Angiotensin II Type 1a Receptor Is Involved in the Occurrence of Reperfusion Arrhythmias

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Background—A growing body of evidence has suggested that the renin-angiotensin system plays an important role in the development of cardiac hypertrophy induced by hemodynamic overload and left ventricular remodeling after myocardial infarction. The role of the renin-angiotensin system in ischemia-reperfusion (IR) injury, however, has not been established.

Methods and Results—To determine the role of angiotensin II (Ang II) in IR injury, we examined infarct size and arrhythmias after IR using Ang II type 1a receptor (AT1a) knockout mice. The left coronary artery was occluded for 30 minutes followed by reperfusion for 120 minutes. There were no significant differences in infarct size between wild-type and knockout mice determined by dual staining with triphenyltetrazolium chloride and Evans blue dye. The number of ventricular premature beats after reperfusion in knockout mice, however, was much less than in wild-type mice. Treatment with a selective AT1 antagonist, CV-11974, before ischemia blocked reperfusion arrhythmias in wild-type mice but had no effects on infarct size.

Conclusions—Ang II may be critically involved in the induction of ventricular arrhythmias but not in the determination of infarct size after IR. (Circulation. 1998;97:315-317.)

Key Words: reperfusion • arrhythmia • angiotensin • myocardial infarction

O cclusion of coronary arteries induces myocardial necrosis. Although restoration of blood flow is the only way to save the myocardium from eventual necrosis, reperfusion often exacerbates myocardial damage. This IR injury includes expansion of the infarction area and the occurrence of life-threatening arrhythmias. Many studies have demonstrated that ACE inhibitors have beneficial effects on IR injury as well as on MI and congestive heart failure. These effects of ACE inhibitors have been attributed to both blockade of Ang II synthesis and a decrease in breakdown of bradykinin, which may stimulate the production of prostaglandin and nitric oxide.

Blockade of the renin-angiotensin system with AT1 antagonists has also been reported to have beneficial effects on MI and congestive heart failure. There are two major subtypes of Ang II receptors, AT1 and AT2, and AT1 receptors are further subdivided into AT1a and AT1b receptors. It is generally accepted that most of the well-known Ang II functions in the cardiovascular system are mediated through AT1. However, recent pharmacological studies have demonstrated that an AT1 antagonist, losartan, has no effect on infarct size after IR in vivo. In isolated heart, however, an AT1 antagonist, TCV 116, reduces release of creatine kinase after global ischemia. Thus, whether Ang II is involved in IR injury is still controversial.

We and others have recently generated AT1a KO mice by gene targeting. The blood pressure of AT1a KO mice was lower than that of WT mice, suggesting that AT1a-mediated Ang II signaling is essential for the maintenance of systemic blood pressure. Recent studies have suggested that AT1b may contribute to the regulation of blood pressure when AT1a is absent. In the present study, to determine the role of Ang II in IR injury, we examined infarct size and arrhythmias after IR in AT1a KO mice.

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Methods

IR Injury Model in Murine Hearts In Vivo

All protocols were approved by local institutional guidelines. Animals were assigned to three groups: AT1a KO mice 10 to 14 weeks of age (n=5), age-matched control (no drug) WT mice (n=7), and AT1 antagonist-treated (CV-11974; 0.1 mg/kg IV) WT mice (n=5) from the same genetic background were bred as previously described. IR was produced by transiently ligating the LCA as previously described. In brief, animals were anesthetized with sodium pentobarbital (50 mg/kg IP) and artificially ventilated. After opening the left chest, the heart was exposed and a reversible snare occluder, consisting of 8–0 nylon surgical suture and a polyethylene tube, was placed around the proximal LCA under ECG monitoring (surface ECG lead I or II). Animals underwent 30 minutes of LCA occlusion followed by 120 minutes of reperfusion. Body temperature was maintained between 35°C and 37°C by heating lamps. The agents were injected into...
the jugular vein 5 minutes before the onset of ischemia and had no effect on blood pressure or heart rate.

Assessment of AAR and Infarct Size After IR Injury
Infarct size was estimated as described previously. In brief, after reperfusion for 120 minutes, the LCA was reoccluded and 100 µL of Evans blue dye was injected into the LV cavity. The heart was excised immediately. The atria and right ventricular free wall were removed, and the LV was cut transversely into five sections. The AAR was the area not stained by the Evans blue dye. Sections of the ventricle were incubated in 1.5% TTC solution for 10 minutes. After TTC staining, viable myocardium was stained brick red; infarct regions were not stained by the TTC and were pale white. Each slice was then photographed with a charge-coupled device (CCD) camera and recording equipment (Atto Corp), weighed, and quantified by use of image analysis software (NIH Image; NIH, Research Service Branch). The fractions of both AAR to total slice size and infarct size to total slice size were calculated and multiplied by the weight of the slice to determine AAR and infarct weight per slice. Infarct size was expressed as a percentage of LV mass and of the AAR.

Electrocardiography During IR Injury
ECGs (lead I or II) were obtained by subcutaneously inserting needle electrodes into the limbs and were recorded for 1 minute during the control period before occlusion, for 1 minute at 15 and 30 minutes after occlusion, and for 120 minutes from the start of reperfusion. The duration of VT and the number of VPBs were analyzed.

Statistical Analyses
All results are expressed as mean±SEM. Multiple comparisons among three groups were carried out by two-way ANOVA and Fisher’s exact test for post hoc analyses.

Results
This study summarizes results from 17 mice (AT1a KO, n=5; control WT, n=7; and AT1 antagonist–treated WT, n=5). Two control WT mice were dead immediately after reperfusion owing to ventricular fibrillation. Fifteen of 17 mice survived the experiments, and 5 mice in each group were used for the following assessment.

Infarct Size After IR
Because we ligated the LCA at the most proximal portion, the coronary artery occlusion consistently created a large AAR. The size of MI in KO mice was almost identical to that in the other two groups. There were no significant differences in

infarct size/LV, AAR/LV, or infarct size/AAR ratios among the three animal groups (Fig 1).

Reperfusion Arrhythmias
VT was defined as a run of three or more consecutive, rapid VPBs that were morphologically similar to each other (Fig 2A). No VPBs were detected during ischemia, and almost all VPBs were observed within 10 minutes after reperfusion in the three animal groups. The number of VPBs was 27±8 in controls, 5±3 in drug-treated WT mice, and 4±2 in KO mice (Fig 2B). Nonsustained VT occurred in all five WT mice, with an average duration of 13±5 seconds, whereas there was no VT or ventricular fibrillation in either the five KO mice or the five AT1 antagonist–treated WT mice (Fig 2C).

Discussion
The cardioprotective efficacy of AT1 antagonists during IR injury has been controversial. In the present study, we tried to clarify the role of AT1 in IR injury using AT1a KO mice. Although there was no difference in infarct size between
control WT mice and KO mice, KO mice showed less postreperfusion ventricular arrhythmias than WT mice. In addition, treatment with an AT1 antagonist, CV-11974, also elicited preventive effects on reperfusion arrhythmias in WT mice. These results suggest that during IR, Ang II is involved in the induction of arrhythmias through AT1.

It has been reported that although ACE inhibitors reduced infarct size after IR, this beneficial effect was abolished by a bradykinin antagonist, Hoe 140. Direct AT1 stimulation by Ang II or AT1 blockade by losartan also did not alter the degree of infarct size in vivo context. All these results suggest that AT1 is not involved in the determination of infarct size after IR. There has been one report, however, that demonstrates that the AT1 antagonist TCV 116 decreases the release of creatine kinase in an isolated heart model after IR. Therefore, the role of AT1 in determining infarct size after IR has not been established. In the present study, there was no difference in infarct size among the three animal groups (Fig 1), strongly suggesting that AT1 is not involved in determining infarct size after IR.

Reperfusion-induced arrhythmias are postulated to be associated with major alterations in [Ca\(^{2+}\)] level. Recent studies have shown an association between an increase in [Ca\(^{2+}\)] and the induction of ventricular arrhythmias. In addition, it has also been reported that the calcium channel blocker verapamil can attenuate electrophysiological alterations and that ryanodine, an inhibitor of calcium release from the sarcoplasmic reticulum, can decrease the occurrence of reperfusion arrhythmias. These observations suggest that an increase in [Ca\(^{2+}\)], plays an important role in reperfusion arrhythmias. However, the underlying mechanism of calcium overload during reperfusion remains unknown. In the present study, we found that both genetic deletion of the AT1a gene and treatment with the AT1 antagonist CV-11974 significantly attenuated reperfusion arrhythmias. It is well known that Ang II not only increases Ca\(^{2+}\) influx through the L-type Ca\(^{2+}\) channel but also induces Ca\(^{2+}\) release from intracellular stores through AT1. These results suggest that Ang II may play a major role in calcium overload during reperfusion, which is closely related to the incidence of reperfusion arrhythmias. In addition, Ang II induces the release of catecholamines, which also may result in an increase of [Ca\(^{2+}\)]. ACE inhibitors have also been shown to exert beneficial effects on inhibition of arrhythmias as well as reduction of infarct size. Because bradykinin, which is increased with the treatment of ACE inhibitors, may release norepinephrine, blockade of AT1 may more completely suppress the release of catecholamines. Consistent with our findings, an AT1 antagonist, losartan, has been recently reported to attenuate ventricular tachyarrhythmias during reperfusion. Finally, there was no significant difference in the incidence of arrhythmias between AT1 antagonist–treated WT mice and KO mice. In addition, in spite of the highly activated renin-angiotensin system in AT1a KO mice, fewer arrhythmias were observed in KO mice than in control mice during reperfusion, suggesting that AT1b may not play a major role in the induction of reperfusion arrhythmias. Together with our findings that infarct size was comparable in three animal groups, the present results suggest that Ang II may be an independent endogenous inducer of arrhythmia in the heart during IR injury and that AT1 antagonists may be useful in preventing reperfusion arrhythmias.

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References

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