Heart failure with reduced ejection fraction (HFrEF) is characterized by increases in sympathetic outflow directed at the heart, kidneys, and skeletal muscle. 1–6 Mechanisms responsible for such activation have yet to be elucidated fully.

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The afferent autonomic disturbance now considered principally responsible for eliciting increased efferent sympathetic discharge to skeletal muscle in human HFrEF is the loss of its inhibition by cardiopulmonary reflexes arising from stretch-sensitive mechanoreceptors sited in ventricles, atria, and the pulmonary veins.1,7,8 The difficulty with this parsimonious interpretation, however, is that it discounts several lines of evidence signaling the emergence of a paradoxical cardiopulmonary sympathetic excitatory reflex elicited by one of heart failure’s fundamental hemodynamic perturbations, an elevation in cardiac filling pressure. Individuals with HFrEF exhibit a positive, rather than an inverse correlation between mean pulmonary artery pressure and either cardiac norepinephrine spillover 5 or muscle sympathetic nerve activity (MSNA). 4 Nonhypotensive lower body negative pressure (LBNP; -10 mm Hg) and nonhypertensive positive pressure (LBPP; +10 mm Hg) in 11 treated HFrEF (left ventricular ejection fraction 25±6% [mean±standard deviation]) patients and 14 similarly aged controls. Single-unit muscle sympathetic nerve activity discharge was termed either anticipated, if firing frequency exhibited classic negative-feedback responses, or paradoxical. LBNP and LBPP had no heart rate, stroke volume, or blood pressure effects (P>0.05). Estimated central venous pressure decreased with LBNP (P<0.05), increased with LBPP (P<0.05), and was consistently higher in HFrEF (P<0.05). During LBNP, the ratio of single units with anticipated and paradoxical discharge was similar in HFrEF (18:7) and controls (27:5), whereas LBPP elicited paradoxical reflex excitation in a greater proportion of HFrEF single units (7:18 versus 24:6; P=0.0001). Consequently, LBPP increased mean single-unit firing frequency (P<0.05) and did not inhibit multiunit muscle sympathetic nerve activity of HFrEF subjects (P<0.05 versus controls). Firing of 12/18 HFrEF (but no control) single units increased during both LBPP and LBNP.

Conclusion—These findings provide the first evidence in human HFrEF for an augmented excitatory cardiopulmonary–muscle sympathetic nerve activity reflex response to increased preload, incorporating 2 distinct single-unit populations with differing firing properties. (Circulation. 2015;131:459-468. DOI: 10.1161/CIRCULATIONAHA.114.010765.)

Key Words: central venous pressure ▪ heart failure ▪ mechanoreceptors ▪ sympathetic nervous system

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From the University Health Network and Mount Sinai Hospital Division of Cardiology, Department of Medicine, University of Toronto, Toronto, Ontario, Canada.
*Drs Millar and Murai contributed equally.
Correspondence to John S. Floras, MD, DPhil, University Health Network and Mount Sinai Hospital Division of Cardiology, Department of Medicine, University of Toronto, Suite 1614, 600 University Ave, Toronto, Ontario, Canada, M5G 1X5; E-mail john.floras@utoronto.ca
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fibers exhibiting opposite firing characteristics in response to acute changes in filling pressure without simultaneous effects on systemic blood pressure, stroke volume, or peripheral resistance, proving this supposition incorrect. Of 21 single units identified, 16 exhibited classical anticipated sympathoinhibitory responses to elevations in central venous pressure induced by nonhypertensive lower body positive pressure (LBPP), whereas 5 responded with paradoxical increases in firing. The behavior of each single unit identified was consistently anticipated or paradoxical when the opposite stimulus of nonhypotensive LBNP lowered filling pressure. The implication of this finding is that the conventional use of multunit preparations to study neurogenic circulatory regulation may obscure the detection of concurrent excitatory cardiopulmonary-reflex modulation of MSNA if present in HFrEF. Yet to be determined is whether the relative proportion of single units exhibiting paradoxical excitatory responses to nonhypotensive LBPP is augmented in HFrEF.

The purpose of the present investigation was to compare cardiopulmonary reflex control of peripheral sympathetic outflow in HFrEF patients and healthy controls of similar age by studying single- and multunit MSNA responses to nonhypotensive LBNP and nonhypertensive LBPP. Our primary hypothesis was that HFrEF patients would demonstrate a greater proportion of single units that respond to LBPP (ie, increased filling pressure) with increased firing rates, resulting in a less-than-anticipated attenuation of multunit MSNA by this stimulus. If confirmed experimentally, this would represent the first direct microneurographic evidence for an augmented excitatory cardiopulmonary-MSNA reflex in human HFrEF.

Methods

Study Subjects

Eleven patients with diagnosed and treated HFrEF (left ventricular ejection fraction 25±5% [mean±standard deviation]; 10 men; 53±11 years) and 14 healthy control subjects (11 men; 56±7 years) participated in this study. Excluded were patients with moderate or severe mitral regurgitation, Canadian Cardiovascular Society class III or IV angina, New York Heart Association class IV dyspnea, implanted cardiac resynchronization devices, autonomic neuropathy, diabetes mellitus, chronic kidney disease, body mass index >30 kg/m², atrial fibrillation, and frequent premature ventricular contractions (>5% of total beats). Control subjects (data from 8 published as proof-of-concept) were screened to ensure the absence of medication known to affect cardiovascular function. The Research Ethics Boards of the University Healthy Network and the Mount Sinai Hospital approved this protocol. Informed written consent was obtained from all participants.

General Procedures

Heart rate was acquired continuously from lead II of the ECG. Blood pressure was recorded continuously from a right hand digit (Portapres, Finapres Medical Systems B.V., The Netherlands) and at timed intervals by using an upper left arm cuff (Dinamap Pro 100, Critikon, Tampa, FL). Respiratory movement was tracked by a pneumobelt connected to a pressure transducer. Central venous pressure was estimated (eCVP) in 9 control subjects and 6 HFrEF patients from a polyethylene catheter inserted in a suitable right antecubital vein. Echocardiography (Vivid 7, GE Healthcare, Pittsburgh, PA) was used to calculate left ventricular ejection fraction (Teichholz method) and stroke volume, permitting the determination of cardiac output and total peripheral resistance.

Postganglionic single- and multunit MSNA was recorded simultaneously from the right fibular nerve with the use of previously reported methods. A high-impedance (10 mΩ) tungsten microelectrode (UNP35G0S; Frederick Haer, Brunswick, ME) was inserted percutaneously into a motor fascicle and then adjusted until spontaneous pulse-synchronous multibursts of sympathetic activity were observed, and large unitary spike discharges could be easily separated from the background noise in the raw nerve recording.

Experimental Protocol

All studies were completed during a single-morning experimental session in a quiet, light- and temperature-controlled room following 12- to 24-hour abstinence from alcohol and caffeine. In HFrEF patients, diuretics (if prescribed) were withheld on the study morning; otherwise, all other medications were taken at usual times.

Subjects lay supine within a custom-built lower body tank sealed at the level of the iliac crest. The tank was constructed with a removable side panel to access the right fibular nerve and a gauge to monitor the gradual adjustment, either positive or negative, of its internal pressure.

After a 15-minute rest interval, heart rate, blood pressure, MSNA, and eCVP were recorded over a 7-minute baseline, and echocardiographic images were acquired. Next, LBPP was applied incrementally over at least 30 seconds, maintained at –10 mm Hg for 7 minutes to reduce selectively eCVP (Figure 1, top), then gradually reversed. After the eCVP and blood pressure reequilibrated, values were recorded over a second 7-minute baseline. Next, LBPP was applied incrementally over at least 30 seconds, maintained at +10 mm Hg for 7 minutes to increase selectively eCVP (Figure 1, bottom), then gradually withdrawn.

Echocardiographic assessment of transmural flow and stroke volume was performed before and during the last 3 minutes of both –10 mm Hg LBPP and +10 mm Hg LBPP. In 5 HFrEF patients and 2 control subjects, this sequence of LBNP and LBPP application was reversed.

Data Acquisition and Analysis

Continuously acquired data were digitized and stored simultaneously by both LabView (National Instruments, Austin TX) and Spike2 (ver.5, Cambridge Electronics Design, Cambridge, UK). Signal output to LabView was sampled at either 1000 Hz (ECG) or 200 Hz (all other signals with the exception of single-unit MSNA). Simultaneous output to Spike2 was sampled at either 12 000 Hz (single-unit MSNA) or 1000 Hz (all other signals).

Multunit MSNA was calculated as burst frequency (bursts/min) and burst incidence (bursts/100 heartbeats). Up to 3 single units were detected in each subject. In brief, candidate single units were selected by isolating large identifiable unitary spikes in the raw neurogram within a distinct discharge amplitude range. Confirmation that these action potentials originated from a single fiber were made by established criteria: spike synchronization with a multibiust burst; triphasic spike morphology with the main phase being negative; and superimposition of candidate action potentials with minimal variation. Single-unit MSNA was calculated as spike frequency (spikes/min) and spike incidence (spikes/100 heartbeats). Single-unit responses were identified as anticipated, if spike frequency increased with LBNP or decreased with LBPP, and paradoxical, if such single-unit discharge decreased with LBNP or increased with LBPP.

Statistical Analysis

Values are presented as mean±standard deviation. Baseline characteristics of subjects with and without HFrEF were compared by using unpaired t tests. The primary hypothesis was tested by comparing the proportion of expected versus observed single-unit MSNA responses in HFrEF and control subjects by using a 2 × 2 contingency table and Fisher exact test. Prior identification, in healthy individuals, of single units within the multibiust preparation exhibiting qualitatively...
Figure 1. **Top**, Representative tracing from 1 patient with heart failure acquired before and during nonhypertensive lower body negative pressure (LBNP; −10 mm Hg). **A**, Typical recording of single- and multiunit MSNA, arterial and estimated central venous pressure (eCVP), and electrocardiogram. Unit 1 is paradoxical, whereas units 2 and 3 exhibit anticipated responses to LBNP. **B**, Identified single units superimposed. **Bottom**, Representative tracing from 1 patient with heart failure (same as **Top**) acquired before and during nonhypertensive lower body positive pressure (LBPP; +10 mm Hg). **A**, Typical recording of single- and multiunit MSNA, arterial and estimated central venous pressure (eCVP), and electrocardiogram. All units exhibit paradoxical responses to LBPP. **B**, Identified single units superimposed. A.U. indicates arbitrary units; and MSNA, muscle sympathetic nerve activity.
Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Control (n=14)</th>
<th>HF (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>56±7</td>
<td>53±11</td>
</tr>
<tr>
<td>Male/female</td>
<td>11/3</td>
<td>10/1</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>66±8</td>
<td>25±6†</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>59±10</td>
<td>71±13</td>
</tr>
<tr>
<td>Estimated central venous pressure, mm Hg</td>
<td>3.1±2.7</td>
<td>5.8±2.6*</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>126±11</td>
<td>108±11†</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>72±9</td>
<td>69±7</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>90±9</td>
<td>82±8*</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>32±16</td>
<td>55±20†</td>
</tr>
<tr>
<td>MSNA, bursts/100 heart cycles</td>
<td>54±24</td>
<td>73±18†</td>
</tr>
</tbody>
</table>

Therapy

- β-blockade: 10
- ACE inhibitor: 9
- Angiotensin-receptor blocker: 1
- Calcium-channel blocker: 1
- Vasodilator: 3
- Statin: 7
- Loop diuretic: 7
- Mineralocorticoid receptor antagonist: 7
- Digoxin: 1

Upper panel values presented as mean±SD. ACE indicates angiotensin-converting enzyme; HF, heart failure; MSNA, muscle sympathetic nerve activity; and SD, standard deviation.

*P≤0.05; †P≤0.01 vs healthy controls.

different discharge properties in response to the same stimulus indicates the independence of single-unit behavior within the same subject. Nonetheless, to account for the possibility of the clustering of anticipated or paradoxical firing responses within an individual, a generalized estimating equation regression model was also used to estimate the relative increase in the proportion of fibers exhibiting paradoxical firing between the heart failure and healthy control groups. The log-binomial generalized estimating equation model used an exchangeable correlation structure.

Secondary hypotheses concerning within-group multiunit and single-unit responses to each independent intervention (LBPP) were evaluated by Wilcoxon signed rank tests. Echocardiographic data were analyzed within each group (HF vs control) for condition (baseline, LBNP, LBPP) by using a Friedman repeated-measures analysis of variance on ranks. Differences between groups were tested using Mann-Whitney rank sum tests. Multi- and single-unit MSNA responses to LBNP or LBPP in heart failure and control subjects were compared by using a 2-way repeated-measures analysis of variance to determine main group (HF vs control) and condition (baseline, LBNP, LBPP) by using a Tukey or Bonferroni post hoc test. Any significant interaction effects were tested with post hoc tests were applied to establish pairwise differences for any significant interaction effects. All data were analyzed with the use of SigmaPlot for Windows (version 10.0; Systat Software Inc, Richmond, CA). An α-level of ≤0.05 was considered statistically significant.

Table 2. Effects of Lower Body Negative and Positive Pressure on Hemodynamic Variables Estimated by Doppler Echocardiography

<table>
<thead>
<tr>
<th>Hemodynamic variable</th>
<th>Baseline</th>
<th>LBNP</th>
<th>LBPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>60±10</td>
<td>60±10</td>
<td>59±10</td>
</tr>
<tr>
<td>Central venous pressure, mm Hg</td>
<td>3.1±2.7</td>
<td>1.4±2.9†</td>
<td>3.0±2.7</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>125±11</td>
<td>126±11</td>
<td>126±11</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73±10</td>
<td>72±10</td>
<td>73±9</td>
</tr>
</tbody>
</table>

Values presented as mean±SD. HB indicates heartbeats; LBNP, lower body negative pressure; LBPP, lower body positive pressure; MSNA, muscle sympathetic nerve activity; and SD, standard deviation.

Table 3. Effects of Lower Body Negative Pressure and Positive Pressure on Hemodynamics and Muscle Sympathetic Nerve Activity in Healthy Controls

<table>
<thead>
<tr>
<th>Hemodynamic variable</th>
<th>Baseline</th>
<th>LBNP</th>
<th>Baseline</th>
<th>LBPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>60±10</td>
<td>60±10</td>
<td>59±10</td>
<td>60±8</td>
</tr>
<tr>
<td>Central venous pressure, mm Hg</td>
<td>3.1±2.7</td>
<td>1.4±2.9†</td>
<td>3.0±2.7</td>
<td>4.6±2.8†</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>125±11</td>
<td>126±11</td>
<td>126±11</td>
<td>127±9</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73±10</td>
<td>72±10</td>
<td>73±9</td>
<td>74±9</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>90±10</td>
<td>90±10</td>
<td>91±9</td>
<td>92±8</td>
</tr>
</tbody>
</table>

Units with anticipated responses

- Number of fibers: 27
- Number of subjects: 14
- Spike frequency, spikes/min: 28±18 47±22‡ 31±18 24±17‡
- Spike incidence, spikes/100 hb: 48±35 78±39‡ 52±34 40±27‡

Units with paradoxical responses

- Number of fibers: 5
- Number of subjects: 5
- Spike frequency, spikes/min: 22±12 19±12 20±12 28±15* 
- Spike incidence, spikes/100 hb: 36±18 30±20 32±19 46±26* 

Values presented as mean±SD. HB indicates heartbeats; LBNP, lower body negative pressure; LBPP, lower body positive pressure; MSNA, muscle sympathetic nerve activity; and SD, standard deviation.

*P≤0.05; †P≤0.01; ‡P≤0.001 compared with baseline.
Table 4. Effects of Lower Body Negative Pressure and Positive Pressure on Hemodynamics and Muscle Sympathetic Nerve Activity in Patients With Heart Failure

<table>
<thead>
<tr>
<th>Hemodynamic variable</th>
<th>Baseline</th>
<th>LBNP</th>
<th>Baseline</th>
<th>LBPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>71±18</td>
<td>72±12</td>
<td>67±12</td>
<td>69±14</td>
</tr>
<tr>
<td>Central venous pressure, mm Hg</td>
<td>6.5±1.9</td>
<td>4.3±1.3*</td>
<td>6.4±1.9</td>
<td>8.9±1.3*</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>110±11</td>
<td>109±12</td>
<td>109±11</td>
<td>110±12</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>67±6</td>
<td>67±6</td>
<td>67±5</td>
<td>67±6</td>
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<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>82±8</td>
<td>81±8</td>
<td>81±7</td>
<td>81±7</td>
</tr>
</tbody>
</table>

**Multiunit MSNA**

| Burst frequency, bursts/min | 55±20 | 59±17† | 54±20§ | 57±22§ |
| Burst incidence, % | 73±18 | 79±17* | 72±18 | 76±20§ |

**Single-unit MSNA**

| Number of fibers | 25 | 25 | 25 | 25 |
| Spike frequency, spikes/min | 35±15 | 39±23 | 34±13 | 44±24*§ |
| Spike incidence, spikes/100 hb | 52±28 | 58±35 | 52±25 | 62±34*§ |

**Units with anticipated responses**

| Number of fibers | 18 | 18 | 7 | 7 |
| Number of subjects | 9 | 9 | 3 | 3 |
| Spike frequency, spikes/min | 35±16 | 48±21‡ | 32±19 | 21±23* |
| Spike incidence, % | 54±30 | 70±32‡ | 54±36 | 36±41* |

**Units with paradoxical responses**

| Number of fibers | 7 | 7 | 18 | 18 |
| Number of subjects | 3 | 3 | 8 | 8 |
| Spike frequency, spikes/min | 35±14 | 17±9* | 35±10 | 53±18‡ |
| Spike incidence, % | 49±21 | 24±13* | 51±19 | 74±24‡ |

Values presented as mean±SD. hb indicates heartbeats; LBNP, lower body negative pressure; LBPP, lower body positive pressure; MSNA, muscle sympathetic nerve activity; and SD, standard deviation.

*P<0.05; †P<0.01; ‡P<0.001 compared with baseline.

§P<0.05 compared with controls at the same time point.

Results

In comparison with healthy controls of similar age, under baseline conditions, HFrEF patients had higher eCVPs (P<0.05) and multiunit MSNA burst frequency (P<0.01) and incidence (P<0.05), and lower systolic (P<0.01) and mean arterial pressure (P<0.05; Table 1).

In both groups, –10 mm Hg LBNP lowered eCVP and +10 mm Hg LBPP increased eCVP without affecting blood pressure, heart rate, stroke volume, cardiac output, or total peripheral resistance (Tables 2 through 4). The relative change in eCVP between controls and HFrEF was similar during LBNP (P=0.77) and LBPP (P=0.17). The interrogation of transmural flow during LBPP did not detect any change in the E relative to the A wave, or any new or worsening mitral regurgitation.

In healthy control subjects, nonhypotensive LBPP, as expected, increased multiunit MSNA burst frequency (P<0.001) and incidence (P<0.001; Table 3). Thirty-two single units were identified. Overall, LBNP increased spike frequency (P<0.001) and incidence (P<0.001; Table 3). Of these single units, 27 or 84% exhibited an anticipated increase in spike frequency (P<0.001) and incidence (P<0.001). Conversely, 5 or 16% of the single units discharged paradoxically, each with a decrease in spike frequency and incidence (both P=0.063).

Nonhypertensive LBPP, as expected, decreased multiunit MSNA burst frequency (P<0.05) and incidence (P=0.064; Table 3). Thirty single units were identified. Overall, LBPP decreased spike frequency (P<0.05) and incidence (P<0.05; Table 3). Of these single units, 24 or 80% exhibited an anticipated decrease in spike frequency (P<0.001) and incidence (P<0.001). Conversely, 6 or 20% of the single units discharged paradoxically increasing spike frequency (P<0.05) and incidence (P<0.05). Of these units displaying paradoxical discharge, 5 fired also paradoxically when LBPP was applied. With respect to the sixth unit, we could not confirm with certainty corresponding firing data for LBPP, because the effect of LBPP displaced the microelectrode, which then was repositioned to obtain a new baseline and LBPP response.

As in the control group, in HFrEF subjects, nonhypotensive LBPP, as expected, increased multiunit MSNA burst frequency (P<0.05) and incidence (P<0.05; Table 4). Twenty-five single units were identified. Overall, and in contrast to the significant increases in control subjects, LBNP did not change spike frequency (P=0.16) or incidence (P=0.29; Table 4). The magnitude of spike frequency and incidence responses in the HFrEF population were less than in the control group (both P<0.05). Of these single units, 18 or 72% exhibited an anticipated increase in spike frequency (P<0.001) and incidence (P<0.001). Conversely, 7 or 28% of the units discharged paradoxically with a decrease in spike frequency (P<0.05) and incidence (P<0.05). The proportion of single units demonstrating anticipated excitatory and paradoxical inhibitory responses to nonhypotensive LBPP was similar between the HFrEF cohort and controls (P>0.05; Figure 2).

In contrast to the reduction in multiunit MSNA documented in controls, in HFrEF subjects, nonhypertensive LBPP did not change multiunit MSNA burst frequency (P=0.28) or incidence (P=0.24; Table 4). Consequently, there was a between-cohort difference in these responses (both P<0.05), a finding observed also for total integrated multiunit MSNA activity. Twenty-five single units were identified. Overall, LBPP increased mean spike frequency (P<0.05) and incidence (P<0.05; Table 4). By contrast, in the control group, mean spike frequency and incidence decreased significantly (Table 3). As a result, there was a between-cohort difference in these responses (both P<0.05) to this stimulus. Of these single units, 7 or 28% exhibited an anticipated decrease in spike frequency (P<0.05) and incidence (P<0.05). Conversely, 18 or 72% of the units discharged paradoxically, resulting in an increase in spike frequency (P<0.001) and incidence (P<0.001). The proportion of single units demonstrating a paradoxical firing increase in response to LBPP was
greater in the HFrEF cohort than in control subjects (and, by default, the proportion demonstrating an anticipated decrease in firing in response to LBPP less in the HFrEF cohort than in control subjects; \( P=0.0001 \), using Fisher exact test; Figure 2). The generalized estimating equation analysis yielded concordant findings. With LBNP, there was a nonstatistically significant relative increase of paradoxical firing in heart failure of 1.7 (95% confidence interval, 0.50–5.7; \( P=0.40 \)). With LBPP, the relative increase was larger and statistically significant at a value of 3.67 (95% confidence interval, 1.9–7.1; \( P<0.0001 \)). Of these paradoxical discharging single units, 1 was detected during LBNP alone, 6 during both LBNP and LBPP, and 12 during LBPP alone (ie, they discharged appropriately during LBNP; Figure 3).

**Discussion**

The present experiments add to our current understanding of afferent neural mechanisms contributing to the increase in efferent muscle sympathetic nerve traffic documented in HFrEF in several novel and important respects.\(^1\) The principal finding was that, in HFrEF patients, compared with healthy subjects of a similar age, a significantly greater proportion of single units within the multiunit MSNA preparation discharged paradoxically in response to the acute stimulation of cardiopulmonary mechanoreceptors by LBPP. Second, as a consequence, the net multiunit response to nonhypotensive LBPP differed significantly between control subjects, who exhibited a significant decrease in MSNA, as anticipated, and HFrEF subjects, who did not. In 6 of these 11 HFrEF subjects, multiunit MSNA increased during LBPP, indicating a paradoxical cardiopulmonary reflex sympathetic excitatory response to an acute increase in filling pressure. Third, this proportionate population difference with respect to single-unit discharge properties was specific to the stimulus of nonhypertensive LBPP. When nonhypotensive LBNP was applied to unload cardiopulmonary mechanoreceptors, the proportion of anticipated and paradoxical single units contributing to the net MSNA response was similar in these 2 cohorts. Finally, and intriguingly, these experiments provide the first evidence, to our knowledge, for the existence in human HFrEF of a
single-unit population exhibiting U-shaped discharge characteristics in response to selective changes in filling pressure elicited by these 2 interventions (Figure 3). In HFpEF subjects, 12 or 48% of the single units identified exhibited paradoxical firing only in response to LBPP; their discharge in response to nonhypotensive LBNP was appropriate. Integration within the mean voltage neurogram of single units exhibiting both appropriate inhibition and paradoxical excitation would account for the attenuated gain of the multiunit MSNA cardiopulmonary reflex response to LBPP and the previously documented loss of reflexive multiunit sympathoexcitation during LBNP. As well, such summation illuminates why elevations in cardiac norepinephrine spillover in mild to moderate HFpEF are not accompanied by parallel increases in multiunit MSNA.

With the weight of evidence arguing for preserved arterial baroreflex regulation of MSNA in human HFpEF, attention has focused on the loss of its cardiopulmonary reflex inhibition as the principal afferent abnormality arising in this condition. Cardiopulmonary mechanoreceptors can be stimulated or unloaded selectively if lower body pressure is applied or reduced gently at low levels (±5–10 mm Hg) that modify venous return without engaging the arterial baroreflex by altering simultaneously systemic blood pressure, cardiac output, or stroke volume (as confirmed in Table 2). Before the introduction of contemporary HFpEF drug therapy, Dunlap et al reported marked attenuation of the multiunit MSNA response to nonhypotensive LBNP in HFpEF patients relative to healthy control subjects. This finding, which assumes that, within the multiunit envelope, all postganglionic sympathetic neurons respond uniformly to an acute change in filling pressure, was interpreted as indicating impaired cardiopulmonary reflex sympathoinhibition. The present findings provide the first definitive evidence that the attenuated gain of cardiopulmonary reflex regulation of multiunit MSNA documented in HFpEF results in part from the summation of reflex discharge from single units exhibiting directionally opposite responses to the identical mechanical stimulus.

The term cardiopulmonary receptor refers to a diverse population of afferent nerve endings with respect to anatomic distribution and neural response initiated. The classic or anticipated cardiopulmonary reflex response (ie, reflex sympathoinhibition elicited by increased cardiac filling pressure, governed primarily by stimulation of unmyelinated vagal afferents located mainly in the left ventricle) is presumed to normally predominate. However, cardiac myelinated vagal afferents located primarily at venoatrial junctions elicit paradoxical reflex cardiac and peripheral sympathetic excitation when stimulated by similar mechanical stretch. Discharge of such cardiac myelinated vagal afferents is augmented if intravascular or atrial volumes increase, as in HFpEF. Cardiac unmyelinated and myelinated sympathetic afferents found primarily within the left ventricle also respond to both mechanical stretch and chemical stimuli by eliciting sympathoexcitation.

Twelve or 48% of the single units recorded from HFpEF subjects displayed U-shaped firing characteristics (see the

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**Figure 4.** Conceptual schematic illustrating the emergence of paradoxical sympathetic reflex activation in human heart failure with reduced ejection fraction (HFpEF). In healthy control subjects (Normal) with normal atrial pressure (P atrial), an increase in P atrial within the normal range (↑) elicits a reflex (−) inhibition of firing (1 single-unit spike) in 80% of efferent single units identified, but a reflex (+) increase in spike frequency (3 single-unit spikes) in 20% of identified single units. The integrated multiunit (MSNA) consequence is sympathoinhibition. In HFpEF with elevated P atrial (↑↑), a further increase in P atrial (↑↑) stimulates a population of normally quiescent sympathoexcitatory (+) mechanoreceptors (thicker line), whereas the gain of the sympathoinhibitory cardiopulmonary reflex (−) is impaired (thinner line). In the present series, lower body positive pressure elicited reflex inhibition of efferent spikes (1 single-unit spike) in only 28% of single units identified; in the remaining 72%, spike frequency increased (3 single-unit spikes). The mean integrated multiunit (MSNA) response differed significantly from control subjects as a consequence of the loss of sympathoinhibition in some HFpEF subjects, net sympathoexcitation. MSNA indicates muscle sympathetic nerve activity.
anticipated unit 2 and 3 with LBNP in Figure 1 [top] and paradoxical unit 2 and 3 with LBPP in Figure 1 [bottom] from the same HFrEF subject). Although this observation was not anticipated, it is known that low-threshold C-tactile skin mechanoreceptors respond in a similar U-shaped discharge pattern to brush stroking.30 If these 12 U-firing single units were extracted from the aggregate data, the ratio of anticipated to paradoxical responses to LBPP would be similar between HFrEF patients and controls. Further investigation is required to determine whether this novel discharge pattern represents the functional emergence of a specific population of cardiopulmonary mechanoreceptors with a higher pressure operating point and unique firing characteristics, or results (in the HFrEF cohort only) perhaps from stimulation of extrathoracic mechanoreceptors31-33 by the rostral volume shift induced by LBPP.

Our novel finding of increased single-unit spike frequency and incidence in response to nonhypertensive LBNP in HFrEF provides direct microneurographic evidence for the existence of a sympathoexcitatory reflex activated preferentially by an increase in cardiac filling pressure (Figure 4) and provides fresh insight into the resolution of several hitherto difficult to explain observations in human HFrEF: forearm vasoconstriction with acute volume expansion34; paradoxical positive correlations between filling pressures and efferent sympathetic activity, plasma norepinephrine, or multiunit MSNA3-5; and acute forearm vasodilation35 and a reduction in cardiac norepinephrine spillover36 with nonhypotensive LBNP.

Although the functional significance of this sympathoexcitatory cardiopulmonary reflex on vasoconstrictor tone and limb blood flow in individuals with HFrEF requires further investigation, from the clinical perspective, recognition of the predominance of paradoxical single units suggests gradual normalization of cardiac filling pressure as 1 means of restoring autonomic balance and improving the clinical course of such patients. Stimulation of such single units by high filling pressure could provoke sympathetically mediated reductions in venous capacitance, a potential mechanism for acute decompensation.36 Activation of this reflex during dynamic exercise, when central venous pressure is increased, may contribute to reflex neurogenic vasoconstriction; multiunit MSNA recorded during 1-legged cycling relates inversely with maximal exercise capacity.37 Single units discharging paradoxically in response to LBPP were detected in only 8 of the 11 HFrEF patients. Whether the presence or absence of such units reflects between-subject differences with respect to atrial hemodynamics, mechanics, or histology; the etiology of heart failure; responsiveness to specific therapies; or prognosis are hypotheses for future consideration.

We acknowledge several limitations. Our HFrEF population was not, on average, volume overloaded, and received optimal medical therapy, including β-adrenoceptor antagonism. This class has no chronic effect on multiunit MSNA in HFrEF,38 but the majority of subjects were receiving also angiotensin-converting enzyme inhibition that has been shown in HFrEF to lower multiunit burst incidence and to improve the sensitivity of the cardiopulmonary reflex multiunit MSNA responses to LBNP.8 Consequently, the present findings may underestimate the magnitude of differences characteristic of the untreated HFrEF state. Because of patient and recruitment considerations, our independent variable was eCVP, not pulmonary capillary wedge pressure. However, in a previous nonhypotensive LBNP study from our laboratory involving similar HFrEF patients also without significant mitral regurgitation, changes in right atrial pressure correlated tightly with simultaneously measured pulmonary capillary wedge pressure.10 Owing to our relatively small sample size, single- and multiunit MSNA responses were analyzed conservatively by using nonparametric statistics. We did not determine the specific anatomic location of mechanoreceptors eliciting these paradoxical responses, but presume these to be myelinated vagal afferents situated at the venoatrial junctions22 stretched by changes in local pressure or volume. Our experimental intervention has been applied extensively as a stimulus selective to the cardiopulmonary baroreflex (notably more so in younger subjects than in the present middle-aged healthy volunteers less prone to changes in stroke volume or blood pressure).8-10,12,17 However, the possibility that changes in venous pressure also stimulated or unloaded sympathoexcitatory mechanoreceptors located in the peripheral venous circulation11,37 or (as suggested by data from a few healthy subjects exposed to substantially higher positive pressure) the abdomen cannot be excluded.35 Regardless, the present observations during LBPP demonstrate the existence in HFrEF of a unique venous volume or pressure-dependent sympathetic excitatory reflex. Finally, subtle movement in the microelectrode position between interventions could have led to recording from an anticipated single unit during LBNP and a paradoxical single unit during LBPP. Although we cannot discount this possibility, we consider it unlikely for 2 reasons. First, during all recordings, we monitored the raw neurogram both audibly and visually. If baseline voltage changed, both interventions were repeated. Second, the U-shaped firing was identified only in HFrEF patients (6 of 11), but not in any of the 14 control subjects. Because only a few fibers can be identified with this method within a single session, we are unable to ascertain precisely the general prevalence of each single-unit population in either HFrEF patients or in control subjects of similar age and sex. However, the present data should provide confidence that the true proportion of single units responding paradoxically in response to increased preload is indeed greater in the population with heart failure.

Conclusions
In HFrEF, in comparison with healthy control subjects, acute increases in central venous pressure induced by nonhypertensive LBPP cause a greater proportion of 2 distinct populations of efferent muscle sympathetic vasoconstrictor single units to paradoxically increase firing frequency. A discrete population, evident only in HFrEF patients, exhibits U-shaped firing properties, with discharge intensifying in response to both increases and decreases in filling pressure. Paradoxical sympathetic activation in response to increasing filling pressure may contribute to the autonomic disturbances of heart failure.
with reduced ejection fraction and provides a mechanism to explain previous findings of impaired cardiopulmonary control of peripheral sympathetic activity.1,7,8 Because increased MSNA is associated with premature mortality,9 this first demonstration in human HFrEF of an augmented sympathoexcitatory cardiac-skeletal muscle vasoconstrictor reflex represents a novel mechanism for sympathoexcitation and provides a potential target for therapy.

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Disclosures

None.

References


CLINICAL PERSPECTIVE

Increases in sympathetic outflow directed at the heart, kidneys, and skeletal muscle characteristic of heart failure with reduced ejection fraction (HFrEF) compromise the function of these organs and ultimately foreshorten survival. The afferent autonomic derangement assumed principally responsible for such excitation is the loss of reflex sympathetic inhibition by cardiopulmonary baroreceptors. However, there is evidence for the emergence in HFrEF of a parallel paradoxical excitatory cardiopulmonary reflex, elicited by increases in filling pressure. Thus far, conclusions concerning reflex regulation of muscle sympathetic nerve activity in HFrEF have been derived from multiunit recordings, assuming that all postganglionic single units within the multiunit envelope discharge concordantly. Using single-unit recordings, we identified subpopulations of efferent postganglionic fibers exhibiting opposite firing characteristics in response to acute changes in central venous pressure. In HFrEF patients, in contrast to healthy matched controls, increases in preload caused the majority of efferent muscle sympathetic vasoconstrictor single units to paradoxically increase, rather than decrease, their firing frequency. Consequently, multiunit muscle sympathetic nerve activity was not inhibited reflexively (P<0.05 versus controls). The emergence in human HFrEF of an augmented excitatory cardiopulmonary-skeletal muscle vasoconstrictor reflex response to increased preload represents a novel mechanism to account for limb vasoconstriction and exercise intolerance observed with volume expansion and for the improved clinical course that often follows decongestion and hemodynamic optimization. Concurrent engagement of renal sympathetic nerves by this reflex would amplify sodium retention. We hypothesize that paradoxical muscle sympathetic reflex activation in response to increased atrial pressure is a neurogenic disturbance common to both HFrEF and heart failure with a preserved ejection fraction.