Angiotensin-(1-7): Cardioprotective Effect in Myocardial Ischemia/Reperfusion

Anderson J. Ferreira, Robson A.S. Santos, Alvair P. Almeida

Abstract—In this study we evaluate the effects of angiotensin-(1-7) on reperfusion arrhythmias in isolated rat hearts. Rat hearts were perfused according to Langendorff technique and maintained in heated (37±1°C) and continuously gassed (95% O₂/5% CO₂) Krebs-Ringer solution at constant pressure (65 mm Hg). The electrical activity was recorded with an ECG (bipolar). Local ischemia was induced by coronary ligation for 15 minutes. After ischemia, hearts were reperfused for 30 minutes. Cardiac arrhythmias were defined as the presence of ventricular tachycardia and/or ventricular fibrillation after the ligation of the coronary artery was released. Angiotensin II (0.20 nmol/L, n=10) produced a significant enhancement of reperfusion arrhythmias. On the other hand, Ang-(1-7) presented in the perfusion solution (0.22 nmol/L, n=11) reduced incidence and duration of arrhythmias. The antiarrhythmic effects of Ang-(1-7) was blocked by the selective Ang-(1-7) antagonist A-779 (2 nmol/L, n=9) and by indomethacin pretreatment (5 mg/kg IP, n=8) but not by the bradykinin B₂ antagonist HOE 140 (100 nmol/L, n=10) or by L-NAME pretreatment (30 mg/kg IP, n=8). These results suggest that the antiarrhythmic effect of low concentrations of Ang-(1-7) is mediated by a specific receptor and that release of endogenous prostaglandins by Ang-(1-7) contributes to the alleviation of reversible and/or irreversible ischemia-reperfusion injury. (Hypertension. 2001;38(part 2):665-668.)

Key Words: heart • angiotensin II • angiotensin antagonist • renin-angiotensin system • nitric oxide • prostaglandins

In the last years, there are both indirect and direct evidence that the renin-angiotensin system (RAS) is involved in the pathogenesis of myocardial injury (ischemia-reperfusion and preconditioning). Besides angiotensin (Ang) II, other endogenous biologically active products of the RAS have been identified, including Ang-(1-7) and Ang-(3-8). Among the biologically active end products of the RAS, the heptapeptide Ang-(1-7) is particularly interesting because it can be formed directly from Ang I by an ACE-independent pathway and is essentially devoid of effects exerted by Ang II through AT₁ receptors, including vasoconstriction and induction of drinking.

ACE inhibitors have beneficial effects on reperfusion arrhythmias, which have been attributed to a reduction of both local Ang II generation and bradykinin (BK) degradation. However, plasma Ang-(1-7) concentration increases several-fold during treatment with ACE inhibitors. Furthermore, Ang-(1-7) has been recently reported to produce vasodilation in canine coronary artery rings preconstricted with the thromboxane A₂ analogue U46619 and to increase the release of [³H]norepinephrine from isolated rat atria. These observations suggest that Ang-(1-7) can participate in the pharmacological effects of ACE inhibitors in reperfusion arrhythmias.

In a previous study, we have shown an enhancement of reperfusion arrhythmias in isolated hearts perfused with normal Krebs-Ringer solution (KRS) containing Ang-(1-7) at a concentration of 27 nmol/L. However, in a recent study, we have shown that Ang-(1-7) in lower concentration (2.2 nmol/L) augments BK-induced vasodilator responses through release of NO and vasodilator prostaglandins. Therefore, the purpose of the present study was to investigate the effect of Ang-(1-7) in low concentration in the isolated perfused rat heart during ischemia and reperfusion.

Methods
Male Wistar rats (240 to 300 g body weight) were decapitated 10 to 15 minutes after intraperitoneal injection of 400 IU heparin. The thorax was opened, and the heart was carefully dissected and perfused with KRS through a 1.0 cm aortic stump. The perfusion fluid was maintained at 37±1°C, with a pressure of 65 mm Hg and constant oxygenation (5% CO₂/95% O₂). A force transducer (model FT 03, Grass) was attached through a heart clip to the apex of the ventricles to record the contractile force (tension, g) on a computer, by a data-acquisition system (Codas, Dataq Instruments Inc). A diastolic tension of 0.5 to 1.0g was applied to the hearts. Electrical activity was recorded with an ECG (Nihon Kohden) with the aid of 2 cotton wicks placed directly on the surface of the right atrium and left ventricle (bipolar lead). Coronary flow was measured by collecting the perfusate over a period of 1 minute at regular intervals. The hearts were perfused for an initial 30-minute period with KRS (control, n=11) or with KRS containing (1) Ang II (0.20 nmol/L, n=10); (2) Ang-(1-7) (0.22 nmol/L, n=11); (3) A-779 (2 nmol/L, n=9); (4) A-779 (2 nmol/L) plus Ang-(1-7) (0.22

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nmol/L, n=10; (5) HOE 140 (100 nmol/L, n=7); and (6) HOE 140 (100 nmol/L) plus Ang-(1-7) (0.22 nmol/L, n=10). After the equilibration period, the left anterior descending coronary artery (LAD) was ligated by the method described by Lubbe et al., beneath the left auricular appendage together with the adjacent veins. The ligature was released after 15 minutes, and reperfusion with different KRS (above) was performed for an additional 30 minutes. Cardiac arrhythmias were defined as the presence of ventricular tachycardia and/or ventricular fibrillation after the ligature of the coronary artery was released. To obtain a quantitative measurement, the arrhythmias were graded arbitrarily by their duration, with a duration of 30 minutes considered as irreversible arrhythmia. Therefore, the occurrence of cardiac arrhythmias for up to 3 minutes was assigned the factor 2; 3 to 6 minutes was assigned the factor 4; 6 to 10 minutes was assigned the factor 6; 10 to 15 minutes was assigned the factor 8; 15 to 20 minutes was assigned the factor 10; 20 to 25 minutes was assigned the factor 11; and 25 to 30 minutes was assigned the factor 12. A value of 0 to 12 was thus obtained in each experiment and is denoted as arrhythmia severity index (ASI).12

To evaluate the role of cyclooxygenase products in the Ang-(1-7) effects, rats received indomethacin (5 mg/kg IP) plus heparin (400 IU IP). After 1 hour, the rats were decapitated, the thorax was opened, and the heart was dissected and perfused with KRS or KRS containing Ang-(1-7) (0.22 nmol/L, n=8). After the equilibration period, the LAD was ligated as described above.

In another experimental group, we determined the role of NO in the Ang-(1-7) effects. Rats received Nω-nitro-L-arginine methyl ester (L-NAME) (30 mg/kg IP) plus heparin (400 IU IP, n=8). The protocol was the same as that described above. Finally, the effects of combined treatment with indomethacin and L-NAME were investigated. Rats received indomethacin (5 mg/kg IP) plus L-NAME (30 mg/kg IP) plus heparin (400 IU IP) and were decapitated 1 hour later. The thorax was opened, and the heart was dissected and perfused with KRS or KRS containing Ang-(1-7) (0.22 nmol/L, n=8). After the equilibration period, experimental procedure was the same as described above. 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.

Statistical Analysis

Data are reported as mean±SEM. Statistical analysis was performed by Student’s t test or ANOVA followed by Bonferroni test. P<0.05 was considered significant.

Results

Figure 1 illustrates the protocol for ischemia-reperfusion in isolated perfused rat hearts. It shows a typical time course of reperfusion-induced arrhythmias in the control group, ie, isolated heart perfused with normal KRS (Figure 1A), and experimental perfusion of isolated heart with KRS containing Ang-(1-7) (Figure 1B). Ventricular tachycardia and/or ventricular fibrillation on reperfusion was observed in both groups. In control hearts, ventricular tachycardia (VT) degenerated into ventricular fibrillation (VF) during the 30 minutes of reperfusion, whereas Ang-(1-7) tended to inhibit the degeneration of VT to VF and to enhance spontaneous reversion to normal sinus rhythm. Figure 1C shows the changes in coronary flow in isolated hearts perfused with normal KRS or KRS containing angiotensin peptides. Occlusion of the coronary artery resulted in a comparable flow reduction (∼50%), which was sustained throughout the ischemic period. On reperfusion, coronary flow increased to values not different from the control period within 5 minutes. After the control and Ang II–treated groups in hearts perfused with Ang-(1-7) were compared, no apparent increase in coronary flow was observed after 20, 25, and 30 minutes of reperfusion. However, statistically significant differences were found between the Ang II–treated and Ang-(1-7)–treated groups.

The antiarrhythmogenic effects of Ang-(1-7) were evidenced by a significant decrease of ∼46% in ASI (4.0±1.2 versus 7.45±1.3 for the control group, Figure 2A). In contrast, an increase of ∼40% in ASI was observed in Ang II–perfused hearts (10.40±1.21 versus 7.45±1.3 for the control group; P<0.05; Figure 2A). In addition, the occurrence of irreversible arrhythmias increased from 45.5% in control hearts to 70% in the hearts perfused with KRS containing Ang II. However, in presence of Ang-(1-7), the occurrence of irreversible arrhythmias was only 18% (Figure 2B). The Ang-(1-7) antagonist A-779 completely blocked the antiarrhythmogenic effect of Ang-(1-7) (Figure 3A). Strikingly, A-779 by itself increased the duration (10.0±1.05 versus 7.45±1.03 for control group) and incidence of reperfusion arrhythmias (67% versus 45% for control group).

The BK-B2 receptor antagonist HOE 140 at 100 nmol/L concentration increased the duration (10.29±1.40 versus 7.45±1.03 for control group) and incidence of reperfusion arrhythmias (71% versus 45% control group, Figure 3B). However, HOE 140 did not block the antiarrhythmogenic effect of Ang-(1-7). As shown in Figure 3 C, the antiarrhythmogenic effect of Ang-(1-7) was significantly blocked by indomethacin pretreatment. However, the effect of Ang-(1-7)
was not changed by L-NAME pretreatment. Combination of indomethacin with L-NAME produced the same effect as observed for indomethacin alone.

Discussion

Ours data show for the first time that, conversely to Ang II, Ang-(1-7) produced an antiarrhythmogenic effect. Ang-(1-7) presented in the perfusion solution at 0.22 nmol/L concentration reduced the incidence and duration of reperfusion arrhythmias. The antiarrhythmogenic effect of this low concentration of Ang-(1-7) contrasts with our previous study in which we showed that Ang-(1-7) at a 100-fold higher concentration (27 nmol/L) decreases coronary flow and has arrhythmogenic effects, probably related to its norpinephrine-releasing activity in the heart at high concentration. At a concentration of 0.22 nmol/L, as used in this study, Ang-(1-7) had no detectable direct myotropic effect on coronary vessels and presented a notable antiarrhythmogenic effect. Taken together, our results indicate that Ang-(1-7) has a biphasic effect on myocardial function being antiarrhythmogenic at a more physiological concentration. This observation is in keeping with several previous studies showing that Ang-(1-7) effects are not always proportional to the dose.

The antiarrhythmogenic effect of Ang-(1-7) appears to be mediated by a specific Ang-(1-7) receptor, because the specific Ang-(1-7) antagonist completely abolished its effects. More important, the Ang-(1-7) antagonist A-779 increased the incidence and duration of reperfusion arrhythmias, suggesting a protective role for endogenous Ang-(1-7) in the ischemia/reperfusion injury.

We have confirmed previous findings showing that blockade of BK-B2 receptors increases myocardial ischemia/reperfusion injury. In some circumstances, Ang-(1-7) has been show to exert its effects by release of BK through a still unknown mechanism. Ang-(1-7) potentiates BK in several preparations, including the isolated rat heart. However, in the present study no evidence was found for a kinin-mediated mechanism in the Ang-(1-7) antiarrhythmogenic effect.

Pretreatment with L-NAME did not significantly change the incidence and duration of reperfusion arrhythmias. Our data are in agreement with previous studies, showing that only using longer periods of ischemia (>35 minutes) L-NAME increases reperfusion arrhythmias. As observed with the BK-B2 antagonist HOE 140, L-NAME treatment did not influence the amelioration of cardiac arrhythmias produced by Ang-(1-7). However, we cannot discard the possibility that at least part of the antiarrhythmogenic effect of Ang-(1-7) was due to a differential effect on NO and O2 production as recently described in cultured bovine aortic endothelial cell. In addition, one may argue that the absence of L-NAME effect was because it was washed out from the
isolated heart. However, we have previously shown that using the same conditions implemented in the present study, L-NAME completely blocked the BK-potentiating activity of Ang-(1-7). The beneficial effects of prostaglandins release on the reperfusion arrhythmias appear to involve stimulation of EP3 receptors, which in turn can induce activation of repolarizing membrane currents and inhibition of damages effects caused by ischemia-induced catecholamine release.

In summary, in the present study we have shown that at a low concentration, Ang-(1-7) decreased the incidence and duration of ischemia-reperfusion arrhythmias in isolated rat hearts. These cardioprotective effects were blocked by the Ang-(1-7) antagonist A-779. Furthermore, our results suggest that release of endogenous prostaglandins contributes to the alleviation of reversible and/or irreversible ischemia-reperfusion injury by Ang-(1-7). However, we must carefully consider other possible mechanisms for the cardioprotective effects of Ang-(1-7), because, the mechanism responsible for ischemia and reperfusion injury are presently uncertain, that is, they have a multifactorial etiology.

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