Transmural action potential and ionic current remodeling in ventricles of failing canine hearts

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HEART FAILURE (HF) is associated with a high mortality (14), with up to 50% of deaths being sudden and unexpected due to ventricular tachycardia or fibrillation (43). The mechanisms underlying ventricular arrhythmogenesis in electrically remodeled failing hearts are not fully understood (32, 44). Studies have consistently found prolongation of cardiac action potential (AP) duration (APD) in myocardial tissues and/or cells from different models of experimental HF (26, 44) and from explanted tissues of humans with terminal HF (3). Abnormalities in repolarization can predispose to dispersion of repolarization, leading to nonexcitable gap reentry (6), and AP prolongation also favors the development of early afterdepolarizations (EADs), which can induce triggered arrhythmias (9).

The ionic current mechanisms underlying AP prolongation in the failing heart have been described in several species, including humans (44). It is generally believed that K+ current (IK) downregulation is involved in the abnormal ventricular repolarization of failing hearts (see reviews) (30, 44). These IK include 4-aminopyridine (4-AP)-sensitive transient outward K+ current (Ito), inward rectifier K+ current (IK1), and the rapid and slow components of the delayed rectifier K+ current (IKr and IKs, respectively), although discrepant observations have been reported (44).

It has been recognized that electrical heterogeneity is an important determinant of normal cardiac electrical function. In the ventricular wall, distinct AP morphologies and APDs have been demonstrated in endocardium, midmyocardial layer, and epicardium of canine (1) and human (8, 16) hearts. The longest APDs have been found in ventricular midmyocardial cells of various species, including the guinea pig (41), pig (42), dog (40), and human (8, 16). Distinctive pharmacological responsiveness has been studied in detail in canine hearts (1). In addition, differential responses of epicardium and endocardium to ischemia were described in canine ventricular tissues and cells (24). However, little is known about how HF-related electrical remodeling is expressed transmurally in ventricles. Rapid ventricular tachypacing induces a cardiomyopathy in canine hearts with hemodynamic changes similar to those seen in human HF (28). The present study used the canine model to determine how transmural heterogeneity of cellular electrophysiology is remodeled and to evaluate how major membrane ionic currents responsible for AP repolarization (e.g., Ito, IK1, IKr, and IKs) are affected by transmural electrical remodeling based on observations in cells isolated from the subendocardium, midmyocardial layer, and subepicardium of failing hearts.

Address for reprint requests and other correspondence: G.-R. Li, L4-55, New Medical Faculty Complex, Univ. of Hong Kong, 21 Sassoon Rd., Pokfulam, Hong Kong SAR, China (E-mail: grli@hkucc.hku.hk). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
MATERIALS AND METHODS

Pacing-induced HF model. All animal care and handling procedures followed the Guidelines of the Canadian Council for Animal Care. Ventricular pacing-induced canine HF was produced with well-established procedures (13, 21). Briefly, adult mongrel dogs of either sex (20–25 kg) were anesthetized, and artificial respiration was maintained via an endotracheal tube connected to a Harvard-type mechanical ventilator. Under sterile conditions, a unipolar endocardial pacing lead (Medtronic; Minneapolis, MN) was inserted through the right jugular vein, and the distal end of the lead was screwed into the right ventricular apex under fluoroscopic guidance and connected to a custom-modified pacemaker (model 8084, Medtronic), which was placed subcutaneously at the base of the neck. After the dogs had recovered from the operation for 2 days, the pacemaker was programmed to stimulate the ventricles at 240 beats/min with the use of 0.42-ms square-wave pulses of 1.5-fold threshold current.

After 4–5 wk of chronic tachycardia, clinical symptoms of terminal HF were evident, including loss of appetite, lethargy, dyspnea, and ascites. Severe HF was confirmed in eight randomly selected dogs by transthoracic two-dimensional echocardiography. The echocardiography was performed before pacemaker stimulation and after 5 wk of ventricular pacing with a standard echocardiography system (Hewlett-Packard; Andover, MA). Rapid ventricular pacing induced significant dilation of the right and left ventricles and led to a decrease in the left ventricular ejection fraction from 54.6 ± 2.6% before pacing to 24.7 ± 1.1% after 5 wk of pacing (P < 0.01). Similar changes in AP and ionic currents were found in HF animals with or without echocardiography. Therefore, the data were combined for the HF group.

Three of twenty-eight programmed animals died suddenly on the fifth week before the experiments, and, therefore, twenty-five animals served as the HF group. In seven other animals, the pacemaker was not activated (sham group). No difference in cardiac electrophysiological properties and ionic currents was found between cells from sham animals [e.g., mean APD at 90% repolarization (APD90) at 1 Hz: 275 ± 28, 332 ± 37, and 237 ± 38 ms in epicardial, midmyocardial, and endocardial cells, respectively] and those from normal non-operated animals (n = 10, mean APD90: 289 ± 31, 325 ± 42, and 247 ± 29 ms in corresponding cell types), so the sham and normal control group data were combined.

Cardiac cell preparation. Left ventricular tissues from isolated control and failing hearts were obtained via a left thoracotomy after dogs were anesthetized with morphine (2 mg/kg sc) and α-chloralose (120 mg/kg iv) under ventilation with room air. Hearts were initially placed in oxygenated Tyrode solution, and the left anterior descending coronary artery was cannulated. Ventricular cells were enzymatically isolated with a procedure described previously (16, 19). Briefly, the free transmural wall of the anterior left ventricle (~30 × 50 mm) was removed along with the coronary artery branch irrigating it. The free wall was perfused with oxygenated, nominally Ca2+-free Tyrode solution for 20–30 min, and the solution was then changed to one containing 200–300 U/ml collagenase (CLS II, Worthington Biochemical; Freehold, NJ) for 60–100 min. Regional cells were separated from the digested tissue. Subendocardial and subepicardial cells were taken from the endocardial and epicardial surfaces (<1 mm thick), whereas midmyocardial cells were dissociated from the midmyocardial layer. The cells were placed in a high-K+ storage solution (see Solutions) and gently trituated with a Pasteur pipette. The isolated cells were kept in the medium at least 1 h (at room temperature) before use. Only quiescent rod-shaped cells showing clear cross-striations were used.

A small aliquot of the solution containing the isolated cells was placed in an open perfusion chamber (1 ml) mounted on the stage of an inverted microscope. Cells were allowed to adhere to the bottom of the dish for 5–10 min and were then superfused at 2–3 ml/min with Tyrode solution. Only quiescent rod-shaped cells showing clear cross-striations were used.

Solutions. The Tyrode solution contained (in mmol/l) 136 NaCl, 5.4 KCl, 1.0 MgCl2, 1.8 CaCl2, 0.33 NaH2PO4, 10 glucose, and 10 HEPES; pH was adjusted to 7.4 with NaOH. The high-K+ storage medium contained (in mmol/l) 20 KCl, 10 KH2PO4, 10 glucose, 70 K-glutamate, 10 β-hydroxybutyric acid, 10 taunine, 0.5 EGTA, and 20 mannitol and 0.1% albumin; pH was adjusted to 7.2 with KOH. The pipette solution contained (in mmol/l) 20 KCl, 110 K-aspartate, 1.0 MgCl2, 10 HEPES, 5.0 EGTA (0.05 for the recording of APs), 0.1 GTP, 5.0 Na2-phosphocreatine, and 5.0 Mg2-ATP; pH was adjusted to 7.2 with KOH. For I K1 determination, external Na+ was replaced by equimolar choline. BaCl2 (0.5 mmol/l) was used to inhibit IK1, and CdCl2 (200 μmol/l) was used to block Ca2+ current (ICa). Ca2+-dependent transient outward chloride current (ICl,ca or I oca) was inhibited by 5 mmol/l EGTA in the pipette solution and by the addition of Cd2+ to the external solution. The experiments were conducted at 36°C (for AP, IK1, and IKC recordings) or room temperature (22°C, for IK1, IKO1, and ICa recordings).

Data acquisition and analysis. The whole cell patch-clamp technique was used. Borosilicate glass electrodes (1.0 mm outer diameter) were pulled with a Brown-Flaming puller (model P-87) and had tip resistances of 2–3 MΩ when filled with pipette solution. The tip potentials were compensated before the pipette touched the cell. A gigaseal (>10 GΩ) was obtained, and the cell membrane was ruptured by gentle suction to establish the whole cell configuration. Liquid junction potentials after membrane rupture between the external and pipette solutions (10.5 ± 0.3 mV) were not corrected except for recordings of APs and IK1. Data were acquired by the use of an Axopatch 200A and/or 200B amplifier (Axon Instruments; Foster City, CA). Command pulses were generated by a 12-bit digital-to-analog converter controlled by pCLAMP software (Axon Instruments). Recordings of the AP and membrane currents were low-pass filtered at 2 kHz and stored on the hard disk of an IBM-compatible computer.

Cell capacitance was calculated by integrating area of the capacitive response to 5-nm hyperpolarizing step from a holding potential of −60 mV divided by the voltage drop. Membrane capacitance was 145.5 ± 6.1, 150.3 ± 7.2, and 148.7 ± 6.9 pF, respectively, in endocardial (n = 76), midmyocardial (n = 73), and epicardial (n = 68) cells from control hearts [P = not significant (NS)] and 151.5 ± 7.6, 157.3 ± 8.1, and 154.4 ± 7.4 pF in endocardial (n = 102), midmyocardial (n = 114), and epicardial (n = 112) cells from failing hearts (P = NS vs. controls). Series resistance was electronically compensated.

Only cells with stable IK for 5 min after membrane rupture were used for study. To further exclude possible effects of IK rundown on the current measurement, the time course of changes of IK was monitored after rupture of the patch membrane in a set of experiments in cells from control and failing hearts. No apparent rundown was observed for at least 15 min after membrane rupture in the three cell types from the two groups. Therefore, IK was determined between 5 and 12 min after rupture of the cell membrane.
Table 1. RMP, APD50, and APD90 at 0.5 Hz in cells from different regions of control and failing canine hearts

<table>
<thead>
<tr>
<th>Region</th>
<th>Control</th>
<th>Heart Failure</th>
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<tr>
<td></td>
<td>n</td>
<td>RMP (mV)</td>
</tr>
<tr>
<td>Epi</td>
<td>32</td>
<td>-82.1 ± 0.6</td>
</tr>
<tr>
<td>M</td>
<td>35</td>
<td>-80.5 ± 0.7</td>
</tr>
<tr>
<td>Endo</td>
<td>31</td>
<td>-81.4 ± 0.5</td>
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Values are means ± SE; n = no. of cells. RMP, resting membrane potential (in mV); APD50 and APD90, action potential duration at 50% and 90% repolarization (in ms), respectively; M, midmyocardium. *P < 0.01 vs. endocardial (Endo) cells; †P < 0.05 vs. epicardial (Epi) cells; §P < 0.05 and $P < 0.01 vs. control.

Nonlinear curve-fitting programs (Clampfit in pCLAMP 6 or SigmaPlot, Jandel Scientific; Rafael, CA) were used to perform curve-fitting procedures. Results are presented as means ± SE. Paired and unpaired Student's t-tests were used as appropriate to evaluate the statistical significance of differences between two group means, and ANOVA was used for multiple groups. Values of P < 0.05 were considered to indicate statistical significance.

RESULTS

Remodeling of heterogeneous AP in ventricular cells of HF. Transmembrane potential was recorded in current clamp mode with normal Tyrode bath solution at 36°C. Cardiac AP characteristics (at 0.5–2 Hz) are illustrated in Fig. 1 in cells isolated from the ventricular epicardium, midmyocardial layer, and endocardium of control and failing canine hearts. In control cardiac cells (Fig. 1A), APs showed only a small phase 1 (top) in endocardial cells, whereas they displayed a prominent phase 1 and a significant "spike-and-dome" configuration in midmyocardial (middle) and epicardial (bottom) cells. APD was substantially shorter in endocardial and epicardial cells than in the midmyocardial cells, especially at the low stimulation rates of 0.5 and 1 Hz. Figure 1B shows that AP characteristics were substantially altered in the three cell types from failing hearts. The phase 1 and spike-and-dome configuration of the AP was clearly diminished in epicardial and midmyocardial cells, and the APD was prolonged in all regions. Average data for APD and resting membrane potential at 0.5 Hz are summarized in Table 1. APD was significantly longer in midmyocardial cells than in endocardial or epicardial cells from control hearts at lower rates, consistent with previous reports (1, 40). The heterogeneous electrophysiology seen in controls was diminished by an inhomogeneous prolongation of APD in failing hearts. Mean values of APD90 at 0.5 Hz were increased by 218 ms (59%), 150 ms (32%), and 295 ms (91%), respectively, in epicardial, midmyocardial, and endocardial cells. Figure 2 illustrates rate-dependent properties of APD at 50% repolarization and APD90 in cells from control and failing hearts.

EADs in cells from failing hearts. Figure 3 displays EADs recorded in endocardial, midmyocardial, and epicardial cells from failing hearts observed during APs recorded with the use of a train of 10 pulses (2-ms duration) at 0.5 Hz. EADs were never observed in cells from control hearts. The incidence of EADs was 17% in endocardial (10 of 59 cells), 34% in midmyocardial (22 of 65 cells), and 20% in epicardial (12 of 60 cells) cells, respectively. In all cell types, EADs appeared as single and/or multiple oscillations of the AP, and oscillations of the AP decreased or disappeared when the frequency increased to 1 or 2 Hz. The mean proportion of APs showing EADs was 0.46, 0.10, and 0.02 at 0.5, 1, and 2 Hz, respectively.

Changes in I_{K1}. I_{K1} was determined in normal Tyrode solution containing 10 μmol/l nifedipine. Figure 4A illustrates whole cell I_{K1} elicited by 300-ms voltage steps between −110 and −30 mV from −40 mV, as shown in the inset, in endocardial cells from control (left top) and failing (right top) hearts. I_{K1} amplitude
was substantially reduced in the cells from the failing heart. The current was fully inhibited by 0.5 mmol/l Ba$^{2+}$ in control (left bottom) and failing (right bottom) cells. Figure 4B shows current-voltage ($I$-$V$) relationships of $I_{K1}$ in cells from control and failing hearts. No difference in inward or outward $I_{K1}$ was found among the three cell types from control and failing hearts. However, $I_{K1}$ density was significantly suppressed for both inward (−110 and −100 mV) and outward (−70 to −30 mV) components in cells from failing hearts ($P < 0.05$ or $P < 0.01$ vs. control). The expanded outward component (Fig. 4C) of the $I_{K1}$-$V$ relations shows that $I_{K1}$ density was significantly decreased in the three cell types of failing hearts at −70 to −30 mV. $I_{K1}$ density at −60 mV was reduced by 40.9%, 40.7%, and 41.1%, respectively, in epicardial (6.1 ± 0.6−3.6 ± 0.3 pA/pF),

Fig. 3. Early afterdepolarizations (EADs) in cells from the failing heart at 0.5 Hz. A: EADs were recorded in an Endo cell. B: EADs were recorded in a M cell. C: EADs were recorded in an Epi cell. D: percentage of EADs over 10 stimulation pulses in all cell types ($n = 44$) with EADs at 0.5, 1, and 2 Hz and EAD category (inset) [single (S) oscillation and multiple (M) oscillations of the AP in all cell types with EADs at the corresponding rate].
The results indicate that $I_{o1}$ was substantially decreased in the cell from the failing heart. Figure 5B displays mean $I-V$ relationships of peak $I_{o1}$ in control (left) and failing (right) cells. Density of $I_{o1}$ is clearly smaller in endocardial cells ($n = 25$) than in epicardial ($n = 23$) and midmyocardial ($n = 21$) cells from control hearts, consistent with previous reports (23). Although the $I_{o1}$ gradient remained, $I_{o1}$ density was reduced in endocardial ($n = 38$), midmyocardial ($n = 45$), and epicardial ($n = 41$) cells from failing hearts ($P < 0.05$ or $P < 0.01$ vs. control at $+10$ to $+60$ mV). At $+40$ mV, $I_{o1}$ was decreased by 43%, 45%, and 43%, respectively, in epicardial ($6.6 \pm 0.3$–$3.8 \pm 0.4$ pA/pF), midmyocardial ($6.3 \pm 1.0$–$3.5 \pm 0.6$ pA/pF), and endocardial ($3.2 \pm 0.3$–$1.8 \pm 0.3$ pA/pF) cells ($P < 0.01$ vs. control). The results indicate that $I_{o1}$ is homogeneously downregulated across the ventricular wall of failing hearts, contributing to the diminished phase 1 of the AP.

Changes in $I_{o1}$.

It is well known that canine ventricular cells exhibit two components of transient outward currents: $I_{o1}$ and $I_{o2}$ (or $I_{Cl, Ca}$) (45). We focused on $I_{o1}$, which is decreased in failing human (3, 29) and canine (13) ventricular cells, to study whether alteration of $I_{o1}$ is transmurally homogeneous across the ventricular wall. $I_{o1}$ was recorded using 300-ms voltage steps between $-30$ and $+60$ mV (see Fig. 5A, inset). Figure 5A illustrates $I_{o1}$ traces in epicardial cells from control (left) and failing (right) hearts. $I_{o1}$ was substantially decreased in the cell from the failing heart. Figure 5B displays mean $I-V$ relationships of peak $I_{o1}$ in control (left) and failing (right) cells. Density of $I_{o1}$ is clearly smaller in endocardial cells ($n = 25$) than in epicardial ($n = 23$) and midmyocardial ($n = 21$) cells from control hearts, consistent with previous reports (23). Although the $I_{o1}$ gradient remained, $I_{o1}$ density was reduced in endocardial ($n = 38$), midmyocardial ($n = 45$), and epicardial ($n = 41$) cells from failing hearts ($P < 0.05$ or $P < 0.01$ vs. control at $+10$ to $+60$ mV). At $+40$ mV, $I_{o1}$ was decreased by 43%, 45%, and 43%, respectively, in epicardial ($6.6 \pm 0.3$–$3.8 \pm 0.4$ pA/pF), midmyocardial ($6.3 \pm 1.0$–$3.5 \pm 0.6$ pA/pF), and endocardial ($3.2 \pm 0.3$–$1.8 \pm 0.3$ pA/pF) cells ($P < 0.01$ vs. control). The results indicate that $I_{o1}$ is homogeneously downregulated across the ventricular wall of failing hearts, contributing to the diminished phase 1 of the AP.
Time- and voltage-dependent kinetics of $I_{to1}$ were not altered by HF in the three cell types. For instance, recovery time constants ($\tau_1$ and $\tau_2$) of $I_{to1}$ were 21.3 ± 9.5 and 289.5 ± 54.2, 25.7 ± 13.4 and 332.1 ± 64.8, and 27.6 ± 14.7 and 258.4 ± 69.2 ms, respectively, in epicardial, midmyocardial, and endocardial myocytes in the control group, whereas in HF $\tau_1$ and $\tau_2$ were 24.1 ± 9.4 and 312.5 ± 59.6, 27.7 ± 14.2 and 341.6 ± 85.7, and 29.3 ± 12.6 and 271.5 ± 68.3 ms in the corresponding cell types ($n = 10$ for each cell type, $P = \text{NS vs. control}$).

Changes in $I_{Ks}$. $I_{Ks}$ is believed to be an important repolarizing current in mammalian hearts, and its lesser expression in the midmyocardium contributes in part to APD heterogeneity in canine ventricles (22). We measured $I_{Ks}$ under conditions of Na+- and extracellular K+-free solution in the presence of 10 mmol/l nifedipine, 5 mmol/l 4-AP, 1 mmol/l atropine, and 5 mmol/l E-4031. Under extracellular K+-free conditions, $I_{Ks}$ is greatly diminished, whereas the density of $I_{Ks}$ is augmented (22, 38). $I_{K1}$ is also practically eliminated (19, 20, 22). Figure 6A illustrates representative $I_{Ks}$ tracings in cells from the epicardium, midmyocardial layer, and endocardium of control hearts recorded with 3-s voltage steps to between −30 and +50 mV from a holding potential of −60 mV, followed by 2-s repolarizations to −30 mV (see Fig. 6A, inset). The developing currents observed during depolarization steps and the tail currents ($I_{Ks,step}$ and $I_{Ks,tail}$, respectively) were smaller in midmyocardial cells than in epicardial or endocardial cells of control hearts. Figure 6B plots complete data for $I_{Ks,tail}$. Shown are current densities measured at −30 mV after a 3-s pulse to +30 mV. Each point represents results from an individual cell. The thick lines indicate the mean values for each group. The density of $I_{Ks,tail}$ was substantially less in midmyocardial cells (1.9 ± 0.2 pA/pF, $n = 31$, $P < 0.05$) than in epicardial (2.8 ± 0.3 pA/pF, $n = 27$) and endocardial (2.6 ± 0.3 pA/pF, $n = 29$) cells, consistent with previous reports (22).

Figure 6C shows $I_{Ks}$ traces in cells isolated from the epicardium, midmyocardial layer, and endocardium of failing hearts using the same protocol and conditions as in Fig. 6A. The amplitudes of $I_{Ks,step}$ and $I_{Ks,tail}$ were substantially decreased by HF in all three cell types. Individual $I_{Ks,tail}$ are plotted in Fig. 6D. $I_{Ks,tail}$ density averaged 1.2 ± 0.1, 0.96 ± 0.1, and 1.1 ± 0.1 pA/pF in epicardial ($n = 48$), midmyocardial ($n = 49$), and endocardial ($n = 45$) cells, respectively. The heterogeneous distribution of $I_{Ks}$ seen in controls disappeared in failing hearts. Mean values of $I_{Ks,tail}$ were decreased by 57%, 49%, and 58% in epicardial, midmyocardial, and endocardial cells, respectively ($P < 0.01$ vs. control).

Figure 7 displays I-V relationships of $I_{Ks,step}$ and $I_{Ks,tail}$ in regional cells from control and failing hearts. $I_{Ks,step}$ was measured from time-dependent current to the end of the depolarization step. As Fig. 7A shows, although $I_{Ks,step}$ density was slightly smaller at +10 to +60 mV in midmyocardial cells, no statistically significant difference was observed ($P = \text{NS vs. epicardial or endocardial cells}$). However, the current was significantly reduced in all the three regions from failing hearts at +10, +30, and +60 mV ($P < 0.05$ or $P < 0.01$ vs. control). Figure 7B shows that $I_{Ks,tail}$ density was significantly smaller in midmyocardial cells at +10, +30, and +60 mV in controls ($P < 0.05$ vs. epicardial or endocardial cells). Failing hearts showed a downregulated $I_{Ks,tail}$ in all three regions ($P < 0.01$ at +10, +30, and +60 mV vs. control). The results indicate that $I_{Ks}$ decreases in all cell types of failing hearts and the distribution of $I_{Ks}$ becomes homogeneous in failing hearts.

![Fig. 6. Slow component of the delayed rectifier K+ current ($I_{Ks}$) in cells from different regions of canine hearts. A: voltage-dependent $I_{Ks}$ tracings recorded in Epi ($C_m = 143.5$ pF), M ($C_m = 150.1$ pF), and Endo ($C_m = 148.5$ pF) cells with the protocol shown in the inset at 0.1 Hz. A smaller amplitude of $I_{Ks}$ was seen in the M cell. B: tail current ($I_{Ks,tail}$) density at +30 mV. The thick lines are the mean data of Epi (□; $n = 27$), M (■; $n = 31$), and Endo (△; $n = 29$) cells. $I_{Ks,tail}$ density was smaller in M cells ($P < 0.05$ vs. Endo or Epi cells). C: voltage-dependent $I_{Ks}$ tracings recorded in Epi ($C_m = 147.6$ pF), M ($C_m = 159.7$ pF), and Endo ($C_m = 155.9$ pF) cells from failing hearts. The amplitude of $I_{Ks}$ was reduced in all regions compared with that of the control as in A. D: density of $I_{Ks,tail}$ at +30 mV in cells from failing hearts. The difference of $I_{Ks,tail}$ density disappeared in Epi (▲; $n = 48$), M (●; $n = 49$), and Endo (△; $n = 45$) cells, but the averaged current density (thick lines) was significantly decreased in all the regions ($P < 0.01$ vs. control in B).
**L-type Ca\(^{2+}\) current.** L-type Ca\(^{2+}\) current (I\(_{\text{Ca,L}}\)) was evoked by voltage steps to between −40 and +50 mV from −50 mV in control and failing cells under Na\(^+\)- and K\(^+\)-free conditions. Figure 9A displays representative I\(_{\text{Ca,L}}\) recorded with the protocol shown in the inset in control (left) and failing (right) cells. I-V relationships of inward peak I\(_{\text{Ca,L}}\) are shown in Fig. 9B (control) and Fig. 9C (HF) with the peak current being observed at +10 mV in the three cell types from control and failing hearts. No significant differences were found in I\(_{\text{Ca,L}}\) density or kinetics as a function of cell type or disease at any voltage. The density of I\(_{\text{Ca,L}}\) at the voltage (+10 mV) corresponding to peak current was −9.2 ± 0.8, −8.9 ± 0.8, and −8.7 ± 0.7 pA/pF, respectively, in epicardial, midmyocardial, and epicardial cells of control hearts (P = NS) and −9.5 ± 0.7, −9.9 ± 1.2, and −9.4 ± 0.8 pA/pF in the corresponding regions of failing hearts (P = NS vs. control).

Time- and voltage-dependent kinetics of I\(_{\text{Ca}}\) were not affected by HF. At +10 mV, the inactivation \(\tau_1\) and \(\tau_2\).

**I\(_{\text{K}}\).** To study whether alteration of I\(_{\text{K}}\) contributes to the prolonged APD, we used the I\(_{\text{K}}\) blocker E-4031 as a tool to define I\(_{\text{K}}\) as the E-4031-sensitive component (37) in control and failing cells. The experiments were performed under conditions similar to those for I\(_{\text{Ks}}\) except for the inclusion of 5.4 mmol/l K\(^+\) in the bath. As Fig. 8A shows, the membrane currents were initially measured at −5 min after rupture using the voltage steps shown in the inset, and recordings were repeated after application of 5 μmol/l E-4031 for 5–7 min. E-4031-sensitive current (I\(_{\text{Kr}}\)) was obtained by digital subtraction of currents before and after the blocker. Figure 8B shows that no regional differences in the I-V relation of E-4031-sensitive I\(_{\text{Kr}}\) were observed in the three cell types of control hearts. No significant change in I\(_{\text{Kr}}\) was found in cells from failing hearts (P = NS vs. control).
of \( I_{\text{Ca}} \) in control hearts were 11.6 ± 1.4 and 73.6 ± 4.4, 11.7 ± 1.7 and 69.3 ± 5.6, and 12.9 ± 1.5 and 75.7 ± 9.7 ms, respectively, in epicardial, midmyocardial, and endocardial cells (\( P = \text{NS} \)), and, in failing hearts, \( \tau_1 \) and \( \tau_2 \) were 12.3 ± 1.5 and 68.4 ± 6.8, 13.7 ± 1.8 and 73.6 ± 8.7, and 11.3 ± 1.5 and 72.1 ± 8.9 ms in corresponding regional cells, respectively (\( n = 11 \) in each cell type, \( P = \text{NS} \) vs. control). Recovery \( \tau_1 \) and \( \tau_2 \) of \( I_{\text{Ca}} \) from inactivation were 27.3 ± 5.9 and 334.1 ± 61.7, 22.5 ± 7.1 and 312.8 ± 47.2, and 24.7 ± 8.7 and 298.9 ± 54.6 ms in control epicardial, midmyocardial, and endocardial cells, respectively. In the HF group, the recovery \( \tau_1 \) and \( \tau_2 \) were 23.5 ± 6.7 and 317.2 ± 49.6, 27.1 ± 7.5 and 294.8 ± 53.6, and 25.3 ± 10.3 and 309.4 ± 48.5 ms in the corresponding cell types (\( n = 12 \) for each cell type, \( P = \text{NS} \) vs. control).

**DISCUSSION**

In the present study, we found the first evidence, to our knowledge, that tachycardia-induced canine HF causes transmural electrical remodeling and attenuates the transmural heterogeneity in APD. EADs were observed in cells from the endocardium, midmyocardial layer, and epicardium of failing hearts, and several ionic currents were remodeled. \( I_{\text{K1}}, I_{\text{to1}}, \) and \( I_{\text{Ks}} \) were reduced in the three cell types of failing hearts; however, \( I_{\text{Kr}} \) and \( I_{\text{Ca.1}} \) were not significantly affected. Reductions in \( I_{\text{K1}} \) and \( I_{\text{to1}} \) were transmurally homogeneous, whereas \( I_{\text{Ks}} \) was affected to a relatively greater extent in the epicardium and endocardium than in the midmyocardium, eliminating the transmural \( I_{\text{Ks}} \) gradient observed in the normal heart.

**Comparison with previous findings of AP remodeling in HF.** APD is significantly prolonged in cells and tissues dissociated from ventricles of different species with HF induced by various mechanisms, including pressure and/or volume overload in rats, cats, and guinea pigs as well as chronic ventricular tachycardia-induced HF in dogs and rabbits (26, 44). In ventricular cells from failing human hearts, the cardiac APD also shows significant prolongation (3). Delayed repolarization is believed to be the cellular mechanism for the prolonged QTc on clinical electrocardiograms observed in HF patients with ventricular hypertrophy and/or dilated cardiomyopathy (5, 15), and the mechanisms of sudden death in patients with HF may be related to those of the acquired long QT syndrome (26). Our results provide the additional information that the transmurally heterogeneous AP morphology and APD seen in the control heart are attenuated in failing hearts (see Figs. 1 and 2 and Table 1). EADs are observed in the three cell types from failing hearts and may induce triggered activity that causes ventricular arrhythmias (1, 44).

The attenuated APD heterogeneity we saw is not consistent with observations in mild cardiac hypertrophy in the guinea pig (4), which increased APD in epicardial and midmyocardial cells but decreased APD in endocardial cells. Studies in vivo exhibit spatial and temporal dispersion of QT intervals on electrocardiograms in humans (2) and of monophasic APs in animals (33). However, these differences may reflect alterations in APD in different zones of the heart (e.g., apex vs. base) while transmural heterogeneity decreases.

**Comparison with previous findings of ionic current remodeling in HF.** It is well known that AP morphology and APD depend on the balance between depolarizing and repolarizing currents. Therefore, both depolarizing and repolarizing currents were studied to clarify the ionic mechanisms of the prolonged AP in failing hearts, and several membrane currents were found to be involved (30, 44). \( I_{\text{to1}} \) has consistently been observed to be downregulated in tachycardia-induced HF in dogs (13) and rabbits (36) and in terminally failing human hearts (3, 29), although discrepant results have been reported in other models and species (44). The present study demonstrated that density of \( I_{\text{to1}} \) was decreased, and kinetics of \( I_{\text{to1}} \) were not changed in failing hearts, consistent with a report from Kaab et al. (13) in the same model and species. We provide further informa-
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ation that the transmural gradient of \( I_{\text{to1}} \) distribution is not changed, but the current density is similarly reduced in all regions (see Fig. 5). Downregulation of \( I_{\text{to1}} \) is related to the decreased phase 1 of the AP clearly present in HF cells (Fig. 1). It has been demonstrated that the mRNA transcripts coding the \( I_{\text{to1}} \) channel in the rat (Kv4.2) (7) are decreased in parallel with reduced \( I_{\text{to1}} \) density in hypertrophied cells (34). The mRNA for Kv4.3 coding for \( I_{\text{to1}} \) in humans (7) was found to be reduced by a similar extent to \( I_{\text{to1}} \) in the failing human left ventricle (12).

Observations regarding changes in \( I_{\text{k1}} \) in cells from hypertrophied and/or failing hearts are discrepant. Some of these differences may be due to the intensity, nature (e.g., hypertrophy vs. failure), initiating stimuli, and duration of cardiac disease (44). However, downregulated \( I_{\text{k1}} \) expression was consistently observed in tachypacing-induced canine HF (13) and in failing human hearts (3). In the present study, we found that \( I_{\text{k1}} \) density was homogeneously reduced (both inward and outward components) in endocardial, midmyocardial, and epicardial cells of failing hearts, which contributes in part to the prolonged APD.

\( I_{\text{kr}} \) and \( I_{\text{ks}} \) are believed to play an important role in repolarization of cardiac APs in mammalian hearts, including dogs (22) and humans (17). The downregulation of \( I_{\text{kr}} \) and/or \( I_{\text{ks}} \) may contribute to delayed repolarization in HF. Decreased \( I_{\text{kr}} \) and \( I_{\text{ks}} \) were recently observed in ventricular cells from rabbits with pacing-induced HF (46). A preliminary study (18) has reported that \( I_{\text{kr}} \) was reduced in cells from terminally failing human hearts compared with mildly diseased cells. In the present study, we found that \( I_{\text{kr}} \), and not \( I_{\text{ks}} \), was significantly reduced in all three regions, consistent with a recent report (48) in hypertrophic rabbit hearts in which only \( I_{\text{ks}} \), not \( I_{\text{kr}} \), was reduced in ventricular endocardial and epicardial cells. Our results showed that the percentage of \( I_{\text{ks}} \) reduction was greater in epicardial and endocardial cells than in midmyocardial cells, which may contribute in part to the inhomogeneous prolongation of APD in the three regions. The elimination of the transmural APD gradient in HF may be due to the associated elimination of the transmural \( I_{\text{ks}} \) gradient.

It is believed that alteration of intracellular Ca\(^{2+}\) handling may account for the abnormalities in excitation-contraction coupling in the failing heart and is involved in the occurrence of EADs (11). However, studies of \( I_{\text{ca,l}} \) have been discrepant. The density of \( I_{\text{ca,l}} \) has been found to be unchanged, increased, or decreased in different models and species (44). In general, \( I_{\text{ca,l}} \) is increased in mild-to-moderate hypertrophy and decreased in severe hypertrophy and HF (44). In hypertrophied guinea pig hearts, inhomogeneous changes in \( I_{\text{ca,l}} \) were considered to contribute to a transmurally heterogeneous prolongation of ventricular APD (4). Schroder et al. (39) found that single Ca\(^{2+}\) channels exhibited increased availability and opening probability in cells isolated from failing human hearts, but whole cell \( I_{\text{ca,l}} \) did not show significant differences, when compared with nonfailing cells, in agreement with a previous report in humans (27). Recently, it has been found that the L-type Ca\(^{2+}\) channel number is reduced and channel open times are increased in ventricular cells from pacing-induced canine failing hearts, so that overall \( I_{\text{ca,l}} \) density is unchanged (10). The results of the present study showed no significant change in \( I_{\text{ca,l}} \) density and kinetics in cells from the endocardium, midmyocardial layer, and epicardium, consistent with other reports in the same model and species (13).

Limitations of the present study. The present study was performed in cells isolated from the endocardium, midmyocardial layer, and epicardium of the left anterior ventricular wall of control and failing canine hearts. We cannot exclude the possibility that heterogeneous electrophysiology still exists in other regions of the ventricle in failing hearts, such as between the base and apex of the left ventricle and between the right and left ventricles and/or septum. Another limitation is that the present study focused only on \( I_{\text{to1}}, I_{\text{k1}}, I_{\text{kr}}, I_{\text{ks}}, \) and \( I_{\text{ca,l}} \). We did not assess other currents, such as the Na\(^+\)/Ca\(^{2+}\) exchange current (\( I_{\text{na,ca}} \)) and late Na\(^+\) current (\( I_{\text{na,x}} \)), which have also been reported to contribute to regional heterogeneity in canine ventricles (50, 51). Whether alteration of \( I_{\text{na,ca}} \) and/or \( I_{\text{na,x}} \) contributes to the higher incidence of EADs in failing midmyocardial cells remains further experimental study. Another limitation is that membrane currents, including \( I_{\text{ca}} \), were studied with 5 mM pipette EGTA, resulting in strong intracellular Ca\(^{2+}\) buffering and very low free Ca\(^{2+}\) concentrations. This approach was necessary to prevent contamination of \( I_{\text{ca}} \) by \( I_{\text{to2}} \) and to optimize cell stability. However, it would have prevented us from detecting any changes due to altered intracellular Ca\(^{2+}\) handling. In addition, membrane currents were measured at a slow rate (0.2 Hz) to prevent possible rate-dependent inactivation of the currents. This rate is much slower than that of spontaneous APs and the APs that we recorded; however, we did not observe any change in current kinetics, suggesting that our results are pertinent to AP changes.

Potential significance of our findings. It is well known that heterogeneity of cellular electrophysiology is present across the ventricular wall in mammalian hearts of several species, including dogs and humans. The present study is the first of which we are aware to study the remodeling of APs and ionic currents in cells across the left ventricular wall in a well-defined canine model of HF. Spontaneous EADs were observed in the three regions of failing hearts, and the incidence of EADs was higher in midmyocardial cells than in endocardial and epicardial cells. It seems that midmyocardial cells are more susceptible to EADs in failing hearts, as in response to cardioactive agents under nondiseased conditions (1). In multicellular preparations and intact hearts, EADs constitute an important cellular mechanism for triggered activity (1, 47). It appears that downregulation of multiple K\(^+\) channel currents, i.e., \( I_{\text{to1}}, I_{\text{k1}}, \) and \( I_{\text{ks}} \) is responsible for the delayed repolarization and favors the genesis of EADs in the three regions, which is consistent with earlier
simulation studies (25, 35). Therefore, pharmacological therapy to activate or potentiate \( I_K \) (48) may be efficacious in controlling life-threatening ventricular arrhythmias in patients with HF. In addition, gene therapy to increase \( I_{K1} \) and/or \( I_{Ks} \) may be effective against arrhythmias induced by electrical remodeling. It has been reported that overexpression of human \( K^+ \) channels may terminate EADs in cultured rabbit ventricular cells (31).

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