The nonpeptide angiotensin-(1–7) receptor Mas agonist AVE-0991 attenuates heart failure induced by myocardial infarction

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Submitted 1 August 2006; accepted in final form 17 October 2006

The nonpeptide angiotensin-(1–7) receptor Mas agonist AVE-0991 attenuates heart failure induced by myocardial infarction. Am J Physiol Heart Circ Physiol 292: H1113–H1119, 2007. First published October 20, 2006; doi:10.1152/ajpheart.00828.2006.—The nonpeptide AVE-0991, which has been reported as a selective ligand for the angiotensin-(1–7) [ANG-(1–7)] receptor Mas, has actions similar to those attributed to the cardioprotective product of the renin-angiotensin system, ANG-(1–7). In this study, we evaluated the cardiac effects of AVE-0991 in normal and infarcted male Wistar rats. Myocardial infarction was induced by left coronary artery ligation. At the end of the treatment, Langendorff technique was used to analyze cardiac function. Left ventricle serial sections were dyed with Giemsa staining to quantify the infarcted area. In normal hearts, AVE-0991 produced a significant decrease in perfusion pressure and an increase in systolic tension, rate of tension rise and fall, and heart rate. These effects were completely blocked by the perfusion of the hearts with a solution containing the selective ANG-(1–7) antagonist A-779. Nω-nitro-L-arginine methyl ester (L-NAME) treatment abolished the AVE-0991-induced vasodilation in isolated hearts. AVE-0991 significantly attenuated the decrease in systolic tension (sham operated, 13.00 ± 1.02 g; infarction, 7.18 ± 0.66 g; AVE treated, 9.23 ± 1.05 g, n = 5).+dT/dt, −dT/dt, and heart rate induced by myocardial infarction. Infarction-induced vasconstriction was completely prevented by AVE-0991 treatment. Furthermore, AVE-0991 significantly decreased the infarcted area (6.98 ± 1.01 vs. 3.94 ± 1.04 mm²) in AVE-treated rats. These data indicate that the compound AVE-0991 produces beneficial effects in isolated perfused rat hearts involving the ANG-(1–7) receptor Mas and the release of nitric oxide. In addition, our results indicate that AVE-0991 attenuates postischemic heart failure.

angiotensin II; renin-angiotensin system; A-779; angiotensin-converting enzyme 2

ANGIOTENSIN-(1–7) [ANG-(1–7)] is an endogenous peptide of the renin-angiotensin system with several beneficial effects that are often opposite to those attributed to ANG II. The recent identification of the ANG-(1–7) forming enzyme ACE2 (7, 23) and of Mas as an ANG-(1–7) receptor (22) has added further support and wider acceptance for the existence of a vasodilator/antiproliferative arm in the renin-angiotensin system, formed by the ACE2-ANG-(1–7)-Mas axis. This arm counterregulates the ACE-ANG II-AT1 receptor axis that induces vasoconstriction, salt and water reabsorption, and proliferative effects (21).

The heart is an important target for ANG-(1–7) actions, where it is formed (2, 18) and presents a cardioprotective role. ANG-(1–7) induces vasodilatation in porcine (11, 16) and canine coronary arteries (5) and potentiates the vasodilatory effect of bradykinin in isolated perfused rat hearts (1). Effects of ANG-(1–7) in the heart function include decrease of the incidence and duration of ischemia-reperfusion arrhythmias and improvement of the postischemic contractile function in isolated perfused rat hearts (9, 10). These latest effects were also observed in transgenic rats (TGR[A1–7]3292), which possess a chronic 2.5-fold increase in circulating ANG-(1–7) levels. These animals also presented a significant increase in first derivative of left ventricular pressure at basal conditions and a less pronounced cardiac hypertrophy induced by isoproterenol (20). Additionally, infusion of ANG-(1–7) for 8 wk improved endothelial aortic function and coronary perfusion and preserved cardiac function in rats with heart failure induced by ligation of the left coronary artery (14).

It has been described that the nonpeptide compound AVE-0991 evokes similar effects to those observed for ANG-(1–7) in cultured bovine aortic endothelial cells (25), kidneys (15), and vessels (8, 13). Recently, Benter et al. (3) showed that chronic administration of ANG-(1–7) or its synthetic analog, AVE-0991, significantly improved the ischemia-reperfusion recovery in isolated perfused spontaneously hypertensive rat hearts treated with Nω-nitro-L-arginine methyl ester (L-NAME).

The purpose of this study was to evaluate the effects of 1-wk treatment with AVE-0991 on the development of heart failure induced by left coronary artery ligation.

MATERIALS AND METHODS

Animals. Male Wistar rats weighting 250–300 g were obtained from the animal facility of the Biological Sciences Institute, Federal University of Minas Gerais (CEBIO). All experimental protocols were performed in accordance with the guidelines for the human use of laboratory animals of our Institute and approved by local authorities.

Isolated heart preparation. Rats were treated either with AVE-0991 (1 mg/kg, n = 9) or vehicle (0.9% NaCl, n = 11) administered orally by gavage. At the end of the 7-day period of AVE-0991 treatment, the animals were decapitated 10–15 min after intraperitoneal injection of 400 IU of heparin. After the thorax was opened, the heart was carefully dissected, removed from the thoracic cavity, and placed in a plate containing ice-cold Krebs-Ringer solution (KRS) to support and wider acceptance for the existence of a vasodilator/antiproliferative arm in the renin-angiotensin system, formed by the ACE2-ANG-(1–7)-Mas axis. This arm counterregulates the ACE-ANG II-AT1 receptor axis that induces vasoconstriction, salt and water reabsorption, and proliferative effects (21).
attenuate any potential cardiac damage during dissection of aorta artery. The hearts were perfused through an aortic stump with KRS containing 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4·7H2O, 2.5 mM CaCl2·2H2O, 11.7 mM glucose, and 26.5 mM NaHCO3. The perfusion flow was maintained constant (7–8 ml/min) at 37°C and constant oxygenation (5% CO2-95% O2). Tension and coronary perfusion pressure were continuously recorded. A force transducer was attached through a heart clip to the apex of the ventricles to record the contractile force (tension, g) on a computer, through a data acquisition system (Biopac Systems, Santa Barbara, CA). A diastolic tension of 0.5–1.0 g was applied to the hearts. Coronary perfusion pressure was measured by means of a pressure transducer connected to the aortic cannula and coupled to the recording system. The heart rate (HR) was derived from the changes in cardiac tension. After 20–30 min of stabilization, the functional parameters were recorded for an additional period of 30 min. To evaluate the involvement of the ANG-(1–7) receptor Mas in the cardiac effects of AVE-0991, an additional set of isolated hearts from rats treated or not with AVE-0991 (AVE) were perfused with normal Krebs-Ringer solution (KRS) or KRS containing A-779. The values of each animal were obtained by the average of 7 values (each value collected at 5-min intervals during the 30-min experimental period). *P < 0.05 compared with control group (two-way ANOVA followed by Bonferroni test).

Myocardial infarction procedures. Under anesthesia with 10% ketamine-2% xylazine (4:3; 0.1 ml/100 g ip), rats were placed in the supine position on a surgical table, tracheotomized, intubated, and ventilated with room air using a respirator for small rodents. Subdermal electrodes were placed to allow the determination of electrocardiogram (ECG). The chest was opened by a left thoracotomy at the
fourth or fifth intercostal space. To expose the heart, a small-sized retractor was used to maintain the ribs separated. After incision of the pericardium, the heart was quickly removed from the thoracic cavity and turned left to allow access to the proximal left anterior descending (LAD) coronary artery. A 4-0 silk suture was snared around the LAD and tightly ligated to occlude the vessel. The heart was then placed back, and the chest was closed with 4-0 silk sutures. Sham-operated rats were treated in the same manner, but the coronary artery was not ligated. After surgical procedures, ECG tracings were obtained to confirm the myocardial ischemia, i.e., ST-segment elevation and increase in R-wave amplitude. Infarcted rats received either AVE-0991 (1 mg/kg, \( n = 5 \)) or vehicle (0.9% NaCl, \( n = 5 \)) administered orally by gavage during 7 days. Sham-operated rats received saline (\( n = 7 \)). Seven days after induction of infarction, cardiac function was evaluated by the Langendorff technique.

Myocardial infarction quantification. At the end of perfusion, left ventricles were kept in 4% Bouin fixative for 24 h at room temperature. The tissues were dehydrated by sequential washes with 70% ethanol, 80% ethanol, 90% ethanol, and 100% ethanol and imbedded in paraffin. Transversal sections (6 \( \mu m \)) were cut starting from the base area of the left ventricle at intervals of 40 \( \mu m \) and dyed with Gomori trichrome. Infarcted area was measured in two tissue sections (one at base area and another at apex area of the left ventricle) of each animal (\( n = 6–7 \)). Images at \( \times10 \) magnification were obtained through a JVC TK-1270/RGB microcamera. The KS300 software built in a Kontron Elektronick/Carl Zeiss image analyzer was used for quantification.
infarcted area quantification using the image segmentation function, where all pixels with green hues were selected for creation of a binary image and subsequent calculation of the total area occupied by infarcted tissue (6). The data were expressed as square millimeters.

Statistical analysis. All data are expressed as means ± SE. The cardiac function values of each animal were obtained by the average of seven values (each value collected at 5-min intervals during the 30-min experimental period). The infarcted area of each animal was obtained by the average of all values acquired in each tissue section. Statistical significance was estimated using two-way ANOVA followed by Bonferroni posttest for cardiac function experiments and Student’s t-test for infarcted area quantification (GraphPad Prism 4.0). The level of significance was set at \( P < 0.05 \).

RESULTS

One week of treatment with AVE-0991 produced a significant decrease in perfusion pressure (56.55 ± 0.86 vs. 68.73 ± 0.69 mmHg in vehicle-treated rats, Fig. 1A) and an increase in systolic tension (11.40 ± 0.05 vs. 9.84 ± 0.15 g in vehicle-treated rats, Fig. 1B), rate of tension rise (+dT/dt; 184.30 ± 0.50 vs. 155.20 ± 1.97 g/s in vehicle-treated rats, Fig. 1D), rate of tension fall (−dT/dt; 179.60 ± 1.39 vs. 150.80 ± 2.42 g/s in vehicle-treated rats, Fig. 1E). A slight increase in HR was also observed (220.40 ± 0.71 vs. 214.20 ± 0.74 beats/min in vehicle-treated rats; Fig. 1F). No significant changes were
observed in diastolic tension (Fig. 1C). These effects were completely blocked by the perfusion of the hearts with a solution containing the selective ANG-(1–7) antagonist A-779. This antagonist alone induced a significant increase in perfusion pressure and a decrease in systolic tension, \(+\text{d}T/\text{d}r\), and \(-\text{d}T/\text{d}r\). In addition, l-NAME cotreatment abolished the AVE-0991-induced vasodilation in isolated hearts (68.73 ± 0.69 vs. 65.30 ± 0.85 mmHg in AVE + l-NAME-treated rats, Fig. 2A). l-NAME alone induced an increase in perfusion pressure and a decrease in HR (Fig. 2, A and F). Furthermore, rats treated with AVE-0991 plus l-NAME showed a significant increase in diastolic tension (Fig. 2E).

We next evaluated the effect of AVE-0991 on the heart function of infarcted rats (Fig. 3). Strikingly, AVE-0991 significantly attenuated the decrease in systolic tension (sham operated, 13.00 ± 0.09 g; infarction, 6.96 ± 0.47 g; AVE treated, 9.23 ± 0.28 g), \(+\text{d}T/\text{d}r\) (sham operated, 209.90 ± 1.09 g/s; infarction, 103.6 ± 5.50 g/s; AVE treated, 136.20 ± 4.66 g/s), \(-\text{d}T/\text{d}r\) (sham operated, 199.70 ± 1.76 g/s; infarction, 89.51 ± 5.18 g/s; AVE treated, 124.40 ± 6.53 g/s), and HR (sham operated, 216.50 ± 2.37 beats/min; infarction, 156.40 ± 1.33 beats/min; AVE treated, 185.90 ± 3.60 beats/min) induced by myocardial infarction. Infarction-induced vasoconstriction was completely prevented by AVE-0991 treatment (sham operated, 95.48 ± 1.60 mmHg; infarction, 115.00 ± 3.94 mmHg; AVE treated, 86.87 ± 2.28 mmHg). Moreover, AVE-0991 significantly decreased the infarcted area (Fig. 4).

**DISCUSSION**

In this work, we have found that the compound AVE-0991 improved the cardiac function of normal rats through a mechanism involving the receptor Mas and release of NO. More important, this ANG-(1–7) mimic induced cardioprotective effects in the model of heart failure induced by left coronary artery ligation and reduced the infarcted area.

AVE-0991 was first described by Wiemer et al. (25) in 2002. These authors reported that AVE-0991 competed with high affinity for the ANG-(1–7) binding in endothelial cells membrane. No significant displacement was observed for the binding of ANG II to AT\(_1\) or AT\(_2\) receptors. Functionally, AVE-0991 stimulated NO and superoxide (O\(_2^-\)) release from bovine aortic endothelial cells with a similar efficiency as observed for ANG-(1–7) (25). Afterward, studies have demonstrated that this compound mimics the ANG-(1–7) effects in the kidney (15) and blood vessels (8, 13) acting on the ANG-(1–7) receptor Mas. Recently, Benter et al. (3) showed that chronic administration of ANG-(1–7) or AVE-0991 significantly improved the ischemia-reperfusion recovery in isolated perfused hearts from spontaneously hypertensive rats treated with l-NAME. Since ANG-(1–7) has been described as a biologically active peptide with many cardiac effects (9, 14, 19), our results are in agreement with those studies showing that AVE-0991 mimics the ANG-(1–7) actions.

AVE-0991 produced a significant decrease in perfusion pressure and an increase in systolic tension and \(+\text{d}T/\text{d}r\) without detectable alterations in diastolic tension. These findings are in keeping with previous reports suggesting that ANG-(1–7) plays beneficial effects in the coronary vasculature (1, 5, 11, 16) and in cardiac function (9, 10, 14, 20). Furthermore, many of the cardiac ANG-(1–7) actions as well as AVE-0991-induced effects were completely blocked by the selective ANG-(1–7) receptor antagonist A-779, suggesting that ANG-(1–7) through interaction with Mas receptor and AVE-0991 play its effects. Moreover, because A-779 does not block AT\(_1\)
or AT2 receptors, a primary involvement of these receptors in its effects is highly unlikely. However, we cannot disregard the possibility of interaction of Mas with AT1 and AT2 receptors in mediating the AVE-0991 effects in the heart (21). Interestingly, -A779 alone elicited a significant increase in perfusion pressure and a decrease in systolic tension and ±dT/dt, suggesting that endogenous ANG-(1–7) is importantly involved in the heart function. The absence of changes in diastolic tension could be due to limitations of the Langendorff preparation to detect slight changes in this parameter. Further studies with in vivo measurements would be necessary to clarify the effect of AVE-0991 on the diastolic tension.

It has been reported that NO release participates in the ANG-(1–7) effects in coronary vessels (1, 5, 11, 16) but not in cardiac function (9, 10). In fact, we have found that -NAME cotreatment abolished the AVE-0991-induced vasodilatation but not the improvement on cardiac function elicited by this compound. In keeping with previous studies, -NAME alone provoked an increase in perfusion pressure (3, 24) and a decrease in HR (12). In addition, long-term administration of -NAME alone increased systolic tension and ±dT/dt, likely due to the left ventricular hypertrophy induced by NO synthase inhibition (17). Strikingly, rats treated with AVE-0991 plus -NAME showed a significant increase in diastolic tension. This effect could not be explained by our present data.

We have observed that treatment with AVE-0991 produced a slight increase in HR. Likewise, long-term increase of plasma ANG-(1–7) levels observed in transgenic rats that overexpress an ANG-(1–7)-producing fusion protein produced higher basal HR in in vivo and in vitro conditions (20). Moreover, isolated hearts from Mas-deficient mice showed a significant decrease in HR compared with wild-type mice (19). In sharp contrast, infusion of ANG-(1–7) for 7 days elicited a slight bradycardia in Wistar rats (4). Differences between strain, species, and duration of the treatment may account for these discrepant findings. Further studies are obviously necessary to clarify the exact effects of ANG-(1–7) on HR.

Treatment with AVE-0991 significantly attenuated the decrease in systolic tension, ±dT/dt, and HR observed in hearts from infarcted rats. In addition, infarction-induced vasoconstriction was completely prevented by AVE-0991 treatment. Accordingly, chronic infusion of ANG-(1–7) during 8 wk decreased the deleterious effects induced by coronary artery ligation in left ventricular end-diastolic pressure, coronary flow, and aortic endothelial function in rats (14). In addition, genetic deletion of Mas produced heart failure in mice (19). The reduction in infarcted area observed in AVE-0991-treated rats is also in line with the study by Loot et al. (14). This latter observation explains in part the beneficial functional effects observed in hearts from AVE-0991-treated rats. Another explanation for the improvement of the cardiac function seemed in AVE-0991-treated infarcted rats could be related to a more viable myocardium. However, the observation that AVE-0991 also improved the heart function of normal rats indicates that other factors in addition to the reduction of the infarcted area played a role in the improved heart function of AVE-0991-treated infarcted rats. It should be mentioned that in the present study, AVE-0991 treatment was initiated just after the induction of the myocardial ischemia. Thus the effects of AVE-0991 in hearts with established ischemia remain to be evaluated. In addition, as blood pressure was not measured in the different groups, we cannot eliminate the participation of changes in blood pressure in some of the alterations observed in cardiac function induced by AVE-0991 treatment. However, at the same dose used in the present study (1 mg/kg), no significant changes in blood pressure were observed in AVE-0991-treated mice (15). Furthermore, infusion of AVE-0991 (11 to 230 pmol/min for 60 min) in Wistar rats did not change the blood pressure (8). The absence of major changes in arterial pressure was expected, once chronic or acute infusion of ANG-(1–7) produces only slight alterations in mean arterial pressure (21).

In summary, these findings demonstrate that the nonpeptide AVE-0991 evoked effects comparable to ANG-(1–7) in the heart. Furthermore, long-term AVE-0991 treatment produces beneficial effects in isolated perfused rat hearts involving the ANG-(1–7) receptor Mas and the release of NO. More important, our results also show that AVE-0991 attenuates postischemic heart failure in rats.

ACKNOWLEDGMENTS

The authors thank Dr. Markus Bleich from Aventis Pharma for kindly providing AVE-0991.

GRANTS

This work was supported in part by Pró-reitoria de pesquisa (PRPq; Federal University of Minas Gerais), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Programa de Apoio a Núcleos de Excelência (PRONEX)), and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG-PRONEX).

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