

Potential of Bradykinin by Angiotensin-(1-7) on Arterioles of Spontaneously Hypertensive Rats Studied In Vivo

Liliam Fernandes, Zuleica Bruno Fortes, Dorothy Nigro, Rita C.A. Tostes, Robson A.S. Santos, Maria Helena Catelli de Carvalho

Abstract—In the present study, we investigated the potentiating effect of angiotensin-(1-7) [Ang-(1-7)] on bradykinin (BK)-induced vasodilation in the mesenteric vascular bed of anesthetized spontaneously hypertensive rats using intravital microscopy. Topical application of BK and Ang-(1-7) induced vasodilation in mesenteric arterioles. The BK-induced effect, but not acetylcholine, sodium nitroprusside, or histamine responses, was potentiated in the presence of Ang-(1-7). This interaction was abolished by BK-B₂ and Ang-(1-7) antagonists (HOE 140 and A-779, respectively), a K⁺ channel blocker (tetraethylammonium), and cyclooxygenase inhibitors (indomethacin and diclofenac); however, nitric oxide synthase inhibition (*N* ω -nitro-L-arginine methyl ester) did not modify the Ang-(1-7)-potentiating activity. Long-term angiotensin-converting enzyme (ACE) inhibition increased BK and Ang-(1-7)-induced vasodilation. The BK potentiation by Ang-(1-7) was preserved after ACE inhibition, Ang II type 1 receptor blockade, or the combination of both treatments. The most striking finding of this study was the unexpected observation that the potentiation of BK vasodilation in spontaneously hypertensive rats treated short- or long-term with ACE inhibitors was reverted by the Ang-(1-7) antagonist A-779. Our results unmasked a key role for an Ang-(1-7)-related mechanism in mediating BK potentiation by ACE inhibitors. (*Hypertension*. 2001;37[part 2]:703-709.)

Key Words: bradykinin ■ angiotensin ■ microcirculation ■ hypertension ■ angiotensin-converting enzyme ■ renin-angiotensin system

Angiotensin-(1-7) [Ang-(1-7)] is a bioactive component of the renin-angiotensin system that can be formed by an angiotensin-converting enzyme (ACE)-independent pathway.¹ Moreover, recent studies suggest that this heptapeptide is an endogenous substrate for ACE.² Several data indicate that the net actions of the renin-angiotensin system in the long-term regulation of blood pressure may depend on a balance between the effects of Ang II and Ang-(1-7).³

Ang-(1-7) potentiates the vasodilatory effect of bradykinin (BK) in isolated dog coronary arteries,⁴ the vasoconstrictor action in rabbit jugular veins,⁵ and the hypotensive effect of BK in normotensive⁶ and hypertensive⁷ rats. In a previous study, we demonstrated, using in vivo/in situ microvessel preparations of normotensive rats, that Ang-(1-7) increases the BK-induced vasodilation through the release of products of nitric oxide (NO) and cyclooxygenase (COX).⁸

Taken together, these observations may be particularly important because long-term ACE inhibition further increases plasma Ang-(1-7) levels.^{9,10} Interestingly, it was demonstrated that systemic administration of an Ang-(1-7) monoclonal antibody¹¹ or short-term inhibition of the endogenous

synthesis of Ang-(1-7) by 2 neprilysin inhibitors¹² partially reversed the antihypertensive response in spontaneously hypertensive rats (SHR) that were treated with lisinopril/losartan long-term, suggesting that Ang-(1-7) may play a role in the mechanism of action of these drugs. One important question that arises from these observations is whether the direct vasodilation and/or BK potentiation of Ang-(1-7) occurs at the level of resistance vessels, the most functionally important site for determining peripheral vascular resistance.¹³ In this study, we addressed this issue by investigating the Ang-(1-7) activity on the BK-induced effect and the possible mechanisms involved within SHR resistance arterioles by using in vivo/in situ mesenteric microvessel preparations. Furthermore, the interaction of Ang-(1-7) and BK was also evaluated after long-term ACE inhibition, Ang II type 1 receptor (AT₁) blockade, and the combination of both treatments.

Methods

Animals

Experiments were performed in male SHR weighing 220 to 250 g (aged 14 to 16 weeks). All animal procedures were done in

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From the Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil, and the Department of Physiology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil (R.A.S.S.).

Correspondence to Maria Helena Catelli de Carvalho, PhD, Avenue Prof Lineu Prestes, 1524, sala 217, Cidade Universitaria CEP 05508-900 Sao Paulo, SP, Brazil. E-mail mahecaca@icb.usp.br

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accordance with the guidelines of the Ethics Committee of the Institute of Biomedical Sciences, University of Sao Paulo, Brazil. Animals were kept in a temperature-controlled room on a 12/12-hour light/dark cycle. For groups that received prolonged treatment, blood pressure was measured indirectly (tail-cuff method) in conscious animals before starting treatment and 24 hours after the last administration.

Drugs and Reagents

Acetylcholine chloride, histamine dihydrochloride, sodium nitroprusside (SNP), tetraethylammonium chloride (TEA), indomethacin, and *N* ω -nitro-L-arginine methyl ester (L-NAME) were obtained from Sigma Chemical Co; BK, Ang-(1-7), and D-Ala⁷-Ang-(1-7) (A-779) were from Bachem-CA; diclofenac potassium salt (Cat-aflan) was from Geigy; and enalapril (Renitec), losartan (Cozaar), and chloral hydrate were from Merck, Sharp, & Döhme. Enalaprilat and HOE 140 were kindly supplied by Merck, Sharp, & Döhme (Whitehouse Station, NJ) and Hoechst Marion Roussel (Frankfurt, Germany), respectively.

Intravital Microscopy

The rats were anesthetized with chloral hydrate (450 mg/kg SC), and the mesentery was arranged for microscopic observation *in situ*.^{14,15} Briefly, the animals were kept on a special board that was heated at 37°C; the board included a transparent plate on which the tissue to be transilluminated was placed. The mesentery was kept moist and warm by irrigating the tissue with warmed (37°C) Ringer Locke's solution (pH 7.4) containing 1% gelatin. A 500-line television camera was combined with a triocular microscope to facilitate observation of the enlarged image (3400 \times) on the video screen. An image-splitting micrometer was adjusted to the phototube of the microscope. The image splitter sheared the optical image into 2 separate images and displaced one with respect to the other. The displacement of one image from the other allowed for the measurement of the vessel diameter.¹⁶ A₂ arterioles (15 to 25 μ m) were selected for study, and any changes in vessel diameter were estimated after the topical application of vasoactive agents. The drugs were added to the preparation in a standard volume of 0.01 mL and were removed by washing with warmed Ringer-Locke's solution.

Experimental Protocols

Effect of Ang-(1-7) on the BK-Induced Vasodilation in Mesenteric Arterioles

In the first set of experiments, different doses of BK (1, 10, and 30 pmol) and Ang-(1-7) (10, 100, and 1000 pmol; *n*=8 for each dose) were applied alone. For potentiation experiments, the dose of 1 pmol of BK was initially applied. After an interval of 3 minutes, each dose (*n*=8 to 12) of Ang-(1-7) was added to the preparation 30 seconds before BK application. On the basis of these preliminary studies, the doses of 1 pmol of BK and 100 pmol of Ang-(1-7) were chosen for the additional experiments. To verify whether 1 pmol of BK would potentiate the vasodilation induced by 100 pmol of Ang-(1-7), the nonapeptide was applied 30 seconds before Ang-(1-7) (*n*=8).

Specificity of the Ang-(1-7) Potentiating Activity

BK-equipotent doses of acetylcholine (ACh; 1.6 nmol), SNP (38 pmol), and histamine (5.4 nmol; *n*=8 to 10 for each drug) were tested in the absence and presence of 100 pmol of Ang-(1-7).

Effect of B₂ and Ang-(1-7) Receptors Antagonists on the Ang-(1-7)-BK Interaction

HOE 140, a specific B₂ receptor antagonist,¹⁷ and A-779, a specific Ang-(1-7) receptor antagonist,¹⁸ were used. The dose and the time delay necessary for the specific effects of these agents were chosen in preliminary experiments. HOE 140 (100 pmol) or A-779 (100 pmol) were added to the preparations 15 seconds and 1 minute, respectively, before test BK (*n*=8) and Ang-(1-7) (*n*=7), alone or in combination (*n*=8).

Participation of Prostanoids, Endothelium-Derived Hyperpolarizing Factor, and NO on the Ang-(1-7)-BK Interaction

To inhibit the COX pathway, animals were treated with indomethacin (5 mg/kg IM) or diclofenac (2.5 mg/kg IM) 30 minutes before the following experiments (*n*=8 for each group). Control animals received vehicle (Tris buffer for indomethacin or saline for diclofenac). The participation of an endothelium-derived hyperpolarizing factor (EDHF) on the potentiation of BK by Ang-(1-7) was evaluated using TEA. Due to the rapid onset of action of this agent,¹⁹ 100 pmol of Ang-(1-7) was applied combined with 90 pmol of TEA (*n*=8). To investigate whether NO could be involved in the interaction of BK and Ang-(1-7), L-NAME (10 nmol), a NO synthase (NOS) inhibitor, was added to the preparations 3 minutes before BK, Ang-(1-7), or both (*n*=9). The dose and the time delay necessary for the effect of this agent were chosen in preliminary experiments. In all groups, BK (1 pmol) and Ang-(1-7) (100 pmol) were tested alone and in combination.

Effect of ACE Inhibition and AT₁ Blockade on Ang-(1-7)-BK Interaction

Animals were treated with the ACE inhibitor enalapril (10 mg \cdot kg⁻¹ \cdot d⁻¹) or with the AT₁ antagonist losartan (15 mg \cdot kg⁻¹ \cdot d⁻¹) by gavage over 21 days (*n*=8 to 10). Animals in a third group (*n*=10) received both drugs in combination (10 mg/kg enalapril plus 15 mg/kg losartan) during the same period. Control animals received vehicle (saline). At the end of the treatment period, animals were anesthetized, and the interaction of Ang-(1-7) and BK was analyzed as described above.

Effect of Ang-(1-7) Receptor Blockade on BK Vasodilation After ACE Inhibition

BK (1 pmol) was tested in the presence of A-779 (100 pmol, topically applied 15 seconds before BK administration) in animals treated short-term with enalaprilat (10 mg/kg IV for 30 minutes before administration; *n*=8) or long-term with enalapril (10 mg/kg each day for 21 days by gavage, *n*=9). Control animals received vehicle (saline).

Statistical Analysis

Comparisons were made by ANOVA followed by the Tukey-Kramer test, Kruskal-Wallis test, Student's paired or unpaired *t* test, and linear regression when appropriate. All values are reported as mean \pm SEM. Statistical significance was set as *P*<0.05.

Results

Vascular Effects of BK and Ang-(1-7) Alone and in Combination

BK and Ang-(1-7) induced vasodilation (expressed as percent increase in initial diameters) in SHR arterioles studied *in vivo* (Figures 1A and 1B). The vasodilation elicited by BK (1 pmol) was potentiated by Ang-(1-7) 10 and 100 pmol, whereas the higher dose of Ang-(1-7) (1000 pmol) did not modify the effect of BK (Figure 1C). On the basis of these results, the doses of 1 pmol of BK and 100 pmol of Ang-(1-7) were chosen for the additional experiments because (1) they elicited a small but significant increase in arteriolar diameter (3% to 5%) when tested alone and (2) these doses used in conjunction elicited the highest potentiating effect of Ang-(1-7) on BK vasodilation (Figure 1C). It is important to mention that (1) the alteration in diameter induced by Ang-(1-7) had already disappeared after 30 seconds, when BK was applied, and (2) the vascular effect of 100 pmol of

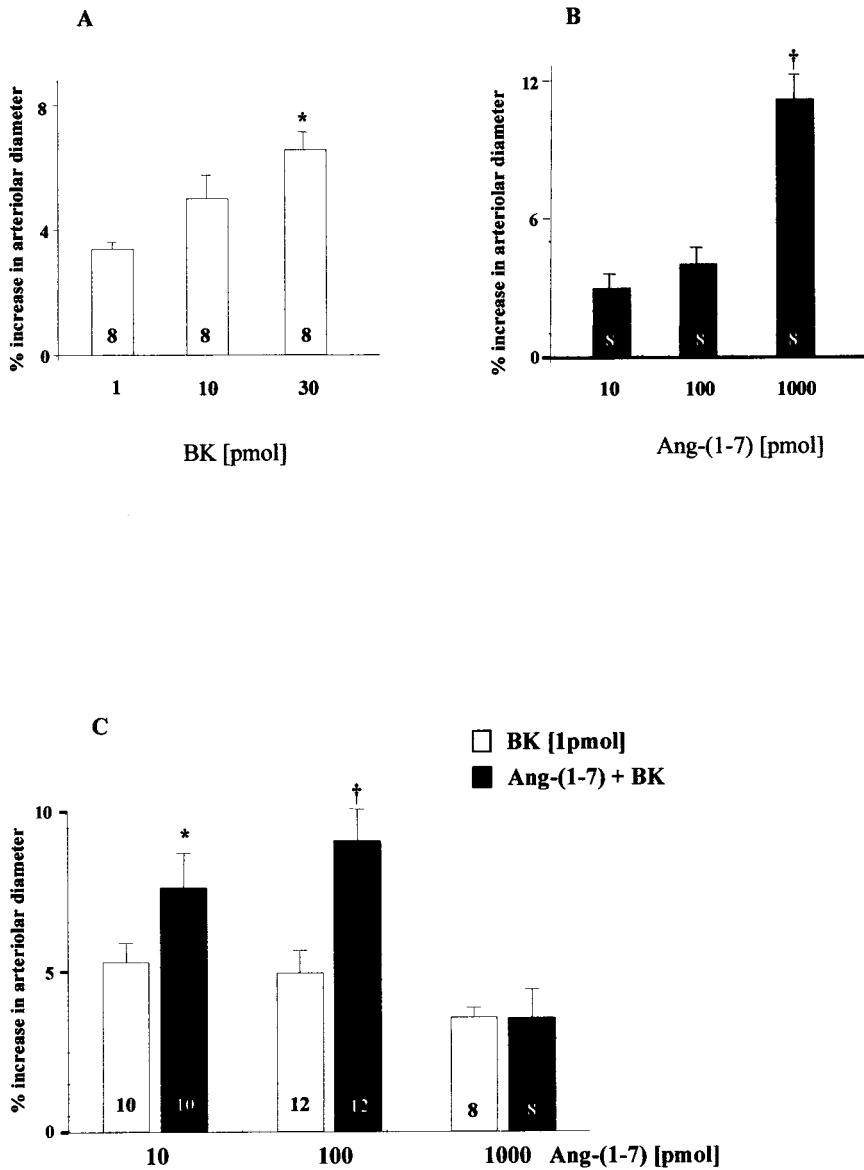


Figure 1. Bar graphs show increase (%) observed in SHR mesenteric arteriolar diameter induced by topical application of BK (A), Ang-(1-7) (B), and BK after Ang-(1-7) (C). Results are expressed as mean ± SEM. Numbers inside bars indicate number of preparations tested. In A, **P* < 0.01 vs BK 1 pmol; in B, †*P* < 0.001 vs Ang-(1-7) 10 and 100 pmol; and in C, **P* < 0.05 and †*P* < 0.01 vs BK.

Ang-(1-7) (4.04 ± 0.6%) was not increased by the prior addition of 1 pmol of BK (3.89 ± 0.6%).

Specificity of the BK Potentiation by Ang-(1-7)

The vascular effects of ACh and SNP were not potentiated in the presence of 100 pmol of Ang-(1-7) (ACh: 2.12 ± 1.0% to 2.99 ± 0.9%; SNP: 5.19 ± 0.3% to 4.21 ± 0.4%). The responses to histamine were reduced with Ang-(1-7) [3.49 ± 0.4% to 1.34 ± 0.3%; *P* < 0.01 versus value in the absence of Ang-(1-7)].

Effect of B₂ and Ang-(1-7) Receptor Blockade on the Ang-(1-7)-BK Interaction

Application of HOE 140 (100 pmol) nearly abolished the vasodilation induced by BK, without any statistically significant effect on Ang-(1-7) dilation. Treatment with A-779 (100 pmol) did not interfere with BK dilation but blocked the effect of Ang-(1-7) (Table). The potentiation of BK by Ang-(1-7) was not observed after B₂ or Ang-(1-7) receptor blockade.

Contribution of Prostanoids, EDHF, and NO to the Potentiation of BK by Ang-(1-7)

After treatments with the COX inhibitors indomethacin (5 mg/kg) or diclofenac (2.5 mg/kg), the vasodilation induced by BK or Ang-(1-7) alone was unaltered (Table), but the potentiating effect of Ang-(1-7) was completely abolished (Figure 2). In the same way, the K⁺ channel blockade (TEA-90 pmol) did not alter the effects of BK and Ang-(1-7) alone (Table), but it abolished the BK potentiation by Ang-(1-7) (Figure 2). NOS inhibition (10 nmol of L-NAME) reduced the effects of BK and Ang-(1-7) (Table), without preventing the Ang-(1-7) potentiating activity on BK vasodilation. The ratio between BK and Ang-(1-7) plus BK was the same before and after NOS inhibition, as seen in Figure 2.

Effects of ACE Inhibition, AT₁ Blockade, or Combination of Both Treatments on the Ang-(1-7)-BK Interaction

Enalapril (ACE inhibitor), losartan (AT₁ antagonist), and enalapril plus losartan treatments decreased blood pressure in

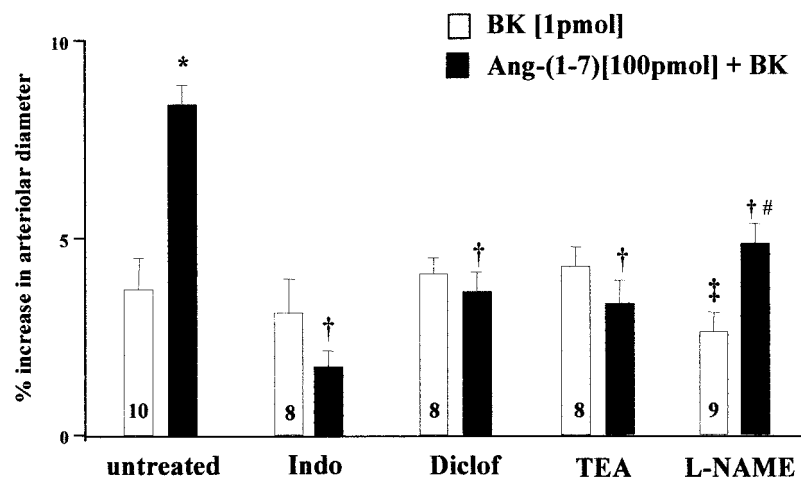


Figure 2. Bar graphs show increase (%) observed in SHR mesenteric arteriolar diameter induced by topical application of BK and BK after Ang-(1-7) in untreated animals and animals treated with indomethacin (Indo; 5 mg/kg), diclofenac (Diclof; 2.5 mg/kg), TEA (90 pmol topically applied), and L-NAME (10 nmol, topically applied). Numbers inside bars indicate number of preparations tested. * $P < 0.01$ vs BK, † $P < 0.001$ vs untreated, ‡ $P < 0.05$ vs BK untreated, and # $P < 0.05$ vs BK after L-NAME.

SHR from 171.7 ± 5.6 to 143.4 ± 5.3 mm Hg, 167.8 ± 2.8 to 147.1 ± 6.9 mm Hg, and 179.8 ± 3.2 to 129.2 ± 6.6 mm Hg, respectively ($P < 0.05$ versus values before treatment for all). ACE inhibition induced an increase in BK and Ang-(1-7) dilation, whereas AT_1 blockade did not modify the effects of each of these agents alone (Table). ACE inhibition (enalapril or enalapril plus losartan) promoted a slight but significant potentiation of BK by Ang-(1-7) (Figure 3A). AT_1 blockade did not modify the Ang-(1-7)-activity on BK (Figure 3A). There was no relation between the decrease in blood pressure caused by these treatments and the vasodilation elicited by Ang-(1-7) plus BK. The correlation coefficient (r) was -0.29 for enalapril, 0.59 for losartan, and 0.18 for enalapril plus losartan.

Effect of Ang-(1-7) Receptor Blockade on the BK Vasodilation

BK (1 pmol) was tested in the presence of A-779 (100 pmol, topically applied 15 seconds before BK) in rats treated short-term with enalaprilat (10 mg/kg) or long-term with enalapril ($10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$). The increased BK vasodilation observed after short- or long-term ACE inhibition was reverted by A-779 in the microvessels of SHR (Figure 3B). It is important to mention that the dose used of A-779 did not

modify the vascular effects of 1 pmol of BK in untreated rats (Table).

Discussion

In the present study, we demonstrated for the first time that in the resistance blood vessels of SHR, Ang-(1-7) induces vasodilation and potentiates BK when topically applied. In addition, we showed that the vasodilation promoted by the heptapeptide may occur through NO release from endothelial cells after the stimulation of a specific receptor. Furthermore, our data unraveled an important contribution of Ang-(1-7) for the increased vasodilation produced by BK in rats treated short- or long-term with ACE inhibitors.

Several studies have described the vascular effects of the heptapeptide Ang-(1-7). Its vasodilatory effect was demonstrated in cat hindlimbs,²⁰ dog coronary arteries,^{4,21} and the microcirculation of normotensive rats.⁸ Interestingly, our experiments showed that Ang-(1-7) produces vasodilation in SHR arterioles, even when applied at low doses (10 pmol).

The BK-potential by Ang-(1-7) was described in conscious normotensive⁶ and hypertensive rats,⁷ isolated dog coronary arteries,^{4,21} and mesenteric microvessels of normotensive rats.⁸ In the present investigation, we demonstrated that in the mesenteric bed of SHR, the effect of 1 pmol of BK

Effect of Antagonists and Enzyme Inhibitors on BK and Ang-(1-7) Vasodilation

Group	BK (1 pmol)		Ang-(1-7) (100 pmol)	
	Untreated	Treated	Untreated	Treated
HOE 140 (100 pmol, TA)	6.52 ± 1.1	$-0.57 \pm 0.9^*$ (8)	3.23 ± 0.7	1.95 ± 0.8 (7)
A-779 (100 pmol TA)	5.59 ± 1.0	5.04 ± 0.5 (7)	4.50 ± 0.7	$-0.44 \pm 0.6^*$ (7)
Indomethacin (5 mg/kg IM)	3.68 ± 0.8	3.08 ± 0.5 (8)	2.83 ± 0.3	2.53 ± 0.6 (8)
Diclofenac (2.5 mg/kg IM)	3.94 ± 0.5	4.08 ± 0.5 (8)	2.88 ± 0.4	2.36 ± 0.3 (8)
TEA (90 pmol TA)	4.32 ± 0.5	3.98 ± 0.7 (8)	3.57 ± 0.4	2.75 ± 0.5 (8)
L-NAME (10 nmol TA)	4.86 ± 0.5	$2.60 \pm 0.5^\ddagger$ (9)	3.79 ± 0.3	$1.29 \pm 0.5^*$ (9)
Enalapril ($10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for 21 d)	3.09 ± 0.4	$8.42 \pm 0.7^\ddagger$ (10)	3.18 ± 0.3	$5.61 \pm 0.5^\ddagger$ (10)
Losartan ($15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for 21 d)	4.93 ± 0.3	3.75 ± 0.2 (10)	3.76 ± 0.9	4.48 ± 0.5 (10)
Enalapril+losartan (10 and $15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for 21 d, respectively)	3.35 ± 0.05	$7.79 \pm 0.3^\ddagger$ (10)	3.31 ± 0.3	$5.68 \pm 0.4^*$ (10)

Values are mean \pm SEM. Number in parentheses indicates number of experiments. TA indicates topical application.

* $P < 0.01$, † $P < 0.05$, ‡ $P < 0.001$ vs values in untreated animals.

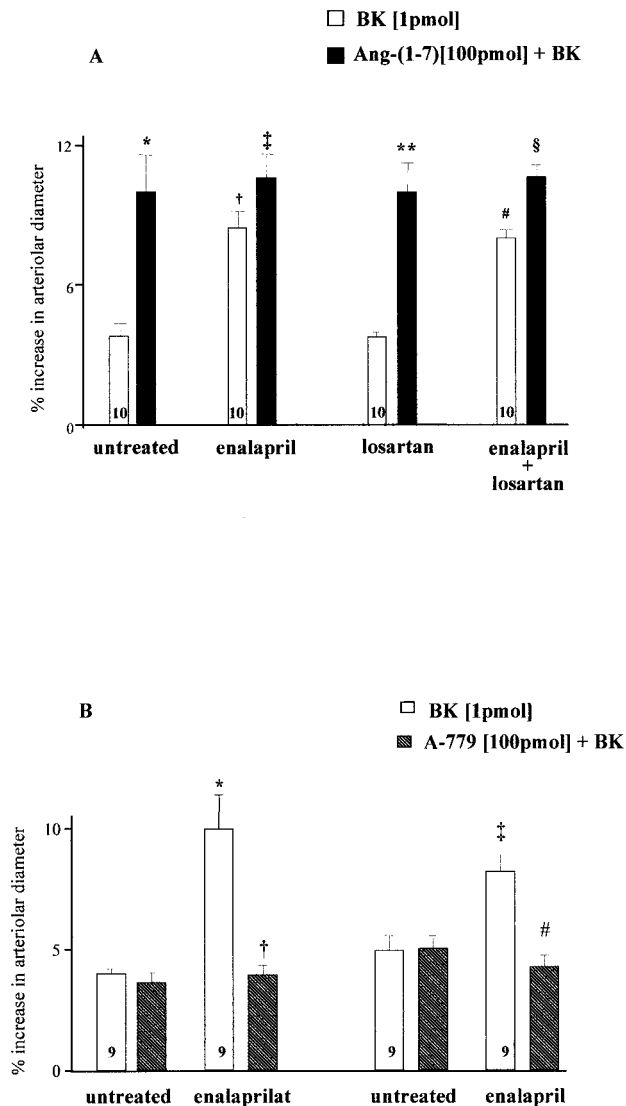


Figure 3. A, Bar graphs show increase (%) observed in SHR mesenteric arteriolar diameter induced by BK and by BK after Ang-(1-7) was topically applied in untreated animals and animals treated with enalapril ($10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$), losartan ($15 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$), or enalapril plus losartan. * $P < 0.01$ vs BK, † $P < 0.05$ vs BK untreated, ‡ $P < 0.05$ vs BK after enalapril, ** $P < 0.001$ vs BK after losartan, # $P < 0.001$ vs BK untreated, and § $P < 0.01$ vs BK after enalapril plus losartan. B, Bar graphs show increase (%) observed in SHR mesenteric arteriolar diameter induced by BK and by BK after Ang-(1-7) antagonist A-779 was topically applied in animals treated short-term with enalaprilat (10 mg/kg) or not (untreated) and in animals treated long-term with enalapril ($10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) or not (untreated). * $P < 0.001$ vs BK, † $P < 0.001$ vs BK in enalaprilat-treated animals, ‡ $P < 0.05$ vs BK, and # $P < 0.01$ vs BK in enalapril-treated rats.

was potentiated in the presence of 10 and 100 pmol of Ang-(1-7). The fact that the alteration in diameter induced by Ang-(1-7) had already disappeared when BK was applied led us to conclude that Ang-(1-7) is, in fact, potentiating BK, excluding a summation of both vasodilation effects. One of the first important aspects of this study is the fact that the potentiation of BK by Ang-(1-7) was observed at low doses but not at high doses of the heptapeptide (1000 pmol). Similar results were reported in the mesenteric microvessels of

normotensive rats.⁸ Curiously, a bell-shaped dose-response curve was obtained for the release of arachidonic acid and 6-keto-prostaglandin F₁α by Ang-(1-7) in cultured vascular smooth muscle cells,²² indicating the existence of an ideal concentration of this agent to activate intracellular pathways. Although our data do not allow the evaluation of this phenomenon, the hypothesis of receptor desensitization cannot be discarded.

One of the possible mechanisms proposed for the BK potentiation by Ang-(1-7) is based on the fact that the heptapeptide, as a substrate for ACE, could inhibit ACE activity and elevate BK levels.^{3,21} Our results are in contrast with this hypothesis, because the Ang-(1-7) receptor blockade by topical application of its antagonist A-779 completely abolished the potentiation of BK by Ang-(1-7), indicating that the binding of the heptapeptide to its own receptor is an essential condition for BK potentiation. Similar observations were reported previously,^{7,8} suggesting that this metabolic effect may not represent the main pathway of BK potentiation by Ang-(1-7).

It has been suggested that the Ang-(1-7) receptor could be linked to a signaling pathway that would stimulate the formation of NO via an intermediate rise in the concentration of vascular kinins.⁴ Our data with the BK B₂ antagonist HOE 140 does not support this hypothesis. The dose of HOE 140 was sufficient to completely block the vasodilation produced by BK and did not significantly change the effect of Ang-(1-7). These data are in agreement with previous findings⁸ and may indicate that, in the vascular bed tested, Ang-(1-7) binds to a specific receptor and activates an intracellular signal pathway that is independent of BK stimulus.

Similar to the observations in normotensive rats,⁸ Ang-(1-7) did not potentiate the vasodilation produced by ACh or SNP in SHR mesenteric microvessels; instead, it reduced histamine dilation. These data might indicate that in hypertensive animals the Ang-(1-7) potentiating activity may be specific for BK and may not be evoked by any agent that releases NO, similar to the data from normotensive vascular preparations.^{6,8,21}

In the mesenteric bed of SHR, pretreatment with L-NAME produced a pronounced reduction of the Ang-(1-7) effect (70%). The vasodilation induced by BK was also significantly reduced in SHR, but in a manner different from the observed reductions in the microvessels of normotensive rats.⁸ BK can induce vasodilation by releasing at least 3 endothelium-derived relaxing factors (NO, prostaglandin I₂, and EDHF); however, the contribution of each factor may vary according to the vascular territory and animal model. It was reported that in hypertensive rats, part of the hypotensive effect of BK was mediated through the NO system, whereas in normotensive rats, the hypotensive effect of BK would be mediated mainly by prostaglandins or EDHF.²³ We also demonstrated that in microvessels of SHR, the BK-potentiation by Ang-(1-7) was preserved after NOS inhibition, in opposition to the observations in normotensive animals.⁸ These data can indicate that, although Ang-(1-7) and BK may stimulate NO production/release in mesenteric microvessels of SHR, the Ang-(1-7)-potentiating activity is

not dependent on the NO system in this animal model of hypertension.

Our experiments showed that in the microcirculation of SHR, the potentiation of BK by Ang-(1-7) depends on prostanoids release, although the vascular effect of each peptide separately was not altered by pretreatments with COX inhibitors such as indomethacin or diclofenac. Concerning the Ang-(1-7) potentiating activity, similar results were previously observed in normotensive rats,^{6,8} suggesting that Ang-(1-7) can potentiate BK, at least in part, by facilitating the production of a vasodilator prostaglandin, such as prostaglandin I₂, or impairing the release of vasoconstrictor prostanoids in the microvessels of hypertensive animals.

It has been described that, in addition to NO and prostaglandin I₂ release, the vasodilation produced by BK could involve EDHF production.^{24,25} In the present study, we demonstrated that the blockade of K⁺ channels with TEA abolished the Ang-(1-7)-potentiating effect on BK vasodilation in the microvessels of SHR, without interfering with the effect of each agent alone. This is the first demonstration that the BK-potentiation by Ang-(1-7) can involve hyperpolarization of the cell membrane, probably elicited by an EDHF. This finding is particularly relevant when taking in account the fact that EDHF seems to have a prevalent role in the vasorelaxation of rat mesenteric microvessels.²⁶

It was demonstrated that some of the Ang-(1-7) actions in the kidney²⁷ and heart²⁸ can be blocked by the AT₁ receptor antagonist losartan and that this blockade can increase plasma levels of the heptapeptide.²⁹ Our data, however, showed no interference of losartan on the Ang-(1-7) effects, which is unlike the involvement of AT₁ receptors on the actions of this substance in SHR microvessels.

In the present study, Ang-(1-7) vasodilation was significantly augmented by long-term treatment with the ACE inhibitor alone or in combination with an AT₁ receptor antagonist, probably due to the fact that ACE is a major Ang-(1-7) metabolizing enzyme.² Similar results were obtained in microvessels of normotensive rats,⁸ in which the vasodilation induced by Ang-(1-7) increased ≈2-fold after the administration of enalaprilat.

It has been suggested that some actions of Ang-(1-7) could be mediated by the potentiation of endogenous BK through the facilitation of a "cross-talk" mechanism between ACE and the B₂ receptor.³⁰ In this way, the binding of Ang-(1-7) to ACE would facilitate the "cross-talk" of ACE and the B₂ receptors, leading to the potentiation of endogenous BK independent of interference with BK hydrolysis. In our experiments, ACE inhibition did not prevent the Ang-(1-7) potentiating activity. This observation and the finding that the Ang-(1-7) antagonist A-779 completely abolished the Ang-(1-7) effects strongly suggest that, in mesenteric microvessels of SHR, the BK-potentiation activity of Ang-(1-7) is a receptor-mediated event that is not dependent on its interaction with ACE.

The most striking finding of our study was the unexpected observation that the potentiation of BK vasodilation in SHR treated short- or long-term with ACE inhibitors was reverted by the Ang-(1-7) antagonist A-779. This effect cannot be attributed to a nonspecific action because A-779 had no

noticeable influence on the vasodilation produced by BK in untreated rats. Our findings strongly suggest that endogenous Ang-(1-7) is an important component of the BK potentiation by ACE inhibition *in vivo*. A-779 [and Ang-(1-7)] could displace the ACE inhibitor from a catalytic ACE site involved in the BK metabolism. However, this possibility is highly unlikely because A-779 does not interfere with the ACE activity measured with Hip-His-Leu,³¹ which is hydrolyzed by either the N- or C- domain of ACE.³² A permissible effect of Ang-(1-7), after binding to its own receptor, for the cross-talk-related transduction mechanism evoked by the binding of enalaprilat to ACE should be considered as well.

In summary, we found that the BK potentiation by Ang-(1-7) also occurs in resistance vessels of SHR through a receptor-mediated mechanism involving vasodilator prostaglandin and membrane hyperpolarization but not NO release. We also demonstrated that the Ang-(1-7) potentiating activity is preserved after long-term ACE inhibition in hypertensive animals. Although further studies are needed for clarifying the mechanism(s) conveying this effect, it is important to emphasize that our results unmasked a key role for an Ang-(1-7)-related mechanism in mediating BK potentiation by ACE inhibitors.

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Liliam Fernandes, Zuleica Bruno Fortes, Dorothy Nigro, Rita C. A. Tostes, Robson A. S. Santos and Maria Helena Catelli de Carvalho

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