

Short-Term Angiotensin(1-7) Receptor Mas Stimulation Improves Endothelial Function in Normotensive Rats

Raphael Faria-Silva, Fernanda V. Duarte, Robson A.S. Santos

Abstract—In this study we evaluated the effect of angiotensin(1-7) and its nonpeptide analog, AVE 0991, on the endothelial function in vivo. The experiments were performed in conscious adult male Wistar rats, with polyethylene catheters implanted into the descending aorta (through left carotid artery), for injection of acetylcholine or sodium nitroprusside, femoral artery for mean arterial pressure and heart rate measurement; and femoral vein for drug administration. Increasing doses of acetylcholine (3.1 ng to 25.0 ng) or nitroprusside (1.0 μ g to 10.0 μ g) were administered before and 30 minutes after the start of the infusion of: angiotensin(1-7) (0.7 and 7.0 pmol/min); A-779 (180 pmol/min); angiotensin(1-7) (7.0 pmol/min) combined with A-779 (180 pmol/min); AVE 0991 (11, 45, and 230 pmol/min); AVE 0991 (45 pmol/min) combined with A-779 (180 pmol/min), or vehicle (6 μ L/min). Baseline mean arterial pressure and heart rate were not altered during angiotensin(1-7) or AVE 0991 infusion. Angiotensin(1-7) (0.7 pmol/min) infusion produced a significant potentiation of the hypotensive effect of acetylcholine (3.1 ng: -9 ± 1 mm Hg before; -18 ± 2 mm Hg after; $P < 0.05$). A similar potentiation was observed with the higher dose of angiotensin(1-7). As observed for angiotensin(1-7), infusion of AVE 0991 at 230 pmol/min potentiated the acetylcholine effect (3.1 ng: -8 ± 2 mm Hg before; -16 ± 2 mm Hg after; $P < 0.05$). The potentiating effect was not observed for nitroprusside. A-779 or L-NAME treatment blocked the potentiation produced by angiotensin(1-7) or AVE 0991. Our data indicate that short-term stimulation of angiotensin(1-7) receptors improve endothelial function through facilitation of nitric oxide release. (*Hypertension*. 2005;46 [part 2]:948-952.)

Key Words: angiotensin ■ endothelium ■ nitric oxide ■ renin-angiotensin system

The renin-angiotensin system (RAS) plays an important role in cardiovascular physiology and cell function.¹ The renin-angiotensin system is usually known as a hormonal and tissular system that releases angiotensin II (Ang II) and is involved in the regulation of blood pressure and salt and fluid homeostasis. It is becoming evident that the renin-angiotensin system has 2 major arms: a vasoconstrictor/proliferative in which the main mediator is Ang II, and a vasodilator/anti-proliferative, in which the major effector is angiotensin(1-7) [Ang(1-7)] acting on the G protein-coupled receptor Mas.²

Angiotensin(1-7) is a biologically active heptapeptide, which can be formed in a pathway independent of the angiotensin-converting enzyme. The blood vessels are an important site for the formation and biological actions of Ang(1-7).³ In contrast to Ang II, Ang(1-7) is neither dipsogen nor an aldosterone secretagogue, but similarly to Ang II, it releases vasopressin, prostaglandins, and nitric oxide.³ Ang(1-7) can improve the baroreceptor reflex^{4,5} and decrease smooth muscle cell growth.⁶ Peripherally, the most important actions of Ang(1-7) appear to be related to the control of hydroelectrolyte balance⁷ and cardiovascular function.⁸ We have recently shown that Ang(1-7) is an

endogenous ligand for the G protein-coupled receptor Mas.² Because Ang(1-7) seems to counteract many of Ang II cardiovascular effects, this peptide and its receptor are potential targets for the development of cardioprotective or anti-hypertensive agents. The receptor Mas is blocked by the heptapeptide D-Ala(7)-Ang(1-7) [A-779], a specific and potent antagonist of Ang(1-7),⁹ disrupting hemodynamic and renal responses to Ang(1-7), such as the antidiuretic effect¹⁰ and bradykinin potentiation in vivo and in mesenteric microvessels.^{11,12}

Recently Wiemer et al¹³ described a novel nonpeptide compound, AVE 0991 (AVE), which was able to evoke effects on endothelial cells similar to those observed for Ang(1-7). AVE and Ang(1-7) stimulated nitric oxide (NO) release from cultured cells caused by the activation of endothelial NO synthase, and this effect was inhibited by the NO synthase inhibitor L-NMMA. This effect seems to be a receptor-mediated event. In this regard, recent studies showed that AVE is an Ang(1-7) receptor Mas agonist.¹⁴

In this study we tested the hypothesis that short-term Ang(1-7) Mas receptor stimulation by Ang(1-7) and AVE could improve endothelial function in vivo, by facilitating NO release.

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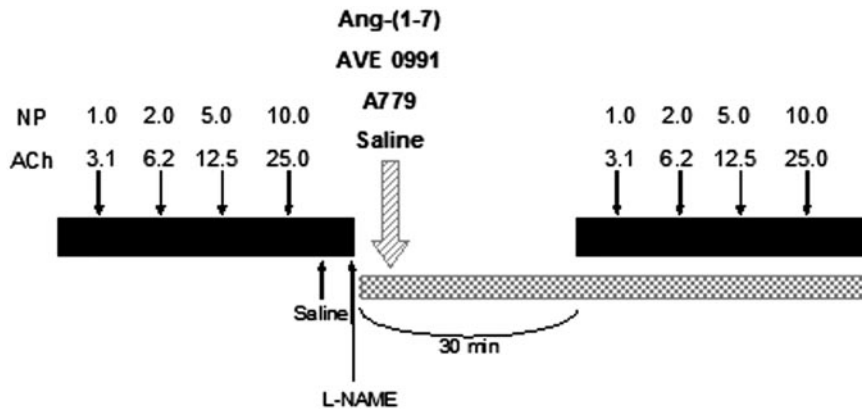


Figure 1. Schematic drawing of the experimental procedures.

Methods

Animals

Experiments were performed in 99 nonanesthetized male Wistar rats, weighing 230 to 330 grams, bred at the animal facility of the Biological Sciences Institute (CEBIO, Federal University of Minas Gerais, Brazil). All animal procedures were performed in accordance with institutional guidelines (Federal University of Minas Gerais, Brazil).

General Surgical Procedures

Twenty-four to 48 hours before the experiment, under anesthesia with 2.5% tribromoethanol (1.0 mL/100 grams) intraperitoneally, a polyethylene catheter (PE-10 connected to PE-50) was introduced into the descending aorta, through the left carotid artery, for intra-arterial injections. The correct position of the catheter was verified by the absence of bradycardia during the first seconds of acetylcholine (ACh) injection, and also during postmortem examination. Other catheters were implanted into abdominal aorta (through femoral artery) for mean arterial pressure (MAP) and heart rate measurements and into the femoral vein for intravenous infusions and injections. After recovery from anesthesia, the rats were kept in individual cages with free access to water and chow until the end of the experiments.

Arterial Pressure Measurements

The arterial pressure and heart rate were monitored by a solid-state strain gauge transducer connected to a computer through a data acquisition system (MP 100; BIOPAC Systems, Inc, Santa Barbara, Calif). The experiments were conducted in conscious rats.

Drugs

ACh, sodium nitroprusside (NP), Ang(1-7), *N*-ω-nitro L-methyl arginine (L-NAME), D-Ala(7)-Ang(1-7) [A-779], and AVE 0991 were dissolved in isotonic saline (0.9% NaCl) immediately before use. Ang(1-7) and A-779 were from Bachem, Germany. ACh, NP, and L-NAME were purchased from Sigma Chemical Co (St Louis, Mo). AVE 0991 was a generous gift from Dr Markus Bleich and Dr Juergen Punter from Aventis Pharma.

Experimental Protocols

Protocol 1: Effect of Ang(1-7) or AVE on the Hypotensive Action of ACh in Wistar Rats

Intra-arterial bolus injections of ACh (3.1, 6.2, 12.5, 25.0 ng) were made before and within 30 minutes of intravenous infusion of: Ang(1-7) (0.7 pmol/min for 60 minutes, *n*=5; or 7.0 pmol/min for 60 minutes, *n*=9); AVE (11 pmol/min for 60 minutes, *n*=6; 45 pmol/min for 60 minutes, *n*=9; or 230 pmol/min for 60 minutes, *n*=7) or vehicle (6 μL/min for 60 minutes, *n*=7). An interval of 3 minutes was allowed between ACh injections. After the first series of ACh injections, the catheter was washed with isotonic saline. The doses of the drugs and the time points for determining its effects were chosen based on preliminary experiments. A schematic drawing of the experimental procedure is shown in Figure 1.

Protocol 2: Effect of Ang(1-7) or AVE on the Hypotensive Action of ACh in Wistar Rats Previously Treated With L-NAME

After the first series of ACh injections (3.1, 6.2, 12.5, 25.0 ng), rats were treated with L-NAME (30 mg/kg IV) just before Ang(1-7) (7.0 pmol/min for 60 minutes, *n*=6), AVE (230 pmol/min for 60 minutes, *n*=6), or vehicle infusion (6 μL/min for 60 minutes, *n*=6).

Protocol 3: Effect of Ang(1-7) or AVE on the Hypotensive Action of NP in Wistar Rats

Intra-arterial bolus injections of NP (1.0, 2.0, 5.0, 10.0 μg) were made before and within 30 minutes of intravenous infusion of: Ang(1-7) (7.0 pmol/min for 60 minutes, *n*=8), AVE (45 pmol/min for 60 minutes, *n*=5), or vehicle (6 μL/min for 60 minutes, *n*=5). An interval of 3 minutes was allowed between NP injections. After the first series of NP injections, the catheter was washed with isotonic saline.

Protocol 4: Effect of A-779 on the ACh-Potentiating Activity of Ang(1-7) or AVE in Wistar Rats

Intra-arterial bolus injections of ACh (3.1, 6.2, 12.5, 25.0 ng) were made before and within 30 minutes of intravenous infusion of: A-779 (180 pmol/min for 60 minutes, *n*=6), Ang(1-7) (7.0 pmol/min for 60 minutes) combined with A-779 (180 pmol/min for 60 minutes)

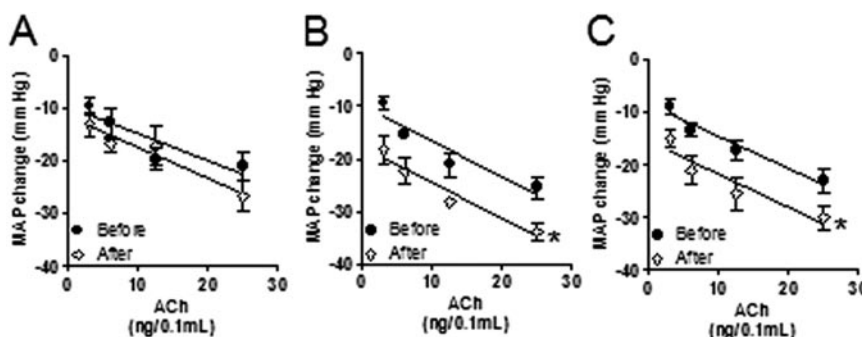


Figure 2. Linear regression showing the dose-response hypotensive effect of ACh in conscious Wistar rats before and within 30 minutes of intravenous infusion of: (A) isotonic saline (6 μL/min, *n*=7); (B) Ang(1-7) (0.7 pmol/min, *n*=5); and (C) Ang(1-7) (7.0 pmol/min, *n*=9). Values are expressed as mean±SEM. **P*<0.05, 30-minute infusion compared with the period before infusion.

Effect of Ang(1-7), A-779, and AVE 0991 Infusion on MAP and HR of Unanesthetized Wistar Rats

	MAP (mm Hg)		HR (bpm)	
	Before	After	Before	After
Control	120±4	121±3	407±25	383±19
Ang(1-7) (0.7 pmol)	118±9	121±6	375±18	373±18
Ang(1-7) (7.0 pmol)	120±5	123±3	378±26	371±22
A-779 (180 pmol)	128±4	127±5	430±32	443±29
AVE0991 (11 pmol)	116±5	117±5	401±7	391±15
AVE0991 (45 pmol)	125±6	131±5	414±21	447±28
AVE0991 (230 pmol)	130±6	129±6	406±21	400±21

Values are expressed as mean±SEM.

HR indicates heart rate; MAP, mean arterial pressure.

(n=7), or AVE (45 pmol/min for 60 minutes) combined with A-779 (180 pmol/min for 60 minutes) (n=7).

Statistical Analysis

Numerical values are given as mean±SEM. Comparisons were made by Student paired *t* test or 2-way ANOVA with Bonferroni post-test when appropriate (GraphPad Prism). The criterion for statistical significance was set at *P*<0.05.

Results

Effect of Ang(1-7) Infusion in Normotensive Wistar Rats

Infusion of Ang(1-7) at 0.7 pmol/min for 60 minutes significantly increased the ACh-evoked hypotension in normotensive Wistar rats (3.1 ng: -9 ± 1 mm Hg before; -18 ± 2 mm Hg after; and 25.0 ng: -25 ± 2 mm Hg before; -34 ± 1 mm Hg after; *P*<0.05). The same effect was observed with a 10-fold increase in Ang(1-7): (3.1 ng: -9 ± 1 mm Hg before; -15 ± 2 mm Hg after; and 25.0 ng: -23 ± 2 mm Hg before; -30 ± 2 mm Hg after; *P*<0.05) (Figure 2), indicating that the maximum effect of Ang(1-7) was reached with the smaller dose. Ang(1-7) infusion did not change baseline MAP or heart rate (Table). No changes were observed in baroreflex. Isotonic saline infusion did not change the hypotensive effect of ACh at any time point.

Effect of AVE Infusion in Normotensive Wistar Rats

Infusion of AVE at 11 pmol/min for 60 minutes did not change the hypotensive effect of ACh (3.1 ng: -9 ± 1 mm Hg before, -10 ± 2 mm Hg after; and 25.0 ng: -25 ± 3 mm Hg before, -25 ± 2 mm Hg after). However, higher doses of

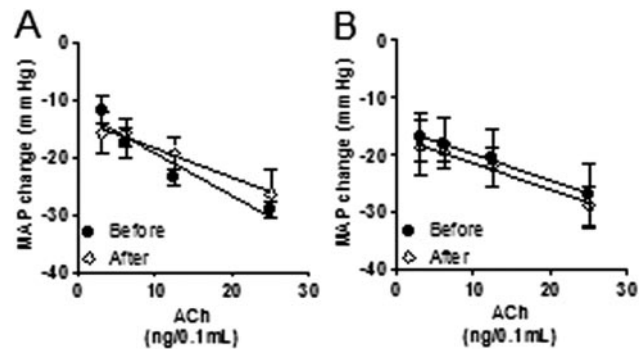


Figure 4. Dose-response hypotensive effect of ACh in conscious Wistar rats previously treated with L-NAME, before and within 30 minutes of intravenous infusion of (A) Ang(1-7) (7.0 pmol/min, n=6) and (B) AVE (230 pmol/min, n=6). Values are expressed as mean±SEM. Isotonic saline infusion did not change the hypotensive effect of ACh in rats pretreated with L-NAME (data not shown).

AVE significantly increased the hypotensive response of small doses of ACh (45 pmol/min: 3.1 ng: -7 ± 1 mm Hg before, -14 ± 1 mm Hg after; and 6.2 ng: -13 ± 2 mm Hg before, -17 ± 1 mm Hg after; *P*<0.05; and 230 pmol/min: 3.1 ng: -8 ± 2 mm Hg before, -16 ± 2 mm Hg after; and 6.2 ng: -13 ± 2 mm Hg before, -20 ± 3 mm Hg after; *P*<0.05) (Figure 3). AVE did not potentiate the effect of the higher dose of ACh (25.0 ng) at any rate used.

Evaluating Ang(1-7) Potentiation of ACh-Evoked Hypotension

Treatment with L-NAME did not abolish the hypotensive effect of ACh in normotensive rats. However, the potentiation of ACh-evoked hypotension by Ang(1-7) or AVE was completely blocked in rats previously treated with L-NAME (30 mg/kg) (Figure 4). Baseline MAP was significantly increased after L-NAME injection (151 ± 4 mm Hg at 30 minutes versus 132 ± 4 mm Hg, before infusion; *P*<0.0001). Isotonic saline infusion in rats pretreated with L-NAME did not change the hypotensive effect of ACh at any time point (data not shown). As shown in Figure 4, the absolute changes in MAP produced by ACh after L-NAME treatment were actually higher than those observed in baseline conditions; however, the proportional changes in blood pressure were not different. Neither Ang(1-7) nor AVE infusion produced potentiation of the hypotensive effect of NP (Figure 5).

To evaluate whether the ACh-potentiating activity of Ang(1-7) was a receptor-mediated response, we also deter-

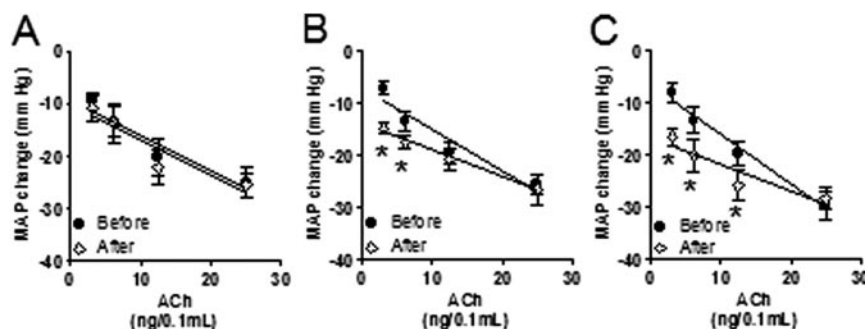


Figure 3. Dose-response hypotensive effect of ACh in conscious Wistar rats before and within 30 minutes of intravenous infusion of: (A) AVE (11 pmol/min, n=6); (B) AVE (45 pmol/min, n=9); and (C) AVE (230 pmol/min, n=7). Values are expressed as mean±SEM. **P*<0.05, 30-minute infusion compared with the period before infusion.

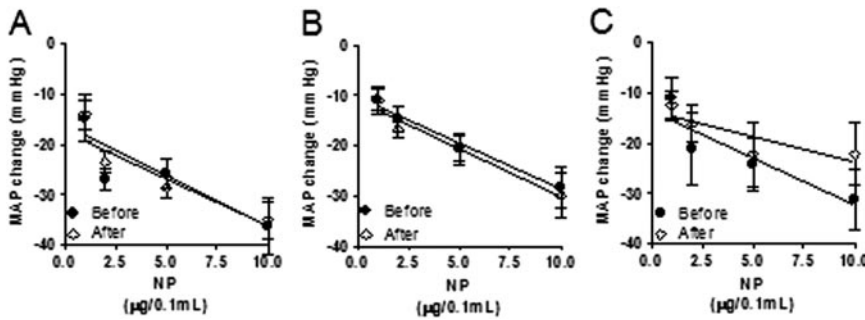


Figure 5. Dose-response hypotensive effect of NP in conscious Wistar rats before and within 30 minutes of intravenous infusion of: (A) isotonic saline (6 μ L/min, $n=5$); (B) Ang(1-7) (7.0 pmol/min, $n=8$); and (C) AVE (45 pmol/min, $n=5$). Values are expressed as mean $\pm$ SEM.

mined the effect of Ang(1-7) combined with its selective receptor antagonist A-779. As previously shown, infusion of Ang(1-7) at 0.7 or 7.0 pmol/min significantly increased the hypotensive effect of ACh. However, infusion of A-779 combined with Ang(1-7) abolished the ACh-potentiating activity of this heptapeptide, as well as of AVE. This effect could not be attributed to an influence of A-779 alone on the hypotensive action of ACh, because no consistent changes in ACh-evoked hypotension were observed when A-779 was infused at this rate (Figure 6). A-779 infusion did not significantly change baseline MAP or heart rate (Table). It should be noted that the MAP values are slightly higher than the normal because of occlusion of the left carotid artery for the ACh injections.

Discussion

The major finding of this study was that short-term Ang(1-7) infusion significantly increased the hypotensive effect of intra-arterial ACh administration in normotensive rats. A similar action was observed with its nonpeptide analog, AVE 0991. This effect was not observed for NP. In addition, the potentiation of ACh responses by Ang(1-7) and AVE was completely blocked by pretreatment with L-NAME or A-779 infusion.

The data taken from the literature suggest that Ang(1-7) exerts its ACh-potentiating effect acting on endothelial cells, once this peptide can be formed locally¹⁵ and it is capable of releasing nitric oxide³ and vasodilatory prostaglandins.⁷ Moreover, Ang(1-7) reduced neointimal thickness, neointimal area, and percentage stenosis in a stent implantation model in rats.¹⁶ Fernandes et al¹² demonstrated that in the resistance blood vessels of spontaneously hypertensive rats, Ang(1-7) induces vasodilation and potentiates BK when topically applied by releasing NO from endothelial cells. However, Ang(1-7) did not potentiate the vasodilation pro-

duced by ACh in spontaneously hypertensive rats mesenteric microvessels.¹²

We have used a dose of AVE $\approx$ 65- and 330-fold the one used for Ang(1-7) to produce potentiation of the ACh response. Although we do not have direct data to explain this difference, it could be speculated that in the case of Ang(1-7), other mechanisms could be involved, such as binding to angiotensin-converting enzyme or conformational differences between rat and bovine endothelial cells receptor Mas, in which the data of Wiemer et al¹³ were obtained. According to this possibility, in rats the actions of Ang(1-7) would be more effective than AVE 0991. It has been described that in vitro AVE 0991 is more potent than Ang(1-7) in promoting NO release¹³ and also led to a smaller generation of superoxide in culture cells when compared with Ang(1-7).¹³ However, we demonstrated that AVE potentiated only the ACh smaller doses (3.1, 6.2, and 12.5 ng). In our study, AVE tended to reduce the hypotensive response produced by NP (Figure 5C), suggesting a different NO/superoxide ratio in culture cells when compared with in vivo studies.

The ACh-potentiating activity of Ang(1-7) or AVE was completely abolished by A-779. We and others have shown that A-779 antagonizes the effects of Ang(1-7) in several preparations.¹⁷ More recently, we have shown that A-779 is an Ang(1-7) Mas receptor antagonist² and suppresses the Ang(1-7) actions centrally and peripherally.⁹ Thereafter, the ACh-potentiating activity of Ang(1-7) in vivo appears to be a receptor Mas-mediated event.

L-NAME treatment abolished the potentiation of the hypotensive effect of ACh by Ang(1-7) and AVE, suggesting that ACh potentiation involves the facilitation of the NO release from the endothelium. We cannot discard, however, the involvement of arachidonic acid derivatives and the endothelium-derived hyperpolarizing factor¹⁸ in this effect.

The knowledge that Ang(1-7) and its analogs improve endothelial function in vivo opens new possibilities for the

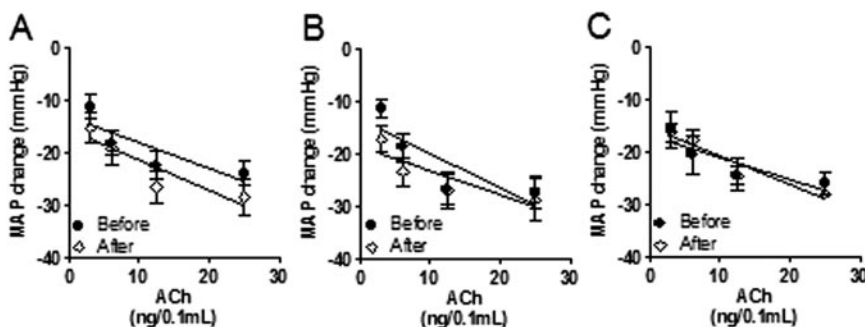


Figure 6. Dose-response hypotensive effect of ACh in conscious Wistar rats before and within 30 minutes of intravenous infusion of: (A) A-779 (180 pmol/min, $n=6$); (B) Ang(1-7) (7.0 pmol/min) associated with A-779 (180 pmol/min) ($n=7$); and (C) AVE 0991 (45 pmol/min) associated with A-779 (180 pmol/min) ($n=7$). Values are expressed as mean $\pm$ SEM.

future treatment of cardiovascular diseases such as atherosclerosis, hypertension, and heart failure.

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