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Original Article

Angiotensin (1-7) Inhibits Ang II-mediated ERK1/2 Activation by Stimulating MKP-1 Activation in Vascular Smooth Muscle Cells

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The renin–angiotensin system (RAS) exerts profound physiological effects on blood pressure regulation and fluid homeostasis, mainly by modulating renal, cardiovascular, and central nervous systems. Angiotensin (Ang)-(1-7), an end-product of RAS, is recognized by its cardiovascular protective properties through stimulation of the Mas receptor, including vasodilation, anti-inflammatory, and antihypertensive actions, and consequently, counter-regulating the well-known Ang II-elicited actions. The overall hypothesis of this study is that Ang-(1-7) inhibits Ang II-induced ERK1/2 activation in vascular smooth muscle cells (VSMCs), via regulation of mitogen-activated protein phosphatase-1 (MKP-1) activity. Aortas from male Wistar rats were incubated with Ang-(1-7) or vehicle. Concentration-response curves to Ang II were performed in endothelium-denuded aortas, in the presence or absence of ERK1/2 (PD98059) inhibitor or Mas receptor (A-779) antagonist. Expression of proteins was assessed by western blot, and immunohistochemistry was conducted in VSMCs. Ang-(1-7) incubation decreased Ang II-induced contractile response in aortas, and this effect was not observed in the presence of PD98059 or A-779. Stimulation of VSMCs with Ang-(1-7) prevented Ang II-induced ERK1/2 phosphorylation, but not C-Raf-activation. Furthermore, Ang II decreased MKP-1 phosphorylation in VSMCs. Interestingly, simultaneous incubation of Ang-(1-7) with Ang II favored MKP-1 phosphorylation, negatively modulating ERK1/2 activation in VSMCs. The results suggest that Ang-(1-7) counter-regulates actions evoked by Ang II overproduction, as observed in cardiovascular diseases, mainly by modulating MKP-1 activity. This evidence suggests that the role of Ang-(1-7) in MKP-1-regulation represents a target for new therapeutic development.

Key words: Angiotensin (1-7), ERK1/2, MKP-1, angiotensin-II, renin-angiotensin system, VSMCs, MAPK phosphatase

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The renin–angiotensin system (RAS) is well recognized for its physiological effects in the regulation of blood pressure and fluid homeostasis. In the vasculature, angiotensin (Ang)-II, one of the most abundant component of this system, promotes several pro-hypertensive actions primarily via activation of vascular-resistance mechanisms (1,2). However, the RAS complexity has been demonstrated, and other members from the RAS also display the capacity to modulate vascular effects, while some others display Ang-II counter actions. For example, Ang-(1-7) is accepted as an important biologically active peptide in the RAS. Ang-(1-7) can be generated by different enzymes, but the most potent and well known Ang-(1–7)-generating enzyme is angiotensin converting enzyme 2 (ACE2), which can generate Ang-(1–7) from the hydrolysis of Ang II or Ang I (3).

Ang-(1–7) has been previously identified in the circulation, kidney, heart, and in the vasculature (4, 5), and this peptide exerts its effects mainly through the Mas receptor activation. Taking into consideration the distinct actions evoked by Ang-(1-7) and Ang II, the overall effect raised by these components from the RAS are mainly driven by the balance between the vasoconstrictor/proliferative and vasodilator/anti-proliferative actions of Ang-II and Ang-(1–7), respectively (5–8).

Chronic elevation of Ang-II contributes to a plethora of cardiovascular deleterious effects (2). In vascular smooth muscle cells (VSMCs), overproduction of Ang-II results in sustained activation of the three major mitogen-activated protein kinases (MAPKs) pathway subfamilies, including extracellular signal-regulated kinases (ERK1/2) (9, 10), which favors vasoconstriction, vascular remodeling, and elevation of blood pressure. Conversely, Ang-(1-7), following stimulation of the Mas receptor, shows cardiovascular protective properties (8,11), such as prevention of vascular hypertrophy (12), thrombosis (13) and fibrosis (14), among others. Such phenotype displayed by Ang-(1-7) has been credited to different mechanisms, including the production of vasodilation-induced factors (15) and attenuation of vasoconstriction-related pathways (e.g., ERK1/2) (16).

Extracellular signal-regulated kinase 1/2 (ERK1/2) is a kinase belonging to the mitogen-activated protein kinase (MAPK) family, and can be activated by various extracellular signals (17). At the molecular level, a cascade of phosphorylation and dephosphorylation modulates the activation status of ERK1/2 (18). Activated ERK1/2 phosphorylates substrates in the cytoplasm or nucleus, and thereby is involved in various physiological and pathological processes, such as cell growth, development, proliferation, and differentiation (19, 20). When activated, the ERK1/2 also modulates the activity of caldesmon and calponin, allowing the actin-myosin interaction, eliciting vascular contraction (20, 21).

Many phosphatases act as major regulators of MAPKs pathway, and are likely to be one of the most energy-efficient controllers in their deactivation process (18, 22). In mammalian cells, the dual-specificity protein phosphatases, also known as the MAP kinase phosphatases (MKPs), are the primary phosphatases responsible for deactivation of ERK1/2 (18). In this regard, MKP-1 was the first MKP identified which belongs to a family of inducible nuclear dual-specificity phosphatases exerting catalytic activity to dephosphorylation and inactivation of MAP kinase isoforms in mammalian cells and tissues (23, 24). Since MKP-1 deactivates MAPKs, this phosphatase regulates a number of physiological and pathophysiological processes including immunity, metabolic homeostasis, cellular responses to anti-cancer drugs, muscle regeneration, and neuronal function (18, 24).

A body of evidence suggests that Ang-II decreases MKP-1 activity (25), favoring ERK1/2 activation. Besides that, the interplay between Ang-

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II and Ang-(1-7), and their action on MKP-1 activity has not been investigated in VSMCs. Therefore, in this study, we test the hypothesis that Ang-(1-7) counterbalances Ang II-mediated ERK1/2 activation, which attenuates the vascular contractile response evoked by Ang II, via modulation of MKP-1 activity.

Materials and methods

Animals

Male Wistar rats, 10-12 weeks-old, obtained from the colony of the Federal University of Mato Grosso, Brazil, were used in this study. All experimental procedures were approved by the Ethics Committee on Animal Research (CEUA) of the Federal University of Mato Grosso (protocol 23108.166477/2016-20) and are in accordance with the Guidelines of the Brazilian College of Animal Experimentation (COBEA). The animals were treated in accordance with Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

Vascular functional studies

After euthanasia, the thoracic aorta was removed and cleaned from fat tissue in an ice-cold physiological salt solution, containing 130 mM NaCl, 14.9 mM $NaHCO_3$, 4.7 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4 \cdot 7H_2O$, 1.56 mM $CaCl_2 \cdot 2H_2O$, 0.026 mM EDTA, and 5.5 mM glucose (Synth Ltd., Brazil), as described previously (26). Endothelium was mechanically removed, and arterial segments were carefully mounted as ring preparations in standard organ chambers for the recording of isometric tension by a PowerLab 8/SP data acquisition system (ADInstruments Pty Ltd., Australia). Vascular segments were immersed in the physiological solution, at 37 °C, and were continuously bubbled with a mix of 95% O_2 and 5% CO_2 under a resting tension of 30 mN. After a 60 min equilibration period, aorta integrity was assessed first by vascular stimulation with a high potassium solution (120 mM KCl; Synth Ltd., Brazil); and after washing and a new stabilization period, the absence of the endothelium was verified by contracting the segments with phenylephrine (1 µM PE; Sigma Chemical, USA) followed by stimulation with acetylcholine [10 µM ACh; Sigma Chemical, USA). The absence of ACh-induced relaxation was assumed as the complete endothelium elimination, as described previously (27). Concentration-responses to Ang II (1 nM to 1 µM; Millipore, USA) were performed in the absence or in the presence of Ang-(1-7) (10 µM; GenOne Biotechnologies, Brazil), incubated for 5 min. As previously described by other authors, in some experiments, arteries also were incubated with PD98059 (10 µM ERK1/2 inhibitor; Tocris, USA) (28), Mas receptor antagonist (10 µM A779; Abcam, USA) (29) or membrane-permeable analog of the cAMP inhibitor (100 µM Rp-AMPS; Tocris Bioscience, USA) (30), for 40 min, prior to the incubation with either Ang-(1-7) or vehicle. To avoid the possibility of Ang II tachyphylatic responses, concentration-response curves to Ang II were performed only once in each vascular preparation, and therefore, incubations with the inhibitors were performed in a parallel vascular preparation from the same animal.

VSMCs isolation and culture

VSMCs were isolated from rat thoracic aortas by the explant technique, as previously described (31). Cultures were maintained in Dulbecco's modified Eagle's medium (Gibco-BRL, USA) supplemented with 10% FBS (Invitrogen, USA) and antibiotics. Immunoreactivity assays were used to characterize the VSMCs and to confirm the absence of other cell types in the cultures. The cells expressed α-smooth muscle actin and calponin, which are contractile proteins and were used to identify VSMCs. No positive immunoreactivity to von Willebrand factor VIII or CD31 (PECAM-1), which are markers of endothelial cells, was detected (data not shown).

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VSMCs from second to third passage were used to avoid significant phenotypic modulation (32). After reaching maximum confluence, and 24 h after serum removal, cells were incubated with Ang II (1 µM; 5 min) or with vehicle (H_2O, similar volume), in the presence or absence of Ang-(1-7) (10 µM, 5 min) or Ang-(1-7) + cAMP antagonist (Rp-AMPS, 100 µM; 5 min).

Western blot analysis

Proteins (40 µg) extracted from VSMCs were separated by electrophoresis on a 10% polyacrylamide gel, and subsequently transferred to a nitrocellulose membrane, and western blots were performed as previously described (33). Non-specific binding sites were blocked with 5% skim milk in Tris-buffered saline solution with Tween (TBS-T) for 1 h at 24 °C. Membranes were then incubated with primary antibodies overnight, at 4 °C, under constant agitation. Antibodies to total forms of proteins were as follow: total MKP-1 (Abcam, USA) and ERK1/2 (Cell Signaling Technology, USA). Immunoblots for non-phosphoproteins were carried out in the same membranes used to evaluate their phosphorylated forms: ERK1/2$^{Thr202/Tyr204}$ (Cell Signaling Technology, USA), DUSP1/MKP-1^{Ser359} (R&D Systems, USA) and C-Raf^{Ser338} antibody (Cell Signaling Technology, USA). After incubation with respective secondary antibodies, signals were visualized using chemiluminescence and images were captured using Image Quant LAS 4000, USA.

Immunocytochemistry

Immunocytochemistry for phosphorylated forms of ERK1/2 and MKP-1 was performed in cultured VSMCs from rat aorta, as previously described, plated on glass coverslips (5000 cells/cm^2). After 24 h in serum-free medium, cells were washed, fixed in 4 % paraformaldehyde for 10 min, permeabilized (0.1 % Nonidet P40; Sigma-USA) and incubated in blocking buffer (1 % (w/v) BSA in PBS) for 30 min at room temperature (25 °C). Cells were incubated with the primary antibodies, anti-phosphorylated ERK 1/2$^{Thr202/Tyr204}$ (Cell Signaling Technology, 1:1000- USA) and anti-phosphorylated DUSP1/MKP-1 ^{Ser359} (Sigma-Aldrich, 1:500 - USA), for 1 h, at 37 °C and counterstained with goat anti-rabbit IgG Alexa Fluor 647) (1:500 dilution; red fluorescence, Abcam- USA) and with goat anti-mouse IgG Alexa Fluor 488 (1:500 dilution, green fluorescence, Abcam- USA), at 4 °C. Cells were then incubated with 5 µl/ml 4',6-diamidino-2-phenyindole (DAPI; Sigma- USA) for 15 min to detect nuclei, as previously described (34, 35). Cover slips were mounted, and labeled cells were examined using a Zeiss microscope and software.

Data Analysis

The results are shown as mean ± SEM (n), where n represents the number of animals used in the experiments (n= 6-8). Contractions were recorded as changes in the displacement from baseline, and are represented as mN. Concentration–response curves were fitted using a non-linear interactive fitting program (GraphPad Prism 5.0; GaphPad software; USA) and two pharmacological parameters were obtained: the maximal effect generated by the agonist (or E_{max}) and pD_2 values were calculated as the − log half maximal effective concentration (EC_{50}). Statistical analyses were performed using one-way ANOVA followed by Tukey post-hoc test or Student's T test. Values of $P<0.05$ were considered statistically significant.

Results

The regulatory role of Ang-(1-7) was tested upon Ang II-induced vasoconstriction. Ang-(1-7) incubation decreased Ang II-induced contraction in aortas (E_{max} (mN) 4.36 ± 0.4 vs 6.0 ± 0.5, Ang-(1-7) and vehicle, respectively; (Figure 1A; Table 1). Ang-(1-7) action is prevented when vessels are simultaneously incubated with A779 (10 µM), a Mas receptor antagonist (E_{max} (mN) 6.0 ± 0.8 vs 5.6 ± 0.5, vehicle and Ang-(1-7), respectively). A

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similar effect was observed with Rp-AMPS (100 µM), a cAMP inhibitor (E_{max} (mN) 7.0 ± 0.3 vs 6.7 ± 0.3, vehicle and Ang-(1-7), respectively; Table 1). The effect of Ang-(1-7) was also determined

Table 1. pD_2 and E_{max} values for angiotensin II-induced contraction in endothelium-denuded rat aortas treated with Angiotensin-(1-7).

	Vehicle (*n*=8)		Ang-(1-7) (*n*=8)	
	pD_2	E_{max}	pD_2	E_{max}
Ang II	7.2 ± 0.17	6.0 ± 0.5	7.78 ± 0.19*	4.36 ± 0.4*
Ang II + A779	7.76 ± 0.22	6.0 ± 0.8	7.95 ± 0.26	5.6 ± 0.5
Ang II + Rp-AMPS	8.33 ± 0.05	7.0 ± 0.3	8.21 ± 0.08	6.7 ± 0.3
Ang II + PD98059	7.91 ± 0.16	4.0 ± 0.2	8.03 ± 0.14	3.6 ± 0.2

The results are presented as mean ± SEM of *n* = 8 in each experimental group. The pD_2 values are $-\log EC_{50}$ and E_{max} values represent the contractions induced by angiotensin II and are represented as mN. A779: Mas receptor antagonist; Rp-AMPS: cAMP inhibitor; PD98059: ERK1/2 inhibitor. * P < 0.05 vs. vehicle (H_2O).

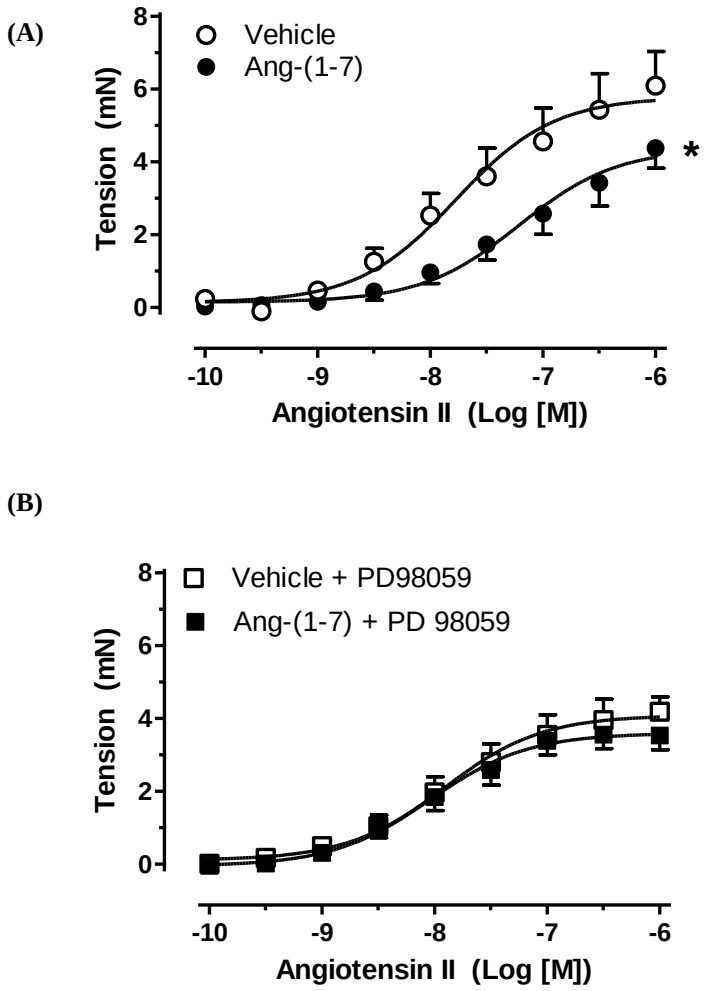


Fig.1. Ang-(1-7) decreases vasoconstriction induced by Ang II and this effect is not observed in the presence of ERK 1/2 inhibitor. A: Incubation with Ang-(1-7) (10 µM), for 5 min, decreases contractions to Ang II in endothelium-denuded rat aortas vs. vehicle (H_2O, n=8 for each group). B: ERK 1/2 inhibitor (PD98059, 10 µM) abolishes differences between Ang-(1-7) and vehicle groups in contractile-response induced by Ang II. The contraction values were calculated in relation to the tension (mN), and corrected by the length (mm) of each vessel. The results are presented as mean ± SEM for each experimental group. The statistical significance of the data was determined using the *Student's* t test. * P < 0.05 vs. vehicle.

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in the presence of PD98059 (10 µM), a pharmacological inhibitor for ERK1/2. As expected, incubation with PD98059 decreased Ang II-induced contraction in both groups (E_{max} (mN) 4.0 ± 0.2 vs 3.6 ± 0.2, vehicle and Ang-(1-7), respectively; Figure 1B).

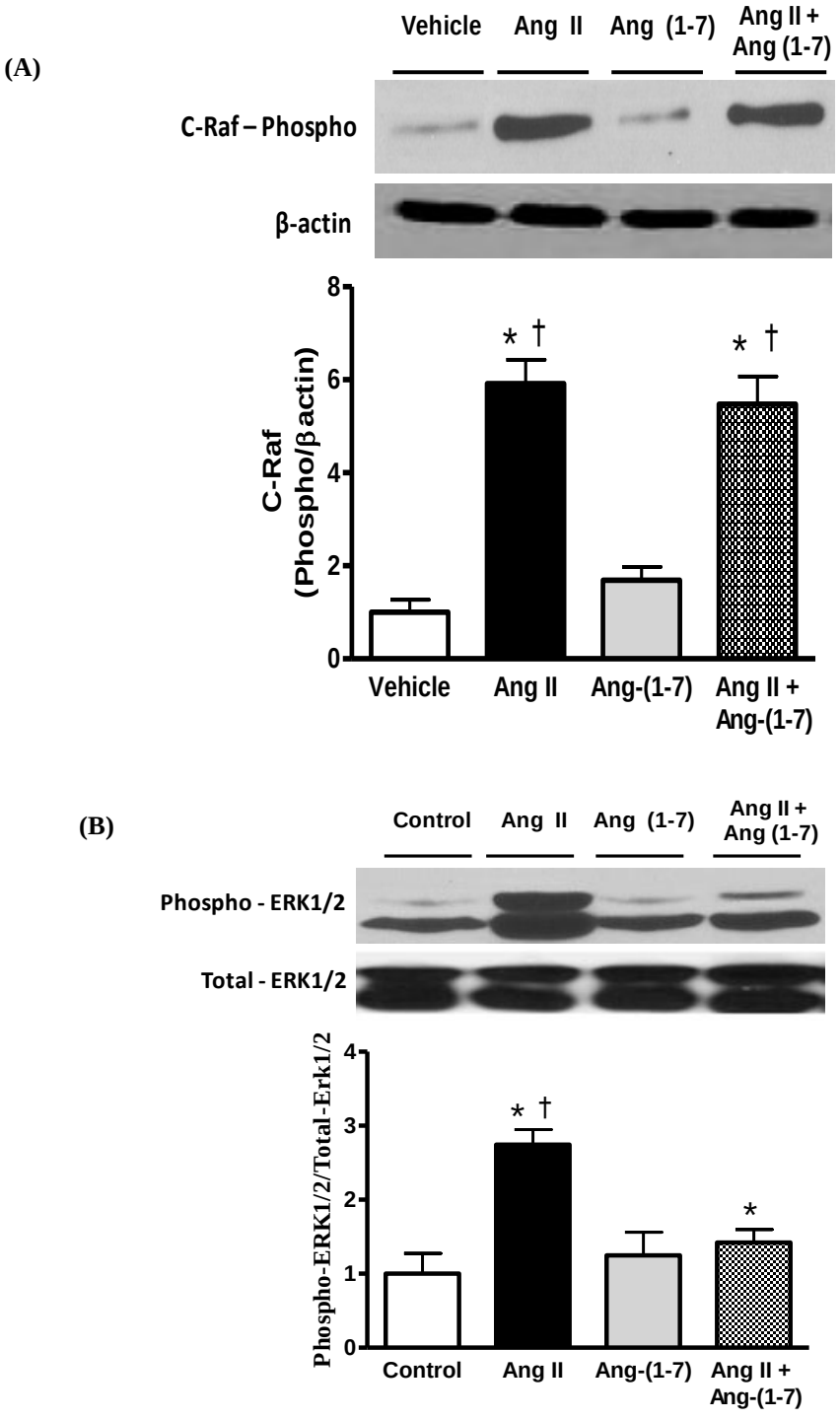


Fig.2. Ang-(1-7) prevents Ang II-induced ERK1/2 phosphorylation, but not C-Raf-phosphorylation. A: Incubation of VSMCs with Ang II (1 µM, for 2 min), increased the phosphorylation of C-Raf, and Ang-(1-7) (10 µM, for 5 min) in the presence of Ang II, did not change this pattern-response. B: Ang II increased vascular ERK1/2 phosphorylation, an effect that was prevented by Ang-(1-7). Bar graphs show the relative expression of phosphorylated C-Raf ^{Ser338} or phosphorylated ERK1/2$^{Thr202/Tyr204}$, after normalization to the corresponding total protein expression (B) or β-actin protein expressed (A) and presented as arbitrary units (n=6). Results are presented as mean ± SEM in each experimental group. * $P < 0.05$ vs. vehicle (H_2O); † $P < 0.05$ vs. Ang-(1-7).

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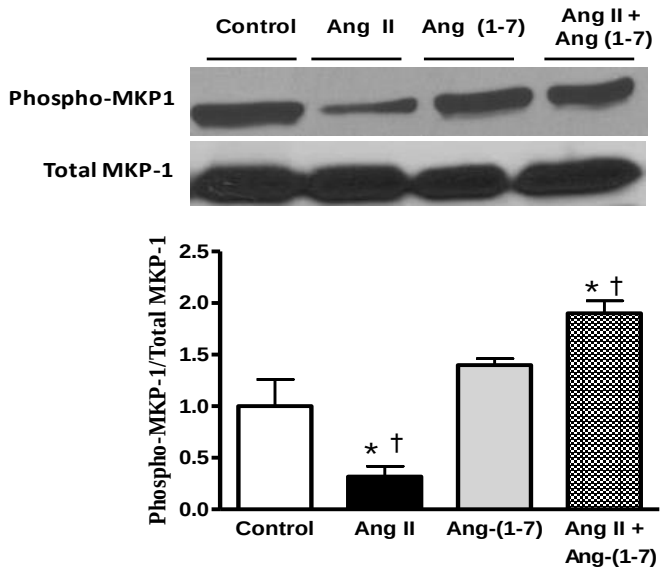


Fig.3. Ang II decreases MKP-1 phosphorylation in VSMCs, and this effect is abolished by Ang-(1-7). Incubation of VSMCs with Ang II (1 µM, for 2 min), decreased the phosphorylation of MKP-1^{Ser359}, and Ang-(1-7) (10 µM, for 5 min) was able to revert this response. Bar graphs show the relative expression of phosphorylated MKP-1^{Ser359} after normalization to the corresponding total protein expression, and are expressed as arbitrary units (n=6). Results are presented as mean ± SEM in each experimental group. * $P < 0.05$ vs. vehicle (H_2O); † $P < 0.05$ vs. Ang-(1-7).

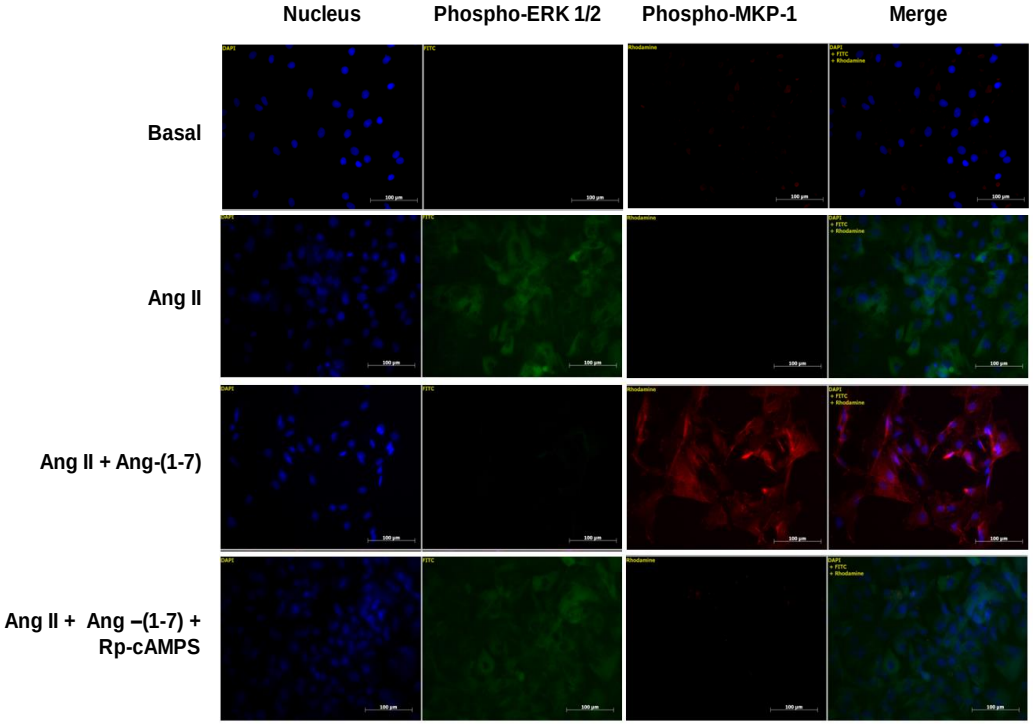


Fig.4. Ang-(1-7) promotes MKP-1 activation and prevents ERK1/2 phosphorylation in VSMCs, upon Ang II stimulation. Immunohistochemistry demonstrating that Ang II increased the phosphorylation of ERK1/2$^{Thr202/Tyr204}$ whereas it decreased MKP-1^{Ser359} phosphorylation in VSMCs. Ang II effects are not observed when cells were previously incubated with Ang-(1-7) (10 µM, n=5 for each group), where augmented phosphorylation of MKP-1^{Ser359} was observed. cAMP antagonist Rp-AMPS (100 µM) abrogated Ang-(1-7) effects on ERK1/2 and MKP-1 phosphorylation. Blue: DAPI stained nuclei; green: ERK1/2$^{Thr202/Tyr204}$ phosphorylation (primary anti-ERK1/2 phosphorylated antibody and secondary anti-mouse IgG antibody Alex Fluor 488); red: MKP-1 ^{Ser359} phosphorylation (primary anti-MKP-1 phosphorylated antibody and secondary anti-rabbit IgG antibody- Alexa Fluor 647). Marked cells were examined using a phase contrast microscope (Zeiss®) with magnification of 20x..

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Stimulation of VSMCs with Ang II increased the phosphorylation of C-Raf and ERK1/2 (Figure 2A-B). Considering the interplay between Ang II and Ang-(1-7), we the determined the effect of Ang-(1-7) in components of the C-Raf-ERK1/2 signaling pathway. While Ang-(1-7) did not prevent C-Raf phosphorylation (Figure 2A), it significantly affected the phophorylation status of ERK1/2 (Figure 2B).

Overexpression of Ang II decreased the phosphorylation of MKP-1 in VSMCs. Single incubation with Ang-(1-7) did not affect MKP-1 phophorylation. Interestingly, simultaneous incubation of Ang-(1-7) and Ang II incremented MKP-1 phosphorylation (Figure 3).

These results were further confirmed with immunohistochemistry analysis. Incubation of VSMCs with Ang II negatively modulated MKP-1 phosphorylation and augmented ERK1/2 phosphorylation. The effect of Ang II on MKP-1-activation was prevented when cells were incubated with Ang-(1-7), and consequently, this peptide attenuated ERK1/2 phosphorylation. The effects of Ang-(1-7) were abolished in the presence of the cAMP antagonist (Rp-AMPS) (Figure 4).

Discussion

Ang-(1-7) is considered as the main antagonist of Ang-II actions (7,8). However, our current understanding of the interplay between Ang-II and Ang-(1-7) and their action on MKP-1 in VSMCs, and consequently in vascular function is still incomplete. Therefore, identifying the role of Ang-(1-7) in MKP-1-regulation directly through the VSMCs may help predict the outcome effects of RAS under physiological and pathological conditions.

Our data provide evidence that Ang-(1-7) decreases Ang II-induced vasoconstriction, and this effect is not observed when arteries are incubated with an ERK1/2 inhibitor. Indeed, Ang-(1-7) attenuates Ang II-induced ERK1/2 activation in VSMCs, through an MKP-1-dependent mechanism. Taken together, our data propose a new mechanism for Ang-(1-7) counter-regulation of the actions evoked by Ang II stimulation, via MKP-1 activation.

Ang-(1-7)-incubation decreases the contractile response induced by Ang II in endothelium-denuded aortas, through a mechanism dependent on Mas receptor activation and cAMP production. The anti-contractile effects displayed by Ang-(1-7) upon Mas receptor activation may, in the long term, contribute to vascular remodeling and blood pressure regulation. Mounting evidence suggests that Ang-(1-7) exerts its effects mainly through the Mas receptor (36), thereby leading to Ang-II-antagonistic effects, such as vasodilation (37) and anti-inflammatory effects (7).

It is a consensus in the literature that Ang-(1-7)/Mas axis induces the relaxation of VSMCs through stimulation of nitric oxide and by the release of prostaglandins and/or endothelial dependent hyperpolarizing relaxation factor (37,38), as well as favoring the potentiation of bradykinin (39). This evidence suggests that a possible mechanism by which Ang-(1-7) counteracts the Ang II-actions could be related to stimulation of vasorelaxation factors derived from endothelial cells.

However, another possibility is that vascular protective effect of Ang-(1-7), on Ang II-induced vasoconstriction, is directly related to the signaling pathways activated in VSMCs. An important observation on this regard was the lack of Ang-(1-7)-evoked response upon Ang II-stimulation, when vessels were previously incubated with an inhibitor for ERK1/2. In VSMCs, Ang II leads to increased phosphorylation of ERK1/2 (40,41), culminating in several vascular events, including vasoconstriction, inflammation, endothelial fibrosis, and vascular dysfunction (41, 42). In accordance with our hypothesis, Ang-(1-7) has been shown to inhibit ERK1/2 in experimental model of allergic asthma

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(43), cardiac remodeling (44), and microvascular damage during hypertension (45). Our results go along with these previous reviews, since Ang-(1-7) was able to prevent Ang II-induced contraction in endothelium-denuded rat aortas, proposing a protective mechanism for Ang-(1-7) directly driven by VSMCs.

The magnitude and duration of ERK1/2 phosphorylation are crucial in determining its biological responses. Therefore, specific protein phosphatases governing its activity play an important role in controlling ERK activation (46). Once activated, C-Ras initiates the sequential phosphorylation events that ultimately result in ERK activation, which regulates the activity of several transcriptional factors, leading to cell-specific biological responses (46, 47). Thus, phosphorylation is an important event for ERK1/2 to become fully active (47). Interestingly, Ang-(1-7) treatment prevented Ang II-induced ERK1/2 phosphorylation but did not affect C-Raf-phosphorylation. Similar results were previously observed in the heart (48), kidney (49) and blood vessels (50). However, the actual mechanism of ERK1/2 inhibition evoked by Ang-(1–7), upon Ang II-stimulation is still unclear.

Considering that Ang-(1-7) can regulate several intracellular signal transduction cascades in VSMCs, the next step was to evaluate the effect of Ang-(1-7) on an important regulatory member of MAPKs, MKP-1. The physiological protein phosphatase for C-Raf is not known, although it can be dephosphorylated *in vitro* by protein phosphatase 1 and protein phosphatase 2A (47). However, MKP-1 is recognized as the dual-specificity MAPK phosphatase, and it is well-established as a negative regulator of ERK1/2 in mammalian cells and tissues. When MKP-1 is phosphorylated, its degradation is inhibited and its half-life grows over two or three folds; consequently, leading to greater activation of this phosphatase (42, 51). Amongst all MKPs, MKP-1 is the most widely studied and it has been suggested that MKP-1 is likely to be one of the most energy-efficient modes for deactivation of MAPK (22). Consequently, some studies indicate that this phosphatase has the potential to serve as a therapeutic strategy for the treatment of diseases (52). In the present study, treatment with Ang-(1-7), itself, did not modify MKP-1 expression. On the other hand Ang II decreases MKP-1 phosphorylation in VSMCs, and this effect was abolished by Ang-(1-7). Thus, one might speculate that Ang II decreases MKP-1 activity favoring ERK1/2 activity, and finally, vasoconstriction. Opposing the Ang II-evoked actions, Ang-(1-7) prevents MKP-1 dephosphorylation induced by Ang II in VSMCs.

The contribution of alternative ways, other than Ang-(1-7), to counter-regulate Ang II-actions via ERK1/2 are under clinic use, reinforcing the importance of this new proposed pathway. It has been reported that the angiotensin type 2 receptor stimulates protein dephosphorylation, which counterbalances protein phosphorylation induced by angiotensin type 1 receptor, thus, affecting the signaling pathways within the cell, leading mainly to the opposite cellular actions (7). Besides that, it has been shown that Ang II evokes ERK1/2 activation via both angiotensin type 1 and type 2 receptors (53). Therefore, the use of renin-angiotensin-aldosterone system inhibitors can also play a relevant role in these signaling pathways, in a synergic manner as Ang-(1-7).

In this sense, studies have been showing that the combination of Ang-(1-7) along with an angiotensin II type 1 receptor blocker (losartan) was more effective in vascular protective effect, regression of glomerulosclerosis, and anti-atherosclerosis effects relative to either separate treatment (54, 55). Therefore, the different renin-angiotensin-aldosterone system inhibitors may promote cardiovascular protective properties through different mechanisms, consequently that

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combined treatment with two renin-angiotensin-aldosterone system inhibitors might have a favorable effect than monotherapy.

Considering that activation of ERK1/2 in vascular tissues is observed in several pathological conditions including hypertension, diabetes, and atherosclerosis, our study provides further evidence that the role of Ang-(1-7) in MKP-1-regulation may represent a target for new therapeutic development. Together, these findings pave the way for the development of new combination strategies that use Ang-(1-7), along with other currently used renin-angiotensin-aldosterone system inhibitors, for the treatment of cardiovascular diseases (56).

In conclusion, these findings show that Ang-(1-7) prevents Ang II-evoked actions, restoring MKP-1-activation, and consequently, attenuating ERK1/2 activity in VSMCs, and consequently, reducing vascular contractility. It is important to mention that MKP-1-activation is not observed when VSMCs are treated with Ang-(1-7), in the absence of Ang-II. This new proposed mechanism of action elucidates the vascular protective effects promoted by Ang-(1-7) directly through the VSMCs upon contractile-stimuli, and opens the possibility for other signaling pathways to be involved in this process.

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Conflict of interest

The authors declare that they have no conflict of interest.

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