Cellular basis of ventricular arrhythmias and abnormal automaticity in heart failure

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We have tested the hypothesis that delayed repolarization in heart failure increases the likelihood for a variety of oscillatory phenomena that can trigger arrhythmias and initiate abnormal automaticity. The implications of delayed repolarization are profound. The terminal repolarization phase of the action potential (AP) is quite labile as membrane resistance is high and small changes in the magnitudes of individual currents can easily initiate a secondary depolarization before full repolarization. These secondary depolarizations, called early afterdepolarizations (EAD), are seen clinically in long Q-T syndrome and can initiate triggered arrhythmias including torsade de pointes (1, 11, 32). By definition, EAD interrupt repolarization during the plateau (or phase 2) of the ventricular AP and involve an unambiguous depolarization (dV/dt > 0; Refs. 11, 26). Transient depolarizations and spontaneous voltage fluctuations that occur after full repolarization or at the resting potential fall into two recognized categories: delayed afterdepolarizations (DAD) and oscillatory prepotentials (OP; Ref. 11). DAD are most prominent at fast stimulation rates and under conditions that increase internal Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) (11). Classically, OP are described in Purkinje fibers as a series of subthreshold oscillations in resting membrane potential that gradually increase in amplitude and subsequently initiate automaticity (10). If these types of oscillatory phenomena occur in single myocytes from failing hearts, then the pathogenesis of fatal ventricular arrhythmias may be linked to cellular electrophysiological abnormalities observed in heart failure.

Ventricular myocytes from failing animal and human hearts consistently demonstrate significant prolongation of the AP (5, 17, 20). Data (5, 20) from ventricular myocytes isolated from normal and failing human and canine hearts suggest that delayed repolarization in heart failure results from reductions in the Ca\(^{2+}\)-independent transient outward K current (I\(_{to}\)) and the outward component of the inward rectifier K current (I\(_{K1}\); Refs. 5, 20). I\(_{K1}\) contributes to the terminal phase of repolarization and establishes a stable resting membrane potential (27, 34). Despite its rapid inactivation, I\(_{to}\) is thought to play a crucial role in the early phases of repolarization by setting the plateau potential, which in turn influences all currents active during the remainder of the AP. In addition to producing similar alterations in repolarizing K currents, canine pacing tachycardia-induced heart failure further mimics human cardiomyopathy in that left ventricular function is depressed and spontaneous ventricular arrhythmias occur (29, 41). Throughout sustained rapid pacing...
~25–35% of dogs die suddenly, and brady- and tachyarrhythmias have been documented in those dogs (29). In addition monophasic AP recorded in anesthetized dogs and Q-Tc intervals measured from surface electrocardiograms are prolonged in the paced animals (29).

The goal of this study was to determine whether oscillatory phenomena are more prevalent in failing myocytes with reduced repolarizing K currents. The appearance of EAD was further provoked by hypokalemia and by exposure to cesium, which has as its major effect the inhibition of K1,3 (and other K currents). Both of these interventions prolong the AP and Q-T interval. We provide evidence that heart failure predisposes ventricular myocytes to EAD and a novel type of spontaneous depolarization (SD). The EAD are favored by elevated external Ca2+ and suppressed by low external Ca2+. Superficially, SD resemble OP insofar as they originate from the resting potential with no requirement for prior electrical activity, but they differ importantly in that SD are sudden, sharp events with no clear periodicity. The SD are suppressed by elevated external Ca2+ and increased in frequency and amplitude at low external Ca2+. The increased propensity for triggered activity (by EAD) and abnormal automaticity (by SD) likely contributes to arrhythmogenesis in heart failure.

**MATERIALS AND METHODS**

Canine pacing tachycardia-induced heart failure model. Investigation of the mechanism of delayed repolarization in heart failure and susceptibility to EAD is ideally conducted in a controlled model that minimizes the variable therapeutic histories and etiologies that typically complicate human studies. The canine pacing tachycardia-induced heart failure model is highly suitable for this purpose as it reproduces many of the electrical, mechanical, and molecular changes of human heart failure, including delayed repolarization (39, 41). Canine hearts paced to failure also exhibit a high incidence of sudden death and malignant arrhythmias, which are also characteristic of human heart failure (29, 39, 41), making this a useful model to study cellular correlates of arrhythmias and abnormal automaticity in heart failure (29). Eight adult mongrel dogs (20–30 kg) were instrumented for rapid ventricular pacing as previously described (29). Dogs were anesthetized with halothane (1% to 2%). Using fluoroscopic guidance, we advanced a bipolar endocardial lead (Medtronic, Minneapolis, MN) through the internal jugular vein and placed at the right ventricular apex. A programmable pacemaker with ventricular sensing (Activitrax, Spectrax, or Legend pacemakers, Medtronic) was connected to the lead and placed subcutaneously at the base of the neck. We initiated pacing after the dogs had recovered from surgery (2 days) and set pacing at a rate of 240 min−1. After 3–4 wk of chronic tachycardia overt clinical symptoms of terminal heart failure were evident. Indications of heart failure such as lethargy, loss of appetite, dyspnea, and ascites were confirmed using hemodynamic monitoring. The cesium-Tyrode solution was prepared by equilibrating the main solution with cesium chloride 10 mM, 100 mM KCl, and 100 mM NaCl while maintaining the concentration of KCl to 2.0 mM in the cesium-Tyrode solution (3 mM CsCl) to further prolong APD and to prevent depolarization of the resting membrane potential and initiation of spontaneous electrical activity. In preliminary experiments the combination of 2 mM KC1 and 3 mM CsCl was selected because it provoked EAD in a number of normal cells while maintaining a near-normal resting membrane potential. The cesium-Tyrode solution contained 2 mM CaCl2 unless otherwise indicated (see Table 1). The pipette filling solution was composed of (in mM) 140 KCl, 1 MgCl2, 4 MgATP, 10 HEPES, and 5 NaCl (pH adjusted to 7.4 with KOH). An agar bridge was used to minimize changes in the junction potential (~1 mV) on solution changes.

**Isolation of ventricular myocytes.** A high percentage of Ca2+-tolerant, rod-shaped, quiescent myocytes with clear striations and crisp edges were routinely isolated from normal and failing canine left ventricles for electrophysiological studies. The myocytes isolation procedure has been reported in detail previously (20). In brief, a portion of the left ventricle was resected and perfused, via the left anterior descending coronary artery, with a Ca2+-free modified Tyrode solution containing collagenase and protease until the tissue became flaccid (typically by 20 min). Individual myocytes were mechanically dissociated from digested segments of the middle one-third of the myocardial wall (to yield midmyocardial cells). Freshly isolated myocytes were allowed to settle by gravity, then resuspended in normal Tyrode solution and maintained at room temperature (22°C) until used. All myocytes were studied within 10 h of isolation. An average of five cells from every animal were included in the study.

**Electrophysiology.** AP were evoked by short depolarizing current pulses (1–2 ms, 100–300 pA) using the voltage-follower mode (bridge circuit) of an Axodamp-2A amplifier with an input impedance of 1011 Ω. Macropatch clamp currents were recorded using the whole cell patch-clamp configuration and discontinuous switch-damp technique while sampling at 10 kHz. All experiments were performed at 37°C. Pipettes were fabricated from borosilicate glass and fire-polished to have final resistances of 2–4 MΩ when filled with internal recording solution. In general, 60–80% of the series resistance was compensated without causing ringing and seal breakdown. Filled pipettes were mounted into an electrode holder that was fitted into the headstage (HS-2L-gain X0.1, Axon Instruments). Uncompensated capacitance currents in response to small hyperpolarizing voltage steps were recorded for off-line integration as a means of measuring cell capacitance. Voltage protocols and data acquisition were accomplished through the use of custom-written software on personal computers and analog-to-digital communications hardware. Continuous trains of AP were also recorded on FM tape (3964A instrumentation recorder, Hewlett-Packard) at 7.75 in./s. Myocytes were stimulated at a basic cycle length of 5 s. AP duration (APD) was measured as the time from the upstroke to 50 and 90% repolarization to resting potential from the overshoot (APD50 and APD90, respectively). We used the classic definition of an EAD: those interruptions in repolarization during the plateau (or phase 2) of the ventricular AP that involve an unambiguous depolarization (dV/dt < 0; Refs. 11, 26). The amplitude of SD during phase 2 of the AP and at the resting potential were required to exceed 2 mV for these events to be counted as EAD and SD, respectively.

**Solutions.** The cells were bathed in a normal Tyrode solution containing (in mM) 136 NaCl, 4 KCl, 10 glucose, 1 MgCl2, 2 Na-pyruvate, 2 CaCl2, and 10 HEPES (pH adjusted to 7.4 with NaOH). The concentration of KCl was reduced to 2 mM in the cesium-Tyrode solution (3 mM CsCl) to further prolong APD and to prevent depolarization of the resting membrane potential. The myocytes isolation procedure has been reported in detail previously (20). In brief, a portion of the left ventricle was resected and perfused, via the left anterior descending coronary artery, with a Ca2+-free modified Tyrode solution containing collagenase and protease until the tissue became flaccid (typically by 20 min). Individual myocytes were mechanically dissociated from digested segments of the middle one-third of the myocardial wall (to yield midmyocardial cells). Freshly isolated myocytes were allowed to settle by gravity, then resuspended in normal Tyrode solution and maintained at room temperature (22°C) until used. All myocytes were studied within 10 h of isolation. An average of five cells from every animal were included in the study.
Statistical analysis. Pooled data are presented as means ± SE. Comparisons of measurements between groups were performed using a Student's t-test. Effects of cesium within one group of cells were evaluated by paired Student's t-test. Differences in the incidence and frequency of EAD and SD between normal and failing cells were evaluated with the chi-square test statistic including the Yates correction (15). Steady-state current-voltage relationships between groups were compared by multivariate ANOVA (Systat, SPSS). Differences in the data were considered statistically significant when P < 0.05.

RESULTS

Delayed repolarization and increased susceptibility to early afterdepolarizations in failing myocytes. Repolarization is delayed in canine tachycardia-induced heart failure (20, 29) as it is in various other models of heart failure and in human cardiomyopathies. On reaching a steady-state condition during continuous low-frequency (0.2 Hz) stimulation in normal Tyrode solution, AP recorded in failing myocytes were prolonged on average compared with normal myocytes (Fig. 1, A and B). Unlike previous AP recordings in normal and failing isolated cardiomyocytes (20), these AP were performed at 37°C without intracellular Ca²⁺ buffers. Under these basal experimental conditions (normokalemia, bradycardia, no added intracellular Ca²⁺ buffers) APD measured at 50% repolarization (APD₅₀) for normal and failing myocytes exposed to normal Tyrode solution (A) and cesium-Tyrode solution (B). In myocytes that exhibited early afterdepolarizations (EAD), APD₅₀ measurements were made for normal AP waveform (–EAD) and also for an AP in which EAD interrupted repolarization (+EAD). Box surrounds middle 50% of the data: bottom of box marks 25th percentile, median line marks 50th percentile, top of box marks 75th percentile (33, 40). The vertical lines extend to 5th and 95th percentile. The bottom symbols mark the minimum value (●) and the 1st percentile (□), the middle symbol indicates the mean (▲), and the top symbols mark the 99th percentile (◆) and maximum value (●). Midmyocardial cells tend to display greater APD at

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Table 1. Experimental recording solutions

Fig. 1. Steady-state action potentials (AP) stimulated (0.2 Hz) in normal (A and C) and failing myocytes (B and D) during perfusion with normal Tyrode solution (A and B) and cesium-Tyrode solution (C and D).

Fig. 2. Box plots of distributions of action potential duration at 50% repolarization (APD₅₀) measurements for normal and failing myocytes exposed to normal Tyrode solution (A) and cesium-Tyrode solution (B). In myocytes that exhibited early afterdepolarizations (EAD), APD₅₀ measurements were made for normal AP waveform (–EAD) and also for an AP in which EAD interrupted repolarization (+EAD). Box surrounds middle 50% of the data: bottom of box marks 25th percentile, median line marks 50th percentile, top of box marks 75th percentile (33, 40). The vertical lines extend to 5th and 95th percentile. The bottom symbols mark the minimum value (●) and the 1st percentile (□), the middle symbol indicates the mean (▲), and the top symbols mark the 99th percentile (◆) and maximum value (●).
slow stimulation rates than do epicardial and endocardial cells in the dog (23). The pooled data presented in the box plots shown in Fig. 2A also indicate increased dispersion of APD50 in failing myocytes. The increase in APD resulting from pacing-induced heart failure under more physiological recording conditions in this study (ΔAPD50 = +31%, ΔAPD90 = +34%) is similar to that achieved previously in the study by Kääb et al. (ΔAPD50 = +32%, ΔAPD90 = +30%, Ref. 20). Thus under identical recording conditions AP are prolonged by similar amounts with the development of heart failure.

The motivation for this study was to test the hypothesis that the slow and labile repolarization that occurs in heart failure predisposes failing hearts and myocytes to EAD. Consistent with this hypothesis, clear-cut phase 2 EAD (as defined in MATERIALS AND METHODS) were observed in 32% of failing cells (n = 8 of 25 cells from 5 of 5 hearts) but in none of the normal cells studied (n = 27, P < 0.01). Of the AP recorded in the eight failing cells that exhibited EAD, those AP waveforms that contained an EAD were the longest of all AP recorded in the normal Tyrode solution (Fig. 1B) with an average APD50 of 1,563 ± 338 ms (Fig. 2A).

Myocytes from both normal and failing hearts were exposed to the inorganic cation cesium to test our hypothesis that failing cells are more predisposed to EAD when the reserve of repolarizing currents is further reduced by cesium blockade, as was found to be the case in in vivo CsCl infusion experiments (29). Switching to cesium-Tyrode solution prolonged AP duration and increased the incidence of EAD in both groups of cells (Figs. 1, C and D, and 2B). Failing myocytes exhibited prolonged APD50 and APD90 values in cesium-Tyrode solution (Fig. 2B) for both AP waveforms with EAD (1,955 ± 167 and 2,257 ± 198 ms, n = 12) and without EAD (1,077 ± 79 and 1,290 ± 82 ms, n = 22) compared with measurements in normal myocytes [with EAD 1,256 ± 351 and 1,469 ± 349 ms, n = 4, P = 0.06 and 0.06; without EAD 960 ± 93 and 1,139 ± 90 ms, n = 27, P = not significant (NS) and NS, respectively]. An increased propensity for EAD was again observed for failing cells in the presence of cesium: 19% of normal myocytes (n = 5 of 27 cells from 3 of 6 hearts) and 55% of failing cells (n = 12 of 22 cells from 5 of 5 hearts) exhibited EAD (P < 0.03). Of the 12 failing cells that exhibited EAD in the cesium-Tyrode solution, EAD occurred in 1 or more cells studied from each of 5 failing hearts; i.e., EAD occurred in a fraction of cells studied from every failing heart. The five normal cells that exhibited EAD in cesium-Tyrode solution were isolated from three different hearts. The findings that APD are longer and EAD occur more frequently in failing myocytes support the idea that delayed repolarization increases susceptibility to EAD.

To quantify the frequency of occurrence of EAD in each myocyte that exhibited EAD, we have defined a function called EAD frequency. EAD frequency is the average number of EAD that occur per AP once a steady-state condition is achieved during continuous stimulation. For example, consider a train of 20 AP stimulated from rest at 0.2 Hz. The first five AP establish a steady-state condition and these are not analyzed. Among the 15 steady-state AP (6 through 20) there are 11 normal AP, 3 AP with 1 EAD, and 1 AP with 2 EAD in the same AP. In this case, there are 5 total EAD per 15 total AP analyzed and the EAD frequency equals 0.33 EAD per AP. Myocytes in which no EAD occurred during a train of AP were excluded, so that this analysis quantifies the frequency of EAD only in the subgroup of cells that exhibited EAD. In contrast, EAD incidence quantifies the fraction of cells in the entire population that exhibited EAD, no matter how often EAD occurred in a particular cell. We have calculated EAD frequency values for trains of AP recorded in both groups of myocytes and plotted the results in Fig. 3B. In failing myocytes EAD frequency equals 0.56 ± 0.14 (n = 8) EAD per AP when perfused with normal Tyrode solution. Exposure to cesium-Tyrode solution increased EAD frequency in failing myocytes (Fig. 3B) and, as mentioned above, the incidence of EAD in both cell types (Fig. 3A). Failing myocytes exposed to cesium-Tyrode solution had in-

Fig. 3. EAD incidence (A) and box plots of EAD frequency distribution (B) data for normal and failing myocytes superfused with normal Tyrode solution (left) and cesium-Tyrode solution (right). A, left: in normal Tyrode solution, EAD were exhibited in 32% of failing myocytes (n = 8 of 25 cells from 5 of 5 hearts) but in none of the normal cells studied (n = 27, P < 0.01). A, right: whereas exposure to cesium-Tyrode solution increased the incidence of EAD in both groups, EAD incidence remained higher in failing myocytes (55%, n = 12 of 22 cells from 5 of 5 hearts) compared with normal myocytes (19%, n = 5 of 27 cells from 3 of 6 hearts, P < 0.03). B, left: EAD frequency in failing myocytes exhibiting EAD in normal Tyrode solution (n = 8) equaled 0.56 ± 0.14 EAD/AP. B, right: of myocytes exhibiting EAD in cesium-Tyrode solution, failing myocytes had increased EAD frequency (0.72 ± 0.17, n = 12) compared with normal myocytes (0.19 ± 0.05, n = 5, P = 0.06).
increased EAD frequency (0.72 ± 0.17, n = 12) compared with normal myocytes (0.19 ± 0.05, n = 5, P = 0.06). Thus EAD occur with greater incidence and frequency in failing myocytes than in normal cells.

Cesium blockade of inward rectifier potassium current. We used cesium to provoke EAD in myocytes (Fig. 3A, normal myocytes, normal Tyrode solution vs. cesium-Tyrode solution, P < 0.05) as a corollary of the use of cesium to evoke EAD and torsade de pointes in vivo (22, 32). Cesium has been used as a tool to separate $I_{K1}$ from the net membrane current in cardiac Purkinje fibers (18). External K$^+$ concentration ($[K^+]_o$) was decreased to 2 mM from 4 mM to prevent spontaneous phase 4 depolarization in the presence of cesium, thereby stabilizing the resting membrane potential and enabling stimulated AP to be recorded. Compared with normal Tyrode solution, the cesium-Tyrode solution hyperpolarized (P < 0.01) normal (−91 ± 1 mV vs. −81 ± 1 mV, n = 27) and failing myocytes (−89 ± 1 mV vs. −81 ± 1 mV, n = 22). Our voltage-clamp data (not shown) indicate that the predominant effect of the cesium-Tyrode solution is a reduction of outward K currents (nonspecific block), which are active during repolarization with the greatest reduction occurring in $I_{K1}$. At −40 mV, the cesium-Tyrode solution reduced net outward current from 1.5 ± 0.6 to 0.4 ± 0.4 pA/pF in normal myocytes (n = 6) and from 0.5 ± 0.3 to 0.1 ± 0.1 pA/pF in failing myocytes (n = 9). Additionally, outward current density was significantly reduced in failing myocytes compared with normal myocytes in normal Tyrode solution (P < 0.01).

Role of Ca$^{2+}$ in EAD induced by cesium. Subgroups of normal and failing myocytes were exposed to cesium-Tyrode solution containing higher and lower concentrations of Ca$^{2+}$ to examine the role of transsarcolemmal Ca$^{2+}$ entry in EAD. When external Ca$^{2+}$ concentration ([Ca$^{2+}]_o$) was tripled (to 6 mM), the incidence of EAD increased in both groups of cells (Figs. 4, A and B, and 5A: P < 0.01 normal myocytes; P = NS failing myocytes). In 6 mM [Ca$^{2+}]_o$, 75% (6 of 8 cells from 2 of 2 hearts) of normal myocytes and 100% (5 of 5 cells from 2 of 2 hearts) of failing myocytes exhibited EAD (P = NS). Lowering [Ca$^{2+}]_o$ to 0.5 mM from 6 mM reduced the incidence of EAD to 25% (2 of 8 cells from 2 of 2 hearts) in normal cells and to 20% (1 of 5 cells from 1 of 2 hearts) in failing cells (Fig. 5A). Switching to the low-Ca$^{2+}$ cesium-Tyrode solution suppressed EAD in five of six normal cells (P = NS) and in four of five

![Fig. 4. Steady-state AP stimulated (0.2 Hz) in normal myocytes (A and C) and failing myocytes (B and D) during perfusion with high-Ca$^{2+}$ cesium-Tyrode solution (A and B) and low-Ca$^{2+}$ cesium-Tyrode solution (C and D). EAD are potentiated when external Ca$^{2+}$ is elevated and suppressed when it is decreased. Spontaneous depolarizations (SD) are prevalent in failing myocytes exposed to low-Ca$^{2+}$ cesium-Tyrode solution (D). Resting potential is stable in normal myocytes bathed in low-Ca$^{2+}$ cesium-Tyrode solution (C).](image-url)
failing cells (P = 0.05) that exhibited EAD in 6 mM [Ca²⁺]₀. Thus elevation of [Ca²⁺]₀ potentiates EAD, whereas lowering [Ca²⁺]₀ suppresses EAD in both groups of cells. Similarly, EAD frequency increased to the highest values obtained in the high-Ca²⁺ cesium-Tyrode solution (1.34 ± 0.29, n = 6 normal myocytes and 1.03 ± 0.28, n = 5 failing cells) and subsequently decreased when [Ca²⁺]₀ was lowered to 0.5 mM (Fig. 5B). These findings suggest that a net inward current carried by or modulated by Ca²⁺ supports the EAD (see DISCUSSION; Refs. 7, 12, 19, 26).

Increased susceptibility to SD in failing myocytes. An unexpected and interesting observation was made during the study of EAD: the resting membrane potential of many failing myocytes was not stable between AP, either after a train of AP or during long periods without stimulation. As shown in Fig. 4D, the resting membrane potential immediately after a train of AP is stable initially; however, as time elapses SD occur that increase in frequency and amplitude. These SD superficially resemble SD observed in canine Purkinje fibers when [K⁺]₀ is lowered to 2.7 mM. In contrast to OP in Purkinje fibers that truly oscillate at some frequency and move away from the baseline potential in both directions the SD we have observed in failing canine ventricular myocytes do not occur repetitively, nor do they occur with a characteristic frequency, but instead they appear as spontaneous random depolarizations from resting potential (Figs. 4D, 7, and 8A).

Interestingly, normal myocytes did not exhibit SD in normal Tyrode solution (0 of 27), whereas 20% (5 of 25 cells from 2 of 5 hearts) of failing cells did (P < 0.05, Fig. 6A). Although cesium increased the incidence of SD in both groups of cells, SD occurred in 50% (11 of 22 cells from 4 of 5 hearts) of failing myocytes and only 4% (1 of 27 cells from 1 of 6 hearts) of normal myocytes (P < 0.01). The 11 failing cells that exhibited SD in cesium-Tyrode solution were isolated from 4 of the 5 failing dogs and at least 2 failing cells studied from each of these 4 failing dogs demonstrated SD. Varying extracellular Ca²⁺ in the cesium-Tyrode solution produced opposite effects on the incidence of SD (Fig. 6A) and EAD (Fig. 5A). When [Ca²⁺]₀ was reduced to 0.5 mM (low-Ca²⁺ cesium-Tyrode solution) the incidence of SD increased to 67% (4 of 6 cells from 2 of 2 hearts) in failing myocytes and to 11% (1 of 9 cells from 1 of 3 hearts) in normal myocytes (P = NS). Conversely, increasing [Ca²⁺]₀ to 6 mM decreased the incidence of SD back to 0% (0 of 8 cells from 3 hearts) in normal myocytes and to 40% (2 of 5 cells from 1 of 2 hearts) in failing cells (Fig. 6A, P = NS).

We calculated SD frequency values to provide a quantitative index of the frequency with which SD reoccur in the same cell. Similar to EAD frequency, we defined SD frequency as the average number of SD that occur between each AP in a continuous train of stimulated AP. If no SD occurred in a cell, that cell was...
excluded so that this analysis compares the frequency of SD in cells exhibiting SD and not the susceptibility of failing or normal myocytes to SD. These results are plotted in Fig. 6B. In failing myocytes, SD frequency equals 0.39 ± 0.05 (n = 5 cells) SD/cycle in normal Tyrode solution that increased to 0.64 ± 0.13 (n = 11 cells, P = NS) SD/cycle in the cesium-Tyrode solution. SD frequency in the single normal myocyte in which such events were observed was smaller (0.19 SD/cycle, cesium-Tyrode solution) than in failing myocytes. In cells where SD occurred, we made two observations: lowering [Ca^{2+}]_i to 0.5 mM increased SD frequency in both cell types (0.4 SD/cycle, n = 1 in normal cells and 1.01 ± 0.28 SD/cycle, n = 4 in failing cells, P = NS) and the incidence and frequency of SD were higher in failing cells under any condition studied (Fig. 6, A and B).

SD initiate abnormal automaticity. The events described above may superficially resemble DAD, but closer inspection reveals important differences. First, there is no clear coupling of the SD to prior stimulated activity. Second, the findings that SD are suppressed by high Ca^{2+} and potentiated by low Ca^{2+} are opposite to the known dependence of DAD on Ca^{2+} (13). Slow pacing rates were used to help distinguish the initial observations of SD from DAD that would be favored at faster pacing rates. The longer interval at resting potential between stimulated AP served to separate SD as unique events that are not initiated immediately after repolarization. Additionally, we recorded membrane potential in the absence of external stimulation to determine whether SD are in some way coupled to final repolarization of the AP or are unique events, which are clearly distinguishable from DAD. Resting membrane potential in normal myocytes exposed to the low-Ca^{2+} cesium-Tyrode solution remained stable for long periods (>3 min) without any evidence of SD (Fig. 7A). In contrast, SD developed in failing myocytes often after switching from normal Tyrode solution to the low-Ca^{2+} cesium-Tyrode solution (Fig. 7, B and C). Low-Ca^{2+} cesium-Tyrode solution was used because it provoked SD in the highest percentage of failing myocytes (Fig. 6A). As shown in Fig. 7B, SD were sometimes of sufficient amplitude to initiate trains of spontaneous activity. In this example, a SD depolarizes the cell beyond the AP threshold and initiates a 28-s period of spontaneous AP and SD that are self-sustaining. The run eventually terminates itself and a stable resting potential is once again established. An excerpt of this run is shown in Fig. 7C at a faster time scale to illustrate that SD do occur randomly, lacking a prescribed oscillatory period.

Mechanism for SD. What is the ionic mechanism responsible for generating SD? We tested the hypothesis that SD are membrane potential responses to spontaneous increases in intracellular Ca^{2+} such as those that underlie DAD (11). However, SD were observed in two failing myocytes without any rise in intracellular Ca^{2+} as indexed by the Ca^{2+} indicator indo 1 (Fig. 8A). Spontaneous releases of Ca^{2+} during intentional Ca^{2+} overload caused by subsequent exposure to ouabain (10^{-3} M) confirmed our ability to detect a rise in [Ca^{2+}]_i (and a corresponding DAD) in these experiments (Fig. 8B). A second hypothesis was that the spontaneous opening and closing of Na^{+} channels are the ionic events causing the SD. To test this idea, we added tetrodotoxin (TTX) to the low-Ca^{2+} cesium-Tyrode solution at a concentration (10 nM) sufficient to block most of the TTX-resistant Na^{+} channels found in heart. Although TTX did not inhibit SD (Fig. 7D, n = 3), it did prevent the initiation of abnormal automaticity triggered by the SD that exceeded the threshold for firing AP.

To characterize further the ionic mechanism of SD, we recorded transmembrane current in failing cells (n = 4) that had exhibited SD while exposed to the low-Ca^{2+} cesium-Tyrode solution. Using voltage-clamp control, whole cell current was recorded continuously while the holding potential was maintained at −90 mV, a voltage near the resting membrane potential of myocytes exposed to the cesium-Tyrode solution. Representative 400-ms segments of such a recording from a failing myocyte are shown in Fig. 9A. Current traces obtained in normal Tyrode solution are stable and show no evidence of transient fluctuations away from the mean current (−857 ± 2 pA). Currents obtained in the low-Ca^{2+} cesium-Tyrode solution do exhibit numerous departures from the mean current (−5 ± 2 pA). These transient currents are always inward but appear non-uniform in amplitude and kinetics (Fig. 9A). All aspects of the kinetics and duration of these currents are consistent with the irregular behavior of the SD that have been observed. Nonstationary fluctuation analysis (20) of 15 consecutive sweeps recorded in normal Tyrode solution and in low-Ca^{2+} cesium-Tyrode solution is shown in Fig. 9B. The variance of the currents obtained in low-Ca^{2+} cesium-Tyrode solution is significantly larger than for the currents recorded in normal Tyrode solution, which is consistent with the occurrence of the transient inward currents only in the low-Ca^{2+} cesium-Tyrode solution and with the high prevalence of SD in cesium-Tyrode solution.

In summary, we observed that SD are transient depolarizations from the resting potential that vary (apparently at random) in frequency of occurrence and in magnitude. SD differ from DAD in that SD may arise during quiescent periods and can even initiate automaticity during quiescence in isolated myocytes. SD are almost exclusively observed in failing myocytes but are also observed in normal myocytes when exposed to cesium and hypokalemia. There is no intracellular Ca^{2+} transient associated with SD, and elevation of external Ca^{2+} reduces the incidence and frequency of SD. Although we have excluded several potential explanations, the precise mechanism underlying SD is as yet undetermined.

DISCUSSION

We have found that heart failure induced by sustained tachycardia-pacing in the dog prolongs the ventricular AP and predisposes the midmyocardial cells to phase 2 EAD and SD. When stimulated at 0.2
Hz, failing myocytes gave rise to EAD more frequently than normal myocytes both at baseline and when stressed with cesium and hypokalemia. EAD were further provoked by elevation of $[Ca^{2+}]_o$ in both groups of cells and suppressed by lowering $[Ca^{2+}]_o$. Failing myocytes were also predisposed to SD, which increased in frequency and amplitude at low $[Ca^{2+}]_o$ and were suppressed when $[Ca^{2+}]_o$ was elevated. Our results indicate that AP prolongation in heart failure favors the occurrence of triggered activity caused by EAD and automaticity caused by SD: both events are potential cellular triggers for arrhythmogenesis in heart failure.

Observations of EAD in hypertrophy and heart failure. Whereas others have found EAD to be more prevalent in hypertrophied myocardium, this is the first observation of increased susceptibility to EAD in heart failure. EAD have been observed in hypertrophied papillary muscles from the left ventricles of rats made hypertensive by unilateral renal artery constriction (2). Exposure of papillary muscles to tetraethylammonium (TEA, 10–30 mM) to decrease outward currents, induced EAD and triggered activity in hypertrophied muscles but not in normal muscles (2).

In an in vivo study of canine left ventricular hypertrophy produced by renovascular hypertension, prolongation of monophasic AP was observed (4). Administration of BAY K 8644, a dihydropyridine agonist for L-type Ca current, produced phase 2 EAD and ventricular tachyarrhythmias more frequently in dogs with hypertrophy compared with controls (4). In contrast
with our in vivo (29) and in vitro (this study) results, Ben-David et al. (4) found that control and hypertrophied hearts developed EAD with equal incidence when exposed to cesium; however, the amount of cesium infused was so large that it induced phase 3 EAD in every dog. In an earlier study, Kass and co-workers (29) found that infusion of CsCl at a dose of 1 meq/kg body wt preferentially prolonged monophasic APD90 in failing canine hearts compared with controls. In addition Holter electrocardiogram recordings during 24-h periods when pacing was discontinued revealed that nonsustained ventricular tachycardia was more frequently observed in dogs with heart failure. In fact the number of episodes per 24 h increased significantly from 0.4 ± 0.6 to 6.7 ± 10.2 with heart failure (P < 0.05; Ref. 29).

Mechanism of phase 2 EAD in heart failure. An EAD requires at least a transient period of zero net current. The complement of inward and outward ionic currents, which shape the transition from the AP plateau to phase 2 repolarization, may become momentarily equal as the result of changes in the magnitude, kinetics, and/or voltage dependence of L-type Ca current, Na/Ca exchange current, and delayed rectifier or inward rectifier K currents. Potential mechanisms for the generation of phase 2 EAD in failing canine myocytes include, but are not limited to, reductions in outward K currents (20), enhanced Na/Ca exchange (14, 28, 31), and reactivation of L-type Ca current (19, 44). Reductions in outward K currents, IK1 in particular, whether as a result of heart failure (5, 20) or of cesium blockade (26) increase susceptibility to EAD. However, the observed potentiation of EAD incidence and frequency at elevated [Ca2+]o (Fig. 5, A and B) is consistent with phase 2 EAD arising as a direct consequence of Ca2+ entry through L-type Ca2+ channels. As proposed by Marbán et al. (26), the likely mechanism for cesium-induced EAD is delayed inactivation or reactivation of L-type Ca current. Our findings are also consistent with electrogenic Na/Ca exchange generating the inward current that sustains the EAD when extruding Ca2+ across the sarcolemma (7, 12). Reports of increased Na/Ca exchange activity in failing myocytes (28) and increased Na/Ca exchanger mRNA and protein in failing human myocardium (14, 31) are consistent with the heightened susceptibility of failing canine myocytes to EAD. Additionally phase 2 EAD, which are dependent on the L-type Ca window current during the AP plateau, should be more sensitive to differences in APD such as occur between normal and failing myocytes (19, 24, 42). The role of APD on EAD formation was examined by retrospective analysis of the AP recordings obtained in the Kääb et al. study (20). In that study intracellular Ca2+ was highly buffered by the dialysis of EGTA (2 mM) from the patch pipette, causing APD to be prolonged and the incidence of EAD to be increased in both failing (37%, n = 30 cells) and normal myocytes (8%, n = 25 cells) compared with the present study. The higher incidence of EAD in cells with increased APD, as a result of intracellular Ca2+ buffering, is consistent

Fig. 8. Membrane potential (top) and intracellular Ca2+ (bottom) recorded in a failing myocyte dialyzed with indo 1 before (A) and during (B) exposure to ouabain (10−5 M). SD occur without an increase in indo 1 ratio. Depolarizations induced by ouabain are associated with a transient elevation in the indo 1 signal. Both recordings were made in same cell.

Fig. 9. A: net membrane currents recorded in a failing myocyte continuously voltage clamped at −90 mV during exposure to normal Tyrode solution (left) and low-Ca2+ cesium-Tyrode solution (right). Similar transient inward currents were recorded in 3 other failing myocytes exposed to low-Ca2+ cesium-Tyrode. B: variance analysis of 15 consecutive sweeps and box plots of variance distributions from myocytes in A.
with delayed repolarization in general as a mechanism predisposing failing myocytes to EAD.

Observations of SD in normal, hypertrophied, or failing hearts. The SD that we have observed in failing myocytes are unique events. Whereas SD may bear some resemblance to OP (37, 38), SD have characteristics that distinguish them from OP as well as from DAD (11, 35). We have ruled out the possibility that SD are an unusual manifestation of DAD related to Ca\(^{2+}\) overload. In contrast with SD, DAD appear after AP, diminish in quiescence, are enhanced by elevation of [Ca\(^{2+}\)]\(_{i}\)o, disappear when [Ca\(^{2+}\)]\(_{i}\) decreases after stimulation, depend on release of Ca\(^{2+}\) from the sarcoplasmic reticulum, and are at least partly caused by the electrogenic extrusion of Ca\(^{2+}\) by Na/Ca exchange (11, 35, 43). Although both SD and OP can be induced or enhanced by lowering [Ca\(^{2+}\)]\(_{i}\)o and tend to be suppressed by elevation of [Ca\(^{2+}\)]\(_{i}\)o, SD in isolated myocytes differ from OP observed in Purkinje fibers (37, 38). OP occur as alternating depolarizing and repolarizing oscillations around the resting potential and do so at some set frequency. SD always occur in the depolarizing direction, whereas the magnitude and frequency of SD suggest that these are random events. The OP described by Spiegler and Vassalle (35) in sheep Purkinje fibers and designated Th\(_{OS}\), because they occur near the threshold for I\(_{Na}\) are perhaps most similar to SD. Both SD and Th\(_{OS}\) appear before AP, occur during quiescence, are facilitated or induced by low K\(^{+}\)o and occur in the absence of Ca\(^{2+}\) overload. However, there are still fundamental differences that set Th\(_{OS}\) apart from SD. Cesium, TTX, and lidocaine were found (35) to suppress Th\(_{OS}\), implicating a TTX-sensitive Na current as the underlying mechanism. On the other hand, SD occur in the presence of cesium (3 mM) and are unaffected by TTX (10 µM, Fig. 7C). Thus SD are distinct from afterdepolarizations and other types of previously described oscillatory phenomena.

Possible mechanism for SD. Our results do not definitively identify the mechanism of SD in failing canine myocytes. A net inward current must generate the SD because SD always transiently depolarize the resting membrane potential. In cells susceptible to SD, we have recorded inward current transients appropriate in size and frequency to underlie the SD. We have considered and excluded several mechanisms by which such inward currents may be spontaneously generated. The underlying inward current could be small transient Na currents created by the sporadic opening of Na channels at the resting potential. If this were the case, the Na channels must be TTX insensitive because TTX did not inhibit SD but did block I\(_{Na}\) sufficiently to prevent excitation of AP (Fig. 7C) despite the occurrence of SD that exceeded the AP stimulus threshold. We have ruled out Ca\(^{2+}\) as the charge carrier because the incidence and frequency of SD are reduced at elevated [Ca\(^{2+}\)]\(_{i}\)o, which would increase the driving force for inward Ca\(^{2+}\) flux. Similarly, we have ruled out Na/Ca exchange current generated by the extrusion of intracellular Ca\(^{2+}\) in exchange for extracellular Na\(^{+}\) as a potential mechanism, because spontaneous Ca\(^{2+}\) releases from the sarcoplasmic reticulum should occur less frequently when [Ca\(^{2+}\)]\(_{i}\)o is reduced, and because SD can occur in the absence of intracellular Ca\(^{2+}\) oscillations (Fig. 8A). Additionally, reanalysis of AP recordings made during the Kååb et al. study (20) uncovered that SD also occurred in 20% of failing myocytes in which [Ca\(^{2+}\)]\(_{i}\)o was highly buffered by EGTA-containing patch pipettes. SD are not likely to be caused by the hyperpolarization activated inward current (I\(_{K1}\)), which is responsible for normal phase 4 depolarization in the sinoatrial node and is also expressed in canine ventricular myocytes (30) because SD occur more frequently when myocytes are exposed to cesium, which blocks I\(_{K1}\) (8). Alternatively, we speculate that SD may reflect instability in the resting membrane potential (as a result of the reduction of I\(_{K1}\), Ref. 20) and that the inward currents arise because of the stochastic opening and closing of a few (as yet unidentified) channels. Finally, we have considered the possibility that SD are an artifact of the patch-clamp technique but this seems unlikely for the following reasons. First, there was no evidence of seal breakdown and the seals remained stable throughout the duration of the experiments (>30 min). Second, SD are reversibly induced and suppressed by changing the external solution to cesium-Tyrode solution and back to normal Tyrode solution. Third, SD increased and decreased in frequency when external Ca\(^{2+}\) was reduced and increased, respectively. The present study identifies SD as unique events that may contribute to abnormal automaticity. Although we have excluded a variety of plausible mechanisms, further study will be required to dissect the precise pathways that give rise to SD.

Physiological relevance. Our results indicate that AP prolongation in heart failure causes enhanced susceptibility to EAD and increased dispersion of repolarization in myocytes: two popular mechanisms for the initiation of arrhythmia and torsade de pointes in heart failure (16). When infusions of CsCl induce long Q-T syndrome (22) and/or bradycardia-dependent polymorphic ventricular tachycardia (6) in intact animals (dogs), the development of afterdepolarizations always preceded the development of ventricular arrhythmias (22). This supports our hypothesis that EAD underlie the development of malignant arrhythmias in heart failure. We also found increased dispersion of AP duration in failing myocytes (Fig. 2A), which could profoundly impact excitability if the pathophysiology of heart failure alters local resistivity. If human ventricular myocardium is as predisposed to developing EAD as we have found myocytes from failing canine ventricles to be, then slowing of the heart rate, hypokalemia, or treatment with drugs that block repolarizing K currents may all predispose the myocytes in failing human hearts to developing EAD (4). A major and unanticipated result of this study is that through our observation of SD in failing myocytes we have identified a potential mechanism for abnormal automaticity in heart failure that originates within the individual myocyte.

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