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Research Article

Repolarization abnormalities in cardiomyocytes of dogs with chronic heart failure: role of sustained inward current

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Abstract. We previously showed that a canine model of chronic heart failure (HF) produced by multiple coronary microembolizations manifests ventricular arrhythmias similar to those observed in patients with chronic HF. In the present study, we used single canine cardiomyocytes isolated from the left ventricle (LV) of normal dogs (n = 13) and dogs with HF (n = 15) to examine the cellular substrate of these arrhythmias. Action potentials (APs) and ion currents were measured by perforated and whole cell patch clamp, respectively. We found prolonged APs and alterations of AP duration resulting in early afterdepolarizations (EADs) at the low pacing rates of 0.5 Hz and 0.2 Hz. Na^+ channel blockers saxitoxin (STX, 100 nM) and lidocaine (90 μM) reduced AP duration dispersion and abolished EADs in HF cardiomyocytes. The steady-state current (I_{ss})-voltage relation, in the voltage range from −25 mV to 25 mV analogous to the AP plateau level, was significantly shifted inward in HF cardiomyocytes. STX and lidocaine shifted the I_{ss}-voltage relationship in an outward direction. The shifts produced by both drugs was significantly greater in cardiomyocytes of dogs with HF, indicating an increase in inward current. In the experimental configuration in which K^+ currents were blocked, the density of the steady-state Ca^{2+} current (I_{Ca}) was found to decrease in HF cardiomyocytes by approximately 33%. In contrast, the density of the steady-state Na^+ current (I_{Na}) significantly ($P < 0.01$) increased in HF cardiomyocytes (0.17 ± 0.06 pA/pF) compared with normal cells (0.08 ± 0.02 pA/pF). The relative contribution of I_{Na} to the net inward current was greater in HF cardiomyocytes, as evident from the increased ratio of I_{Na}/I_{Ca} (from 0.22 to 0.68). These observations support a hypothesis that anomalous repolarization of HF cardiomyocytes is due, at least in part, to an increased steady-state inward Na^+ current.

Key words. Cardiomyocyte; action potential; saxitoxin; lidocaine; nifedipine; patch clamp; sodium channel; calcium channel.

Heart failure (HF) is associated with profound abnormalities of both cardiac rhythm and contractile function. Despite recent progress in the treatment of congestive HF, mortality remains high. Approximately 40% of these patients die suddenly due to sudden cardiac death syndrome [1–6]. Ventricular tachycardia and fibrillation were documented in ~80% of patients with HF in whom electrocardiogram (ECG) Holter recordings were being obtained at the time of sudden death [7–9]. Cellular mechanisms that could generate abnormal rhythmic activity in HF include afterpotentials, reentry and enhanced automaticity (see for review [10]). Studies in isolated single cardiomyocytes from failing human and animal hearts have consistently shown im-

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paired repolarization, independent of the etiology of HF [11–14]. It is believed that abnormal repolarization provides an arrhythmogenic substrate in HF. Ion current balance change resulting from augmented inward depolarizing current or reduced outward repolarizing currents may produce action potential prolongation leading to triggered activity arising from afterpotentials. While new concepts of the ionic mechanisms of the HF-induced impaired repolarization implicate outward K^+ current downregulation [12–15], the role of the inward currents has not been studied in detail. A persistent inward Na^+ current that exceeded the duration of a typical action potential (AP) has been consistently observed in Purkinje fibers and ventricular cardiomyocytes of various mammalian species [16–21], but its potential significance in HF has not been reported.

Because of varying etiologies, concomitant pharmacological treatment and difficulty in obtaining tissue from patients with HF, except in end-stage disease, a reliable animal model of HF can provide an opportunity to investigate cellular mechanisms underlying malignant arrhythmias. We previously developed a reproducible canine model of chronic HF which manifests marked and sustained depression of ventricular function, dense ventricular ectopy and sudden death in $\sim 13\%$ of animals [22, 23]. In a previous study we showed prolongation of AP duration in ventricular cardiomyocytes in this canine model of HF. AP duration was normalized by Na^+ channel blockers saxitoxin (STX) and lidocaine, suggesting a possible role of Na^+ inward current in AP prolongation in HF. In the present study, we characterized repolarization abnormalities and the ionic current balance change underlying these alterations in APs of HF cardiomyocytes. We tested the hypothesis that an increase in sustained inward Na^+ current is responsible, at least in part, for a defective balance of ion currents that produces the observed abnormalities in APs. Preliminary data from this study have been reported previously in abstract form [25, 26].

Materials and methods

Dog HF model. The dog model of chronic HF was previously described in detail. In the present study, 17 healthy mongrel dogs, weighing between 24 and 31 kg, underwent sequential coronary microembolization to produce HF. Embolizations were performed 1 to 3 weeks apart and were discontinued when the left ventricle (LV) ejection fraction, determined angiographically, was $\leq 35\%$. Two dogs died suddenly in the course of embolization, and experiments were performed on remaining 15 animals. At the time of harvesting the heart for cardiomyocyte isolation (approximately 3 months after the last microembolization), LV ejection fraction was $27 \pm 2\%$. The protocol for this study was approved by the institutional Care of Experimental Animals Committee and conformed to the "Position of the American Heart Association on Research Animal Use" and the guiding principles of the American Physiological Society.

Cardiomyocyte isolation. Two methods were used to isolate cardiomyocytes, namely (i) coronary artery perfusion of a wedge of left ventricle (LV) tissue [24] (5 normal and 5 failed hearts), and (ii) tissue slices of LV (8 normal and 10 failed hearts). For the slice method, the dog heart was rapidly removed under general anesthesia and placed in ice-cold cardioplegic solution consisting of (in mM): NaCl 110, $CaCl_2$ 1.2, $MgCl_2$ 16, KCl 16, pH 7.8 (adjusted with Na_2HCO_3). A transmural tissue block was dissected from the left ventricle apex. Five to eight mid-myocardium longitudinal slices, $\sim 10 \times 20$ mm and 0.5–1 mm thick, were dissected using a blade and rinsed in oxygenated trituration solution (TTS) at room temperature. The composition of TTS was (in mmol/l): NaCl 140, KCl 5.4, $MgCl_2$ 2, glucose 5, HEPES 10 (pH 7.4). All subsequent procedures were performed in O_2-saturated and constantly triturated TTS at 37 °C. To remove interstitial Ca^{2+}, specimens were immersed in 100 ml of TTS for 20 min, and the procedure was repeated twice. Slices were transferred into TTS containing 25 μmol/l of Ca^{2+} and protease type XXIV (Sigma), 4 U/ml for 3–10 min and subsequently treated with a mixture of collagenase (Worthington, type II, 291 U/mg) and hyaluronidase (Sigma, type IV-S) 0.5 mg/ml for 15–20 min. Finally, slices were incubated for 20 min with collagenase only. The cell suspension was centrifuged for 1 min at 100*g*, and the cardiomyocyte pellet was resuspended in minimum essential media Eagle MEM (Sigma) with 200 μmol/l Ca^{2+}. The yield of viable, Ca^{2+}-tolerant, rod-shape myocytes varied from 30% to 50%.

Patch-clamp technique and data acquisition. Transmembrane ion currents were recorded under voltage clamp by whole cell patch-clamp technique [27] using Axopatch 200A patch-clamp amplifier (Axon Instruments Inc.). The patch pipette tip resistance was 0.8–1.2 MΩ. Signals from the patch-clamp amplifier were filtered at 5 kHz (-3 dB) using a four-pole low-pass Bessel filter and then digitized at a sampling rate of 10 kHz (Digidata 1200, Axon Instruments). pClamp 6.0 software (Axon Instruments) was used to run voltage/current-clamp protocols.

Measurements of ion currents. The steady-state balance of ion currents was evaluated by measuring the net whole-cell current as the average current within 480–500 ms of the depolarization step of different membrane voltages. The patch pipette solution contained (in mM): KCl 143, NaCl 10, EGTA 5, MgATP 2, HEPES 10 (pH 7.4 with KOH). The bath solution contained (in mM):

KCl 5.4, NaCl 140, $MgCl_2$ 2, $CaCl_2$ 1.8, HEPES 10 (pH 7.4 with NaOH). With the aim of studying the contribution of different ions in inward current, in some experiments the content of the pipette and extracellular solutions was chosen to suppress K^+ currents. The pipette solution contained (in mM): CsCl 133, EGTA 10, MgATP 5, tetraethylammonium 20, HEPES 5 (pH 7.3 with CsOH). The bath solution contained (in mM): NaCl 140, CsCl 5.4, $MgCl_2$ 2, $CaCl_2$ 1.8, HEPES 5 (pH 7.3 with NaOH).

Ion currents were recorded at room temperatures of 22–24 °C and normalized to membrane capacitance. The holding potential was -80 mV. The quality of the voltage clamp was controlled in each cardiomyocyte as previously described [28]. Briefly, the deviation (V_{dev}) from voltage command associated with series resistance (R_s) and ion current (I) was estimated to $V_{dev} = I \cdot R_s$. First, we determined the noncompensated R_s as

$$R_s = 20 \text{ mV}/I_c \quad (1)$$

where I_c is the peak value of capacitive current evoked by a square shape voltage pulse of 20 mV amplitude applied from -80 mV. In all experiments the noncompensated R_s was less than 2 MΩ. Electronic series resistance compensation (K_s) was imposed to a point just before oscillations occurred. The final setting of the K_s value (on Axopatch 200A amplifier) varied from 75% to 95%. With R_s compensation, V_{dev} was estimated as

$$V_{dev} = I_{max} \cdot R_s \cdot (100\% - K_s)/100\% \quad (2)$$

Where I_{max} is maximum ion current measured. In all experiments I_{max} did not exceed 1 nA. Satisfactory voltage control was assumed if V_{dev} was < 2 mV, and only these cells were included in the study.

Recording of action potentials by perforated patch clamp. Action potentials of single cardiomyocytes were recorded in current-clamp mode in an amphotericin-B perforated patch-clamp configuration as previously described [24]. To achieve perforation of the membrane patch, the patch pipette solution was supplemented with 0.32 mM amphotericin-B (Sigma Chemical). Cardiomyocytes were stimulated by current pulses of 0.05–0.1 ms duration with an amplitude of 2.5 times the excitation threshold (~ 20 nA). This current was sufficient to evoke APs when the Na^+ current was partially blocked by STX or lidocaine. APs were recorded at 37 °C.

Drug concentration justification. It was shown that the affinity to STX is different for cardiac Na^+ channel compared with the nerve and skeletal muscle isoforms [29]. Namely, the cardiac isoform is almost 10^2 times less sensitive to STX ($IC_{50} = 100$ nM) [30] as compared with nerve and skeletal muscle isoforms. In studies of STX effect on AP we used 100 nM of STX to block approximately 50% of Na^+ channels so that unblocked Na^+ channels were sufficient to evoke cardiomyocyte excitation. Lidocaine is known as a state-dependent blocker of the cardiac Na^+ channels. There are two binding sites for lidocaine within the cardiac Na^+ channel. The low affinity binding site is associated with the resting state and open channel (tonic) block ($K_d \sim 300$–800 μM) [31–35]. The high-affinity binding ($K_d \sim 10$ μM) [32, 33, 35] site (inactivated state) is thought to be responsible for the use-dependent block. In human cardiomyocytes, 100 μM of lidocaine produced $\sim 20\%$ of tonic block of Na^+ channels [34]. Accordingly, we anticipated a similar tonic block of Na^+ channels by 90 μM of lidocaine upon low-frequency stimulation. L-type Ca^{2+} current was blocked by 2 μM of nifedipine [46].

Data analysis. Data were analyzed using pClamp 6 software (Axon Instruments). AP duration was measured at the membrane potential level (baseline), corresponding to 90% of AP repolarization (APD_{90}). Zero time for APD_{90} was chosen as the intercept point of the AP upstroke and the baseline. In voltage-clamp experiments, the membrane capacitance (C) was measured by using a voltage ramp as previously described [28]. Statistical significance among mean data was determined using Student's *t*-test for unpaired data. A probability of $P < 0.01$ was considered significant. The mean APD_{90}s for the two groups at five frequencies were analyzed by repeated measures analysis of variance (ANOVA). The SAS procedure MIXED was used for the analysis, since it permits all observations to be included, even if data for one or more frequencies may be missing for a dog. In the analysis it was assumed that observations for different frequencies for a given dog were correlated, but that the correlation decreased as a function of the difference in the frequencies. Three tests were performed: one for a group effect, one for a time effect and one for a time-by-group interaction. In the presence of a significant test for interaction, individual *t*-tests were performed between the two groups at each frequency. A requirement for a significant test for interaction or for a main effect for group, prior to comparisons at individual frequencies, was used to address the issue of multiple comparisons. All data are reported as the mean ± SD.

Results

Characterization of action potentials

APs of HF cardiomyocytes exhibited repolarization abnormalities. We have previously shown prolongation of AP in cardiomyocytes of dogs with chronic HF [24]. In the present study we compared APs in cardiomyocytes of normal and HF dogs in more detail. We found a small but significant shift of the resting potential (RP)

towards depolarization (-78.2 ± 4.7 mV in normal, n = 15 versus -75.3 ± 3.1, n = 63, in HF, $P < 0.01$). The APs of cardiomyocytes of dogs with HF exhibited the following abnormalities regardless of the cell isolation method:

1) At low stimulation rates, the repolarization in cardiomyocytes of failed hearts was prolonged (see examples in fig. 1). We compared action potential duration in normal and HF cardiomyocytes at five different stimulation frequencies from 0.2 to 3 Hz (fig. 2). The action potential was significantly prolonged in HF cardiomyocytes at stimulation rates of 0.2 Hz and 0.5 Hz.

2) APs of individual cardiomyocytes of HF dogs showed larger variations of AP duration as evidenced by a greater standard deviation for APD_{90} values compared with normal cardiomyocytes (fig. 3). The distribution of APD_{90} values in normal cardiomyocytes was almost symmetrical with only one narrow dominant peak (fig. 3A–C). In contrast to normal cardiomyocytes, the distribution of AP duration in HF cardiomyocytes was asymmetrical, with a skew towards greater values of AP duration (fig. 3D–F). Alterations of AP duration in HF cardiomyocytes were greater at lower pacing rates (0.2 Hz versus 0.5 Hz and 1 Hz) as reflected by a larger standard deviation and complex AP duration distribution containing multiple dominant peaks (fig. 3).

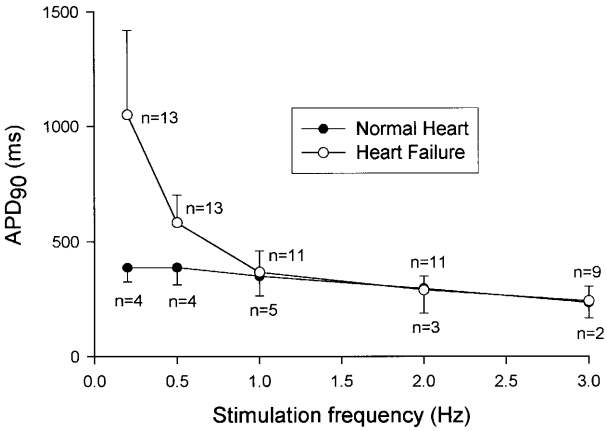

Figure 2. Action potential duration as a function of stimulation frequency in cardiomyocytes from normal dogs (closed circles) and in cardiomyocytes from dogs with heart failure (open circles). Shown are mean ± SD. Number of dogs (n) is indicated at each data point. The repeated measures ANOVA tests were significant ($P \leq 0.001$). Since the interaction test was significant, separate *t*-tests were performed for each frequency. At the two lowest frequencies (0.2 and 0.5 Hz) the HF cardiomyocytes group had significantly higher APD_{90} values than the normal cardiomyocytes group ($P < 0.001$ and $P = 0.009$, respectively). The differences at the higher frequencies (1.0–3 Hz) were not statistically significant.

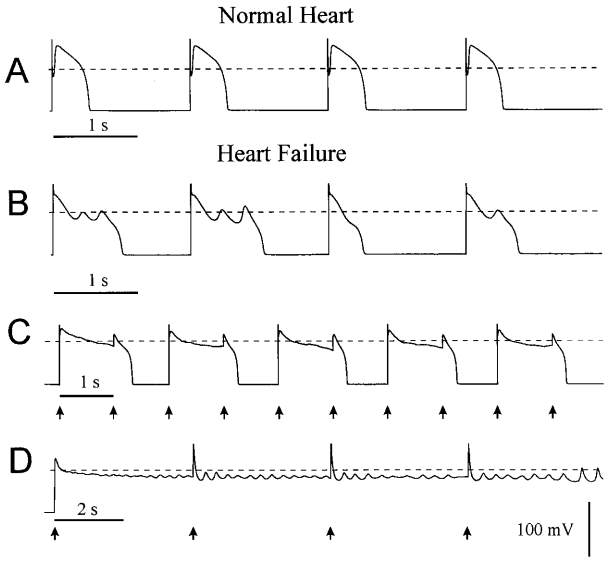

Figure 1. Repolarization abnormalities in cardiomyocytes isolated from dogs with heart failure. Representative examples of action potentials in cardiomyocytes isolated from normal (*A*) and failed (*B–D*) myocardium. Action potentials in cardiomyocytes of failed hearts were prolonged, displayed a reduced notch, duration alterations and early afterdepolarizations. In some cardiomyocytes AP duration was greater than the stimulation interval (*C*). An extreme case of abnormally prolonged action potential was a complete loss of membrane repolarization (*D*). Arrows indicate stimulation onset.

3) APs of HF cardiomyocytes frequently exhibited early afterdepolarizations (EADs) identified as oscillations on the AP plateau (fig. 1B). The occurrence of EADs was more common at lower frequencies of stimulation. At 0.5 Hz pacing, 11.5% of APs had EADs (344 of 2988 AP recordings in 62 cardiomyocytes of 13 dogs), whereas at 0.2 Hz, APs with EADs occurred in 35% of recordings (488 of 1389 APs). In contrast to HF cardiomyocytes, normal cardiomyocytes exhibited no EADs at any stimulation frequency.

4) In some HF cardiomyocytes the AP duration exceeded the stimulation period, resulting in AP alternations, for example only one AP was generated in response to every two stimuli (fig. 1C).

5) An extreme case of abnormal prolongation of the AP was a complete loss of membrane repolarization observed in some cardiomyocytes (4 of 54 cardiomyocytes, fig. 1C).

6) The notch of phase I of the AP characteristic of midmyocardial cardiomyocytes [36] was prominent in all normal cardiomyocytes (fig. 1A) but was significantly reduced or completely absent in HF cardiomyocytes (fig. 1B,C).

STX and lidocaine reduced dispersion of APs in HF cardiomyocytes. To test the contribution of Na^+ chan-

nel function to the AP abnormalities described above, we used the Na^+ channel blockers STX and lidocaine as tools. Both drugs reversibly reduced AP duration (see also [24]) and dispersion and, in addition, abolished EADs (fig. 4 and fig. 5). In the presence of STX or lidocaine the resting potential changed insignificantly (not shown).

Characterization of ion currents

HF cardiomyocytes had the ion current balance defect within the voltage range of the AP plateau. To investigate the ionic mechanism of abnormal repolarization in HF cardiomyocytes, we compared a net steady-state ion current (I_{ss}) in cardiomyocytes of normal dogs and dogs with chronic HF. In our experimental conditions, I_{ss} in cardiomyocytes of normal dogs were close to zero within the voltage range of the AP plateau from -25 mV to 25 mV (fig. 6A). In contrast, the I_{ss}-voltage relationship of cardiomyocytes isolated from HF dogs had an inward limb that was found at these voltages (fig. 6B). Since the mean value of membrane capacity ($C = 216 \pm 51$ pF, $n = 45$) in hypertrophied cardiomyocytes of HF dogs [37] was significantly larger than in normal cardiomyocytes (148 ± 40 pF, $n = 42$), the balance of the net steady-state ion currents was characterized as the I_{ss} density (I_{ss}/C). Statistical data on net current density are shown in fig. 6C. The current density measured in HF cardiomyocytes (averaged data from 28 cardiomyocytes of eight dogs) within the voltage range from -25 mV to 25 mV was found to be significantly different compared with normal cardiomyocytes (26 cardiomyocytes of eight dogs), suggesting a defective balance of ion currents in the cardiomyocytes. For example, at 0 mV, the current density in HF cardiomyocytes was -0.344 ± 0.122 pA/pF, whereas it was close to zero in cardiomyocytes of normal dogs (-0.05 ± 0.137 pA/pF). The I_{ss}/C values at positive voltages were outward in cardiomyocytes of normal dogs but were inward in cardiomyocytes of HF dogs within a voltage range up to 25 mV.

Na channel blockade produced a greater change in steady-state ion current balance in HF cardiomyocytes. Whereas a specific Na^+ channel blocker STX (1 μM) had little effect on the I_{ss}-voltage relation in normal cardiomyocytes (fig. 7A), it produced a significant outward shift of the I_{ss}-voltage relation in HF cardiomyocytes within the voltage range of AP plateau from -25 mV to 25 mV (fig. 7B). Statistical analysis revealed that the average current blocked by STX at 0 mV was significantly larger in HF cardiomyocytes (0.22 ± 0.03 pA/pF, 29 cardiomyocytes from five dogs) as compared with normal cardiomyocytes (0.12 ± 0.06 pA/pF, 26 cardiomyocytes from four dogs). Another Na^+ channel blocker, lidocaine (90 μM), a class 1B antiarrhythmic drug, also produced an outward shift of the I_{ss}-voltage relation in HF cardiomyocytes (fig. 7C). The average current blocked by lidocaine in HF cardiomyocytes (0.18 ± 0.02 pA/pF) was a little smaller than that blocked by STX but still significantly greater than in normal cardiomyocytes (0.10 ± 0.04, fig. 7F).

A pharmacological dissection of the inward currents. To further dissect out the contribution of different ions to the increased inward current in HF cardiomyocytes, in the next series of experiments the content of the pipette and extracellular solutions was chosen to suppress K^+

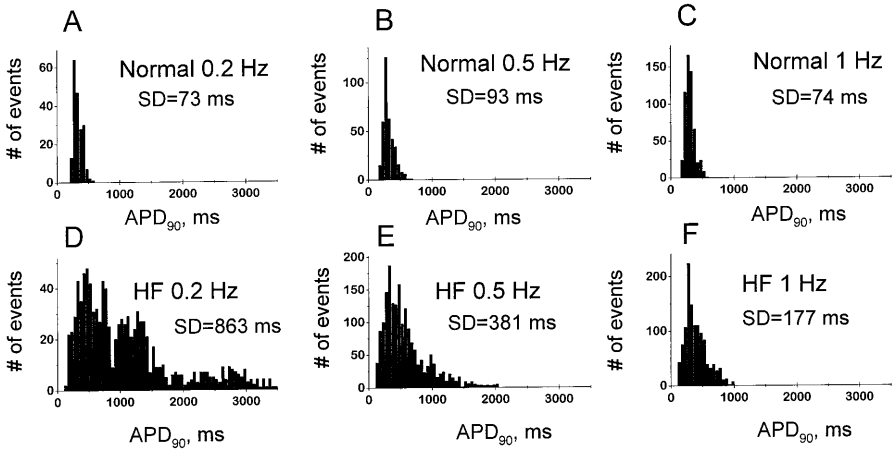

Figure 3. Dispersion of action potential duration in cardiomyocytes isolated from normal and failed dog hearts. Shown are distributions of action potential duration (APD_{90}) measured at different stimulation frequency in 4 normal dogs (*A–C*) and 13 dogs with HF (*D–E*). The stimulation frequencies (0.2, 0.5, 1 Hz) and SD reflecting AP duration variability are indicated at the histograms. A bin size for all histograms was 50 ms.

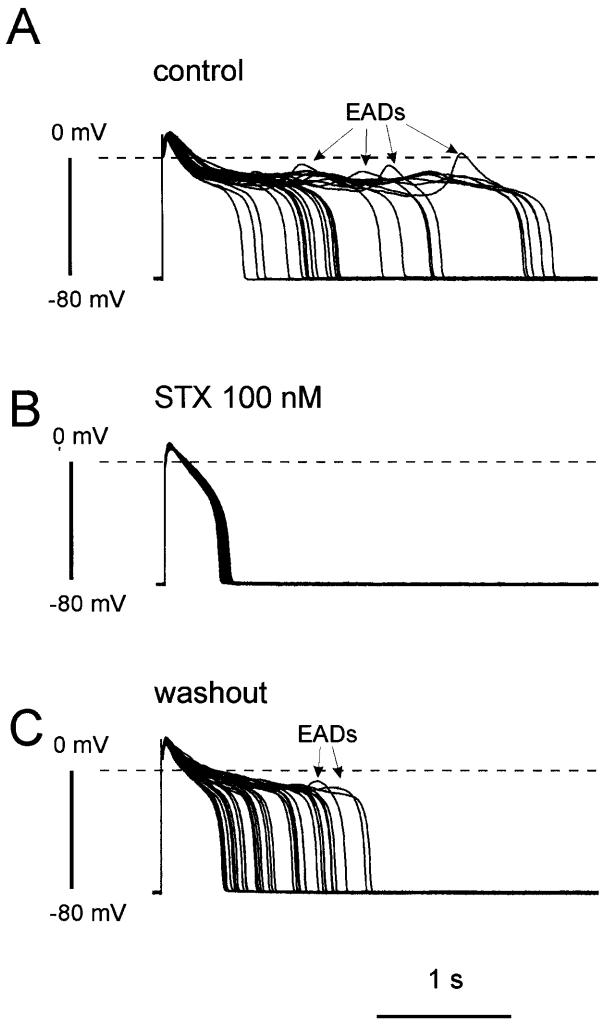

Figure 4. Example of the effect of STX (100 nM) on action potential dispersion and EADs in failed cardiomyocytes. Shown are superimposed traces of action potential before STX application (control, *A*), 3 min after STX application (*B*), and after 5 min STX washout (*C*). Arrows indicate occurrence of EADs in control and after STX washout. Stimulation frequency 0.25 Hz; temperature 37 °C.

currents (see 'Materials and methods'). In our experimental protocol we first applied nifedipine (2 μM) to block L-type Ca^{2+} current; the rest of the inward current was almost completely blocked by STX (1 μM). Representative examples of the effects of these drugs on ion current density (at 0 mV) together with statistical data are shown in figure 8. We found that the density of the nifedipine-sensitive component decreased ($P < 0.05$) in HF cardiomyocytes (0.25 ± 0.09 pA/pF, seven dogs, 31 cells) compared with normal cells (0.37 ± 0.08 pA/pF, five dogs, 28 cells) by approximately 33%. In contrast, the density of the STX-sensitive component significantly ($P < 0.01$) increased in HF cardiomyocytes (0.17 ± 0.06 pA/pF, four dogs, 22 cells) compared with normal cells (0.08 ± 0.02 pA/pF, four dogs, 26 cells). The relative contribution of Na^+ current to the net inward current was greater in HF cardiomyocytes, as evident from the increased ratio of Na^+ current to Ca^{2+} current (from 0.22 to 0.68) (see fig. 8C).

Discussion

Anomalous repolarization is a distinct feature of the electrophysiological characterization of cardiomyocytes isolated from dogs with coronary microembolization-induced chronic heart failure. The changes include a significant prolongation of AP duration (see also [24]), a large AP duration dispersion, partial and/or complete absence of excitability and absence or reduction of notch in early repolarization phase I of the AP. These AP disturbances were accompanied by increased sustained inward current in the voltage range of the AP plateau in HF LV cardiomyocytes. In the present study

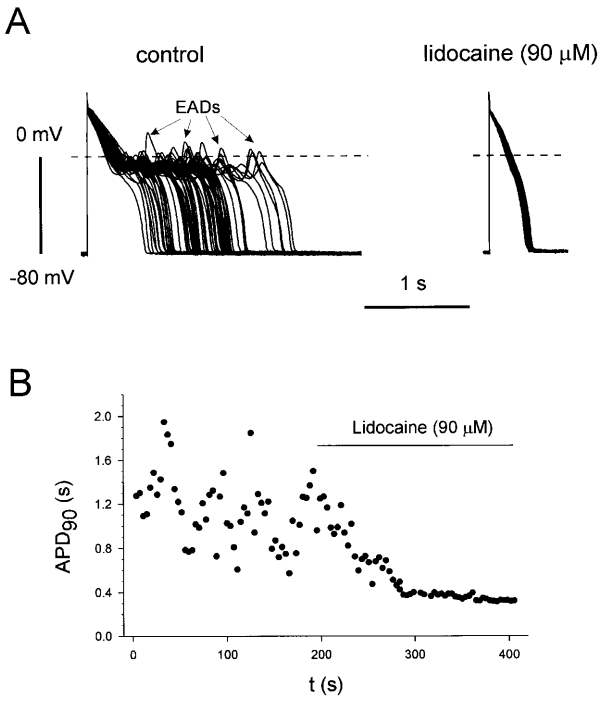

Figure 5. Example of the effect of lidocaine (90 μM) on action potential dispersion and early EADs in failed cardiomyocytes. Shown are superimposed action potential recordings before (control) and 4 min after lidocaine application. Arrows indicate occurrence of EADs before drug application. (*B*) shows the time course of the lidocaine effect on action potential duration (APD_{90}). Stimulation frequency 0.25 Hz; temperature 37 °C.

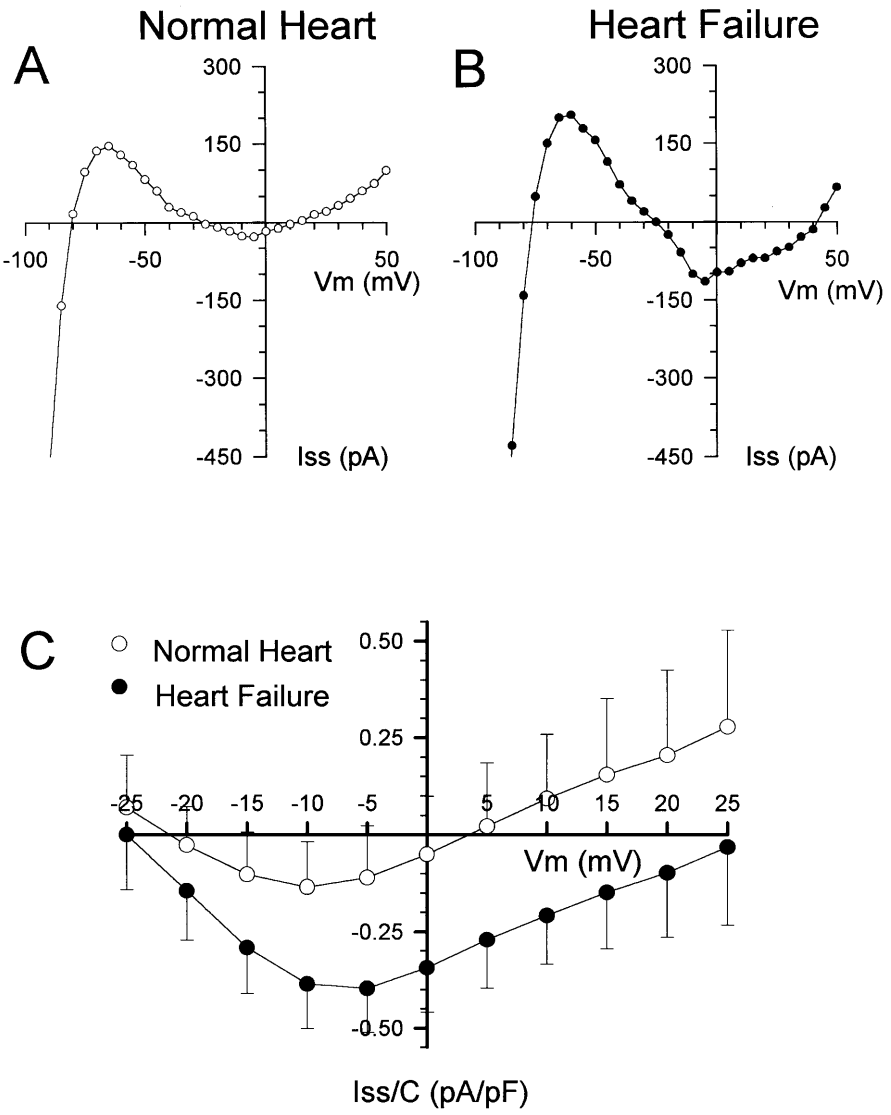

Figure 6. Cardiomyocytes isolated from failed hearts displayed an increased sustained inward current within the voltage range analogous to the action potential plateau level. Representative examples of steady-state current-voltage relationship in cardiomyocytes of normal dog (*A*) and dog with heart failure (*B*). (*C*) shows statistical data on the net steady-state current density (I_{ss}/C) measured in 28 cardiomyocytes of eight dogs with HF and in 26 cardiomyocytes of eight normal dogs within the voltage range from -25 mV to 25 mV. The ion current balance was close to zero in normal cardiomyocytes but was significantly shifted inward in cardiomyocytes isolated from failed hearts. Shown is mean $\pm$ SD. Mean I_{ss}/C values in cardiomyocytes of normal and failed hearts were statistically different within the entire voltage range from -25 mV to 25 mV ($P < 0.01$).

we describe a new mechanism for the abnormal repolarization in HF, namely the presence of an inward current possibly related to Na^+ channel activity.

Ion currents responsible for action potential remodeling in heart failure

Repolarizing currents. The transient outward current (I_{to}) shows rapid activation followed by inactivation; the latter is responsible for its transient nature. This current plays a dominant role in the initial repolarization ('notch') following the action potential upstroke formation. The reduction of I_{to} has been reported by many investigators and is now generally accepted as a common feature of HF [12–14] and is in line with our finding. Indeed, we documented a reduced AP notch (fig. 1) as an indication of diminished I_{to}.

The delayed rectifier current (I_K) is activated upon depolarization and does not undergo inactivation. Activation of I_K during the AP plateau initiates the onset of final repolarization [38]. I_K has not been implicated in HF [12, 39].

Inwardly rectifying K current (I_{K1}) is activated during AP repolarization, facilitating rapid final repolarization. I_{K1} is also responsible for maintaining the resting membrane potential. We found a small but significant depolarization of HF cardiomyocytes that could be explained by an alteration in I_{K1}. Indeed, Koumi and colleagues [40] demonstrated that I_{K1} channels in patients with dilated cardiomyopathy exhibited electrophysiological properties distinct from I_{K1} channels found in patients with ischemic cardiomyopathy and in donor hearts. By contrast, Beuckelmann and colleagues [12] reported decreased density of whole-cell I_{K1} in ventricular cardiomyocytes from patients with terminal HF.

Depolarizing currents. L-type Ca^{2+} channel activation during the initial phase of the AP elicits the inward current (I_{CaL}) that extends over the entire duration of the plateau. The data on L-type Ca^{2+} channel expression in HF is controversial. Both unchanged [41, 14] and decreased [42, 43] density of the peak I_{CaL} have been reported in HF. Decreased density of the peak I_{CaL} was also described in our dog chronic HF model [44]. The persistent component of I_{CaL} has been described in the present study and found to be also decreased in HF (see fig. 8).

Activation of the Na^+ channel is responsible for the upstroke of the action potential. Under normal conditions, most of the Na^+ channels undergo fast inactivation, but a small portion does not seem to inactivate and carry inward current for the entire duration of the plateau [19–21]. No differences in transient Na^+ current characteristics between ventricular cardiomyocytes isolated from normal and failing human or animal hearts have been reported [14, 45]. In the present study, we show a significant increase in steady-state Na^+ current component which may counterbalance the decreased steady-state Ca^{2+} current in HF cardiomyocytes (fig. 8). In concert with decreased K^+ and Ca^{2+} currents, the increase of Na^+ current can play a significant role in repolarization abnormalities in HF cardiomyocytes.

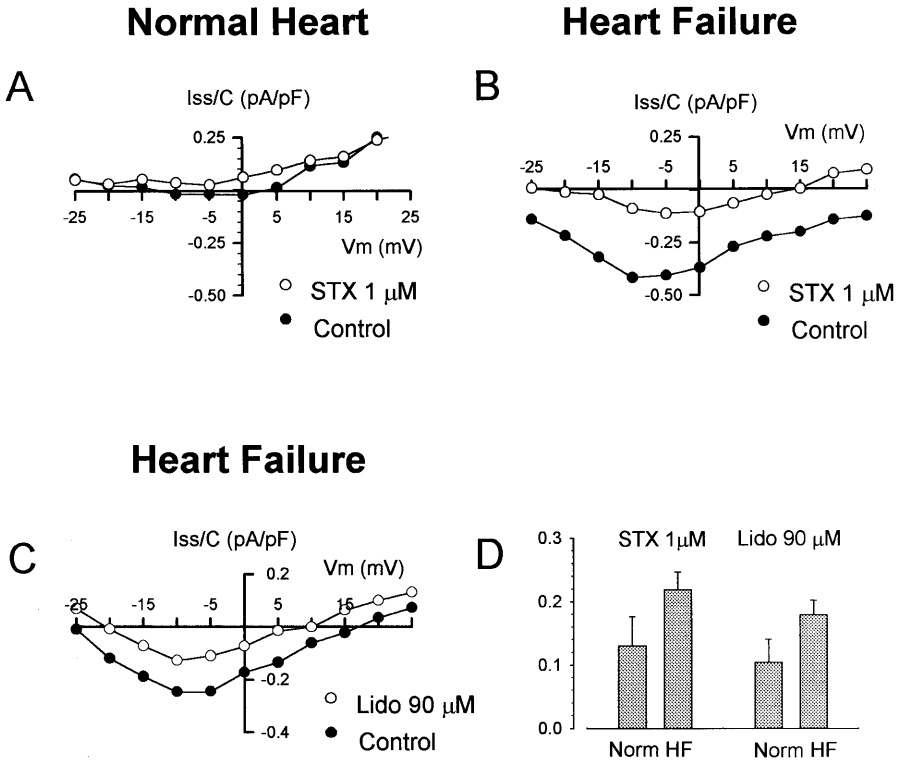

Figure 7. Effect of Na^+ channel blockers, STX (1 μM) and lidocaine (90 μM), on the net steady-state membrane current (I_{ss}) was greater in cardiomyocytes of dogs with heart failure compared with that of normal dogs. Shown are examples of STX effect on the current density (I_{ss}/C)-voltage relationships in normal (*A*) and failed (*B*) cardiomyocytes. (*C*) shows an example of lidocaine effect on the I_{ss}/C-voltage relationships in failed cardiomyocytes. (*D*) shows average data of STX and lidocaine effect on I_{ss} density measured at 0 mV in 26 cardiomyocytes of four normal dogs (Norm) and 29 cardiomyocytes of five dogs with HF. The effect of both drugs was significantly greater ($P < 0.01$) in cardiomyocytes of HF dogs.

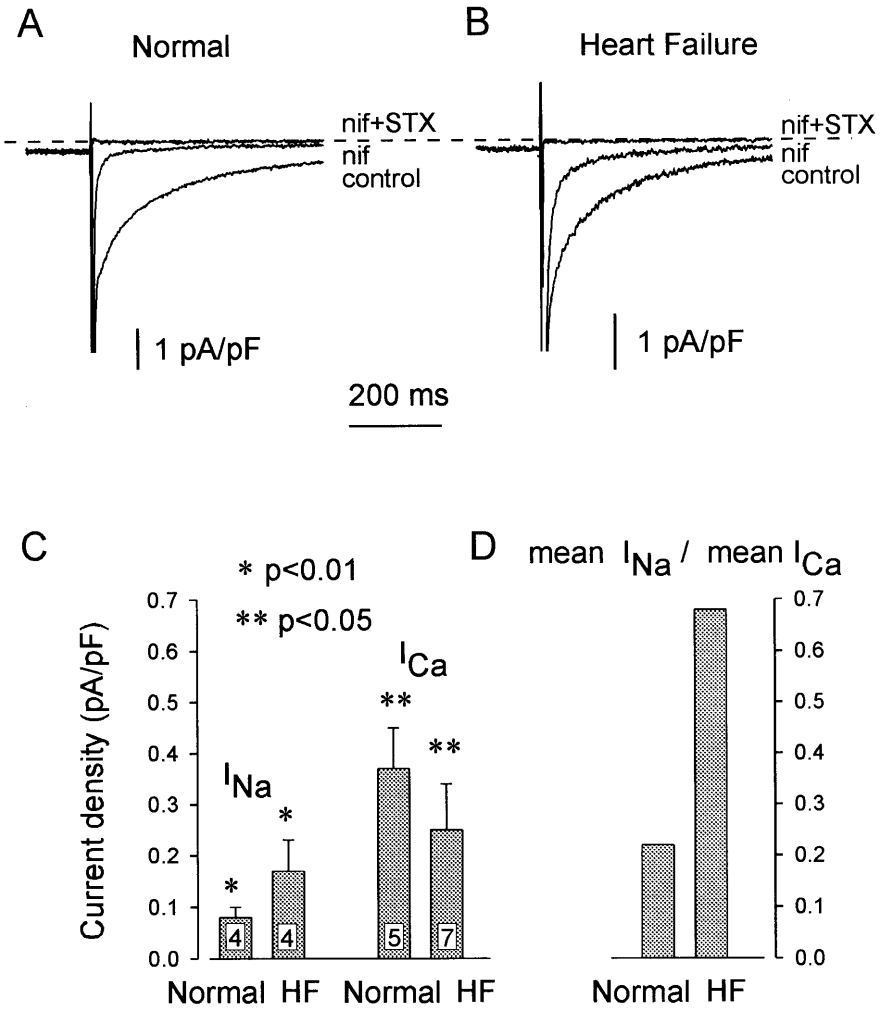

Figure 8. Contribution of Ca and Na ions to the net steady-state inward current in cardiomyocytes isolated from normal dogs and dogs with heart failure (HF). The content of the pipette and extracellular solutions was chosen to suppress K^+ currents (see 'Materials and methods'). (*A*) and (*B*) show raw traces of the inward current recorded in normal and HF cardiomyocyte, respectively. Consecutive application of Ca^{2+} channel blocker, nifedipine (nif, 2 μM), and Na^+ channel blocker, STX (1 μM), completely blocked the inward current (nif + STX). Recordings were performed at 0 mV 5 min after the drug application. The peak current was truncated. Dashed line indicates zero current. (*C*) shows average data of nifedipine-sensitive (I_{Ca}) and STX-sensitive (I_{Na}) current density measured at 0 mV 500 ms after depolarization onset. The numbers within the bars indicate the number of dogs. Note the density of I_{Ca} decreased, whereas I_{Na} density increased in HF cardiomyocytes, resulting in increased ratio I_{Na}/I_{Ca} (*D*).

Mechanisms of the sustained inward Na^+ current. One possibility that explains the increase of sustained inward current in HF cardiomyocytes is a modification of Na^+ channel activity induced by the membrane environment changes. We have previously shown that modification of Na^+ channel gating can be induced by changes in the lipid bilayer composition or cytoskeleton, resulting in a significant slowing of Na^+ current inactivation and/or the appearance of a sustained Na^+ current [20, 47, 48].

Na^+ channel inactivation can also be modulated by channel protein phosphorylation [49]. Dephosphorylation of multiple intracellular proteins, including ion channels, resulting in outburst of Na^+ channels with modified inactivation, could occur in HF and may be mediated by increased expression of cardiac protein phosphatases [50]. Other possibilities would be the expression in HF of an unknown ion channel or an isoform of the Na^+ channel. Although all of the mechanisms discussed above might play a contributory role in the origin of the late inward Na^+ current, the exact mechanism(s) awaits further study.

Physiological significance and clinical relevance. Experimental and clinical studies suggested that dispersion of repolarization and EADs in the Purkinje

and ventricular muscle fibers are two major mechanisms underlying *torsades de pointes* (twisting of the points) [51, 52]. These types of arrhythmias are induced by pause or bradycardia [53], which is in line with our findings. Indeed, the AP prolongation and dispersion of duration as well as the incidence of EADs in HF cardiomyocytes were higher at the lower pacing rates (figs 2 and 3). EADs, in turn, can be a substrate for triggered arrhythmias described in patients with HF [10, 36, 51]. The major role in initiation of EADs is believed to be L-type Ca^{2+} channel window current [54, 55]. In the present study, we demonstrated that inward Na^+ current may facilitate the generation of EADs resulting from AP prolongation in HF cardiomyocytes.

The inverse relationship between action potential duration and stimulation frequencies (fig. 2) might be due, in part, to a slow recovery of the late Na^+ current [46], which explains why the current is less pronounced at elevated frequencies. Shortening of the action potential duration is thus partially due to a smaller inward Na^+ current. Although AP prolongation was not evident at physiological frequencies (1 Hz and higher, fig. 2), dispersion of AP duration was still substantially larger in HF cardiomyocytes (fig. 3C,F), possibly reflecting the beat-to-beat QT interval variability documented in HF [56, 57]. The dispersion of repolarization along with EADs in HF cardiomyocytes might also contribute to the development of reentry by creating conditions necessary to support reentrant excitation [58]. We demonstrate complete loss of repolarization of some HF cardiomyocytes (fig. 1D). Accordingly, groups of cardiomyocytes within the failing ventricle may potentially form areas of excitation block and/or abnormal conduction. In turn, the failure of these areas to repolarize simultaneously with adjacent tissue may also provide a substrate for reentrant excitation.

Involvement of inward currents in the development of cardiac rhythm abnormalities was recently demonstrated by the discovery of a genetic mutation in the cardiac Na^+ channel resulting in defective Na^+ current inactivation in some patients with congenital heart disease [59, 60]. An important observation was that only a small fraction of the Na^+ channels with impaired inactivation was sufficient to produce a dramatic effect on cardiomyocyte repolarization because of a precise balance of ion currents at the AP plateau level [59]. We speculate that modified and/or novel Na^+ channels might be involved not only in congenital heart disease but also in HF [46]. The inward current described in this study is sensitive to the class 1B antiarrhythmic drug lidocaine and may have some clinical relevance.

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