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
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

![Figure 1](image-url). Infarct size after IR. The LCA was occluded for 30 minutes followed by reperfusion for 120 minutes. There were no significant differences in infarct and ischemic risk areas among the three animal groups. Control indicates control WT mice; CV-11974, CV-11974–treated WT mice.

![Figure 2](image-url). A, Examples of ventricular arrhythmias in WT mice. Paper speed: 25 mm/s during sinus rhythm and episodes of VT and VPBs (arrowheads). B, The incidence of VPBs during 10 minutes of reperfusion. *P<.01 versus controls. C, Duration of VT (in seconds). **P<.01 versus AT1a KO mice and CV-11974. VF indicates ventricular fibrillation; Control, control WT mice; and CV-11974, CV-11974–treated WT mice.
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\(^{2+}\)]\(_i\) levels. Recent studies have shown an association between an increase in [Ca\(^{2+}\)]\(_i\)\(_i\) 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 ryano- dine, an inhibitor of calcium release from the sarcoplasmic reticulum, can decrease the occurrence of reperfusion arrhythmias. These observations suggest that an increase in [Ca\(^{2+}\)]\(_i\) 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 

![Image]

References


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