Repolarization abnormalities and afterdepolarizations in a canine model of sudden cardiac death

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Sudden cardiac death (SCD) is a major cause of cardiovascular mortality in the United States, accounting for ~80,000 deaths annually. Ambulatory ECG recordings have established that the vast majority (>80%) of these deaths result from tachyarrhythmias that culminate in ventricular fibrillation (VF; Refs. 1, 5, 19, 21). Postmortem examinations indicate that scar tissue due to a previous myocardial infarction (MI) is present in approximately one-third of SCD subjects (51). It has been estimated that up to 80% of SCD results from myocardial ischemia or its sequelae (40). Ventricular arrhythmias often occur when there is an underlying electrophysiological substrate; alterations in repolarizing potassium currents are known to contribute to arrhythmogenesis (32). Specifically, alterations in inward rectifier K+ current (I_K1), and the repolarizing K+ currents: transient outward current (I_O1) and/or the delayed rectifier K+ currents (I_Kr and I_Ks), are arrhythmogenic. The specific abnormalities in repolarization that predispose to arrhythmias in the setting of a healed MI have not been well defined. Numerous studies (13, 34, 35) have identified electrophysiological abnormalities in the hours to days after MI in canine models. At 5 and 14 days postinfarction, there is shortening of the action potential duration (APD) in the peri-infarct epicardial border zone (EBZ; Ref. 10). Specifically, post-MI decrements in I_O1 occur at 5 and 14 days postinfarction in the peri-infarct EBZ; however, at 2 mo postinfarction, I_O1 reportedly returns to control values (13). Jiang et al. (25) reported a reduction in both I_Kr and I_Ks in myocytes isolated from the EBZ of 5 day post-MI canine hearts. Notably in these previous studies, stratification for, and the occurrence of, lethal arrhythmias were not described, and the specific mechanisms predisposing to post-MI SCD during recurrent ischemia remain poorly defined.

In this study, we utilized a well-characterized, highly reproducible post-MI canine model of SCD (6, 41). In this model, after recovery from an MI (3–4 wk), the animals are risk stratified for susceptibility to sustained ventricular tachyarrhythmias. Arrhythmia susceptibility is assessed by a submaximal exercise plus ischemia test to stratify animals as either susceptible or resistant to sustained ventricular tachyarrhythmias; thus the model simulates post-MI patients who may or may not have residual susceptibility to lethal ventricular arrhythmias during recurrent ischemia or adrenergic activation. Furthermore, there is a significant increase in spontaneous ventricular arrhythmias and sudden death in animals stratified as arrhythmia susceptible by the exercise plus ischemia test (6). During arrhythmia testing, susceptible animals have a longer QT interval and abnormal T wave morphology compared with resistant animals. During arrhythmia testing, resistant animals have a shorter QT interval and normal T wave morphology compared with susceptible animals.
the animals resistant to induction of ventricular arrhythmias (6). While there are ample in vivo data in this model, the cellular electrophysiological mechanisms of arrhythmia susceptibility have not been defined in this model. In this study, we tested the hypothesis that susceptibility to ventricular tachyarrhythmias results from alterations in repolarization. Our results suggest that downregulation of repolarizing K⁺ currents and afterdepolarizations provide a substrate for initiation of ventricular arrhythmias after MI.

MATERIALS AND METHODS

All procedures were approved by the Ohio State University Institutional Animal Care and Use Committee and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Sixteen mongrel dogs (male/female; 2–3 years of age) had a surgically induced MI by occlusion of the left anterior descending coronary artery, as described previously (6). Two D-, B-, and M-mode echocardiograms were performed in a subset of dogs (n = 5) while the animals were under butorphanol sedation (0.5 mg/kg im) at baseline and 8 wk after surgery. In the same subset, 24-h ambulatory ECG was recorded by Holter monitor. Ten age-matched dogs served as controls (i.e., dogs without MI).

In vivo preparation. A left ventricular anterior infarction was induced by the occlusion of the left anterior descending coronary artery, as described previously (6, 8, 41). At the time of surgery, a pulsed Doppler flow transducer and vascular occluder were placed on the left circumflex artery. After recovery, arrhythmia susceptibility was determined using a combined exercise with ischemia test, where animals exercised on a treadmill at a target heart rate (~210 beats/min or 70% of the maximum heart rate). During the final minute of exercise, the circumflex artery was occluded via the implanted occluder; the occlusion was maintained for one additional minute after the cessation of exercise (6). This method reproducibly induced VF in ~60% of the animals, which were classified as “VF⁺”; the remainder of the animals were classified as “VF⁻”.

Myocyte isolation. Myocytes were isolated 8–10 wk postinfarction from the anterolateral left ventricular midmyocardial wall. To eliminate the possibility of any effects due to acute ischemia during the experiment, the hearts were rapidly excised and perfused with cold cardioplegic solution (containing 5% glucose, 0.68 glutamine, 10 glucose, 5 pyruvate, 1 CaCl₂, along with 0.65 mg/ml collagenase (Worthington type 2; 0.65 mg/ml) and protease-free BSA (0.65 mg/ml) were added to the perfusate. The left main coronary artery was cannulated for myocyte isolation as described previously (28). In hearts from the post-MI animals, a clear margin of the infarct was visible as scar tissue. After 30–45 min of perfusion, the digested tissue with 4-aminopyridine (44).

Electrophysiological protocols. Myocytes were placed in a laminin-coated cell chamber (Cell Microcontrols, Norfolk, VA) and superfused with bath solution containing the following (in mM): 135 NaCl, 5 MgCl₂, 5 KC1, 10 mM glucose, 1 mM CaCl₂, and 5 mM HEPES, pH adjusted to 7.40 with NaOH, at a temperature of 36 ± 0.5°C. For action potential (AP) recordings, the concentration of CaCl₂ in the bath solution was increased to 1.8 mM. During potassium current measurements, L-type calcium current was blocked by the addition of 2 μM nifedipine to the superfusate. Solutions were changed with a six-port gravity flow system (~1 ml/min). Borosilicate glass micropipettes (tip resistance of 1.5–3 MΩ) were filled with pipette solution containing the following (in mM): 100 K⁺-aspartate, 40 KC1, 5 MgCl₂, 5 EGTA, and 5 HEPES, pH adjusted to 7.2 with KOH. Perforated whole cell patch clamp (using amphotericin B) was used to minimize alterations in intracellular milieu. For voltage clamp experiments, only recordings with an access resistance <20 MΩ were included in the analyses. Series resistance compensation (~70%) was used for current recordings. For determination of drug-sensitive currents, only cells with less than a 20% change in access resistance were included in the analyses. All drug-sensitive currents were recorded after 3–5 min of drug superfusion, which in our pilot experiments resulted in steady-state current blockade.

APs were recorded with perforated whole cell patch techniques, as described above. APs were measured as the average of the last 10 (steady state) APs, obtained during a train of 25 APs at each stimulation rate. To analyze beat-to-beat variability in the AP recordings, the SD of the APD at 90% repolarization in each myocyte was calculated for beats 15 to 25. The amplitude of phase 2 was measured as the maximal potential after phase 1 of the AP. We observed arrhythmias in some cells; myocytes exhibiting cellular arrhythmias were excluded from APD measurements. In a second series of current clamp experiments, cellular arrhythmias were quantified by recording APs in the presence and absence of isoproterenol.

Transient outward potassium current (Iₒ) was elicited from a holding potential of ~60 mV by a series of 100-ms test potentials from −20 to +50 mV and measured as peak current minus steady-state current. I₉₋₁ was elicited by voltage steps from −140 to +40 mV from a holding potential of −40 mV. The current was measured at the end of each 100-ms test pulse. I₉₋₁ inward conductance (mS/cm²) was determined by calculating the slope of the linear portion of the current density-voltage relationship from −140 mV to −100 mV (11). Peak outward I₉₋₁ density was measured as the current at −60 mV (Iₒ−o).

Rand (Iₒ₋K) and slow (I₉₋₁₋K) components of the delayed rectifier current were elicited using 10-mV incremental voltage steps from −40 to +50 mV from a holding potential of −50 mV. Iₒ₋-K was measured as the d-sotalol-sensitive (100 μM) current, while d-sotalol-insensitive tail currents were used to measure I₉₋₁₋K (18, 27). During Iₒ₋-K and I₉₋₁₋K recordings, the bath solution also contained 4-aminoopyridine (100 μM) to prevent any potential contamination by “Kᵢᵣᵣ-like” plateau current (44).

Sustained outward potassium current was elicited from a holding potential of −40 mV using 10-mV voltage steps from −20 to +50 mV. A combination of a −40 mV holding potential and an 80-ms prepulse to −10 mV was used to inactivate Iₒ₋-K. A sustained 4-aminoopyridine-sensitive plateau current was measured as the steady-state difference current, recorded after a minimum of 4 min of superfusion with 4-aminoopyridine (44).

A sustained 40-mV holding potential and a 80-ms prepulse to −10 mV was used to inactivate Iₒ₋-K. A sustained 4-aminoopyridine-sensitive plateau current was measured as the steady-state difference current, recorded after a minimum of 4 min of superfusion with 4-aminoopyridine (44).

D-sotalol (30 and 100 μM) was used to inhibit Iₒ₋-K during AP recordings in VF⁺ myocytes and control myocytes. These concentrations selectively inhibit Iₒ₋-K in canine ventricular myocytes (43, 50).

Data acquisition was performed with Clampex 8.0 software (Axon Instruments, Sunnyvale, CA) and an Axopatch 200A patch-clamp amplifier.

Solutions and chemicals. All chemicals for buffer and stock solution preparation were purchased from Fisher Scientific, Sigma-Aldrich (St. Louis, MO), and Invitrogen (Carlsbad, CA). Stock solutions of nifedipine, amphotericin, and 4-aminoopyridine were prepared daily.

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Isoproterenol solutions were prepared daily from commercially available injectable solutions (Sanofi Winthrop Pharmaceuticals, New York, NY). d-sotalol was obtained from Merck Research Laboratories (West Point, PA). All nifedipine, isoproterenol, and amphotericin B solutions were protected from exposure to light.

**K+ channel protein subunit measurements.** hERG was detected using left ventricular tissue homogenates. Tissue samples were pulverized using an ice-cold mortar and pestle. Integral membrane proteins were isolated as described previously (26). Isolated membrane proteins (3 mg) were cleared with protein G-Sepharose beads (Amersham Biosciences) for 1–2 h at 4 °C. Cleared lysates were then incubated with 15 μL of mouse anti-hERG antibody (Axxora, San Diego, CA) on a rotating platform for 12–16 h at 4 °C. Thirty microfilters of protein G beads were added to the samples and incubated for an additional 2 h at 4 °C. Beads were collected by centrifuging at 1,000 g and washed three times in 150 mM NaCl, 25 mM Tris-HCl, pH 7.4, 5 mM NaEDTA, and 1% (vol/vol) Triton X-100. Proteins were eluted by incubating beads at 65 °C for 10 min in 30 μL 4× LSB [25 mM Tris-HCl, pH 6.8, 2% (vol/vol) SDS, 10% glycerol, and 200 mM DTT]. Eluted proteins were separated by 7.5% SDS-PAGE using the Criterion system (Bio-Rad Laboratories). Proteins were transferred onto Immobilon-P polyvinylidene difluoride membrane (Millipore, Bedford, MA) with the use of Criterion transfer membrane (Millipore, Bedford, MA) on a rotating platform for 12–16 h at 4 °C. Thirty microfilters of protein G beads were added to the samples and incubated for an additional 2 h at 4 °C. Beads were collected by centrifuging at 1,000 g and washed three times in 150 mM NaCl, 25 mM Tris-HCl, pH 7.4, 5 mM NaEDTA, and 1% (vol/vol) Triton X-100. Proteins were eluted by incubating beads at 65 °C for 10 min in 30 μL 4× LSB [25 mM Tris-HCl, pH 6.8, 2% (vol/vol) SDS, 10% glycerol, and 200 mM DTT]. Eluted proteins were separated by 7.5% SDS-PAGE using the Criterion system (Bio-Rad Laboratories). Proteins were transferred onto Immobilon-P polyvinylidene difluoride membrane (Millipore, Bedford, MA) with the use of Criterion transfer system (Bio-Rad). hERG protein was visualized by Western blot analysis with rabbit anti-hERG antibody, as described previously (39).

All other K+ channel subunits were assessed by immunoblot analysis. Myocytes were lysed with RIPA buffer (Santa Cruz Biotechnology, Santa Cruz, CA). Cell lysate proteins (10 μg) were subjected to 4–20% SDS-PAGE and blotted onto nitrocellulose membranes (Bio-Rad, Hercules, CA). Anti-Kv1.5, -Kv4.3, -KChQ1, -KChP2, and -Kir2.1 antibodies were from Santa Cruz. Anti-GAPDH antibody was from Abcam (Cambridge, MA). Each sample had total protein assayed to provide equal loading on the gels, and GAPDH was assayed to provide equal loading on the gels, and GAPDH was measured as an internal control. Blots were developed with SuperSignal West Pico (Pierce) and quantified using ImageJ (National Institutes of Health) and Origin 7 (OriginLab, Northampton, MA) software.

**Statistical analysis.** Acquired electrophysiological data were analyzed using Clampfit 8.0 (Axon Instruments) and Origin 6.1 (OriginLab). Currents were normalized to cell capacitance and are expressed as pA/pF. APD, current densities, and channel expression levels were analyzed by ANOVA with post hoc least significant difference testing as appropriate (SAS for Windows v9.1, Cary, NC). All data are presented as means ± SE.

**RESULTS**

There was no evidence of impaired global left ventricular structure or function after infarction, as has been previously reported in this model (6, 7, 22). Left ventricular fractional shortening was unchanged after MI (41 ± 1.6 vs. 40.6 ± 0.9%, baseline and 8 wk post-MI, respectively; P = NS). Left ventricular diameters at end systole (2.43 ± 0.05 vs. 2.40 ± 0.03 cm, baseline and 8 wk post-MI, respectively; P = NS) and end diastole (4.14 ± 0.14 vs. 4.13 ± 0.05 cm, baseline and 8 wk post-MI, respectively; P = NS) were unchanged. However, myocyte capacitance was significantly increased post-MI in both VF+ and VF− groups (P < 0.05) compared with controls (controls: 157 ± 19; VF+: 202 ± 14; and VF−: 191 ± 8 pF) but did not differ as a function of susceptibility to VF.

VF+ myocytes exhibited a significant increase in action potential durations at 50% (APD50) and 90% (APD90) repolarization at both 0.5 and 1 Hz (P < 0.05), while the VF− myocytes did not differ from controls (Fig. 1, A–C). The plateau potential did not differ significantly among the VF+, VF−, and control myocytes (27.3 ± 0.8, 35.6 ± 2.8, and 31 ± 1.6 mV at 0.5 Hz in control, VF+, and VF− groups, respectively; P = 0.08). The resting membrane potential was also similar in the three groups (−82 ± 2.6 mV in control, −79.6 ± 0.9 mV in VF+ and −79.8 ± 0.4 mV in VF− group, respectively).

There was increased beat-to-beat variability in the APD90 from the VF+ myocytes, quantified as the SD of APD90 (Fig. 1D; Ref. 3). The VF+ myocytes exhibited a significantly increased variability in APD90 at both 0.5 and 1 Hz compared with both control and VF− myocytes (P < 0.05).

I0 was reduced to a similar extent in both the VF+ and VF− groups (Fig. 2) compared with control values (P < 0.05). No differences in the kinetics of inactivation or recovery from

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**Fig. 1.** VF+ myocytes exhibit prolonged action potential duration at 50 and 90% repolarization (APD50 and APD90) and increased variability in APD90. A and B: representative action potential tracings from control (black), VF+ (red), and VF− (blue) recorded at 0.5 and 1 Hz, respectively. Line indicates 0 mV potential. C: summary APD50 and APD90 values at the 2 stimulation rates in the 3 groups. D: averaged SD in APD90 measured from each myocyte in the 3 groups plotted as the function of stimulation frequency (*P < 0.05 vs. control; §P < 0.05 vs VF−). VF+ or VF−: susceptible or resistant, respectively, to sustained ventricular tachyarrhythmias.
inactivation of $I_{to}$ were found between groups (data not shown). Inward $I_{K1}$ slope conductance was significantly reduced in the VF$^+$ group compared with either the control or the VF$^-$ groups (Fig. 3). The peak outward component of $I_{K1}$ did not differ among the three groups.

$I_{Kr}$ was significantly reduced in myocytes from the VF$^+$ group to levels that were almost undetectable ($P \leq 0.05$; Fig. 4); $I_{Kr}$ density was unchanged in VF$^-$ compared with controls. The slow component of the delayed rectifier current ($I_{Ks}$) did not differ among the three groups (Fig. 5A). We recently identified a 4-aminopyridine-sensitive "$I_{Kur}$-like" plateau current in a majority (70\%) of left ventricular midmyocardial myocytes (44). The VF$^+$ myocytes had a reduced density of "$I_{Kur}$-like" plateau current compared with controls and the VF$^-$ groups ($P < 0.05$; Fig. 5B). The VF$^-$ myocytes did not differ from control values in 4-aminopyridine-sensitive plateau current density.

In experiments to quantify cellular arrhythmias, we observed early afterdepolarizations (EADs) in 8 out of 12 VF$^+$ myocytes (Fig. 6A). We observed no evidence of EADs in 11 control and 8 VF$^-$ myocytes. When treated with isoproterenol, there was no significant change in the number of cells showing EADs (9 out of 12 in presence of isoproterenol vs. 8 out of 12 at baseline in the VF$^+$ group). No delayed afterdepolarizations were observed in any experiments. In ambulatory ECGs, we observed frequent ventricular premature depolarizations and episodes of non sustained ventricular tachycardia in dogs from the VF$^+$ group (Fig. 6B), specifically those with myocytes exhibiting EADs.

To verify that the loss of $I_{Kr}$ is a critical contributor to AP variability and afterdepolarizations, a series of separate exper-
ments was conducted in control and VF− myocytes. $I_{Kr}$ was inhibited by superfusion of $\beta$-sotalol, a selective $I_{Kr}$ blocker (Fig. 7). In control myocytes, both concentrations of $\beta$-sotalol resulted in APD prolongation (data not shown). In control myocytes, 30 $\mu$M sotalol did not induce any EADs ($n = 6$) at 0.5, 1, or 2 Hz; 100 $\mu$M $\beta$-sotalol resulted in only a single isolated EAD in one of six myocytes at 0.5 Hz. In the VF− myocytes, superfusion with 30 or 100 $\mu$M $\beta$-sotalol consistently resulted in early afterdepolarizations at all frequencies (6 of 8 myocytes at both concentrations tested, compared with 0 of 8 at baseline; Fig. 7).

Consistent with the reduction in $I_{Ks}$ in the VF− myocytes, we observed a significant reduction in Kir2.1 expression (Fig. 8). KChIP2 was significantly reduced to a similar extent in both VF+ and VF− myocytes compared with controls. hERG1α was expressed in control, VF+, and VF− left ventricular tissues (data not shown); both mature and immature forms of the subunit were found in all groups. There were no

Fig. 4. $I_{Kr}$ is significantly reduced in VF+ myocytes. A: representative current traces from the 3 groups, elicited by the protocol shown in inset. B: summary I-V relationships from myocytes in the 3 groups. $I_{Kr}$ is substantially reduced in myocytes from VF+ animals, while VF− myocytes are comparable with controls (*P < 0.05 vs. control; §P < 0.05 vs. VF−).

Fig. 5. $I_{Ks}$ is unchanged, while the 4-aminopyridine sensitive plateau current is reduced in the VF+ myocytes. A: $I_{Ks}$ traces obtained from myocytes from the 3 groups with the summary current density-voltage relationships shown at right (P = NS). B: 4-aminopyridine sensitive plateau current, which is reduced in VF+ myocytes; the summary current density-voltage relationships shown at right (*P < 0.05 vs. control; §P < 0.05 vs. VF−). The proportion of cells expressing the 4-aminopyridine-sensitive current is shown for each group and did not differ among groups.
significant between group differences in the expression of KCNQ1, Kv1.5, or Kv4.3.

**DISCUSSION**

SCD is a leading cause of cardiovascular mortality, and preexisting coronary artery disease is a major risk factor for SCD. In the present study, a distinct form of electrophysiological remodeling was observed in myocytes isolated from the hearts of animals with reproducible ischemia-induced sustained ventricular tachyarrhythmias (6, 46). Specifically, myocytes from dogs with healed MIs and lethal arrhythmias also exhibited early afterdepolarizations, AP prolongation, and increased beat-to-beat variability in repolarization. These electrophysiological changes resulted from specific K⁺ current abnormalities. Two discrete mechanisms appear to contribute to the observed abnormalities in K⁺ currents: 1) altered function due to altered expression of ion channel subunits (e.g., KChIP2 and I<sub>Kr</sub>), and 2) posttranslational modification of function (e.g., hERG1a and I<sub>Ko</sub>).

Our data suggest that early afterdepolarizations may trigger reentrant ventricular tachyarrhythmias in this model of sudden death.
death. Early afterdepolarizations occur when repolarization is prolonged; repolarization depends on the balance between multiple currents (K⁺ currents, late Na⁺ current, NCX, and Iₐ), and alterations in this balance can result in prolongation of the AP. Reductions in a single repolarizing K⁺ current, Iₖr, may be sufficient to result in AP prolongation in the canine ventricle (50). Recently, it was shown that block of Iₖr in isolation is insufficient to prolong the APD unless Iₖr is also inhibited in normal canine ventricular myocytes (50). This requirement for the block of multiple currents has been attributed to the presence of a “repolarization reserve,” where multiple repolarizing K⁺ currents can compensate for the absence of a single repolarizing current (36, 37). In the present study, early afterdepolarizations were observed in the VF⁺ myocytes on the background of reduced Iₖ to, Iₖr, and a 4-aminopyridine-sensitive “Iₖur-like” current. As such, it was not clear which defect or defects in repolarization were required for the initiation of the afterdepolarizations in this model of SCD. However, the observation that block of Iₖr in the VF⁺ myocytes prolonged APD and provoked afterdepolarizations (i.e., converted these cells to the VF⁺ phenotype) strongly suggests that inhibition of Iₖr is required for arrhythmogenesis. Furthermore, since d-sotalol (30 μM) did not induce EADs in the control myocytes, it appears that APD prolongation due to Iₖr inhibition alone is not sufficient to induce EADs. Multiple repolarizing currents must therefore be impaired before “repolarization reserve” is sufficiently compromised to allow for the induction of cellular arrhythmias. As a consequence, repolarization reserve in normal canine ventricular myocytes prevents induction of EADs at physiologically relevant stimulation rates (0.5 and 1 Hz) after inhibition of Iₖr in isolation. As previously noted, EADs were induced with Iₖr blockade in the VF⁻ myocytes (where Iₖ is reduced; Fig. 7). Thus the combination of the inhibition of Iₖ and Iₖr is sufficient to induce EADs. When considered together, these data suggest that in the VF⁺ myocytes the pathophysiological inhibition of multiple repolarizing K⁺ currents results in APD prolongation, AP variability, and induction of EADs, thereby providing a substrate for the initiation of ventricular tachyarrhythmias.

The myocytes in the present study were derived from the left ventricular anterior midmyocardium, and Iₖr blockers have been shown to produce greater AP prolongation in the midmyocardium compared with the epicardium or the endocardium. While AP prolongation is more prominent at slower

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**Fig. 8.** Expression of K⁺ channel subunits. A: representative Western blots of K⁺ channel subunits in left ventricular myocytes from control, VF⁻, and VF⁺ animals. GAPDH was measured as an internal control. B: KChIP2 is reduced in both VF⁺ and VF⁻ groups relative to control (P < 0.05). C: Kir2.1 is reduced in the VF⁺ group relative to control and the VF⁻ group (P < 0.05). There were no significant differences in the expression of Kv1.5 (D), KCNQ1 (E), or Kv4.3 (F) among groups. Data were averaged from 4 samples in each group.
stimulation rates, an elegant study by Hua and Gilmour (23) demonstrated the contribution of \( I_{Kr} \) to rate-dependent AP dynamics in canine endocardial myocytes, with “baseline” \( I_{Kr} \) (current activated preceding the upstroke of the AP during continuous AP clamp stimulation) increasing significantly at faster stimulation rates. In contrast, they observed that “peak” \( I_{Kr} \) increased at slower stimulation rates (23). Furthermore, decreasing \( I_{Kr} \), both in isolated myocytes and in silico, increased the amplitude of APD alternans. In a separate study (24), these authors demonstrated the converse, that hERG overexpression in canine ventricular myocytes successfully abolished APD alternans. Additional studies (12, 16, 17) have demonstrated the converse, that hERG overexpression in canine ventricular myocytes successfully abolished APD alternans. Additional studies (12, 16, 17) have linked the loss of \( I_{Kr} \) with the reduction in the QT interval was associated with a significantly increased risk of sudden death.

Previous studies with this model by other investigators found prolongation of the QT interval in susceptible dogs (2, 46). Interestingly, electroanatomic mapping of the left ventricular endocardium revealed marked intraventricular heterogeneity in VF+, animals, with discrete regions demonstrating longer repolarization times (46). This regional dispersion of repolarization suggests that a substrate for reentry may result from dispersion of refractoriness (i.e., the possibility of unidirectional block of premature beats). The afterdepolarizations and triggered beats observed may contribute to the initiation of reentry, given the underlying substrate. The relevance of our findings is underscored by a clinical study (42) that reported that in patients with a previous MI, consistent prolongation of the QT interval was associated with a significantly increased risk of sudden death.

Comparisons to other experimental canine MI studies. Multiple studies have documented the cellular electrophysiological remodeling process occurring within hours to days after MI in dogs. Most of the original studies (4, 34) in this area have focused on the epicardial border zone and the Purkinje fibers as sites of reentry initiation. In contrast to these previous studies, we studied dogs with healed MIs and a known predisposition to ischemia-induced sudden death, and our AP measurements were restricted to the midmyocardial layer where we found increases in APD90 and APD99. It is possible that additional regional differences in repolarization within the left ventricular, either transmural or regional, could contribute to arrhythmogenesis.

Independent of arrhythmia risk, we found a decrement in \( I_{to} \) density in both post-MI groups. This reduction is consistent with a previous study (31) of reduced \( I_{to} \) on day 5 postinfarction in the EBZ. In a separate study (13) from these investigators, \( I_{to} \) was also reduced at 14 days postinfarction in EBZ myocytes but restored to control values 2 mo postinfarction. In contrast to this study, we found a significant reduction in \( I_{to} \) at 8–10 wk post-MI in both the VF+ and VF− groups. However, our data suggest that \( I_{to} \) plays only a very minor role in modulating APD, as has been previously suggested (20). This interpretation is supported by the observation that \( I_{to} \) density was reduced in the VF− myocytes, yet these cells exhibited normal APDs. In fact, comparable reductions in \( I_{to} \) were noted for both VF+ and VF− myocytes, while only the VF+ myocytes had APD prolongation.

There was no difference in the expression in Kv4.3 in the VF+ and VF− myocytes. The reduction in \( I_{to} \) may be explained by the known modulatory effects of KChIP2 on Kv4-encoded \( I_{to} \) (29, 33, 38), and a similar pattern of Kv4.3 and KChIP2 subunit expression with reduced \( I_{to} \) has recently been described in a canine model of ischemic cardiomyopathy (30). Thus the reduction in \( I_{to} \) observed may be attributed to the reduction in KChIP2 expression.

Delayed rectifier currents have also been studied previously (14, 25) in canine postinfarction models. Jiang et al. (25) reported a reduction in both \( I_{Kr} \) and \( I_{Ks} \) in myocytes isolated from the EBZ of 5-day post-MI canine hearts, which was associated with reduced mRNA for the subunits encoding \( I_{Kr} \) and \( I_{Ks} \). While we observed a lack of \( I_{Kr} \), in the VF+ myocytes, there was not an associated absence of hERG1a in these tissues. This observation may be consistent with posttranslational modulation of hERG channel function. In fact, oxidative modulation of hERG channels has been recently reported to alter \( I_{Kr} \) (45).

In the aforementioned canine model (EBZ 5 days post-MI), Dun et al. (13) described a more complex form of remodeling in myocytes from the infarcted EBZ. They reported an upregulation in the TEA-sensitive component of the delayed rectifier current (possibly Kv2.1). Notably, similar to our finding in the midmyocardial region of a 4-aminopyridine-sensitive plateau current, Dun et al. reported a C93S-sensitive (C93S is a selective blocker of Kv1.5 channels) current in myocytes from both the EBZ and normal zone of canine hearts. We (44) recently reported the presence of a similar current that activates at plateau voltages and modulates canine midmyocardial APD. Because of its similarities with canine atrial \( I_{Kur} \) (both in properties and inhibition with micromolar 4-aminopyridine), we suggest that this is a 4-aminopyridine sensitive “\( I_{Kur} \)-like” plateau current. “\( I_{Kur} \)-like” current was selectively reduced only in myocytes from the VF+ group, which has the potential to alter the plateau potential as well as the APD.

Limitations

The myocyte studies were limited to those isolated from the left ventricular midmyocardium. Therefore, the contribution of electrophysiological abnormalities in other ventricular regions to the arrhythmic phenotype remains undefined. The present studies focused on repolarizing \( K^+ \) currents as modulators of the AP, additional studies will be required to determine the potential roles of \( Na^+ \) current or abnormalities in \( Ca^{2+} \) cycling to arrhythmogenesis in this model.

The myocardiun undergoes a process of dynamic remodeling after a MI. We only studied animals that were 8–10 wk postinfarction. This time point was selected as one where ventricular remodeling is complete (9, 15, 48). Time-dependent pathological remodeling of myocyte electrophysiology was beyond the scope of the present study.
In humans, medications are typically given post-MI to minimize ventricular remodeling and sudden death. In our cohort of animals, no such medications were administered. Additional studies would be required to examine the effects of such medications (e.g., angiotensin-converting enzyme inhibitors and β-adrenergic antagonists) on arrhythmogenic electrophysiological remodeling.

**Perspectives and Significance**

In the present study, an increased risk of lethal arrhythmias in the late phase after infarction were associated with prolonged APs, increased beat-to-beat variability in repolarization, and cellular arrhythmias. The repolarization abnormalities that predispose to lethal arrhythmias were associated with a profound reduction in \( I_{Kr} \). In the normal canine ventricle, multiple repolarizing potassium currents provide repolarization reserve. After a MI, susceptibility to lethal arrhythmias arises from abnormalities in multiple repolarizing currents; at the myocyte level these abnormalities are manifested as repolarization variability, afterdepolarizations and triggered activity. Further studies are warranted to examine other potential contributors to arrhythmogenesis (e.g., abnormalities in myocyte calcium handling or autonomic modulation of electrophysiology) in this model.

**REFERENCES**


