Thrombin Receptor and Ventricular Arrhythmias after Acute Myocardial Infarction

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The mechanism mediating the development of ventricular arrhythmia (VA) after acute myocardial infarction (AMI) is still uncertain. Thrombin receptor (TR) activation has been proven to be arrhythmogenic in many other situations, and we hypothesize that it may participate in the genesis of post-AMI VA. Using a left coronary artery ligation rat model of AMI, we found that a local injection of hirudin into the left ventricle (LV) significantly reduced the ratio of VA durations to infarction sizing, whereas injection of thrombin receptor–activating peptide (TRAP) increased the ratios of VA duration to infarction sizing. The effects of TR activation on whole-cell currents were investigated in isolated myocytes. TRAP increased a glibenclamide-sensitive outward current. Pretreatment of rats with glibenclamide (4 mg/kg intraperitoneally) eliminated the effects of a local injection of TRAP on the ratios of VA durations to infarction sizing. TR mRNA and protein expression in the ischemic left ventricle had reached its peak by 20 min postligation in the rat AMI model ($P < 0.05$). TR-immunoreactive myocytes were observed in infarcted LV but were seldom seen in the right ventricle or in the normal heart. By 60 min, TR transcript levels had returned to control levels. We conclude that increased TR activation and expression in the infarcted LV after AMI may contribute to VA through a mechanism involving glibenclamide-sensitive potassium channels.

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INTRODUCTION

Acute myocardial infarction (AMI) has been a major public health problem for decades. Nearly 1 million patients annually suffer from AMI in the U.S. (1). AMI mortality has declined significantly over the past decade with the application of new procedures such as hemodynamic monitoring, defibrillation, thrombolysis, and emergency percutaneous coronary intervention. Nevertheless, data from the National Registry of Myocardial Infarction and Cooperative Cardiovascular Project indicated that the mortality rate in AMI patients still approaches 20% (2). Because >50% of these deaths occur within 1 h of the event, most of these patients die before reaching a hospital, making it difficult to determine precisely the cause of death. Clinical experience and evidence from research with animal models of AMI in the rat, dog, cat, and pig suggested that the mechanism of death in these instances is likely to be arrhythmia, most often ventricular arrhythmia (3–5). Interestingly, continuous electrocardiogram (ECG) recordings after left coronary artery ligation in a rat model of AMI showed two distinctly active arrhythmogenic periods: one from immediately after ligation (time 0) to 30 min, another extending from 90 min to 9 h postligation. Each period was followed by a quiescent phase of low ectopy (6). The mechanism underlying these ventricular arrhythmias has not been totally resolved.

Thrombin has numerous cellular effects, including mediation of cell growth and development, in addition to its essential role in hemostasis. Thrombin mediates these effects via proteolytic activation of the thrombin receptor (TR) (7). Thrombin and its receptor contribute to coronary atherosclerosis and AMI initiation. The evidence also suggests that they may affect cardiac myocytes in addition to the vessels (8). In other situations such as in perfused isolated heart, TR activation by thrombin has been shown to be arrhythmogenic (9–12). The present study was designed to test the

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hypothesis that TR participates in the genesis of post-AMI ventricular arrhythmias and to examine TR expression and activation after AMI.

MATERIALS AND METHODS

Animal Model

All experimental protocols complied with the guidelines of the Animal Care and Use Committees of St. John Medical Center and Sun Yat-sen University. Charles River rats (male, 18 weeks) were used for all experiments. Each rat was anesthetized with sodium pentobarbital (20–30 mg/kg), intubated, and ventilated throughout the procedure. A left thoracotomy was performed to provide quick access to the heart by gentle pressure on the right side of the thorax. Thereafter, a suture was placed under the left coronary artery between the pulmonary artery outflow tract and the left atrium. If the ST-T in lead I was elevated upon tightening of the suture but returned to normal when the suture was relaxed, then the suture was tightened and tied. Upon satisfactory suturing, the animal model for AMI was considered successful. In sham AMI controls, the suture was left in place without tightening (3-6).

In Vivo Experimental Protocol for Arrhythmias

For the first set of in vivo experiments, rats were divided into four groups (seven rats per group): TRAP, hirudin, vehicle [0.011 g/mL trifluoroacetic acid [TFA]) for TRAP, vehicle (0.036% mannitol) for hirudin. TRAP (150 nM, 100 μL) and hirudin (4 AIU, 100 μL) were injected locally in the left ventricular area 1–2 min before the ligature around the left coronary artery was tightened. In control groups, the vehicle solutions (100 μL) for TRAP and hirudin were injected in the same ways as were active agents in TRAP and hirudin groups.

For the second group of in vivo experiments, rats were divided into three groups (five rats per group): vehicle for glibenclamide and vehicle for TRAP (Vg + Vt); glibenclamide and TRAP (Gli + TRAP); and glibenclamide and vehicle for TRAP (Gli + Vt). Each rat in the Gli + TRAP and Gli + Vt groups received an intraperitoneal injection of glibenclamide at a dose of 4 mg/kg; 10–15 min later a local injection of the TRAP (150 nM, 100 μL) or vehicle (100 μL) for TRAP solution was administered into the left ventricular area. The left coronary artery was occluded 1–2 min after the local injection. Meanwhile each rat in the Vg + Vt group received an intraperitoneal injection of vector for glibenclamide followed 10–15 min later by a local injection of the vector (100 μL) for TRAP into the left ventricular area. The left coronary artery was also occluded 1–2 min after the local injection in Vg + Vt animals.

Infarction Sizing

Rats were injected with 3% Evans blue (5 mL per rat) through tail vein 3–5 min before rats were euthanized. Thereafter, the hearts were hand-cut into nine to ten slices. Nonperfused zones were dissected from each slice and weighted. Infarction sizing were estimated from nonperfused zone and then expressed as the ratio of nonperfused tissue to total heart (weight).

Evaluation of Ventricular Arrhythmias

An ECG was continuously recorded throughout the experimental period with the use of three stainless steel electrodes such that one was attached to the right forelimb, one to the left upper forelimb, and one to the right leg. The ECG signal was sent to a Pentium II computer running Real-Time Linux for analysis. Arrhythmias were characterized according to the following features: (a) ventricular extrasystoles (VES), defined as single and couplet ventricular responses without a preceding P wave; (b) ventricular tachycardia (VT), defined as three or more consecutive, morphologically similar ventricular complexes without a preceding P wave; and (c) ventricular fibrillation (VF), rapid, abnormal activity of at least six or more ventricular responses without recurring amplitude or baseline voltage. The duration of each VES, VT, and VF episode was determined and assigned to one of the following postinjection intervals: 0–5, 5–20, or 20–30 min. Because the ventricular arrhythmias after AMI are closely positively related to the infarcted area after AMI, and the infarcted areas in each rat were different; therefore, the ratios of VA durations to infarction sizing, which we used for evaluation of VA, in different groups were comparable.

Chemicals and Solutions

Hirudin was purchased from Kekan (Naning, China). Glibenclamide, TRAP, and all other chemicals used in our experiments were purchased from Sigma (USA). Glibenclamide was dissolved in DMSO as a 10-mM stock solution and diluted in the working solution to produce a concentration of 0.011% to which cells were exposed. TRAP was dissolved in 0.1% TFA to form a 50-mM stock solution and diluted in working solution to produce a final solution with a concentration of 0.005% to which cells were exposed. Hirudin was dissolved in working solution to produce a concentration of 0.016% mannitol.

The buffer solution for the cell preparation above contained (in mM): NaCl 123; KCl 5.4; $NaHCO_3$ 5; NaH_2PO_4 2; $MgCl_2$ 1.6; glucose 10; taurine 20; and HEPES 20. The pH at 25°C was adjusted to 7.35 with 1 M NaOH. The high-K^+/low-Cl^- solution was (in mM): taurine 10; oxalic acid 10; glutamic acid 70; KCl 25; KH_2PO_4 10; glucose 11; EGTA 0.5; and HEPES-KOH buffer 10 (pH 7.3–7.4). The standard external control solution (extracellular bath solution in whole-cell patch-clamp technique) contained (in mM): NaCl 140; KCl 5; $MgCl_2$ 1; $CaCl_2$ 1; and HEPES 10 (pH 7.35–7.40 at 25°C adjusted with 1 M NaOH). The standard internal solution (intracellular pipette solution in whole-cell patch-clamp technique) contained (in mM): KCl 140, EGTA 5, HEPES 5, KOH 10, and $MgCl_2$ 1 (pH 7.2 adjusted with 1 M KOH) was supplemented by 0.1 mM Na_2ATP and 0.2 mM GTP lithium salt.

Cardiomyocyte Preparation

Single ventricular myocytes were enzymatically dissociated from 250- to 350-g male Wistar rats. Briefly, collagenase I (0.5 mg/L) in normally Ca^{2+}-free bathing solution was perfused through the coronary artery using a Langendorff apparatus for 25 to 30 min (37°C). The ventricles were then separated from the atria and gently dissociated by pipetting. Cells were allowed to precipitate in enzyme-free solution, and the supernatant was discarded. The cells were stored in a high-K^+/low-Cl^- solution at 4°C for later experiments. Rod-shaped relaxed ventricular myocytes with clear striations were used for experiments.

Whole-Cell Current Recordings and Data Analysis

The giga-Ω–seal patch-clamp technique was used in the whole-cell configurations. The currents were measured using a patch-clamp amplifier (Axopatch-1C; Axon Instuments Inc., Foster City, CA, USA). All data were recorded and analyzed by computer using Pclamp software (Axon Instruments). Electrodes were pulled to a pipette resistance of 2–5 MΩ. The electrode resistance (3–5 MΩ) in series to the cell membrane was compensated. Experiments were performed with broken patches in the whole cell configuration using the voltage clamp mode. Cells were clamped at –40 mV and switched at 1-s intervals through a series of voltage steps beginning at –40 mV and proceeding at 15-mV increments to 40 mV. The duration of each step was 0.3 s. (See Figures 3 and 4.) The duration of each step at –40, –25, –10, 5, 20, 35, 40 mV was 0.3 s; but after these voltages, the voltages came to zero and hold at zero for a while; the whole duration was 1 s.

Detection of TR mRNA Level

Total RNA was isolated from infarcted left ventricles at different time points in the experimental group and from the matching left ventricular areas at the same time points in the control group. cDNA was then synthesized from the total RNA samples. Thereafter, cDNA was amplified using primers based on the rat TR sequence. Rat TR primers (sense: 5′-CCTATGAGACAGCCA GAATC-3′; antisense: 5′-GCTTCT TGACC TTCATCC-3′) were chosen to amplify a 355-bp fragment from nucleotides 146–500 in M81642, Genbank (13); rat GAPDH primers (sense: 5′-CGGAG TCAACGGATTTGGTCGTAT-3′ and antisense, 5′-AGCCTTCTCCATGGTGGTGA AGA C-3′) were chosen to amplify a 305-bp fragment (14,15). Reverse-transcriptase polymerase chain reactions (RT-PCRs) were conducted under the following amplification conditions: denaturation for 5 min at 94°C, 35 cycles at 94°C for 1 min, 56°C for 1 min, 72°C for 2 min, and elongation at 72°C for 5 min. PCR products were analyzed using 1.5% agarose gels. The amplification fragments were confirmed by sequencing (16). Rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control. TR mRNA was normalized relative to the GAPDH mRNA in each sample.

Detection of TR by Immunohistochemistry and Western Blot

Briefly, thin paraffin sections (5 μm) were deparaffinized with xylene, immediately immersed in acetone, and washed in PBS (pH 7.4). The sections were blocked with 5% bovine serum albumin at 4°C for 60 min and then incubated with anti–PAR-1 primary antibody (Santa Crutz Biotech, Santa Crutz, CA, USA) for 60 min at 4°C. Immunolabeling was amplified with the avidin-biotin-peroxidase complex (ABC) method (Vectastin Kits; Vector Laboratories, Burlingame, CA, USA) and visualized by reaction with DAB. Phenol-soluble proteins from homogenized infarcted LV in different time points after the ligation of left coronary arteries were separated by SDS-PAGE and transferred to nitrocellulose membranes. The immunoreactive bands were detected by enhanced chemiluminescence (Amersham).

Statistical Analysis

The data were summarized as means ± SE and compared between experimental and control groups using Student t test. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of Hirudin on VA after Left Coronary Occlusion

In the first study, there were no deaths from VA in the hirudin and vehicle control groups. As shown in Figure 1, in AMI rats treated by local cardiac injection of hirudin (4 AIU), the ratio of VA durations to infarction sizing were significantly decreased (316.3 ± 41.3 vs. 39.7 ± 23.3, $n = 7$, $P < 0.05$) compared with the vehicle control group during the period between 5 and 20 min after coronary occlusion. This substantial decrease indicates that hirudin can reduce the durations of VA after AMI. The VA durations in all groups during the period between 0 and 5 min after coronary occlusion were very variable and did not show any relationship with the infarction sizing (data did not show); however, the variety of VA durations had been decreased 5 min later after coronary occlusion. In sham AMI control groups, no VA were found in local cardiac injection of hirudin and its vehicle.

Effect of TRAP on VA after Left Coronary Occlusion

In the second study, two of seven rats in the TRAP group died from VA, and no vehicle control rats died from VT or VF. As shown in Figure 2, in AMI rats injected intracardially with TRAP (150 μM, 100 μL), the ratios of VA durations/infarction sizing were significantly increased compared with the vehicle-only group during the period between 0 and 5 min (309 ± 28.2, $n = 5$, vs. 117.8 ± 45.6, $n = 7$; $P < 0.05$) and 5 and 20 min (603 ± 36.8, $n = 5$, vs. 330.2 ± 38.1, $n = 7$; $P < 0.05$) after coronary occlusion. These results indicate that TRAP can increase the duration of ventricular arrhythmias after

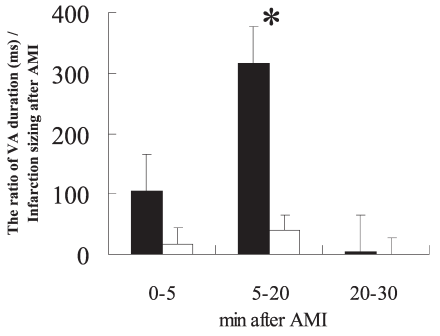

Figure 1. Local injection of hirudin (5 AIU) decreases the ratio of VA durations (ms)/ infarction sizing. The ratio of VA durations/ infarction sizing after AMI was significantly shorter in hirudin-treated rats during the 5- to 20-min time periods after coronary artery occlusion. *$P < 0.05$ compared with vehicle controls.

AMI. Interestingly, in sham AMI control group, VA durations (ms) were 0.778 ± 0.027, 3.22 ± 1.92, and 0.0272 ± 0.027 ($n = 5$) 0–5, 5–20, and 20–30 min, respectively, after local injection of TRAP, but no VA were found in local injection of vehicle group.

Effects of TRAP on Whole-Cell Membrane Currents of Cardiac Ventricular Myocytes

The typical effects of TRAP treatment on ventricular myocyte membrane currents at various membrane potentials are presented in Figure 3. Command voltage steps (300-ms duration) were applied from –140 to 40 mV every 3 s. Basal potassium currents were elicited in the absence of TRAP (Figure 3A). Decreased inward currents were observed at the holding potential and during depolarizing pulses 3 min after TRAP application to the bath (Figure 3B). However, at 6 min, prominent inward currents were recorded (Figure 3C). Interestingly, prominent outward and inward currents were observed 9 min after TRAP application (Figure 3D), and the induced outward currents were almost completely suppressed by glibenclamide (1 μM) (Figure 3E). Thereafter, TRAP-induced currents could be washed out by applying drug-free bath solution. The cells remained responsive to TRAP after washout, though with a slower onset and a slight decrease in the current amplitude. Similar results were obtained in five independent experiments.

Figure 3 (F and G) shows current-voltage (I-V) relationships of the average Ik density values from five experiments. Mean current density (–9.45 ± 1.0 $pApF_{-1}$ vs. –13.5 ± 1.28 $pApF_{-1}$) was significantly suppressed at –140 mV 3 min after TRAP was added to the bath solution ($n = 5$, $P < 0.05$ compared with baseline). Mean inward current density recorded at –140 mv was increased by about 2- and 2.4-fold at 6 min (26.43 ± 1.34 $pApF_{-1}$) and 9 min (32.10 ± 2.16 $pApF_{-1}$), respectively, after 150 μM TRAP was added to the bath solution (compared with baseline 13.5 ± 1.28 $pApF_{-1}$, $n = 5$, $P < 0.05$); outward current density did not significantly change until 9 min after the addition of TRAP. Mean outward current density was significantly increased during depolarizing voltage steps to potentials positive to –65 mV 9 min after TRAP was added to the bath solution (at 40 mV: 42.2 ± 2.66 $pApF_{-1}$ compared with 2.3 ± 0.37 $pApF_{-1}$ [baseline], $n = 5$, $P < 0.05$). These outward currents were blocked by 1 μM glibenclamide (at 40 mV: 2.22 ± 0.73 $pApF_{-1}$ vs. 2.3 ± 0.37 $pApF_{-1}$ [baseline], $n = 5$, $P < 0.05$), indicating that glibenclamide-sensitive K_{ATP} channels were mainly responsible for the TRAP-induced prominent outward currents. The mean inward current density greatly diminished in the presence of 0.2 mM Ba^{2+} (at –140 mV: 2.05 ± 0.612 $pApF_{-1}$ vs. 13.5 ± 0.88 $pApF_{-1}$ [baseline], $n = 5$, $P > 0.05$) (Figure 4A, B, C, D), indicating that Ba^{2+}-sensitive inward rectifying potassium channels were mainly responsible for the TRAP-induced inward current changes. The typical whole-cell current responses to TRAP described here (Figure 3) are representative of approximately one-half of the cells in each rat heart in our experiments. About one-third of cells in each heart demonstrated only increased inward TRAP-responsive currents (Figure 3C). A few cells demonstrated only decreased inward currents in response to TRAP (as shown in Figure 3B).

Effect of Glibenclamide on TRAP-Increased Ratio of VA Durations/ Infarction Sizing after Left Coronary Occlusion

As summarized in Figure 5, pretreatment with glibenclamide (4 mg/kg intraperitoneally) reduced the ratio of VA durations to infarction sizing at 0–5 min and 5–20 min after AMI. The ratio of VA durations to infarction sizing of subjects treated with TRAP (150 μmol/L) after the glibenclamide pretreatment (Gli + TRAP: 17.3 ± 2.9 [0–5 min], 45.4 ± 6.8 [5–20 min], and 19 ± 3.2 [20–30 min]) did not differ from that of subjects given only vehicle after the glibenclamide pretreatment (Gli + Vt: 21.7 ± 4.7 [0–5 min], 38.8 ± 12.7 [5–20 min], and 12.9 ± 3.0 [20–30 min]; $P > 0.05$, $n = 5$ per group). Meanwhile, both the Gli + Vt and the Gli + TRAP groups exhibited reduced ratios of VA duration/infarction sizing relative to the no drug control groups (Vg + Vt: 182.3 ± 40.5 [0–5 min], 375.2 ± 35.5 [5–20 min], and 20.16 ± 1.6 [20–30 min], all $P < .05$). These results indicate that glibenclamide-sensitive K_{ATP} channels are responsible for the ventricular arrhythmias that follow AMI as well as the

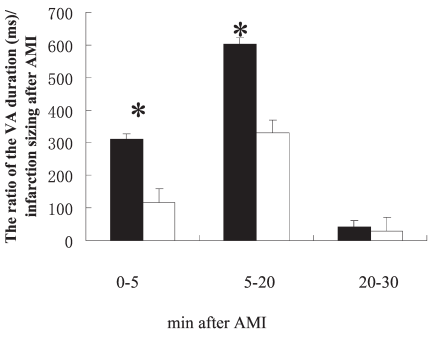

Figure 2. Local injection of TRAP (150 μmol/L) increases the ratio of VA durations (ms)/ infarction sizing. The ratio of VA durations/ infarction sizing after AMI was longer in TRAP-treated rats during the 0- to 5-min and 5- to 20-min time periods after coronary artery occlusion. *$P < 0.05$ vs. vehicle controls.

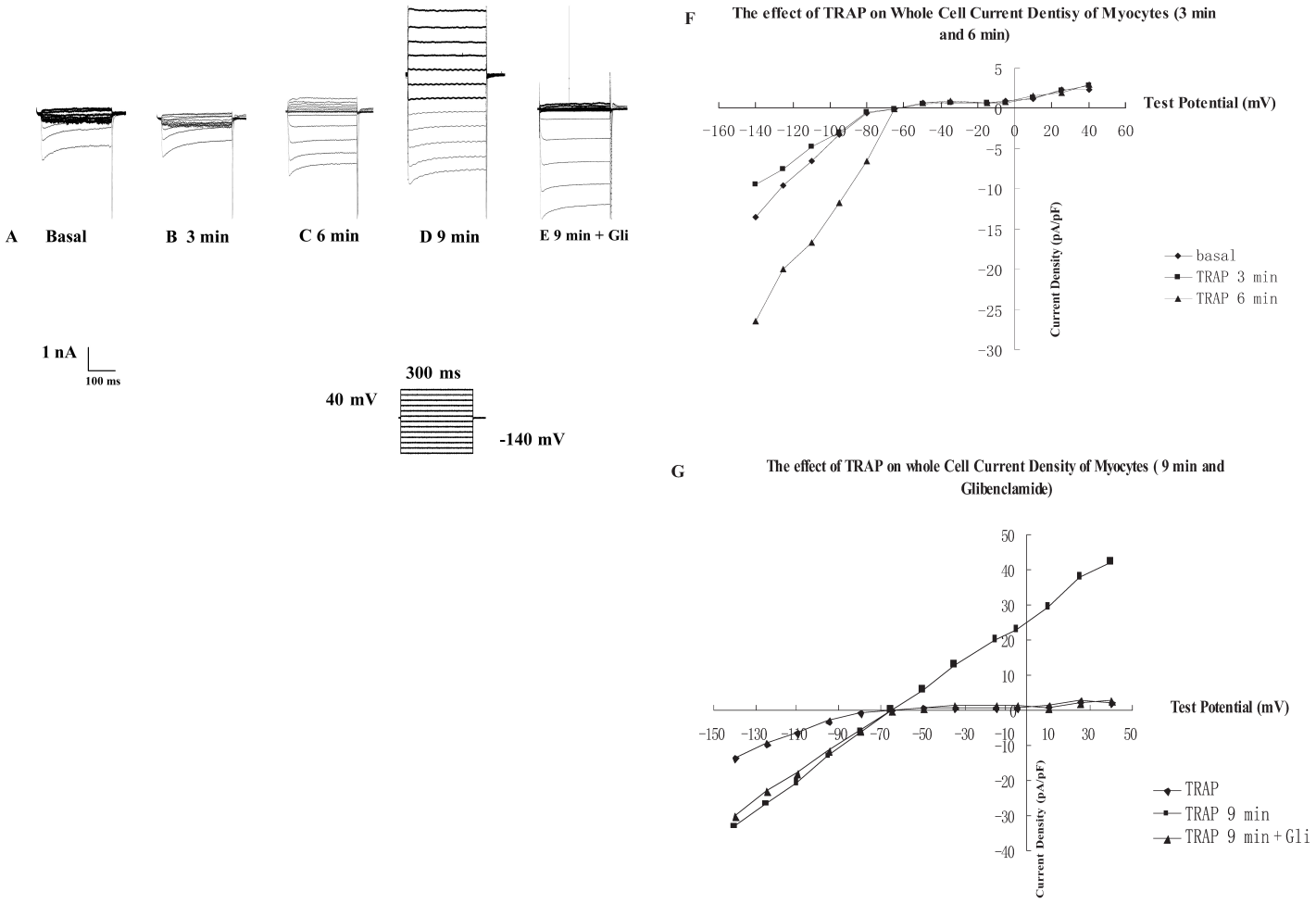

Figure 3. Effect of TRAP on whole-cell transmembrane currents in cardiac ventricular myocytes. Cells were suspended in bath solution and then sealed to the tip of a patch electrode. After break-in by suction, voltage was clamped at –40 mV and stepped up in 15-mV increments from –140 to 40 mV. The current that flowed across the plasma membrane in response to the applied voltage steps was then recorded. (A) One representative cell from the approximately one-half of cells in each heart that responded to TRAP. After baseline current was measured, currents were developed at 3, 6, and 9 min after perfusion with bath solution containing TRAP (150 μmol/L) and thereafter glibenclamide (1–2 min after 1 μmol/L glibenclamide was added). (B) I-V curves from five experiments at basal, 3 and 6 min after perfusion with bath solution containing TRAP. (C) I-V curves from five experiments at basal and 9 min after perfusion with bath solution containing TRAP and thereafter glibenclamide (TRAP 9 min + Gli) was added in the bath solution.

ventricular arrhythmias induced by TRAP after AMI.

TR Changes after Acute Myocardial Infarction

RT-PCR analysis revealed that TR mRNA levels from infarcted left ventricles gradually increased after ligation of left coronary arteries and reached a peak of 2.02 ± 0.35 times baseline levels (n = 4, P < 0.05) at 20 min after the infarction; thereafter TR mRNA returned to near-normal levels by 60 min after AMI (Figure 6A, B); Western blot analysis revealed that the same changes occurred in protein level. TR from infarcted left ventricles reached a peak of 3.01 ± 0.17 times baseline levels (n = 5, P < 0.05) at 20 min after infarction. Likewise, immunohistochemistry showed that TR expression of some cardiac myocytes in the infarcted area increased within 30 min and then returned to near-normal levels by 60 min after AMI (Figure 7A–G). In normal hearts, TR was found in some endothelial cells of capillaries and arterioles but seldom, if ever, in cardiac myocytes. In the infarcted hearts, TR immunoreactivity was observed in some cardiac myocytes in almost all areas of infarcted left ventricles, often near the surface 20 min after AMI. However, TR expression was seldom found in the right ventricle when the left coronary arteries were ligated. RT-PCR showed that TR was restored to a normal level by 60 min after AMI, and TR remained in a few cardiac myocytes of the infarcted left ventricles. Interestingly, TR most

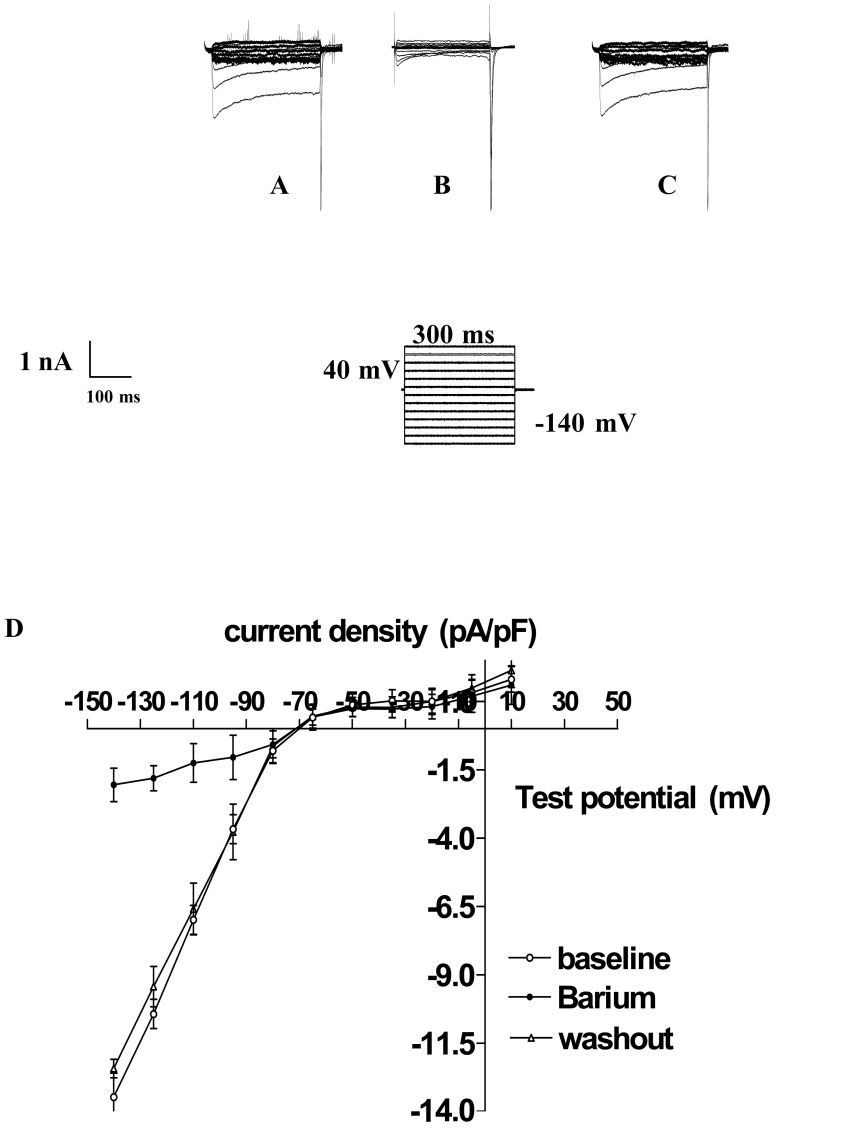

Figure 4. Effect of $BaCl_2$ on the whole-cell transmembrane currents in ventricular cardiac myocytes. After measuring baseline currents (A), the cell was perfused with bath solution containing $BaCl_2$ (0.2 mmol/L) (B), and then with Ba^{2+}-free bath solution (C). (D) I-V curve from five experiments.

often, but not always, appeared in cardiac myocytes near capillaries and arterioles in the infarcted ventricles.

DISCUSSION

Our initial studies demonstrated that the thrombin antagonist hirudin decreased the ratios of VA durations to infarction sizing and that TRAP treatment increased the ratios of VA durations to infarction sizing after AMI. In sham AMI control, TRAP caused some VA but the durations of VA were transient and very variable. Thereafter, we found that TRAP could alter glibenclamide-sensitive K_{ATP} and barium-sensitive inward rectifying potassium currents. TRAP-induced VA was associated with glibenclamide-sensitive potassium channels. Finally, we found TR expression after AMI in some cardiac myocytes, especially the myocytes near the capillaries and arterioles of the infarcted areas. The post-AMI increases in TR expression increased within 30 min of infarction and returned to near-baseline levels by 60 min post-AMI. Our work provides evidence to understand the mechanism of ventricular arrhythmia after AMI and supports the use of hirudin in AMI not only for thrombolytic therapy but also for ventricular arrhythmia.

Our results directly implicated TR in the genesis of VA after AMI: TRAP can increase the ratios of VA durations to infarction sizing, whereas thrombin antagonist, hirudin, could decrease the ratios of VA durations to infarction sizing after AMI. Interestingly, the VA durations in all control groups during the period 0–5 min after AMI were very variable and did not show any relationship to the infarction sizing. However, the VA durations during the period 5–20 min after AMI in all control groups were positively related to infarction sizing. VA durations were significantly decreased during the period 20–30 min after AMI in all groups. This phenomenon may indicate that the VA during early stage in this model is related to the mechanical stimulation of surgery and unstable cellular electrical activities in the initial ischemic stage of AMI. As a result, we divided the 30 min after AMI into three stages, so we could see the real effect of the interventional factors on VA during the period 5–20 min after AMI. In addition, in sham AMI control group, TRAP did not significantly increase the VA durations. This phenomenon may be because TRAP locally injected in sham AMI group stayed too briefly and then was washed out by blood. Finally, we did not use the incidence of VA in our work. The incidences of VES, VT, and VF cannot be readily compared between groups because the shapes and widths of the QRS complexes varied between individual rats and there were no measurable QRS waves in VF.

TR activation has been shown to be the basis of arrhythmias in situations other than AMI. For example, TR activation can increase 1,4,5-inositol triphosphate and calcium mobilization in cardiomy-

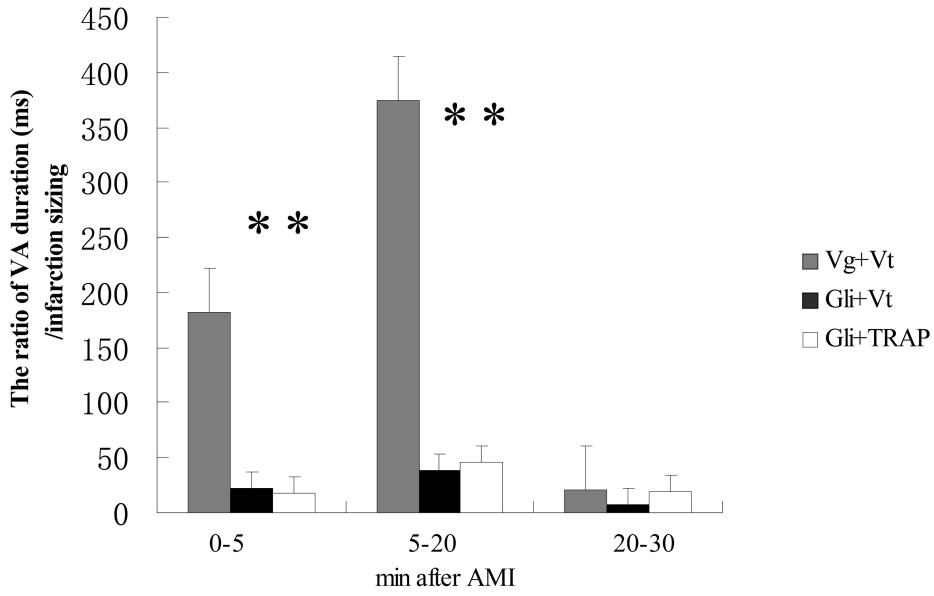

Figure 5. Effect of glibenclamide (4 mg/kg) on TRAP (150 μmol/L)-induced ratio of VA duration (ms)/infarction sizing during 30 min of coronary artery occlusion in rats. *Statistical significance compared with control (vehicles) ($P < 0.05$) for ratio of VA duration/infarction sizing at 0- to 5-min and 5- to 20-min time periods. $n = 5$ per group. Vg, vehicle for glibenclamide; Vt, vehicle for TRAP; Gli, glibenclamide.

ocytes (13,16). Generation of inositol 1,4,5-triphosphate can produce ventricular arrhythmias in the perfused isolated heart (17–19). TR activation may have an arrhythmogenic action during myocardial reperfusion via the release of inositol 1,4,5-triphosphate (9). Clinical evidence indirectly suggests that TR activation may underlie malignant ventricular arrhythmia after AMI. Acute ischemia induced by nonthrombotic balloon occlusion, which rarely induces thrombus formation, rarely elicits acute malignant ventricular arrhythmias; in contrast, acute ischemia induced by thrombotic coronary occlusion results in a greater incidence of malignant ventricular arrhythmias both in humans and in experimental animals (20,21). Moreover, administration of conjunctive antithrombin reagents with fibrinolytic therapy can decrease mortality in the very early stages of AMI more than fibrinolytic therapy alone (22,23). This is not surprising given that fibrinolytic drugs can expose clot-bound thrombin, which becomes activated and produces more thrombin during fibrinolytic therapy. This thrombin can activate its receptors and cause ectopic rhythms, which may be responsible for the mortality in the early stages of AMI. Accordingly, conjunctive antithrombin therapy may play a beneficial anti-arrhythmic role in addition to preventing further coagulation. In contrast, when spasm and thrombosis occur simultaneously in many patients with acute coronary syndromes, the treatment directed against vasospasm usually relieves pain but does not reduce the incidence of sudden death (24,25).

In addition to showing that TR activation is responsible for the arrhythmia after AMI, our study has also demonstrated that the mechanism involved in development of post-AMI arrhythmia involves the opening of glibenclamide-sensitive cardiac K_{ATP} channels. Our finding that only half of the myocytes had increased glibenclamide-sensitive outward current densities is not problematic for our interpretation because the beating rate of cardiac myocytes in the intact heart always follows the rate of the fastest beating cells. It is well established that activation of K_{ATP} channels during cardiac ischemia is an important adaptive mechanism, protecting the heart against stunning and infarction, as well as participating in preconditioning protection (26,27). On the other hand, there are disputes about the role of K_{ATP} in cardiac arrhythmias during an acute ischemic insult. Most of the evidence supports a scenario whereby opening of myocardial K_{ATP} channels by hypoxia and ischemia subsequently increases potassium efflux, which leads to a shortening of the action potential duration, a depolarization of the membrane by extracellular potassium accumulation, and inhomogeneities in repolarization; this scenario creates a substrate for reentry (28–31). These potentially arrhythmogenic mechanisms are implicated in the genesis of ischemic arrhythmias. Many Vaughan Williams class I anti-arrhythmic drugs inhibit K_{ATP} channels in cardiac muscles (32–34). However, some investigators did not find effects of K_{ATP} channel blockers, such as glibenclamide and 5-hydroxydecanoate, on the incidence of arrhythmia after ischemic insult (35–37). These controversial observations might be due to different experimental conditions or protocols, but it cannot be excluded that the effects of glibenclamide on mitochondrial channels differ in different animal models or conditions.

Our finding that TR activation increased Ba^{2+}-sensitive potassium currents in only about one-third of the cells in each heart, and decreased currents in some cells, might be a result of differing physiological status of the cardiac myocytes or different anatomic positions of the cells in the hearts. Different cellular responses to TR activation have been observed previously (38). The inward rectifying K current is crucial in stabilizing the resting membrane potential (E_m) of cardiac myocytes. Reduced inward rectifying K current has been shown to allow $I_{Na/Ca}$ to produce greater depolarization, which alters threshold and propensity for triggered arrhythmia in cardiac myocytes (39). In contrast, increased inward

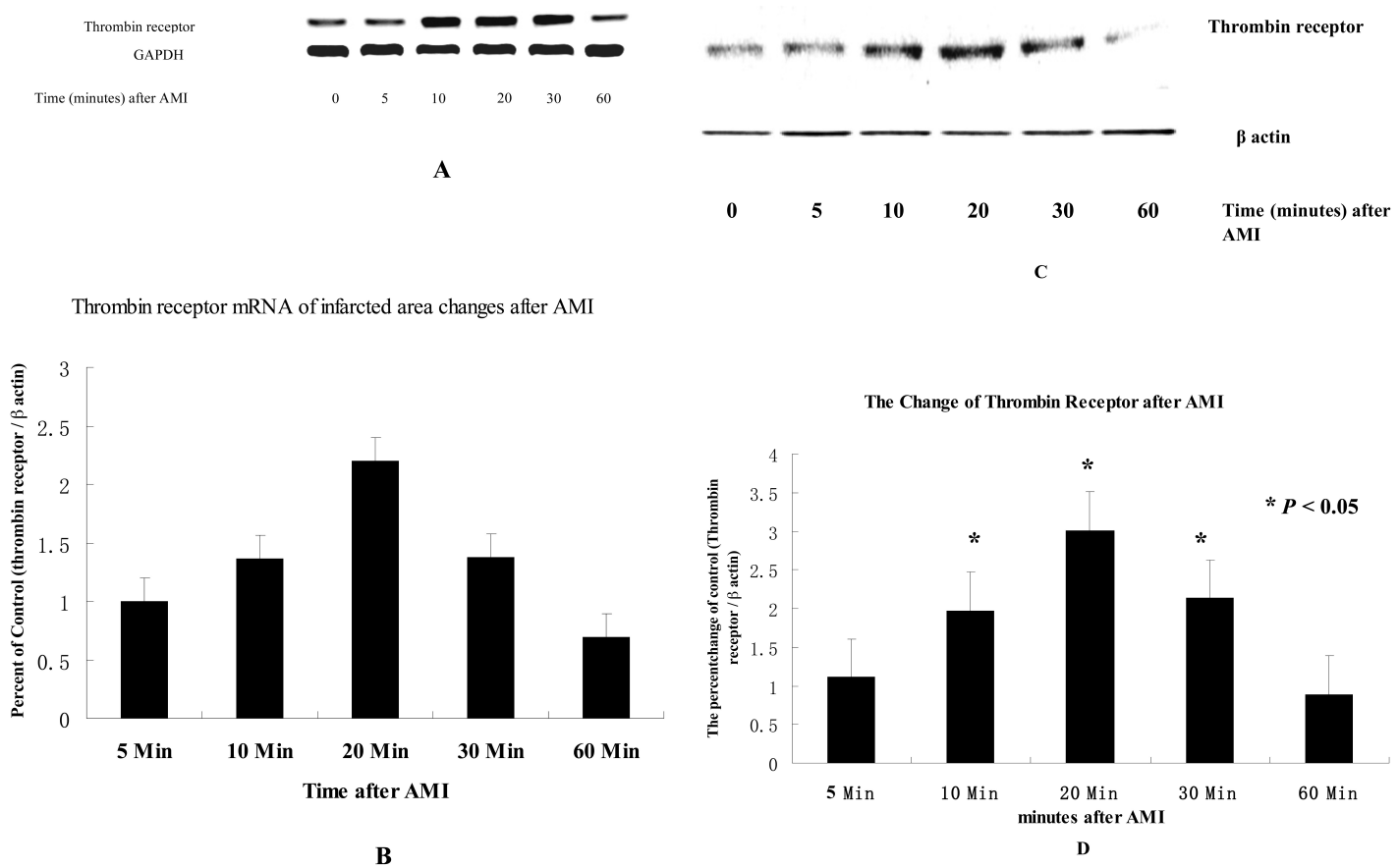

Figure 6. (A) Representative RT-PCR analysis of TR in the infarcted left ventricles at different time points after ligation of left coronary arteries. (B) Percent of control of the ratio of thrombin receptor intensity to GAPDH intensity in RT-PCR (n = 4). (C) Representative Western blot analysis of TR in the infarcted left ventricles at different time points after ligation of left coronary arteries. (D) Percent of control of the ratio of thrombin receptor intensity to β-actin intensity in Western blot (n = 5).

rectifying K current can stabilize the resting E_m of cardiac myocytes and reduce the excitability of the cells (40). Thus the literature includes mixed results and the role of TR activation on inward rectifying K current after AMI remains to be resolved.

The most important cause of ischemic damage to cardiomyocytes during AMI is hypoxia. Although hypoxia causes downregulation of TR gene expression in cardiomyocytes (41), the pathophysiological processes occurring in the first few hours of AMI are more complicated than simple hypoxia, involving thrombin generation, thrombus formation, hypoglycemic stress, and neurohumoral factor stimulation. Thrombin can induce TR gene expression in endothelial cells (42,43). Moreover, in hypoxic cardiomyocytes, thrombin stimulation can overcome the downregulation of TR gene expression induced by hypoxia (41). Our observation that most cardiac myocytes with TR expression were located near capillaries and arterioles is consistent with the possibility that these cells were stimulated by extracellular factors, for example, thrombin, carried in blood vessels. TR expression is very important for cardiomyocytes. Thrombin-induced receptor cleavage leads to thrombin receptor endocytosis and degradation in lysosomes; as a result, the activated thrombin receptors are unable to recycle (44,45). The replenishment of the consumed thrombin receptors on cardiomyocytes is the key to the continuous effect of thrombin. However, the increased expression of TR after AMI does not mean the receptor activation. Although we did use TRAP to activate the TR, we will investigate binding assays for TR after AMI in further work.

In summary, the present study demonstrated that hirudin treatment decreased the ratio of VA durations to infarction sizing and TRAP-induced ventricular arrhythmias after AMI. TRAP induced changes in Ba^{2+}-sensitive inward rectifying currents and glibenclamide-sensitive outward K_{ATP} currents. TRAP-induced ventricular arrhythmia could be eliminated by pre-AMI injection of glibenclamide. TR expression was increased in some cardiac myocytes in the acutely

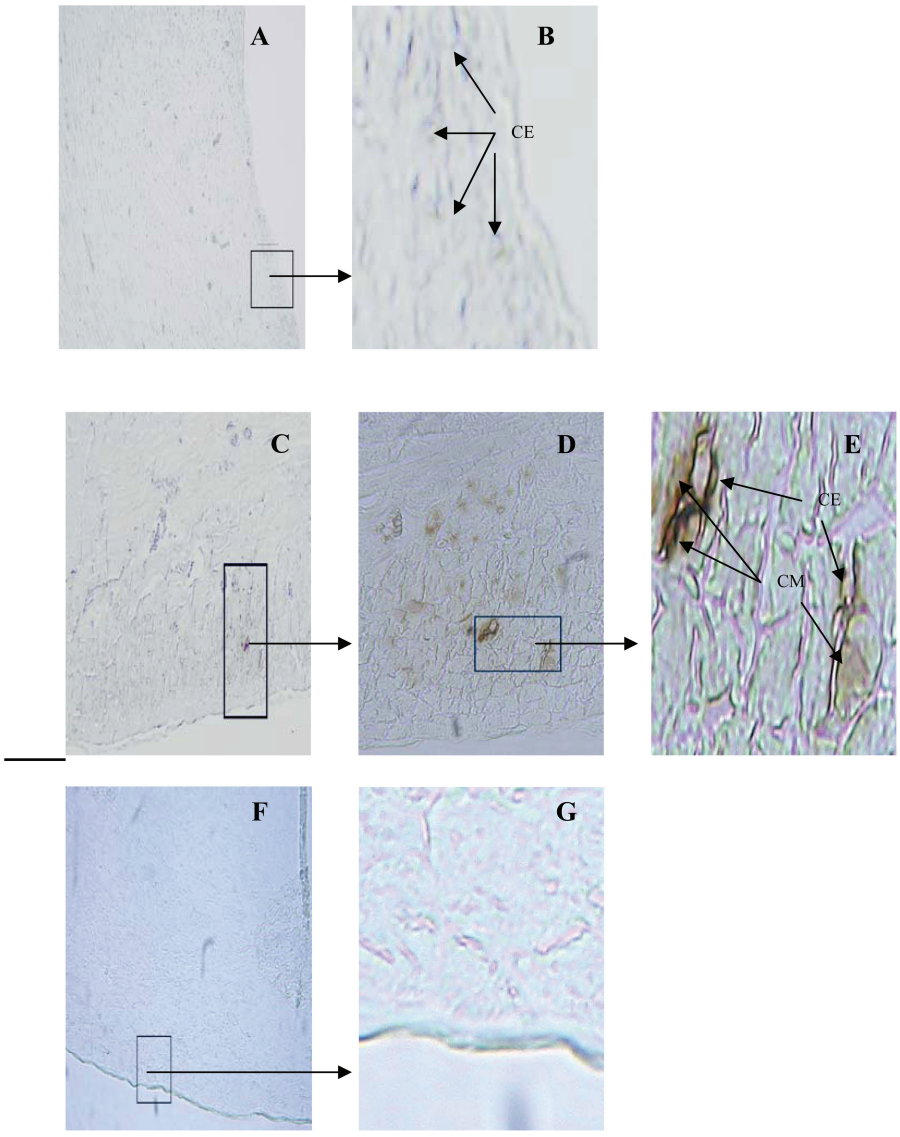

Figure 7. Representative immunostaining for TR in infarcted left ventricles at different time points. (A) Control left ventricle (LV). (B) An enlarged rectangular area (indicated in A) from control LV. Some capillary endothelial cells (CE) were very weakly positive. (C) Left ventricle at 20 min after ligation of left coronary artery. (D) An enlarged rectangular area indicated in C. (E) An enlarged rectangular area indicated in D. CE showed a very strongly positive response and cardiac myocytes (CM) around CE also had strong positive signals. (F) Left ventricle at 60 min after ligation of left coronary artery. (G) An enlarged rectangular area indicated in F.

infarcted LV. Our work focused on rats; however, other species such as humans may have different electrophysiological bases. For instance, guinea pigs have different action potential from rats, so further clinical evidences should be more carefully observed.

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