Calcium-dependent arrhythmias in transgenic mice with heart failure

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London, Barry, Linda C. Baker, Joon S. Lee, Vladimir Shusterman, Bum-Rak Choi, Toru Kubota, Charles F. McTierman, Arthur M. Feldman, and Guy Salama. Calcium-dependent arrhythmias in transgenic mice with heart failure. Am J Physiol Heart Circ Physiol 284: H431–H441, 2003. First published October 17, 2002; 10.1152/ajpheart.00431.2002. —Transgenic mice overexpressing the inflammatory cytokine tumor necrosis factor (TNF-α) (TNF-α mice) in the heart develop a progressive heart failure syndrome characterized by biventricular dilatation, decreased ejection fraction, atrial and ventricular arrhythmias on ambulatory telemetry monitoring, and decreased survival compared with nontransgenic littermates. Programmed stimulation in vitro with single extra beats elicits reentrant ventricular arrhythmias in TNF-α (n = 12 of 13 hearts) but not in control hearts. We performed optical mapping of voltage and Ca²⁺ in isolated perfused ventricles of TNF-α mice to study the mechanisms that lead to the initiation and maintenance of the arrhythmias. When compared with controls, hearts from TNF-α mice have prolonged of action potential durations (action potential duration at 90% repolarization: 23 ± 2 ms, n = 7, vs. 18 ± 1 ms, n = 5; P < 0.05), no increased dispersion of refractoriness between apex and base, elevated diastolic and depressed systolic [Ca²⁺], and prolonged Ca²⁺ transients (72 ± 6 ms, n = 10, vs. 54 ± 5 ms, n = 8; P < 0.01). Premature beats have diminished action potential amplitudes and conduct in a slow, heterogeneous manner. Lowering extracellular [Ca²⁺] normalizes conduction and prevents inducible arrhythmias. Thus both action potential prolongation and abnormal Ca²⁺ handling may contribute to the initiation of reentrant arrhythmias in this heart failure model by mechanisms distinct from enhanced dispersion of refractoriness or triggered activity.

Arrhythmias remain a major health problem in cardiomyopathies of both ischemic and nonischemic origin. As many as 50% of patients with congestive heart failure (CHF) die suddenly, and this accounts for at least 250,000 annual deaths in the United States (42). Pharmacological treatments of arrhythmias can do more harm than good, and device therapies are limited by high cost and the limitations that they bring to quality of life (5). Prolonged action potential duration (APD) and downregulation of the repolarizing transient outward K+ current (Ito) and inward rectifier K+ currents (IK1) are present in tissue and cardiac myocytes isolated from patients and animal models with CHF (3, 16, 25, 39). This delayed repolarization, along with enhanced dispersion of repolarization, may contribute to arrhythmias and sudden death (10, 12, 40). Altered intracellular Ca²⁺ handling in the heart, including decreased peak systolic Ca²⁺, elevated diastolic Ca²⁺, and prolongation of the Ca²⁺ transient are also present in CHF (4, 15, 27, 29). These alterations in Ca²⁺ handling can lead to triggered activity and arrhythmias (11). The relative contributions of abnormalities in depolarization and Ca²⁺ handling to the genesis of arrhythmias has yet to be determined. Inflammatory cytokines, including TNF-α, are increased in the serum and hearts of patients with CHF and may contribute to the pathophysiology of the disease (22, 37, 38). We recently engineered mice that overexpress TNF-α in the heart under the control of the α-myosin heavy chain promoter (TNF-α mice) and develop a cardiomyopathy characterized by biventricular dilatation, decreased left ventricular ejection fraction, and decreased survival compared with nontransgenic littermates (19). Most of the mice exhibit symptoms of CHF before death (tachypnea, cyanosis, and ascites) and show evidence of compensated heart failure at autopsy (pleural effusions, hepatic congestion, and severe atrial and ventricular dilatation). We now show that these mice develop arrhythmias on ambulatory telemetry monitoring and use optical mapping of voltage and Ca²⁺ in hearts isolated from the mice to demonstrate that abnormalities in both voltage and Ca²⁺ may contribute to the initiation of reentrant arrhythmias by mechanisms distinct from enhanced dispersion of refractoriness or triggered activity.

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MATERIALS AND METHODS

Breeding of TNF-α transgenic mice. All studies were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Heterozygous TNF-α transgenic mice were bred with FVB controls to generate TNF-α mice and wild-type littermate controls. Mice aged 3 to 9 mo were used for the current studies. For all studies, TNF-α and control mice were age, sex, and strain matched.

Ambulatory recording of arrhythmias in mice. Radiotelemetry electrocardiogram monitors (Data Sciences) were implanted subcutaneously on the backs of TNF- and control mice with the use of tribromoethanol (Avertin) anesthesia as previously described (23). The mice were allowed to recover for at least 6 days, at which point 24 h of telemetry was recorded and saved on disk at 400 Hz. Monitoring was then continued for up to 4 mo. All telemetry data were scanned by hand for atrial and ventricular arrhythmias. At least 95% of the data for each mouse was adequate for analysis. A computer-based arrhythmia detection system was simultaneously developed and tested using this telemetry.

Optical mapping of action potentials. Mice were anesthetized with fluothane, heparinized (50 units ip), and then euthanized. The heart was then rapidly excised, cannulated, and placed in a chamber specifically designed to immobilize, pace, and focus an image of the left ventricular free wall on a photodiode array as previously described (2). The perfusate contained (in mM) 141 NaCl, 25 NaHCO3, 5 HEPES, 1.2 NaH2PO4, 1.0 MgSO4, 5.0 KCl, 50 dextrose, and 1.8 CaCl2 (pH 7.4), bubbled with 95% O2-5% CO2. Perfusion pressure was maintained at 60-80 mmHg, and the temperature of the perfusate was maintained at 37°C by feedback control as previously described (2). Hearts were stained with the voltage-sensitive dye 1-(3-sulfonatopropyl)-8-[2-(di-n-butylamino)-6-naphthyl]vinyl]pyridinium betaine (di-4-ANEPPS, 10-15 µM of a 3 mM stock solution in DMSO) delivered as a bolus through the port of a bubble trap, which resulted in homogeneous dye loading throughout the heart and action potentials that were stable for up to 4 h (2). Atrial and ventricular contraction were initiated by programmed stimulation from one of the Te electrodes, digitized with temporal resolution 1 kHz, and recorded using this telemetry. Here, [Ca2+]i was calibrated by measuring the Rhod 2 fluorescence at 520 nm when the dye is bound to Ca2+ (Fmax) and the fluorescence when all the dye is free (Fmin), as previously described (7). In heart muscle homogenates, disocciation constant equaled 720 nM for Rhod 2/Ca2+. Calibration was performed in a dedicated subset of hearts because of concerns that Fmax would decrease during prolonged experiments.

Table 1. Frequency of arrhythmias in TNF-α transgenic mice

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Control Mice</th>
<th>Transgenic Mice</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature atrial beats (&gt;5/hr)</td>
<td>6/2 (10%)</td>
<td>5/2 (10%)</td>
<td>NS</td>
</tr>
<tr>
<td>Atrial flutter/fibrillation</td>
<td>0/10 (0%)</td>
<td>9/15 (60%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Premature ventricular beats (&gt;5/10sec)</td>
<td>1/10 (10%)</td>
<td>12/15 (80%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Coupled, nonsustained VT</td>
<td>0/10 (0%)</td>
<td>8/15 (53%)</td>
<td>0.034</td>
</tr>
<tr>
<td>Nonsustained VT</td>
<td>8/15 (53%)</td>
<td>5/15 (33%)</td>
<td>0.057</td>
</tr>
</tbody>
</table>

Optical mapping of intracellular [Ca2+]. For mapping of Ca2+ transients, hearts were stained with the Ca2+-sensitive dye Rhod 2-AM (25 µg dissolved in 25 µl of DMSO added to the coronary perfusate). Rhod 2-AM is membrane permeable and becomes Ca2+ sensitive and trapped in the cytosol when esterified to Rhod 2 intracellularly. With excitation light (λem = 520 ± 20 nm), Rhod 2 exhibits a more than 100-fold increase in fluorescence at its emission wavelength (λem = 585 nm) on binding Ca2+ and is typically used as a single excitation and single emission wavelength dye. We have shown that the total dye concentration in myocardial samples can be determined from the absorption of the dye and dye-Ca2+ complex (dye-Ca2+):2) through the fluorescence emission (8). Thus a single emission dye can be accurately calibrated by simultaneous measurements of the dye's absorption (total dye content) and fluorescence (dye-Ca2+). Here, [Ca2+]i was calibrated by measuring the Rhod 2 fluorescence when all the dye is bound to Ca2+ (Fmax) and the fluorescence when all the dye is free (Fmin), as previously described (7). In heart muscle homogenates, disocciation constant equaled 720 nM for Rhod 2/Ca2+. Calibration was performed in a dedicated subset of hearts because of concerns that Fmax would decrease during prolonged experiments.

Data analysis. Data are presented as means ± SD. The number of experiments (n) indicates the number of hearts (or mice) used. APD and calcium transient parameters for each heart were measured as the average of several contiguous action potential durations (APD) at 75% and 90% repolarization (APD75 and APD90, respectively). Action potentials with signal-to-noise ratios of <10 or excessive movement artifacts were eliminated, and the remaining activation 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 (2, 34). For each diode, a gradient vector was calculated to each of the eight adjacent diodes. The distance between adjacent diodes was used to determine local velocity vectors. Average velocity was calculated from the vectoral average of 124 local velocity vectors oriented between 0 and π radians.

Optical mapping of isochronal maps as previously described (2, 34). Near the ventricular dissociation.
Arrhythmia and optical mapping data for TNF-α mice and controls are compared by Student’s t-test for paired and unpaired data as appropriate. Continuous variables were compared by ANOVA with Scheffé’s multiple-range test. Isochronal activation maps of basic and premature beats are constructed with contour lines drawn at 1-ms apart. The activation time point is determined by the peak time derivative of the action potential upstroke. Gradients of APD are compared with gradients of conduction, and the locations of lines of block are determined as a function of the coupling intervals during programmed premature stimulation. Data before and after an intervention and comparison of rate-dependent changes are compared using analysis of variance using repeated measures.

**RESULTS**

**Arrhythmias in TNF-α transgenic mice.** Ambulatory telemetry monitoring of the TNF-α mice showed atrial and ventricular arrhythmias not found in FVB controls (Table 1). Examples of premature atrial beats, runs of atrial fibrillation or flutter, premature ventricular beats (PVBs), and runs of ventricular tachycardia (VT) are shown in Fig. 1. Couplets and runs of ventricular tachycardia were more common in male TNF-α mice (4 of 7) than in female TNF-α mice (1 of 8), consistent with the prior findings of more severe CHF symptoms and decreased survival in males (17).

**Fig. 1.** Arrhythmias in tumor necrosis factor (TNF)-α transgenic mice. A: telemetry traces from a wild-type FVB and a TNF-α mouse. Note clear P wave, QRS complex, and ST segment in these mice in sinus rhythms. B: atrial arrhythmias, including a run of a supraventricular tachycardia at a rate of 850 beats/min (a), and an irregularly irregular rhythm that appears to be atrial fibrillation (b). C: ventricular arrhythmias, including a ventricular couplet (a) and an 8-beat run of nonsustained ventricular tachycardia (b). D: death of a TNF-α mouse. Baseline heart rhythm with a rate of 540 beats/min several weeks before death is shown (a). With symptomatic heart failure, rhythm became junctional at a rate of 280 beats/min, slow for the mouse (b). Near the time of death, the rhythm became agonal and wider complex, and ventricular ectopy was present (c).
In contrast to human heart failure, mean heart rate of age- and sex-matched mice was lower in TNF-α mice than in controls (620 ± 64 beats/min, n = 15, vs. 679 ± 32 beats/min, n = 10; P = 0.01). This finding was less pronounced if mice not in sinus rhythm were excluded (632 ± 59, n = 12; P = 0.04). Serial 24-h telemetry recordings on three TNF-α mice showed a progressive decrease in heart rate with increasing age. Prolonged episodes of bradycardia with junctional rhythms preceded and accompanied death (Fig. 1D). No episodes of tachyarrhythmias resulted in death during continuous monitoring of mice for up to 4 mo.

Optical mapping of ventricular action potentials in TNF-α transgenic hearts. We used the voltage-sensitive dye di-4ANEPPS to record action potentials from a 4-mm × 4-mm area on the epicardial surface of the left ventricle of Langendorff-perfused isolated mouse hearts paced near the apex at a cycle length (CL) of 200 ms (Fig. 2A). The output of the 124-element array, representative optical action potentials from several channels, and isochronal activation maps are shown for hearts isolated from a TNF-α mouse and a littermate control. APD75 and APD90 were prolonged by 14% and 28% in TNF-α transgenic hearts (17 ± 1 and 23 ± 2 ms, n = 7) compared with wild-type hearts (15 ± 1 and 18 ± 1 ms, n = 5; P < 0.05 for each; Fig. 2B). Mean conduction velocity was similar for wild-type and transgenic hearts (0.51 ± 0.03 vs. 0.50 ± 0.05 m/s; n = 3 each) and decreased with faster stimulation frequencies (CL = 100 ms; 0.40 ± 0.02 vs. 0.40 ± 0.03 m/s; n = 3 each; Fig. 2C).

Premature stimuli were applied to the ventricle following a train of 10 stimuli at a CL of 200 ms (Fig. 3, A and B). The refractory periods measured at the base (53 ± 6 ms) and at the apex (45 ± 7 ms) of hearts from the TNF-α mice (n = 7) were nearly identical to those measured at the base (52 ± 9 ms) and at the apex (44 ± 10 ms) of hearts from wild-type mice (n = 7). In contrast, restitution kinetics for action potential amplitude (APA) showed significant differences between TNF-α and wild-type hearts (Fig. 3C). The APAs of premature S2 beats normalized to the preceding S1 beat and plotted as a function of the S1-S2 interval were significantly smaller for S1-S2 intervals <100 ms in TNF-α hearts compared with controls (n = 5 mice each; P < 0.001). Similarly, the conduction velocities of the premature beats were markedly slower at S1-S2 intervals <80 ms in TNF-α hearts (P < 0.0001; Fig. 3, B, D, and E) and areas with very slow conduction were apparent.

Programmed stimulation of ventricular arrhythmias in TNF-α hearts. Single extra stimuli elicited VT when applied at the apex of 12 of 13 and at the base of 2 of 4 transgenic mouse hearts compared with only 1 of 7 wild-type FVB hearts (Fig. 4A). The first tachycardia in any given heart usually lasted 12–15 beats and self-terminated, whereas subsequent inductions typically lasted for ~15 min. Some of the tachycardias were monomorphic, whereas others showed varying directions and velocities indicative of polymorphic arrhythmias. Activation maps showed that the slow, heterogeneous conduction of the premature impulse leads to functional lines of block and the initiation of a reentrant ventricular tachycardia (Fig. 4B). The tachycardias then propagated around the perimeter of the mouse heart as previously described (2).

Optical mapping of Ca2+ transients in TNF-α mice. Hearts from TNF-α mice and FVB controls were stained with the Ca2+-sensitive dye Rhod 2-AM, and parameters of the calcium transient were measured (Figs. 5 and 6A). At a CL = 200 ms, peak systolic [Ca2+] was decreased in TNF-α mice compared with controls (635 ± 21 vs. 746 ± 46 nM; n = 4 each; P < 0.05), diastolic [Ca2+] was increased (334 ± 37 vs. 257 ± 30 nM; n = 4 each; P < 0.01), and the Ca2+ transient was markedly prolonged (72 ± 6 ms, n = 10, vs. 54 ± 5 ms, n = 8; P < 0.01). The time from stimulation to the onset of the Ca2+ transient was not different in the TNF-α hearts compared with wild-type hearts (16 ± 2 vs. 14 ± 1 ms; n = 5 each; P = not significant). Hearts from TNF-α mice developed Ca2+ alternans at longer CLs than control hearts (Fig. 6B), and periods of rapid pacing (CL = 80 ms) raised diastolic Ca2+ and elicited runs of nonsustained polymorphic VT in transgenic but not control hearts (n = 3 each; Fig. 6C).

Lower [Ca2+] prevents arrhythmias in TNF-α mice. Single premature ventricular stimuli in hearts loaded with Rhod 2 did not elicit VT (n = 0/6). We speculated that the difference between voltage- and Ca2+-sensitive dyes might be due to the Ca2+-buffering effect of Rhod 2.

To test the dependence of arrhythmias on elevated Ca2+, we repeated the voltage- and calcium-mapping experiments and lowered extracellular [Ca2+] from 1.8 to 1.0 mM. Lowering extracellular [Ca2+] prolonged APD90 from 20 ± 2 to 26 ± 0.3 ms in wild-type hearts and from 24 ± 2 to 34 ± 2 ms in TNF-α hearts (n = 3 each; Fig. 7, A and C). Similarly, APD75 lengthened from 18 ± 2 to 21 ± 2 ms in wild-type hearts and from
20 ± 1 to 25 ± 0.3 ms in TNF-α hearts. Lowering extracellular [Ca\(^{2+}\)] decreased the magnitude of the intracellular Ca\(^{2+}\) transient to a similar extent in control and TNF-α hearts (20 ± 3% vs. 24 ± 3%, n = 3 each; Fig. 7B). The duration of the Ca\(^{2+}\) transient decreased with lowering of the extracellular [Ca\(^{2+}\)] to a somewhat greater extent in hearts from TNF-α mice versus wild-type mice (14 vs. 9 ms; Fig. 7D).
No arrhythmias could be induced in the control hearts at the lower extracellular [Ca\textsuperscript{2+}] despite the APD prolongation. Inducible ventricular arrhythmias in the TNF-\(\alpha\) hearts were eliminated by the lower [Ca\textsuperscript{2+}] in the extracellular solution (\(n = 3\) each). Of note, the fall of conduction velocity seen with ventricular PVBs was also attenuated by the low Ca\textsuperscript{2+} solution (\(n = 3\), \(P = 0.02\); Fig. 3E). The lower extracellular [Ca\textsuperscript{2+}] solution did not lead to a significant change in conduction velocity at the basal CL of 200 ms (data not shown).

**DISCUSSION**

Repolarization abnormalities, cellular coupling, and arrhythmias. Abnormalities in repolarization are present in patients with CHF and in a number of large animal models (3, 16, 25, 29). Decreases in the repolarizing potassium currents \(I_{K1}\) and \(I_{Ks}\) have been documented. The prolongation of APD and QT interval may promote arrhythmias directly via early afterdepolarizations or indirectly through enhanced dispersion of repolarization (10, 12, 41). Abnormalities in conduction are also present in the hearts of patients with CHF and result from structural changes such as fibrosis and decreased connexin43 gap junction protein (30, 31).

Similar findings are present in genetically engineered mice. Mice lacking both fast and slow \(I_{to}\) (\(I_{to,f}\) and \(I_{to,s}\), respectively) have marked prolongation of APD, early afterdepolarizations, and ventricular arrhythmias (13). Transgenic mice overexpressing a truncated K\textsuperscript{+} channel fragment have increased vulnerability to reentrant VT due to enhanced dispersion of repolarization and refractoriness (2, 24). Disruption of the K\textsuperscript{+} channel-interacting protein 2 leads to loss of \(I_{to}\) and susceptibility to ventricular arrhythmias (20). Mice homozygous for disruption of the transcription factor HF-1b have conduction defects and arrhythmias, and those homozygous for a cardiac-restricted connexin43 knockout also have enhanced arrhythmogenessis and sudden death from tachyarrhythmias (14, 26).

The TNF-\(\alpha\) mice described here have a modest prolongation of APD compared with mice with disrupted K\textsuperscript{+} channel expression, without any significant increase in refractory periods. Lowering extracellular [Ca\textsuperscript{2+}] further prolonged APD, possibly due to a decrease in Ca\textsuperscript{2+}-dependent inactivation of the L-type Ca\textsuperscript{2+} channel (16). Of note, arrhythmias were suppressed under these conditions. In addition, conduction velocity is similar for transgenic and control mice at CLs down to 100 ms. Thus changes in repolarization and refractoriness alone do not seem sufficient to explain the increase in reentrant arrhythmias that result from single premature beats.

Calcium abnormalities and arrhythmias. Abnormalities of Ca\textsuperscript{2+} handling may contribute to arrhythmias. Human heart failure is usually characterized by decreased expression of the sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase (SERCA), variable decreases in phospholamban, and increased expression of the Na/Ca exchanger (1). The resulting prolongation of the Ca\textsuperscript{2+} transient and intracellular Ca\textsuperscript{2+} overload could lead to premature sarcoplasmic reticulum Ca\textsuperscript{2+} release and delayed afterdepolarizations (11). Mutations in the cardiac sarcoplasmic reticulum Ca\textsuperscript{2+} release channel (hRyR2) have recently been described in patients with catecholaminergic polymorphic ventricular tachycardia and in patients with arrhythmogenic right ventricular dysplasia (32, 36). These genetic syndromes further suggest a direct link between abnormalities in Ca\textsuperscript{2+} handling and arrhythmias.

TNF-\(\alpha\) mice have abnormal expression of the transcripts encoding Ca\textsuperscript{2+} regulatory proteins (18), possibly as a direct effect of cytokine overexpression (42). The decreased expression of SERCA and of the SERCA-to-phospholamban ratio would be predicted to decrease sarcoplasmic reticulum Ca\textsuperscript{2+} uptake. Here, we show that hearts from wild-type FVB mice have systolic and diastolic intracellular [Ca\textsuperscript{2+}] similar to those reported previously in mice and in other species (7, 9). In contrast, hearts from TNF-\(\alpha\) mice have an elevated diastolic [Ca\textsuperscript{2+}], a decreased systolic [Ca\textsuperscript{2+}], and marked prolongation of the Ca\textsuperscript{2+} transient. In addition, I) premature beats triggered during the tail of the prior Ca\textsuperscript{2+} transient propagate slowly and heterogeneously, leading to functional lines of conduction block and the initiation of reentrant arrhythmias; 2)
rapid pacing of the TNF-α mouse hearts leads to an elevated diastolic \([\text{Ca}^{2+}]\), \(\text{Ca}^{2+}\) alternans, and arrhythmias; and 3) interventions that lower intracellular \([\text{Ca}^{2+}]\), including buffering by Rhod 2 and lowering extracellular \([\text{Ca}^{2+}]\), normalize conduction and prevented the inducible arrhythmias. \(\text{Ca}^{2+}\) sparks result from the quantal release on \(\text{Ca}^{2+}\) from the sarcoplasmic reticulum (6). Spontaneous, asynchronous \(\text{Ca}^{2+}\) release from the sarcoplasmic reticulum is more common in the setting of \(\text{Ca}^{2+}\) overload and can lead to the propagated diastolic \(\text{Ca}^{2+}\) oscillations and contractions seen in isolated myocytes and intact tissue (21). Synchronization of the sparks and \(\text{Ca}^{2+}\) release can lead to delayed afterdepolarizations and triggered arrhythmias, at least in part through activation of the electrogenic Na/Ca exchange current. Diastolic \(\text{Ca}^{2+}\) oscillations could contribute to the measured increase in global diastolic \([\text{Ca}^{2+}]\) that we see in the TNF-α mice. The nonuniform elevations in intracellular \([\text{Ca}^{2+}]\) and membrane potential could also contribute to the initiation and maintenance of reentrant arrhythmias by causing slow, heterogeneous conduction of premature beats. If true, this demonstrates an additional mechanism by which \(\text{Ca}^{2+}\) overload in heart failure can promote reentrant arrhythmias, independent of increasing the frequency of premature beats via initiation of triggered activity (afterdepolarizations).

Previous studies on transgenic mice with elevated intracellular \([\text{Ca}^{2+}]\) have shown diminished responsiveness to \(\beta\)-adrenergic stimulation (35). Lowering the extracellular \([\text{Ca}^{2+}]\) reversed the defect. It is tempting to speculate that similar mechanisms may lead to both the abnormalities in \(\beta\)-receptor responsiveness and arrhythmias.

How do elevations in intracellular \([\text{Ca}^{2+}]\) lead to changes in the conduction velocity of premature beats but not in the refractory period? Elevated intracellular \([\text{Ca}^{2+}]\) following a premature beat or local spontaneous

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**Fig. 4.** Optical mapping of ventricular arrhythmias. A: induction of ventricular tachycardia (VT) using a single premature stimulus (S2) applied to the posteroapical wall of a TNF-α transgenic heart during pacing at a CL of 200 ms. Note the rate of the tachycardia exceeds 1,000 beats/min. B: map showing isochronal lines of activation for the last paced S1 beat (left), the premature S2 beat that leads to the initiation of VT (middle), and the first two beats (VT1 and VT2) during the tachycardia (right). Note that slow, heterogeneous conduction of the premature beat (S2) leads to a functional line of conduction block. This leads to the initiation of a reentrant circuit (arrow) that propagates around the outer perimeter of the heart (beats VT1 and VT2). As in the previous figures, the heart is viewed from the left anterior oblique position, the apex of each heart is at the bottom, and isochronal lines of activation are 1-ms apart.

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**Fig. 5.** Calcium transients in TNF-α and control mice. A: systolic \([\text{Ca}^{2+}]\) is lower and diastolic \([\text{Ca}^{2+}]\) is higher in hearts from TNF-α compared with control mice. *\(P < 0.05\). **\(P < 0.01\). B: calcium transient duration measured from the upstroke to 75% return to baseline. **\(P < 0.01\).
sarcoplasmic reticulum Ca\(^{2+}\) release could depolarize the membrane potential through Ca\(^{2+}\)-dependent leak currents, Ca\(^{2+}\)-dependent Cl\(^{-}\) currents, or via the electrogenic Na/Ca exchange. A small change in membrane potential could affect the time- and voltage-dependent recovery from inactivation of fast Na\(^{+}\) channels, leading to slow propagation of premature impulses but not conduction failure. In support of this hypothesis, APA restitution was markedly abnormal during premature beats in TNF-\(\alpha\) mice (Fig. 3C). Thus interactions between voltage- and Ca\(^{2+}\)-dependent abnormalities may contribute to arrhythmias in this mouse heart failure model.

It is also possible that dynamic alterations in cell-to-cell coupling, such as reduced gap junction conduction in the presence of high intracellular [Ca\(^{2+}\)], could explain the initiation of reentrant VT in these mice (28, 40). These seem less likely to occur within the time course of the action potential, however. Alternatively, changes in Ca\(^{2+}\)-dependent intracellular processes via cAMP- or calmodulin-dependent kinases could affect arrhythmogenesis (35).

In this isolated perfused mouse heart model, we found that staining with Rhod 2 suppressed arrhythmias. Lowering extracellular [Ca\(^{2+}\)] also suppressed arrhythmias, suggesting Ca\(^{2+}\) buffering as the mecha-
nism. In a guinea pig model with long QT, staining with equivalent concentrations of Rhod 2 did not suppress ventricular arrhythmias (7). Thus the potential effects of cytoplasmic buffering by Ca$^{2+}$-sensitive dyes on cardiac electrophysiology are model dependent and must be considered. We also cannot exclude other differences in the handling of Rhod 2 in the TNF-α mice compared with controls.

The mouse as a model for arrhythmias. Many structural and electrophysiological differences exist between the mouse and human heart, and findings in the mouse must be interpreted with caution. The slower basal heart rate of the TNF-α mice compared with controls is one example. However, the TNF-α mice do develop many of the structural and biochemical findings present in human heart failure. Here, we show that these mice also develop atrial and ventricular arrhythmias and have used optical mapping with voltage- and Ca$^{2+}$-sensitive dyes to dissect the mechanisms of the arrhythmias. Our findings implicate changes in intracellular Ca$^{2+}$ handling as critical to the genesis of arrhythmias. These findings complement those of other transgenic mice with alterations in electrophysiological pathways that lead to arrhythmias (2, 13, 14, 20, 24, 26, 33). Further exploration of the pathways that couple changes in intracellular [Ca$^{2+}$] to arrhythmias is warranted.

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