The Endothelium-Dependent Vasodilator Effect of the Nonpeptide Ang(1–7) Mimic AVE 0991 Is Abolished in the Aorta of *Mas*-Knockout Mice

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Abstract: Recently, we demonstrated that the endothelium-dependent vasodilator effect of angiotensin(1–7) in the mouse aorta is abolished by genetic deletion of the G protein-coupled receptor encoded by the *Mas* protooncogene. To circumvent the limitations posed by the possible metabolism of Ang(1–7) in this vessel, in this work we studied the mechanism underlying the vasorelaxant effect of AVE 0991, a nonpeptide mimic of the effects of Ang(1–7), using wild-type and *Mas*-deficient mice. Ang(1–7) and AVE 0991 induced an equipotent concentration-dependent vasodilator effect in aortic rings from wild-type mice that was dependent on the presence of endothelium. The vasodilator effect of Ang(1–7) and AVE 0991 was completely blocked by 2 specific Ang(1–7) receptor antagonists, A-779 and D-Pro^7-Ang(1–7), and by inhibition of NO synthase with L-NAME. Moreover, in aortic rings from *Mas*-deficient mice, the vasodilator effect of both Ang(1–7) and AVE 0991 was abolished. In contrast, the vasodilator effect of acetylcholine and substance P were preserved in *Mas*-null mice. In addition, the vasoconstriction effect induced by Ang II was slightly increased, and the vasodilation induced by the AT_2 agonist CGP 42112A was not altered in *Mas*-deficient mice. Our results show that Ang(1–7) and AVE 0991 produced an NO-dependent vasodilator effect in the mouse aorta that is mediated by the G protein-coupled receptor Mas.

Key Words: angiotensin(1–7), AVE 0991, Mas receptor, Ang(1–7) receptor, angiotensin II, mouse aorta

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The heptapeptide angiotensin(1–7) [Ang(1–7)] is a biologically active component of the renin–angiotensin system (RAS) originated from angiotensin I and angiotensin II (Ang II) by the action of peptidases.[1–3] Unlike Ang II, which causes vasoconstriction, proliferation, and hypertrophy, Ang(1–7) has essentially the opposite effects,[3] promoting vasodilatation[4] and antiproliferation.[5]

The existence of specific binding sites for Ang(1–7) in bovine endothelial cells[6] and studies using the selective antagonist A-779[7–9] have provided evidence for the existence of an Ang(1–7) receptor distinct from the classical Ang II receptors AT_1 and AT_2. More recently, we have reported that Ang(1–7) is an endogenous ligand for the G protein-coupled receptor Mas.[10] Mas deficiency in mice produces specific loss of Ang(1–7) binding in kidney slices. Furthermore, *Mas*-transfected CHO cells bound [^{125}I]Ang(1–7) with high affinity (K_d 0.83 nM). The [^{125}I]Ang(1–7) binding in CHO-transfected cells was displaced with high affinity by Ang(1–7) and the Ang(1–7) antagonist A-779 but not by the AT_1 antagonist CV11974 or the AT_2 antagonist PD 123,319. Furthermore, Ang(1–7) induced release of arachidonic acid from *Mas*-transfected CHO or COS cells.[10] Accordingly, the antidiuretic effect of Ang(1–7) in water-loaded mice and its direct vasodilator effect in aortic rings of wild-type mice were abolished in *Mas*-knockout mice.[10,11]

AVE 0991 is a recently described nonpeptide mimic of the effect of Ang(1–7) on the endothelium.[12] The availability of a nonpeptide agonist might be useful to confirm that the Mas-mediated effects of Ang(1–7) are not caused by its possible enzymatic products. In addition, AVE 0991 and related compounds have the potential to be used as cardiovascular drugs. Therefore, in this study we investigated the mechanism involved in the vasodilator effect of Ang(1–7) and AVE 0991 in the aorta using wild-type and *Mas*-deficient mice. We hypothesized that, as recently shown for Ang(1–7), AVE 0991 is a Mas agonist in the mouse aorta.

METHODS

Animals

We used 12- to 14-week-old male homozygous *Mas*-deficient mice (n = 10, 26.5 ± 2.2 g) on the pure genetic background C57BL/6, and age-matched wild-type C57BL/6 (n = 34, 28.0 ± 2.6 g) control mice. Animals were originally obtained from Dr Michael Bader at the Max-Delbruck Center

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for Molecular Medicine and bred in our animal facilities at the Department of Physiology and Biophysics of the Federal University of Minas Gerais. The animals were maintained in collective cages in an appropriate room with controlled temperature and with a 12-hour light cycle. The animal feeding and treatment protocols were reviewed and approved by the Animal Care Committee of the Institute of Biological Sciences, UFMG, Brazil. Mice were killed by cervical dislocation and exsanguinations, and tissues were rapidly removed.

Mouse Aortic Ring Preparation and Mounting

Rings (2–3 mm) from the descending thoracic aorta, free of adipose and connective tissue, were set up in gassed (95% O_2 and 5% CO_2) Krebs-Henseleit solution (mmol/L): NaCl 110.8, KCl 5.9, $NaHCO_3$ 25.0, $MgSO_4$ 1.07, $CaCl_2$ 2.49, NaH_2PO_4 2.33, and glucose 11.51, at 37 °C, under a tension of 0.5 g, for 1 hour to equilibrate. The presence of a functional endothelium was assessed by the ability of acetylcholine (Ach; 10 μM; Sigma, St Louis, MO) to induce more than 50% relaxation of vessels precontracted with phenylephrine (0.1 μM; Sigma, St Louis, MO). When necessary, the endothelium was removed by rubbing the intimal surface with a wooden stick. Ang(1–7) (Bachem, Torrance, CA) and AVE 0991 (a generous gift from Dr Markus Bleich, Aventis Pharma, Frankfurt, Germany) were added in increasing cumulative concentrations (0.0001 to 0.3 μM) once the response to 0.1 μM phenylephrine had stabilized. L-NAME (Sigma, St Louis, MO), A-779 (Bachem, Torrance, CA), and D-Pro^7-Ang(1–7) (ByoSynthan, Berlin-Bush, Germany) were added to the bath 20 minutes before the addition of phenylephrine. As a control for the above-mentioned protocol, another vessel segment from each mouse was simultaneously monitored for Ang(1–7) and AVE 0991 effects alone. In experiments performed in vessels without functional endothelium or in the presence of L-NAME, vessels were precontracted with 0.03 μM phenylephrine to achieve the same tension level as the others. The other inhibitors did not affect the contraction induced by 0.1 μM phenylephrine (not shown). In additional experiments the vasodilator effect of substance P (Bachem, Torrance, CA; 0.1 pM to 0.3 μM), acetylcholine (Sigma, St Louis, MO; 0.001 to 100 μM), and CGP42112 (0.0001 to 0.3 μM), an AT_2 agonist, was tested. The vasoconstriction effect of Ang II (Bachem, Torrance, CA; 0.00001 to 1.0 μM) was also evaluated. Mechanical activity, recorded isometrically by a force transducer (World Precision Instruments, Inc, Sarasota, FL), was fed to an amplifier-recorder (Model TMB-4; World Precision Instruments, Inc) and to a personal computer equipped with an analogue-to-digital converter board (AD16JR; World Precision Instruments, Inc), using CVMS data acquisition/recording software (World Precision Instruments, Inc).

Statistical Analysis

Results are presented as means ± SEM. Two-way ANOVA with Bonferroni multiple comparison posttest was used to compare concentration–response curves obtained in aortic rings. The vasodilator effect of Ang(1–7), AVE 0991,

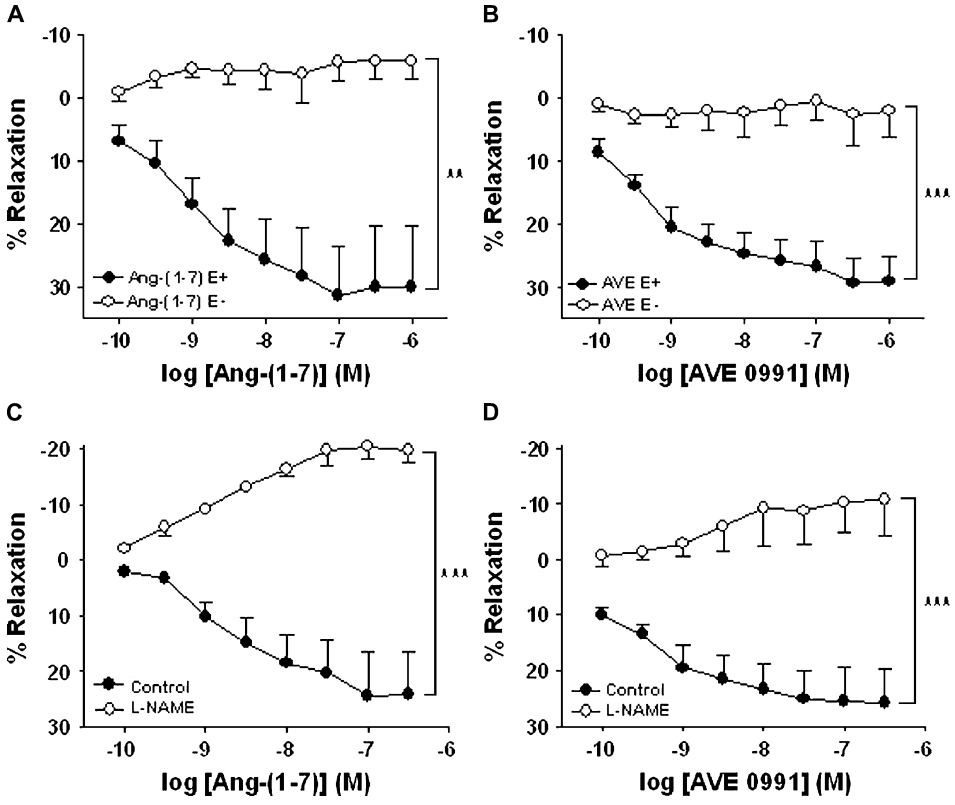

FIGURE 1. Vasodilator effect of Ang(1–7) (A) and AVE 0991 (B) in aortic rings from wild-type mice containing (E+) or lacking functional endothelium (E−). Treatment of wild-type mouse aorta with L-NAME (100 μM) abolishes the vasodilator effect of Ang(1–7) (C) and AVE 0991 (D). Each point represents the mean ± SEM generated from at least 7 separate experiments. $^{**}P < 0.01$; $^{***}P < 0.001$.

acetylcholine, substance P, and CGP42112A was expressed as percentage decrease in maximal contraction induced by phenylephrine. The vasoconstriction effect of Ang II was expressed in millinewtons (mN). Student *t*-test was used to compare maximal values for the relaxant effect (E_{max}) and concentration required to produce 50% of the maximum response (EC_{50}). All statistical analyses were considered significant when $P < 0.05$. Values of EC_{50} were calculated graphically from the individual concentration–response curves by nonlinear curve fitting.

RESULTS

Vasorelaxant Effect of Ang(1–7) and AVE 0991 in Endothelium-Intact and Endothelium-Denuded Aortic Rings From Wild-Type Mice

In endothelium-intact aortic rings from wild-type mice, Ang(1–7) induced a concentration-dependent vasodilator effect (Fig. 1A), which was completely blocked by pretreatment of the vessels with 100 μM L-NAME (Fig. 1C). AVE 0991 induced an equipotent vasodilator effect (Fig. 1B), which was also abolished by 100 μM L-NAME (Fig. 1D). Maximal values for the relaxant effect (E_{max}) were 31.4 ± 7.7 and 29.1 ± 3.9 for Ang(1–7) and AVE 0991, respectively. EC_{50} values were 2.0 ± 0.5 nM and 1.3 ± 0.6 nM. In endothelium-denuded vessels the vasorelaxant effect of both Ang(1–7) and AVE 0991 was completely abolished (Fig. 1A,B).

Effect of Selective Ang(1–7) Antagonists on the Vasodilator Effect of Ang(1–7) and AVE 0991

The vasorelaxant effect of Ang(1–7) was abolished when endothelium-intact aortic rings from wild-type mice were pretreated with 2 selective antagonists of Ang(1–7) receptors, A-779 (Fig. 2A) or D-Pro^7-Ang(1–7) (Fig. 2C). In a similar way, A-779 (Fig. 2B) and D-Pro^7-Ang(1–7) (Fig. 2D) blocked the vasorelaxation induced by AVE 0991.

Effect of Ang(1–7) and AVE 0991 in Aortic Rings from Mas-Deficient Mice

In aortas from *Mas*-deficient mice containing a functional endothelium, we found a total abolition of the vasorelaxant effect of both Ang(1–7) (Fig. 3A) and AVE 0991 (Fig. 3B). In contrast, the vasodilator effects of ACh (Fig. 4A) and substance P (Fig. 4B) were preserved in both strains. Maximal values for the relaxant effect of ACh (E_{max}, %) were 91.5 ± 3.2 and 94.5 ± 1.9 in the aortas of wild-type mice and *Mas*-deficient mice, respectively. EC_{50} values were 0.14 ± 0.02 nM and 0.23 ± 0.05 nM. Maximal values for the relaxant effect of substance P (E_{max}) were 17.6 ± 1.5 and 21.6 ± 2.2 in aortas of wild-type mice and *Mas*-deficient mice, respectively. EC_{50} values were 0.00107 ± 0.0006 nM and 0.0074 ± 0.004 nM, respectively.

Effect of Ang II and CGP42112A in Aortic Rings from Mas-Deficient Mice

To evaluate the functionality of the Ang II receptors AT_1 and AT_2 in aortas from *Mas*-deficient mice,

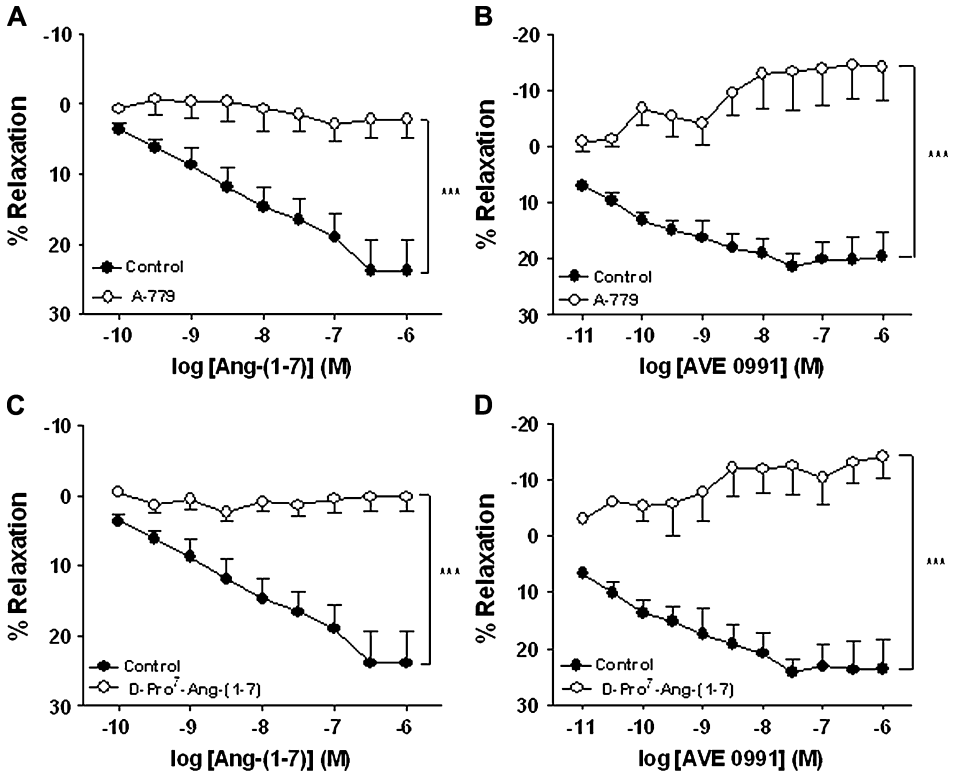

FIGURE 2. Effect of Ang(1–7) receptor blockade by A-779 (A, B) and D-Pro^7-Ang(1–7) (C, D) on the vasodilator effect of Ang(1–7) (A, C) and AVE 0991 (B, D). Each point represents the mean ± SEM generated from at least 4 separate experiments. ***$P < 0.001$.

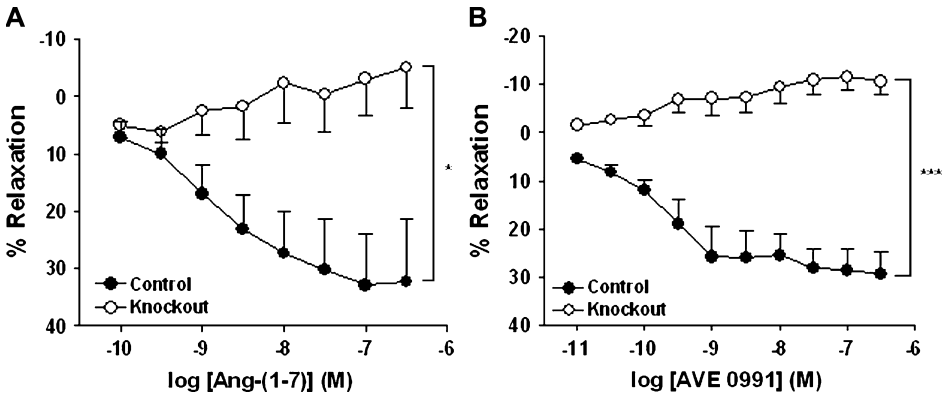

FIGURE 3. The vasodilator effect of Ang(1–7) (A) and AVE 0991 (B) is abolished in aortas from *Mas*-deficient mice. Each point represents the mean ± SEM generated from 5 separate experiments. $^{**}P < 0.01$; $^{***}P < 0.001$.

concentration–response curves to Ang II and CGP42112A were constructed in aortas from *Mas*-null mice containing a functional endothelium. As seen in Figure 5A, the vasoconstrictor effect induced by Ang II in *Mas*-knockout mice was slightly increased. On the other hand, the vasodilator effect induced by CGP42112A in *Mas*-deficient mice was similar to the response observed in control mice (Fig. 5B). Maximal values for the contraction effect (mN) induced by Ang II were (E_{max}) 0.32 ± 0.06 and 0.26 ± 0.07 in the aorta of wild-type mice and *Mas*-deficient mice, respectively. Maximal values for the relaxant (%) effect of CGP42112A were (E_{max}) 24.56 ± 3.68 and 19.74 ± 4.25 in aortas of wild-type mice and *Mas*-deficient mice, respectively.

DISCUSSION

In this study we have observed that the novel nonpeptide compound AVE 0991 as well as Ang(1–7) produced a nitric oxide (NO)- and endothelium-dependent vasodilator effect on the aorta of wild-type mice that was completely abolished by blockade of Ang(1–7) receptors and after genetic deletion of *Mas*.

Removal of endothelium or pretreatment of the vessels with L-NAME completely blocked the vasorelaxation induced by both Ang(1–7) and AVE 0991, indicating that endothelial NO mediates their vasodilator effect in the mouse aorta. Our results are consistent with previous reports showing that Ang(1–7)[13] and AVE 0991[12] induce release of bioactive NO from cultured endothelial cells and from *Mas*-transfected CHO cells.[14] A direct vasodilator action to Ang(1–7) has been described in several preparations including rabbit afferent arterioles,[15] canine[16] and porcine coronary arteries,[17] and rat aorta.[18] A potent vasodilator effect of Ang(1–7) in several preparations in vivo has also been recently described in anesthetized rats.[19] Several mechanisms have been proposed to explain the vasodilator effect of Ang(1–7): stimulation of vasodilator prostaglandins, increased production of nitric oxide, or both, via stimulation of a specific receptor[6,3] or by potentiating the vasorelaxant effect of bradykinin.[4,20–23]

Our data clearly indicate that in the mouse aorta NO release plays a major role in the vasodilator effect of Ang(1–7) and AVE 0991. Our data also support the possibility that both Ang(1–7) and AVE 0991 act through stimulation of specific endothelial receptors. Removal of the endothelium abolished Ang(1–7)- and AVE 0991-induced vasorelaxation. Moreover, pretreatment of control mouse aortic rings with the Mas antagonist A-779 and the newly described Ang(1–7) selective antagonist D-Pro^7-Ang(1–7)[24] completely blocked the vasorelaxant effects of both agonists.

To verify the functionality of AT_1 and AT_2 receptors in aortas from *Mas*-knockout mice, we have evaluated the contractile response to Ang II and the vasodilator effect induced by CGP42112A, a selective AT_2 agonist. The vascular responses to Ang II and CGP42112A were similar in both strains. Together, our results clearly show that the Mas receptor is not important for Ang II effects in mouse aorta. More

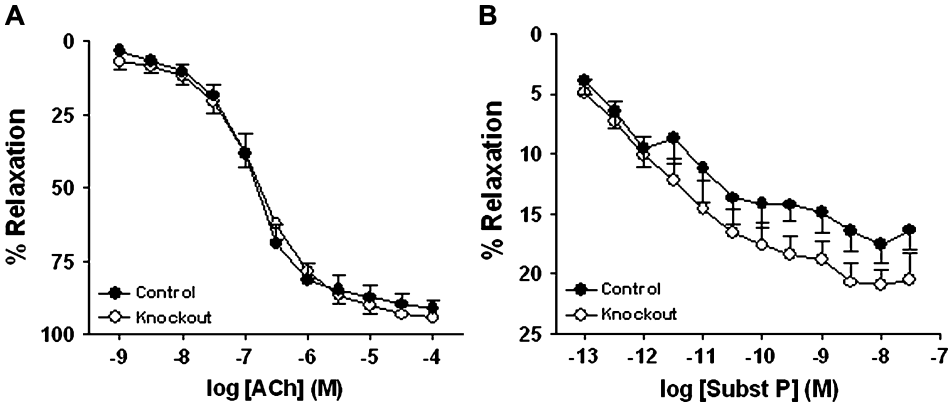

FIGURE 4. Vasodilator effect of acetylcholine (A) and substance P (B) in aortic rings from wild-type mice and *Mas*-deficient mice precontracted with phenylephrine (0.1 μM). Each point represents the mean ± SEM generated from 4 separate experiments.

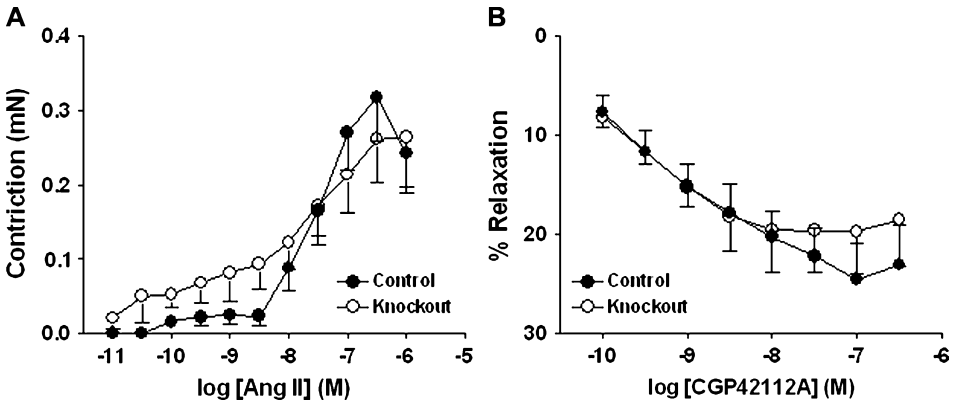

FIGURE 5. Vasoconstriction effect of Ang II (A) and the vasodilator effect of CGP42112A in aortic rings from wild-type mice and *Mas*-deficient mice containing a functional endothelium. Each point represents the mean ± SEM generated from 4 separate experiments.

importantly, because the effect of Ang(1–7) is completely abolished in *Mas*-knockout mice, the above results rule out the possibility that vasodilation induced by Ang(1–7) in mouse aorta is mediated by stimulation of Ang II receptors. These observations are in keeping with our previous findings in kidney slices showing that AT_1 and AT_2 levels are preserved in *Mas*-knockout mice.[10] In addition, we have recently shown that AVE 0991 induces NO production in *Mas*-transfected CHO cells, which is completely blocked by A-779 but not by AT_1 or AT_2 blockers.[14]

It should be pointed out that the nitric oxide release induced by Ang(1–7) and AVE 0991 in bovine endothelial cells is only partially (50%) blocked by A-779.[12] In addition, the effect of both agonists were partially blocked (50%) by the losartan metabolite EXP 3174 and essentially abolished (~90% blockade) by the AT_2 antagonist PD 123177. Besides, differences in the level of Mas expression, bovine angiotensins possess a valine instead of isoleucine in the 5 position. This suggests that the angiotensin receptors in this species may have structural differences from the angiotensin receptors in the ones expressing Ile^5-angiotensins such as humans and rodents.[25] The fact that the effects of Ile^5-angiotensin II and Val^5-angiotensin II are not always the same in rodents[26] is in keeping with this possibility. The possibility of oligomerization and functional interactions between angiotensin receptors should also be considered.[27,28] Indeed, ongoing experiments in our laboratory indicate the existence of a functional interaction between Mas and AT_1[29] and AT_2 receptors. It has been observed, for instance, that A-779 attenuates the vasodilator effect of CGP42112A in the aorta of C57BL/6, and a similar attenuation was observed for Ang(1–7) with PD123319 (Silva DM, Lemos VS, Santos RA, unpublished results).

Furthermore, species and regional differences in the responses to Ang(1–7) and angiotensin antagonists have been extensively described and reviewed.[3,27]

We have recently shown that the G protein-coupled receptor Mas mediates biological effects of Ang(1–7), including the vasorelaxation of mouse aorta.[10] We now show that Mas deficiency abolished the vasodilator effect of Ang(1–7) as well as of the nonpeptide agonist AVE 0991, indicating that the vasodilator effect of AVE 0991 in the mouse aorta is mediated by Mas. The observation that the relaxation produced by acetylcholine and substance P is preserved in *Mas*-knockout animals ruled out the possibility that the absence of relaxation in response to Ang(1–7) and AVE 0991 is caused by a nonspecific vasodilation dysfunction in *Mas*-deficient mice.

Perspectives

A question that arises from our studies is whether the interaction with Mas would explain all Ang(1–7) effects. Probably not. Many previous studies suggest that, in addition to the interaction with the A-779-sensitive receptor Mas,[7–10,14] Ang(1–7) is capable of interacting with ACE[4] and AT_1[3,30] and AT_2-like receptors[31] or, more likely, with Mas-AT_1 and/or Mas-AT_2 oligomers.[14,32]

Several studies have shown that Ang(1–7) may play an important hemodynamic role by increasing baroreflex sensitivity,[33] modulating vascular reactivity to angiotensin II[27,28] and bradykinin,[4,19,20,23] and influencing cardiac output.[19] The development of a nonpeptide and orally active Ang(1–7) agonist open new research and therapeutic possibilities in the field of cardiovascular and cardiovascular-related diseases. More important, our findings further strengthen the evidence for the existence of a Mas–Ang(1–7) axis that might be involved in the renal,[14,34,35] cardioprotective,[36,37] and antihypertensive[38] actions of Ang(1–7).

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REFERENCES

1. Santos RAS, Campagnole-Santos MJ. Central and peripheral actions of angiotensin-(1–7). *Braz J Med Biol Res*. 1994;27:1033–1047.
2. Ferrario CM, Iyer SN. Angiotensin-(1–7): a bioactive fragment of the renin–angiotensin system. *Regul Peptides*. 1998;78:13–18.
3. Santos RAS, Campagnole-Santos MJ, Andrade SP. Angiotensin-(1–7): an update. *Regul Pept*. 2000;91:45–62.
4. Li P, Chappell MC, Ferrario CM, et al. Angiotensin-(1–7) augments bradykinin-induced vasodilation by competing with ACE and releasing nitric oxide. *Hypertension*. 1997;29:394–400.
5. Freeman EJ, Chisolm GM, Ferrario CM, et al. Angiotensin-(1–7) inhibits vascular smooth muscle cell growth. *Hypertension*. 1996;28:104–108.
6. Tallant EA, Lu X, Weiss RB, et al. Bovine aortic endothelial cells contain an angiotensin-(1–7) receptor. *Hypertension*. 1997;29:388–393.
7. Fontes MAP, Silva LCS, Campagnole-Santos MJ, et al. Evidence that angiotensin-(1–7) plays a role in the central control of blood pressure at

the ventro-lateral medulla acting through specific receptor. *Brain Res*. 1994;665:175–180.
8. Santos RAS, Campagnole-Santos MJ, Baracho NCV, et al. Characterization of a new angiotensin antagonist selective for angiotensin-(1–7): evidence that the actions of angiotensin-(1–7) are mediated by specific angiotensin receptors. *Brain Res Bull*. 1994;35:293–298.
9. Ambühl P, Felix D, Khosla MC. [7-D-Ala]-Angiotensin-(1–7): selective antagonism of angiotensin-(1–7) in the rat paraventricular nucleus. *Brain Res Bull*. 1994;35:289–291.
10. Santos RA, Silva AC, Maric C, et al. Angiotensin-(1–7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci USA*. 2003;100:8258–8263.
11. Lemos VS, Silva DMR, Walther T, et al. The endothelium-dependent vasodilator effect of Ang(1–7) is abolished in the aorta of Mas knockout mice. *Hypertension*. 2002;40:43. Abstract.
12. Wiemer G, Dobrucki LW, Louka FR, et al. AVE 0991, a nonpeptide mimic of the effects of angiotensin-(1–7) on the endothelium. *Hypertension*. 2002;40:847–852.
13. Heitsch H, Brovkovych S, Malinsky T, et al. Angiotensin-(1–7)-stimulated nitric oxide and superoxide release from endothelial cells. *Hypertension*. 2001;37:72–76.
14. Pinheiro SVB, Simões e Silva AC, Sampaio WO, et al. The nonpeptide AVE 0991 is an Angiotensin-(1–7) receptor Mas agonist in the mouse kidney. *Hypertension*. 2004;44:490–496.
15. Ren Y, Garvin J, Carretero OA. Mechanism involved in bradykinin-induced efferent arteriole dilation. *Kidney Int*. 2002;62:544–549.
16. Brosnihan KB, Li P, Ferrario CM. Angiotensin-(1–7) dilates canine coronary arteries through kinins and nitric oxide. *Hypertension*. 1996;27: 523–528.
17. Pörsti I, Bara AT, Busse R, et al. Release of oxide nitric by angiotensin-(1–7) from porcine coronary endothelium: implications for a novel angiotensin receptor. *Br J Pharmacol*. 1994;111:652–654.
18. Le tran Y, Foster C. Angiotensin-(1–7) and the rat aorta: modulation by the endothelium. *J Cardiovasc Pharmacol*. 1997;30:676–682.
19. Sampaio WO, Nascimento AA, Santos RAS. Systemic and regional hemodynamic effects of angiotensin-(1–7) in rats. *Am J Physiol Heart Circ Physiol*. 2003;284:H1985–H1994.
20. Paula RD, Lima CV, Khosla MC, et al. Angiotensin-(1–7) potentiates the hypotensive effect of bradykinin in conscious rats. *Hypertension*. 1995; 26:1154–1159.
21. Abbas A, Gorelik G, Carbini LA, et al. Angiotensin-(1–7) induces bradykinin-mediated hypotensive responses in anesthetized rats. *Hypertension*. 1997;30:217–221.
22. Gorelik G, Carbini LA, Scicli AG. Angiotensin 1–7 induces bradykinin-mediated relaxation in porcine coronary artery. *J Pharmacol Exp Ther*. 1998;286:403–410.
23. Fernandes L, Fortes ZB, Nigro D, et al. Potentiation of bradykinin by angiotensin-(1–7) on arterioles of spontaneously hypertensive rats studied in vivo. *Hypertension*. 2001;37:703–709.
24. Santos RAS, Haibara AS, Campagnole-Santos MJ, et al. Characterization of a new selective antagonist for angiotensin-(1–7), D-Pro^7-angiotensin-(1–7). *Hypertension*. 2003;41:737–743.
25. Zou LX, Imig JD, Hymel A, et al. Renal uptake of circulating angiotensin II in Val^5-angiotensin II infused rats is mediated by AT_1 receptor. *Eur J Pharmacol*. 1994;256:73–78.
26. Speth RC, Khosla MC, Spech MM, et al. Rat (Ile^5) but not bovine (Val^5) angiotensin raises plasma norepinephrine in rats. *Hypertension*. 1981;3:II-25–29.
27. de Gasparo M, Catt KJ, Inagami T, et al. International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacol Rev*. 2000;52: 415–472.
28. Roks AMJ, Van Geel PP, Pinto YM, et al. Angiotensin-(1–7) is a modulator of the human renin angiotensin system. *Hypertension*. 1999; 34:296–301.
29. Matos de Moura M, Santos RAS, Fontes MAP. Evidence for a functional cardiac interaction between losartan and angiotensin-(1–7) receptors revealed by orthostatic tilting test in rats. *Br J Pharmacol*. 2005;144:755–760.
30. Ueda S, Masumori-Maemoto S, Ashino K, et al. Angiotensin-(1–7) attenuates vasoconstriction evoked by angiotensin II but not by noradrenaline in man. *Hypertension*. 2000;35:998–1001.
31. Gironacci MM, Vatta M, Rodriguez-Fermepin M, et al. Angiotensin-(1–7) reduces norepinephrine release through a nitric oxide mechanism in rat hypothalamus. *Hypertension*. 2000;35:1248–1252.
32. Kostenis E, Milligan G, Christopoulos A, et al. G-protein-coupled receptor Mas is a physiological antagonist of the Angiotensin II type 1 receptor. *Circulation*. 2005;111:1806–1813.
33. Campagnole-Santos MJ, Heringer SB, Batista EN, et al. Differential baroreceptor reflex modulation by centrally infused angiotensin peptides. *Am J Physiol*. 1992;263:R89–R94.
34. Santos RA, Simões E, Silva AC, et al. Evidence for a physiological role of angiotensin-(1–7) in control of hydroelectrolyte balance. *Hypertension*. 1996;27:875–884.
35. DelliPizzi AM, Hilchey SD, Bell-Quilley CP. Natriuretic action of angiotensin(1–7). *Br J Pharmacol*. 1994;111:1–3.
36. Ferreira AJ, Santos RA, Almeida AP. Angiotensin-(1–7): cardioprotective effect in myocardial ischemia/reperfusion. *Hypertension*. 2001;38:665–668.
37. Loot AE, Roks AJ, Henning RH, et al. Angiotensin-(1–7) attenuates the development of heart failure after myocardial infarction in rats. *Circulation*. 2002;105:1548–1550.
38. Iyer SN, Ferrario CM, Chappell MC. Angiotensin-(1–7) contributes to the antihypertensive effect of the blockade of the renin-angiotensin system. *Hypertension*. 1998;31:356–361.

