Recruitment pattern of sympathetic muscle neurons during premature ventricular contractions in heart failure patients and controls

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A premature ventricular contraction (PVC) produces a prolonged diastolic period, diminished stroke volume and transient decrease of diastolic blood pressure (DBP) that represents an acute physiological stress resulting in baroreceptor-mediated changes in muscle sympathetic nerve activity (MSNA) (34). During programmed stimulation-induced PVCs in healthy humans, significant modifications in neural discharge were presented as an increase in the amplitude and duration of the sympathetic burst immediately after the PVC (post-PVC burst) (9).

The size of a postganglionic sympathetic burst reflects the number of active axons firing in that multiunit recording (22, 26), as well as the size of the action potentials (APs). Recent work from our laboratories indicates the presence of a latent subpopulation of postganglionic sympathetic axons that generate large APs, are present during larger bursts, reflect faster conduction velocities, and are recruited with higher probability during severe chemoreflex (30) or baroreflex stress (26). The expression of such a subpopulation of latent, but large, APs during post-PVC sympathetic bursts remains to be established.

High sympathetic nervous system outflow accompanies chronic heart failure (CHF) and tends to increase with disease severity. This heightened sympathetic drive is recognized as an important prognostic factor in these patients (1, 10). In addition, the prevalence of PVC increases in CHF patients (9, 34) and a higher rate of PVCs can contribute to a state of elevated sympathetic discharge both in peripheral tissues (MSNA) and within the heart (29). PVCs are also encountered periodically in healthy people with a prevalence that increases with age.

Under pathologic conditions of high sympathetic activity, such as CHF, a chronic increase in AP firing frequency and/or recruitment of postganglionic sympathetic neurons may already be achieved at baseline. The very high levels of sympathetic discharge in CHF have led to the question of whether or not levels can be increased further during severe stress and if so, is the increase in sympathetic burst integral due to recruitment of already active axons and/or of additional axons.

Single-unit recordings from the multiunit bursts of MSNA (4, 18, 19) demonstrate increased firing frequency and firing probability of individual sympathetic fibers in CHF patients during sinus rhythm and also an increased incidence of multiple firings of already active fibers within post-PVC bursts in CHF (19), supporting the view that increased firing of already active axons can contribute to post-PVC burst enhancement in CHF. However, the ability to recruit latent subpopulations of MSNA may be limited in CHF. The heightened prevalence of post-PVC sympathetic bursts in CHF provided an opportunity to examine whether CHF limits sympathetic reserve and reduces the ability to increase MSNA discharge through recruitment of new and larger APs.

Therefore, the purpose of this study was to test the hypothesis that CHF patients display sympathetic reserve through recruitment of an additional, subpopulation of larger axons by studying the AP peak-to-peak amplitude and discharge patterns during post-PVC bursts.

METHODS

Subjects. Data were obtained from six CHF patients and from six non-heart failure control subjects whose anthropometric and clinical characteristics are listed in Table 1. These subjects were selected on the basis of their PVC occurrence from a larger number of CHF patients and on the basis of age and sex-matched controls that were recruited for a larger study. All subjects gave written informed consent to participate in the study that was conducted in accordance with the Declaration of Helsinki and was approved by research ethics board at The University of Split, School of Medicine. CHF patients were recruited from the Department of Cardiology, University Hos-
Table 1. Anthropometric and clinical data for subjects

<table>
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<tr>
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<th>CHF Patients (n = 6)</th>
<th>Controls (n × 6)</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>62 ± 11</td>
<td>59 ± 5</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>4/2</td>
<td>3/3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174 ± 0.1</td>
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<tr>
<td>Weight, kg</td>
<td>83 ± 16</td>
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<tr>
<td>BMI</td>
<td>27 ± 2</td>
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<tr>
<td>LVEF, %</td>
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<td>LVDD, mm</td>
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<td></td>
<td>III: 1</td>
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<tr>
<td></td>
<td>MSNA</td>
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<tr>
<td>Burst frequency, burst/min</td>
<td>55* 30</td>
<td></td>
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<tr>
<td>Burst incidence, burst/100 heart beats</td>
<td>83* 43</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. *P < 0.05 different from controls.

CHF, congestive heart failure; BMI, body mass index; LVEF, left ventricular ejection fraction; LVDD, left ventricular diastolic diameter; NYHA, New York Heart Association; ACEi, angiotensin-converting enzyme inhibitor; MSNA, muscle sympathetic nerve activity.

Patients were asked to continue their normal medication protocol on the morning of the study. Subjects arrived at the laboratory in the morning hours, 2 h postprandial, having abstained from caffeine, or other stimulants for 12 h. While supine, they were given 10 min of quiet rest after instrumentation to allow stabilization of hemodynamic parameters. Data were collected during a 10–15-min period of rest and quiet breathing.

Integrated bursts of MSNA were identified as exhibiting pulse synchrony, having a signal-to-noise ratio (SNR) of least 2:1 with respect to a previous period of neural silence and having characteristic rising and falling slopes. Burst occurrence was confirmed by visually inspecting the corresponding raw neurogram. Integrated activity was expressed as burst frequency (number of bursts per minute) and burst incidence (number of bursts per 100 heartbeats). Burst size was expressed as burst integral (amplitude × duration/2) and normalized to the largest burst in sinus rhythm. APs were detected and extracted from the filtered raw MSNA signal using techniques reported previously (2, 25, 26, 30). Briefly, this technique uses a continuous wavelet transform (CWT) for AP detection. The CWT uses a “mother wavelet” adapted to an average AP waveform constructed from (and hence with the same peak-to-peak amplitude as) physiological recordings of postganglionic sympathetic APs in our laboratory (25). The CWT is applied to the filtered MSNA to provide a wavelet coefficient between the signal of interest (i.e., an action potential) and the mother wavelet, such that the largest wavelet coefficient occurs in the presence of the APs, but negligible coefficients occur when applied to noise. A thresholding analysis separates the wavelet coefficients related to APs and those related to noise (13). Isolating the largest suprathreshold wavelet coefficient provides the exact location of the negative peak for each AP. Using this location, we obtained the AP waveforms from their exact location within the original filtered raw signal by putting the estimated location of APs in the center of a predefined window (3.2 ms). In this way, the amplitude and peak-to-peak amplitude of each extracted AP remain unaltered.

Action potential data were quantified in two ways. First, the number of APs in each burst (AP/burst) and their frequency (i.e., number of APs per second) were established. Second, extracted APs were then ordered based on peak-to-peak amplitude and grouped into clusters based on similar peak-to-peak amplitude. Cluster bin widths are automatically defined based on Scott’s Rule, which balances bin width, data bias, and variance to minimize the integrated mean square error (28). In the text, the clusters are identified sequentially on the basis of the relative size within a given individual for the data set being interrogated. With this approach, the number of total clusters (i.e., groups of APs with similar peak-to-peak amplitudes) varies by subject. Not all clusters are present in every integrated burst. Therefore, the term “active cluster” relates to those clusters present for a defined condition, such as sinus rhythm bursts or a post-PVC burst in the current context.

The amplitude of the negative peak of the mean action potential over the standard deviation of the background noise (i.e., during sympathetic silence) defines the SNR for a data segment. To enhance AP detection, data were selected when SNR was ≥3 (25). The average raw MSNA SNR for CHF patients was 3.74 ± 0.2, and for controls, it was 4.39 ± 0.5. According to previous analysis (25), we expect that the level of SNR ~3.74 would produce a 91 ± 11% correct detection rate for AP and a false-positive detection rate of 3%.

Because of the frequency of PVCs in some patients and to provide similar levels of data from each individual, APs were analyzed for maximum periods of six heartbeats before a PVC, during which time at least two sinus rhythm bursts were present. In total, 164 sinus rhythm bursts and 35 post-PVC bursts were selected in the 6 CHF patients, as well as 121 sinus rhythm bursts and 35 post-PVC bursts from the control group. Even though CHF patients exhibited a higher number of PVC than controls, a similar number of PVCs was selected in both groups to enable statistical comparison of MSNA and action potential (AP) parameters. After PVCs were visually detected, corresponding BP and integrated MSNA data were analyzed, and AP information was quantified as outlined above.
**Statistical analysis.** Results are expressed as means ± SD, and level of significance is set at \( P < 0.05 \). The effects of burst type (sinus rhythm vs. post-PVC) and group (CHF group vs. control group) were assessed with a factorial ANOVA. Differences between groups in the terms of the increase of AP parameters and in hemodynamic parameters from sinus rhythm to PVC were assessed using Student’s two-tailed, unpaired *t*-test. Distribution analysis was used to assess the proportionate shift in peak-to-peak amplitude of APs between the sinus rhythm and post-PVC bursts in both groups.

**RESULTS**

**Integral MSNA burst analysis.** As shown in Fig. 1, spontaneous PVCs in CHF patients and in control subjects were characterized by prolonged diastole and large post-PVC bursts. Compared with controls, and considering only the sinus rhythm bursts, the CHF group had higher integrated MSNA burst frequency (30 ± 6 vs. 55 ± 6 bursts/min; \( P < 0.05 \)) and higher burst incidence (43 ± 7 vs. 83 ± 7 bursts/100 heart beats; \( P < 0.05 \)). Compared with bursts observed during sinus rhythm, the post-PVC burst integral was greater than bursts that occurred during sinus rhythm by ∼365% in CHF patients, \( (P < 0.05) \) and ∼632% in controls \( (P < 0.05) \). A group (CHF vs. control) by burst type (sinus rhythm vs. post-PVC) interaction was observed for burst integral; \( F(1,140) = 11.28, P < 0.05 \).

**AP analysis.** Figure 2 illustrates the occurrence of postganglionic sympathetic APs for each AP cluster as a function of burst size between sinus rhythm bursts and the post-PVC burst. This visual representation illustrates the presence of small- and medium-sized APs in sinus rhythm bursts but the higher probability of a large AP subpopulation during the post-PVC burst.

Figures 3–5 illustrate the mean AP data. Main effects of group \( (P < 0.001) \) and burst type \( (P < 0.001) \) were observed for AP/burst (Fig. 3). In a point-wise contrast, the CHF group (17 ± 8 APs/burst) had greater AP content per burst during sinus rhythm bursts than controls (9 ± 3 APs/burst; \( P < 0.05 \); Fig. 3). Also, the sinus rhythm burst AP frequency was greater...
in CHF (14 ± 8 AP/s) compared with controls (7 ± 2 AP/s; Fig. 3; P < 0.05). This contrast formed the basis of an interaction effect between group and burst type for AP frequency $F(1,140) = 7.642; P = 0.01$. During sinus rhythm bursts, the number of active clusters per burst was greater in CHF patients (5 ± 1 clusters/burst) compared with controls (3 ± 1 clusters/burst; $P = <0.05$; Fig. 3).

The increase in AP/burst between sinus rhythm and post-PVC bursts was less in CHF patients (10 ± 8 AP/burst) than in controls (15 ± 7 AP/burst $P < 0.01$) (Fig. 4). Similarly, the change in AP frequency between sinus rhythm and post-PVC bursts was less in CHF (4 ± 6 AP/s) compared with the control group (10 ± 5 AP/s, $P = 0.01$) (Fig. 5). The increase in number of active AP clusters on going from sinus rhythm to post-PVC bursts was the same between CHF and control (Fig. 3).

Figure 5 represents the distributions of sinus rhythm bursts and post-PVC bursts in terms of their unique AP clusters (based on their peak-to-peak amplitude). Both groups exhibited a rightward shift in the distribution toward larger APs in the post-PVC burst compared with sinus rhythm.

**DISCUSSION**

The main findings of the present study are as follows: 1) in sinus rhythm, CHF patients had higher multiunit MSNA outflow in terms of burst frequency and burst incidence, more active clusters of sympathetic neurons, a greater number of APs per burst, as well as higher AP firing frequency than non-heart failure controls; 2) AP content increased within post-PVC bursts in both groups, but to a greater extent in controls vs. CHF; and 3) similar increases in active clusters with the post-PVC burst were observed in both groups. These results indicate that moderate CHF patients exhibit some sympathetic reserve with retention of the ability to increase MSNA through recruitment of additional neurons.

**Integrated burst analysis (multiunit MSNA).** Heightened baseline sympathetic drive represents a hallmark of chronic heart failure (6, 14, 32). Although the central mechanisms determin-
ing sympathoexcitation in CHF are not known, they probably reflect the net balance and interaction between augmented excitatory and diminished inhibitory influences (6). The lower diastolic blood pressure during sinus rhythm in CHF group may contribute to the greater overall SNA in CHF patients through the baroreflex pathway. Given the critical role of the baroreflex in sympathetic inhibition, the long pause and decay in DBP that follows a PVC should result in increased sympathetic burst amplitude, duration, and area (7). As reported earlier (9), augmentation of sympathetic burst size and duration occurs in response to a PVC in CHF patients. However, the change in burst size was lower in CHF group compared with controls in the current study, largely due to smaller overall AP content, not the ability to recruit larger APs. Yet, the post-PVC fall in DBP was the same in CHF and control participants. Thus, these CHF patients demonstrated an attenuated ability to reflexively increase sympathetic outflow. Although this difference cannot be explained mechanistically in the current study, one might investigate the possibility that heightened arterial stiffness in CHF (23) impairs the baroreflex sensitivity to a fall in DBP.

AP analysis. Several mechanisms have been described to explain short-term sympathoexcitatory responses in humans (18, 21). These include an increase in the frequency of sympathetic neurons that are already active producing a higher firing probability across bursts, repeated firing of the same neuron within the same burst, and the recruitment of additional, previously silent neurons (2, 3, 30). Previously, Macefield and colleagues (3, 17, 19, 20) demonstrated the increased firing probability of particular single neurons in CHF patients, both across and within bursts. These investigators used a single-unit recording method to quantify sympathetic outflow, a method
that assesses the firing frequency of a single sympathetic fiber over time. Although this single-unit approach suggests whether or not a neuron becomes more or less active, it cannot address the question about latent populations of sympathetic neurons that express a low firing probability and/or become active in response to physiological stress, such as the PVC. The approach used in the current study, while unable to track the firing patterns of single axons, emphasizes the patterns of activity in all APs comprising the burst, with information regarding the size of each AP and the appearance of new and larger APs on a burst-by-burst basis. The potential problem of complete overlap and summation of concurrent spikes has been established in this approach (25), being <1% and apparent summed APs are deleted. Thus, the method offers the opportunity to observe the presence of different sizes of APs and their firing probabilities. Large axons have larger APs, and this principle has enabled the observations in healthy individuals, that larger bursts often contain larger APs that are not present in smaller bursts and the increased probability of their recruitment during severe chemoreflex (30) or baroreflex (26) stress.

During sinus rhythm, the CHF group had higher total AP frequency compared with controls. Higher AP frequency in CHF patients was caused by higher burst incidence and also by a greater number of APs within a single burst. Moreover, sinus rhythm bursts of MSNA in CHF patients contained a greater number of AP clusters. Therefore, both a greater frequency of integrated bursts and an increased AP content within each burst, contribute to the heightened baseline sympathetic outflow in CHF. Previous observations that the high-probability APs in sinus rhythm bursts of healthy individuals are related strongly to a baroreflex mechanism (27) supports the conjecture outlined above that lower diastolic blood pressure during the sinus rhythm may contribute to the larger number of APs per burst and active clusters in CHF patients than in controls. Inasmuch as the number of APs determine the size of an integrated burst and different central mechanisms are proposed to affect burst frequency (gating) vs. size (15), these data indicate that CHF produces aberrations in both features of control, such that more efferent APs/burst and bursts/min are emitted.

**Sinus rhythm-related bursts vs. post-PVC burst.** Compared with sinus rhythm sympathetic bursts, the post-PVC bursts expressed greater total AP content but more in control than CHF patients. Some of the additional APs in the post-PVC burst will be repeated firings of the same neuron (3). However, the post-PVC bursts from both groups were also marked by the emergence of larger AP clusters that were not observed frequently during sinus rhythm bursts. Thus, neurons that were normally latent during sinus rhythm bursts were recruited during the post-PVC burst. Importantly, inspection of Fig. 4 indicates that CHF had a larger effect on the ability to increase APs/burst but not AP clusters. These data indicate that the ability to recruit previously latent neurons was not affected by CHF. Rather, and by inference, the reduced ability to increase the integrated size and AP content of a post-PVC burst might be due to an attenuated ability to enhance further the repeated firing of already active neurons.

**Hemodynamic analysis: diastolic blood pressure fall and augmentation of sympathetic activity.** CHF impairs arterial baroreflex regulation of heart rate, but not MSNA burst frequency (5, 7). Also, a normal increase in total body norepinephrine spillover was observed in CHF patients during sodium nitroprusside infusion (6, 12). The fall in DBP following a PVC provides an analogous baroreflex hemodynamic stress indicated by the lack of post-PVC burst in one patient in whom DBP did not fall following the PVC (data not shown). However, the additional analysis of burst size and AP content provides information not apparent when the frequency of bursts in the integrated MSNA signal forms the sole basis of baroreflex sensitivity. Certainly, a burst occurs during a post-PVC period if DBP falls. But, the size of the burst represents the number of APs contributing to the integrated signal (22). In this regard, the current data expose an element of potential baroreflex dysregulation of MSNA control in CHF. CHF patients started with higher AP parameters during sinus rhythm bursts and, thereby, a ceiling effect may be expressed that limits the overall increase in total sympathetic outflow for the same fall in DBP.

**Conclusion.** The increases in AP frequency and active clusters during a post-PVC sympathetic burst indicate that moderate CHF patients have the capacity to increase sympathetic discharge through recruitment of an additional subpopulation of neurons during transient, acute baroreflex unloading. However, the increase in AP frequency was smaller in CHF vs. control individuals, suggesting that CHF limits the ability to increase firing of already active fibers.

**Limitations of the study.** The CHF patients retained their standard pharmacological treatment during the study, and this
practice may interfere with MSNA. We choose this strategy to avoid rebound cardiovascular responses and associated baro-receptor-mediated effects on sympathetic nerve traffic and also to maintain comparative consistency with previous work in this population (19, 31, 34). Second, microneurography measures peripheral sympathetic outflow and may not reflect cardiac sympathetic drive. However, cardiac norepinephrine spillover correlates with muscle sympathetic nerve traffic and increases in patients with major ventricular arrhythmias (8).

**Perspectives and Significance**

The clinical significance of the sympathetic AP recruitment patterns in CHF lies in the correlation between MSNA and cardiac norepinephrine spillover (33) and the mechanistic basis of sudden death after periods of frequent ectopic beats (24). These data indicate that examination of AP discharge patterns may provide a complementary view at aberrant sympathetic activation in disease states. The chronic elevation in sympathetic outflow may be arrhythmogenic, decreasing the threshold for ventricular fibrillation (16, 29). The current observations that CHF produces aberrations in both integrated burst frequency and in AP recruitment within each burst suggest that central control of these two discharge features are modified in CHF. Establishment of the brain and brain stem regions that determine the differential control of burst frequency and AP recruitment may contribute to the formation of strategies that modulate chronic sympathoexcitatory states.

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AUTHOR CONTRIBUTIONS


DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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Author contributions: P.Z.M., T.B., D.N.B., J.S., and Z.D. approved final version of manuscript; P.Z.M., D.N.B., J.S., and Z.D. edited and revised manuscript; P.Z.M., T.B., D.N.B., J.S., and Z.D. approved final version of manuscript.

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