Ca$^{2+}$ clock malfunction in a canine model of pacing-induced heart failure

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Shinohara T, Park HW, Han S, Shen MJ, Maruyama M, Kim D, Chen PS, Lin SF. Ca$^{2+}$ clock malfunction in a canine model of pacing-induced heart failure. *Am J Physiol Heart Circ Physiol* 299: H1805–H1811, 2010. First published October 1, 2010; doi:10.1152/ajpheart.00723.2010.—The mechanisms of sinoatrial node (SAN) dysfunction in heart failure (HF) remain unclear. We hypothesized that impaired rhythmic spontaneous sarcoplasmic reticulum Ca$^{2+}$ release (Ca$^{2+}$ clock) plays an important role in SAN dysfunction in HF. HF was induced in canine hearts by rapid ventricular pacing. The location of pacemaking sites was determined in vivo using computerized electrical mapping in acute open-chest preparations (normal, *n* = 3; and HF, *n* = 4). Isoproterenol (Iso, 0.2 μg·kg$^{-1}$·min$^{-1}$) infusion increased heart rate and shifted the pacemaking site to the superior SAN in all normal hearts. However, in failing hearts, Iso did not induce superior shift of the pacemaking site despite heart rate acceleration. Simultaneous optical recording of intracellular Ca$^{2+}$ and membrane potential was performed in Langendorff-perfused isolated right atrium (RA) preparations from normal (*n* = 7) and failing hearts (*n* = 6). Iso increased sinus rate, enhanced late diastolic Ca$^{2+}$ elevation (LDCAE), and shifted the pacemaking sites to the superior SAN in all normal but in none of the HF RAs. Caffeine (2 ml, 20 mmol/l) caused LDCAE and increased heart rate in four normal RAs but in none of the three HF RAs. Iso induced ectopic beats from lower crista terminals in five of six HF RAs. These ectopic beats were suppressed by ZD-7288, a specific pacemaker current (*I*_f) blocker. We conclude that HF results in the suppression of Ca$^{2+}$ clock, resulting in the unresponsiveness of superior SAN to Iso and caffeine. HF also increases the ectopic pacemaking activity by activating the *I*_f at the latent pacemaking sites in lower crista terminals.

Sinoatrial node (SAN) remodeling and reduced SAN reserve occur frequently in patients with heart failure (HF) (20). However, the mechanisms of SAN dysfunction in HF remain unclear. The experimental and clinical studies in advanced HF have demonstrated widespread structural and electrophysiological remodeling of the atria (12, 21). In addition, HF is also known to result in the downregulation of the hyperpolarization-activated pacemaker current in the SAN (*I*_f) (23) but significant upregulation of the *I*_f in the right atrium (RA) (26). This differential remodeling was thought to underlie the mechanisms of SAN dysfunction and increased atrial arrhythmias in HF. However, multiple studies showed that the spontaneous diastolic depolarization in SAN occurs because of a synergistic interaction between the voltage clock mediated by voltage-sensitive membrane ion currents, such as *I*_f (2, 3), and Ca$^{2+}$ clock mediated by rhythmic spontaneous sarcoplasmic reticulum (SR) Ca$^{2+}$ release and Na$^+$/Ca$^{2+}$ exchanger current activation (10, 11, 13). Using simultaneous membrane potential (*V*_m) and intracellular Ca$^{2+}$ (Ca$^{2+}$) mapping, our laboratory recently demonstrated that the acceleration of the Ca$^{2+}$ clock in the superior SAN plays an important role in SAN rate acceleration during β-adrenergic stimulation in an isolated, Langendorff-perfused RA preparation (8). HF is known to be associated with significant abnormalities of Ca$^{2+}$ handling (1, 19), which may result in Ca$^{2+}$ clock dysfunction. Consistent with this possibility, we showed that in ambulatory dogs, the increased sympathetic nerve discharges from the stellate ganglion is associated with abnormal heart rate responses, including tachybradycardia (17). The power spectral analysis of heart rate variability and autonomic nervous system activity demonstrated that the HF SAN is unable to respond properly to the tonic sympathetic activation in HF (18). We hypothesize that in HF, the impaired Ca$^{2+}$ clock within the SAN works synergistically with reduced *I*_f to prevent SAN rate response to sympathetic stimulation, whereas the continued presence of *I*_f in the latent pacemaking sites is responsible for ectopic atrial activity. The purpose of the present study was to test these hypotheses in a canine model of pacing-induced HF.

**MATERIALS AND METHODS**

**Canine model of pacing-induced HF.** All animal study protocols were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and the Methodist Research Institute. We studied 23 adult mongrel dogs weighing 22 to 28 kg. The pacing-induced HF was produced in 10 dogs with the use of previously described techniques (17). In short, after anesthesia with isoflurane (1.5 to 2%), a pacemaker system was implanted to the right ventricular apex. After 2 wk, the pacemaker was activated to pace at 150 beats/min for 3 days, at 200 beats/min for 3 days, and at 250 beats/min for 3 wk to induce HF. Transthoracic echocardiography was performed at baseline and after cessation of rapid pacing to confirm that high-rate ventricular pacing induced HF. A two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscles to estimate left ventricular ejection fraction. Left ventricular ejection fraction was 73.1 ± 2.9% at baseline and 35.0 ± 3.2% at HF (*P* < 0.001).

**Computerized electrical mapping in vivo.** A Unemap computerized mapping system (Auckland, NZ) was used in the study (25). We used an electrode patch with 448 electrodes with 1-mm interelectrode distance covering a 1.5 × 2.7-cm area to study the effect of β-adrenergic stimulation at the earliest pacemaker sites in normal (*n* = 3) and HF (*n* = 4) dogs. We mapped the activation pattern of the SAN and surrounding RA according to methods previously published (25).

**Langendorff-perfused canine isolated RA preparation.** We used isolated RAs from 10 normal dogs and 6 dogs with pacing-induced HF for optical mapping study. The heart was rapidly excised under general anesthesia, and the right coronary artery was perfused with a 37°C Tyrode solution equilibrated with 95% O$_2$-5% CO$_2$ to maintain a pH of 7.4. The composition of Tyrode solution was (in mmol/l) 125 NaCl, 4.5 KCl, 0.25 MgCl$_2$, 24 NaHCO$_3$, 1.8 NaH$_2$PO$_4$, 1.8 CaCl$_2$, and 5.5 glucose. The coronary perfusion pressure was regulated between 50 and 60 mmHg. Both ventricles and left atrium were...
removed, and all visible coronary artery branches were tied off. During optical recordings, contractility was inhibited by 10–17 μmol/l of blebbistatin, and the motion artifact was negligible even after isoproterenol (Iso) infusion (8). Pseudo-ECG was recorded with widely spaced bipolar RA electrodes using Iso-DAM8A (World Precision Instruments).

Assessment of SAN function in vitro. Sinus node recovery time (SNRT) was determined by bipolar pacing with a programmable stimulator (Bloom Associates, Reading, PA) for 30 s at progressively shorter pacing cycle lengths (400, 350, and 300 ms) with the two electrodes placed near the SAN. The longest time interval from the last paced atrial depolarization to the first spontaneous sinus beat was recorded as the SNRT. The corrected SNRT was determined by subtracting an average of three sinus cycle lengths before the commencement of atrial pacing from the SNRT. The sinoatrial conduction time (SACT) was measured by the method described by Narula et al. (15): SACT = (return cycle length – basic cycle length)/2.

Dual V_m and Ca^{2+} recordings. The simultaneous V_m and Ca^{2+} mapping was performed using previously described techniques (8). Briefly, the RAs were stained with Rhod-2 AM and RH237 (Molecular Probes, Eugene, OR) and excited with laser light at 532 nm. Fluorescence was collected using two cameras (MiCAM Ultima, BrainVision, Tokyo, Japan) at 1 ms/frame and 100 × 100 pixels with spatial resolution of 0.35 × 0.35 mm²/pixel for 2 s. After dual V_m and Ca^{2+} optical mapping of baseline spontaneous heart beats, pharmacological intervention was performed. The heart rate response to Iso (0.01, 0.03, 0.1, 0.3, and 1.0 μmol/l) was tested in normal and HF dog RAs. A bolus injection of 2 ml caffeine (20 mmol/l) within 1 s was performed in normal and HF dog RAs. ZD-7288 (3 μmol/l), a selective If blocker, was administered to evaluate the characteristic of Iso-induced ectopic beats in normal (n = 3) and HF (n = 5) dogs.

Data analysis. The Ca^{2+} and V_m traces were normalized to their respective peak-to-peak amplitude for comparison in timing and morphology. The slope of enhanced late diastolic Ca^{2+} elevation (LDCAE) was measured by the previously described method (8).

Data were expressed as means ± SE. Statistical analysis was performed using paired Student’s t-test or one-way analyses of variance (ANOVA) with Bonferroni’s post hoc analysis. The Pearson’s χ²-tests were used to compare two categorical variables. The repeated-measure ANOVA model was used to compare Iso- or ZD-7288-induced heart rate response between control and HF dogs. A P value of ≤0.05 was considered significant.

RESULTS

Effects of β-adrenergic stimulation on SAN function in vivo. We used an electrode patch with 448 electrodes (Fig. 1A) to study the effect of β-adrenergic stimulation on the earliest pacemaker sites in normal (n = 3) and HF (n = 4) dogs. Examples of isochronal maps of electrical activation before and after Iso infusion in normal and HF dogs are shown in Fig. 1, C and D. The earliest pacemaker site is located on the middle (n = 2) or inferior (n = 1) SAN at baseline in normal dog hearts. β-Adrenergic stimulation with Iso infusion (0.2 μg·kg⁻¹·min⁻¹) shifted the earliest pacemaker site (red) to

Fig. 1. Computerized electrical mapping of sinoatrial node (SAN) activations in normal and heart failure (HF) dogs. A: picture of the 448-channel electrode patch. B: pictures of electrode location. Rectangle surrounded by dotted line (B, left) represents electrode patch location. C: isochronal maps at baseline (BSL; left) and during isoproterenol (Iso) perfusion (right) in normal dog. D: isochronal maps at BSL (left) and during Iso perfusion (right) in HF dog. SVC, superior vena cava; IVC, inferior vena cava; RAA, right atrial appendage; RAV, right atrial ventral; HR, heart rate; bpm, beats/min. Bars are 10 mm.
the superior SAN with heart rate acceleration in all three normal hearts (Fig. 1C). In all four failing hearts, the earliest pacemaker site was located on the inferior SAN at baseline, but treatment with Iso did not shift the early activation site to the superior SAN despite heart rate acceleration in any HF hearts (Fig. 1D). The distribution of the pacemaking site during Iso infusion showed a significant difference between normal and HF dogs (P = 0.008). Iso (0.02 μmol/l) significantly shifted the earliest pacemaker site upward in normal dogs compared with HF (8.6 ± 1.0 vs. 1.7 ± 0.7 mm, P = 0.002).

SAN dysfunction of HF dogs in vitro. Figure 2 shows the assessment of SAN function in normal and HF RAs. The RAs of HF dogs had longer SNRT (1,277 ± 149 vs. 842 ± 57 ms, P = 0.021, Fig. 2A), and longer corrected SNRT (485 ± 97 vs. 238 ± 31 ms, P = 0.036, Fig. 2B) than those of normal dogs. SACT was also significantly longer in HF dogs than in normal dogs (179 ± 24 vs. 110 ± 17 ms, P = 0.045, Fig. 2C). Figure 3 shows the activation pattern on SAN and the surrounding RA during spontaneous sinus rhythm during baseline in normal and HF dogs. The pacemaking sites at baseline in RAs of normal dogs (n = 7) were located at the middle and inferior SANs in four and three dogs, respectively. All of RAs from HF dogs (n = 6) were the inferior SAN. Normal dogs showed small LDCAEs in the pacemaking sites at baseline, whereas the LDCAEs did not occur in any HF dogs [1.45 ± 0.20 vs. 0.11 ± 0.05 arbitrary units (AU)/s, P < 0.001, Fig. 3, C and D].

Impaired Ca^{2+} clock activation at superior SAN with β-adrenergic stimulation and caffeine injection in HF dogs. Figure 4A shows examples of activation patterns at Iso (0.1 μmol/l) infusion in normal and HF RAs. In a normal RA, a robust LDCAE (arrows in Ca^{2+} tracing of Fig. 4A,a) occurred at the pacemaking site of superior SAN. The earliest Vm activation site shifted to superior SAN after Iso infusion as shown in a previous study (8). This finding was consistently observed in all six normal RAs during Iso infusion. However, none of the six RAs from HF dogs showed LDCAE in superior SAN (Fig. 4A,b), and the earliest Vm activation site did not shift to superior SAN. The distribution of the pacemaking site during Iso infusion showed a significant difference between normal and HF RAs (P < 0.001). The slope of the LDCAE was significantly depressed in HF compared with normal dogs (0.52 ± 0.13 vs. 3.87 ± 0.50 AU/s at Iso 0.01 μmol/l, P < 0.001, Fig. 4B). Figure 4C shows the Iso-dose response curve of heart rate obtained from RAs of six normal and six HF dogs, respectively. In normal hearts, Iso dose-dependently increased heart rate. The heart rate response to Iso reduced significantly after the induction of HF (P < 0.001).

When Iso dosage was increased, Iso induced ectopic beats from the lower crista terminalis in five of six HF dogs (Fig. 5A) but none in the normal RAs. There were no LDCAEs in the ectopic beat sites. Figure 5C shows heart rate changes associated with I_{r} blocker (ZD-7288) treatment in normal and HF RAs. Iso induced ectopic beats in HF, and ZD-7288 (3 μmol/l) suppressed the rate of ectopic beats, resulting in bradycardia at 61 ± 3 beats/min (n = 5). ZD-7288 significantly reduced heart rate in HF RAs compared with that in normal RA (P < 0.001, Fig. 5C).

Figure 6A,a shows the typical caffeine response in normal RA. Caffeine injection shifted the pacemaking site to superior SAN with concomitant LDCAE (arrows in Ca^{2+} tracing of Fig. 6A,a). This finding was consistently observed in all four normal RAs that were perfused with caffeine. In HF RAs, however, caffeine did not result in LDCAE compared with that in normal RA (0.78 ± 0.32 vs. 4.18 ± 0.20 AU/s, P < 0.001, Fig. 6B) and did not shift the pacemaking site upward in any of the three HF dogs (P = 0.03). Furthermore, the heart rate response to caffeine injection reduced significantly in HF RAs (118 ± 4 vs. 159 ± 7 beats/min, P = 0.005, Fig. 6C).

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DISCUSSION

The present study is the first that documents Ca\(^{2+}\) clock dysfunction in dogs with pacing-induced HF. The major findings of the present study include the following: 1) Iso-induced LDCAE was impaired in the superior SAN in HF; 2) response of LDCAE and heart rate to \(\beta\)-adrenergic stimulation are impaired in the superior SAN of failing hearts, suggesting a role for dysfunctional Ca\(^{2+}\) cycling and SAN function in HF; 3) caffeine also failed to increase the rate of SR Ca\(^{2+}\) release in the superior SAN in HF; and 4) Iso-induced ectopic beats from the lower crista terminalis worked as the leading pacemaker in HF. This ectopic pacemaker was suppressed by a specific \(I_f\) blocker. These findings indicate that a defective Ca\(^{2+}\) clock at the superior SAN is responsible for the unresponsiveness of SAN to sympathetic stimulation in HF. HF also increases the ectopic pacemaking activity by downregulating \(I_f\) (23) in the SAN. Zicha et al. (26) reported that hyperpolarization activated cyclic nucleotide-gated potassium channel 4 (HCN4), which encodes \(I_f\), downregulates in the SAN in HF dogs. The suppression of heart rate acceleration in response to \(\beta\)-adrenergic stimulation in HF may be caused by \(I_f\) downregulation. However, Herrmann et al. (6) recently studied mice with deleted HCN4. They reported that HCN4 is not required to mediate the acceleration of the heart rate in response to \(\beta\)-adrenergic stimulation, although HCN4 is necessary for maintaining a stable cardiac rhythm. Their data suggest that HF-induced Ca\(^{2+}\) clock dysfunction is an important factor that limits SAN responsiveness to autonomic modulation. The present study demonstrated a significantly reduced LDCAE at the pacemaking sites of SAN in HF compared with normal SAN, and the slope of the LDCAE during \(\beta\)-adrenergic stimulation was shallower in HF than in normal SANs. These results suggest that spontaneous SR Ca\(^{2+}\) release was impaired in SANs of HF dogs. In addition to Ca\(^{2+}\) clock malfunction, HF also impairs the membrane voltage clock by downregulating \(I_f\) (23) in the SAN. Zicha et al. (26) reported that hyperpolarization activated cyclic nucleotide-gated potassium channel 4 (HCN4), which encodes \(I_f\), downregulates in the SAN in HF dogs. The suppression of heart rate acceleration in response to \(\beta\)-adrenergic stimulation in HF may be caused by \(I_f\) downregulation. However, Herrmann et al. (6) recently studied mice with deleted HCN4. They reported that HCN4 is not required to mediate the acceleration of the heart rate in response to \(\beta\)-adrenergic stimulation, although HCN4 is necessary for maintaining a stable cardiac rhythm. Their data suggest that HF-induced Ca\(^{2+}\) clock dysfunction is an important factor that limits SAN responsiveness to autonomic modulation.

Impaired response to caffeine. Caffeine sensitizes the ryanodine receptor to activation, resulting in an increased SR Ca\(^{2+}\) release (24). The present study revealed that caffeine failed to increase Ca\(^{2+}\) in HF hearts, suggesting these findings may be
explained by the depletion of SR Ca\(^{2+}\) content on the SR. Previous studies have reported the alterations in Ca\(^{2+}\) handling were found in failing myocardium (5). Several possible mechanisms could explain these phenomena. First, a decreased sarcoplasmic reticulum Ca\(^{2+}\)-ATPase expression and function are present in HF and may result in a reduced SR Ca\(^{2+}\) accumulation (16). Second, an increased forward-mode Na\(^+\)/Ca\(^{2+}\) exchanger current activity opposes SR Ca\(^{2+}\) accumula-

Fig. 4. Effect of Iso on activation pattern of SAN in normal and HF RAs. A: activation patterns in normal and HF RAs during Iso perfusion. Isochronal map and Ca\(^{2+}\) (red) and V_m (black) tracings from S, M, and I SANs at Iso 0.1 \(\mu\)mol/l for normal (a) and HF (b) dogs. Arrows show robust LDCAEs in normal RA. B: slope of LDCAE at Iso 0.1 \(\mu\)mol/l in normal and HF RAs. Slope of LDCAE in superior SAN is significantly suppressed in HF \((P < 0.001)\). C: effect of Iso to HR in normal and HF dogs. Iso-induced HR increase is impaired in RAs from HF dogs. *\(P < 0.05\) vs. corresponding normal group.

Fig. 5. Iso-induced atrial ectopic pacemaker in HF RAs. A: locations of ectopic beats in HF RAs. Red circles show the sites of Iso-induced ectopic beats. All of Iso-induced ectopic beats occurred from the lower crista terminals. Solid line and dotted circles correspond to isochronal map field of B.a and B.b, respectively. B: isochronal maps during ectopic beats appearance. C: change of HR with ZD-7288, pacemaker current (I_f) blocker, to Iso-induced ectopic beats in normal and HF dogs. ZD-7288 (3 \(\mu\)mol/l) significantly suppressed HR in HF compared with normal dogs \((P < 0.001)\). *\(P < 0.01\) vs. corresponding normal group. Bar is 10 mm.
tion (16). The decreased SR Ca\(^{2+}\) content may reduce spontaneous SR Ca\(^{2+}\) release, leading to Ca\(^{2+}\) clock dysfunction. Third, type-2 ryanodine receptor function is increased in HF, probably because of hyperphosphorylation and reduced FKBP12.6 binding (14). These changes may contribute to reduced SR Ca\(^{2+}\) content and further impair the Ca\(^{2+}\) handling in HF.

Iso-induced ectopic beats in HF. Iso caused ectopic beats from the lower crista terminals in HF dogs. Because these ectopic beats were suppressed by ZD-7288, \(I_f\) activation most likely underlined the increased ectopic activity. These results are consistent with our previous report that \(I_f\) is intimately involved in the generation of ectopic beats from the lower crista terminals when the Ca\(^{2+}\) clock in SAN is impaired (22). Honjo et al. (7) reported that myocardial sleeves of pulmonary veins have the potential to generate spontaneous activity, and such arrhythmogenic activity is uncovered by the modulation of Ca\(^{2+}\) dynamics. In the lower crista terminalis area, the same mechanisms might have caused ectopic beats as well. HF decreases HCN4 expression at both mRNA and protein levels in SAN, whereas HCN4 is significantly upregulated in RA from the HF dog (26). The HCN4 upregulation in RA may play a role in atrial arrhythmogenesis in HF. Thus we suggest that the Ca\(^{2+}\) clock dysfunction and the HCN4 upregulation in atrium are associated with atrial ectopic beats in HF.

Clinical implications. HF is associated with SAN dysfunction and increased ectopic pacemaker activity. Lower crista terminalis is a frequent origin of ectopic atrial tachycardia in patients without structural heart diseases (9) and is also a dominant ectopic pacemaking site in dogs with pacing-induced HF (4). Our data provide new insights into the combination of SAN dysfunction and increased ectopic pacemaking activity during sympathetic activation in patients with HF.

Conclusion. SAN dysfunction in a canine model of HF is associated with Ca\(^{2+}\) clock malfunction, characterized by unresponsiveness of SAN to Iso and caffeine. These findings suggest reduced SR Ca\(^{2+}\) release in the superior SAN is a mechanism of SAN dysfunction in HF. HF also increases the ectopic pacemaking activity by activating the \(I_f\) at the latent pacemaking sites in lower crista terminalis.

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DISCLOSURES

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

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Fig. 6. Effect of caffeine on activation pattern of SAN in HF dogs. A: effects of caffeine in normal and HF RAs. A.a: caffeine response in normal RA. Caffeine injection (20 mmol/l, 2-ml bolus given within 1 s) induces LDCAE in normal RA. Arrows show robust LDCAEs. A.b: caffeine responses in HF RA. There is no LDCAE. B: slope of LDCAE in normal and HF RAs. Caffeine-induced robust LDCAE is significantly suppressed in HF (\(P < 0.001\)). C: HR in normal and HF RAs after caffeine injection. Caffeine-induced HR increase is significantly suppressed in HF (\(P = 0.005\)).


