide Ang-(1–7) analog, AVE 0991, is recognized as having beneficial cardiovascular effects similar to those induced by Ang-(1–7). In this study, we evaluated the effects of AVE 0991 on cardiovascular functions and on cardiac and renal remodeling in rats with 2K1C renovascular hypertension.
Main methods: Fisher rats underwent surgery to induce 2K1C renovascular hypertension and were then treated with AVE 0991 (1 or 3 mg/kg) for 28 days. At the end of treatment, the blood pressure (BP), heart rate (HR), and baroreflex sensitivity were evaluated, in conscious animals. The rats were then euthanized and the heart and kidneys removed for subsequent histological analysis.
Key findings: Treatment with AVE 0991 in 2K1C rats restored the baroreflex sensitivity of both bradycardic and tachycardic components to levels comparable to those of normotensive SHAM rats. At a higher dose (3 mg/kg), AVE 0991 was also anti-hypertensive in 2K1C rats. Furthermore, AVE 0991 reduced the heart weight, thickness of myocardial fibers, number of inflammatory cells, and area of collagen deposition in the hearts of 2K1C rats compared to SHAM rats. The inflammatory process and tissue area of collagen deposition were decreased in the clipped kidney of AVE 0091-treated 2K1C rats.
Significance: Our data showed that oral treatment with AVE 0991 reduces blood-pressure cardiac remodeling and improves baroreflex sensitivity in 2K1C renovascular hypertensive rats.

Introduction

Hypertension is characterized by increased activity of the sympathetic nervous system and overactivity of the renin angiotensin system (RAS), accompanied by reduced sensitivity of baroreflex control of arterial pressure (Korner et al., 1974; McCubbin et al., 1956), cardiac hypertrophy and remodeling, and renal lesion (Frohlich et al., 1999; Grobe et al., 2006; Sadjadi et al., 2005a,b).

Cardiac hypertrophy when accompanied by fibrosis may lead to loss of function (Frohlich et al., 1999; Grobe et al., 2006; Schaper, 1998). In addition to affecting cardiac function, hypertension may cause kidney damage, compromising the blood vessels, glomeruli and renal interstitium, causing fibrosis and loss of renal function (Klag et al., 1996; Ljutić and Kes, 2003; Soares et al., 2011). The hyperactivity of some components of the RAS is important in determining the progression of this disease. Angiotensin (Ang) II participates in cardiac remodeling by stimulating myocyte hypertrophy

and fibroblast proliferation (González et al., 2002; Kawano et al., 2000). Conversely, the antitrophic and antifibrotic activity of Ang-(1–7) counteracts these effects (Grobe et al., 2007; Pei et al., 2010; Tallant et al., 2005).

Loot et al. (2002) found that in rats with heart failure, chronic treatment with Ang-(1–7) preserved cardiac function, coronary perfusion and aortic endothelial function. Iwata et al. (2005) demonstrated that in cardiac fibroblasts of adult rats, Ang-(1–7) inhibited collagen synthesis and decreased mRNA expression of growth factors, suggesting an important role of Ang-(1–7) in the regulation of cardiac remodeling. Additionally, Grobe et al. (2007) showed that chronic infusion of Ang-(1–7) prevented myocardial cell hypertrophy of the left ventricle (LV) and interstitial fibrosis induced by infusion of Ang II; and Pei et al. (2010) showed that treatment with Ang-(1–7) in SHR attenuated cardiac hypertrophy and deposition of collagen during hypertensive states.

The renovascular hypertension two-kidney one-clip (2K1C) model is characterized by a hyperactivity of the RAS. In this model, the levels of Ang II increase in both kidneys, clipped and unclipped, thereby increasing plasma levels of Ang II, aldosterone and sympathetic tone, and consequently the blood pressure (BP) (Navar et al., 1998; Von Thun et al., 1994; Zou et al., 1996). Additionally, renal

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production of Ang II modulates the transport of salt and water by the renal tubules as well as the glomerular filtration process and collagen deposition (Ferrario and Varagic, 2010).

An important advance in studies of the effects of Ang-(1–7) was the discovery of non-peptide analog AVE 0991 (Wiemer et al., 2002). Several studies have shown that AVE 0991 acts as a Mas receptor agonist in the kidneys and blood vessels. AVE 0991 also induces release of nitric oxide (Lemos et al., 2005; Santos and Ferreira, 2006) and has a cardioprotective effect by improving cardiac function, reducing atherosclerosis, cardiac hypertrophy and remodeling (Benter et al., 2006; Ferreira et al., 2007b; Wiemer et al., 2002). In addition, recent studies have shown that treatment with AVE 0991 prevented myocardial hypertrophy induced by Ang II (He et al., 2010) and attenuated ventricular remodeling in rats with myocardial infarction (Zeng et al., 2012) through inhibition of inflammatory markers such as signaling TGF-β1/TNF-α and TGF-β1/Smad2.

Given these considerations, this study evaluated the effect of chronic administration of AVE 0991 on BP, HR, sensitivity of the reflex control of HR, cardiac remodeling and renal injury in conscious rats with 2K1C renovascular hypertension.

Materials and methods

Animals

Experiments were performed on male Fisher rats from ENUT, Universidade Federal de Ouro Preto, Brazil. The animals were housed in separate cages in groups of four (2K1C or SHAM), with free access to rat chow and tap water in a temperature- and light-controlled room. All animal procedures were in accordance with the Guidelines for Ethical Care of Experimental Animals, and were performed as approved by the Institutional Ethics Committee of the Federal University of Ouro Preto (Protocol # 2010/55).

Induction of renovascular hypertension

Goldblatt renovascular hypertension was induced as described by Goldblatt et al. (1934). Briefly, the rats (weighing 150–180 g) were anesthetized with a mixture of ketamine and xylazine (50 mg/kg and 10 mg/kg respectively, *ip*), and a silver clip (0.20 mm ID) was placed around the left renal artery through a midline incision (Goldblatt renovascular hypertension, 2-kidney, 1-clip model; 2K1C). Other rats were submitted to similar procedures but without the renal-artery clip placement (SHAM group or normotensive rats). Cardiovascular and histological measurements were carried out 30 days after the surgery.

Arterial pressure measurements

2K1C and SHAM rats were anesthetized with a mixture of ketamine and xylazine (50 mg/kg and 10 mg/kg respectively, *ip*) and 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 drug injections in order to evaluate the baroreflex sensitivity. Pulsatile arterial pressure was monitored by a Gould pressure transducer (PM-1000, CWE) coupled to a blood pressure signal amplifier (UIM100A, Powerlab System). Mean arterial pressure (MAP) and heart rate (HR) were determined from the arterial pressure wave. All variables were continuously recorded with a PowerLab digital acquisition system (PowerLab 4/20, ADInstruments) with an 800 Hz sampling rate.

Intragastric treatment with AVE 0991 or vehicle

Three days after the surgery to induce 2K1C or SHAM, intragastric administration of AVE 0991 at a dose of 1 mg/kg or 3 mg/kg, or of the Vehicle (KOH, 1 ml of 10 mM KOH added to 9 ml of distilled water)

by gavage was started and continued daily for 28 days, always at the same time of day. The animals received around 0.2 ml solution of AVE 0991 (1 or 3 mg/kg) or vehicle.

Evaluation of baroreflex sensitivity

The sensitivity of the baroreflex control of HR was determined by recording reflex HR changes in response to transient increases (baroreflex bradycardia) or decreases (baroreflex tachycardia) in MAP produced by repeated bolus injections of graded doses of phenylephrine (0.5 to 50.0 μg, iv) or sodium nitroprusside (0.5 to 50.0 μg, iv), respectively, in conscious rats. Evaluations of the sensitivity of reflex bradycardia or tachycardia were performed randomly, with a 15-min interval between them. The HR was converted to pulse interval (PI, ms) by the formula: 60,000/HR. A best-fit regression line was drawn from MAP and HR changes obtained with the different doses of phenylephrine or sodium nitroprusside for each animal. The slope of the regression line was used as an index of baroreflex sensitivity (baroreflex gain), as in previous studies (Alzamora et al., 2006).

Analysis of cardiac and renal structures

For the histopathological analysis, hearts and kidneys were collected and fixed in 10% neutral-buffered formalin solution. After 72 h of fixation, the hearts and kidneys were dehydrated, cleared, and embedded in paraffin. The paraffin block was cut into 4–5-μm
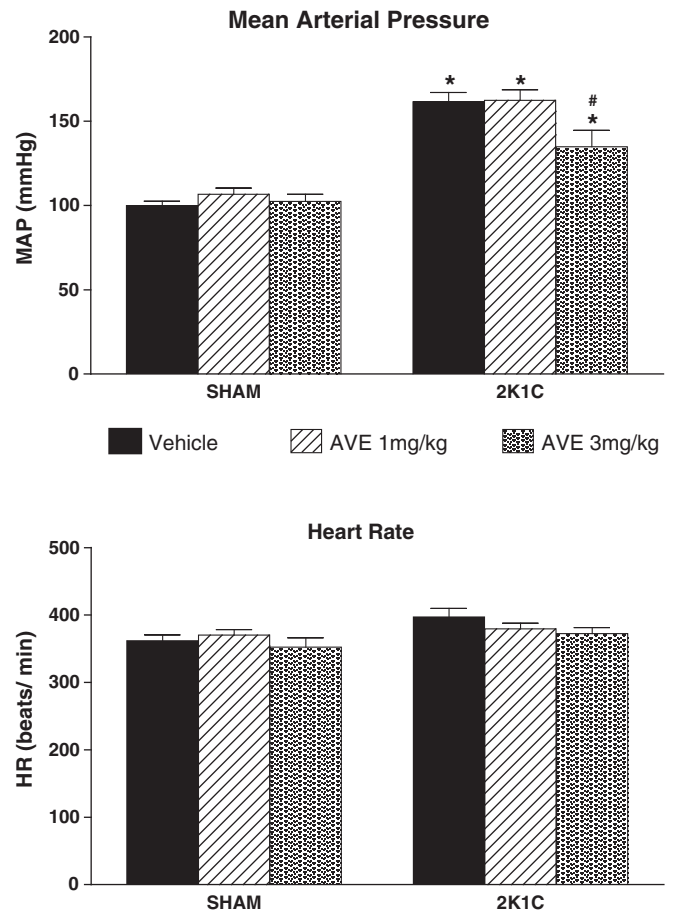

Fig. 1. Baseline levels of mean arterial pressure (MAP, mm Hg) and heart rate (HR, beats/minute) in normotensive rats (SHAM, n = 5–7) and hypertensive rats (2K1C, n = 7–9) treated, per gavage, for 28 days with vehicle or AVE 0991 (1.0 mg/kg or AVE 3.0 mg/kg). $^*p < 0.05$ compared to SHAM vehicle group; $^\#p < 0.05$ compared to 2K1C vehicle group (two-way ANOVA, followed by Bonferroni).
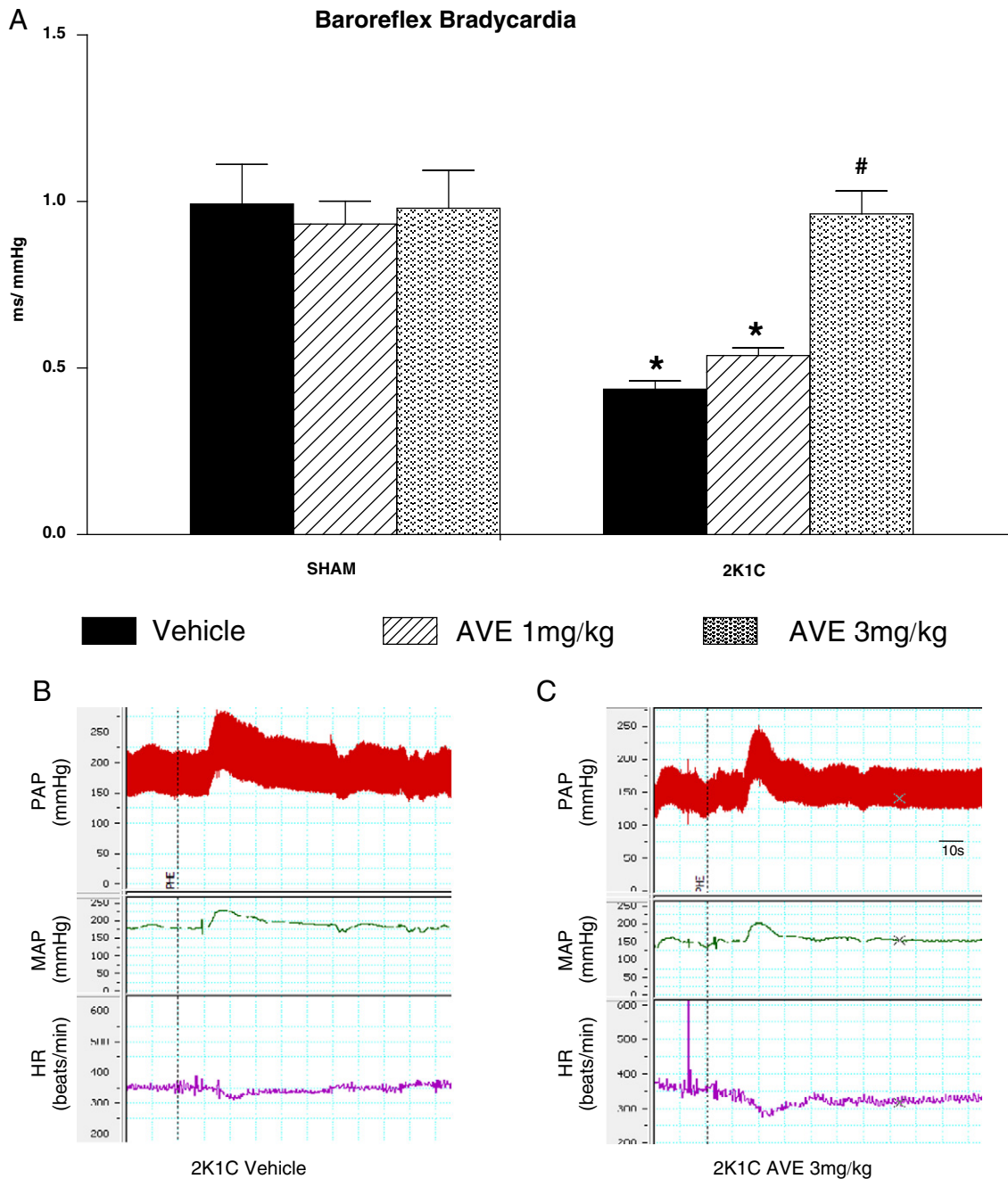
Fig. 2. Index of the sensitivity of the baroreflex bradycardia (ms/mm Hg, panel A) in normotensive rats (SHAM, n = 5–7) or in hypertensive rats (2K1C, n = 7–9) treated, per gavage, with vehicle or AVE 0991 (1.0 mg/kg or 3.0 mg/kg). Blood-pressure recording showing a typical response induced by injection of phenylephrine (4.0 μg, i.v.) in 2K1C vehicle rats (panel B) and in 2K1C treated with AVE 0991 at the dose of 3.0 mg/Kg (panel C). PAP = pulsate arterial pressure, MAP = mean arterial pressure, and HR = heart rate. The dashed lines indicate the moment of the injection of phenylephrine. *p < 0.05 compared to SHAM groups. #p < 0.05 compared to 2K1C vehicle group (two-way ANOVA, followed by Bonferroni).

thick sections, and adjacent sections were stained with hematoxylin/eosin for evaluation of general myocardial and renal damage, or by Masson's trichrome for quantification of collagen-tissue deposition. Morphometric evaluations were made in tissue sections under an optical microscope (Leica DM5000) and analyzed with the Leica Qwin Image Processing and Analysis Software (Germany). In hearts, the cardiomyocyte diameter was measured by the method described by Caliari et al. (2002) and Soares et al. (2011) in 20 optical-microscope images at 40× magnification. For hearts and kidneys, the cardiac and renal inflammatory process and tissue collagen deposition were also quantified, as described by Soares et al. (2011).

Drugs

AVE 0991 was generously donated by Dr. Juergen Puenter from Aventis Pharma; KOH (85% potassium hydroxide ACS from Reagen, Paraná, Brazil).

Statistical analysis

The results are expressed as means ± SEM. The data were analyzed for Kolmogorov–Smirnov normality and followed standard normal distribution; they were subsequently assessed by two-way ANOVA,
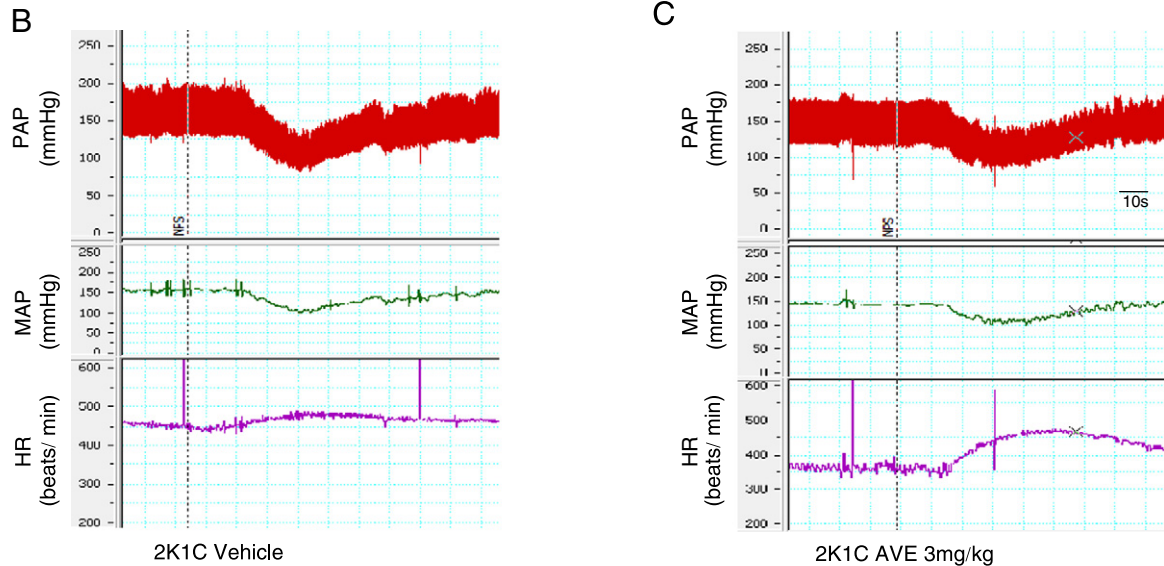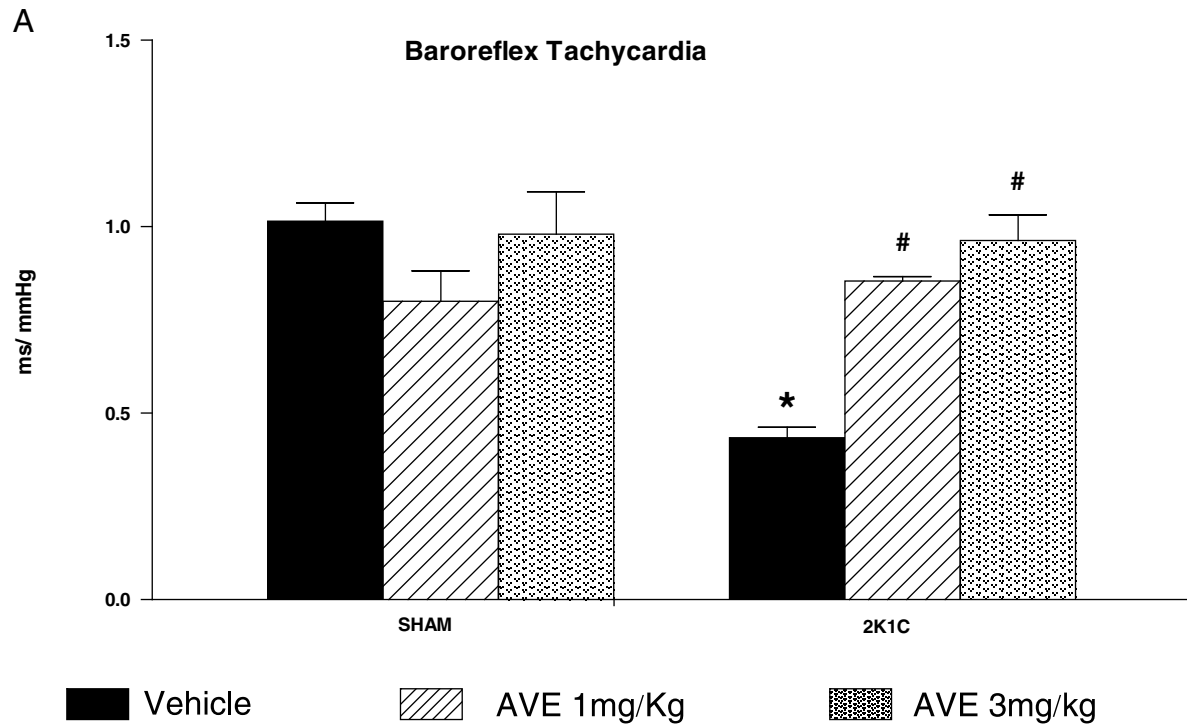
Fig. 3. Index of the sensitivity of the baroreflex tachycardia (ms/mm Hg, panel A) in normotensive rats (SHAM, n = 5–7) or in hypertensive rats (2K1C, n = 7–9) treated, per gavage, with vehicle or AVE 0991 (1.0 mg/kg or 3.0 mg/kg). Blood-pressure recording showing a typical response induced by injection of sodium nitroprusside (4.0 μg, i.v.) in 2K1C vehicle rats (panel B) and in 2K1C treated with AVE 0991 at the dose of 3.0 mg/kg (panel C). PAP = pulsate arterial pressure, MAP = mean arterial pressure, and HR = heart rate. The dashed lines indicate the moment of injection of sodium nitroprusside. *p<0.05 compared to SHAM groups. #p<0.05 compared to 2K1C vehicle group (two-way ANOVA, followed by Bonferroni).

followed by the Bonferroni post-test. Statistical analyses were performed with the software GraphPad Prism (version 5.0, San Diego, USA). The criterion for statistical significance was set at p<0.05.

Results

Baseline MAP and HR levels

The baseline MAP level for all 2K1C vehicle rats (160 ± 5 mm Hg, n = 14) was higher (p<0.05) than the baseline MAP of all SHAM vehicle rats (100 ± 3 mm Hg, n = 12). The 2K1C AVE 1 mg/kg rats (n = 7) also had a higher baseline MAP (p<0.05) than the SHAM vehicle group (n = 6), and similar to that of the 2K1C vehicle rats (n = 7) (p>0.05) (Fig. 1). However, in the treatment with AVE 3 mg/kg, the baseline MAP of 2K1C AVE 3 mg/kg rats (n = 9) was lower (p<0.05) than the MAP of 2K1C vehicle rats (n = 7), but the baseline MAP of 2K1C AVE 3 mg/kg rats was higher (p<0.05) than the baseline MAP of the SHAM vehicle group (n = 6) (Fig. 1). The treatment with AVE in doses of 1 and 3 mg/kg did not alter the baseline MAP levels of SHAM rats (n = 5–7) compared to SHAM vehicle
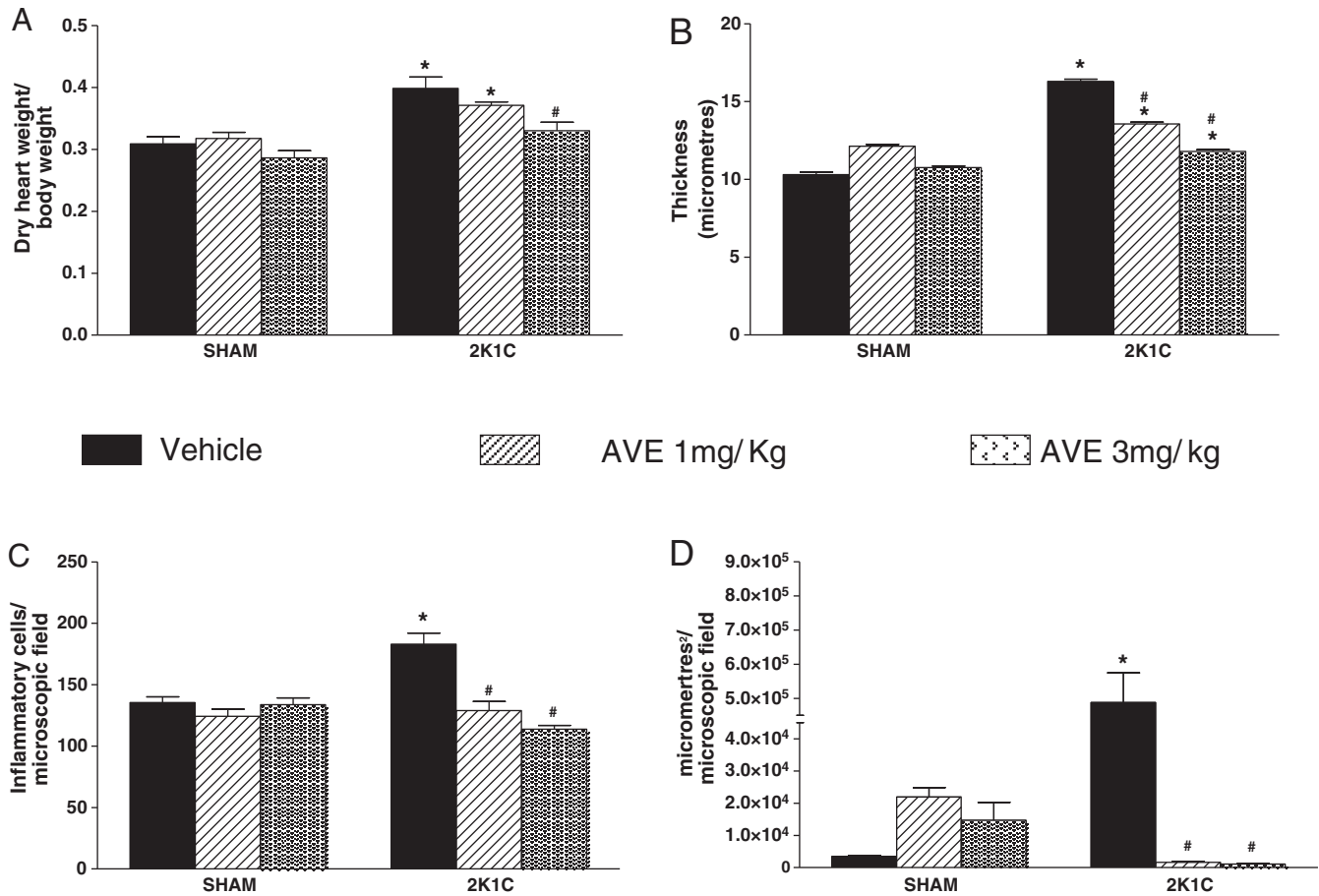
Fig. 4. Panel A: relative heart dry weight (weight/body weight) in SHAM rats (n = 6–9) and 2K1C rats (n = 8–10). Panel B: thickness of the myocardial fibers in the left ventricle (micrometers) in SHAM rats (n = 3–4) and 2K1C rats (n = 3–4). Panel C: number of inflammatory cells in the left myocardium in SHAM rats (n = 3–4) and 2K1C rats (n = 3–4). Panel D: total area of collagen deposition (μm²/microscopic field) in the left ventricle in SHAM rats (n = 3–4) and 2K1C rats (n = 3–4). Sham and 2K1C rats were treated, per gavage, for 28 days with AVE 0991 (1.0 mg/kg or 3.0 mg/kg) or vehicle. *p < 0.05 compared to SHAM group. $^{\#}$p < 0.05 compared to the 2K1C vehicle group (two-way ANOVA, followed by Bonferroni).

animals (n = 6) (Fig. 1). Baseline HR levels did not differ between the 2K1C or SHAM groups treated with vehicle or AVE 0991 at doses of 1 mg/kg or 3 mg/kg (Fig. 1).

Evaluation of baroreflex sensitivity

As expected, the sensitivity of reflex bradycardia in all 2K1C vehicle animals (0.58 ± 0.14 ms/mm Hg, n = 14) was lower (p < 0.05) than the sensitivity of reflex bradycardia in all SHAM vehicle animals (1.03 ± 0.05 ms/mm Hg, n = 12). Regarding the treatment with AVE 1 mg/kg, the sensitivity of reflex bradycardia in 2K1C AVE 1 mg/kg (n = 7) was lower (p < 0.05) than the reflex bradycardia in the SHAM vehicle group (n = 6) (Fig. 2A). Furthermore, the hypertensive 2K1C vehicle animals (n = 7) and 2K1C AVE 1 mg/kg (n = 7) showed similar levels of the sensitivity of reflex bradycardia (p > 0.05) (Fig. 2A). The bradycardiac component of the reflex control of HR in SHAM rats treated with AVE 1 or 3 mg/kg was similar (p > 0.05) to the reflex bradycardia of SHAM rats (Fig. 2A). However, animals treated with 2K1C AVE 3 mg/kg (n = 9) had a higher sensitivity of reflex bradycardia (p < 0.05) compared to 2K1C vehicle rats (n = 8), and similar (p > 0.05) to that of the SHAM vehicle rats (Fig. 2A, B and C).

The sensitivity of the reflex tachycardia in all 2K1C vehicle animals (0.54 ± 0.012 ms/mm Hg, n = 15) was lower (p < 0.05) than the sensitivity of reflex tachycardia in the SHAM vehicle group (1.01 ± 0.04 ms/mm Hg, n = 12). Treatments with AVE in doses of 1 and 3 mg/kg improved the sensitivity of the reflex tachycardia in rats with 2K1C hypertension. The 2K1C AVE 1 mg/kg animals (n = 7)

were similar (p > 0.05) to the SHAM AVE 1 mg/kg group (n = 5) and higher (p < 0.05) than the 2K1C vehicle group (Fig. 3A). Likewise, the 2K1C AVE 3 mg/kg group (n = 9) showed similar levels of sensitivity reflex tachycardia (p < 0.05) to those of the SHAM vehicle group (n = 6), and higher (p < 0.05) than the 2K1C vehicle group (n = 8) (Fig. 3A, B and C).

Our data also showed that baseline levels of BP correlated inversely with the sensitivity of the reflex control of HR (AVE 1 mg/kg, r = − 0.9527 and AVE 3 mg/kg, r = − 0.8804).

Analysis of cardiac structure

Morphological effects of intragastric administration of AVE 0991 in rats with renovascular hypertension were evaluated morphometrically in cardiac tissue. Renovascular hypertension increased the (p < 0.05) cardiac weight in all 2K1C groups, compared with non-hypertensive animals (SHAM groups). Moreover, 2K1C AVE 0991 3.0 mg/kg rats had a lower cardiac weight (p < 0.05) than 2K1C vehicle and 2K1C AVE 0991 1.0 mg/kg rats (Fig. 4A). The cardiomyocyte diameter increased (p < 0.05) in all 2K1C (vehicle or AVE 0991) groups compared with SHAM rats. Again, 2K1C vehicle rats showed an increase (p < 0.05) in cardiomyocyte diameter compared with 2K1C AVE 0991 1.0 mg/kg and AVE 0991 3.0 mg/kg rats (Fig. 4B). Intragastric administration of AVE 0991 prevented the increase of inflammatory cells (p < 0.05) in 2K1C AVE 1.0 mg/kg and 2K1C AVE 3.0 mg/kg compared with 2R1C VEHICLE rats (Figs. 4C and 5). The collagen deposition area also increased (p < 0.05) in
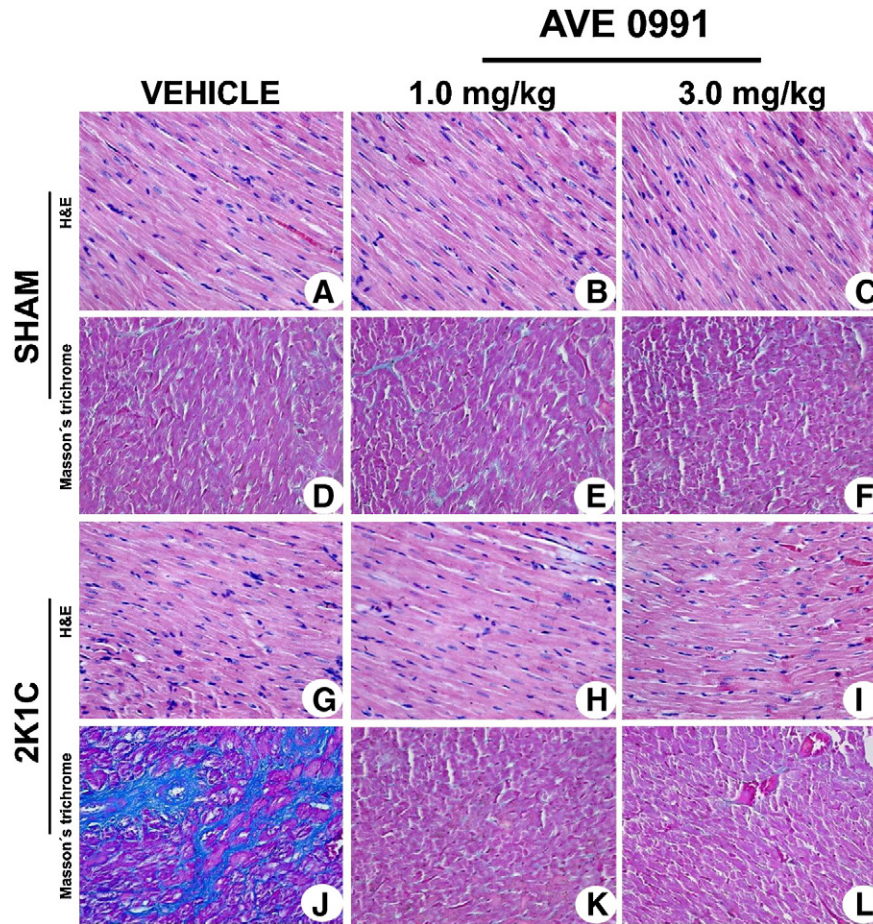
Fig. 5. Photomicrographs of left ventricle of normotensive rats (SHAM) and hypertensive rats (2K1C) treated, per gavage, for 28 days with AVE 0991 (1.0 mg/kg or 3.0 mg/kg) or Vehicle. A–C and G–I: Hematoxylin Eosin staining, 440× magnification. D–F and J–F: Masson's trichromic staining, 440× magnification. 2K1C vehicle rats showing a larger number of inflammatory cells of myocardium (G), together with greater collagen deposition (blue area in J). These histological changes were not observed in SHAM or 2K1C rats treated with AVE 0991.

2K1C vehicle rats over 2K1C AVE 0991 1.0 mg/kg, 2K1C AVE 0991 3.0 mg/kg ($1082 \pm 164 \ \mu m^2$, $p < 0.05$) or SHAM groups (Figs. 4D and 5).

Analysis of renal structure

The histopathological changes in the right (non-clipped) and left (clipped) kidneys were determined by morphometric analysis. In the right kidney, renovascular hypertension caused an increase ($p < 0.05$) of inflammatory cells in 2K1C vehicle, 2K1C AVE 0991 1.0 mg/kg and in 2K1C AVE 0991 3.0 mg/kg compared with the SHAM groups (Figs. 6A and 7). Moreover, no effects of renovascular hypertension or intragastric administration of AVE 0991 on collagen-tissue area were observed in the right kidney (Figs. 6B and 7).

Clipped kidneys also showed an increase ($p < 0.05$) of inflammatory cells in 2K1C vehicle, 2K1C AVE 0991 1.0 mg/kg and in 2K1C AVE 0991 3.0 mg/kg rats compared with SHAM groups (Figs. 6C and 7). The collagen-tissue area increased ($p < 0.05$) in 2K1C vehicle rats compared with SHAM groups, 2K1C AVE 0991 1.0 mg/kg and 2K1C AVE 0991 3.0 mg/kg rats. In addition, the collagen-tissue area in 2K1C AVE 0991 1.0 mg/kg and in 2K1C AVE 0991 3.0 mg/kg was similar to that in the SHAM groups (Figs. 6D and 7).

Discussion

The results of this study showed that an orally active Mas receptor agonist, AVE 0991, was effective in preventing different parameters

that are altered in 2K1C renovascular hypertension. In summary, four weeks of treatment with AVE 0991 improved the sensitivity of the baroreflex control of HR, and reduced the diameter of cardiomyocytes, the number of inflammatory cells, and cardiac fibrosis in 2K1C hypertensive rats. Furthermore, AVE 0991 decreased the number of inflammatory cells and fibrosis in the clipped kidney. Our data also showed that treatment with AVE 0991, at a dose of 3 mg/kg, induced a hypotensive effect in 2K1C hypertensive rats. There was no effect of AVE 0991 on baseline BP in normotensive SHAM rats. Together, the data from this study showed renoprotective and cardioprotective effects induced by treatment with AVE 0991 during development of 2K1C renovascular hypertension.

It is well established that Ang-(1–7) exerts important effects related to the tonic or reflex regulation of BP (Benter et al., 1995; Ferrario, 2003; Santos et al., 2000) and has antitrophic and antifibrotic effects (Santos et al., 2003; Tallant et al., 2005). The discovery of AVE 0991 (Wiemer et al., 2002) as a biologically active compound, resistant to gastric enzymes and showing effects similar to those of Ang-(1–7), made it possible to explore the role of Ang-(1–7) in different pathologies such as cardiovascular diseases. Studies on normotensive animals have shown that short-term treatment with AVE 0991 (1 mg/kg; for 7 or 4 weeks) did not change baseline BP and HR in rats subjected to isoproterenol-induced cardiac hypertrophy and dysfunction (Ferreira et al., 2007a) or in rats that underwent left coronary ligation (Zeng et al., 2012). In keeping with these observations, our present results showed that treatment with AVE 0991 1 or 3 mg/kg did not alter the
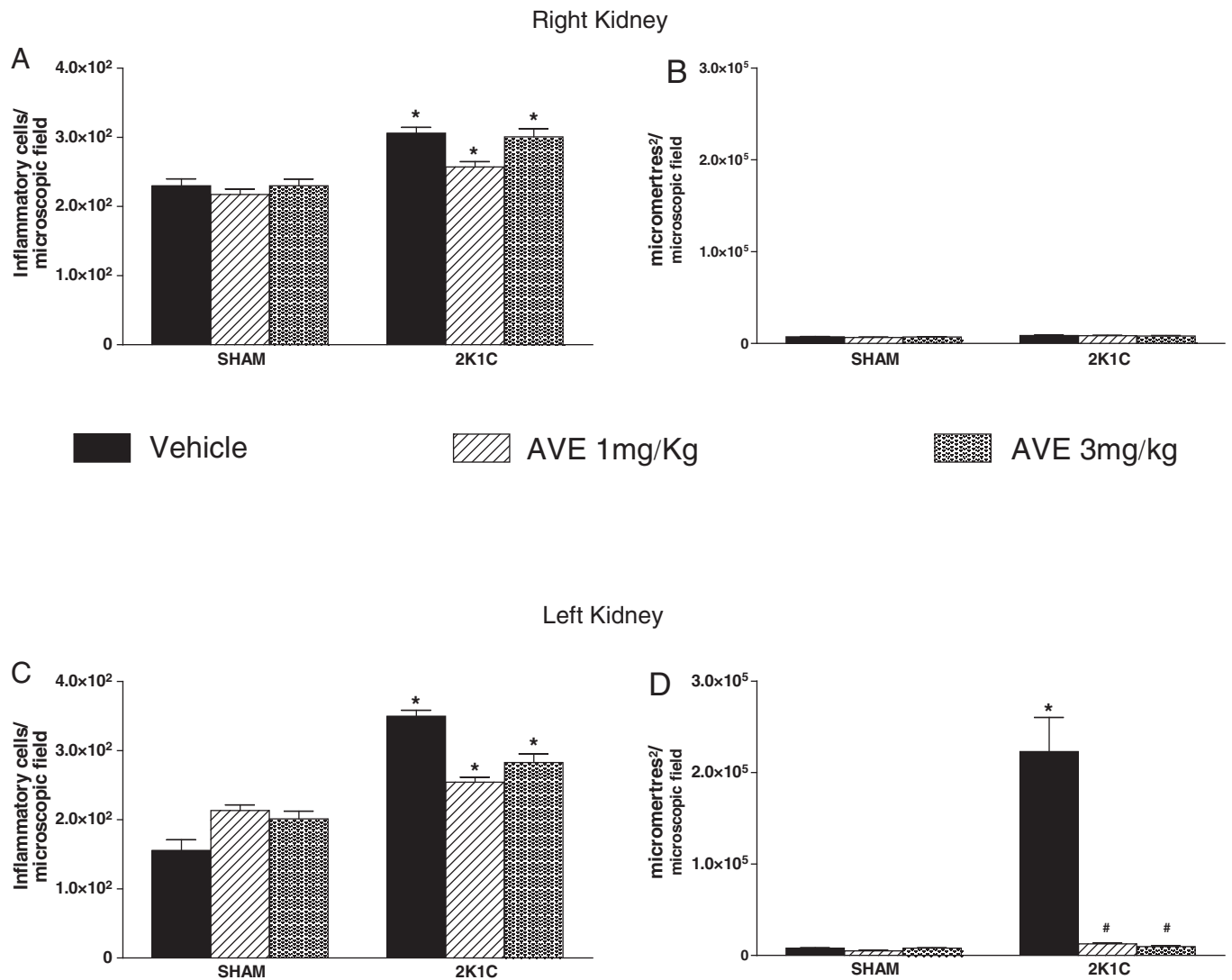
Right Kidney

Left Kidney

Fig. 6. Panels A and C: median number of cells per microscopic field (inflammatory cells) in the right and left kidneys, respectively, in SHAM rats ($n = 6$–9) and 2K1C rats ($n = 8$–10). Panels B and D: total area of collagen-tissue deposition (μm^2/microscope field), in the right and left kidneys, respectively, in SHAM rats ($n = 6$–9) and 2K1C rats ($n = 8$–10). Sham and 2K1C rats were treated, per gavage, for 28 days with AVE 0991 (1.0 mg/kg or 3.0 mg/kg) or vehicle. *$p < 0.05$ compared to SHAM group. #$p < 0.05$ compared to 2K1C vehicle group (two-way ANOVA, followed by Bonferroni).

baseline BP in normotensive SHAM rats. In addition, our data also showed that treatment with AVE 0991 1 mg/kg dose did not alter the baseline BP in 2K1C hypertensive rats.

Moreover, Benter et al. (2006) showed that Ang-(1–7) and AVE 0991 prevented, but did not abolish the L-NAME hypertension induced in SHR. Similarly, our results showed that treatment with AVE 0991 at a dose of 3 mg/kg reduced the baseline levels of BP in 2K1C rats. Reductions in baseline levels of BP are considered important to reduce cardiac afterload and consequently the development of cardiac hypertrophy, and to reduce endothelial and kidney dysfunctions (Hammond and Janes, 1998; Savage et al., 1979).

The reduced sensitivity of baroreflex control of HR is considered to be a risk factor for the development of LV hypertrophy, heart failure and sudden death (Fei et al., 1994; Piratello et al., 2010). There is a consensus, both for animals and for human patients, that hypertension is accompanied by attenuation of baroreflex sensitivity (Korner et al., 1974; McCubbin et al., 1956). Furthermore, our data showed that baseline levels of BP correlated inversely with the sensitivity of the reflex control of HR. Accordingly, only the treatment with AVE 0991 at a dose of 3 mg/kg induced a fall on baseline BP in 2K1C hypertensive that was sufficient to improve both baroreflex

bradycardia and tachycardia. In addition, the treatment with AVE 0991 at a dose of 1 mg/kg, which did not reduce the baseline BP of 2K1C rats, did not induce improvement in sensitivity of the reflex bradycardia.

Ang II and Ang-(1–7) have opposite effects on the control of the baroreflex in normotensive (Campagnole-Santos et al., 1992; Ferrario et al., 1997; Polson et al., 2007) and hypertensive rats (Benter et al., 1995; Britto et al., 1997; Chaves et al., 2000; Heringer-Walther et al., 2001). While Ang II reduces baroreflex sensitivity (Casto and Phillips, 1985; Head, 1996), Ang-(1–7) induces a facilitation of the baroreflex, after both peripheral administration (Santos et al., 2000) and intracerebroventricular (ICV) administration (Campagnole-Santos et al., 1992). In addition, Benter et al. (1995) showed in SHR infused with Ang-(1–7) that there was an improvement in sensitivity of the reflex control of heart rate to levels similar to untreated WKY. Britto et al. (1997) showed that ICV infusion of the selective antagonist of Ang-(1–7), A-779 produces attenuation of the baroreflex control of HR in 2K1C renovascular hypertensive rats subjected to chronic treatment with enalapril (ACE inhibitor), suggesting that part of the beneficial effect induced by ACE inhibition may be related to effects of Ang-(1–7) on the CNS. Later, Heringer-Walther et al. (2001) suggested
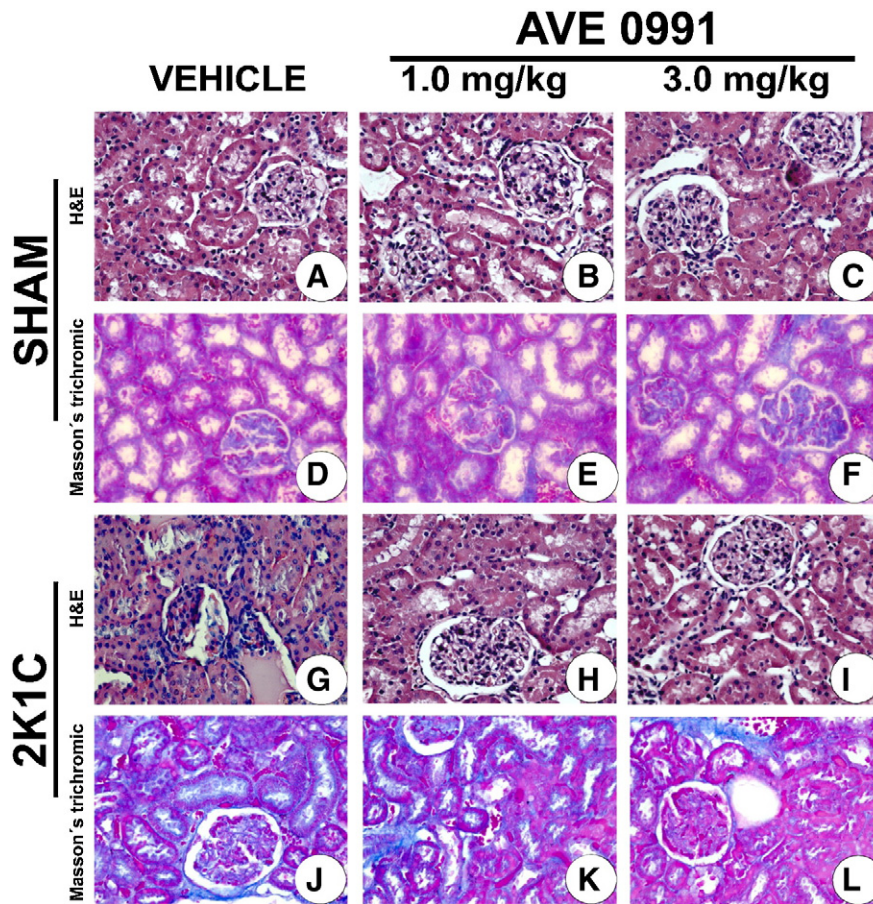
Fig. 7. Photomicrographs of left kidney of normotensive rats (SHAM) and hypertensive rats (2K1C) treated, per gavage, for 28 days with AVE 0991 (1.0 mg/kg or 3.0 mg/kg) or vehicle. A–C and G–I: Hematoxylin eosin staining, 440× magnification. D–F and J–L: Masson's trichromic staining, 440× magnification. 2K1C vehicle rats showing a larger number of inflammatory cells (G) together with greater collagen deposition (blue area in J). This histological change was not observed in SHAM or 2K1C rats treated with AVE 0991.

that Ang-(1–7) is involved in the improvement of reflex bradycardia sensitivity in SHR after ICV infusion of an ACE inhibitor (ramipril). In concordance with these findings, our present data showed that treatment with AVE 0991 improved baroreflex sensitivity in 2K1C hypertensive rats, similar to that described for Ang-(1–7). Further studies will be necessary to determine whether the rise in baroreflex control induced by AVE 0991 is due to peripheral or central elements of the baroreceptor circuitry.

A methodological aspect to be considered in the interpretation of our results is related to the sodium nitroprusside used to evaluate the baroreflex sensitivity of the tachycardia. According to Musialek and Casadei (2000), the nitrovasodilators, nitric-oxide donors, have extra-vascular effects that may interfere with the evaluation of reflex control of HR. The increase in HR induced by sodium nitroprusside may not be directly due to changes in transmission of baroreflex and autonomic nervous-system activity (Musialek and Casadei, 2000). The literature is controversial, but a possible interference relates to a positive chronotropic effect, independent of changes in BP (Casadei and Paterson, 2000; Hogan et al., 1999; Musialek and Casadei, 2000). As already mentioned, it has been reported that the AVE 0991 acts by releasing nitric oxide (Wiemer et al., 2002), allowing a probable interaction with sodium nitroprusside, which may explain the improvement in sensitivity of the reflex control of HR for the tachycardic component at both doses of AVE 0991.

Previous studies showed that Ang-(1–7) can be synthesized in the heart (Zisman et al., 2003) and that this heptapeptide has beneficial effects by reducing cardiac remodeling and attenuating the

development of cardiac arrhythmias (Grobe et al., 2006; Tallant et al., 2005; Trask and Ferrario, 2007). In our present study, the mean dry weight of the heart of 2K1C animals treated with AVE 0991 (3 mg/kg) was lower than that of 2K1C or 2K1C treated with 1 mg/kg AVE. However, AVE 0991 induced a reduction in the thickness of myocardial fibers of the LV in 2K1C rats at both 1 and 3 mg/kg doses. In addition, treatment with AVE 0991 at both doses reduced the number of inflammatory cells in the heart of 2K1C rats. Treatment with both doses of AVE 0991 significantly reduced collagen deposition in the heart of 2K1C rats, reinforcing an antifibrotic and antitrophic effect induced by AVE 0991.

Benter et al. (2006) showed that AVE and Ang-(1–7) were equipotent in reducing cardiac hypertrophy and cardiac collagen deposition in SHR rats treated with the nitric oxide synthase inhibitor, L-NAME. Ferreira et al. (2007a) showed that treatment with AVE 0991 (1 mg/kg/week) during the development of heart failure reduced the size of the infarcted area. In another study, Ferreira et al. (2007b) showed that treatment with AVE 0991 (1 mg/kg) for 7 days in a model of cardiac dysfunction induced by isoproterenol, reduced the weight of the heart, collagen deposition and the diameter of the cardiomyocytes. Our data advanced these observations by showing that AVE 0991 reduced the number of inflammatory cells, collagen deposition and the diameter of cardiomyocytes in 2K1C rats.

2K1C renovascular hypertension is characterized by hyperactivity of the RAS (Ferrario and Varagic, 2010; Pinheiro et al., 2009). The clipped kidney, due to reduction of renal blood flow, loses weight, while the unclipped contralateral kidney increases its weight and

size, probably due to a compensatory hyperfunction (Navar et al., 1995; Navar et al., 1998; Rodrigues et al., 2007). Collagen deposition increases only in the clipped kidney (Soares et al., 2011). Recently, Prieto et al. (2011) evaluated the component of RAS axes [ACE, ACE2, Ang II and Ang-(1–7)] in cortical and medullary regions of both kidneys, clipped and unclipped, in 2K1C hypertensive rats (three weeks after clipping). The levels of Ang II and Ang I increased, while the levels of Ang-(1–7) decreased in both kidneys. However, Ang II levels were higher in the clipped compared to the unclipped kidney. Moreover, in both kidneys the levels of ACE mRNA increased, while ACE2 mRNA levels decreased. These alterations in RAS enzymes probably contribute to change the content of intrarenal RAS peptides, favoring the accumulation of Ang II. This concords with the increase in the number of inflammatory cells and collagen deposition in the clipped kidney of 2K1C rats in our present study. Our results also showed that treatment with both doses of AVE 0991 reduced the number of inflammatory cells and collagen deposition in the clipped kidney. Our data concord with those of Pinheiro et al. (2009), who showed an increase in the expression of collagen and fibronectin in the kidney of mice with genetic deletion of the Mas receptor, suggesting that the decreased levels of Ang-(1–7) or a lack of its effect, combined with increased levels of Ang II, may induce pre-fibrotic lesion in the kidney. Furthermore, our data showed that treatment with AVE 0991 was effective in preventing the inflammatory effect and deposition of collagen in the clipped kidney, probably by an opposition to the high levels of Ang II.

Importantly, the anti-inflammatory and anti-prefibrotic lesions in the heart and kidney were observed with both doses of AVE 0991, even at the dose of 1 mg/kg which did not change the level of hypertension. These data indicate a direct effect, independent of BP alteration, of the Mas receptor agonist AVE 0991 in attenuating kidney and cardiac fibrosis induced by hypertension.

Conclusion

The data from the present study extend previous observations by showing that AVE 0991, an orally active nonpeptide Ang-(1–7) mimic, can attenuate the deleterious effects induced by chronic increase in Ang II on the blood pressure, baroreflex control, and in heart and kidney fibrosis. Futher, our data showed that treatment with AVE 0991 has a direct antitrophic and antifibrotic effect on the heart and kidney in the 2K1C renovascular hypertension model.

Conflict of Interest

None of the authors has a conflict of interest.

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