Optical mapping of late myocardial infarction in rats

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Optical mapping techniques have been used to study the cellular mechanisms of arrhythmias that are reentrant (5, 10, 14, 15, 24) or triggered (18) with very high spatial and temporal resolution (14a). Importantly, optical mapping can be used to determine underlying cellular mechanisms at the level of the whole heart (16) and, therefore, offers several distinct advantages over traditional electrophysiological techniques. Despite these advantages and the high prevalence of MI, optical mapping remains underutilized as a tool to better understand the mechanisms of sudden cardiac death in such patients. In this report, we describe the successful implementation of a novel optical mapping technique specifically designed to investigate the cellular mechanisms of arrhythmogenesis associated with late MI in the whole heart. In addition, we describe a causal relationship between the site of infarction, abnormal action potential conduction (i.e., block and slow conduction), and arrhythmogenesis. The results of this study may significantly enhance our ability to understand the interplay between abnormal cellular electrophysiology and conduction abnormalities associated with MI.

MATERIALS AND METHODS

Experimental model. This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). This study was reviewed and approved by the Animal Care and Use Committees of Case Western Reserve University and the Cleveland Clinic Foundation. To create the model of late MI, we performed permanent ligation of the left anterior descending coronary artery (LAD) in rats (2, 3). Male Lewis rats (250–300 g) were anesthetized with 0.8 ml of a mixture of 2.5 ml ketamine (50 mg/kg) and 0.8 ml xylazine (20 mg/kg). Animals were ventilated with room air at 80 breaths/min with the use of a pressure-cycled rodent ventilator (RSPI002; Kent Scientific). The chest was sterilized with betadine, and a sternotomy was performed. The left atrium was retracted and the LAD was identified. The proximal LAD was ligated by using 6-0 silk. Successful ligation of the LAD was confirmed by blanching and dysfunction of the anterior wall. The sternum and skin were then closed by using 4-0 silk with interrupted sutures. Any residual pneumothorax was reduced by using negative pressure generated by a 20-gauge needle placed in the closed chest, attached to a 10-ml syringe. Each rat was then weaned from the ventilator over 10–15 min.

Echocardiography. To confirm the presence of significant MI, two-dimensional echocardiography was performed by using a 15-MHz linear-array transducer interfaced with an Acuson Sequoia C256 (Siemens Medical Solutions, Malvern, PA) (2, 3). Animals were lightly sedated with ketamine (50 mg/kg ip) for each echocardiogram. As a measure of left ventricular function, the shortening fraction was

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calculated from M-mode recordings. Dimensions were measured between the anterior wall and the posterior wall from the short-axis view at the level of papillary muscles. The presence of significant anterior MI was confirmed by anterior wall akinesis present in at least two adjacent segments on two-dimensional echocardiography (21).

**Optical mapping.** Optical mapping was performed 7 days after MI. Normal rats (n = 9) and rats with late MI (n = 7) were injected with heparin (300 U ip) 15 min before the heart was removed. Rats were then anesthetized with pentobarbital sodium (50 mg/kg ip). After attainment of adequate anesthesia, the heart was immediately excised and arrested by direct aortic perfusion of cold cardioplegia solution (10 ml). Extravascular tissue was carefully dissected away from the heart, which was then placed in a beaker containing 20 ml of oxygenated ice-cold cardioplegia and 180 μM of 4-[(β-[2-(di-n-butyramino)-6-naphthyl]vinyl)pyridium (di-4-ANEPPS) for 20 min. The beaker was shaken gently every 60 s to prevent any inhomogeneity in heart tissue staining. After completion of dye superfusion staining, the aorta was cannulated, and perfusion with 37°C Tyrode solution containing (in mM) 121.7 NaCl, 25.0 NaHCO3, 2.74 MgSO4, 4.81 KCl, 5.0 dextrose, and 2.0 CaCl2 (pH 7.4) was started at ascending flow rates. Perfusion pressure in the cannula was monitored with a pressure transducer (Harvard Apparatus, South Natick, MA), allowing for a ramp up to a target pressure of 70–90 mmHg over a 5-min period. After the target perfusion pressure was achieved, the cannulated heart was immersed in 37°C Tyrode solution in a specialized optical recording chamber for the duration of each experiment. ECG, perfusion pressure, and bath temperature were measured continuously during all experiments.

The optical mapping system used has been described in detail previously (19, 20). Briefly, action potentials were optically recorded at a magnification of 2.1× from 256 sites within a 8.3 mm × 8.3 mm mapping field (0.52-mm interpixel resolution) on the anterior epicardial surface of the ventricle. Fluorescence was excited with a 270-W tungsten-halogen light source [filtered 514 nm (SD 20)] and transmitted to a 16 × 16-element photodiode array detector through a tandem-lens imaging system (emission filter >610 nm). Photocurrent from each photodiode underwent current-to-voltage conversion, amplification (∼200), and band-pass filtering (0.1 to 500 Hz) and was multiplexed and digitized (1,000 samples·s⁻¹·channel⁻¹) with 12-bit precision.

**Data analysis.** With the use of previously described automated algorithms (1), activation times were assigned for each action potential by identifying the greatest positive change in fluorescence (maximum derivative) during each beat. All assigned times were verified visually by an experienced investigator. From local depolarization activation time.

**Experimental protocol.** After being stained with di-4-ANEPPS, normal and infarcted hearts were placed with their anterior left ventricular wall abutted against the optical imaging window. A similar mapping location was chosen for all hearts with MI to ensure that normal tissue, border zone, and the infarct zone were included in the mapping field. Each heart was initially perfused with normal Tyrode solution for 20 min to allow for stabilization and to ensure viability. Perfusion with Tyrode plus 20 mM of diacetyl monoxime was then begun to eliminate motion artifact. Normal hearts were paced just above threshold [0.5 mA (SD 0.2)] at a steady-state cycle length of 150 ms by using a silver unipolar electrode placed on the epicardial surface of the midanterior left ventricle. Infarcted hearts were paced from normal tissue just above threshold [0.6 mA (SD 0.2)], near the infarct zone. Programmed stimulation, with up to three premature stimuli, was used to assess arrhythmia inducibility. Inducibility was defined as the ability to provoke nonsustained or sustained (>30 s) ventricular tachycardia (VT) or ventricular fibrillation. Optical recordings were made during steady-state pacing and during programmed stimulation. All preparations remained viable for the entirety of each optical mapping experiment. Coronary perfusion pressure was maintained at 80 mmHg (SD 10) for all experiments. At the conclusion of optical mapping, hearts were stained with triphenyltetrazolium chloride by using the method of Downey (8). Fluorescence microscopy, with the use of specialized di-4-ANEPPS fluorescence filters, was then performed on ventricular cross sections from each heart to ensure homogenous optical dye staining of normal and infarcted tissue.

**Histological analysis and immunostaining.** To precisely localize each myocardial infarct, histology and confocal immunofluorescent microscopy were used to determine regions of fibrosis and viable cardiac myocytes, respectively, from ventricular cross sections obtained after optical mapping. Such analysis was required because the presence of a thin rim of surviving epicardial tissue within the infarct zone prevented precise localization of the full extent of the infarct from charge-coupled device (CCD) images of the epicardium alone. Perfusion fixation with HistoChoice (Amresco, Solon, OH) was performed, and the hearts were sectioned into three equal divisions perpendicular to the left ventricular long axis. The midventricular and apical segments were paraffin-embedded, and 5-μm-thick sections were stained with hematoxylin and eosin. Photomicrographs of each section were acquired by using an AxioCam HRc digital camera (Microtek, Carson, CA) with a Nikon E600 microscope (Nikon, Tokyo, Japan). The stained sections were analyzed using Adobe Photoshop (Adobe, San Jose, CA).

Per immunostaining, paraffin-embedded tissue sections 5 μm thick were applied to glass slides, oven-dried, and then deparaffinized in xylene. The slides were then hydrated gradually by using graded alcohols and washed in Dulbecco’s phosphate-buffered saline (1× D-PBS) for 5 min. Slides were then placed in a container and covered with 10 mM sodium citrate buffer (pH 6.0) and heated at 95°C for 5 min. The buffer was replaced with fresh buffer and reheated for an additional 5 min and then cooled for ∼30 min. The slides were then washed in D-PBS three times for 5 min each. Specimens were then incubated with blocking serum (1% bovine serum albumin and 1% normal goat serum in D-PBS) for 30 min. Slides were then incubated overnight at 4°C with mouse anti-breast milk-epidermal growth factor (EGF)-conjugated secondary antibody diluted to 1.5 μg/ml in blocking buffer in a dark humidified chamber at 37°C. After being washed extensively with D-PBS, coverslips were mounted with aqueous mounting medium using 4′,6-diamidino-2-phenylindole (DAPI) (Vectorshiel Mounting Medium, H-1200; Vector Laboratories, Burlington, CA). An upright spectral laser scanning confocal microscope (model TCS-SP; Leica Microsystems, Heidelberg, Germany) equipped with blue argon (for DAPI), green argon (for Alexxa Fluor 488), and red krypton (for Alexxa Fluor 594) laser was used for confocal analysis. Data were collected by sequential excitation to minimize “bleed-through.” Image processing, analysis, and the extent of colocalization were evaluated by using Leica Confocal software. Optical sectioning was averaged over four frames, and the image size was set at 1,024 × 1,024 pixels. There were no digital adjustments made to the images.

**Statistics.** Statistical comparisons were made between groups by using the Student’s t-test, except where noted otherwise. All values are reported as means (SD). P < 0.05 was considered significant.
Fig. 1. A: charge-coupled device (CCD) image of a Langendorff-perfused normal rat heart stained by rapid dye superfusion technique. White box depicts the optical mapping field; 5 of 256 action potentials recorded from sites depicted in A are shown in center of figure. B: isochrone activation map illustrating normal anisotropic conduction from pacing site (center). In this example, conduction velocity measured in the transverse direction (white arrow at 1 o’clock position) is 0.38 m/s and 0.62 m/s in the longitudinal direction (white arrow at 4 o’clock position). C: optical action potential amplitude map of mapping field illustrating a radial decrease in amplitude occurring with distance from the center of the array.

RESULTS

Electrophysiology of normal hearts. Optically recorded action potentials from control rats with the use of the rapid dye superfusion technique are shown in Fig. 1A. All optically recorded action potentials recorded within the mapping field (white box), five of which are shown, exhibited a rapid depolarization phase followed by early repolarization. Each control heart demonstrated normal, anisotropic impulse conduction that spread radially from the site of pacing (Fig. 1B). Mean conduction velocity (CV) along the transverse axis [0.44 m/s (SD 0.08)] was significantly (P < 0.05) slower than that in the longitudinal axis [0.61 m/s (SD 0.10); n = 9]. Importantly, no evidence of abnormal conduction (i.e., slow conduction or impulse block) was observed. In each control heart, the amplitude of optically recorded action potentials (i.e., fluorescence amplitude) exhibited a smooth and uniform, radial decrease from the location of maximum excitation light intensity (Fig. 1C). In control hearts, no arrhythmias were observed during the initial 20-min perfusion/stabilization period or during steady-state pacing, and none (0/9) had inducible ventricular arrhythmias during programmed stimulation.

Electrophysiology of hearts with MI. In all rats with late MI, echocardiography confirmed the presence of anterior wall akinesis and decreased cardiac function (shortening fraction 25.2% (SD 8.4) compared with normal 50.4% (SD 6.2)). When hearts with late MI were removed for optical mapping, a zone of infarction was visually apparent from CCD images taken during optical mapping (Fig. 2A). In addition, histological analysis (hematoxylin and eosin staining; Fig. 2, B and C) and immunofluorescence confocal microscopy (troponin I; Fig. 2D) of cross sections verified the full extent of the infarct zone and confirmed that infarcted and normal tissue was present in the optical mapping field. In each heart with MI, a thin rim of surviving subepicardial myocardium was observed that varied in thickness from 250 μm (Fig. 2B, black arrow) to regions where infarcted tissue appears to break through the epicardium (Fig. 2B, red arrow). The surviving tissue surrounding the infarct was defined as the border zone region. In regions where histological sections and optical action potentials were both available, the amplitude of the optical action potential was consistent with the thickness of surviving tissue. This is demonstrated in Fig. 2C, which shows a higher-magnification view of the section shown in Fig. 2B along with optical action potentials recorded from the same location. At site a, in the absence of infarcted tissue, a normal optical action potential amplitude was observed. In contrast, at site b in the border zone, where viable tissue overlies infarcted tissue (i.e., surviving epicardial rim, 250 μm), a much smaller action potential was observed. Finally, at site c where almost no viable tissue was observed (<50 μm), no action potential was recorded.

Figure 3 shows isochrone activation maps and action potentials recorded from two infarcted hearts. Optically recorded action potentials obtained from the sites labeled a–e are shown with their respective activation times. In both maps shown in Fig. 3, the dashed yellow line represents the extent of infarcted tissue, where the infarct zone is to the right of the yellow line. Action potentials recorded from tissue outside the infarct zone (to the left of the yellow line) exhibited rapid depolarization followed by early repolarization, similar to recordings from controls. In contrast, action potentials recorded from within the infarct zone (to the right of the yellow line) were either absent or had slow upstrokes and small amplitude. Signals recorded from the heart shown in Fig. 3A exhibited an abrupt decrease in action potential amplitude (decreasing to flat line signals as seen in sites d and e) and slowing of the action potential upstroke (sites b and c) occurring at the infarct border (dashed yellow line). The region from which flat line signals were recorded was defined as an area of conduction block (gray shaded area). In contrast, the heart shown in Fig. 3B exhibited low-amplitude action potentials that propagated (albeit slowly) throughout the infarct zone. In this example (Fig. 3B), analysis of confocal images and histology in the infarct zone indicated...
heart (see Table 1). CV in the normal zone, as indicated by the white arrows to the left of the dashed yellow line in Fig. 3, was similar to that in controls [0.40 m/s (SD 0.05) in the transverse direction and 0.49 m/s (SD 0.07) in the longitudinal direction]. However, CV in the longitudinal direction was not statistically different from that in the transverse direction, possibly due to the limited number of recording sites near the infarct zone from which to measure longitudinal CV. In the four hearts in which conduction in the border zone spread radially from the pacing site, it was possible to measure CV (as for the heart shown in Fig. 6). CV in the border zone was significantly slower in the transverse direction 0.24 (SD 0.08) (n = 4) of infarcted hearts compared with controls. Because we did not pace from the

Fig. 2. A: whole heart view of a 7-day-old anterior myocardial infarction (MI) showing a unipolar electrode pacing from normal tissue near infarct zone. Horizontal black line represents area from which midventricular cross section shown in B was taken. B: hematoxylin and eosin-stained ventricular cross section, indicating the presence (black arrow) or absence (red arrow) of surviving subepicardial myocardium within infarct zone. Black box in B encompasses boundary between infarct and normal zones, which are shown in higher magnification in C. C: action potentials recorded from sites a–c. Note decreased amplitude of the action potential recorded from site b (area with ~250 μM of viable myocardium overlying infarcted myocardium) compared with the normal action potential recorded from site a. In areas with <50 μM of viable myocardium overlying the infarct zone (site c), flat line signals were recorded. D: troponin I antibody staining of normal myocardium (red, at left) and unstained infarct zone (4,6-diamidino-2-phenylindole nuclear stain at right) confirming that the border zone was within the optical mapping field.

CV and action potential duration were measured in the normal and border zones (where possible) of each infarcted

Fig. 3. Isochrone activation maps and action potentials recorded from 2 infarcted hearts. Optically recorded action potentials, shown with their respective activation times relative to stimulation time beneath each signal, were obtained from sites labeled a–e. A: signals recorded from the heart exhibited an abrupt decrease in action potential amplitude (decreasing to flat line signals as seen in sites d and e) and slowing of action potential upstroke (sites b and c) occurring at infarct border (dashed yellow line). Gray shaded area to right of thick black line depicts area of conduction block. Conduction velocity in noninfarcted zone was measured from sites marked with large white arrows (transverse at 1 o’clock position, 0.36 m/s; longitudinal at 4 o’clock position 0.34 m/s). B: heart exhibiting low-amplitude signals that slowly propagated into the infarct zone. Conduction velocity in the noninfarcted zone was measured from site marked with large white arrow in longitudinal direction (0.52 m/s). In this region, conduction velocity could not be measured in the transverse direction. Because of the nonradial spread of conduction in the border zone in this example, conduction velocity could not be determined in this region. Note the extreme slowing that occurred at the infarct border (between sites a and b). In both A and B, action potentials from each heart are drawn on the same scale so that signal amplitude can be compared. C: action potentials recorded from normal zone (top signal, in black) and border zone (bottom signal, in red) that have been amplitude-normalized to allow comparison of action potential duration.
Table 1. APD and CV measured in normal and infarcted rat hearts utilizing voltage-sensitive dye superfusion technique

<table>
<thead>
<tr>
<th></th>
<th>CV Transverse, m/s</th>
<th>CV Longitudinal, m/s</th>
<th>APD90, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.44 (0.08)</td>
<td>0.61 (0.11)</td>
<td>92.2 (11.8)</td>
</tr>
<tr>
<td>Normal zone of MI</td>
<td>0.40 (0.05)</td>
<td>0.49 (0.07)</td>
<td>102.2 (12.1)</td>
</tr>
<tr>
<td>Border zone of MI</td>
<td>0.24 (0.08)</td>
<td>0.21 (0.08)</td>
<td>104.8 (7.5)</td>
</tr>
<tr>
<td>Inducible MI</td>
<td>0.21 (0.08)</td>
<td>0.21 (0.08)</td>
<td>104.5 (9.2)</td>
</tr>
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Values are means (SD); n, number of rats. In normal hearts, conduction velocity (CV) was significantly faster in longitudinal direction than in transverse direction (*P < 0.05). In myocardial infarction (MI) group, significant slowing of CV was observed in infarct border tissue compared with normal tissue in same hearts (†P < 0.05). No difference in CV in border zones of inducible versus noninducible hearts was found. Action potential duration at 90% duration (APD90) was measured in normal and infarcted animals (average of 5 sites from within each region), and no significant differences were found. APD90 measured from border zone was similar to that measured in normal zone in infarct group.

Infarct zone and due to the presence of abnormal conduction patterns in the border zone, CV in the longitudinal direction could not be reliably assessed. We found no difference in APD90 in the border zone [104.8 ms (SD 7.5)] compared with the normal zone [102.2 ms (SD 12.1)] of infarcted hearts (see Table 1; P = 0.66). Shown in Fig. 3C is an example of action potentials recorded from normal tissue and the border zone.

We investigated whether heterogeneous di-4-ANEPPS staining may have caused the rapid decrease in optical action potential amplitude. Fluorescence microscopy was performed in ventricular cross sections from normal and infarcted hearts to quantify di-4-ANEPPS staining. As shown in Fig. 4, a cross section from an infarcted heart shows homogeneous di-4-ANEPPS staining despite the presence of a large anterior left ventricular wall infarction (indicated by lack of triphenyltetrazolium chloride staining) (13). Similar homogeneous staining was observed in all heart sections from both MI and control hearts.

Arrhythmias in hearts with MI. In hearts with late MI, no spontaneous arrhythmias were observed during the initial 20-min perfusion/stabilization period or during steady-state pacing; however, in two of seven hearts (29%), sustained VT (≥30 s duration) was induced by two premature stimuli. Figure 5 shows the ECG, action potentials, and activation maps during the induction of sustained monomorphic VT in a heart with late MI. During steady-state pacing (S1), a stable line of conduction block was observed within the infarct zone. The full extent of the infarct zone is to the right of the dashed yellow line in Fig. 5, whereas conduction block is shown by the solid black line in the isochrone maps. Signals recorded from within the infarct zone indicate the presence of small action potentials (site b) recorded from border zone tissue and a lack of action potentials nearer to the center of the infarct zone during steady-state pacing (site c). During premature stimulation, conduction slowing became apparent in the border zone (site b), depicted as crowding of isochrone lines and low action potential upstroke (see activation panels for S2 and S3). The line of conduction block during premature stimulation, however, was comparable to that during baseline pacing. Once VT was initiated (V1, V2, and V18), a reversal of the activation pattern occurred. This was accompanied by the persistence of a stable line of conduction block in the infarct zone (see V18 activation map). The amount of conduction slowing was similar in inducible [0.21 m/s (SD 0.08); n = 2] and noninducible [0.25 m/s (SD 0.10); n = 2] (P = 0.68) preparations. In addition, there was no difference in APD90 between inducible [104.5 ms (SD 9.2)] and noninducible [105.0 ms (SD 8.0); P = 0.95] preparations.

Figure 6 shows an example of functional conduction block associated with reentrant monomorphic VT. Figure 6A is an image of the mapping area, where the yellow line depicts the full extent of the infarct zone (to the right of dashed yellow line) as determined by histological analysis and projected onto the epicardial surface. During steady-state pacing (Fig. 6B), normal conduction velocity (0.46 m/s) and action potential amplitude (site a) were observed in the region of normal tissue (to the right of the yellow dashed line in Fig. 6A). In contrast, conduction slowing (0.21 m/s) and low-amplitude action potentials (sites b and c) were observed in the border zone (to the right of the yellow dashed line). In this example, conduction block was not observed during pacing. Figure 6C is a representative map of VT recorded from the same region. Note the presence of conduction block in the border zone (thick black line) that was not present during steady-state pacing. Signals recorded from sites a–e depict reentrant excitation with slow conduction at the ends of the line of functional block. These data demonstrate slow conduction in border zone tissue during prematurity.

Fig. 4. A: triphenyltetrazolium chloride (TTC)-stained ventricular cross section from the midwall of a rat heart with a 7-day-old MI, illustrating a large area of nonviability in the anterior wall corresponding to the infarct zone. B: Fluorescence photograph illustrating homogeneity of 4-[(β-[2(di-n-butylamino)-6-naphthyl]vinyl)pyridinium (di-4-ANEPPS) staining with use of our dye superfusion technique (di-4-ANEPPS filter).
steady-state pacing that was associated with reentrant excitation caused by functional block.

DISCUSSION

To our knowledge, this is the first study to use surface loading (i.e., superfusion) of di-4-ANEPPS to study MI in the whole heart. We have shown that 1) it is possible to optically record high-fidelity action potentials from whole hearts with late MI by using a rapid dye superfusion technique and 2) optical mapping can be used to characterize the cellular electrophysiology of MI, which in our model includes many of the same electrophysiological abnormalities observed in patients (e.g., slow conduction, block, and inducible ventricular arrhythmias).

Optical mapping with use of rapid dye superfusion. Optical mapping in Langendorff-perfused whole rat hearts has been reported recently in a study by Nygren et al. (22), in which voltage-sensitive dye was delivered via the coronary perfusate. The morphology of the optically recorded action potentials obtained in our study by using rapid dye superfusion were of similar morphology to those obtained by Nygren et al. (22). In addition, we obtained similar activation patterns and conduction velocity by using rapid dye superfusion. These data suggest that the rapid dye superfusion technique does not adversely affect action potential morphology and conduction. In addition, one potential problem with di-4-ANEPPS perfusion observed by Nygren et al. (22) was the incidence of irrecoverable arrhythmias with the use of dye concentrations above 1 mM or at physiological temperature, necessitating the use of low concentration and low-temperature dye perfusion. Even under these conditions, atrioventricular conduction delay and coronary perfusion pressure instability were observed. In contrast, the rapid dye superfusion technique used in the present study was not associated with any arrhythmias, atrioventricular delay, or coronary perfusion pressure instability. One possible reason we did not observe arrhythmias or coronary pressure instability in normal hearts with the use of rapid dye superfusion is because only the outermost tissue layer was exposed to optical dye staining for a short time (20 min) by using this technique. Therefore, most of the heart, including nodal and specialized conduction tissue, should not be affected.

Malignant ventricular arrhythmias are responsible for the majority of deaths associated with MI. Optical mapping can be a powerful tool to investigate regional and global cellular electrophysiology and arrhythmogenesis associated with MI. Previously, a major limiting factor associated with optical mapping in the study of MI was that standard whole heart optical dye delivery methods (i.e., coronary perfusion) may introduce apparent heterogeneity due to occluded coronary vasculature. Moreover, the absence or presence of collateral flow could introduce further uncertainty in dye delivery. There-
eral electrophysiological substrates associated with late MI dye superfusion technique, we were able to demonstrate sev-
zone is to the right of the dashed yellow line. It is con-
the study of MI using optical mapping techniques.
method for homogeneous optical dye delivery are essential for
fore, we believe that the development and validation of a
Electrophysiological characteristics of MI. Using the rapid
dye superfusion technique, we were able to demonstrate sev-
Several electrophysiological substrates associated with late MI
using optical mapping techniques. We observed slow conduc-
conduction, unidirectional block (functional and anatomical), and
reentrant excitation. We also observed low-amplitude action potentials and the absence of action potentials (i.e., flat line) from within the infarct zone (see Limitations). CCD images, histology, and confocal analysis indicated that low-amplitude action potentials originated from the infarct border zone and were associated with the presence of a thin epicardial rim of surviving myocardium (i.e., border zone tissue). This is consistent with reduced levels of fluorescence emanating from regions with fewer viable myocardial cells. Low-amplitude action potentials recorded from border zone tissue also exhibited slow upstrokes and were accompanied by the presence of slow conduction (Fig. 3B), both of which have been shown previously. Regions within the infarct zone that exhibited no action potentials (i.e., flat line) corresponded to regions where a surviving rim was absent.

Interpretation of optically recorded action potential amplitudes must be made with caution, because amplitude depends on several factors such as dye staining, excitation light, and the number of electrically viable cells in a given area. Because our rapid superfusion technique resulted in uniform dye staining (Fig. 4), unlike coronary perfusion in the presence of an occluded artery, it is doubtful that dye staining heterogeneity could explain the abrupt decrease in action potential amplitude that we observed in the infarct zone. Light intensity is also heterogeneous and typically decreases uniformly with distance from its center of maximal excitation (28) and thus could also produce spatial differences in optically recorded action potential amplitude. To address this possibility, we positioned the highest intensity of excitation light closer to infarct zone than to normal tissue. This served to ensure that the low-amplitude signals we observed near the infarct zone were not due to a fall off in excitation light. Finally, to avoid signal amplitude decreases at the corners of the array (due to vignetting), all measurements were made with the boundary between the infarct and normal zones positioned in the center of the array. Therefore, the decrease in optical action potential amplitude that we observed in hearts with MI is likely due to changes in the electrophysiological substrate or a decrease in viable myocytes (see Limitations).

Using novel optical mapping techniques, we were able to demonstrate many of the electrophysiological characteristics associated previously with MI (4). For example, in two hearts, sustained VT was induced by programmed stimulation. In each case, slow conduction and conduction block were seen within the infarct zone. These data and the example of VT shown in Fig. 6 suggest that reentry is the likely mechanism of arrhythmogenesis reported in our study. Previous studies in canines with subacute MI have typically shown reentrant excitation with regions of functional block in the infarct border zone (12, 14, 17, 23, 31). We did observe functional block in some cases (Fig. 6); however, conduction block that persisted during steady-state pacing and VT (i.e., anatomical block) as shown in Figs. 3 and 5 was more common.

Recent studies by Takahashi et al. (29) and Qian et al. (26) have used standard di-4-ANEPPS perfusion techniques to optically map electrophysiological changes occurring during acute ischemia. In contrast, our study is the first to utilize a rapid di-4-ANEPPS superfusion technique to optically map action potentials in a whole heart model of late MI. Takahashi
et al. (29) used programmed stimulation to induce and characterize reentry in an isolated canine left ventricular wedge model of acute MI. By delivering optical dye via the perfusate before coronary occlusion, they demonstrated a reduction of optical action potential amplitude in the ischemic zone after ligation. The magnitude of optical action potential amplitude reduction that Takahashi et al. (29) observed during acute MI is similar to the decrease in action potential amplitude we observed in late MI. In addition, we observed slow conduction in border zone tissue, as did Takahashi et al. (29). In summary, our method of optical mapping late MI enabled us to observe similar MI-associated electrophysiological characteristics (e.g., slow and blocked conduction) as reported by Takahashi et al. (29) in acute MI.

Limitations. Given that optical mapping measures fluorescence from an aggregate of cells, the decrease in optical action potential amplitude that we observed in border zone tissue could be due to a decrease in the number of viable cells, a reduction in actual action potential amplitude, or a combination of both. It is also possible that when no optical action potential (i.e., flat line) was observed, the surviving epicardial rim was so thin that the resulting fluorescence could not be measured with our optical mapping techniques. Therefore, interpretation of such signals (i.e., low amplitude, flat line) is difficult unless, for example, microelectrode recordings are performed from the border zone.

In conclusion, we report a novel, rapid dye superfusion technique to enable whole heart, high-resolution optical mapping of late MI. We have shown that this experimental model reproduces many of the electrophysiological characteristics associated with MI in patients (e.g., slow conduction, block, and reentrant excitation) and can be used to illuminate the mechanistic relationship between abnormal cellular electrophysiology and arrhythmogenesis associated with late MI. These techniques may also be useful in the study of other electrical and anatomic heterogeneities associated with sudden cardiac death.

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