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Original Paper

Angiotensin II type 1 receptor blockade restores angiotensin-(1–7)-induced coronary vasodilation in hypertrophic rat hearts

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Abstract

The aim of the present study was to investigate the coronary effects of Ang-(1–7) [angiotensin-(1–7)] in hypertrophic rat hearts. Heart hypertrophy was induced by abdominal aorta CoA (coarctation). Ang-(1–7) and AVE 0991, a non-peptide Mas-receptor agonist, at picomolar concentration, induced a significant vasodilation in hearts from sham-operated rats. These effects were blocked by the Mas receptor antagonist A-779. Pre-treatment with L-NAME (N^G-nitro-L-arginine methyl ester) or ODQ (1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinozalin-1-one) [NOS (NO synthase) and soluble guanylate cyclase inhibitors respectively] also abolished the effect of Ang-(1–7) in control hearts. The coronary vasodilation produced by Ang-(1–7) and AVE 0991 was completely blunted in hypertrophic hearts. Chronic oral administration of losartan in CoA rats restored the coronary vasodilation effect of Ang-(1–7). This effect was blocked by A-779 and AT_2 receptor (angiotensin II type 2 receptor) antagonist PD123319. Acute pre-incubation with losartan also restored the Ang-(1–7)-induced, but not BK (bradykinin)-induced, coronary vasodilation in hypertrophic hearts. This effect was inhibited by A-779, PD123319 and L-NAME. Chronic treatment with losartan did not change the protein expression of Mas and AT_2 receptor and ACE (angiotensin-converting enzyme) and ACE2 in coronary arteries from CoA rats, but induced a slight increase in AT_2 receptor in aorta of these animals. Ang-(1–7)-induced relaxation in aortas from sham-operated rats was absent in aortas from CoA rats. *In vitro* pre-treatment with losartan restored the Ang-(1–7)-induced relaxation in aortic rings of CoA rats, which was blocked by the Mas antagonist A-779 and L-NAME. These data demonstrate that Mas is strongly involved in coronary vasodilation and that AT_1 receptor (angiotensin II type 1 receptor) blockade potentiates the vasodilatory effects of Ang-(1–7) in the coronary beds of pressure-overloaded rat hearts through NO-related AT_2- and Mas-receptor-dependent mechanisms. These data suggest the association of Ang-(1–7) and AT_1 receptor antagonists as a potential therapeutic avenue for coronary artery diseases.

Key words: angiotensin-(1–7), angiotensin II type 1 receptor (AT_1 receptor), coronary vasodilation, hypertrophic heart, Mas receptor

INTRODUCTION

LVH (left ventricular hypertrophy) is a powerful risk factor for sudden cardiac death, at least in part because of myocardial ischaemia [1–3]. Previous findings suggest that both coronary hypertension and myocardial hypertrophy contribute to the global impairment of coronary circulation in LVH [4,5]. In line with these data, it was demonstrated that abnormalities in the

Abbreviations: ACE, angiotensin-converting enzyme; AngII, angitoensin II; Ang-(1–7), angiotensin-(1–7); AT_1 receptor, angiotensin II type 1 receptor; AT_2 receptor, angiotensin II type 2 receptor; A.U., arbitrary units; BK, bradykinin; BP, blood pressure; CoA, coarctation; KRS, Krebs–Ringer solution; L-NAME, N^G-nitro-L-arginine methyl ester; LSD, least significant difference; LVH, left ventricular hypertrophy; MAP, mean arterial pressure; NOS, NO synthase; ODQ, 1*H*-[1,2, 4]oxadiazolo[4,3-*a*]quinozalin-1-one; RAS, renin–angiotensin system; SNP, sodium nitroprusside.

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coronary circulation in pressure-overloaded hearts was induced by hypertensive vascular changes [6,7] as well as by the presence of myocardial hypertrophy [8,9].

A balance of relaxing and contracting factors ensures appropriate heart perfusion under physiological conditions. However, this balance is altered in disease conditions, such as hypertension [10], most probably because of impaired coronary endothelial NO production [11].

Abnormalities in the RAS (renin–angiotensin system) have been strongly implicated in hypertension [12] as well as coronary dysfunction [13]. For example, AngII (angiotensin II) participates in the development of LVH through haemodynamic and non-haemodynamic mechanisms [14,15], and it may also be directly involved in coronary artery dysfunction [16].

Ang-(1–7) is a biologically active component of the RAS that binds to Mas, inducing effects that oppose those elicited by AngII, and has many beneficial activities in the cardiovascular system, including vasodilatation in several vascular beds [17–21].

Previously, van Esch et al. [22] demonstrated that Ang-(1–7) blocked AT_1 receptor (AngII type 1 receptor)-induced vasoconstriction but did not affect the coronary circulation when applied alone at nanomolar or micromolar concentrations in isolated rat hearts. However, our previous results showed that Ang-(1–7) at picomolar concentrations was able to decrease the perfusion pressure in isolated perfused mice hearts in the presence of losartan [21], which suggests that this peptide has a powerful effect on the coronary circulation and may involve other angiotensinergic receptors.

Several studies have proposed that Ang-(1–7) may have therapeutic potential for the treatment of CVDs (cardiovascular diseases) [23,24]. However, the effect of Mas receptor activation in the coronary bed of the heart affected with overload-induced hypertrophy is unknown. Therefore the aim of the present study was to clarify the coronary effects of Ang-(1–7) and AVE 0991, a non-peptide Mas-receptor agonist, in rat hearts and to investigate possible changes in these effects in heart hypertrophy. In addition, we sought to evaluate the roles of angiotensinergic receptors in the effects of Ang-(1–7).

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250–300 g were provided by the animal facilities of the Federal University of Goiás. All animals were kept in temperature-controlled rooms with a 12/12 h light/dark cycle and had free access to water and food. All animal procedures were performed in accordance with institutional guidelines approved by local authorities.

BP (blood pressure) measurement

The rats were anaesthetized [ketamine (70 mg/kg of body weight) and xylazine (30 mg/kg of body weight)], and a polyethylene catheter (PE-50) was inserted into the right carotid artery, tunnelled under the skin and exteriorized at the neck. MAP (mean arterial pressure) measurements were made in conscious rats 24 h after recovery from anaesthesia. The data were recorded on a data acquisition system (Dataq Instruments).

Hypertrophic heart rat model

Cardiac hypertrophy was induced by abdominal aortic CoA (coarctation). After anaesthesia was induced with ketamine/xylazine solution (70 and 30 mg/kg of body weight respectively), a left laparotomy was performed, the descending aorta was isolated and a bent 21-gauge needle was placed next to the aorta. The suture was tied around the needle and the aorta at the level of abdominal aorta above the celiac artery. After ligation, the needle was quickly removed. In the sham group, age-matched animals underwent the same procedure without the placement of the aortic banding. The wet weights of the left ventricles were recorded, normalized for tibia length and then expressed as ventricular mass index.

Chronic treatment

To evaluate the role of RAS components in the effects of Ang-(1–7), some CoA rats were treated with subdepressor doses of the AT_1 receptor antagonist losartan (1 mg/kg of body weight per day) by gavage.

Isolated heart preparation

The rats were decapitated 10–15 min after the intraperitoneal injection of 200 units of heparin. The thorax was opened, and the heart was carefully dissected and perfused through an aortic stump with KRS (Krebs–Ringer solution) containing 118.4 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4 \cdot 7\ H_2O$, 1.25 mmol/l $CaCl_2 \cdot 2\ H_2O$, 11.7 mmol/l glucose and 26.5 mmol/l $NaHCO_3$. The perfusion flow was maintained constant (8–10 ml/min) at 37 °C and constant oxygenation (5% CO_2 and 95% O_2). Coronary perfusion pressure was measured with a pressure transducer that was connected to the aortic cannula and coupled to a data-acquisition system (Biopac Systems). After a basal period (30–40 min), the hearts from sham or CoA rats were perfused for an additional 15 min with KRS containing (i) Ang-(1–7) (20 pmol/l), (ii) AVE 0991 (20 pmol/l) or (iii) BK (bradykinin; 50 nmol/l). To evaluate the mechanisms involved in the effects of Ang-(1–7), the hearts were perfused for a basal period with KRS containing (iv) the Mas receptor antagonist A-779 (2 nmol/l); (v) the AT_1 receptor antagonist losartan (1 μmol/l); (vi) the NOS (NO synthase) inhibitor L-NAME (N^G-nitro-L-arginine methyl ester; 10 μmol/l), (vii) the selective soluble guanylate cyclase inhibitor ODQ (1*H*-[1,2,4] oxadiazolo [4,3-*a*]quinozalin-1-one; 200 nmol/l), (viii) losartan plus A-779; (ix) losartan plus PD123319 or (x) losartan plus L-NAME. After this period, Ang-(1–7) (20 pmol/l) was added to the perfusion solution containing the antagonists and/or inhibitors, and the hearts were perfused for an additional period of approximately 15 min.

Isolated aortic ring preparation

Isolated aortic rings were used to evaluate the effect of Ang-(1–7) in thoracic aorta under pressure overload. Aortic rings (4 mm) from the descending thoracic aorta above the constriction or sham procedure site were set up in gassed (95% O_2 and

Table 1 BP and cardiac morphometry parameters
Results are means ± S.E.M. *$P < 0.05$ compared with the sham group. Los, losartan.

Parameter	SHAM ($n = 10$)	CoA ($n = 11$)	CoA + chronic Los ($n = 8$)
MAP (mmHg)	99.8 ± 3.38	127.1 ± 7.64*	119.5 ± 6.403
Heart weight (g)	0.857 ± 0.028	1.026 ± 0.051*	0.980 ± 0.060
Left ventricular weight (g)	0.586 ± 0.017	0.734 ± 0.040*	0.739 ± 0.028
Ventricular mass index (g/cm)	0.173 ± 0.004	0.223 ± 0.016*	0.212 ± 0.008

5% CO_2) Krebs–Henseleit solution containing 118.06 mmol/l NaCl, 4.6 mmol/l KCl, 24.9 mmol/l $NaHCO_3$, 2.4 mmol/l $MgSO_4.7H_2O$, 3.3 mmol/l $CaCl_2 \cdot 2\ H_2O$, 0.9 mmol/l KH_2PO_4 and 11.1 mmol/l glucose at 37 °C. The rings were kept under a tension of 1.5 g for 1 h to equilibrate. Mechanical activity was recorded isometrically using a data-acquisition system (Dataq Instruments). The effects of acetylcholine (10^{-9}–10^{-5} mol/l) and Ang-(1–7) (10^{-10}–10^{-6} mol/l) were evaluated in aortic rings pre-constricted with phenylephrine (0.1 μmol/l). To evaluate the role of Mas and/or AT_1 receptor and NOS, the vessels were pre-treated (10 min) with A-779 (10^{-6} mol/l) or L-NAME (10^{-6} mol/l) in association with losartan (10^{-6} mol/l) before the addition of Ang-(1–7). The vasorelaxant response to acetylcholine (10^{-5} mol/l) was used to evaluate the endothelial function of the rat aortic rings.

Immunohistochemistry

Expression of RAS proteins in coronary arteries and aorta were evaluated by immunohistochemistry. Rat hearts and aorta were fixed with 10% formalin, embedded in paraffin and sectioned at 3-μm thickness. Endogenous peroxidase was blocked with 3% H_2O_2. Sections were incubated either with rabbit polyclonal primary antibody against Mas (1:100 dilution; Abcam), AT_2 receptor (AngII type 2 receptor; 1:500 dilution; Abcam), ACE (angiotensin-converting enzyme; 1:500; Abcam) and ACE2 (1:500; Gene Tex) overnight at 4 °C. Subsequently, biotinylated secondary antibody followed by streptavidin–biotin conjugate (Biocare Medical) was applied. Positive staining was visualized using DAB (diaminobenzidine), and nuclei were counterstained with haematoxylin. All images were taken at the same light intensity using a light microscope (Olympus, BX43) at ×400 (coronary arteries) or ×200 (aortas) magnification. The immunostaining results were quantified in different coronary arteries and aortas using the ImageTool 2.0 image analysis program. After images were captured, the vessels wall was selected and analysed in the grey scale range of 0–255. Staining intensity was measured as an average of the area (i.e. the sum of grey values of all pixels divided by the number of pixels in the area) and values recorded as A.U. (arbitrary units). Microscopy setting (light power) were determined at the beginning of each imaging session and then held constant during the analysis of all the samples for each antibody.

Data analysis

The results are the means ± S.E.M. One-way ANOVA followed by the Newman–Keuls post-test was used to analyse the BP, morphometric parameters, immunohistochemical analysis and basal parameters in isolated hearts. Two-way ANOVA with LSD (least significant difference) (Fisher) multiple comparison post-test was used to compare the curves obtained in isolated hearts and aortic ring preparation protocols. All statistical analyses were considered significant at $P < 0.05$.

Table 2 Influence of chronic and acute treatments on the basal coronary perfusion pressure
Results are means ± S.E.M. *$P < 0.05$ compared with the sham group; †$P < 0.05$ compared with the CoA group. Los, losartan.

Group	Basal perfusion pressure (mmHg)	n
Sham	101.00 ± 6.46	13
Sham + A-779	108.7 ± 19.77	4
Sham + L-NAME	144.7 ± 17.02*	5
Sham + ODQ	143.0 ± 9.08*	4
CoA	76.10 ± 7.74*	11
CoA + Los chronic	78.44 ± 14.25	5
CoA + Los chronic + A-779	73.82 ± 8.78	4
CoA + Los chronic + PD123319	66.49 ± 4.52	5
CoA + Los acute	100.4 ± 8.48	6
CoA + Los acute + A-779	93.1 ± 19.63	5
CoA + Los acute + PD123319	86.4 ± 7.39	4
CoA + Los acute + L-NAME	130.0 ± 21.16†	6

RESULTS

BP and cardiac morphometric parameters

BP was measured in the carotid artery 21 days after abdominal aortic banding. The MAP was significantly higher in CoA rats compared with the sham-operated group. Chronic treatment with subdepressor doses of losartan did not change the BP in CoA rats (Table 1). To confirm the cardiac hypertrophy animal model, morphometric analysis of the hearts was performed. As shown in Table 2, the abdominal aortic banding procedure induced a significant increase in whole heart and left ventricular weight and ventricular mass index. Treatment with losartan was unable to reduce the pressure overload-induced LVH (Table 1).

Basal parameters of coronary perfusion pressure

Table 2 shows the basal values of coronary perfusion pressure in the presence or absence of antagonists/inhibitors before the

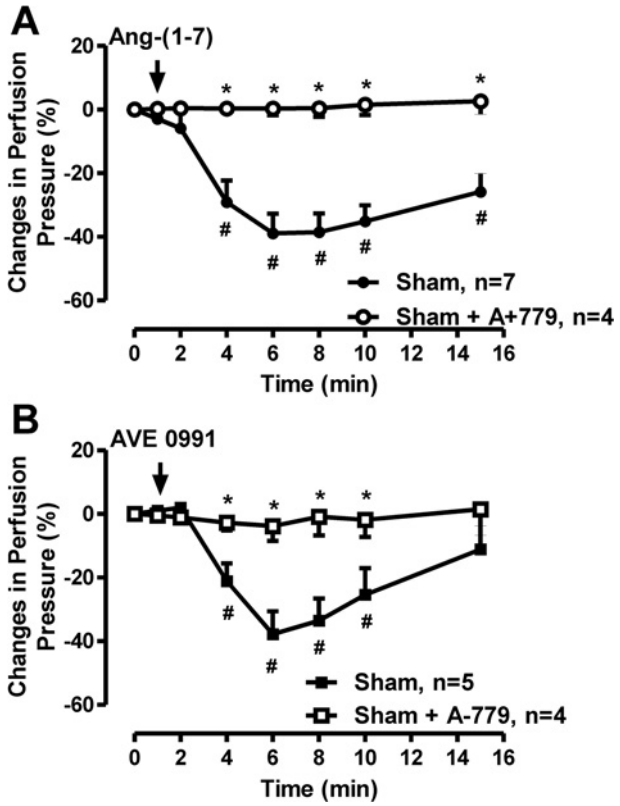

Figure 1 Effects of (A) Ang-(1–7) (20 pmol/l) and (B) AVE 0991 (20 pmol/l) on coronary perfusion pressure in isolated perfused hearts from sham rats

The hearts were perfused in the absence or presence of A-779 (2 nmol/l). $^{\#}P < 0.01$ compared with the basal levels; $^{*}P < 0.01$ between time points. Statistical significance was calculated by two-way ANOVA followed by Fisher's LSD. Each point represents the mean ± S.E.M.

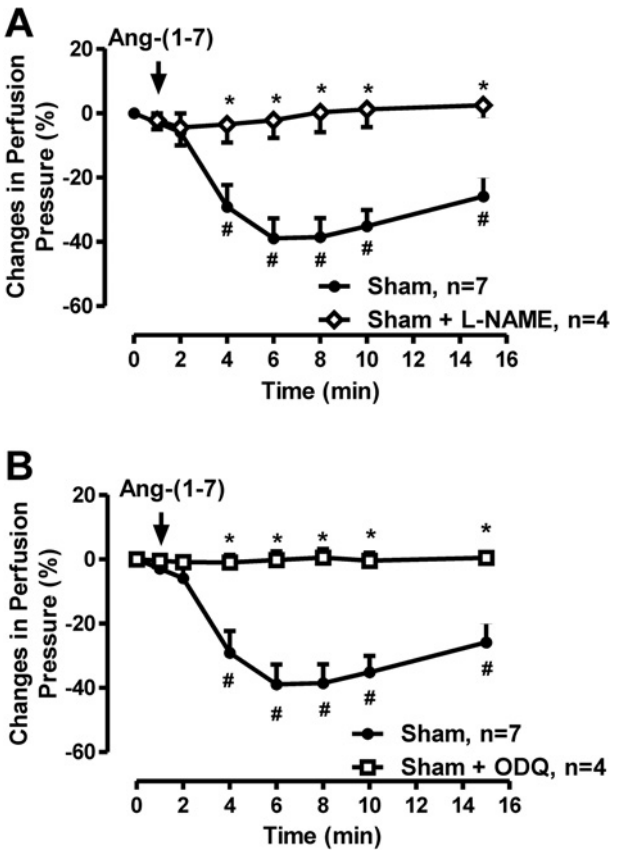

Figure 2 Effects of Ang-(1–7) (20 pmol/l) on coronary perfusion pressure in the absence or presence of (A) L-NAME (1 μmol/l) or (B) ODQ (0.2 μmol/l) in isolated perfused hearts from sham rats

$^{\#}P < 0.01$ compared with the basal level; $^{*}P < 0.01$ between time points. Statistical significance was calculated by two-way ANOVA followed by Fisher's LSD. Each point represents the mean ± S.E.M.

addition of Ang-(1–7) or AVE 0991. During the basal period, perfusion pressure was lower in hypertrophic hearts compared with the sham group. *In vivo* or *in vitro* treatment with losartan had no effect on perfusion pressure during the basal period. The presence of L-NAME in KRS induced an increase in perfusion pressure in sham and hypertrophic hearts. Similarly, the perfusion pressure was also higher in hearts perfused with guanylate cyclase inhibitors.

Coronary vasodilation induced by Mas receptor activation

To evaluate the involvement of the Mas receptor in the coronary haemodynamics, the normal hearts were perfused with Ang-(1–7) and AVE 0991 after the basal period. Both Mas agonists induced a significant coronary vasodilation indicated by a decrease in perfusion pressure. These effects were blocked by the Mas receptor antagonist A-779 (Figure 1).

NO has been implicated in several mechanisms involved in the vascular effects of Ang-(1–7). Therefore we evaluated the role of NOS and soluble guanylate cyclase in Ang-(1–7)-induced coronary vasodilation. As observed in Figure 2, pre-treatment with L-NAME or ODQ abolished the effect of Ang-(1–7) in hearts from sham-operated rats.

To evaluate the effect of Mas receptor activation in the coronary bed under pathological conditions, pressure-overloaded hearts were perfused with Ang-(1–7) or AVE 0991. The coronary vasodilation produced by both Mas agonists, Ang-(1–7) and AVE 0991, was completely blunted in hypertrophic hearts (Figures 3).

Effect of chronic AT_1 receptor blockade in the coronary actions of Ang-(1–7)

We evaluated the role of the angiotensinergic receptors on the coronary effects of Ang-(1–7). First, we focused on the role of the AT_1 receptor in Ang-(1–7)-induced coronary vasodilation. Strikingly, chronic losartan treatment in CoA rats restored the coronary vasodilatory effect of Ang-(1–7) in hypertrophic hearts (Figure 4A). This effect was blocked by the Mas receptor antagonist A-779 (Figure 4B).

The AT_2 receptor has been suggested to be implicated in some of the effects of Ang-(1–7) [21,25,26]. Thus we evaluated the effect of AT_2 receptor blockade on the vasodilatory action of Ang-(1–7) in hypertrophic rat hearts chronically treated with losartan. Pre-treatment of the isolated hearts with PD123319 abolished the Ang-(1–7)-induced coronary vasodilation in these hearts (Figure 4C).

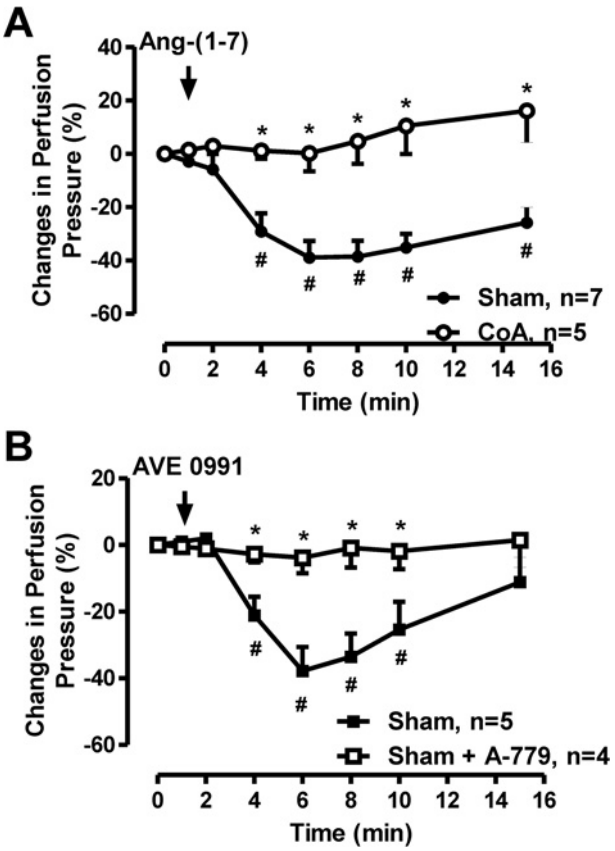

Figure 3 Effects of (A) Ang-(1–7) (20 pmol/l) and (B) AVE 0991 (20 pmol/l) on coronary perfusion pressure in isolated perfused hearts from CoA rats

$^{\#}P < 0.01$ against basal level; $^{*}P < 0.01$ between time points. Statistical significance was calculated by two-way ANOVA followed by Fisher's LSD. Each point represents the mean ± S.E.M.

Effect of acute AT_1 receptor blockade in the coronary actions of the Ang-(1–7)

To evaluate whether the effect of losartan was due to systemic or local changes, isolated hypertrophic hearts were perfused with losartan before the addition of Ang-(1–7). Interestingly, acute treatment with losartan also restored the Ang-(1–7)-induced coronary vasodilation (Figure 5A). This effect was also blocked by A-779 (Figure 5B) and PD123319 (Figure 5C). Acute treatment with losartan did not change the effect of Ang-(1–7) in hearts from sham-operated rats (results not shown). Thereafter, we evaluated the role of NOS in the Ang-(1–7)-induced coronary vasodilation in hypertrophic hearts. As observed in Figure 5(D), pre-treatment with L-NAME abolished the effect of Ang-(1–7) in hypertrophic hearts after acute losartan treatment.

Involvement of the AT_1 receptor in the coronary effects of BK in isolated hypertrophic hearts

To evaluate whether the coronary vasodilation abnormalities observed in hypertrophic hearts were associated with endothelial dysfunction, the hearts were perfused with BK, a known endothelium-dependent vasodilator. Nanomolar concentrations of BK produced a decrease in perfusion pressure in sham hearts

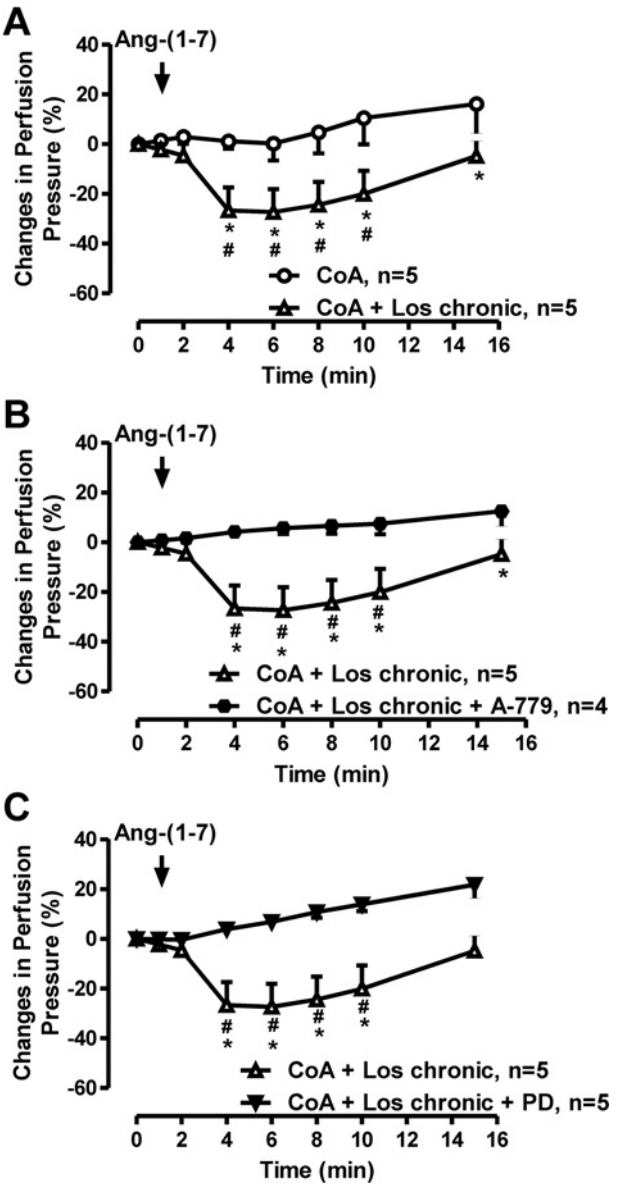

Figure 4 Effects of Ang-(1–7) (20 pmol/l) on coronary perfusion pressure in isolated perfused hearts from CoA rats chronically treated with losartan (1 mg/kg of body weight per day)

The hearts were perfused in the (**A**) absence or (**B**) presence of A-779 (2 nmol/l) or (**C**) PD123319 (2 nmol/l). $^{\#}P < 0.01$ compared with the basal levels; $^{*}P < 0.01$ between time points. Statistical significance was calculated by two-way ANOVA followed by Fisher's LSD. Each point represents the mean ± S.E.M. Los, losartan.

but not in hypertrophic hearts. AT_1 receptor blockade did not change the coronary effects of the BK in hypertrophic hearts (Figure 6).

Involvement of the AT_1 receptor in Ang-(1–7) effects in isolated aortic rings from pressure-overloaded rats

It is well known that Ang-(1–7) is able to induce aortic ring relaxation [19,27]. In the present study, to determine whether the deterioration of the Ang-(1–7) vasodilator effect was restricted to

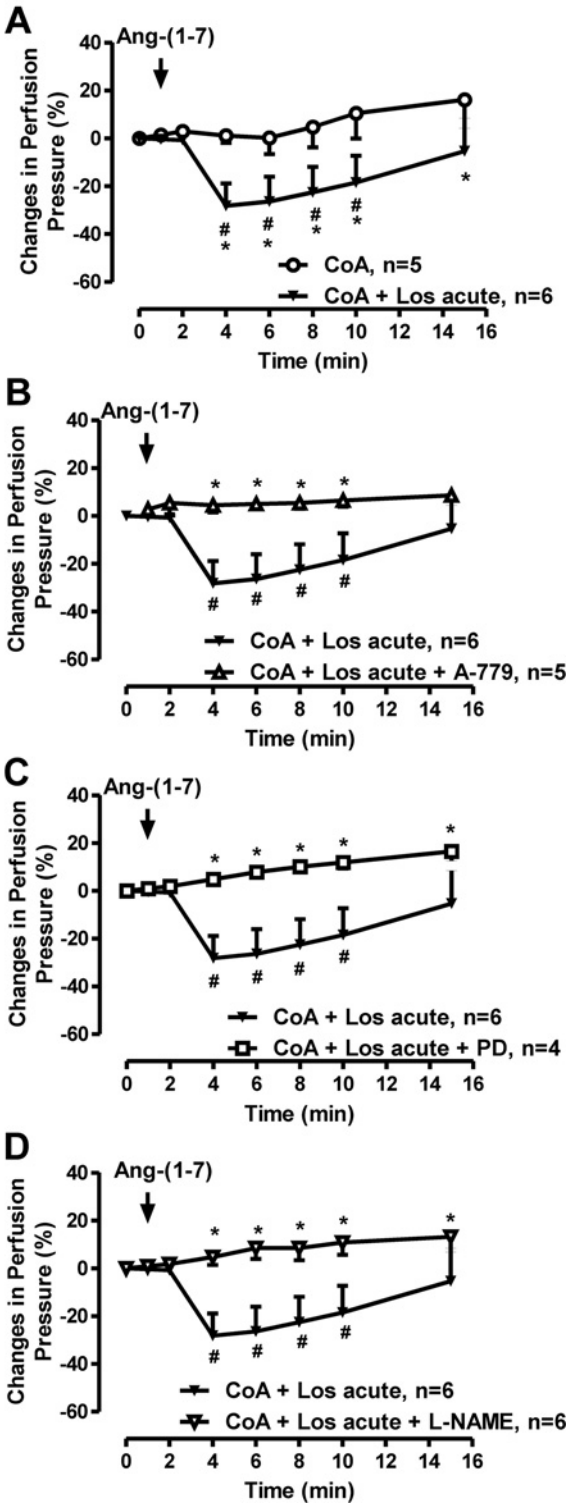

Figure 5 Effects of Ang-(1–7) (20 pmol/l) on coronary perfusion pressure in isolated perfused hearts acutely treated with losartan (1 μmol/l) (A) alone or in association with (B) A-779 (2 nmol/l), (C) PD 123319 (2 nmol/l) or (D) L-NAME (1 μmol/l)
$^{\#}P < 0.01$ against basal level; $^{*}P < 0.01$ between time points. Statistical significance was calculated by two-way ANOVA followed by Fisher's LSD. Each point represents the mean ± S.E.M. CoA, aortic coarcted rats; Los, losartan.

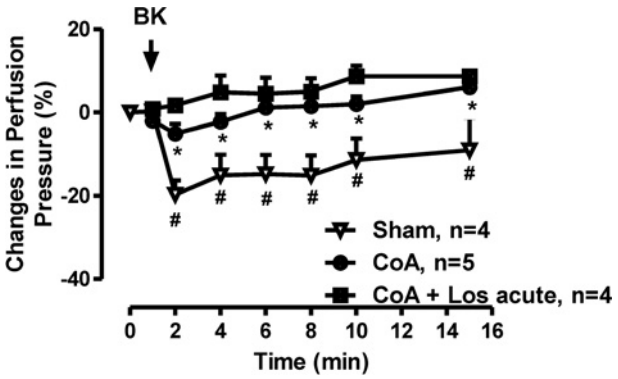

Figure 6 Effects of BK (50 nmol/l) on coronary perfusion pressure in isolated perfused hearts from sham and CoA rats
The CoA hearts were perfused in the absence or presence of losartan (1 μmol/l). $^{\#}P < 0.01$ compared with the basal level; $^{*}P < 0.01$ between time points. Statistical significance was calculated by two-way ANOVA followed by Fisher's LSD. Each point represents the mean ± S.E.M. Los, losartan.

the coronary bed in hypertrophic hearts, we assessed the effects of Ang-(1–7) in aortic rings isolated from CoA rats. As expected, Ang-(1–7) induced a significant relaxation in the aorta from sham animals. This effect was significantly reduced in the aortas of CoA rats (Figure 7A). Chronic treatment with losartan was not able to restore the Ang-(1–7)-induced aorta relaxation in these rats (Figure 7A). However, acute pre-treatment with the AT_1 receptor antagonist losartan restored the Ang-(1–7)-induced relaxation in aortic rings from CoA rats (Figure 7B), and this effect was blocked by treatment with the Mas antagonist A-779 (Figure 7C) and L-NAME (Figure 7D).

We then determined whether these animals presented endothelial dysfunction. Therefore the acetylcholine relaxant response was evaluated in aortic rings from sham and CoA rats. The acetylcholine-induced vasorelaxant effect was attenuated in CoA aortic rings (results not shown).

Effect of chronic AT_1 receptor blockade on expression of RAS proteins in coronary arteries and aorta

To evaluate whether the effect of chronic AT_1 receptor blockade on Ang-(1–7)-induced vasodilation was related to changes in the expression of RAS proteins, we performed immunohistochemical analysis in coronary arteries and aorta. Mas and AT_2 receptors, ACE and ACE2 expression were unchanged in pressure-overloaded hearts. Chronic losartan treatment did not alter the expression of these proteins in coronary arteries from hypertrophic hearts (Figure 8). In order to verify if the lack of effect of chronic losartan treatment on Ang-(1–7)-induced aortic dilatation was related to changes in the expression of the angiotensin receptors, we analysed the Mas and AT_2 receptor expression in aorta. The expression of these proteins was also unchanged in pressure-overloaded aortas. Losartan treatment induced a slight increase in AT_2 receptor, but not in Mas receptor expression (Figure 9).

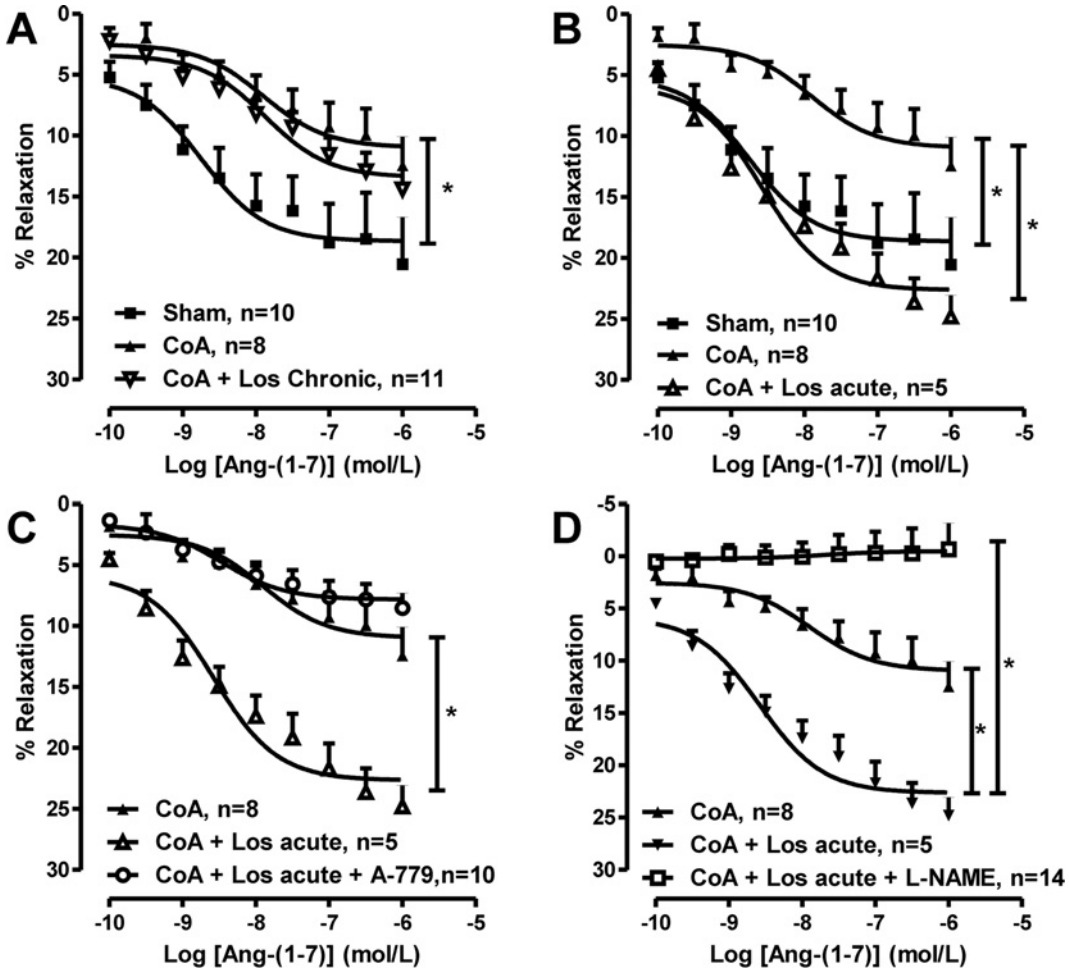

Figure 7 **Effect of losartan on the vasorelaxation in response to Ang-(1–7)**

(**A**) Effect of chronic treatment with losartan (1 mg/kg of body weight per day) on the vasorelaxation response to Ang-(1–7) in aortic rings from CoA rats. (**B**–**D**) Effects of pre-treatment (10 min) with losartan (10^{-6} mol/l) (**B**) alone or in association with (**C**) A-779 (10^{-6} mol/l) or (**D**) L-NAME (10^{-6} mol/l) in the vasorelaxation response to Ang-(1–7) in aortic rings from CoA rats. $^{*}P < 0.01$. Statistical significance was calculated by two-way ANOVA followed by Fisher's LSD. Each point represents the mean ± S.E.M. Los, losartan.

DISCUSSION

The major findings of the present study were that both Ang-(1–7) and AVE 0991, in picomolar concentrations, induced potent coronary vasodilation in normal hearts through Mas activation and the NO–guanylate cyclase pathway. This vasodilation was blunted in hypertrophic hearts. However, AT_1 receptor blockade completely restored the Ang-(1–7)-induced coronary vasodilation. Indeed, the Ang-(1–7) vasorelaxant activity was reduced in aortas from CoA rats, and acute AT_1 receptor blockade treatment also restored the Ang-(1–7) effect.

Ang-(1–7) has been shown to exert vasodilator activity in various microvascular beds, including coronary arteries [19,28–33]. The coronary vasodilation effect of Ang-(1–7) was first demonstrated in porcine coronary arteries [29]. Later, this effect was also demonstrated in coronary arteries from dogs [28] and mice [21]. Almeida et al. [34] described that Ang-(1–7) induced an increase in the vasodilatory effect of BK by NO and prostaglandin release-related mechanisms. Indeed, the overexpression of Ang-(1–7) in the plasma [35] and heart [36] enhanced coronary flow after ischaemia. However, previous studies did not observe any direct vasodilatory effect for Ang-(1–7) in the rat coronary bed [22,37], most probably due to the peptide concentration used. A number of studies have demonstrated that peptides can cause vasoconstriction or vasodilation in the coronary arterioles depending on their concentrations [38,39].

ACE inhibitors and AT_1 receptor blockers were able to improve coronary circulation in LVH [4]. Moreover, valsartan not only reduced high BP but improved coronary flow in hypertensive patients [40]. These findings clearly show that angiotensin receptors are involved in coronary modulation in cardiac hypertrophy. In this study, we observed that chronic treatment with a subdepressor dose of losartan potentiates the Ang-(1–7)-induced coronary vasodilation in isolated hypertrophic hearts. This beneficial effect of losartan may be related to improvements in coronary endothelial function [41] because endothelium dysfunction

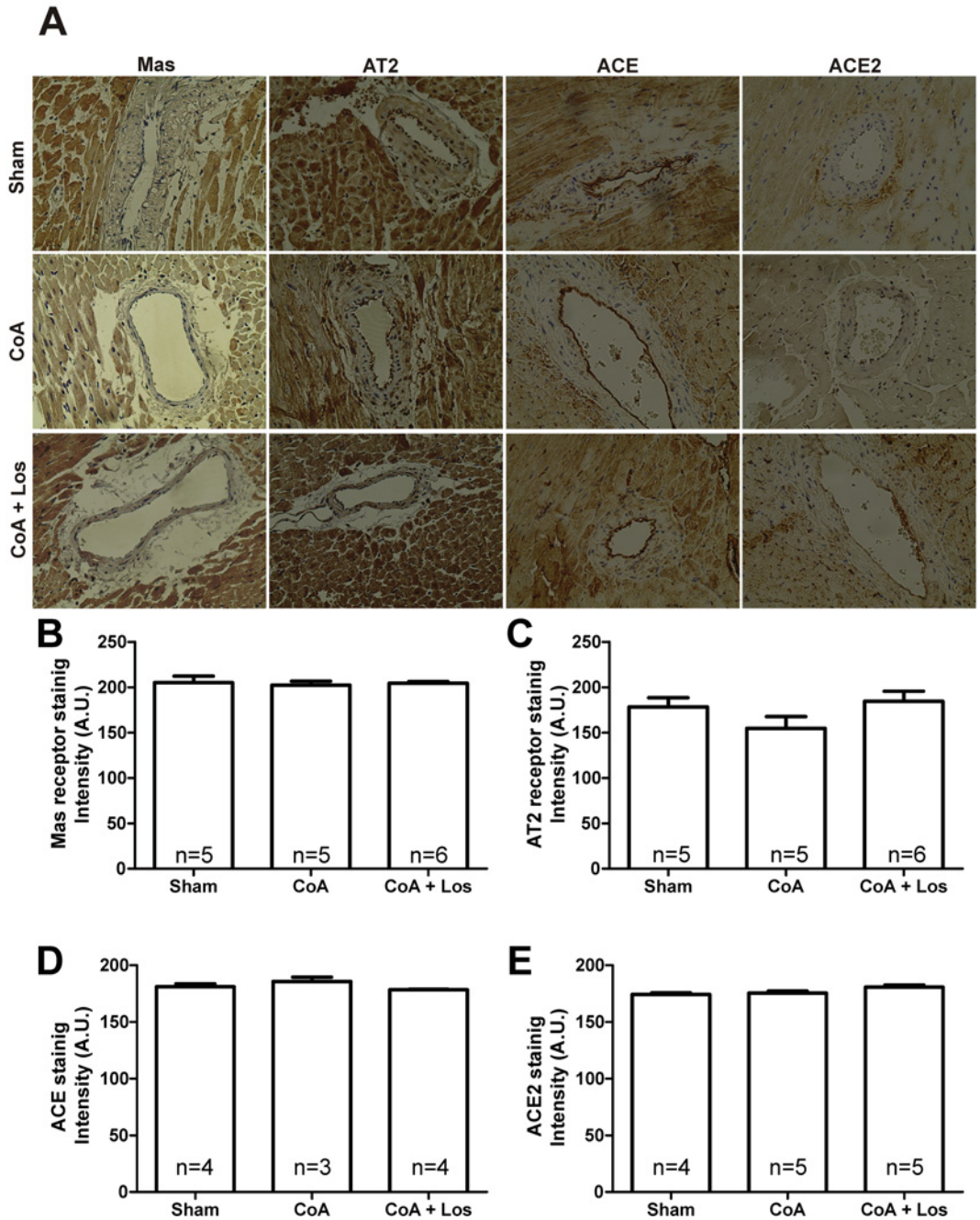

Figure 8 **Mas receptor, AT_2 receptor, ACE and ACE2 expression in coronary arteries**
(**A**) Representative images showing the immunohistochemical staining of Mas and AT_2 receptors and ACE and ACE2 from sham, CoA-, and CoA plus chronic losartan-treated coronary arteries. Images are shown at $\times$400 magnification. Quantification of (**B**) Mas and (**C**) AT_2 receptors, (**D**) ACE and (**E**) ACE2 in the coronary arteries of each group. Results are means $\pm$ S.E.M. Los, losartan.

has been demonstrated in several hypertensive animal models [42–44]. Accordingly, endothelial dysfunction was observed in the coronary vascular bed; BK was unable to induce coronary vasodilation in hypertrophic hearts and coronary vasodilation induced by SNP (sodium nitroprusside) was not different between control and hypertrophic rats. In addition, we also observed a significant reduction in acetylcholine-induced, but not in SNP-induced, relaxation in aorta rings from CoA rats (results not shown), suggesting systemic endothelium dysfunction. In contrast with the coronary findings, chronic treatment with losartan was not able to improve the Ang-(1–7)-induced relaxation in aorta rings from CoA rats. These observations indicate that the coronary bed has a greater responsiveness to losartan. However, the mechanisms for the lack of the effect of Ang-(1–7) in hypertrophied heart or in aorta from rats chronically treated with losartan remain to be elucidated.

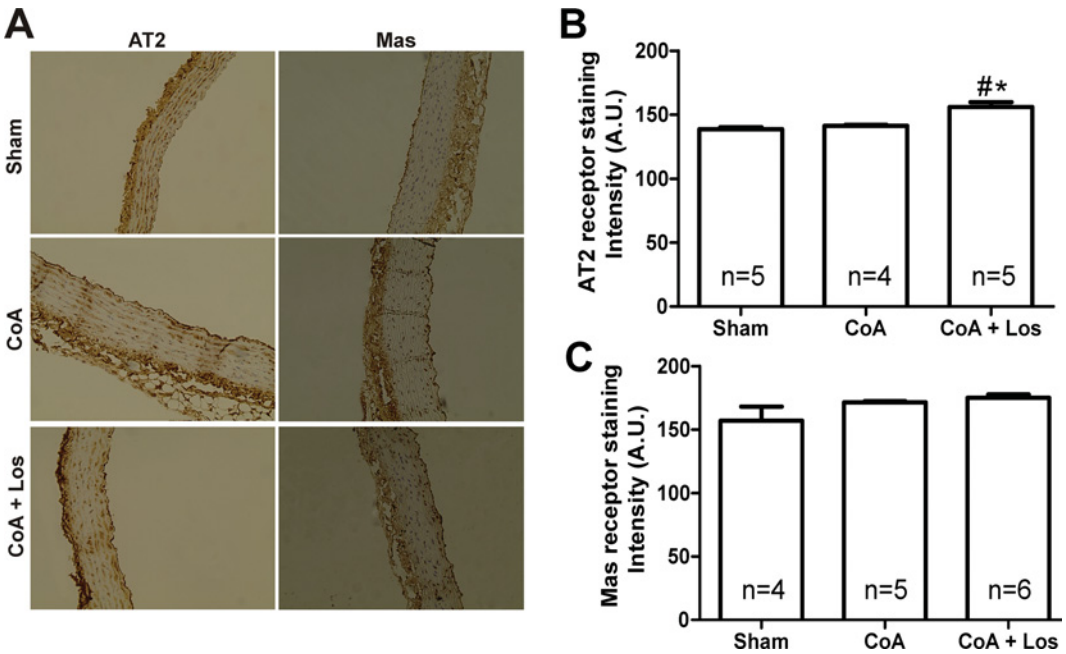

Figure 9 Mas receptor and AT_2 receptor expression in aortas

(**A**) Representative images showing the immunohistochemical staining of Mas and AT_2 receptors from sham, CoA- and CoA plus chronic losartan-treated aortas. Images are shown at ×200 magnification. Quantification of (**B**) AT_2 and (**C**) Mas receptors in the aorta of each group. Results are means ± S.E.M. $^{\#}P < 0.01$ compared with the sham group; $^{*}P < 0.01$ compared with the CoA group. Los, losartan.

Although a systemic contribution of losartan to the effects of Ang-(1–7) should be considered, a local mechanism cannot be rejected in view of the fact that losartan effects were also observed in acutely treated hearts. Accordingly, acute treatment with losartan also restored Ang-(1–7)-induced relaxation in aortic rings from CoA rats. In addition, A-779 was completely able to inhibit the effects of Ang-(1–7) in all conditions tested. It could be argued that losartan exerts a non-specific effect in potentiating vasodilation in hypertrophic hearts. However, losartan was not able to restore the BK-induced coronary vasodilation in these hearts. Taken together, these findings suggest a possible interaction between angiotensin receptors.

Angiotensin receptor interactions have been proposed by different groups [45,46], including functional antagonism, cross-talk and oligomerization. Kostenis et al. [46] reported that the Mas receptor can hetero-oligomerize with the AT_1 receptor in transfected mammalian cells. The functional role of this interaction was observed in Mas knockout mice, which showed enhanced AngII-mediated vasoconstriction in mesenteric arteries [46]. Our previous findings suggest that Ang-(1–7) produces coronary effects in isolated perfused mouse hearts through mechanisms involving interaction of Mas with AT_1 and AT_2 receptors [21].

A number of studies have described changes in angiotensin receptor expression in hypertrophic hearts. Akers et al. [47] observed an increase in AT_1 receptor density in pressure overload-induced LVH. Moreover, spontaneous hypertensive rats or renovascular hypertensive rats also presented higher ventricular levels of AT_1 and AT_2 receptor mRNA and receptor density [48]. Indeed, increases in AT_2 receptor levels were also observed in the vasculature under pathological conditions [49,50]. In contrast, we did not observe any between-group differences in AT_2 receptor as well as Mas receptor, ACE or ACE2 expression in coronary arteries. However, we demonstrated that an AT_2 receptor antagonist was able to inhibit Ang-(1–7)-induced coronary vasodilation in losartan-perfused hypertrophic hearts. Therefore we can hypothesize that AT_1 receptor blockade potentiates Ang-(1–7)-induced coronary vasodilation by modulating Mas and AT_2 receptor interactions in an expression-independent manner. In aorta, we observed only a small increase in expression of AT_2 receptor in coarcted rats treated with losartan, but this change was not able to improve the Ang-(1–7)-induced vasorelaxation.

The present study also demonstrated that L-NAME and ODQ inhibited the vasodilatory effects of Ang-(1–7) in normal hearts. Similarly, L-NAME inhibited the coronary vasodilation in losartan-treated hypertrophic hearts. These results show that NO and guanylate cyclase are involved in the effects of Ang-(1–7). Accordingly, several studies have demonstrated that the vascular effects of Ang-(1–7) are endothelium dependent and involve NO production [19,51]. Furthermore, a short-term infusion of Ang-(1–7) improved endothelial function through a mechanism involving NO release in rats [52], and Mas receptor deletion in mice results in endothelium dysfunction [53].

In conclusion, our data demonstrate that the Mas receptor is strongly involved in coronary vasodilation in physiological conditions. Indeed, acute and chronic AT_1 receptor blockade potentiates the vasodilatory effect of Ang-(1–7) in coronary bed of the pressure-overloaded rat heart through AT_2- and Mas-receptor-related mechanisms involving NO release. Thus the association of Ang-(1–7) and AT_1 receptor antagonists may have potential as a therapeutic treatment for coronary artery diseases.

CLINICAL PERSPECTIVES

- In the present study, we have used an isolated heart preparation to evaluate the coronary effects of Ang-(1–7) in hypertrophic rat hearts. We observed that Ang-(1–7) and AVE 0991, an Ang-(1–7) agonist, induced a significant vasodilation in coronary and aorta vessels of sham-operated rats. These effects were completely absent in pressure overloaded rats. Acute and chronic treatment with an AT_1 receptor antagonist potentiated the Ang-(1–7)-induced coronary vasodilation in isolated hypertrophic hearts and this effect was inhibited by Mas receptor antagonism.
- Many studies have shown that RAS blockers are able to ameliorate the coronary flow in pathological conditions through mechanisms related to improvements of the endothelial function.
- Thus our present data indicate that the association of Mas receptor agonists with AT_1 receptor antagonists might be a new therapeutic strategy to improve the management of coronary artery diseases.

AUTHOR CONTRIBUTION

Álvaro Souza performed the isolated vessel and heart experiments and carried out the chronic treatment. Deny Sobrinho and Gisele Alves carried out the isolated heart experiments. Jônathas Almeida and Larissa Macedo carried out the chronic treatment and performed the isolated hearts experiments. Juliana Porto, Eneida Vêncio and Diego Colugnati developed and interpreted the immunohistochemical analyses, and Robson Santos and Anderson Ferreira designed and revised the paper. Elizabeth Mendes designed the experiments, interpreted the data and revised the paper. Carlos Castro designed the study, interpreted the data and wrote the paper.

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