

Baroreflex Improvement in SHR After ACE Inhibition Involves Angiotensin-(1-7)

Silvia Heringer-Walther, Érica N. Batista, Thomas Walther, Mahesh C. Khosla, Robson A.S. Santos, Maria J. Campagnole-Santos

Abstract—ACE inhibitors are extensively used in the treatment of hypertension mainly because of their efficiency in reducing blood pressure levels and decreasing vascular and cardiac hypertrophy. In addition, ACE inhibitors improve baroreceptor reflex control. Chronic inhibition of ACE produces (in addition to decreased angiotensin II levels) a severe increase in angiotensin-(1-7) [Ang-(1-7)] levels in several species. We have previously shown that Ang-(1-7) produces a facilitation of the baroreflex control of heart rate. In this study, we evaluated the participation of endogenous Ang-(1-7) in the improvement of baroreflex sensitivity in spontaneously hypertensive rats after central infusion of ramiprilat, an ACE inhibitor. Reflex changes in heart rate were elicited, in conscious rats, by bolus injections of phenylephrine (baroreflex bradycardia) before and after intracerebroventricular infusion of (1) saline (8 μ L/h), 4 hours (n=5); (2) ramiprilat (14 μ g/h), 4 hours (n=6); (3) ramiprilat for 2 hours, followed by ramiprilat combined with A-779 (4 μ g/h), a selective Ang-(1-7) antagonist, for an additional 2 hours (n=6); and (4) A-779 for 2 hours, followed by A-779 combined with ramiprilat for an additional 2 hours (n=5). Intracerebroventricular infusion of ramiprilat produced an important increase (\approx 40%) in baroreflex sensitivity (evaluated as the ratio between changes in heart rate and changes in mean arterial pressure) that was completely reversed by A-779. Furthermore, intracerebroventricular infusion of A-779 prevented the improvement of the baroreflex sensitivity produced by ramiprilat. Intracerebroventricular infusion of saline or A-779 alone did not significantly alter the baroreflex sensitivity. These results suggest that endogenous Ang-(1-7) is involved in the improvement of baroreflex sensitivity observed in spontaneously hypertensive rats during central ACE inhibition. (*Hypertension*. 2001;37:1309-1314.)

Key Words: angiotensin-(1-7) ■ baroreceptors ■ angiotensin-converting enzyme inhibitors ■ renin-angiotensin system ■ rats, inbred SHR ■ A-779

In the last decade, several studies have shown that angiotensin-(1-7) [Ang-(1-7)] constitutes an important functional end-product of the renin-angiotensin system.^{1,2} Because the C-terminal phenylalanine is absent, the Ang-(1-7) actions are distinct from the pressor and drinking responses induced by angiotensin II (Ang II) and angiotensin III (Ang III).^{1,2} Peripherally, Ang-(1-7) itself produces a potent antidiuretic effect on water-loaded rats and potentiates bradykinin (BK).² Centrally, Ang-(1-7) produces cardiovascular responses when microinjected into the dorsomedial^{3,4} or ventrolateral^{5,6} medulla, and lateral ventricular intracerebroventricular infusion of Ang-(1-7) increases the sensitivity of the baroreceptor reflex (baroreflex bradycardia) in normotensive rats, in contrast to the decreased sensitivity produced by Ang II or Ang III infusion.⁷

We have recently characterized the Ang-(1-7) analogue, D-Ala⁷-Ang-(1-7) (A-779), as a selective Ang-(1-7) antagonist without intrinsic agonistic activity in several experimen-

tal approaches.⁸ A-779 was shown to antagonize the Ang-(1-7) actions centrally^{6,8,9} and peripherally⁸ and to displace the binding of ¹²⁵I-Ang-(1-7) to membranes of endothelial cells from bovine aorta¹⁰ and kidney slices.¹¹ In addition, A-779 did not interfere with the pressor, myotropic, or dipsogenic effect of Ang II or with the binding of ¹²⁵I-Ang II to cortical or adrenal medullary membranes, which are rich in angiotensin type 1 (AT₁) and angiotensin type 2 (AT₂) receptors, respectively.⁸ Using this compound, we^{2,4,6,12} and others^{1,9,13} have provided evidence that the effects of Ang-(1-7) are mediated through a specific receptor different from the classic Ang II receptors, AT₁ or AT₂. In addition, intracerebroventricular infusion of A-779 in Wistar rats significantly blunted the baroreceptor control of heart rate (HR), whereas the AT₁ receptor antagonist, losartan, facilitated the baroreflex,¹² suggesting a differential role for endogenous angiotensin peptides on baroreflex modulation in the brain.

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From the Department of Physiology and Biophysics, Biological Sciences Institute, Federal University of Minas Gerais, Belo Horizonte (S.H.-W., E.N.B., R.A.S.S., M.J.C.-S.), Brazil; the Department of Cardiology and Pneumology, University Hospital Benjamin Franklin, Free University of Berlin (S.H.-W., T.W.), Berlin, Germany; and the Department of Neurosciences, Cleveland Clinic Foundation (M.C.K.), Cleveland, Ohio.

Correspondence to Maria Jose Campagnole-Santos, PhD, Departamento de Fisiologia e Biofísica, Av. Antonio Carlos, 6627-ICB-UFMG, 31270-901 Belo Horizonte, MG, Brazil. E-mail mjes@icb.ufmg.br

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ACE inhibitors, which are largely used in the treatment of human hypertension, produce (among their important cardiovascular effects) a noticeable improvement of baroreceptor reflex.^{14,15} This facilitatory effect of ACE inhibitors is probably due to the blockade of ACE in the brain.^{16,17} It is well known that ACE inhibition decreases the production of Ang II and increases the circulating concentration of Ang-(1-7) in spontaneous hypertensive rats (SHR)¹⁸ and humans.¹⁹ The Ang-(1-7) buildup after ACE inhibition can be due to the accumulation of Ang I and/or decrease in its inactivation, because ACE is an important route for the metabolism of Ang-(1-7).²⁰ In the present study, we tested the hypothesis that the enhancement in baroreflex sensitivity produced by brain inhibition of ACE in SHR could involve the participation of central Ang-(1-7).

Methods

Animals

Experiments were performed in male SHR, 14 to 20 weeks old, (SHR/M/EPM) obtained from the animal facility (CEDEME) of the Escola Paulista de Medicina of the Federal University of São Paulo, Brazil. Those animals were originally imported from the National Institutes of Health and bred at CEDEME. On arrival in the animal facility of our institute, the animals were kept in a temperature-controlled room with a 14/10-hour light/dark cycle for 1 month.

Surgical Procedures

A metallic cannula (25-gauge butterfly needle) with one end connected to polyethylene tubing (PE-10) was stereotaxically inserted into the right lateral ventricle of animals anesthetized with thiobarbital (40 to 60 mg/kg IP), as described previously.^{7,12} One day before the experiments, polyethylene catheters were inserted into the femoral artery and vein and were tunneled subcutaneously to the back of the neck, with the animals under ether anesthesia. At the end of each experiment, 5 μ L Evans blue dye (5%) was injected through the intracerebroventricular cannula. The brain was then removed, and the position of the cannula in the lateral ventricle was confirmed by the diffusion of only the dye throughout the ventricular system.

Arterial Blood Pressure Measurements

Arterial pressure was monitored by a solid-state strain-gauge transducer (model TP-200T, Nihon Kohden); HR was determined with a counter (model AT-601G, Nihon Kohden) triggered by the arterial pressure wave. All variables were recorded continuously, in freely moving rats, on a direct-writing Nihon Kohden polygraph (model CP-640G).

Intracerebroventricular Infusion Procedure

Continuous intracerebroventricular infusions of saline (vehicle) or drugs were carried out with a Hamilton syringe (10 μ L, Hamilton Co) connected to a Harvard pump (No. 11, Harvard Apparatus) at a rate of 8 μ L/h for \approx 4.5 hours. Before the beginning of the infusion, 2.5 to 3.0 μ L of the infusion solution was injected into the intracerebroventricular cannula (for 3 minutes) to fill up the dead space, measured in each animal before cannula implantation. Infusions were performed in 4 different groups: (1) saline alone, 8 μ L/h for 4 hours (n=4); (2) ramiprilat alone, 14 μ g/h for 4 hours (n=6); (3) ramiprilat alone, 14 μ g/h for 2 hours, followed by ramiprilat, 14 μ g/h, combined with A-779, 4 μ g/h for an additional 2 hours (n=6); and (4) A-779, 4 μ g/h for 2 hours, followed by A-779, 4 μ g/h, combined with ramiprilat, 14 μ g/h for additional 2 hours (n=5). The effectiveness of ACE blockade was confirmed at the end of the experiments by demonstrating a complete blockade of the pressor and drinking effect produced by intracerebroventricular injection of 100 ng of angiotensin I (Ang I).

Drugs

The ACE inhibitor ramiprilat and the Ang-(1-7) antagonist A-779 were dissolved in sterile isotonic saline (0.9% NaCl) immediately before use. The Ang-(1-7) antagonist was provided by Dr M.C. Khosla or purchased from Bachem. Ramiprilat was obtained from Hoechst AG. The infusion rate of ramiprilat was chosen on the basis of preliminary experiments, in which it was shown to result in 100% blockade of the pressor and drinking responses induced by intracerebroventricular injection of 100 ng Ang I. The infusion rate of A-779 was chosen on the basis of preliminary experiments in which this rate was found to be effective in causing a baroreflex change in Wistar rats.

Baroreceptor Reflex Test

Baroreceptor reflex control of HR was determined in each rat by recording reflex HR changes in response to mean arterial pressure (MAP) changes produced by repeated bolus injections of graded doses of phenylephrine (0.2 to 40 μ g/kg IV). Peak changes in HR occurring during the initial 5 seconds of the corresponding maximum change in MAP produced with phenylephrine were recorded. HR (in beats per minute) was converted to pulse interval (in milliseconds) by the following equation: 60 000/HR. The baroreceptor reflex sensitivity was calculated by dividing changes in pulse interval by changes in MAP obtained for each dose of phenylephrine and was called the baroreflex sensitivity index. The average of the baroreflex indexes for each dose was estimated in each rat before and at the end of the second and fourth hour of continuous intracerebroventricular infusions. For illustration purposes, the data were also plotted by the best-fit regression line drawn from the mean \pm SEM of pressure and pulse interval changes for each dose of phenylephrine.

Statistical Analysis

Comparisons among the different time points were assessed by repeated-measures 1-way ANOVA, followed by the multiple comparison Dunnett test, with use of the statistics program PRISM (version 3.0, Graphpad Prism Software). The criterion for statistical significance was set at $P < 0.05$. Numerical values are given as mean \pm SEM.

Results

Effect of Intracerebroventricular Infusions on Baseline MAP and HR

As shown in the Table, intracerebroventricular infusion of saline or of ramiprilat alone or in combination with A-779 did not significantly modify the baseline levels of MAP or HR in most SHR groups. A small decrease in MAP in the animals infused with ramiprilat was significant in the fourth hour of ramiprilat infusion (154 ± 6.8 mm Hg, group 2; Table) when the MAP value was compared with the value before infusion (168 ± 5.6 mm Hg) by use of the paired Student *t* test. Even though that is not the most appropriate statistical analysis, the data suggest a tendency for acute intracerebroventricular infusion of the ACE inhibitor to decrease MAP. No significant changes in baseline HR were observed after any of the treatments (Table).

Effect of Intracerebroventricular Infusions on Baroreflex Control of HR

Intracerebroventricular infusion of ramiprilat produced a significant increase ($\approx 40\%$) in baroreflex sensitivity at the second hour of infusion that was maintained until the fourth hour. The baroreflex sensitivity index at the end of the second hour (0.98 ± 0.09 ms/mm Hg; Table and Figure 1, inset) and at the end of the fourth hour (1.07 ± 0.19 ms/mm Hg; Table and Figure 1, inset) was significantly higher than that before

Baseline MAP and HR and Baroreflex Sensitivity Index (Δ PI/ Δ MAP) Before and At End of 2nd and 4th Hour of Intracerebroventricular Infusions

Groups	n	Period	Baseline		Baroreflex Sensitivity Index
			MAP, mm Hg	HR, bpm	Δ PI/ Δ MAP, ms/mm Hg
Group 1: saline	4	Before	181±8.5	327±7	0.68±0.02
		2nd h	180±4.1	324±11	0.62±0.04
		4th h	171±4.3	316±26	0.70±0.01
Group 2: ramiprilat	6	Before	168±5.6	317±17	0.68±0.08
		2nd h	159±4.6	342±22	0.98±0.09*
		4th h	154±6.8	336±11	1.07±0.19*
Group 3: ramiprilat/A-779	6	Before	164±8.1	333±21	0.87±0.10
		2nd h	162±6.9	334±21	1.24±0.03*
		4th h	153±7.6	349±22	0.88±0.11
Group 4: A-779/ramiprilat	5	Before	176±5.8	358±12	0.85±0.16
		2nd h	170±9.3	357±23	0.76±0.19
		4th h	162±11.7	348±29	0.81±0.20

Values are mean±SE. The baroreflex sensitivity index represents the averaged relationship between reflex changes in pulse interval (Δ PI) and changes in MAP (Δ MAP) produced by graded doses of phenylephrine, which were calculated in each animal, before and at different periods during intracerebroventricular infusions. Groups are as follows: for group 1, saline was infused at a rate of 8 μ L/h for 4 h; for group 2, ramiprilat was infused at a rate of 14 μ g/h for 4 h; for group 3, ramiprilat was infused at a rate of 14 μ g/h for 2 h, followed by ramiprilat at 14 μ g/h combined with A-779 at 4 μ g/h in the 3rd and 4th h; and for group 4, A-779 was infused at a rate of 4 μ g/h for 2 h, followed by A-779 at 4 μ g/h combined with ramiprilat at 14 μ g/h in the 3rd and 4th h.

* P <0.05 vs before infusion (repeated-measures ANOVA followed by Dunnett multiple comparison test).

infusion (0.68 ms/mm Hg; Table 1 and Figure 1, inset). This effect can also be seen in Figure 1 by the shift to the left of the lines that correlate reflex changes in HR (as pulse intervals) and the changes in MAP induced by phenylephrine in the second and fourth hours of infusion.

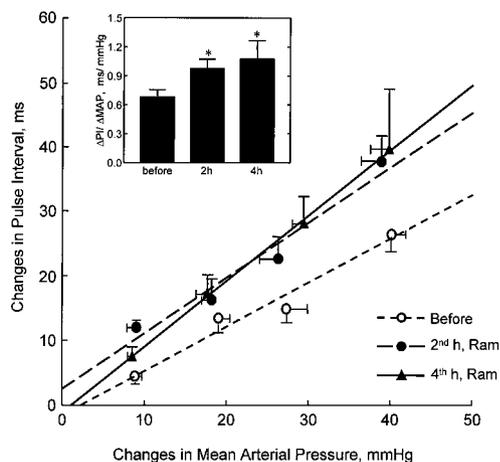


Figure 1. Reflex changes in HR (expressed as pulse interval) in response to changes in MAP produced by graded doses of phenylephrine before and at end of second and fourth hours of continuous intracerebroventricular infusion of ramiprilat (Ram, 14 μ g/h). Lines represent least-squares regression equation fit through the averaged points. Inset corresponds to sensitivity index of baroreflex bradycardia expressed by averaged ratio between reflex changes in HR (as pulse interval, Δ PI) and changes in MAP (Δ MAP) calculated in each animal. * P <0.05 vs before infusion (repeated-measures ANOVA followed by Dunnett multiple comparison test).

The enhancement of the baroreflex bradycardia produced by ramiprilat infusion (baroreflex sensitivity index, 1.24±0.03 ms/mm Hg after 2 hours versus 0.87±0.10 ms/mm Hg before infusion; Figure 2, inset) was completely attenuated by the addition of A-779 to the infusion (0.88±0.11 ms/mm Hg in the fourth hour of infusion; Figure 2, inset). Similarly, the shift to the left of the line that correlates reflex changes in pulse intervals and changes in MAP in the second hour of ramiprilat infusion was reversed in the fourth hour with the combination of A-779 and ramiprilat (Figure 2). Moreover, in other group of rats, the infusion of A-779 prevented the modulatory effect of ramiprilat on baroreflex bradycardia (baroreflex sensitivity index, 0.85±0.16 ms/mm Hg before infusion, 0.76±0.19 ms/mm Hg after 2 hours of infusion of A-779 alone, and 0.81±0.2 ms/mm Hg after an additional 2 hours of infusion of A-779 combined with ramiprilat; Figure 3). Intracerebroventricular infusion of A-779 alone for 2 hours in SHR did not significantly change the baroreflex control of HR (Figure 3). In addition, 4 hours infusion of saline did not alter the baroreflex bradycardia (Figure 4).

Discussion

In the present study, we added further evidence to support the hypothesis that Ang-(1-7) is involved in the central regulation of baroreflex by using the peptide A-779. We and others^{4,6,8,9,12} have shown that A-779 is a potent and selective Ang-(1-7) antagonist with no agonistic properties; antagonistic properties were unrelated to the AT₁ and AT₂ receptor subtypes in several preparations tested.^{8,10,11} Our results demonstrate that central infusion of A-779 produces a signif-

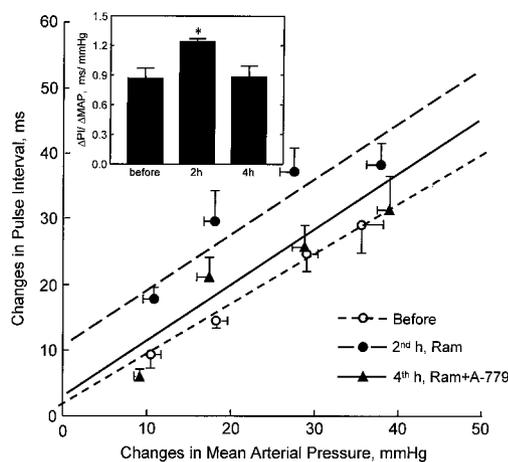


Figure 2. Reflex changes in HR (expressed as pulse interval) in response to changes in MAP produced by graded doses of phenylephrine before and at end of second and fourth hours of continuous intracerebroventricular infusion. In the first 2 hours, animals were infused with Ram (14 $\mu\text{g}/\text{h}$) alone. In the third and fourth hours, the animals were infused with Ram (14 $\mu\text{g}/\text{h}$) combined with the selective Ang-(1-7) antagonist (A-779, 4 $\mu\text{g}/\text{h}$). Lines represent least-squares regression equation fit through averaged points. Inset corresponds to sensitivity index of baroreflex bradycardia expressed by averaged ratio between reflex changes in HR (as pulse interval, ΔPI) and changes in MAP (ΔMAP) calculated in each animal. * $P < 0.05$ vs before infusion (repeated-measures ANOVA followed by Dunnett multiple comparison test).

icant reversal of the improvement of the baroreflex sensitivity induced in SHR by treatment with ramiprilat. These data are in keeping with the observation that central Ang-(1-7) differentially modulates baroreceptor reflex sensitivity: whereas intracerebroventricular infusion of Ang II or Ang III pro-

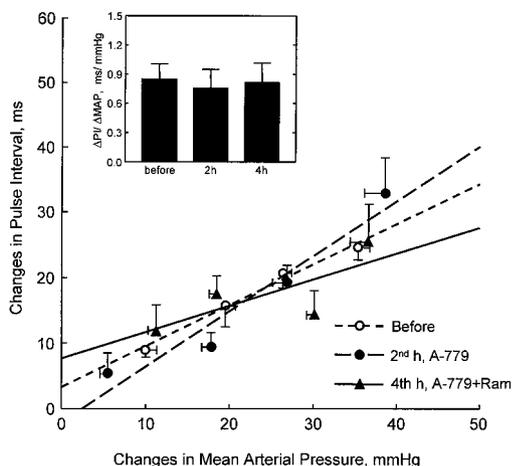


Figure 3. Reflex changes in HR (expressed as pulse interval) in response to changes in MAP produced by graded doses of phenylephrine before and at end of second and fourth hours of continuous intracerebroventricular infusion. In the first 2 hours, animals were infused with selective Ang-(1-7) antagonist (A-779, 4 $\mu\text{g}/\text{h}$) alone. In the third and fourth hours, the animals were infused with A-779 (4 $\mu\text{g}/\text{h}$) combined with Ram (14 $\mu\text{g}/\text{h}$). Lines represent least-squares regression equation fit through averaged points. Inset corresponds to sensitivity index of baroreflex bradycardia expressed by averaged ratio between reflex changes in HR (as pulse interval, ΔPI) and changes in MAP (ΔMAP) calculated in each animal.

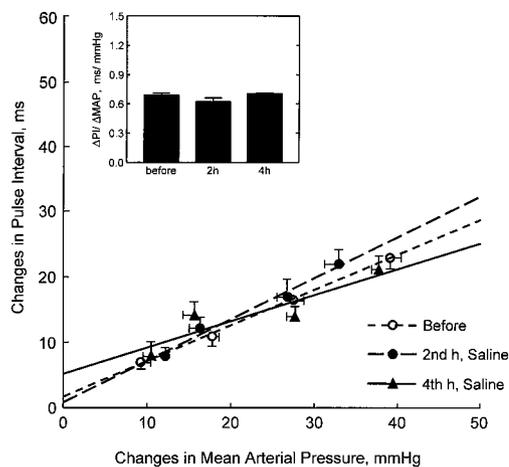


Figure 4. Reflex changes in HR (expressed as pulse interval) in response to changes in MAP produced by graded doses of phenylephrine before and at end of second and fourth hours of intracerebroventricular continuous infusion of saline (8 $\mu\text{L}/\text{h}$). Lines represent least-squares regression equation fit through averaged points. Inset corresponds to sensitivity index of baroreflex bradycardia expressed by averaged ratio between reflex changes in HR (as pulse interval, ΔPI) and changes in MAP (ΔMAP) calculated in each animal.

duced the well-known attenuation of baroreflex sensitivity, intracerebroventricular infusion of an equal dose of Ang-(1-7) produced a significant facilitation of the baroreflex control of HR in normotensive rats.⁷ Furthermore, our data are in accordance with a previous study of Britto et al,²¹ who demonstrated in renovascular hypertensive (Goldblatt 2-kidney, 1-clip) rats that the intracerebroventricular infusion of A-779 reversed the improvement in baroreflex sensitivity produced by chronic oral enalapril treatment.

ACE inhibitors have been largely used in the treatment of human hypertension and in studies with different models of experimental hypertension.¹⁴ ACE inhibitors interfere with the metabolism of the renin-angiotensin system and other peptides known to affect blood pressure, such as BK, the opioid peptides, and substance P.^{14,22} The mechanism of action of ACE inhibitors has been attributed to the inhibition of Ang II formation and/or BK inactivation.²² In addition, it has been shown that ACE inhibition produces an increase in Ang-(1-7) levels in SHR¹⁸ and in humans.¹⁹ The increase in Ang-(1-7) concentration after ACE inhibition may be due to the accumulation of Ang I and/or decrease in Ang-(1-7) inactivation, inasmuch as ACE is an important route in the metabolism of Ang-(1-7).^{1,2,20} Ang-(1-7) has been shown to contribute to the antihypertensive actions of ACE inhibition either alone or in combination with AT₁ receptor antagonists.^{21,23} In addition, changes in the angiotensin and kinin metabolism may be involved in the beneficial cardiovascular effects of ACE inhibition, particularly including the baroreceptor control of HR.^{14,22} Accordingly, previous studies in our laboratory have shown that the central administration of Ang-(1-7)^{4,7} or BK^{24,25} produces facilitation of the baroreflex. In addition, in a subsequent study, we have shown a direct interaction between Ang-(1-7) and BK: intracerebroventricular infusion of BK at a subeffective rate combined with a

subeffective rate of Ang-(1-7) produced a significant enhancement of baroreflex sensitivity.²⁶

We have previously shown that in SHR, which demonstrate impaired baroreflex sensitivity, intracerebroventricular infusion of A-779 was not effective in changing the baroreflex.¹² This effect is probably not linked to the high levels of blood pressure, because in renovascular hypertensive (Goldblatt, 2-kidney, 1-clip) rats, acute intracerebroventricular infusion of A-779 produced a small but significant attenuation of the already low baroreflex sensitivity.²¹ Differences in the endogenous levels of peptides could be responsible for the lower effectiveness of A-779 in SHR. However, the data available in the literature concerning this possibility are inconclusive.^{18,19} In the plasma, Kohara et al¹⁸ have shown a higher level of Ang-(1-7) in SHR (≈ 3 -fold). These authors have not accessed tissue levels of angiotensin peptides. However, Campbell et al¹⁹ did not find alterations in Ang-(1-7) levels in plasma or brain (whole brain) of SHR. Although these authors found no differences, it does not exclude the possibility of regional changes in different brain areas.

In the present study, the improvement in baroreflex sensitivity observed after ramiprilat treatment cannot be ascribed to an alteration in the baseline level of MAP. Even though there was a tendency for MAP to decrease, the values after 4 hours of any infusion combination were still in the hypertensive range. In addition, these data rule out the possibility that the effect observed after ramiprilat could be due to leakage to the periphery.¹⁴ The possibility that the A-779 effect could be due to access to the peripheral circulation is also very unlikely. We have previously shown that 3 hours of intravenous infusion of Ang-(1-7) does not affect baroreflex control of HR.⁷ Thus, the results observed in the present study indicate that ramiprilat and A-779 are acting by changing local angiotensin metabolism and action in the brain. The possibility that the central effect of A-779 on baroreflex modulation could be due to interference with other peptides is unlikely, because we found that A-779 does not influence the biological activity of several peptides, including Ang III, substance P, vasopressin, and BK, even at a molar ratio of 2500:1.⁸ In addition, it is also unlikely that A-779 was acting as a partial agonist on AT₁ receptors, because A-779 does not mimic or interfere with the effects of Ang II centrally^{6,8,12} or peripherally.^{2,8}

It has been shown that different cardiovascular areas in the central nervous system contain receptor sites and all the proteins required for local synthesis of angiotensin peptides, including the nucleus tractus solitarius (nTS), a key region for the baroreceptor reflex.¹⁵ The nTS borders the area postrema and contains the primary synapse of the baroreceptor fibers, which both inhibit the tonic activity of the vasomotor neurons and excite the preganglionic fibers of the parasympathetic system.¹⁵ Microinjection of Ang-(1-7) into the nTS produces, in addition to the hypotensive and bradycardic effects,³ a facilitation of the baroreflex control of HR,⁴ whereas microinjection of Ang II induces hypotension, bradycardia, and an attenuation of the baroreflex mediated by the AT₁ receptor subtype.¹⁵ Even though the pharmacological characteristics of the Ang-(1-7) receptor are not fully determined, initial

studies by Diz and Ferrario²⁷ have provided evidence for an Ang-(1-7) receptor binding at the rostral nTS. In that study, it was shown that Ang-(1-7) was effective in displacing ¹²⁵I-Ang II binding only in the rostral nTS. Moreover, a specific binding site for ¹²⁵I-Ang-(1-7) was also shown in this subarea of the nTS,²⁷ suggesting that angiotensin receptors in the dorsomedial medulla recognize Ang-(1-7) with an affinity similar to Ang II. Therefore, the nTS is a candidate for a central site for the modulatory actions of angiotensins on baroreflex control. Future studies should test the possibility that imbalances in the local formation of angiotensin peptides caused by ACE inhibitors, given peripherally or centrally,¹⁷ can change local levels of angiotensins and other peptides at this site, leading to an improvement of baroreceptor reflex sensitivity.

In summary, our data provide new evidence of an important role of Ang-(1-7) in the central modulation of baroreflex control of HR and show clear evidence that changes in Ang-(1-7) formation in the brain contribute to the improvement of baroreflex sensitivity produced by central administration of ACE inhibitors in SHR.

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