

Thrombin and Its Receptor Enhance ST-Segment Elevation in Acute Myocardial Infarction by Activating the K_{ATP} Channel

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ST-segment elevation is the major clinical criterion for committing patients with chest pain to have emergent coronary revascularizations; however, the mechanism responsible for ST-segment elevation in acute myocardial infarction (AMI), local application of hirudin, a thrombin antagonist, significantly decreased AMI-induced ST-segment elevation in a dose-dependent manner. Hirudin-induced (5 anti-thrombin units (ATU)) decrease in ST elevation was reversed by 250 nmol/L thrombin receptor activator peptide (TRAP). TRAP (250 nmol/L (100 μ L)) significantly induced ST-segment elevation in hearts without AMI. The TRAP effect was blocked by 4 mg/kg glibenclamide and 4 mg/kg HMR1098 and partially blocked by 3 mg/kg 5HD. Pinacidil (0.45 mg/kg) simulated the effect of TRAP (250 nmol/L (100 μ L)) on hearts without AMI. Moreover, single-channel recordings showed that TRAP induced ATP-sensitive K^+ channel (K_{ATP} channel) activity, and this effect was blocked by HMR1098 but not 5HD. Finally, TRAP significantly shortened the monophasic action potential (MAP) at 90% repolarization (MAP₉₀) and epicardial MAP (EpiMAP) duration. These effects of TRAP were completely reversed by HMR1098 and partially reversed by 5HD. Thrombin and its receptor activation enhanced ST-segment elevation in an AMI model by activating the sarcolemmal K_{ATP} channel.

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INTRODUCTION

ST-segment elevation is a typical hallmark to diagnose AMI and is the major clinical criterion for committing patients with chest pain to emergent coronary revascularizations. In spite of this knowledge, the mechanism of ST-segment elevation remains unclear. For decades, two mechanistic theories, the “injury current” theory and the “transmural potential gradient” theory, have aimed to explain ST-segment elevation. However, neither theory can explain how the transmural voltage gradient that leads to ST-segment elevation is generated during ventricular repolarization or explain its ionic basis. Evidence indicates that the potassium

channel may be critical for this process. For example, potassium current activation is sufficient to cause ST elevation during acute ischemia (1). Activation of the ATP-sensitive K^+ channel (K_{ATP} channel) owing to hypoxia is generally hypothesized to result in ST-segment elevation in AMI (2). Additionally, Kir6.2 was shown to be responsible for ST-segment elevation in Kir6.2-knockout mice (3).

Thrombin, a serine protease, is generated at sites of vascular injury and is a key molecule in the pathogenesis of AMI because it is involved in thrombus formation. The generation of thrombin during AMI is a long-lasting process, which extends beyond the acute phase of myo-

cardial infarction (4). Thrombin binds to its receptor and cleaves it in the NH₂-terminal portion. The exposed new NH₂-terminus has been proposed to function as a “tethered peptide ligand” binding to this receptor in hirudinlike regions to cause receptor activation. Hirudin could combine the hirudinlike region of the receptor to compete with tethered peptide ligand binding to this region. Thrombin receptor activating peptide (TRAP), the synthetic peptide of 5-14 amino acids, correspond to the tethered ligand sequence. TRAPs have been found to be agonists for receptor activation (5,6). Thrombin and the thrombin receptor have been shown to play important roles in diverse biological processes, such as platelet aggregation, inflammation and cell proliferation, in a wide variety of tissues (7). Because ST-segment elevation is the major criterion for thrombolytic therapy, we aimed to examine the role of thrombin and its receptor activation in ST-segment elevation during AMI.

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MATERIALS AND METHODS

Animal Model

All experimental protocols complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and the regulations of the Animal Care and Use Committees at Sun Yat-sen University. Albino male guinea pigs (Experimental Animal Center, Guangdong, China) weighing 350–400 g were used in our experiments. The animal AMI model was created as described (8). Briefly, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (40–50 mg/kg). The guinea pigs were then restrained in a supine position, and a tracheotomy was performed. The animals were ventilated with oxygen-enriched room air (90 breaths/min, 5 mL/kg). Electrocardiogram (ECG) limb electrodes were positioned to record in the standard lead II configuration, and each needle electrode was placed under the skin. A left thoracotomy was performed, and then the proximal left coronary artery (LCA) between the pulmonary artery outflow tract and the left atrium was occluded with a silk suture. Myocardial ischemia was confirmed by the presence of regional cyanosis and ST-segment elevation in the ECG and was further confirmed by Evans blue perfusion after every experiment. Only the data from animals with infarct indices between 15% and 25% were analyzed. In the sham control animals, a silk suture was placed under the LCA without ligation. The ECG was recorded at least 5 min after opening the chest for acclimatization and immediately after the LCA occlusion to allow continuous monitoring of changes in the ST segment during ischemia. In some experiments, reagents with restricted volumes of 100 μ L were injected into the midmyocardial wall of infarcted or uninfarcted left ventricles. ST-segment elevation in animal models can be variable. For instance, ST-segment elevation can be caused by a vector change

that results from ischemia, a mechanical alteration of the heart geometry or other factors. Therefore, we analyzed only stable ST segment changes recorded after surgery.

Infarction Sizing

At the end of each experiment, 1.5 mL of 1% Evans blue was slowly injected into the right jugular vein to delineate the myocardial nonperfused zone. The left ventricle was cooled at -20°C for 30 min and cut into 5–10 transverse slices with a custom-made tool composed of five parallel razor blades. The slices were weighed, and both sides of each slice were digitally photographed using a high-resolution digital camera. The size of the nonperfused zones and normal zones were outlined in each section (Figure 1A) (9) and measured by planimetry using Adobe Photoshop CS 8.0.1. Areas were quantified on both sides of each slice and averaged by an investigator blinded to the treatment protocols. The weight of the nonperfused zone (WNZ) in every slice was calculated using the following formula: WNZ in each slice = (nonperfused area in every slice/total area of each slice) \times weight of each slice. The total weight of the nonperfused zone was: $\text{WNZ} = (\text{WN}_1 + \text{WN}_2 \dots + \text{WN}_{10})$, and the infarct index was calculated as follows: $\text{infarct index} = 100 \times \text{WNZ}/\text{weight of both ventricles}$.

Ventricular Myocyte Preparation and Patch-Clamp Experiments

A single ventricular myocyte was enzymatically dissociated (online Data Supplement). Single-channel current recordings were carried out at room temperature using the conventional cell-attached patch configuration, where the patch pipette was filled with the pipette solution and the bath contained Tyrode's solution. An Axon 200B patch-clamp amplifier was used to record channel current. The current was low-pass filtered at 0.2–0.5 kHz and digitized with an Axon interface (DigiData 1320). Data were collected by

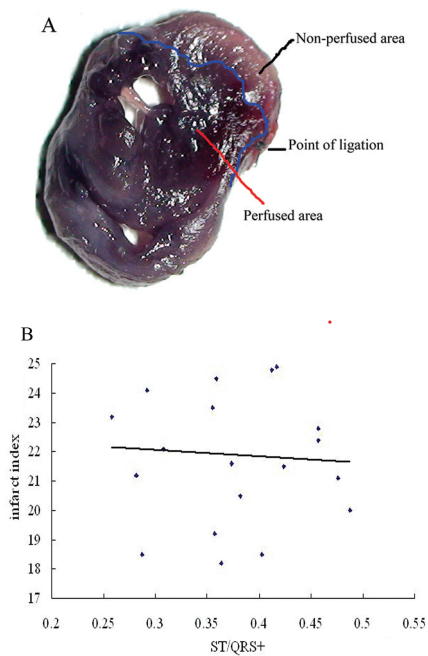


Figure 1. (A) Planimetry of myocardial infarct size. Digital photographs of a mid-ventricular slice after Evans blue staining. Nonperfused zones are clearly identifiable from the normal zones. (B) There was no correlation between the ratios of ST/QRS⁺ and the infarct index ($r = -0.065$, $n = 20$).

an Acer-compatible Pentium computer at a rate of 2 kHz and analyzed using pClamp software system 9.2 (Axon Instruments, Burlingame, CA, USA). Channel activity (NP_o) was calculated by multiplying the channel open probability (P_o) and channel number (N). NP_o was calculated from 30- to 60-sec data samples of 30~60 sec duration recorded at the end of each experiment in the steady state as follows:

$$NP_o = \leq (1t_1 + 2t_2 + \dots it_i)$$

where t_i is the fractional open time spent in each of the observed current levels. An all-point histogram (pClamp 9.2) was used to measure channel activity after determining the closed channel level. No efforts were made to determine whether the change was induced by alterations in channel number or P_o . The channel slope conductance was determined by measur-

ing the K⁺ current at different holding potentials.

Action Potential Recordings in Langendorff-Perfused Hearts

Isolated guinea pig hearts were perfused as described (10). Briefly, guinea pigs were anesthetized with sodium pentobarbital (40–50 mg/kg) and given 200 IU heparin. The heart was quickly excised and submerged in ice-cold Krebs-Henseleit solution and equilibrated with 95% O₂/5% CO₂ (pH 7.4, 37°C). For endocardial recordings, a small access window was created in the interventricular septum to gain access to the left ventricular endocardium. Each heart was perfused retrogradely using the Langendorff method and was provided a constant flow rate of 15 mL/min modified Krebs-Henseleit solution before the right ventricular free wall was partly removed and a small access window in the septum was created. Thereafter, the perfused heart was stabilized and monophasic action potentials (MAPs) were recorded.

The MAP recording protocols used were described by Killeen *et al.* (11). Briefly, two custom-made MAP electrodes were constructed from two spring-shaped, twisted strands of Teflon-coated silver wire (1-mm diameter, 99.99% purity). The electrodes were positioned onto the left ventricular free wall under a stable contact pressure until MAP signals were reproducibly recorded on the BL-420E⁺ Biological Function Analysis System (Taimeng, Chengdu, China). MAPs were amplified, band-pass filtered and digitized before they were extracted and analyzed. Recordings were considered reproducible if they had a stable baseline, a rapid upstroke phase with a consistent amplitude, a smooth contoured repolarization phase and a stable duration.

MAPs were simultaneously recorded from epicardial (EpiMAP) and endocardial (EndoMAP) sites in the center of left ventricular free walls (10). A transmural ECG was recorded with two silver chloride electrodes (2-mm internal diameter

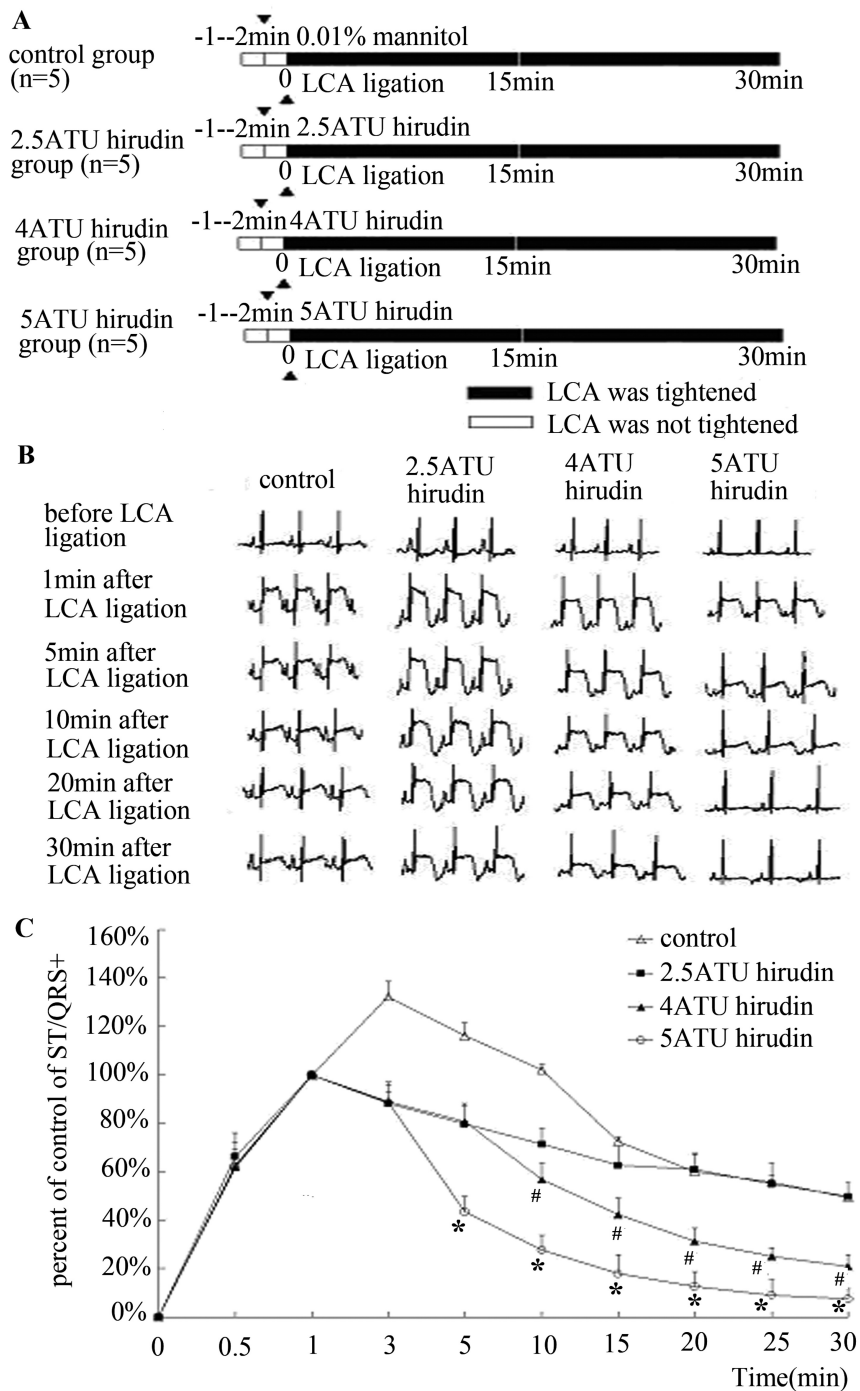


Figure 2. (A) Experimental protocol. The guinea pigs were divided into four groups: 2.5 ATU hirudin (100 μL), 4 ATU hirudin (100 μL), 5 ATU hirudin (100 μL), and vehicle (0.01% mannitol) for hirudin were locally injected in the left ventricular area 1–2 mm below the LCA ligation. The different doses of hirudin were injected 1–2 min before the LCA ligation. (B) Representative electrocardiographic recordings of different hirudin dosages at different time points after LCA ligation. (C) Summary percent of control of the ST/QRS⁺ at different time points for each group. Data are mean ± SEM from five animals per group. *P < 0.05, 5 ATU hirudin group versus 2.5 ATU hirudin group, 4 ATU hirudin group, and control group. #P < 0.05, 4 ATU hirudin group versus 2.5 ATU hirudin group and control group.

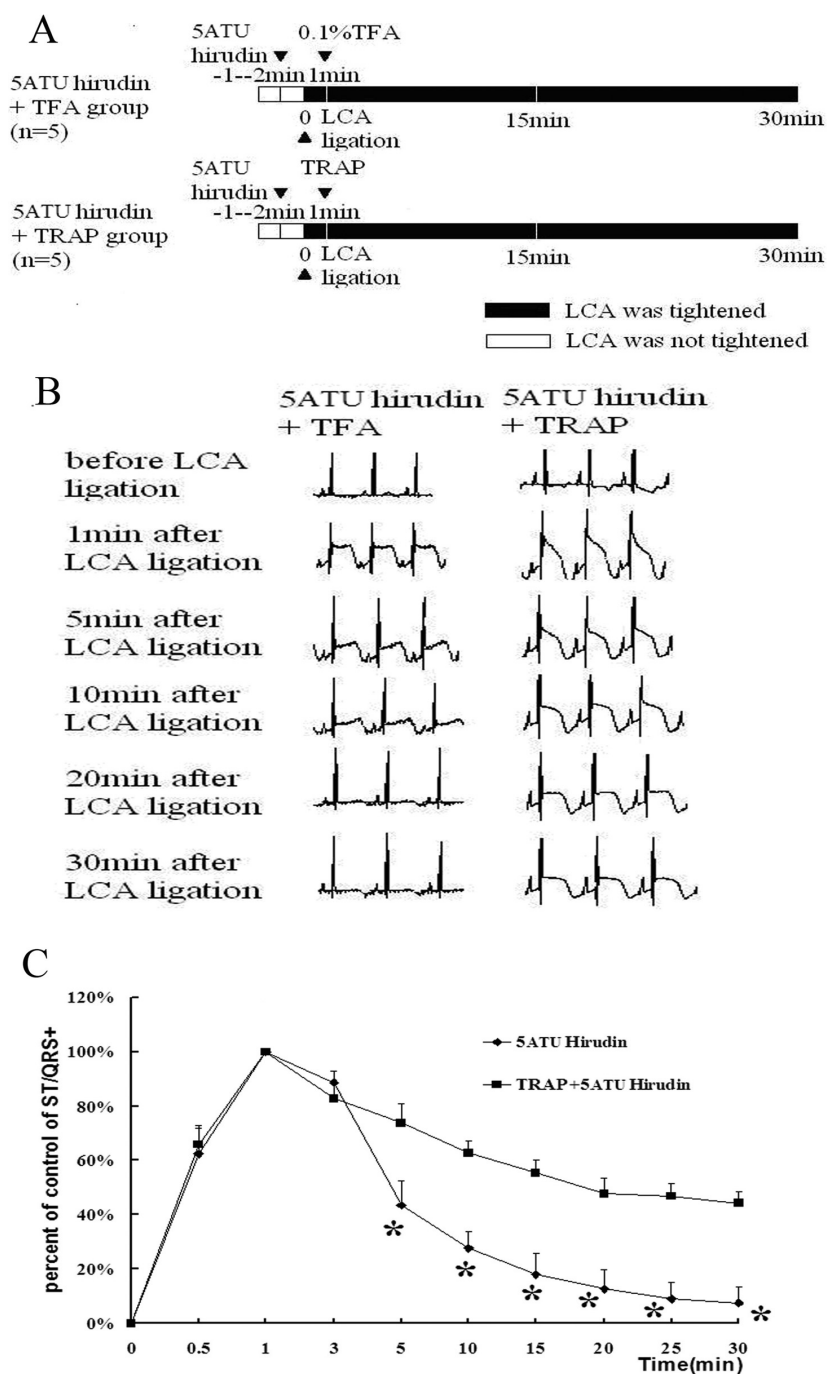


Figure 3. (A) Experimental protocol. Guinea pigs were divided into two groups: 5 ATU hirudin + TFA and 5 ATU hirudin + TRAP. In the 5 ATU hirudin + TRAP group, 5 ATU hirudin was locally injected in the left ventricular wall 1–2 mm below ligation of LCA 1–2 min before the LCA ligation. Thereafter, TRAP (250 nmol/L (100 μ L)) or TFA was locally injected in the same area 1–2 min after the LCA ligation. (B) Representative electrocardiographic recordings of locally injected TRAP or TFA at different time points after LCA ligation. (C) Summary percent of control of the ST/QRSt⁺ at different time points in five cases from each group. Data are mean \pm SEM from five animals per group. * $P < 0.05$ versus 5 ATU hirudin group.

with fishhook-shaped tip) placed at the epicardial (+) and endocardial (–) surfaces of the left ventricular free wall along approximately the same axis as the transmural recordings. The duration of the MAP was measured at 90% repolarization (MAP₉₀).

Statistical Analysis

All values are presented as means \pm SEM. Pearson correlation analysis was used to evaluate the relationship between variables. Differences were evaluated using analysis of variance followed by the Student *t* test (paired or unpaired). Differences were considered to be statistically significant at $P < 0.05$.

All supplementary materials are available online at www.molmed.org.

RESULTS

Animal Model for ST-Segment Elevation

Studies were conducted on an animal model of ST-segment elevation after LCA ligation. As shown in Figure 1A, the nonperfused zones were clearly identified. ST-segment elevation following AMI gradually returned to baseline in a time-dependent manner. ST-segment elevation had no relationship to the infarct index (Figure 1B). In addition, thrombin activity in the infarcted left ventricular wall significantly increased 5 min after LCA ligation in the model (Figure, online Data Supplement).

Effect of Hirudin and TRAP on the ST Segment after AMI

To determine the influence of endogenous thrombin on ST-segment elevation after AMI, different doses of hirudin (2.5, 4 and 5 ATU), an antagonist to thrombin, were locally injected into the infarcted left ventricular walls (Figures 2A, 3A). The ratios of ST-segment elevation to positive QRS amplitude (ST/QRSt⁺) were compared at different time points. As shown in Figure 2B, C, hirudin significantly de-

creased ST/QRS⁺ in a dose-dependent manner. The infarct index in the control (22.6% ± 2.15%), 2.5 ATU hirudin (21.5% ± 1.88%), 4 ATU hirudin (18.9% ± 2.01%) and 5 ATU hirudin (18.6% ± 2.33%) groups showed no significant differences.

To further examine the effect of the thrombin on the ST segment after AMI, the thrombin receptor was activated with a local injection of exogenous TRAP (250 nmol/L [100 μL]) into the infarcted left ventricular as shown in Figure 3A. Interestingly, as shown in Figure 3B, C, TRAP (250 nmol/L [100 μL]) can significantly reverse the effect of 5 ATU hirudin, which decreased ST/QRS⁺. The infarct index in the 5 ATU hirudin group (18.6% ± 2.33%) and 5 ATU hirudin + TRAP group (19.5% ± 2.33%) showed no significant differences.

Effect of the K_{ATP} Channel on TRAP-Induced ST-Segment Elevation

Studies have shown that local activation of the K_{ATP} channel due to depletion of intracellular ATP in infarcted areas of AMI subjects leads to ST-segment elevation in ECG recordings after AMI (12,13). We hypothesized that the effect of thrombin observed in our study was due to the K_{ATP} channel. To eliminate the interference of ATP depletion inducing K_{ATP} channel activation in the AMI model and TRAP-induced vessel constriction, we designed an experiment that directly locally injected TRAP into uninfarcted left ventricles. As described in the experimental protocol (Figure 4A), our data showed that TRAP (250 nmol/L [100 μL]) significantly increased ST/QRS⁺ in normal left ventricular walls. Glibenclamide (4 mg/kg [100 μL]) and HMR1098 (4 mg/kg [100 μL]) nearly blocked the increase in ST/QRS⁺ induced by TRAP. 5HD (3 mg/kg [100 μL]) significantly decreased the TRAP-induced change in ST/QRS⁺ (Figure 4B, C). To further exclude the effect of ATP depletion on TRAP-induced vessel constriction, we used the K_{ATP} channel opener, pinacidil,

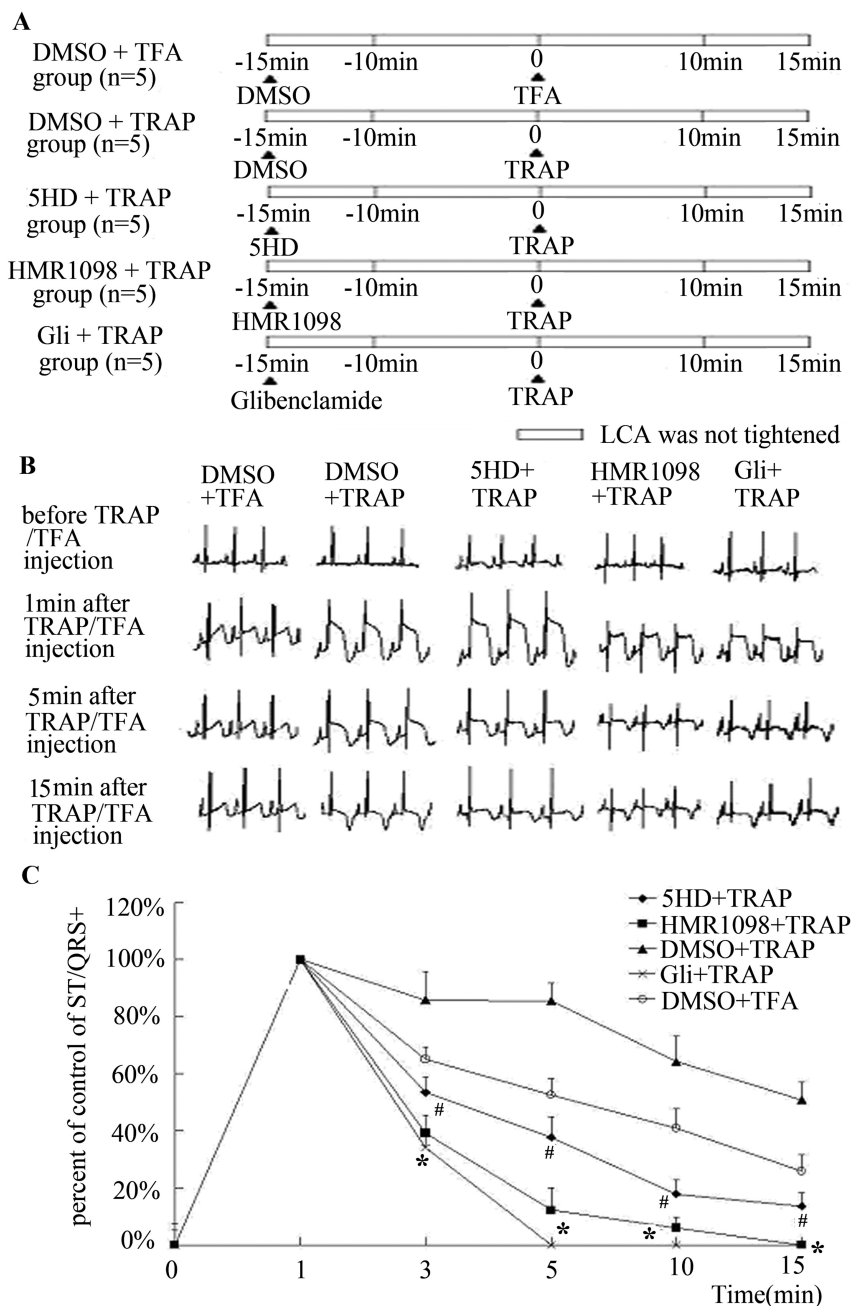


Figure 4. (A) Experimental protocol in guinea pigs that did not undergo LCA ligation. The animals were divided into five groups: 5HD and TRAP, HMR1098 and TRAP, glibenclamide (Gli) and TRAP; vehicle for glibenclamide (DMSO) and TRAP, and vehicle for glibenclamide (0.011% DMSO) and vehicle (0.1% TFA) for TRAP. Each animal received an intravenous injection in the right jugular vein of 5HD (3 mg/kg [100 μL]), HMR1098 (4 mg/kg [100 μL]), glibenclamide (4 mg/kg [100 μL]) or 0.011% DMSO (100 μL), according to their respective groupings 14–15 min before TRAP (250 nmol/L [100 μL]) or TFA (100 μL) was locally injected into the left ventricular area. (B) Representative electrocardiographic recordings. (C) Summary percent of control of ST/QRS⁺ at different time points for each group. Data are mean ± SEM from five animals per group. *P < 0.05, Gli + TRAP group versus 5HD + TRAP group, DMSO + TFA group, and DMSO + TRAP group. #P < 0.05, HMR1098 + TRAP group versus 5HD + TRAP group.

to mimic TRAP in the same model (Figure 5A). Interestingly, pinacidil had an effect similar to that of TRAP (Figure 5B, C). As shown in Figure 5, administration of pinacidil activated K_{ATP} channels in the uninfarcted hearts, which significantly increased ST/QRS⁺ in the same manner that ischemia increases ST/QRS⁺. PBS, the vehicle for pinacidil, did not show any effect on ST/QRS⁺. The increase in ST/QRS⁺ by pinacidil was nearly completely blocked by 4 mg/kg glibenclamide (100 μ L) and was significantly decreased by 4 mg/kg HMR1098 (100 μ L). 5HD (3 mg/kg [100 μ L]) partially decreased the pinacidil-induced change in ST/QRS⁺.

Effect of TRAP on K_{ATP} Channels

To further clarify the effect of TRAP on cardiac myocytes, we used single-channel patch-clamp electrophysiology to observe the effect of TRAP on K_{ATP} channels. After getting current trace from cell-attached patch, we pulled the pipette away from the cell and kept the voltage at 0 mV, then got the inside-out trace. As shown in Figure 6A1, A2, typical K_{ATP} channel traces were recorded from cell-attached patches. These currents were inhibited by glibenclamide (10 μ M), indicating that the channels were K_{ATP} channels. Next, we investigated the effect of TRAP on K_{ATP} channel activity. TRAP (100 μ M) significantly increased NPo from 0.33 ± 0.083 to 0.88 ± 0.094 ($n = 7$, $P < 0.01$) (Figure 6B1, B3). This effect was mimicked by using pinacidil (Figure 6B2, B3). To determine whether the mitochondrial K_{ATP} (mito K_{ATP}) or sarcolemmal K_{ATP} (sarc K_{ATP}) channels were involved in the effect of TRAP, we tested the effects of HMR1098 and 5HD on TRAP-induced K_{ATP} channel activities. As shown in Figure 6C, TRAP (100 μ M) and HMR1098 (100 μ M) significantly decreased NPo from 0.65 ± 0.10 to 0.42 ± 0.13 ($n = 6$, $P < 0.01$). Treatment with 5HD (10 μ M) did not significantly alter NPo (0.33 ± 0.08 to 0.25 ± 0.04 , $n = 6$, $P = 0.133698$).

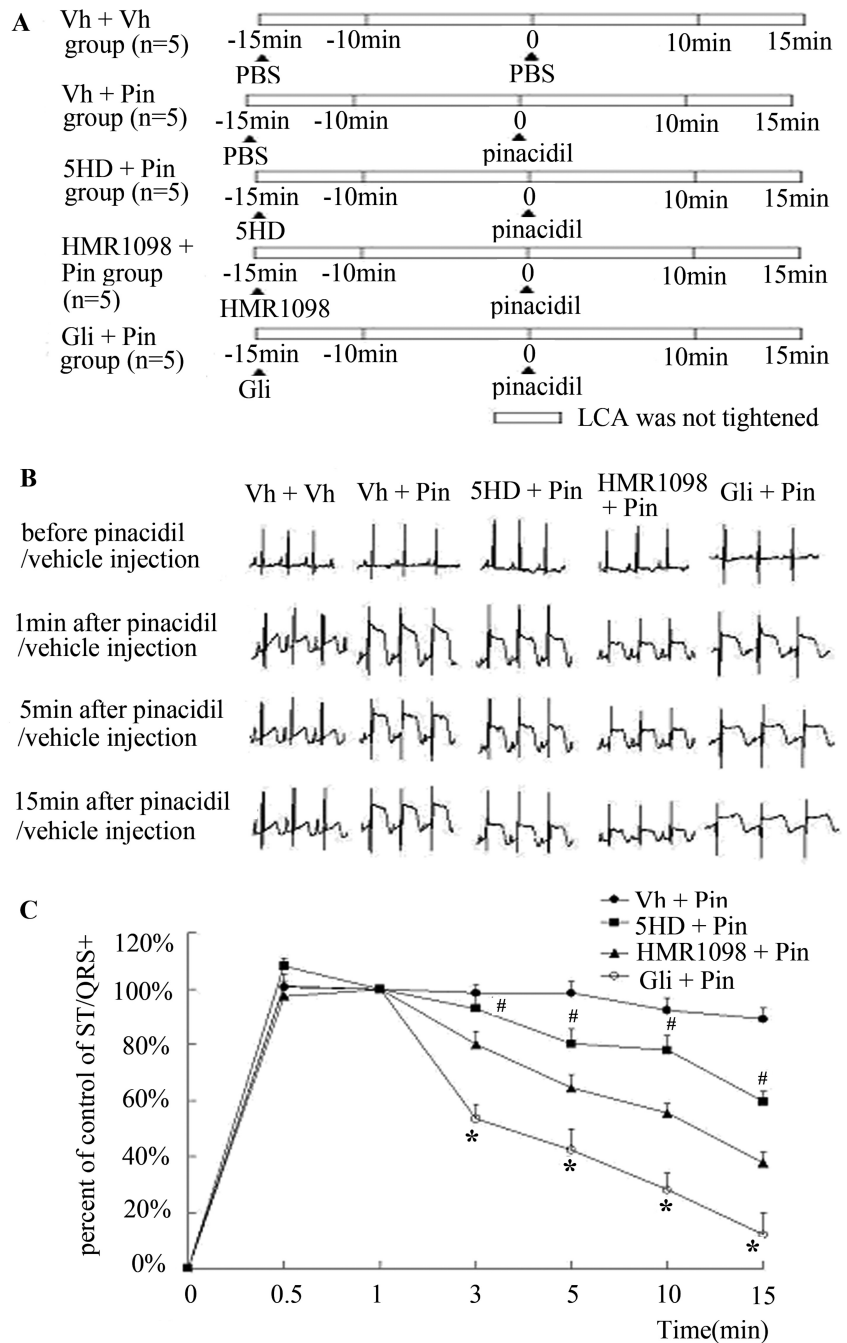


Figure 5. (A) Experimental protocol. All animals were divided into five groups, and the LCA was not tightened: vehicle (Vh (PBS)) for 5HD or HMR1098 and pinacidil (Pin), 5HD and Pin, HMR1098 and Pin, vehicle (PBS) for 5HD or HMR1098 and vehicle (PBS) for pinacidil (Vh and Vh), and glibenclamide (Gli) and Pin. The guinea pig was intravenously injected in the right jugular vein with PBS (100 μ L), 5-HD (3 mg/kg (100 μ L)), HMR1098 (4 mg/kg (100 μ L)) or glibenclamide (4 mg/kg (100 μ L)), according to their respective grouping 14–15 min before pinacidil (0.45 mg/kg (100 μ L)) or PBS (100 μ L) was locally injected into the left ventricular area. (B) Representative electrocardiographic recordings. (C) Summary percent of control of ST/QRS⁺ at different time points for each group. Data are mean \pm SEM from five animals per group. * $P < 0.05$, Gli + Pin group versus 5HD + Pin group, PBS + PBS group, and PBS + Pin group. # $P < 0.05$, 5HD + Pin group versus HMR1098 + Pin group.

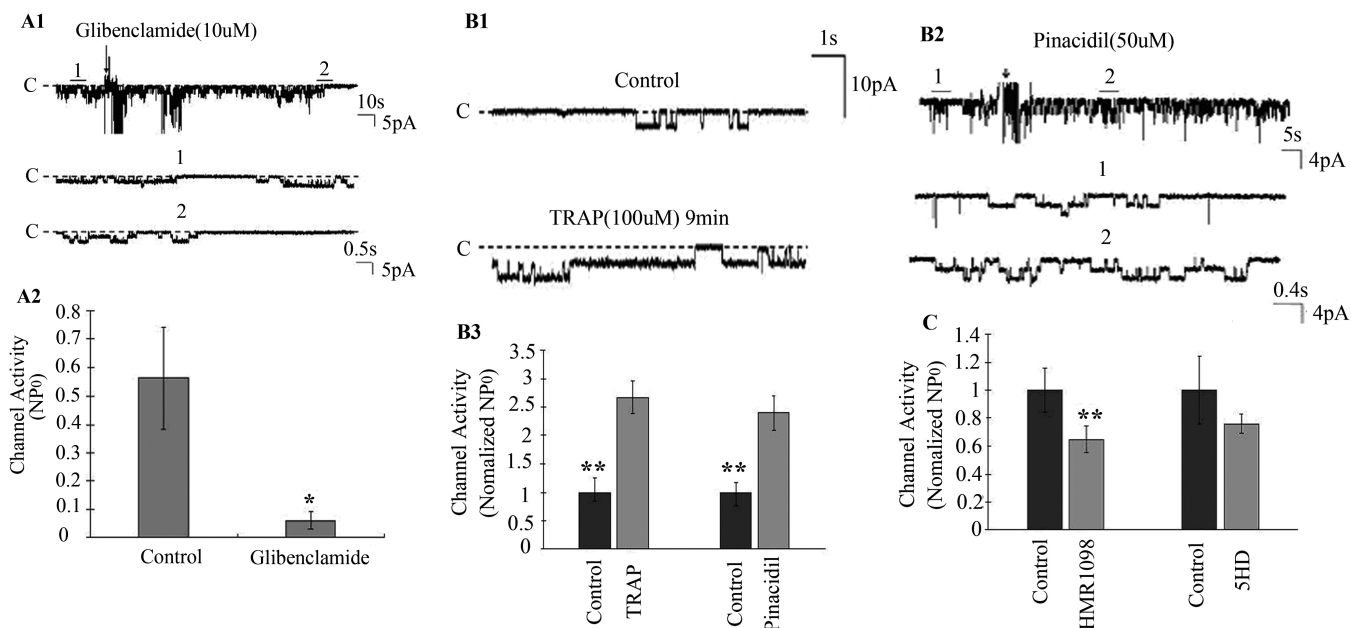


Figure 6. Effects of treatments on K_{ATP} currents. For all the traces, the holding potential was 0 mV, and the closed channel current is indicated by C. (A1) Inhibition of K_{ATP} channel activity by 10 μ M glibenclamide. The top trace shows the experimental time course. Two parts of the trace, indicated by numbers, are extended to show the fast time resolution. (A2) Bar graph showing the mean and SEM of NPo . Glibenclamide (100 μ M) significantly decreased NPo from 0.56 ± 0.18 to 0.06 ± 0.03 ($n = 5$, $*P < 0.05$). (B1) Effect of TRAP (100 μ M) on the K_{ATP} channel in the ventricular myocytes. (B2) Effect of pinacidil (50 μ M) on the K_{ATP} channel in the ventricular myocytes. (B3) Bar graph showing the normalized NPo with control. TRAP (100 μ M) significantly increased channel activity to $266.7 \pm 26.6\%$ ($n = 7$, $*P < 0.01$), and pinacidil (50 μ M) significantly increased channel activity to $239.1 \pm 30.4\%$ ($n = 6$, $**P < 0.01$). (C) Bar graph showing the normalized NPo with control. In the presence of 100 μ M TRAP, HMR1098 (100 μ M) significantly decreased channel activity to $64.6 \pm 10.0\%$ ($n = 6$, $**P < 0.01$), and 5HD (10 μ M) nonsignificantly decreased channel activity to $75.8 \pm 17.1\%$ ($n = 5$, $P > 0.05$).

ST-Segment Elevation in ECG, EpiMAP and EndoMAP in Left Ventricle Walls

To further clarify the electrophysiological mechanism by which TRAP increased ST/QRS⁺, we tested the effect of TRAP on EpiMAP and EndoMAP. The experimental protocols are shown in Figure 7A. The results, shown in Table 1, demonstrate that 200 nmol/L TRAP significantly decreased MAP₉₀ in the epicardial walls (153 ± 4 versus 135 ± 3 , $n = 5$, $P < 0.05$) but not in the endocardial walls (154 ± 2 versus 156 ± 4 , $n = 5$, $P > 0.05$) of left ventricles. In addition, as shown in Figure 7B1, the shortened EpiMAP duration underlies the apparent ST-segment elevation in ECG, which was simultaneously recorded with their MAPs. TFA (0.1%), the vehicle for TRAP, did not show such an effect when administered alone (Figure 7B2 and Table 1). HMR1098 appeared to be

able to reverse the TRAP-induced EpiMAP changes. After perfusion with HMR1098, TRAP-induced EpiMAP duration shortening and TRAP-induced ST-segment elevation in simultaneously recorded ECGs almost disappeared (Figure 7C and Table 1). The MAP₉₀ in the epicardial and endocardial walls did not change significantly under HMR1098 perfusion. 5HD partially reversed the TRAP-induced EpiMAP duration shortening, and the TRAP-induced ST-segment elevation in the simultaneously recorded ECG partially recovered (Figure 7D). The MAP₉₀ measured in the epicardial walls partially decreased following 5HD and TRAP perfusion (Table 1B).

DISCUSSION

Our studies demonstrated that eliminating endogenous thrombin with

hirudin significantly decreased ST-segment elevation, which was induced by AMI in our guinea pig model. Some previous studies demonstrated that tying a single vessel off for a few days to weeks did not cause myocardial infarction in guinea pig hearts (14,15). However, other researchers showed that LCA ligation in guinea pigs resulted in infarction within 3 days of surgery (16–18). Our data indicate that LCA ligation resulted in a nonperfused zone within 1 hour in nearly all the animals. Possible reasons for the differences among these studies include the following: (a) Ligation of LCA does not ligate only a single vessel. The area between pulmonary artery outflow tract and left atrium was ligated, which may include the left descending artery and left circumflex artery. (b) Establishing collateral vessels takes time. Collateral

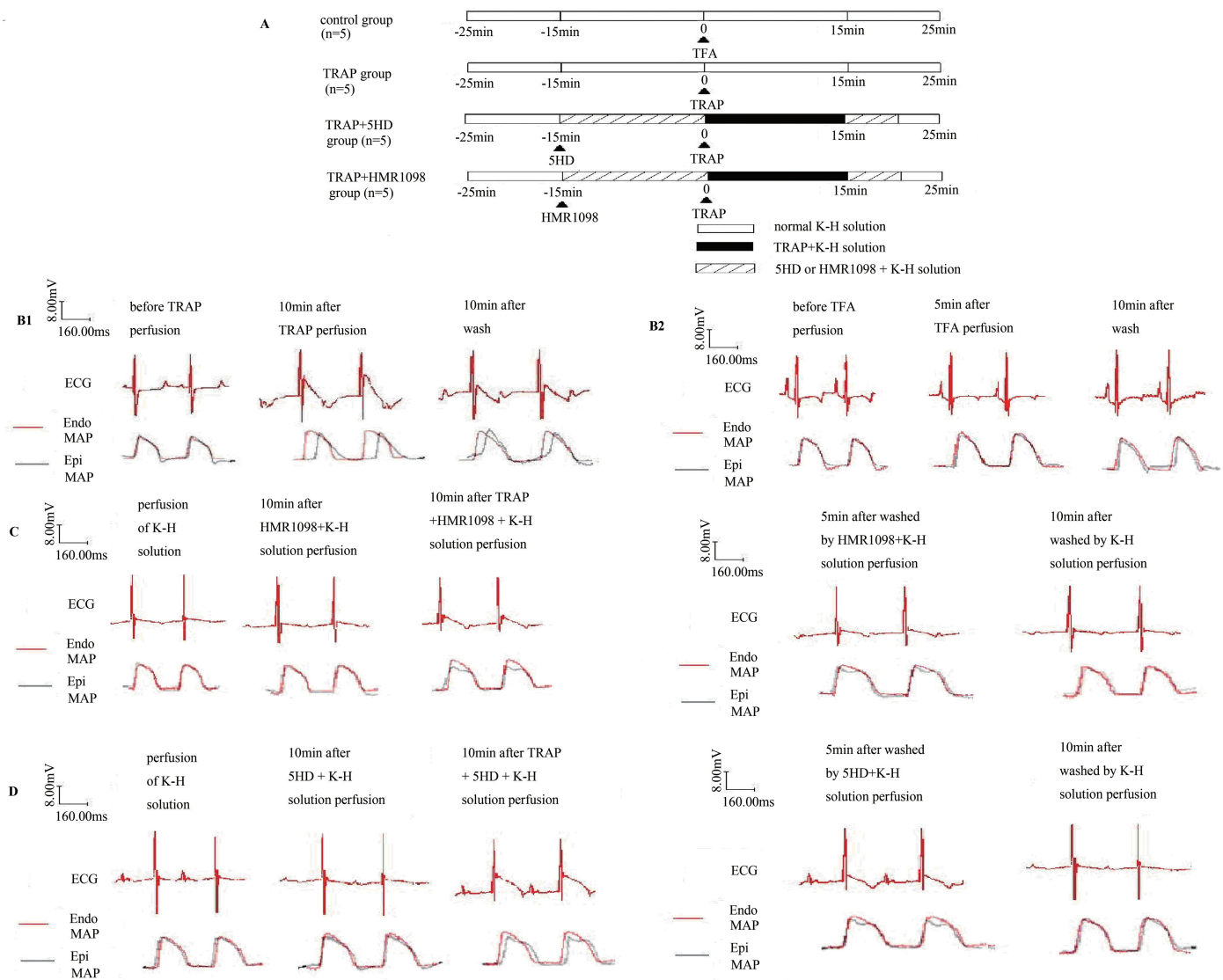


Figure 7. (A) Experimental protocol. In the control and TRAP groups, all hearts were perfused with Krebs-Henseleit solution for 25 min just before TRAP was added in perfusion. After 15 min, TRAP (200 nmol/L) or TFA was washed out with Krebs-Henseleit solution. In the TRAP + 5HD group and TRAP + HMR1098 group, the hearts were perfused with Krebs-Henseleit solution for 10 min before 5HD or HMR1098, respectively, were perfused. Then, 15 min later, TRAP was perfused for another 15 min before being washed out. (B) Representative tracings measuring the electrophysiological effect of TRAP on the left ventricular walls. Each panel shows (from top to bottom) simultaneous recordings of the ECG recorded across the left ventricular, EpiMAP and EndoMAP. (B1) The effect of TRAP (200 nmol/L). (B2) The effect of TFA. (C) Representative electrophysiological tracings showing the effect of TRAP (200 nmol/L) and HMR1098 on the left ventricular walls. (D) Representative tracings showing the electrophysiological effect of TRAP + 5HD.

vessels usually do not open in a normal situation, but open only when the main artery is narrowed or blocked, and this process is very slow. We can see such phenomena very commonly in our clinical activities. Slowly developing, high-grade coronary artery stenoses may progress to complete oc-

clusion without precipitating an AMI because of the development of a rich collateral network over time. However, if plaque fissuring and rupturing occur during the natural evolution of lipid-laden atherosclerotic plaques, this can result in thrombus formation and lead to AMI (19).

We observed no relationship between ST-segment elevation and the infarct index in our study. Previously, infarct size was reported to have a low correlation with ST-segment elevation (20). We used the ratio of ST-segment elevation to positive QRS amplitude rather than absolute ST-segment elevation in our study

Table 1. EpiMAP₉₀ and EndoMAP₉₀ duration.

A. After TRAP and vehicle (TFA) perfusion to the left ventricular walls

	Before perfusion		10 min after perfusion		10 min after wash	
	EpiMAP ₉₀	EndoMAP ₉₀	EpiMAP ₉₀	EndoMAP ₉₀	EpiMAP ₉₀	EndoMAP ₉₀
TRAP	153 ± 4	154 ± 2	135 ± 3 ^{a#}	156 ± 4	154 ± 2	159 ± 5
TFA	151 ± 2	152 ± 3	159 ± 2	156 ± 5	154 ± 4	153 ± 5

B. Before and after perfusion with HMR1098 + TRAP or 5-HD + TRAP

MAP ₉₀	5- HD		HMR1098	
	EpiMAP ₉₀	EndoMAP ₉₀	EpiMAP ₉₀	EndoMAP ₉₀
Perfusion of K-H solution	149 ± 2	152 ± 3	150 ± 2	151 ± 3
10 min after HMR1098/5HD + K-H solution perfusion	162 ± 3	164 ± 5	171 ± 4	173 ± 4
10 min after TRAP + HMR1098/5HD perfusion	152 ± 3 ^{a,c}	168 ± 3	165 ± 5	170 ± 3
5 min after washed by HMR1098/5HD + K-H solution perfusion	162 ± 4	165 ± 5	168 ± 2	171 ± 3
10 min after washed by K-H solution	151 ± 5	150 ± 1	156 ± 3	156 ± 4

Each value represents the mean ± SEM of five animals. *P* < 0.05 ^aEpiMAP₉₀ versus EndoMAP₉₀, ^bEpiMAP₉₀ in TRAP group versus EpiMAP₉₀ in TFA group, ^cEpiMAP₉₀ in 5HD + TRAP group versus EpiMAP₉₀ in HMR1098 + TRAP group.

because absolute ST-segment elevation is dramatically variable in animals with different weights.

Our study showed that thrombin receptor activation plays a very important role in ST-segment elevation in our AMI model. TRAP perfusion was shown to induce ST-segment elevation in uninfarcted hearts (7), but this effect was attributed to TRAP-induced coronary artery constriction and heart dysfunction (21). To exclude the disturbances caused by ATP depletion and TRAP-induced vessel constriction, we locally injected TRAP in left ventricles without AMI, and we observed that TRAP directly affected ST-segment elevation by sarcK_{ATP} channels, although the duration of TRAP-induced ST-segment elevation is much shorter than that of AMI-induced ST-segment elevation and the concentration of TRAP is also much higher than the upper limitation of physiological thrombin concentration. We also applied pinacidil, a K_{ATP} channel opener that relaxes coronary arteries (22), and observed that the effect of TRAP was nearly replicated by pinacidil. Therefore, TRAP-induced ST-segment elevation was not related to coronary artery constriction. Furthermore, our single-

channel recordings indicated that TRAP increased K_{ATP} channel activity, and this effect could be blocked by HMR1098 but not by the mitochondrial K_{ATP} channel blocker 5HD (10 μM), as reported in other cell types (23). 5HD is usually considered a mitochondrial K_{ATP} channel blocker. Notsu *et al.* reported that 5HD at concentrations >1 μM had an effect similar to that of glibenclamide (24). Therefore, sarcK_{ATP} channels play an important role in TRAP-induced ST-segment elevation. The mechanism of increased K_{ATP} channel activity by TRAP is unclear. TRAP not only stimulates accumulation of IP₂ and IP₃ in cardiac myocytes but also modulates cardiac contractile function by the protein kinase C (PKC) pathway (24). Increased PKC in cardiac cells enhances K_{ATP} activity (25).

Our work clearly demonstrates that TRAP induced MAP duration change by activating sarcK_{ATP} channels, which resulted in ST-segment elevation. MAP recorded in cardiac ventricular walls in our experiment is different from transmembrane action potential recorded in single cells. MAP is measured from a group of cells with extracellular electrodes that have a diameter of 1 to 2

mm (26). Therefore, a number of dissimilarities exist between the MAP and transmembrane action potential from single cells. For instance, MAP has less diastolic and action potential amplitude than transmembrane action potential from single cells. The maximum upstroke velocity of MAP is much smaller than that measured in a single cell (26). A notch, the remnant of the “intrinsic deflection,” may appear in the MAP plateau phase, mimicking “phase I repolarization.” This should not represent predominant K_{It0} (a transient outward current) channel activity of epicardial myocardium (27). TRAP induced different electrophysiological responses, including a shortened MAP duration and EpiMAP duration but not EndoMAP duration, by activating sarcK_{ATP} channels and causing ST-segment elevation. This indicates that the K_{ATP} channels in the endocardial and epicardial cells are different, as suggested by previous studies (28–30). Studies have reported that ST-segment elevation was always accompanied by the shortening of action potential after AMI (31,32). There is a general consensus, though not universal, that I_{KATP} is the major current responsible for MAP duration shortening

during acute ischemia (33). In the ECG, a ST-segment elevation is related to shortened EpiMAP duration but not EndoMAP duration (34).

Thrombin can be produced only during thrombus formation during AMI. Therefore, thrombin-induced ST-segment elevation during AMI indicates fresh thrombus forming in the coronary arteries. In addition, thrombin is released during revascularization treatments, and antithrombin therapy with bivalirudin has begun to be used as an important adjuvant therapy during revascularization treatments (35). Decrease in ST-segment elevation following coronary artery recanalization is a major clinical criterion used to judge the effect of revascularization treatments. Therefore, our work indicates that decreases in ST-segment elevation following revascularization treatments with strong antithrombin therapies should be carefully evaluated for their effectiveness.

In conclusion, our study demonstrates that thrombin and its receptor activation significantly enhances ST-segment elevation during AMI. The activation of the thrombin receptor opens sarcK_{ATP} channels, which shortens the MAP duration and EpiMAP duration.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

REFERENCES

- Litovsky SH, Antzelevitch C. (1989) Rate dependence of action potential duration and refractoriness in canine ventricular endocardium differs from that of epicardium: the role of the transient outward current. *J. Am. Coll. Cardiol.* 14:1053.
- Kubota I, et al. (1993) Role of ATP-sensitive K⁺ channel on ECG ST segment elevation during a bout of myocardial ischemia: a study on epicardial mapping in dogs. *Circulation* 88:1845–1851.
- Li RA, Leppo M, Miki T, Seino S, Marbán E. (2000) Molecular basis of electrocardiography ST segment elevation. *Circ. Res.* 87:837–839.
- Szczeklik A, Dropinski J, Radwan J, Krzanowski M. (1992) Persistent generation of thrombin after acute myocardial infarction. *Arterioscler. Thromb. Vasc. Biol.* 12:548–553.
- Vu TKH, Hung DT, Wheaton VI, Coughlin SR. (1991) Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* 1991; 64:1057–68.
- Nanevich T, et al. (1995) Mechanism of thrombin receptor agonist specificity: chimeric receptors and complementary mutations identify a Nanagonist recognition site. *J. Biol. Chem.* 270:21619–25.
- Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. (2001) Proteinase-activated receptors. *Pharmacol. Rev.* 53:245–282.
- Tang L, et al. (2008) Thrombin receptor and ventricular arrhythmias after acute myocardial infarction. *Mol. Med.* 14:131–140.
- Redel A, et al. (2008) Impact of ischemia and reperfusion times on myocardial infarct size in mice in vivo. *Exp. Biol. Med.* 233:84–93.
- Bai C-X, Kurokawa J, Tamagawa M, Nakaya H, Furukawa T. (2005) Nontranscriptional regulation of cardiac repolarization currents by testosterone. *Circulation* 112:1701–1710.
- Killeen MJ, et al. (2008) Effects of potassium channel openers in the isolated perfused hypokalaemic murine heart. *Acta Physiol.* 193:25–36.
- Kleber AG. (1984) Extracellular potassium accumulation in acute myocardial ischemia. *J. Mol. Cell Cardiol.* 16:389–394.
- Wilde AAM, et al. (1990) Potassium accumulation in the globally ischemic mammalian heart: a role for the ATP-sensitive potassium channel. *Circ. Res.* 67:835–843.
- Hearse DJ. (2000) The elusive coypu: the importance of collateral flow and the search for an alternative to the dog. *Cardiovasc. Res.* 45:215–219.
- Rösen R, Marsen A, Klaus W. (1984) Local myocardial perfusion and epicardial NADH-fluorescence after coronary artery ligation in the isolated guinea pig heart. *Basic Res. Cardiol.* 79:59–67.
- Johns TNP, Olson BJ. (1954) Experimental myocardial infarction: I. A method of coronary occlusion in small animals. *Ann. Surg.* 140:675–682.
- Dawson TA, et al. (2008) Cardiac cholinergic NO-cGMP signaling following acute myocardial infarction and nNOS gene transfer. *Am. J. Physiol. Heart Circ. Physiol.* 295:H990–H998.
- du Toit EF, Genis A, Opie LH, Pollesello P, Lochner A. (2008) A role for the RISK pathway and KATP channels in pre- and post-conditioning induced by levosimendan in the isolated guinea pig heart. *Br. J. Pharmacol.* 154:41–50.
- Falk E. (1983) Plaque rupture with severe pre-existing stenosis precipitating coronary thrombosis: characteristics of coronary atherosclerotic plaque underlying fatal occlusive thrombi. *Br. Heart J.* 50:127–131.
- Sciagra R, et al. (2006) ST-segment analysis to predict infarct size and functional outcome in acute myocardial infarction treated with primary coronary intervention and adjunctive abciximab therapy. *Am. J. Cardiol.* 97:48–54.
- Damiano BP, Cheng WM, Mitchell JA, Falotico R. (1996) Cardiovascular actions of thrombin receptor activation in vivo. *J. Pharmacol. Exp. Ther.* 279:1365–1378.
- Deka DK, Raviprakash V, Mishra SK. (1998) Basal nitric oxide release differentially modulates vasodilations by pinacidil and levcromakalim in goat coronary artery. *Eur. J. Pharmacol.* 348:11–23.
- Moritani K, et al. (1994) Blockade of ATP-sensitive potassium channels by 5-hydroxydecanoate suppresses monophasic action potential shortening during regional myocardial ischemia. *Cardiovasc. Drugs Ther.* 8:749–756.
- Notsu T, Tanaka I, Takano M, Noma A. (1992) Blockade of the ATP-sensitive K⁺ channel by 5-hydroxydecanoate in guinea pig ventricular myocytes. *J. Pharmacol. Exp. Ther.* 260:702–708.
- Jiang T, et al. (1996) Thrombin receptor actions in neonatal rat ventricular myocytes. *Circ. Res.* 78:553–563.
- Franz MR. (1999) Current status of monophasic action potential recording: theories, measurements and interpretations. *Cardiovasc. Res.* 41:25–40.
- Tande PM, Mortensen E, Refsum H. (1991) Rate-dependent differences in dog epi- and endocardial monophasic action potential configuration in vivo. *Am. J. Physiol.* 261:H1387–H1391.
- Furukawa T, Kimura S, Furukawa N, Bassett AL, Myerburg RJ. (1991) Role of cardiac ATP-regulated potassium channels in differential responses of endocardial and epicardial cells to ischemia. *Circ. Res.* 68:1693–1702.
- Gilmour RF Jr, Zipes DP. (1980) Different electrophysiological responses of canine endocardium and epicardium to combined hyperkalemia, hypoxia, and acidosis. *Circ. Res.* 46:814–825.
- Kimura S, Bassett AL, Kohya T, Kozlovskis PL, Myerburg RJ. (1986) Simultaneous recording of action potentials from endocardium and epicardium during ischemia in the isolated cat ventricle: relation of temporal electrophysiological heterogeneities to arrhythmias. *Circulation* 74:401–409.
- Samson WE, Scher AM. (1960) Mechanism of ST-segment alteration during acute myocardial injury. *Circ. Res.* 8:780–787.

32. Franz MR, Flaherty JT, Platia EV, Bulkley BH, Weisfeldt ML. (1984) Localization of regional myocardial ischemia by recording of monophasic action potentials. *Circulation* 69:593-604.
33. Shaw RM, Rudy Y. (1997) Electrophysiologic effects of acute myocardial ischemia: a theoretical study of altered cell excitability and action potential duration. *Cardiovasc. Res.* 35:256-272.
34. Kingaby RO, Lab MJ, Cole AWG, Palmer NT. (1986) Relation between monophasic action potential duration, ST segment elevation, and regional myocardial blood flow after coronary occlusion in the pig. *Cardiovasc. Res.* 20:740-751.
35. Stone GW, *et al.* (2008) Bivalirudin during primary PCI in acute myocardial infarction. *N. Engl. J. Med.* 358:2218-2230.