Enhanced Dispersion of Repolarization and Refractoriness in Transgenic Mouse Hearts Promotes Reentrant Ventricular Tachycardia


Abstract—The heterogeneous distribution of ion channels in ventricular muscle gives rise to spatial variations in action potential (AP) duration (APD) and contributes to the repolarization sequence in healthy hearts. It has been proposed that enhanced dispersion of repolarization may underlie arrhythmias in diseases with markedly different causes. We engineered dominant negative transgenic mice that have prolonged QT intervals and arrhythmias due to the loss of a slowly inactivating K⁺ current. Optical techniques are now applied to map APDs and investigate the mechanisms underlying these arrhythmias. Hearts from transgenic and control mice were isolated, perfused, stained with di-4-ANEPPS, and paced at multiple sites to optically map APs, activation, and repolarization sequences at baseline and during arrhythmias. Transgenic hearts exhibited a 2-fold prolongation of APD, less shortening (8% versus 40%) of APDs with decreasing cycle length, altered restitution kinetics, and greater gradients of refractoriness from apex to base compared with control hearts. A premature impulse applied at the apex of transgenic hearts produced sustained reentrant ventricular tachycardia (n=14 of 15 hearts) that did not occur with stimulation at the base (n=8) or at any location in control hearts (n=12). In transgenic hearts, premature impulses initiated reentry by encountering functional lines of conduction block caused by enhanced dispersion of refractoriness. Reentrant VT had stable (>30 minutes) alternating long/short APDs associated with long/short cycle lengths and T wave alternans. Thus, optical mapping of genetically engineered mice may help elucidate some electrophysiological mechanisms that underlie arrhythmias and sudden death in human cardiac disorders. (Circ Res. 2000;86:396-407.)

Key Words: arrhythmia ■ sudden death ■ mice, transgenic ■ di-4-ANEPPS ■ action potential ■ mapping

Cardiac arrhythmias and sudden death can result from rare congenital conditions and damage to the heart and its conduction system induced by ischemia, infarction, or cardiac surgery; drug therapies; and assorted noncardiac diseases. Many of the ion channels responsible for the generation of the cardiac action potential (AP) have recently been identified and cloned. A link between anomalies of repolarization and arrhythmias is most evident in patients with the congenital long QT syndrome, in whom the mutations of cardiac ionic channels prolong AP duration (APD), increase electrical heterogeneity, and lead to ventricular tachycardia (VT) and sudden death. Abnormalities in ion channel expression and ionic currents have also been reported in ischemia and congestive heart failure, which emphasizes their potential importance in the genesis of arrhythmias. A unifying hypothesis to account for arrhythmias in a broad range of cardiac conditions is that enhanced spatiotemporal dispersion of repolarization alters the myocardial substrate to initiate and sustain reentrant rhythms. In this setting, a premature beat can encounter a zone of conduction block and propagate unidirectionally to initiate reentry.

We developed a dominant negative transgenic mouse that overexpresses an N-terminal fragment of the K⁺ channel Kv1.1 in the heart under control of the α-myosin heavy chain promoter. This truncated channel fragment coassembles with wild-type Kv1.x subunits, traps heteromeric channels in the endoplasmic reticulum, and inhibits Kv1.x currents in a dominant negative manner in heterologous expression systems. Ventricular myocytes isolated from the dominant negative transgenic mice exhibited prolonged APDs due to the loss of a slowly inactivating 4-aminopyridine–sensitive current, I_DNI, that is likely encoded, at least in part, by Kv1.5. These mice have prolonged QT intervals, spontaneous nonsustained VT during ambulatory telemetry monitoring, and inducible polymorphic VT during right ventricular programmed stimulation in anesthetized, open-chest preparations.

Here, we applied voltage-sensitive dyes and optical mapping techniques to demonstrate that the elimination of specific ionic currents involved in the repolarization of the cardiac AP increases the dispersion of repolarization and...
refractoriness, alters restitution kinetics, and results in an enhanced vulnerability to reentrant VT. A preliminary report of these findings was presented in abstract form.20

Materials and Methods

Perfusion of Mouse Hearts

Animal procedures were approved by the IACUC of the University of Pittsburgh. Mice were anesthetized with fluothane. Hearts were cannulated and perfused (≈2.5 mL/min) with standard oxygenated Tyrode’s solution, producing 60 to 80 mm Hg of aortic pressure. Hearts were stained with 10 μL of di-4-ANEPPS (2 mmol/L in DMSO) during ≈5 minutes to monitor cardiac APs.21 Except for control studies with diacetyl monoxime (DAM; 15 mmol/L) and cytochalasin D (cyto-D; 5 μmol/L), APs were measured in 1.2 mmol/L free Ca2+ in the absence of chemicals that block contraction to avoid adverse effects on AP characteristics and intercellular coupling. A chamber was used to mechanically stabilize the heart (Figure 1A) and to minimize optical artifacts relative to AP amplitudes.22,23 The cannula was used to minimize resistance (~2 to 5 mm Hg) to flow (Figure 1B) and to allow a compliance chamber close to the heart.

Optical Apparatus

The optical apparatus (Figure 1C) and computer interface have been previously described.22,24,25 Epifluorescence was used to maximize light collection with a short working distance lens. An image of the heart was focused on a 12-element photodiode array, and 124 diodes were monitored. Image magnification was ×4.5, and 4×4 mm2 tissue was mapped. Each diode detected APs from an area of 312×312 μm2 (dead space between diodes=0.1 mm) with a depth of field of ≈70 μm.26

Analysis of Optical APs

Activation and repolarization times were determined from the 124 activation and repolarization time points and displayed as isochronal maps.22,23,25 Activation times were calculated from dF/dtmax of AP upstrokes.22,23 Optical APs and dF/dt were signal processed as previously described.24 An activation time was accepted if dF/dtmax was more than the SD of the background noise. Repolarization times taken at 75% or 90% recovery to baseline were used to determine APD75 and APD90 (see Figure 2). APs with signal-to-noise ratios of <10 or excessive movement artifact were eliminated, and the remaining activation (repolarization) times were triangulated with the use of Delaunay’s triangulation algorithm to overcome spatially irregular data sets. Isochronal lines were drawn from these triangles with linear interpolation and connecting points by lines. Local conduction velocities were calculated from dF/dtmax at 124 sites as previously described.23 Average velocity was calculated from the vectorial average of 124 local velocity vectors oriented between 0 and π radians. Maximum (θmax) and minimum (θmin) conduction velocities are measured relative to a cartesian coordinate system after pacing of the heart at the center of the image to elicit elliptical propagation waves.25 All analysis was automated with in-house software based on IDL 5.1 (Research Systems).26

Statistical Analysis

All values are reported as mean±SD. Student’s t tests were performed on APD and velocities to compare control and transgenic mice; P<0.05 was considered statistically significant. APDs (and refractory periods) from the same heart were averaged from 4 diodes at the base and apex and compared with the use of paired Student’s t test to determine gradients of repolarization (and refractoriness). Grouped t tests were used to determine the statistical significance of gradients of repolarization and refractoriness between control and transgenic mice.

Results

A number of control studies were performed to investigate the validity and interpretation of the data. Hearts stained with di-4-ANEPPS exhibited no changes in the intrinsic heart rate, amplitude of electrograms, or excitability measured as the threshold. In 5 hearts, optical APs and electrograms were recorded every 5 minutes for up to 5 hours to determine the stability of the preparations and the incidence of spontaneous arrhythmias. Heart rate and signal amplitudes remained stable for an average of 3.5 hours (maximum 5 hours, minimum 2.9 hours), and none had spontaneous arrhythmias. These control experiments confirm that di-4-ANEPPS has no overt toxic effects on the heart and that the preparation was stable for several hours.

Motion artifact was suppressed by placement of the heart in a chamber to mechanically stabilize the surface of the heart viewed with the array. One concern is that the isolation, perfusion, and immobilization of the hearts in the chamber could make them ischemic and arrhythmic. In mammalian hearts, ischemia produces a marked decrease in APD, conduction velocity, and force of contraction and often elicits electrical and mechanical alternans.27 Aortic perfusion pressure, bipolar electrograms (recorded from 1 to 3 sites), APD, and conduction velocity were measured before and after placement of the hearts in the chamber. In most experiments, aortic pressure increased by ≈5 mm Hg when the chamber suppressed force, whereas electrogram recordings were not altered, and APDs remained stable for several hours. In contrast, low-flow ischemia (10% to 15% of normal flow) markedly decreased the conduction velocity and dF/dt of AP upstrokes within 5 minutes. In addition, changes indicative of ischemia were apparent if the chamber was tightened. For these reasons, conduction velocity, dF/dt, and aortic pressure changes were regularly measured to ensure the chamber did not by itself produce cardiac ischemia or enhance the vulnerability to arrhythmias.

As an alternative approach to arrest motion artifact, we perfused several control hearts with agents that interfere with force development, such as diacetyl monoxime (DAM, 15 mmol/L) and cytochalasin-D (cyto-D, 5 μmol/L).28–31 Figures 2A and 2B show APs before (trace 0) and after (traces 1 to 3) exposure to DAM and cyto-D. DAM (n=5) produced a 2-fold prolongation of APD within 5 minutes (Figure 2A) and caused a time-dependent decrease in conduction velocity of ≈15% in 75 minutes. The DAM-induced decrease in conduction velocity is consistent with previous reports that DAM also blocks gap junctional communication.32 Cyto-D (n=4) also prolonged APD ≈2-fold within 15 minutes, associated with a prominent plateau phase (Figure 2B). With both agents, APDs remained prolonged but stable for up to 2 hours. At the concentrations used, both agents arrested contraction in 5 minutes and caused only minor changes in the voltage signals immediately after repolarization (<2% on phase IV). This suggests that the low-amplitude motion artifact that remains after stabilization of the heart in the chamber is unlikely to significantly and reproducibly affect the measurement of APD75 or APD90. It also demonstrates that DAM and cyto-D must be avoided in studies of repolarization and arrhythmia mechanisms in the mouse.
Figure 1. Chamber and optical apparatus. a, Digital image of a mouse heart in the perfusion chamber, with stimulating electrodes placed at the apex, base, and anterior edge of the left ventricle. The heart was perfused in a Langendorf apparatus, stained with a voltage-sensitive dye, instrumented with stimulating and recording electrodes, and placed in a fluid-tight, temperature-regulated chamber. The chamber consists of a cylindrical Delran main frame that slides and locks onto a stainless steel post that holds the aortic cannula. The main frame fastens a thermistor, recording and stimulating electrodes firmly in place on the heart, and a glass window locks onto the front of the main frame with a bayonet mount (one-eighth turn) to make a fluid seal with the main frame. The distance between the main frame and the cannula can be adjusted to permit an accurate control of the distance between the epicardium and the glass window to suppress motion artifacts while maintaining normal perfusion pressure to avoid ischemia. A cylindrical Teflon frame was inserted in the rear of the main frame to make a fluid seal such that the heart was bathed by the coronary outflow before it exits through a port on the top of the chamber. Temperature was regulated with a thermistor that controls a heating coil (located in the Teflon cylinder) via a feedback circuit; a roving thermistor (0.25-mm diameter) was used to verify the temperature was uniform along the epicardium of the heart. Several bipolar Ag/AgCl electrodes (0.25 mm diameter) were placed on the anterior, posterior, and right ventricular surfaces and used to pace or record electrograms simultaneously during optical mapping. A silhouette of the photodiode array (4×4 mm) is overlaid on the heart to identify the region of heart that was optically mapped. b, Design of the cannula used to minimize resistance to flow. c, Design of the optical apparatus. Light from a tungsten halogen lamp (100 W) was passed through a 520±30-nm interference filter (the excitation wavelength of di-4-ANEPPS; Omega Optica), reflected by a 45-degree dichroic mirror (<620 nm) and focused on the mouse heart with a macrocamera lens (50-mm, f 1:1.4; Nikon). A fluorescence image from the heart was collected with the same macro lens, filtered through a 630-nm long-pass Schott glass, and focused on the photodiode array. The photocurrent from each diode was passed through a current-to-voltage converter (50 MΩ feedback resistor), AC or DC coupled, amplified (×1, 50, 200, or 1000), digitized (2.0 kHz per channel, maximum of 3.9 kHz) at 12-bit resolution (DAP 3200e/214; MicroStar Laboratories), and stored in computer memory.
Figure 2. Effects of DAM and cyto-D on optical APs. A, Hearts from FVB control mice were perfused, stained with di-4-ANEPPS, and paced on the left ventricular epicardium (CL=80 to 300 ms). APs were recorded in the absence of DAM (15 mmol/L) and then after perfusion with the uncoupler every 5 minutes (for 1 to 2 hours). Traces recorded at the same CL and from the same diode were superimposed by aligning the time axis to illustrate the effects of chemical uncouplers on the time course of APs. Representative APs are shown before (trace 0) and after perfusion with DAM for 5 (trace 1), 10 (trace 2), and 30 (trace 3) minutes. Longer perfusion with DAM (1 to 2 hours) did not cause further prolongation of APD but triggered decrease in conduction velocity by 15% (n=5 hearts). At a CL of 120 ms, APD75 increased from 20±3.2 to 46.6±5.0 ms in the presence of DAM (15 mmol/L) for 10 minutes (average of 8 diodes and 3 hearts). B, APs recorded with the same diode are superimposed before (trace 0) and after perfusion with cyto-D (5 mmol/L) for 5 (trace 1) and 15 (trace 2) minutes. Longer perfusions with cyto-D (1 to 2 hours) did not cause further prolongation of APD but triggered spontaneous polymorphic VT (n=2 of 4 hearts). At a CL of 2 hours) did not cause further prolongation of APD but triggered spontaneous polymorphic VT (n=2 of 4 hearts). At a CL of 120 ms, APD75 increased from 20±3.2 to 46.6±5.0 ms in the presence of DAM (15 mmol/L) for 10 minutes (average of 8 diodes and 3 hearts). Note that the amplitude and signal-to-noise ratios of optical AP recordings were highly stable and show no signs of rundown. C, Analysis of APs from mouse ventricles: APs were filtered, and the first derivative was taken to measure dI/dtmax, the activation time point at which most of the cells viewed by that diode depolarized. APD75 and APD90 were calculated with an automated computer algorithm, which annotated the downstrokes to mark the repolarization time points at 75% and 90% recovery to baseline.

Figure 2C illustrates the analysis of a single AP trace with a computer algorithm that automatically identified the activation and repolarization (at 75% and 90% recovery to baseline) time points. The automated algorithm annotated each AP on the map to identify these time points. A manual override made it possible to correct or delete (typically <5% of the APs) from the analysis errors in the identification of repolarization time points caused by motion artifact or high background noise.

Figures 3A and 3B illustrate symbolic maps of the array; each diode is represented by a square box with the AP recorded by that diode in its respective location for a control and a transgenic heart. Mouse optical APs have the expected “triangular” shape (ie, no plateau phase) with rapidly rising upstrokes and brief durations. When examined on an expanded time base (Figures 3C and 3D), marked prolongation of APD is apparent in transgenic hearts.

In sinus rhythm, activation of the ventricles is driven by the Purkinje fibers of the conduction system of the heart, resulting in a rapid depolarization of the ventricles in 5 to 6 ms for both control and transgenic hearts (Figure 4A). When paced at the center of the left ventricle (300 bpm), activation spread elliptically, with maximum (θmax=0.85±0.13 m/s) and minimum (θmin=0.33±0.17 m/s) conduction velocities at 135/315- and 45/225-degree angles, respectively (n=4 hearts, Figure 4B). The orientation of θmax and θmin was similar to that of guinea pig left ventricle, where they corresponded to longitudinal and transverse axes of epicardial fiber orientations. The average anisotropy of conduction velocities taken as the ratio of θmax to θmin was 2.25 to 1. It should be noted that pacing produces a “virtual electrode” effect that appears as a zone of synchronous depolarization around the electrode site (Figure 4B). Local velocity vectors from this zone were automatically removed from the calculations of conduction velocity.

When paced at the apex, activation spread from apex to base (Figure 4C). The mean conduction velocity (mostly transverse, with a component of longitudinal velocity) was similar for control (θc=0.51±0.17 m/s [mean±SD], n=5) and transgenic (θt=0.49±0.13 m/s, n=5) mouse hearts. In sinus rhythm or when pacing at various sites on the epicar-
ventricular myocytes from these hearts with the use of patch (Figure 5A). Similar findings were reported in isolated hearts when measured at 75% or 90% recovery to baseline ms apart. Activation and repolarization patterns were similar in control and transgenic hearts.

A. Sinus Rhythm

B. Activation, Center Stimulation

C. Activation, Apex Stimulation

D. Repolarization

Figure 4. Patterns of activation and repolarization in the control heart. a, Isochronal lines of activation during sinus rhythm. In this and subsequent activation maps, the first site to depolarize is depicted in “white” (ie, time t=0.0 ms) and subsequent depolarizations are depicted in increasingly darker shades, with isochronal lines 1 ms apart. b, Activation pattern during pacing at the center of the left ventricular free wall, which is positioned at the center of the field-of-view of the array. Conduction velocities were calculated from dF/dt at 124 sites.23 For each diode, a local velocity vector was calculated from the activation time delays between that diode and its 8 nearest neighbors and the interdiode distance. The average velocity was calculated from the vectorial average of 124 local velocity vectors oriented between 0 and π radians. Average velocities along different directions were calculated from the projection of the average vector onto a set of 36 orientations from 0 to 180 degrees, with a bin width of 5 degrees. The maximum (θmax) and minimum (θmin) scalar amplitudes of vectors oriented from 0 to 180 degrees represented the fastest and slowest conduction velocities, respectively, measured relative to a cartesian coordinate system. Its ordinate was oriented base to apex, and its abscissa was oriented in the anterior-to-posterior direction. To determine θmax and θmin, the heart was paced at the center of the area viewed by the array to elicit an elliptical propagation wave. To calculate an “apparent” conduction velocity during sinus rhythm and maximum and minimum velocities during pacing at the center of the heart, the algorithm required some threshold limits during various phases of the calculation. The spatial and temporal resolutions are 0.33×0.33 mm and 0.5 ms, respectively, and the minimum distance and local activation time delay between diodes are 0.66 mm and 0.5 ms, respectively. Thus, the maximum local conduction velocity that can be measured is 0.66/0.5, or 1.3 m/s. At the stimulus site (the center of the ellipse), the electrode distorts the background fluorescence and produces a “virtual electrode” effect. There is no detectable time delay among the 2 or 3 diodes (3-point lagrangian), as would be expected if a small region of tissue is synchronously activated. These points are then automatically removed from the calculation of local vectors and averaged conduction velocity. Thus, the analysis of propagation begins outside the “virtual electrode” zone. c, Activation pattern during pacing at the apex of the left ventricle. In paced hearts, activation occurred in 10 to 18 ms. d, Repolarization pattern of a heart paced at the apex was determined from the repolarization time points measured at APD75. Repolarization patterns were found to be similar in sinus rhythm and in hearts paced at the center or apex of the left ventricle. Repolarization spread from apex to base; the first site to repolarize was depicted in “white” (ie, time t=0.0 ms), and repolarization spreads toward increasingly darker shades, with isochronal lines 1 ms apart. Activation and repolarization patterns were similar in control and transgenic hearts.

APD35 and APD90 were longer in transgenic than in control hearts when measured at 75% or 90% recovery to baseline (Figure 5A). Similar findings were reported in isolated ventricular myocytes from these hearts with the use of patch microelectrodes.13 At any site on the heart, the APD depends on the basic cycle length (CL) and the local restitution kinetics of the AP after an abrupt change in CL or a premature impulse. Figure 5B compares the changes in APD as a function of CL for transgenic and control hearts. The classic shortening of APD with decreased CL was not observed in transgenic hearts. The “flat” APD-versus-CL relationship in transgenic hearts suggests that the “missing” current, I_{slow}, plays an important role in the adaptation of APD to changes in heart rate, especially at short CLs.

As in guinea pig hearts,25 APDs in mouse hearts were heterogeneous along the epicardium, with APD_{35} being shorter at the apex (19±3.3 ms) than at the base (29±2.2 ms, n=6, P<0.05). Gradients of APD from apex to base were also found in the presence of the chemical uncouplers 15 mmol/L DAM and 5 μmol/L cyto-D. From apex to base, APD_{35} increased from 63±4 ms to 69±5 ms (n=3) with DAM and from 45±5 to 50±8 ms (n=3) with cyto-D. The heterogeneity of APDs was more pronounced in transgenic hearts than in control hearts, with APD_{35} of 32±4.3 ms at the apex and APD_{35} of 54±5.3 ms at the base (n=7, P<0.005). The spatial dispersion of repolarization from apex to base was also greater in transgenic (10±3 ms, n=7) than control (6±2.7 ms, n=7) hearts.

Dynamic (ie, temporal) heterogeneities of AP amplitude (APA) and duration are produced by abrupt changes in heart rate or by a premature impulse.27 Figure 5C demonstrates the response characteristics of control APs with decreasing S1-S2 interval. Figure 5D plots the restitution kinetics of APA for the apex and base of control and transgenic mouse hearts. In control hearts, the restitution curves at the apex and base decreased gradually with decreasing S1-S2 interval, until the premature impulse fired during the refractory period and failed to capture. In contrast, transgenic hearts had steep
restitution curves, particularly at the apex, and failed to capture at considerably longer S1-S2 intervals (Table). The enhanced dispersion of repolarization and refractoriness resulted in a highly arrhythmogenic substrate. A single premature stimulus applied near the apex of transgenic hearts reproducibly elicited long-lasting (≥30 minutes) reentrant VT (n = 14/14 hearts). Similar stimulation at the apex of control hearts failed to elicit reentrant VT (n = 12), except for 1 control heart in which a premature pulse at the apex elicited a brief run of nonsustained VT (5 beats). Figure 6A shows optical recordings from the array captured during the initiation of a representative arrhythmia, and Figure 6B illustrates traces from 3 locations on an expanded time scale. Each diode recorded 2 basic beats (CL = 200 ms), the premature impulse (S1-S2 = 60 ms), and the first 5 beats of the VT. Activation of the basic paced beat (S1) propagated from apex to base in 17.0 ms (Figure 6C). The premature impulse applied at the apex triggered APs of lower amplitude (as in Figures 5C and 5D, bottom trace) that propagated 1 mm toward the base before encountering a line of refractory myocardium (Figure

Figure 5. Spatiotemporal heterogeneities of APs in control and transgenic hearts. a, Histogram of mean APD<sub>75</sub> and APD<sub>90</sub> for control (n = 6) and transgenic (n = 7) myocardium (APD<sub>75</sub> 39±8 ms [n = 6] in transgenic hearts; 23±3 ms [n = 6] in control hearts, P<0.05; APD<sub>90</sub> 52±6 ms [n = 7] in transgenic hearts; 29±2 ms [n = 6] in controls, P<0.01). b, Percent change in APD<sub>75</sub> versus CL for transgenic and control hearts. Each data point represents the mean±SD of APDs measured from 8 diodes at the center of the array for either control or 5 transgenic hearts. The plots demonstrate decreased adaptation of transgenic APD as a function of rate. c, Response characteristics of the ventricular AP for a control heart after a premature impulse (S2). The heart was paced at a basic CL (S1-S1 = 200 ms), and at every 10th beat, a premature impulse was applied at varying S1-S2 intervals. The subsequent AP elicited by the premature impulse had an increasingly smaller amplitude and a concurrent decrease in APD and APA with decreasing S1-S2 intervals. The basic and premature impulses were set at 1.2 times the threshold voltage to ensure that the capture remained sensitive to the relative refractory period. d, Restitution kinetics of AP amplitude (APA) for control and transgenic hearts were measured by pacing the ventricle at a basic CL (S1-S1 = 200 ms) for 10 beats, followed by a premature pulse (S2) applied at varying (S1-S2) intervals. S1-S2 varied in steps of 5 ms and then steps of 1 ms as S1-S2 approached the refractory period, until the premature pulse fired during the absolute refractory period and failed to capture. Restitution kinetics were taken as a quantitative measurement of the degree of variation of the AP amplitude (APA) at a given site on the heart, as a function of diastolic interval. The dispersion of refractoriness between apex and base was 10 ms (Table). In the transgenic heart, there was a steep decrease in APA when S1-S2 was <70 ms for apex stimulation and a refractory period that was a maximum of 80 ms at the base. The restitution curves shown here were reproduced in 5 control and 5 transgenic mice, and the dispersion of refractoriness in transgenic mouse hearts was 2-fold greater than that measured in control (Table). Restitution curves were plotted as a function of S1-S2 instead of diastolic interval to better distinguish differences between apex and base.

The enhanced dispersion of repolarization and refractoriness resulted in a highly arrhythmogenic substrate. A single premature stimulus applied near the apex of transgenic hearts reproducibly elicited long-lasting (≥30 minutes) reentrant VT (n = 14/14 hearts). Similar stimulation at the apex of control hearts failed to elicit reentrant VT (n = 12), except for 1 control heart in which a premature pulse at the apex elicited a brief run of nonsustained VT (5 beats). Figure 6A shows optical recordings from the array captured during the initiation of a representative arrhythmia, and Figure 6B illustrates traces from 3 locations on an expanded time scale. Each diode recorded 2 basic beats (CL = 200 ms), the premature impulse (S1-S2 = 60 ms), and the first 5 beats of the VT. Activation of the basic paced beat (S1) propagated from apex to base in 17.0 ms (Figure 6C). The premature impulse applied at the apex triggered APs of lower amplitude (as in Figures 5C and 5D, bottom trace) that propagated 1 mm toward the base before encountering a line of refractory myocardium (Figure

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Refractory periods (RPs) were measured by applying a programmed stimulation protocol at the base and apex of each heart. The refractory period was determined from the average of 6 photodiodes or sites located at the base or apex. Difference in RPs between apex and base was significant for transgenic hearts (P=0.018) and borderline for controls (P=0.06). Difference in RPs between control and transgenic hearts was also significant (P<0.02), and the gradient of refractoriness was ~3-fold greater in transgenic than control hearts.

6D, black arrow). APs propagated below the functional line of conduction block from the anterior to posterior edge of the heart (horizontal white arrow), reemerged above the line of block ~12 ms later (curved arrow), and spread toward the base of the heart (Figure 6D, diagonal white arrow). Optical recordings from the region of conduction block detected 2 distinct depolarizations (seen as a double “hump”) during the premature pulse, corresponding to membrane depolarization below and above the line of block (Figure 6B, trace 2). The subsequent APs during VT propagated from the anterior aspect of the apex toward the posterior aspect base (Figure 6E), demonstrating that the conduction block was dynamic and not caused by a fixed anatomical barrier. The wavelength of the reentrant circuit (conduction velocity of the leading edge (0.27±0.02 m/s, n=4) multiplied by the mean APD₉₀ (39.8±4.3 ms, n=4) was ~10.8 mm, which is consistent with a reentrant circuit around the perimeter of the mouse heart. Activation patterns during the circus movement (Figure 6E, white arrow) were noteworthy for the stability of the AP amplitude, direction, and rate for >45 minutes and for the reproducibility from heart to heart.

The ability of the premature pulse to trigger a circus movement arrhythmia depended on its location. Stimuli at the base of transgenic hearts never elicited VT (n=8 hearts) because when a premature pulse captures near the base of the heart, the ensuing AP propagated toward the apex and always encountered excitable myocardium with shorter refractory periods. Under these conditions, the activation wave failed to encounter conduction blocks and to initiate VT. On the other hand, the ability of premature stimuli at the apex to elicit VT was independent of the basic activation pattern. For instance, in a heart in sinus rhythm (Figure 6F), a premature pulse applied on the anteroapical edge of the heart encountered a functional arc of block and propagated as a clockwise rotor around the block (Figure 6G). Although the activation pattern produced by the premature pulse was dependent on its location, the subsequent reentry patterns typically spread apex to base across the epicardium (Figure 6H).

A detailed analysis of APs and activation patterns during VT revealed the presence of long-lasting, stable alternans (n=6 of 7). VT beats oscillated between large-amplitude, long-duration APs that propagated at rapid conduction velocities and low-amplitude, short-duration APs that propagated at slower conduction velocities (Figure 7A). In 1 heart, alternans occurred at a 1:2 ratio, with the large-amplitude, rapidly conducting APs occurring every third beat. Electrogram recordings from the opposite side of the heart showed...
that electrical alternations on the right ventricle had the same oscillations in CL but were out of phase with those recorded on the left ventricle. The similar frequencies of electrical alternations on the right ventricle had the same amplitude and long duration. Note the presence of CL and T wave alternans with APs of large amplitude and short durations followed by APs of short amplitudes and long duration. The present optical measurements of APD alternans at CLs of 79±1 and 87±1.5 ms (n=5 beats each), \( I_n \), is the interactivation interval at beat n of VT.

slower VT beat propagates around the circuit and allows for greater recovery from refractoriness, thus ensuring that the tachycardia does not self-extinguish. The steep slope of the restitution curve in transgenic mice may also play a role in the maintenance of stable reentrant VT. Variations in the amplitude of APD alternans from heart to heart may reflect differences in the length of the reentry pathway, differences in conduction velocity oscillations, or both.

**Discussion**

The production of mice with specific genetic modifications of cardiac ion channels, cell coupling proteins, and \( \text{Ca}^{2+} \) transport proteins has generated a growing interest in methods to investigate the electrophysiology of the intact mouse heart. Optical mapping offers a unique approach to map APs and characterize its electrical properties. In the present study, we refined optical mapping techniques to record high-fidelity APs from multiple sites on the epicardium of control and genetically engineered mouse hearts.

**Electrophysiology of the Mouse Ventricle**

Optical mapping of APD is particularly challenging in the mouse ventricle because of the short and triangulated shape of the AP. In guinea pig hearts, we had shown that the second derivative of the AP downstroke, \( \frac{d^2F}{dt^2} \), can be relied on to detect the repolarization time point at 97% recovery to baseline. In the approach, however, is only applicable to APs with a stable plateau and rapid downstroke. DAM, verapamil, and cyto-D have been used to abate contractions and diminish optical movement artifacts. In hearts from larger mammals, these uncouplers shorten APD, reduce intercellular coupling, alter restitution kinetics, and act as antiarrhythmic agents. In mouse ventricle, we found that DAM and cyto-D blocked contractions, as expected, but markedly prolonged APD. Hence, in the present study, we relied on mechanical suppression motion artifact. This, along with the high signal-to-noise ratio, made it possible to measure APD\(_{50}\), APD\(_{75}\), and APD\(_{90}\) with high accuracy and to map repolarization as well as activation.

**APD Measurements**

The mouse ventricular AP is triangulated, has no plateau phase, and is shorter than that of most other mammals. The APD that we measured with optical techniques (eg, APD\(_{50}=23±3\) ms) is similar to that reported in mouse whole-cell voltage-clamp studies. In addition, our measurements of APD\(_{50}\) at CL=120 ms in the presence of 15 mmol/L DAM (47±5 ms) agree with a recent report in which \( \text{di-4-ANEPPS} \) and a CCD camera (44±3 ms) were used. The gradient of APD from apex to base is also similar to that found in the guinea pig.

**Activation and Conduction Velocities**

The present optical measurements of mean conduction velocity (\( \Theta_{\text{CL}}=0.51±0.17\) m/s [mean±SD], n=5) in control mouse hearts are in excellent agreement with those obtained with electrodes (0.4±0.1 m/s). In hearts paced at the center of the field-of-view of the array, activation spread elliptically on the left ventricle with maximum (\( \Theta_{\text{max}}=0.85±0.13\) m/s) and
minimum ($\Theta_{\text{min}}=0.33\pm0.17 \text{ m/s}$) velocities. Our results are similar to those of a recent report in which mouse hearts were perfused with DAM and optically mapped with a CCD camera ($\Theta_{\text{min}}=0.63\pm0.04 \text{ m/s}$, $\Theta_{\text{min}}=0.38\pm0.02 \text{ m/s}$) when the 15% to 20% reduction in conduction velocities caused by 15 mmol/L DAM is taken into account. Conduction velocities in mouse hearts are fast but within the range reported in guinea pig, rabbit, and dog hearts. In addition, the orientations of $\Theta_{\text{min}}$ and $\Theta_{\text{min}}$ were closely aligned with the longitudinal and transverse axes of epicardial fibers, as shown for guinea pig hearts. This implies that there is a similar fiber orientation on the mouse and guinea pig epicardium.

**Postpolarizational Refractoriness**

Mouse ventricular APs have refractory periods that are considerably longer than their APDs. At a CL of 200 ms, the refractory period at the base of the heart was 53.8±12.7 ms compared with an APD$_0$ of 29±2 ms. This surprisingly long postpolarizational refractory period was observed in all regions of the ventricles. Experiments in open-chest anesthetized mice with programmed stimulation showed even longer refractory periods (96.7±24.0 ms at 200-ms CL). Postpolarizational refractoriness is likely due to the slow recovery from inactivation of inward currents ($I_{\text{Na}}$ and $I_{\text{Ca}}$), which depends in a complex way on the time and voltage changes of the previous AP downstroke. The refractory period in the mouse may also be influenced by an increase in $K^+$ conductance due to the slow inactivation kinetics of $K^+$ currents that control repolarization. In contrast with other mammals, normal mouse hearts have a marked postpolarizational refractoriness, which could be due to a slower recovery from inactivation of inward currents ($I_{\text{Na}}$ and $I_{\text{Ca}}$) or a small increase in resting membrane potential compared with other mammals. The latter is analogous to the effect of ischemia on other mammalian hearts, where a depolarization of the resting membrane potential results in a postpolarizational refractory period.

**Restitution Kinetics**

The most common method of measuring restitution is to plot the APD of the premature beat as a function of the diastolic interval. The short duration of the mouse heart AP made it difficult to measure statistically significant decreases in APDs of the premature beats, particularly during the steep slope of the restitution curve. We therefore plotted the restitution of APA, which depends on the restitution of inward currents ($I_{\text{Na}}$ and $I_{\text{Ca}}$) and indirectly on $K^+$ repolarizing currents. Previous studies had shown that APA decreased with decreasing S1-S2 intervals. We found similar results with the mouse.

**Control Versus Transgenic Hearts**

We showed that disruption of a component of the repolarizing $K^+$ current in transgenic mice produces APD prolongation, increased refractoriness, and enhanced dispersion of repolarization and refractoriness, resulting in a highly arrhythmogenic substrate. Alterations in the $K^+$ repolarizing currents then indirectly affect excitability through changes in $I_{\text{Na}}$ (or $I_{\text{Ca}}$ or both). Several lines of evidence support the primary role of the $K^+$ channel alterations as a cause of enhanced vulnerability to arrhythmia. (1) The rise time of APs, dF/dt$_{\text{max}}$, and conduction velocities in control and transgenic were similar through a broad range of heart rates (CL = 100 to 300 ms), suggesting that no unexpected anomalies in Na$^+$ channel properties occurred in transgenic mice. (2) The period of postpolarizational refractoriness (refractory period minus APD$_0$) was the same in hearts from control (13.9±4.9 ms) and transgenic (12.24±3.4 ms) mice (n = 4 each). The dispersion of repolarization is not necessarily the same as the dispersion of refractoriness, particularly in conditions such as ischemia that promote postpolarizational refractoriness. In control mice, we found that postpolarizational refractory periods were uniform at the apex and base of the ventricles and that the dispersions of repolarization and refractoriness were alike. Moreover, postpolarizational refractory periods are similar in control and transgenic mice, suggesting that the recovery of inward currents ($I_{\text{Na}}$ and $I_{\text{Ca}}$) was not modified in transgenic mice. Hence, the enhanced dispersion of refractoriness found in transgenic mice is primarily a consequence of the enhanced dispersion of repolarization. The highly arrhythmogenic substrate in transgenic mice may be formed by the steep slope of APA restitution (ie, a prolonged relative refractory period) at the apex in combination with the enhanced gradient of refractory periods from apex to base.

Prolongation of APD has been proposed as a strategy to protect the heart from triggered activity and arrhythmias through a lengthening of the refractory period. More recent data have shown that drugs that prolonged APD and QT interval lead to arrhythmias such as torsade de points. The precise mechanism by which APD and QT prolongation leads to arrhythmias is not fully understood, but enhanced dispersion of refractoriness has been proposed as a possible explanation. In principle, APD prolongation should be antiarrhythmogenic if homogeneous on the ventricle, but when accompanied by an enhanced spatial dispersion of repolarization and refractoriness, it may become proarrhythmic.

Isolated hearts from control or transgenic mice did not exhibit episodes of spontaneous arrhythmias during optical mapping experiments. On the other hand, programmed stimulation with a single premature pulse elicited monomorphic reentrant VT in transgenic (14 of 14) but not control mouse hearts. The location of the premature impulse was critical to its ability to elicit VT. An extra pulse applied near the apex of the heart triggered VT, but pulses applied near the base had no effect. The initiation of VT was characterized by capture of the extra pulse near the apex and its slow propagation due to the relative refractoriness of the tissue. The premature wavefront spread toward the base, encountered a functional line of block, and spread below, then around above the block.

The larger gradient of APD and refractoriness from apex to base (≈2-fold) in transgenic mice may result from the direct loss of nonuniformly distributed $K^+$ channels of the Kv1.x family or from the increased contribution of other nonuniformly distributed channels (in the absence of $I_{\text{Na}}$) to the total current. Of note, nonuniform distribution of $I_{\text{K}}$ in the Kv4.x channels has been described, and transgenic mice that overexpress a mutated Kv4.2 subunit lack $I_{\text{Na}}$,
similar prolongation of APD and QT interval but do not have arrhythmias.\textsuperscript{51} Thus, although APD prolongation may lead to increased early and delayed afterdepolarizations in mammalian hearts with long APDs, spatial and temporal dispersions of repolarization and gradients of refractoriness may be of greater importance in the initiation of reentrant arrhythmias.

Mechanisms Underlying Stable VT in Transgenic Mice

Spatiotemporal analysis of ventricular fibrillation (VF) in hearts of larger animals (dog, rabbit, and sheep) has revealed that VF previously thought to be frenzied and chaotic actually consists of several reentry circuits or rotors with frequencies of 8 or 10 Hz.\textsuperscript{52,53} The reentry circuits in VF, elicited by premature impulses, appeared to span 2 to 3 mm of epicardium (unknown depth) and to maintain relatively stable patterns for 5 to 10 minutes. Thus, reentrant waves are responsible for both VT and VF, and the elucidation of factors that initiate and stabilize reentry is fundamental to our understanding of the causes of sudden cardiac death.

The transgenic mouse described here offers an experimental model of stable VT alternans. The interplay of prolonged APD and enhanced gradient of refractoriness adjusted the wavelength of reentrant circuits (APD×conduction velocity) to match the reentry pathway or the approximate dimensions of the heart. Each rapidly propagating reentry wavefront catches up to its tail and encounters relatively refractory tissue, resulting in a slowing down of conduction. The slow reentrant wavefront propagates through a cycle and then encounters excitable tissue to initiate a fast reentrant beat; hence, the VT alternans are perpetuated. The stable alternans shown in the transgenic mouse hearts in VT is in sharp contrast to reentrant circus movement VT in rings of atrial tissue where CL oscillations preceded termination of VT.\textsuperscript{54} Simulations of spiral wave breakup predict that electrical alternans leads to spatially disorganized wave activity and a transition from VT to VF.\textsuperscript{55}

The results of the present study emphasize that changes in distribution and abundance of ionic currents alone, in the absence of metabolic injury of anatomical barriers, can increase the vulnerability to reentrant VT. Changes in ion channel abundance and cardiac ionic currents are well documented in pathological conditions such as congestive heart failure.\textsuperscript{7,8} Clearly, substantial differences exist between humans and mice with respect to the ionic basis of the cardiac AP, the distribution of ion channels, and the dimensions of the heart. Despite this, studies on molecularly engineered mice may help to elucidate common mechanisms that lead to arrhythmias and sudden cardiac death in human disease states.

Study Limitations

A major concern is that motion artifact might limit the validity and interpretation of APD and repolarization maps in the mouse. All methods that were used to measure APDs, whether intracellular electrodes, extracellular electrodes, or voltage-sensitive dyes, encounter problems caused by muscle contractions, which distort AP downstrokes and the time course of repolarization. We were unable to use agents that inhibit contraction such as DAM or cyto-D due to the changes they caused in APD. We therefore relied on a chamber to inhibit motion.

Several lines of evidence indicate that we have sufficiently eliminated motion artifact to minimize significant errors in repolarization time points. (1) Optical mapping techniques rely on extensive repetition to ensure the validity and reproducibility of APDs and are based on simultaneous measurements from multiple sites. We found remarkable consistency in our measurements, including identical findings with APD\textsubscript{75} and APD\textsubscript{90}. (2) Minimal baseline drift was seen after repolarization of the AP (\(<2\%\) estimated error in APD\textsubscript{90}), and only minor changes were seen after the addition of DAM or cyto-D. In the absence of the mechanical stabilizing chamber, appreciable errors were seen. (3) A reproducible pattern of repolarization was seen (Figure 4D). This would be unlikely if major errors of repolarization were present. (4) We found that the refractory period gradients were similar to those of APD. The former are not subject to motion artifacts. (5) Gradients of APD between apex and base remained in the presence of DAM and cyto-D, despite marked prolongation of APD. Thus, although we cannot absolutely exclude some component of error due to motion artifacts, we believe that repolarization maps were quantitatively accurate and that enhanced dispersion of repolarization underlies the reentrant arrhythmia in transgenic mice.

To measure restitution kinetics, the stimulating electrodes used to apply the basic or premature impulses, or both, must have small dimensions (\(\approx250-\mu\text{m}\) diameter) and the shock strength must be \(\approx1.5\) times the threshold voltage. Otherwise, the stimuli can readily depolarize a large percentage of the mouse ventricle and effectively shock rather than pace these small hearts. We took care to maintain these conditions.

The optical technique used in the present study imaged the initiation and maintenance of reentrant VT on a 4×4-mm\textsuperscript{2} area of the epicardial surface of the left ventricle. We did not simultaneously visualize the septum or right ventricular surface, leading to uncertainty regarding the complete path of the reentrant arrhythmia. Bipolar electrograms from the right ventricular surface were monitored, however, and demonstrated continuity with the arrhythmia on the left ventricular epicardial surface. Similarly, optical recordings and programmed stimulation were not performed in the midmyocardium or on the endocardium. Differences in ion channel distribution have been documented across the myocardial layers of larger mammals,\textsuperscript{50} and we cannot exclude differences in the electrophysiological properties across the myocardium of the mouse heart. We doubt that this will affect the conclusions of the present study, however, given the thinness of the mouse heart wall (\(<1\) mm) and the similar results obtained during epicardial pacing and sinus rhythm with endocardial activation.

An N-terminal fragment of the K\textsuperscript{+} channel Kv1.1 was used to disrupt repolarizing K\textsuperscript{+} currents of the Kv1.x family in the hearts of the mice that we studied. Kv1.2, Kv1.4, and Kv1.5 are expressed in the heart;\textsuperscript{2} the relative extent and importance of these channels in cardiac repolarization remain uncertain. Thus, the detailed molecular mechanism by which the transgene leads to QT prolongation and arrhythmias remains under
investigation. In addition, it is possible that the transgene may affect repolarization in other ways (eg, by binding β-subunits). Despite these concerns, the results shown here directly link changes in the dispersion of repolarization and refractoriness to an enhanced susceptibility to reentrant VT. The mouse is not an ideal model for human cardiac electrophysiology or for the human long-QT syndrome. Its small size may limit reentrant circuits, its basal heart rate is >600 bpm, and its APD is short even when corrected for heart rate. However, the mouse model provides the unique advantage of molecular engineering to genetically manipulate ionic currents involved in the repolarization of the AP. In this system, we have shown that manipulations that increase the dispersion of repolarization and refractoriness from apex to base increase susceptibility to reentrant VT. We speculate that in disease states like ischemia, similar changes in repolarization and postrepolarization refractoriness may enhance the propensity to arrhythmias and sudden death in larger mammals, including humans.

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