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PRECLINICAL STUDIES

Left Stellate Ganglion and Vagal Nerve Activity and Cardiac Arrhythmias in Ambulatory Dogs With Pacing-Induced Congestive Heart Failure

Masahiro Ogawa, MD, PHD,* Shengmei Zhou, MD,* Alex Y. Tan, MD,* Juan Song, PHD,* Ghassan Gholmieh, MD, PHD,† Michael C. Fishbein, MD, FACC,‡ Huai Luo, MD,* Robert J. Siegel, MD, FACC,* Hrayr S. Karagueuzian, PHD,* Lan S. Chen, MD,† Shien-Fong Lin, PHD,* Peng-Sheng Chen, MD, FACC*

Los Angeles, California

Objectives The purpose of this study was to determine the patterns of autonomic nerve activity in congestive heart failure (CHF).

Background The relationship between autonomic nerve activity and cardiac arrhythmias in CHF is unclear.

Methods We implanted radiotransmitters in 6 dogs for continuous (24/7) simultaneous monitoring of left stellate ganglion nerve activity (SGNA), vagal nerve activity (VNA), and electrocardiography before and after pacing-induced CHF.

Results Congestive heart failure increased both SGNA and VNA. The SGNA but not VNA manifested a circadian variation pattern. There was extensive sinus node fibrosis. We analyzed 2,263 episodes of prolonged (>3 s) sinus pauses (PSP) and 1,420 long (>10 s) episodes of paroxysmal atrial tachycardia (PAT). Most (95.3%) PSP episodes occurred at night, and 56% were preceded by a short burst of SGNA that induced transient sinus tachycardia. Long PAT episodes were typically (83%) induced by simultaneous SGNA and VNA discharge, followed by VNA withdrawal. Premature ventricular contractions and ventricular tachycardia were preceded by elevated SGNA.

Conclusions The reduction of sympathovagal balance at night in ambulatory dogs was due to reduced sympathetic discharge rather than a net increase of vagal discharge. The tachybrady syndrome in CHF might be triggered by an intermittent short burst of SGNA that resulted in tachycardia and sinus node suppression. Simultaneous sympathovagal discharge is a cause of long PAT episodes. These data indicate that there is an association between the specific patterns of autonomic nerve discharges and cardiac arrhythmia during CHF. (J Am Coll Cardiol 2007;50: 335–43) © 2007 by the American College of Cardiology Foundation

Congestive heart failure (CHF) is associated with structural, electrophysiological, and neural remodeling (1). Because the autonomic nervous system (ANS) activity regulates cardiac ion channel function, it is possible that specific ANS activity might be responsible for triggering cardiac arrhythmias in CHF. Our laboratory recently developed methods for continuous (24 h/day, 7 days/week) and direct recording from the stellate ganglion nerve activity (SGNA) in normal ambulatory dogs over several months (2). We hypothesized that the same techniques could be applied for continuous recording of vagal nerve activity (VNA) and that simultaneous SGNA and VNA recording in dogs with CHF would provide an insight into the neural mechanisms of cardiac arrhythmias. Furthermore, we hypothesized that specific patterns of ANS activities (signatures) are responsible for triggering specific cardiac arrhythmias in CHF. The purpose of the present study was to test these hypotheses.

Methods

Surgical preparation. The animal experiments were performed with approval of the Institutional Animal Care and Use Committee. A pacing lead was implanted to the right

From the *Division of Cardiology, Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, California; †Division of Neurology, Department of Pediatrics, Children's Hospital, Los Angeles and USC Keck School of Medicine, Los Angeles, California; and the ‡Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, UCLA, Los Angeles, California. This study was supported by the Chun Hwang Fellowship in Cardiac Arrhythmias honoring Dr. Asher Kimchi; the American Heart Association (AHA) Scientist Development grant 0435135N; a Heart Rhythm Society Postdoctoral Fellowship Award; the National Institutes of Health grants P01 HL78931, R01s HL78932, 58533, 66389, and 71140; an AHA Established Investigator Award (#0540093N); an AHA GIA (0555057Y); a University of California Tobacco Related Disease Research Program grant (141T-0001); and a Pauline and Harold Price Endowment.

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Abbreviations and Acronyms

ANS = autonomic nervous system
CHF = congestive heart failure
ECG = electrocardiography
HASDA = high amplitude spike discharge activity
LABDA = low amplitude burst discharge activity
PAT = paroxysmal atrial tachycardia
PSP = prolonged sinus pause
SGNA = stellate ganglion nerve activity
VNA = vagal nerve activity

ventricular apex and connected to an Itrel neurostimulator (Medtronic, Minneapolis, Minnesota) (3). We then implanted a Data Sciences International D70-EEE transmitter (2) with 3 bipolar recording channels to record SGNA from left stellate ganglion, VNA from the left thoracic vagus nerve located above the aortic arch, and subcutaneous electrocardiography (ECG). After 2 weeks of baseline monitoring, the right ventricle was paced at 150 beats/min for 3 days, 200 beats/min for 3 days, and then 250 beats/min for 3 weeks to induce CHF (3). The pacemaker was then turned off to allow an additional 2 weeks of ambulatory monitoring. Echocardiograms were performed to document the left ventricular ejection fraction. The dogs were then killed, and the hearts were harvested for histological studies.

Manual analyses of arrhythmia events. Long episodes of paroxysmal atrial tachycardia (PAT) were identified when there was an abrupt (>50 beats/min) increase in the atrial rate to >160 beats/min that persisted for at least 10 s. A short PAT episode was identified as lasting for <10 s. We also identified premature atrial contractions, premature ventricular contractions, and prolonged (>3 s) sinus pauses (PSP) by manually analyzing all episodes in 1 baseline day (the day before the commencement of pacing) and in days 1, 7, and 14 after cessation of rapid pacing. The premature ventricular contractions are diagnosed by the absence of preceding P-wave, the wide QRS complex, and the compensatory pause.

Association between arrhythmic events and nerve activity. In addition to manual analyses, we also used custom-designed software to perform automatic computer based analyses. The computer first identified the amplitude of baseline noise and then identified as ANS activity all signals that were 3 times higher than the baseline noise. The ANS activity was high-pass (125 Hz) filtered, rectified, and summed over fixed time segments to represent the total nerve activity. These analyses were done for each dog at 1 baseline day (the day before commencement of pacing) and 3 heart failure days including days 1, 7, and 14 after cessation of rapid pacing. The computer then analyzed the total duration of time during which there was only SGNA, only VNA, and simultaneous SGNA and VNA. We determined the duration of nerve activity associated with arrhythmia and not associated with arrhythmia. We then determined the number of arrhythmic episodes with and without simultaneous nerve activity.

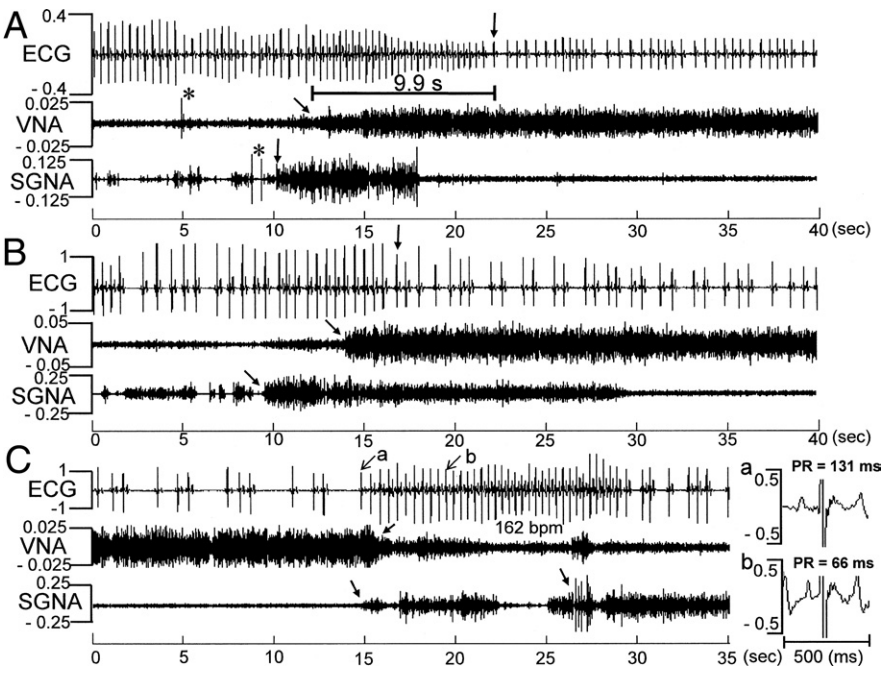

Figure 1 Examples of Autonomic Nervous System Activity From Dog #1

(A) Simultaneous stellate ganglion nerve activity (SGNA) and vagal nerve activity (VNA) discharges cause bradycardia at baseline. **(B)** Similar phenomenon after the induction of congestive heart failure (CHF). **(C)** A brief period of sympathovagal discharge **(between first and second arrows)** induced long paroxysmal atrial tachycardia during CHF. ECG = electrocardiography.

Statistical analyses. The Fisher exact test was used to determine the association between sympathovagal discharges and arrhythmia. Cosinor tests (4) were used to determine whether there were significant circadian variations. Analyses of variance (ANOVAs) were used to determine whether the number of long PAT episodes (>10 s) and the magnitudes of nerve discharges were different at baseline and at days 1, 7, and 14 after cessation of rapid pacing. If the p value was ≤0.05, Newman-Keul tests were then used to compare the means between groups. The same test was used to compare the means of norepinephrine levels at baseline, at the end of rapid pacing, and 14 days after termination of pacing. Nonparametric Newman-Keuls multiple comparisons were used to compare N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels among the 3 different groups. The results were presented as mean ± SD. A p value of ≤0.05 was considered significant.

Results

The dogs were followed up for a total of 60 ± 8 days. Among them, 29 ± 4 days were used for rapid pacing.

Simultaneous recording of SGNA and VNA at baseline and during CHF. Figure 1 shows examples that document successful simultaneous SGNA and VNA recordings. Figure 1A shows 2 kinds of nerve activity. The first kind (asterisks) is high amplitude spike discharge activity (HASDA), and the remaining nerve activity is low amplitude burst discharge activity (LABDA). We define HASDA as spike discharges with peak-to-peak amplitudes of at least 0.2 mV. The LABDA is continuous nerve activity with amplitudes at least 3 times higher than baseline noise. Abrupt increase of SGNA in the form of LABDA (first arrow) was followed by sinus tachycardia. The second arrow indicates increased VNA that corresponds to a period of simultaneous sympathovagal discharge and tachycardia. This was then followed by SGNA withdrawal and bradycardia (third arrow). In all dogs studied, the latency between SGNA (first arrow) and VNA (second arrow, panel A) averaged 0.8 ± 0.5 s. Among 38 selected episodes, the average latency between onset of VNA (second arrow) and bradycardia (third arrow) was 4.8 ± 2.2 s (range 1.6 to 9.9 s). Among 39 selected episodes during CHF, the average latency between VNA (second arrow) and bradycardia (third arrow) was 4.1 ± 1.3 s (range 1.8 to 6.2 s). Figure 1C shows an example of ANS discharge 5 days after cessation of rapid pacing. Persistent and large VNA discharges were associated with sinus bradycardia and sinus arrhythmia between 0 and 20 s. The onset of SGNA (first arrow) resulted in a brief period (2 s) of simultaneous VNA and SGNA activity during which PAT began. The onset of

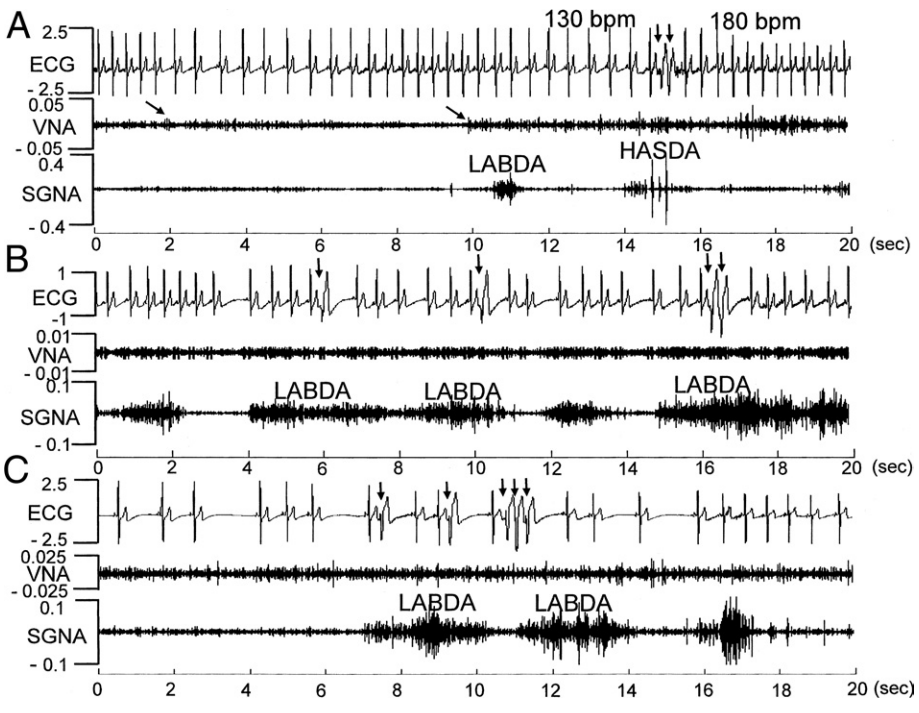

Figure 2 LABDA and HASDA

(A) High amplitude spike discharge activity (HASDA) induced couplets and abruptly increased heart rate 6 days after cessation of rapid pacing in dog #2. **(B)** Low amplitude burst discharge activity (LABDA) episodes associated with isolated premature ventricular contraction and couplets 1 day after cessation of rapid pacing in dog #3. **(C)** LABDA episodes associated with isolated premature ventricular contraction and triplets (ventricular tachycardia) 1 day after cessation of rapid pacing in dog #2. Abbreviations as in Figure 1.

tachycardia was then followed by offset of VNA (second arrow) and PAT at 162 beats/min that persisted for >10 s. A third arrow indicates an episode of HASDA, which coincided with a short (<2 s) burst of increased VNA. The PAT then terminated. The magnified ECG (right panels of Figure 1C) shows the P waves and the PR intervals before the onset of PAT ("a") and during PAT ("b") were significantly different.

SGNA and ventricular arrhythmia. Figure 2A shows the relationship between SGNA and ventricular arrhythmia 6 days after cessation of rapid pacing. Note that increased VNA (first and second arrows, VNA channel) was associated with a reduction of heart rate. The onset of LABDA on SGNA recording with continuous vagal activity was associated with further reduction of heart rate to 130 beats/min, suggesting that vagal discharges slowed heart rhythm more efficiently during increased sympathetic activity (accentuated antagonism) (5). The HASDA was followed immediately by 2 consecutive premature ventricular contractions (downward arrows) and further heart rate acceleration. Figures 2B and 2C show multiple episodes of LABDA associated with premature ventricular contractions, couplets, or ventricular tachycardia.

HASDA versus LABDA. The HASDA probably represents synchronized neuronal discharges, whereas LABDA represents the ordinary and nonsynchronized discharges. Both of these discharges apparently resulted in cardiac catecholamine release, because their presence can increase heart rate and precede the development of arrhythmia. The HASDA was much less common than LABDA at baseline and during CHF. We selected 215 runs of HASDA from 1 baseline day and 1 CHF day of all dogs for manual analyses. There were 14.7 ± 12.9 episodes of HASDA/day/dog at baseline and 16.0 ± 14.4 episodes/day/dog during CHF (p = NS). The number of spikes/run of HASDA was 6.7 ± 2.2 (range 3 to 19) at baseline. This number was slightly larger than the number of spikes/HASDA episode after the induction of CHF (5.9 ± 1.7, range 3 to 13, $p = 0.002$). However, the average HASDA amplitude (1.41 ± 0.80 mV) and the frequency (6.6 ± 0.6 Hz) in CHF were significantly higher than the amplitude (0.64 ± 0.22 mV, $p < 0.0001$) and frequency (5.8 ± 0.7 Hz, $p < 0.0001$) at baseline. The HASDA episodes always occurred either immediately before or after LABDA episodes. Figure 3 shows typical examples of induction of premature atrial contraction (arrow on ECG channel in Figure 3A) and premature ventricular contraction (arrow on ECG channel in Figure 3B) associated with HASDA. Overall, 19 of 215 (8.8%) HASDA episodes induced either premature atrial contractions or premature ventricular contractions. These 19 episodes included 6 of 103 at baseline and 13 of 112 during CHF (p = NS). Most HASDA episodes occurred in daytime (from 6:00 AM to 6:00 PM) at baseline (70 of 103) as well as during CHF (87 of 112). Figure 4 shows the

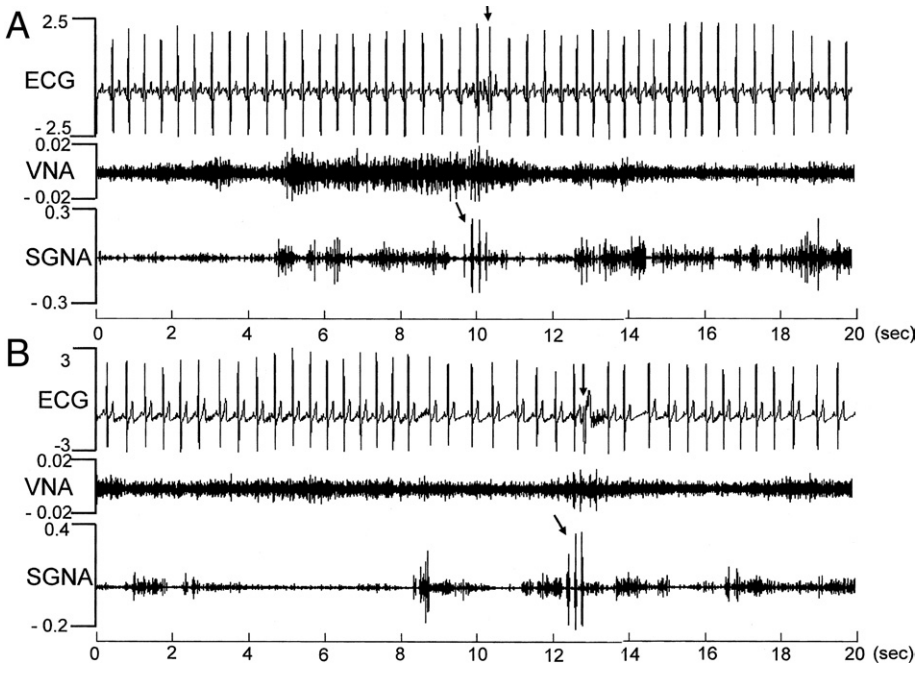

Figure 3 HASDA and Premature Contractions

(A) HASDA induced isolated premature atrial contraction 1 day after cessation of rapid pacing in dog #2. **(B)** HASDA induced isolated premature ventricular contraction 7 days after cessation of rapid pacing in the same dog. Abbreviations as in Figures 1 and 2.

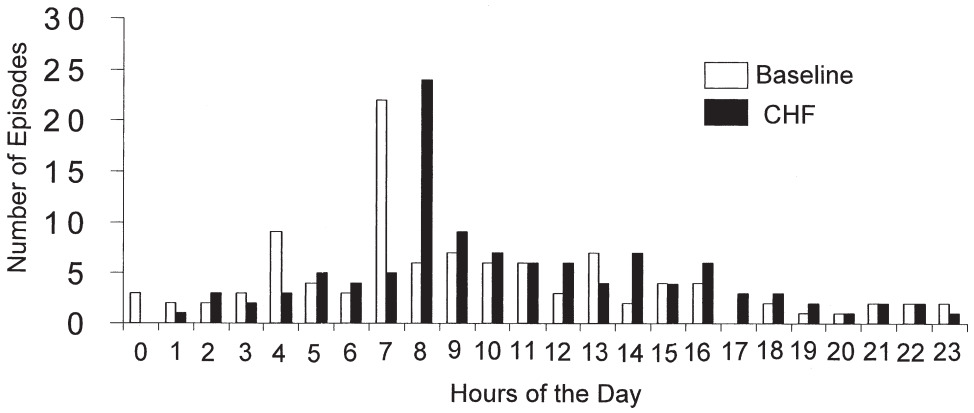

Figure 4 **Circadian Variations of HASDA**

We manually analyzed the number of high amplitude spike discharge activity (HASDA) episodes at baseline (1 day before commencement of rapid pacing) and during congestive heart failure (CHF) (1 day after cessation of pacing). The data of all dogs were then pooled together. There were circadian variations of HASDA, with peak incidence 7:00 AM to 8:00 AM.

number of HASDA episodes/h over a 24-h period. The 7:00 AM to 8:00 AM peaks correlated with the time of awakening and the arrival of the vivarium staff. Cosinor tests showed significant circadian variation of the HASDA episodes at baseline ($p = 0.0019$) and during CHF ($p = 0.0008$). The HASDA is rarely seen on VNA (Figure 1C).

Prolonged sinus pauses. There were no PSP episodes in any dog at baseline. The PSP episodes (/dog) were 5 ± 6, 250 ± 424, and 123 ± 206, respectively, at days 1, 7, and 14 after cessation of rapid pacing (p = NS). Among a total of 2,263 PSP episodes, 2,156 (95.3%) occurred between 6:00 PM and 6:00 AM. In 994 PSP episodes, no obvious changes of SGNA or VNA were observed before and after PSP. In the remaining 1,269 episodes (56%), the PSP occurred owing to short bursts of LABDA and tachycardia (Fig. 5A). Abrupt withdrawal of the LABDA was associated with lengthening of RR interval (arrow) followed by a 5-s pause. Figure 5B shows intermittent LABDA (arrows). The final LABDA episode was large (asterisk). Its abrupt withdrawal was followed by prolonged RR interval (upward arrow) and PSP of 5.5 s. The data within the dotted line segments are enlarged in Figure 5C. The shortening of the RR interval preceded the onset of the LABDA. In contrast, the withdrawal of LABDA preceded the termination of the tachycardia. These data are best explained by a bidirectional coupling between tachycardia and LABDA. Shortening of the RR interval triggered LABDA, probably through baroreflex. A long and large LABDA episode was associated with prolonged tachycardia. The increased tachycardia duration might have resulted in sufficient sinus node suppression to cause PSP, followed by a regular rhythm. Note that there was very little change of VNA in these episodes. In 96 randomly selected PSP episodes, the integrated SGNA 3 s before the onset of PSP averaged 2.6 ± 1.0 μV, higher than the SGNA 3 s after the onset of PSP (2.1 ± 0.8 μV, $p < 0.0001$).

PAT at baseline and during heart failure. We documented a total of 568 episodes of long (>10 s) PAT (95 ± 21 episodes/dog) at 1 baseline day. The number of long PAT episodes were 195 (33 ± 25/dog), 283 (47 ± 27/dog), and 374 (62 ± 43/dog), respectively, in days 1, 7, and 14 after the cessation of rapid pacing ($p = 0.0129$ by ANOVA). The low number of episodes in day 1 was probably due to persistent high sinus rate, which made it difficult for a tachycardia episode to fulfill the "abrupt onset" criterion. The differences between baseline and day 1 ($p < 0.01$) and day 7 ($p < 0.05$) were statistically significant by Newman-Keul test. Among a total of 1,420 episodes, 1,172 episodes were preceded by a brief period of sympathovagal discharge, including 454 (76 ± 17/dog), 176 (29 ± 22/dog), 223 (37 ± 15/dog), and 319 (53 ± 37/dog) for baseline and days 1, 7, and 14 after cessation of rapid pacing, respectively.

We also analyzed the proportion of times that sympathovagal discharges resulted in a change of rhythm. We found that 20 of 152 (13.2%) episodes of simultaneous sympathovagal discharge at baseline, 17 of 163 (10.4%) at day 1 after cessation of rapid pacing, and 16 of 163 (9.8%) episodes 14 days after the cessation of rapid pacing induced PAT. Overall, 53 (11%) of 478 simultaneous sympathovagal discharge episodes induced PAT.

To further determine whether there is a positive association between simultaneous sympathovagal discharges and PAT, we analyzed the frequency of sympathetic alone, vagal alone, and simultaneous sympathovagal discharges throughout the day (Table 1). There was significantly increased SGNA in CHF day 1 as compared with baseline, CHF day 7, and CHF day 14. There was no difference of VNA and sympathovagal discharges in different days. Because PAT accounts for <2% of the 24-h period, the data shown in

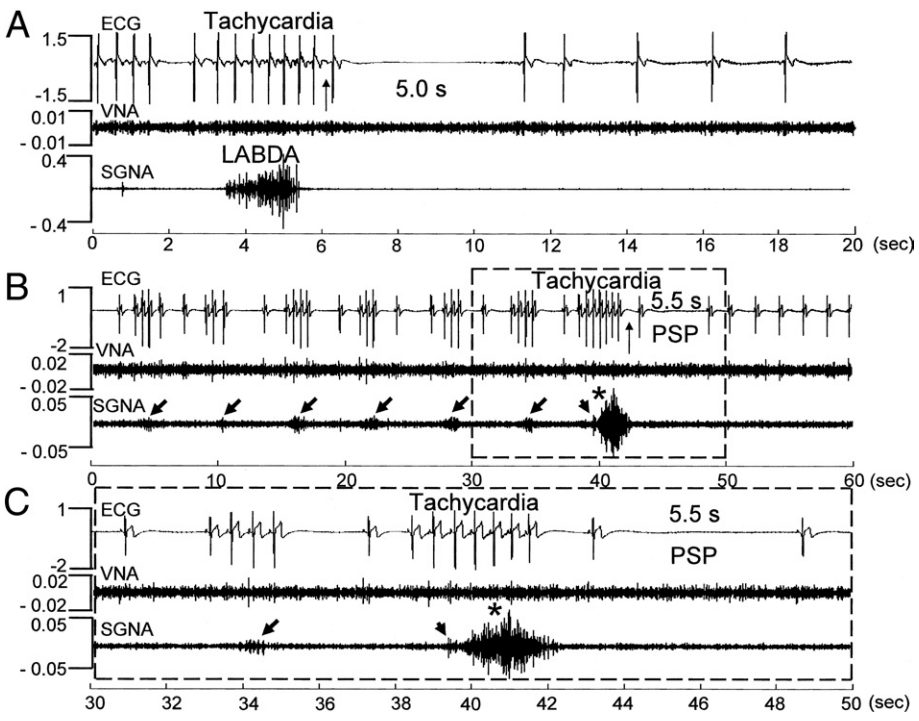

Figure 5 SGNA and Prolonged Sinus Pause

(A) Abrupt termination of LABDA preceded a prolonged sinus pause (PSP) episode. **(B)** Intermittent LABDA **(arrows)** associated with intermittently increased heart rate, and a large LABDA **(*)** induced tachycardia. Sudden SGNA withdrawal resulted in 5.5-s PSP. **(C)** The data within the dotted line in **panel B**. Abbreviations as in Figures 1 and 2.

Table 1 represents the expected nerve discharge patterns in the absence of PAT. Table 1 shows the results of Fisher exact test between 2 categorical data: presence (+) or absence (−) of PAT versus presence (+) or absence (−) of sympathovagal discharges. The results show highly significant positive association between PAT and sympathovagal discharges at a baseline day and at days 1, 7, and 14 after the induction of CHF ($p < 0.0001$ for all days).

Table 1 Sympathovagal Discharges and PAT

	Percent of Time Over a 24-h Period During Which Nerve Activity Was Present		
	SGNA Alone	VNA Alone	Sympathovagal Discharge
Baseline	44.2 ± 5.8%	9.3 ± 4.7%	8.4 ± 4.9%
CHF day 1	51.6 ± 9.5%*	10.5 ± 6.7%	10.4 ± 6.9%
CHF day 7	38.3 ± 6.9%	7.0 ± 3.1%	6.8 ± 3.1%
CHF day 14	36.5 ± 7.0%	8.2 ± 5.8%	8.0 ± 5.4%

		Positive Association Between Sympathovagal Discharges and PAT	
	Sympathovagal Discharge	PAT(+)	PAT(−)
Baseline	+	79.9%	8.4%
($p < 0.0001$)	−	20.1%	91.6%
CHF day 1	+	90.3%	10.4%
($p < 0.0001$)	−	9.7%	89.6%
CHF day 7	+	78.8%	6.8%
($p < 0.0001$)	−	21.2%	93.2%
CHF day 14	+	85.3%	8.0%
($p < 0.0001$)	−	14.7%	92.0%

*$p < 0.05$ compared with baseline, $p < 0.01$ compared with congestive heart failure (CHF) day 7 and CHF day 14.
PAT = paroxysmal atrial tachycardia; SGNA = stellate ganglion nerve activity; VNA = vagal nerve activity.

Premature ventricular contraction in normal and heart failure dogs. We found a total of 105 episodes of premature ventricular contractions, including 35 at baseline and 70 in CHF, in 3 of the 6 dogs studied. In 82 (78%) episodes, the premature ventricular contractions were preceded by an increased SGNA. The patterns of SGNA in these 82 episodes could be either LABDA or HASDA and could occur either at baseline (26 of 35) or during CHF (56 of 70). Among them, 68 of 82 premature ventricular contractions were preceded by LABDA and 14 of 82 were preceded by HASDA. The coupling intervals between premature ventricular contraction and the preceding sinus beat was significantly shorter during CHF (277 ± 43 ms) than at baseline (309 ± 76 ms, $p = 0.0073$).

Effects of CHF on integrated SGNA and VNA. Figures 6A and 6B show that the SGNA during CHF (7.8 ± 4.9 mV) was significantly ($p < 0.0001$) higher than at baseline (5.9 ± 2.9 mV). The VNA during CHF (3.5 ± 1.6 mV) was also significantly ($p < 0.0001$) higher than at baseline (2.8 ± 1.1 mV). We also found that average heart rate decreased from 102 ± 9 beats/min at baseline to 93 ± 12 beats/min after the induction of CHF due to increased PSP episodes. Cosinor tests showed significant ($p < 0.001$) circadian variation of SGNA (but not VNA) at baseline and during CHF.

Changes associated with the induction of CHF. Left ventricular ejection fraction was 57 ± 4% at baseline and 26 ± 7% during CHF ($p < 0.0001$). The peripheral blood norepinephrine levels were 76 ± 33 pg/ml at baseline, 265 ± 200 pg/ml at the end of rapid pacing, and 48 ± 23 pg/ml at CHF day 14 ($p < 0.01$). The NT-proBNP levels were between <180 to 273 pmol/l at baseline, 558 to >3,000 pmol/l at CHF day 1, and 405.5 ± 167.0 pmol/l (243 to 687 pmol/l) at CHF day 14 ($p < 0.05$).

Histological studies. Trichrome staining showed a significant increase in interstitial fibrosis around the sinus nodal artery (Fig. 7A) and in the right atrial free wall (Fig. 7B) in all dogs. The percentage of fibrosis in the right atrium was 10.57 ± 2.89% ($n = 6$), which was approximately 10-fold greater than the percentage of fibrosis reported in normal dogs by

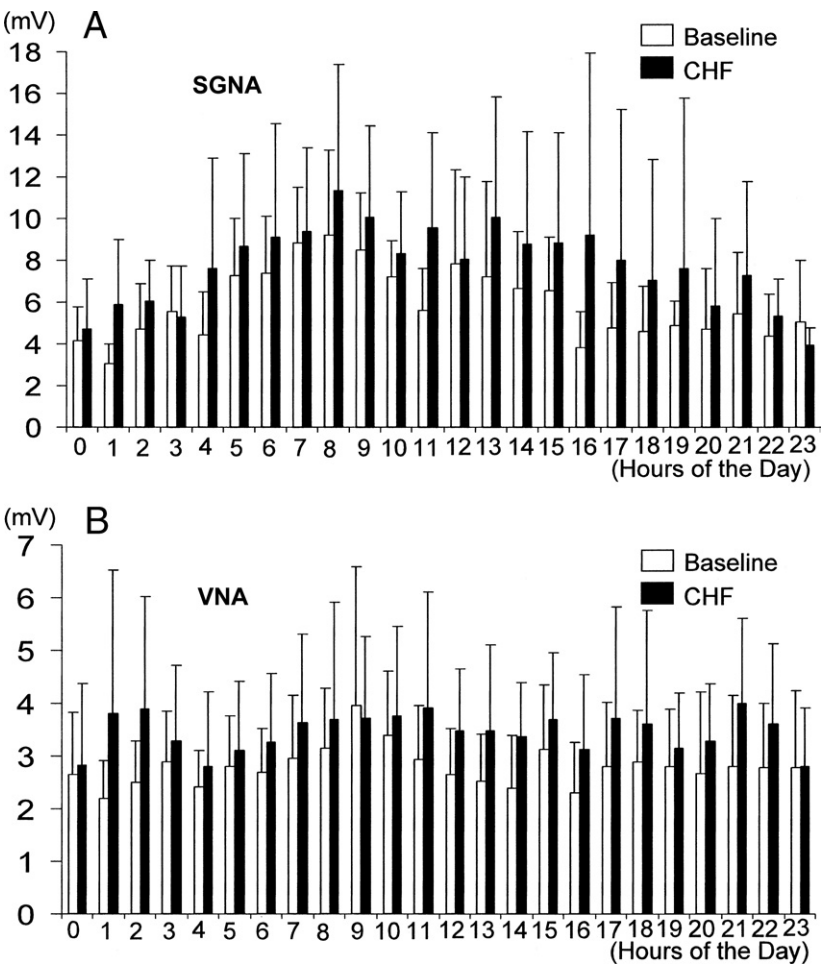

Figure 6 Heart Failure Increases Autonomic Nerve Activity

This figure shows 24-h distributions of integrated SGNA and VNA at baseline and after cessation of rapid pacing in all dogs. Abbreviations as in Figure 1.

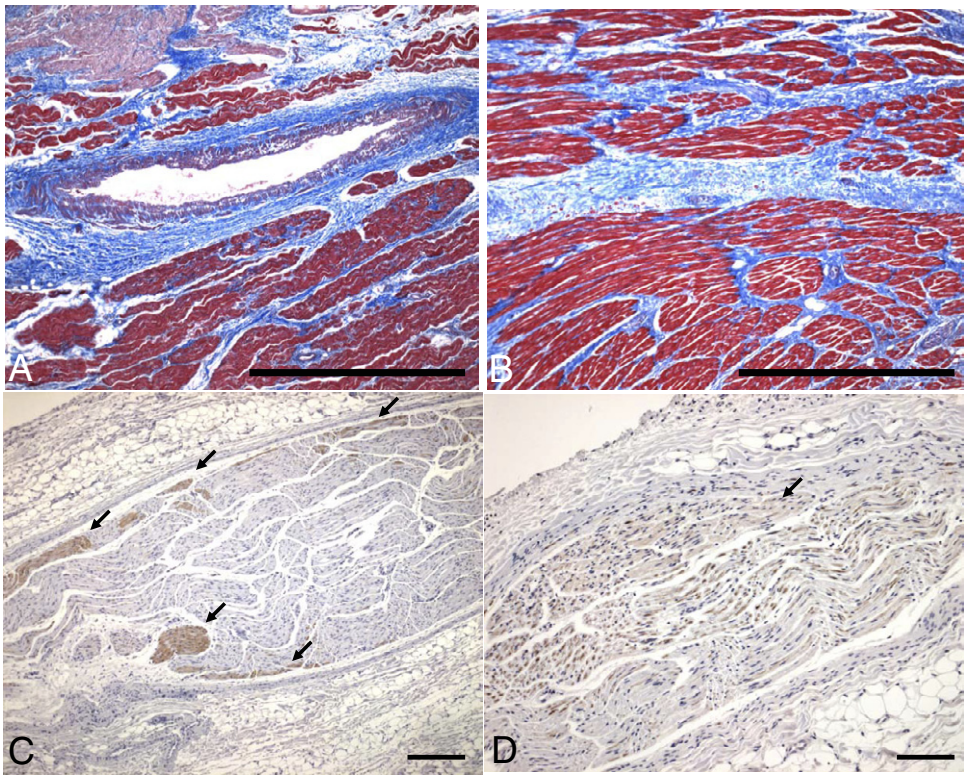

Figure 7 Histological Studies

(A and B) Masson's trichrome stain of sinus node and right atrium, respectively. There was increased fibrosis **(blue)** in these tissues. The objective was 4×, and the calibration bar is 1 mm. **(C and D)** TH and ChAT staining, respectively, of the vagal nerves. **Arrows** point to positively stained structures in brown color. The objective was 10×, and the calibration bar is 0.1 mm.

Miyauchi et al (6). Immunohistochemical staining of the vagal nerve showed coexistence of TH-positive nerves (Fig. 7C) and ChAT-positive nerves (Fig. 7D).

Discussion

Consequences of sympathovagal discharges. We found that simultaneous sympathovagal discharge followed by abrupt vagal withdrawal is a specific ANS activation pattern that preceded the onset of long lasting PAT episodes. Inoue and Zipes (7) showed that that simultaneous sympathetic and vagal stimulation on the right atrial refractoriness was not only additive but rather synergistic. Sharifov et al. (8) showed that acetylcholine-mediated atrial fibrillation was facilitated by simultaneous infusion of isoproterenol. These findings suggest that sympathovagal discharge might shorten action potential duration, which might promote both re-entrant excitation and the development of late phase 3 early afterdepolarizations induced by diastolic elevation of intracellular calcium (9,10).

SGNA and sick sinus (tachybrady) syndrome. Sinus node dysfunction might occur in CHF owing to structural and ion channel remodeling (11,12) and is a major cause of morbidity and mortality in patients with CHF (13). We found in this study that PSP episodes occurred either spontaneously (without elevated ANS activity) or followed a short burst of SGNA and short episode of tachycardia. Because most episodes occurred at night, the nocturnal reduction of sympathetic tone might have induced relatively increased vagal tone, causing bradycardia. A second mode of onset is that increased SGNA might have induced tachycardia, which resulted in overdrive suppression of the sinus node that was not able to recover after SGNA withdrawal.

Absence of circadian variation of vagal nerve discharges. On the basis of the results of heart rate variability analyses (14), there is a circadian variation of sympathovagal balance in humans. Although we documented a significant circadian variation of sympathetic discharges (2), we found no evidence of nocturnal dominance of vagal discharges in this study. These data suggest that reduced sympathetic discharge at night has changed the sympathovagal balance, resulting in a relative predominance of vagal tone on heart rate variability analyses without an absolute increase of vagal discharges.

Study limitations. Because the Data Sciences International transmitters have only 3 recording channels, we did not record the right VNA in this study. It is possible that the right VNA has significantly different patterns of activation than the left VNA. Therefore, it remains unclear whether arrhythmias and

PSP in this model occurred without ANS activity or were preceded by ANS activity in the right vagal or sympathetic nerves. The results of immunohistochemical studies showed that the left vagus nerve, at the level studied, contained both TH-positive and ChAT-positive nerve fibers. Therefore, even though we have recorded the vagal nerve, we cannot attribute all VNA exclusively to parasympathetic discharges. Finally, a single channel ECG does not allow the most accurate determination of the P-wave morphology. In addition, we did not perform any mapping studies during the arrhythmia. The mechanisms of these PATs remain unclear.

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Reprint requests and correspondence: Dr. Peng-Sheng Chen, Krannert Institute of Cardiology, 1801 North Capitol Avenue, E475, Indianapolis, Indiana 46202. E-mail: chenpp@iupui.edu.

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