A growing body of evidence suggests that heterogeneity of electrophysiological characteristics is an important property of the ventricular myocardium. Importantly, a subpopulation of cells, called mid-myocardial cells (M cells), have been described in the ventricular wall of the guinea pig, canine and human. The M cells display a longer action potential duration (APD) and a steeper dependence of APD on rate than epicardial or endocardial cells. This electrophysiological difference between M cells and the other cell types is the main source of the heterogeneity observed in the normal heart.

Transmural and regional heterogeneities in the electrical properties of cardiac cells contribute to the normal function of the heart. They are susceptible to many pathological factors, such as ischemia, hypertrophy, or heart failure, and any significant alternation in these electrical heterogeneities can lead to the development of life-threatening cardiac arrhythmias and sudden death. The heterogeneity of the ventricular electrical properties is reflected in aspects such as APD, effective refractory period (ERP) and conduction velocity, and is more marked in vitro than in the intact heart. In the setting of ischemia, hypertrophy, or heart failure, the altered electrophysiological heterogeneity of the ventricle may have significance in the development of ventricular arrhythmias, but the dynamic variations of the electrophysiological characteristics of the ventricle under pathological conditions are poorly understood.

Myocardial infarction (MI) can create abnormal electrophysiological substrates that trigger ventricular arrhythmias and at different stages of MI, the mechanism underlying the ventricular arrhythmias vary. The study by Anyukhovsky et al suggested that myocardial injury may in fact permit dispersion of repolarization to be manifested transmurally in vivo, and in the setting of Ca2+ overload the transmural electrophysiological dispersion of ventricle wall may be further exaggerated.

Consequently, the aims of the present study were two-fold. First, to investigate the effects of MI on transmural electrophysiological characteristics in the rabbit heart in vivo and second, to compare the changing trends of the monophasic APD (MAPD) and ERP of the endocardial, M and epicardial cells after MI in vivo.

Methods

Animal Preparation

Forty New Zealand white rabbits, including both male and female, weighing 2–2.5 kg, were randomized into either a sham operation (SO) group (n=10) or MI group (n=30). All rabbits were anesthetized with sodium pentobarbital (30 mg/kg iv). Additional smaller doses were given as needed to maintain deep anesthesia. Under controlled ventilation, a thoracotomy through a left parasternal incision...
sion was performed, the pericardium was incised and the anterior wall of the left ventricle was exposed. The left anterior descending (LAD) coronary artery of the animals in the MI group was identified and carefully separated, then ligated downstream 2 mm from where the first diagonal artery branches out. The animals in the SO group underwent thoracotomy without ligation of the LAD. Postoperatively, each rabbit received 400,000 IU penicillin intramuscularly twice daily for 2 days. After successfully producing an anterior MI, which was confirmed by elevation of the ST segment by more than 0.2 mV in leads I, II and aVL, the MI group was randomized further into 3 subgroups that were post MI 2 days (n=10), post MI 14 days (n=10) and post MI 60 days (n=10). The 3 subgroups underwent another thoracotomy at 2, 14, and 60 days after the induction of MI, respectively. In order to artificially stimulate the heart, a slow intrinsic heart rate was needed, so the sinoatrial node was reversibly destroyed by injection of 0.1–0.3 ml of 100 ml/L formaldehyde into the region between the right atrial appendage and superior vena cava. Continual epicardium bipolar pacing with a cycle length (CL) of 300 ms was performed at the high right atrium except when conducting programmed stimulation to determine the ERP.

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the PRC National Department of Health.

Data Acquisition and Analysis

During data acquisition, the pericardial cavity was filled with 39°C physiological saline solution, which was refreshed at 15 ml/min, to keep the heart’s surface temperature constant.

As shown in Fig 1, the custom-made electrodes for recording the monophasic action potential (MAP) comprised an epicardial, midmyocardial and endocardial electrode. Each electrode consisted of a Teflon-coated stainless steel wire (0.1-mm diameter), the distal tip of which was 0.5 mm long, naked and conductive. Because the thickness of the free wall of the rabbit left ventricle is approximately 4 mm, the distance between the tips of the 3 electrodes was 2 mm: the distal, middle and proximal electrodes were designated Endo, Mid and Epi, respectively. The MAP electrodes penetrated the region supplied by the LAD and the reference electrode was placed on the thorax. Leads I, II, III, aVr, aVt, and aVf were attached to record the surface electrocardiogram (SECG). All signals were recorded with a polygraph (LEAD2000B, Jinjiang Ltd) and were filtered to record frequencies between 0.05 Hz and 30 Hz for the MAP and between 30 Hz and 600 Hz for the SECG.

In order to determine the ERP of the 3 layers of myocytes, the pacing threshold of each site was determined first and then programmed stimulation was conducted through bipolar electrodes (as used for MAP recording and labeled with a depth marker so that the tips of the 2 electrodes were at the same level) introduced into the corresponding layer 5 mm from the recording site. Using square wave impulses of 2-ms duration at twice the diastolic threshold, which were generated by a constant voltage stimulator (LEAD2000B, Jinjiang Ltd) and following a train of 8 regular stimuli (S1) with a CL of 300 ms, an early extrastimulus (S2) was introduced and subsequently introduced progressively in 5-ms steps until it failed to trigger an action potential. The ERP was defined as the longest S1S2 interval at which S2 failed to produce a propagated ventricular response. The determination of pacing threshold and ERP of each of the 3 layers of myocardium was completed within 2 min.

For the SO group, the electrophysiological examinations...
were performed during the sham procedure and at 2 days after the procedure. For the MI group, the electrophysiological examinations were performed before the induction of MI, and at 5, 15, and 30 min after coronary occlusion (CO) during the first thoracotomy procedure; for the 3 post MI groups, the examinations were performed at 2, 14, and 60 days after MI, respectively.

All data are presented as mean ± standard deviation. The statistical technique was ANOVA and significance was determined at p<0.05.

## Results

In the MI group, after 30 min of LAD ligation, all animals showed ST-segment elevation in leads I, II, and aVL (average ΔSTI=0.31±0.02 mV, ΔSTII=0.31±0.01 mV, and ΔSTavL=0.30±0.02 mV). There was no change in the ST segment in the SO group. The MAPs recorded by the custom-made electrodes are shown in Fig. 2.

### Dynamic and Characteristic Variation of the MAPD90

Table 1 shows the MAPD90 (CL=300 ms) and MAP amplitude (MAPA) of each of the 3 layers of myocardium in both groups. In the MI group, MAPD90Endo shortened sharply after 5 min of occlusion from 243 ms (baseline) to 124 ms, and reached the lowest point of 116 ms at 30 min after LAD ligation. MAPD90Mid and MAPD90Epi shortened slightly from 228 ms and 236 ms at baseline to the lowest points of 195 ms and 190 ms, respectively, at 30 min after LAD occlusion. The MAPD90 of each of the 3 layers of myocardium recovered slowly during the 2–60 days after MI, but did not return to the baseline values even at 60 days after MI. At baseline, the sequence of the MAPD90 was Endo > Epi > Mid. Within the first 30 min of MI, the average difference in MAPD90 between the midlayer and the other 2 layers was approximately 80 ms. The average MAPA of the 3 layers of myocardium was approximately 80 mV at baseline, and during the 5–30 min after CO the value for each layer decreased slightly and equivalently, then gradually recovered from 14 days after MI. No significant changes in MAPA were observed in the SO group.

### Dynamic and Characteristic Variation of the ERP

The pacing threshold of each site increased shortly after CO, then declined during the 2–60 days after MI and the trend for the change in the ERP was similar to that of the corresponding MAPD90 for all 3 MAP recording locations except for the M cells (Table 2). The ERPEndo and ERP_epi showed a significant correlation with the MAPD90Endo.
Within the first 30 min of MI, the shortening in the ERPmid was relatively smaller than that in the MAPD90mid and as a result, the ERPmid exceeded the MAPD90mid and there was postrepolarization refractoriness (PRR) in the M cells at that stage (Fig 3). The PRR phenomenon lasted throughout the first 30 min of MI, and disappeared at 2 days after MI. Similar to the MAPD90, the ERP recovered partially during the 2–60 days after MI, and the sequence of the ERP of each of the 3 layers changed from Mid(Epi) > Endo at baseline to Epi(Endo) > Mid at 60 days after MI. The biggest difference in the ERP between the midlayer and the other 2 layers was 22 ms (Endo) and 24 ms (Epi), both of which occurred at 5 min after MI.

**Discussion**

There are intrinsic electrical differences between the myocytes from different regions or different layers of the ventricle (most notably among the endocardium, midmyocardium, and epicardium), which are the result of the different contributions of the ionic currents to the transmembrane action potential. All aspects of this electrical heterogeneity can be affected by different pathological stimuli, such as myocardial ischemia or cardiac hypertrophy. In particular, the different responses of the various myocyte populations to these pathological stimuli, as well as a marked increase in nonuniform anisotropy, may be responsible for the increased pro-arrhythmic potential in these conditions.

Techniques for recording the MAP bridge the gap between cellular and clinical cardiac electrophysiology. Using both a MAP recording technique and a programmed stimulation technique, we observed differences in the changing trends of the MAPD90 and ERP of the left ventricular free wall of the rabbit in a pathological setting. Both values showed different changing trends during the course of acute and chronic MI in vivo.

Whereas electrophysiological heterogeneity is an important feature of cardiac tissue, there is variation in the reported experimental data for both the magnitude and spatial extent of the observed APD gradients needed to produce unidirectional block and reentry. In the present study, the plateau phase of MAPmid diminished rapidly after the beginning of ischemia and the average difference in MAPD90 and ERP between the midlayer and other 2 layers was approximately 80 ms and 20 ms, respectively, during the 5–30 min after CO. These marked transmural gradients of MAPD and ERP that appear in the ventricular wall under ischemia conditions may represent the substrate of functional reentry and are in agreement with previous reports in isolated cells from the 3 layers of the ventricular wall in which acute MI shortened the APD.

In this study, although the MAPD and ERP were shortened proportionately in the epicardium and endocardium after MI, they separated and PRR was observed in the M cells during the 5–30 min after CO. Commonly, the ERP of the myocardium shows a significant correlation with the MAPD90 but in some pathological conditions, the ERP extends relatively and the PRR phenomenon appears. The mechanism underlying PRR and its clinical significance is poorly understood, but it is currently thought to have 2 side effects. One is the prevention of arrhythmia by protecting the myocardium from premature excitation depressing the transmural propagation of ectopic activation and avoiding the formation of reentry, and this mechanism may underlie the antiarrhythmia effect of some agents. However, PRR also facilitates reentry by reducing conduction velocity and may even cause conduction block. Therefore, PRR can be also thought of as altered excitability of myocardium and its 2 effects may result from this characteristic.

The differential sensitivity of endocardial, midmyocardial and epicardial cells to the electrophysiological consequences of ischemia suggests that a transmural gradient also exists in the density and/or kinetics of the outward ionic currents, such as the ATP sensitive potassium (KATP) channels. KATP channels are masked by the high intracellular ATP concentration under normal conditions and are opened by cardiac ischemia, which depletes the myocyte of ATP. Opening the KATP channels increases the total outward K+ current, which in turn shortens the APD. Furukawa et al have reported that the epicardial cells are more susceptible to the electrophysiological effects of ischemia than are endocardial cells, and concluded that the differential ATP sensitivity of KATP channels in endocardial and epicardial cells may play an underlying role.

Our results support this notion, although we think the M cells are the most sensitive of the 3 cell types under ischemic conditions.

The cellular and ionic mechanisms of the recovery of the MAPD during the 2–60 days after MI are still unknown, but the construction of compensatory coronary circulation or the inhibition and/or down regulation of KATP channels may be related to it.

MI is an important cause of ventricular arrhythmias, such as ventricular tachycardia and ventricular fibrillation, that often result in sudden cardiac death. Most ventricular arrhythmias occur during the first 10–15 min of MI and are often caused by transmural ventricular reentry. The MI-induced transmural gradient of excitability provides the substrate for reentry and both preexisting and dynamically induced dispersion of refractoriness independently increase the vulnerability to reentrant arrhythmias. Reduction of the dynamically induced dispersion by appropriate alteration of electrical restitution holds promise as an antiarrhythmic strategy.

The main limitation of our study is that we did not simultaneously measure the MAPD and ERP of both the normal and border zones of the MI. However, in the in vivo study, these zones are usually located at the lateral and posterior walls, which makes it very difficult to record the MAP from those sites without altering the position and status of the heart.

**Conclusions**

The MAPD and ERP of each of the 3 layers of the left ventricular free wall show different changing trends during the course of MI, especially the midmyocardium. We think that this may underlie the main mechanism of the various ventricular arrhythmias that occur at different stages of MI.

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References