Rate-dependent shortening of action potential duration increases ventricular vulnerability in failing rabbit heart

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1 Department of Electrophysiology, Research Center of Montreal Heart Institute, Montreal, Quebec, Canada; 2 Department of Cardiovascular Research, Research Institute of Environmental Medicine, Nagoya University; 3 Department of Cardiology, Nagoya University Graduate School of Medicine, Nagoya; and 4 Graduate School of Engineering, University of Tokyo, Tokyo, Japan

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Harada M, Tsuji Y, Ishiguro YS, Takanari H, Okuno Y, Inden Y, Honjo H, Lee JK, Murohara T, Sakuma I, Kamiya K, Kodama I. Rate-dependent shortening of action potential duration increases ventricular vulnerability in failing rabbit heart. Am J Physiol Heart Circ Physiol 300: H565–H573, 2011. First published December 10, 2010; doi:10.1152/ajpheart.00209.2010.—Congestive heart failure (CHF) predisposes to ventricular fibrillation (VF) in association with electrical remodeling of the ventricle. However, much remains unknown about the rate-dependent electrophysiological properties in a failing heart. Action potential properties in the left ventricular subepicardial muscles during dynamic pacing were examined with optical mapping in pacing-induced CHF (n = 18) and control (n = 17) rabbit hearts perfused in vitro. Action potential durations (APDs) in CHF were significantly longer than those observed for controls at basic cycle lengths (BCLs) >1,000 ms but significantly shorter at BCLs <400 ms. Spatial APD dispersions were significantly increased in CHF versus control (by 17–81%), and conduction velocity was significantly decreased in CHF (by 6–20%). In both groups, high-frequency stimulation (BCLs <150 ms) always caused spatial APD alternans; spatially concordant alternans and spatially discordant alternans (SDA) were induced at 60% and 40% in control, respectively, whereas 18% and 82% in CHF. SDA in CHF caused wavebreaks followed by reentrant excitations, giving rise to VF. Incidence of ventricular tachycardia/VFs elicited by high-frequency dynamic pacing (BCLs <150 ms) was significantly higher in CHF versus control (93% vs. 20%). In CHF, left ventricular subepicardial muscles show significant APD shortenings at short BCLs favoring reentry formations following wavebreaks in association with SDA. High-frequency excitation itself may increase the vulnerability to VF in CHF.

METHODS

Animal model. Animal handling conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). Procedures were approved by the Institutional Animal Care and Use Committee at Nagoya University. Japanese white rabbits (2.0 to 3.0 kg) were used for the experiments. Pacing-induced CHF was created as previously described (26, 27). In brief, rabbits were anesthetized with ketamine-xylazine and artificially ventilated with room air supplemented with oxygen-halothane. After an open-chest procedure, a unipolar pacing-lead fixed to the right ventricular free wall was connected to a pacemaker (Medtronic, 5985) implanted subcutaneously in the back. After a full recovery from the surgery, tachycardia pacing (350 beats/min) was programmed and then continued for 3 to 4 wk. The pacemaker implantation was carried out in a total of 37 rabbits; 33 were paced to induce CHF, the remaining four were not (sham-operated group). The data obtained from the four latter animals were pooled together with those of 27 normal rabbits (control group) because the results were similar in both subsets. Compatible with

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previous echocardiographic findings in these models (26), all 33 of the paced rabbits showed physical signs of CHF (ascites, pleural effusion, biventricular dilatation) at the end of tachycardia period. The rabbits were euthanized (pentobarbital 40 mg/kg iv), and the hearts were then excised and perfused with modified Krebs-Ringer solution at 37°C on a Langendorff apparatus. A complete atrioventricular block was produced with a ligature of His bundle for electrophysiological measurements as described below.

**Optical recordings.** The procedure of optical action potential mapping was essentially the same as previously described (7, 10). The heart was stained with a voltage-sensitive dye, 4-[6-[2-(di-n-butylamino)-6-naphthyl][vinyl]]pyridium (di-4-ANEPPS). To minimize motion artifacts, 5 μM cytochalasin D was added. Bipolar ECG was recorded with widely spaced electrodes to monitor the whole ventricular excitations. The signals were filtered from 1.5 to 1,000 Hz and digitized at 1,000 Hz.

The heart was illuminated with bluish-green light-emitting diodes; the emitted fluorescence was recorded with a solid-state image-sensing digital video camera (Fastcam-Max, Photron, Japan) to acquire 10-bit grayscale images from 256 × 256 sites simultaneously, at a speed of 1,000 frames/s. The image acquired (30 × 30 mm) covered the left ventricular anterolateral surface. The action potential signals were temporally and spatially filtered to reduce the noise by using the method as reported by Lee et al. (12).

The dynamic pacing protocol was used to examine the rate-dependent changes of APD. The heart was initially paced from the left ventricular apex at a BCL of 2,000 ms, and the BCL was progressively shortened to 150 ms. A minimum of 200 stimuli was delivered at a given BCL to reach a steady state. APDs at 50% and 90% repolarization (APD50 and APD90) of four consecutive beats were measured and averaged at 16 recording sites covering an 18 × 18 mm square (white dots in Fig. 2A). This was to avoid a distortion of the APD-BCL relationship by APD alternans at short BCLs. Spatial APD dispersion was defined as the maximal difference of APD90 among the 16 recording sites.

Another pacing lead placed at the center of the left ventricular free wall was used to measure conduction velocity (CV) in a square (24 × 16 mm) around the stimulation site. The longitudinal and transverse directions of propagation images from 256 × 256 sites simultaneously, at a speed of 1,000 frames/s. The image acquired (30 × 30 mm) covered the left ventricular anterolateral surface. The action potential signals were temporally and spatially filtered to reduce the noise by using the method as reported by Lee et al. (12).

The dynamic pacing protocol was used to examine the rate-dependent changes of CVs. The heart was initially paced from the left ventricular apex at a BCL of 2,000 ms, and the BCL was progressively shortened to 150 ms. A minimum of 200 stimuli was delivered at a given BCL to reach a steady state. APDs at 50% and 90% repolarization (APD50 and APD90) of four consecutive beats were measured and averaged at 16 recording sites covering an 18 × 18 mm square (white dots in Fig. 2A). This was to avoid a distortion of the APD-BCL relationship by APD alternans at short BCLs. Spatial APD dispersion was defined as the maximal difference of APD90 among the 16 recording sites.

Floating microelectrode recordings. The heart was horizontally placed in a tissue bath and constantly stimulated from the left ventricular apex with a bipolar electrode at BCLs of 600, 1,000, and 2,000 ms. The pulses employed were 2 ms in duration and twice the diastolic threshold in intensity. Action potentials were recorded from the center of the left ventricular anterolateral epicardial surface with a floating microelectrode (direct current resistance 10–20 MΩ; 2.7 mmol/l KCl) in the absence of excitation-contraction uncoupler (cytochalasin D). APD was measured at 90% repolarization. The values obtained from five consecutive beats at a given BCL were averaged.

**L-type Ca2+ current recordings.** Ventricular myocytes were isolated enzymatically from a subepicardial layer of the left ventricular anterolateral wall. The whole-cell patch-clamp method was used for the recording of L-type Ca2+ current (ICa,L). The external solution was composed of (in mM): 143 NaCl, 5.4 CsCl, 0.5 CaCl2, 0.25 NaH2PO4, 5.0 HEPES, and 5.6 glucose, pH adjusted to 7.4 with NaOH. The pipette solution contained (in mM): 80 CsCl, 60 CsOH, 40 aspartate, 5 HEPES, 10 EGTA, 5 MgATP, 5 sodium creatinine phosphate, and 0.65 CaCl2 (pH 7.2, pCa 8.0). The bath temperature was maintained at 37°C. Cell capacitance was determined by applying a ramp voltage pulse of 0.5 V/s at a potential between −40 and +70 mV. To obtain the current-voltage relationship of the peak ICa,L density, the membrane was depolarized to a level between −40 and +60 mV for 225 ms in 10-mV steps from a holding potential of −80 mV at cycle length (CL) 10 s. Each depolarizing pulse was preceded by a brief (75 ms) prepulse to −50 mV to inactivate the fast sodium inward current. Frequency-dependent changes of the peak ICa,L density were estimated by application of 20 depolarizing pulses to 0 mV for 225 ms, each of which was preceded by a prepulse to −50 mV at CLs ranging from 500 to 5,000 ms (23). The current densities in the last four among the 20 depolarizing pulses were measured and then averaged.

**Statistical analysis.** Group data were expressed as means or means ± SD. Two-way repeated-measures ANOVAs were conducted for rate-dependent changes of APD, APD dispersion, and CV. If the interaction between the group and the within-factor effect was statistically significant, pairwise comparison between the groups was performed at each level of the within-factor effect, and each probability value was multiplied by the number of comparisons (Bonferroni correction). χ2-square tests were performed for incidence of VT/VF and SCA/SDA. One-way ANOVA with multiple comparisons was performed for APD90 at a BCL of 150 ms in the SDA group. For the other results, statistical comparisons were performed with unpaired Student’s t-tests. Differences were considered significant when the probability value was <0.05.

**RESULTS**

Rate dependence of basal electrophysiological properties. The rate-dependent changes of APD were examined with optical mapping. Color-gradient maps of APD90 in the left ventricular anterolateral surface at a BCL of 400 ms are shown in Fig. 1A. Figure 1B shows optical action potential signals obtained from a single recording site close to the center of the observation area (a red point in Fig. 1A). At this BCL, the CHF heart had an appreciably shorter APD90 than a control heart. The CHF heart also showed more inhomogeneous APD90 distribution than the control (Fig. 1A).

Group data were obtained from eight CHF and eight control hearts. The APD90 in CHF were significantly longer than those measured for control at a BCL >1,000 ms but significantly shorter at BCLs <400 ms (Fig. 1C). Similarly, APD50 was significantly longer in CHF at BCLs >800 ms but significantly shorter at BCLs <400 ms (Fig. 1D). APD dispersion was signif-
The restitution curve of APD$_{90}$ in CHF was steeper than in control (CTL) and a congestive heart failure (CHF) heart. The APD$_{90}$ at a BCL of 2,000 ms in CHF were significantly shorter than control (181 ± 12 ms vs. 197 ± 11 ms; *P < 0.01). APD$_{90}$ at a BCL of 1,000 ms in CHF were comparable with those in control (231 ± 18 ms vs. 223 ± 16 ms; *P = 0.15). This confirms that the CHF-induced modification of the APD restitution in rabbit ventricular muscle is independent of cytochasin D.

Figure 2 shows the rate-dependent changes of CV. CVs in the longitudinal direction in CHF tended to be less than those in control (by 6–11%), but the differences did not reach statistical significance (Fig. 2A). CVs in the transverse direction in CHF were significantly less than controls (by 12–20%) at all BCLs tested (Fig. 2B).

Figure 3 shows dynamic restitution properties of APD and CV. The restitution curve of APD$_{90}$ in CHF was steeper than that for control. This gave rise to significantly greater breadth and depth of the APD$_{90}$ restitution curve in CHF than those in controls (Fig. 3, A and D). In contrast, the slope of CV restitution (both for CVs in the longitudinal and transverse directions) in CHF was similar to that observed for control (Fig. 3, B and C); there were no significant differences in the breadth and depth of the restitution curves between CHF and control (Fig. 3, E and F).

Floating microelectrode recordings. In all of the optical mapping experiments, action potential signals were recorded in the presence of excitation-contraction uncoupler (cytochasin D). This compound is known to alter APD and the restitution properties in rabbit ventricular muscle (8). To address this concern, we carried out transmembrane action potential recording by using floating microelectrodes in seven CHF and six control rabbit hearts in the absence of cytochasin D and examined the stimulation rate dependency of APD (Supplementary Fig. 1). The APD$_{90}$ at a BCL of 2,000 ms in CHF were significantly longer than that of control (263 ± 28 ms vs. 227 ± 16 ms; *P < 0.01). In contrast, APD$_{90}$ at BCL of 600 ms in CHF were significantly shorter than control (181 ± 12 ms vs. 197 ± 11 ms; *P < 0.01). APD$_{90}$ at a BCL of 1,000 ms in CHF were comparable with those in control (231 ± 18 ms vs. 223 ± 16 ms; *P = 0.15). This confirms that the CHF-induced modification of the APD restitution in rabbit ventricular muscle is independent of cytochasin D.

Rate-dependent decrease of $I_{Ca,L}$. Among a wide variety of ion channel remodeling in CHF reported to date, $I_{Ca,L}$ reduction (25, 26) is the most likely mechanism underlying the APD shortening in pacing-induced failing heart, because reductions of potassium outward currents are all expected to prolong APD. First, we examined the current-voltage relationship of $I_{Ca,L}$ at a long CL (10 s) in each of eight ventricular myocytes from control and CHF hearts. $I_{Ca,L}$ density during depolarization to −10 ~ +40 mV was significantly decreased in the failing myocytes by 33–44%.

We next examined the rate-dependent changes in $I_{Ca,L}$ by applying 20 test pulses (0 mV, 200 ms) at CLs ranging from 500 to 5,000 ms. Figure 4A shows representative current traces during the last four test pulses at CL 500 and 5,000 ms. The $I_{Ca,L}$ amplitude of a CHF myocyte was smaller than a control myocyte at either CL, but the reduction was more prominent at a short CL of 500 ms. Pooled data are shown in Fig. 4, B and C. The $I_{Ca,L}$ density in CHF was significantly smaller than the control at the entire range of CLs tested; the shorter the BCLs, the greater the percent reduction was observed.
shortened progressively (<150 ms) until a 2:1 conduction block or VT/VF was induced. The pacing was stopped within 2 s if a 2:1 conduction block or VT/VF was induced. VT/VFs were categorized into two types in terms of their duration: nonsustained (lasting ≥5 beats and <30 s) or sustained (lasting ≥30 s). In terms of ECG morphology, VT was categorized as a monomorphic or polymorphic type. Polymorphic VT lasting ≥30 s was defined as VF. In control, 2:1 conduction blocks were elicited in 12/15 hearts (80%) and VT/VFs did so in the remaining three hearts (20%; 1 nonsustained polymorphic VT and 2 VF). In CHF, a 2:1 block was elicited only in 1/15 hearts (7%) and VT/VFs did so in the remaining 14 hearts (93%; all VF). CHF showed a significantly higher incidence of VT/VF than control ($P < 0.05$). The critical BCL causing VT/VF in CHF was 123 ± 21 ms ($n = 14$), whereas that in control was 111 ± 11 ms ($n = 3$). The former values tended to be longer than the latter ones, but the differences did not reach statistical significance. The critical BCL causing a 2:1 block in a CHF heart was 130 ms ($n = 1$), and that for control was 143 ± 20 ms ($n = 12$).

**Spatial APD alternans.** Spatial APD alternans was investigated by constructing an APD alternans map during dynamic pacing. The threshold BCL causing APD alternans in CHF hearts (194 ± 35 ms, $n = 15$) was significantly longer than those in controls (156 ± 22 ms, $n = 15$) ($P < 0.05$). We first compared spatial APD alternans at a BCL of 150 ms. In a control heart 2:1 block was induced at BCL of 170 ms, and in a CHF heart VF was induced at BCL of 180 ms. Accordingly, the data obtained from each of 14 hearts were analyzed. In control, three of 14 hearts (21%) showed no spatial APD alternans, 9 of 14 (64%) showed SCA, and the remaining two (14%) showed SDA. In CHF, seven of 14 hearts (50%) showed SCA and the remaining seven (50%) showed SDA; the incidence of SDA in CHF was significantly higher than control ($P < 0.05$). We also compared the spatial APD alternans at the shortest BCL just before the occurrence of a 2:1 conduction block or VT/VF (Fig. 5A). In control (BCLs at 142 ± 23 ms), nine of 15 hearts (60%) showed SCA and the remaining six (40%) showed SDA. In CHF (BCLs at 132 ± 19 ms), two of 11 hearts (18%) showed SCA and the remaining nine (82%) showed SDA. CHF had a significantly higher incidence of SDA than control ($P < 0.05$). We failed to create alternans maps in four CHF hearts at short BCLs (<150 ms) because of incomplete repolarization. In control, nine SCAs were all followed by a 2:1 block, three of six SDAs were followed by a 2:1 block, one SDA was followed by nonsustained polymorphic VT, and the remaining two SDAs were followed by VF (Fig. 5B). In contrast, in CHF one of the two SCAs was followed by a 2:1 block and one SCA was followed by VF; all nine SDAs were followed by VF (Fig. 5B). The VT/VF incidence among the cases of SDA in CHF (9/9, 100%) was significantly higher than that in control (3/6, 50%) ($P < 0.05$).

Figure 5C compares APD$_{90}$ at a BCL of 150 ms in six control and eight CHF hearts with SDA at the shortest BCL just before the occurrence of 2:1 block or VT/VF. The APDs in three control hearts exhibiting VT/VF were significantly shorter than those in three controls with a 2:1 conduction block. The values in eight CHF hearts exhibiting VF were also significantly shorter than those in three controls with a 2:1 block. These results suggest that shorter APDs at high stimulation frequencies may increase the vulnerability to VT/VF.

In SDA, areas of long-short APD alternation are adjacent to areas with short-long APD alternation. This would cause a markedly increased dispersion of refractoriness, which predisposes to wavebreak and initiation reentry. To obtain more insight into this issue, we carried out phase mapping analysis of optical action potentials at the onset of VF following SDA. Representative results in a CHF heart are shown in Fig. 6 (9 sequential phase maps) and Supplementary Movie 1. A wavebreak appeared at 2,629 ms in the left ventricular anterolateral surface at the vicinity of the nodal line shown in the preceding APD alternans map (Fig. 6A, top left). This wavebreak generated a pair of counter-rotating phase singularities (PS1, PS2). PS1 was maintained (lasting more than 1 revolution period, giving rise to a rotor), but PS2 disappeared soon after collision with the atrioventricular groove. Multiple PSs (PS3~7) were then generated; PS3 appeared from the left margin (2,834 ms), and PS4 and PS5 appeared from the inside (2,837 ms). PS3 and PS4 were soon pushed out of the left margin. PS6 and PS7 were generated from a new wavebreak created by a meandering rotor around PS1 (2,841 ms). PS1 and PS6 then disappeared after mutual annihilation (2,848 ms). Figure 7B illustrates trajectories of the seven PSs from 2,613 to 2,848 ms. Thus the generation of rotating excitations from a wavebreak in the vicinity of nodal line was followed by VF. A similar sequence of events was observed in phase maps of other four CHF hearts at the initiation of VF preceded by SDA (Supplementary Fig. 2). Rotors initiating VT/VF were not recognized...
in the observation area in the remaining four SDAs of CHF and three SDAs of control hearts.

VF dynamics. Multiple reentrant activities rotating around functional obstacles and their complex interactions are supposed to be an essential feature to maintain the VF. The APD shortening associated with CV reduction under high stimulation rates, a shortening of wave length, might facilitate such multiple reentrant activities. To test this possibility, we analyzed rotation wave (rotor) dynamics during VT/VF by phase mapping. The PSs lasting more than one revolution period were defined as rotors. Figure 7 shows representative phase maps (sequential snap shots) during a 5-s period of VF in a CHF and control heart. In the CHF heart, a total of 11 rotors (life span ranging 76–449 ms) were recognized during 5 s in the whole observation area. In the control heart, in contrast, only three rotors (life span ranging 72–118 ms) were recognized. Pooled data obtained from eight CHF hearts (all VFs) and three control hearts (2 VFs and 1 nonsustained polymorphic VT) are shown in Fig. 7, B and C. Rotors recognized in CHF had significantly higher density and longer life span compared with those seen in controls.

DISCUSSION

The major findings in the present study are as follows. First, left ventricular subepicardial muscle of CHF hearts has a longer APD at low stimulation rates but a shorter APD at high stimulation rates compared with control hearts. Second, CHF hearts also have larger spatial APD dispersion and slower CV than controls. Third, CHF hearts are more vulnerable to VF than controls in association with higher incidence of SDA under high stimulation rates. Finally, multiple rotors of higher density and longer life span generated by wavebreaks in association with SDA are involved in the high vulnerability of CHF hearts.

Rate-dependent electrophysiological properties. Numerous experimental studies have shown APD prolongation in ventricular myocytes of CHF hearts independent of etiologies (1, 14, 21, 26). In most of these experiments, however, the APD changes were examined only at slow stimulation rates. Data obtained under physiological rates or under tachycardia condition are still limited. In the present study herein, we first demonstrated in a pacing-induced rabbit CHF model that
subepicardial ventricular action potentials show dual responses to stimulation frequencies: longer and shorter APD in CHF compared with controls at slow and fast stimulation rates, respectively. In a similar rabbit CHF model, we had reported before that action potentials recorded from single ventricular myocytes with suction pipettes were prolonged at CLs ranging from 333 to 10,000 ms, although the prolongation was less prominent at short CLs (26). The discrepancies between previous and present observations might be attributable to different preparations, i.e., isolated single cells and whole hearts. Electrotonic interactions between cardiac myocytes through gap junctions are known to modulate action potential configuration (5). Ventricular transmural heterogeneity should also be taken into account, since the myocytes used in our previous study were obtained from all layers of the left ventricular free wall (26).

In the pacing-induced rabbit CHF model, we previously reported that the reductions of transient outward current, delayed rectifier potassium currents, and L-type calcium current ($I_{Ca,L}$) (26, 27). Among these ionic current remodeling, $I_{Ca,L}$ reduction is the most likely mechanism for the APD shortening. The present voltage-clamp data on $I_{Ca,L}$ recordings are consistent with this interpretation, because the $I_{Ca,L}$ reduction in CHF hearts was enhanced remarkably at shorter CLs. However, we cannot rule out other possible ionic mechanisms, and further experiments will be required to clarify the point.

Fig. 4. Rate-dependent change of L-type Ca$^{2+}$ current ($I_{Ca,L}$). Myocytes were isolated from the left ventricular subepicardial layer. A: representative current traces of $I_{Ca,L}$ at cycle length (CL) of 5,000 ms (top) and 500 ms (bottom) in a control (left) and a CHF (right) myocyte (the last 4 among each 20 pulses). The pulse protocol for the voltage clamp is shown on the top. B: peak current density of $I_{Ca,L}$ at CLs ranging 500–5,000 ms. Values are means ± SD (CHF, n = 8; control, n = 8, *P < 0.05 vs. control). C: percent reduction of $I_{Ca,L}$ density in CHF compared with control at CLs tested.

Fig. 5. Spatially concordant/discordant alternans of APD. A: representative spatial APD$_{90}$ alternans map on the left ventricular anterolateral surface in a control and a CHF heart. Positive and negative APD alternans phases are represented by blue and red, respectively. Out-of-phase nodal lines are colored by black. SCA, spatially concordant alternans; SDA, spatially discordant alternans. The BCL causing APD alternans is shown in the bottom. B: incidence of SCA/SDA and the subsequent episodes of a 2:1 conduction block or ventricular tachycardia/ventricular fibrillation (VT/ VF) (CHF, n = 11; control, n = 15, P < 0.05). C: APD$_{90}$ at a BCL 150 ms in the SDA group (2:1 block in control, n = 3; VT/VF in control, n = 3; VF in CHF; n = 8; *P < 0.05 vs. 2:1 block in control).
We should be careful in extending the present results to CHF of other animal species including humans and of other pathogenesis. There are considerable species differences in the outward currents responsible for repolarization of ventricular myocytes (13, 17). Substantial differences in the density and distribution of L-type calcium channels were also demonstrated in rabbits and rats (24). Even in the same species, different CHF models were reported to cause different electrophysiological remodeling of the heart. In a rabbit CHF model induced by combined pressure and volume overload, subepicardial and midmyocardial refractory periods of the left ventricle under stimulation at CL of 250 ms were shown to be increased compared with controls (29). The study also showed that APD at the same CL did not differ in isolated ventricular myocytes in the CHF and control rabbits (29).

Fig. 6. Initiation pattern of VF in a CHF heart. A: APD alternans map (left end) during pacing at a BCL of 125 ms and the subsequent 9 snapshots of phase maps at the VF initiation. Distant bipolar ECG is shown on top. Phase singularities (PSs) are indicated by circles (PS1, black; PS2–7, white). A white asterisk and a triangle indicate wavebreaks. The pound sign and cross sign indicate a site of PS emergence and PS collision, respectively. B: trajectories of PS1–PS7 in the observation area. AV, atrioventricular.

Fig. 7. Dynamics of wave propagation during VF. A: representative snapshots of phase maps exhibiting clockwise/counterclockwise rotors (black circle in highlighted squares) during VF (5-s recording) in a CHF (top) and a control (bottom) heart. PSs lasting for less than 1 revolution period are shown by a white circle. Distant bipolar ECG are shown at the top. B: density of rotors recognized in the observation area (in rotors per centimeters squared per seconds). The results were obtained from 8 CHF (8 VF episodes) and 3 control hearts (2 VF and 1 polymorphic VT episodes). Values are means ± SD (*P < 0.05 vs. control). C: rotor life span (in milliseconds). Values are means ± SD of 71 rotors in 8 CHF hearts and 10 rotors in 3 control hearts (*P < 0.05 vs. control).
Ventricular vulnerability in CHF. The present study revealed that the incidence of VT/VF induction by dynamic pacing in CHF hearts was significantly higher than that in controls. This high vulnerability of CHF hearts may be interpreted in part by the high prevalence of SDA (82% in CHF vs. 40% in control). Many theoretical and experimental studies have shown that SDA causes marked dispersion of refractoriness leading to multiple wavebreaks (9, 15, 18, 20, 22). This situation has been confirmed in our optical mapping experiments. In the CHF hearts, a wavebreak emerged at the vicinity of nodal line of SDA generated rotors responsible for complex reentrant activities at the initiation of VF (Fig. 6 Supplementary Fig. 2). A steeper APD restitution in combination with larger APD dispersion in the CHF hearts may facilitate the occurrence of SDA (18, 20).

However, the high vulnerability of CHF hearts cannot be attributed solely to SDA. In six control hearts exhibiting SDA, only three were followed by VT or VF, whereas nine of nine CHF hearts exhibiting SDA were all followed by VF. This means that other factors should also be taken into account. In a simulation study on a two-dimensional sheet of cardiac tissue, Qu et al. (20) have proposed the three conditions required for the genesis of reentrant arrhythmias initiating from breakup following SDA: 1) sufficient slow propagating wave back to cause conduction failure (relevant with slow CV property), 2) inhomogeneity to cause conduction failure locally (relevant with increased APD dispersion/SDA), and 3) alternation of wavelength or refractory period to facilitate reentry (relevant with short APD/slow CV properties) (20). The CHF model in the present study under high stimulation rates meets these three conditions: the CV was significantly slower than control, the APD dispersion was significantly larger than control, and the prevalence of SDA was significantly higher than control. The shorter APD under high stimulation rates in CHF may also facilitate the occurrence of reentry through a shortening of wavelength. Among the six control hearts with SDA, three exhibiting VT/VF had significantly shorter APD90 at a given wavelength. Among the six control hearts with SDA, the CV was significantly slower than control, and the prevalence of SDA was significantly higher than control. In the present study under high stimulation rates meets these three conditions: the CV was significantly slower than control, the APD dispersion was significantly larger than control, and the prevalence of SDA was significantly higher than control.

VF dynamics in CHF. Our phase mapping analysis during VT/VF showed that VF in CHF hearts was characterized by higher density and longer life span of rotors in the observation area compared with VT/VF in controls (Fig. 7). This may reflect more frequent generation of new rotors of longer life span in the CHF hearts. Kay et al. (11) have demonstrated in optical mapping experiments in normal pig hearts that continuous generation of new rotors, rather than a single driving rotor, is critical for VF maintenance. The present phase mapping data during VT/VF are compatible with this concept.

Moreno et al. (16) have demonstrated in an optical mapping study on pacing-induced sheep CHF model that VF in the failing heart is characterized by low density of PSs/rotors and by long life span of the rotors. These observations suggest more organized activation during VF in CHF than the control. The discrepancy between their data and the present results might be ascribed to different procedures of CHF creation in different animal species. Limitations. Our observations are restricted to the LV epicardial surface of rabbit hearts. Electrophysiological remodeling of endocardial and intramural layers of LV could be different from those observed in the epicardial layers. Cardiac arrest following VF in animals in vivo or in humans causes global ischemia and an increase in intraventricular pressure. These metabolic disorders and mechanical stress are known to have serious effects on the electrophysiological properties of ventricular myocytes and VF dynamics (30). We used cytochalasin D as an excitation-contraction uncoupler in the optical mapping experiments. Optical action potential recording using blebbistatin, an alternative excitation-contraction uncoupler having minimal effects on the action potential configuration (6), will be a subject of future studies. Despite these limitations, the present results may provide a new perspective for better mechanistic understanding of high vulnerability to VF in CHF hearts.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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