Paradoxical Muscle Sympathetic Reflex Activation in Human Heart Failure

Running title: Millar et al.; Paradoxical sympathoexcitation in heart failure

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Abstract

**Background**—Muscle sympathetic activation in heart failure with reduced ejection fraction (HFrEF) has been attributed, on the basis of multi-unit recordings, to attenuated inhibitory feedback from stretch-sensitive cardiopulmonary mechanoreceptors. However, such preparations integrate two populations of single-units exhibiting directionally opposite firing when atrial pressure is perturbed. We tested the hypothesis that the proportion of single-units firing paradoxically when filling pressure increases is augmented in HFrEF.

**Methods and Results**—Muscle sympathetic nerve activity (MSNA) and estimated central venous pressure (eCVP) were recorded during non-hypotensive lower body negative pressure (LBNP; -10mmHg) and non-hypertensive positive pressure (LBPP; +10mmHg) in 11 treated HFrEF (LVEF 25±6% [mean±SD]) patients and 14 similarly-aged controls. Single-unit MSNA 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 ($P\textgreater0.05$), eCVP 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 vs. 24:6; $P=0.0001$). Consequently, LBPP increased mean single-unit firing frequency ($P<0.05$) and did not inhibit multi-unit MSNA of HFrEF subjects ($P<0.05$ versus controls). Firing of 12/18 HFrEF (but no control) single-units increased during both LBPP and LBNP.

**Conclusions**—These findings provide the first evidence in human HFrEF for an augmented excitatory cardiopulmonary-MSNA reflex response to increased preload, incorporating two distinct single-unit populations with differing firing properties.

**Key words:** heart failure, sympathetic nerve activity, cardiopulmonary receptors, microneurography, central venous pressure
Introduction

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

The afferent autonomic disturbance now considered principally responsible for eliciting increased efferent sympathetic discharge to skeletal muscle in human HFrEF is loss of its inhibition by cardiopulmonary reflexes arising from stretch-sensitive mechanoreceptors sited in ventricles, atria, and the pulmonary veins.\textsuperscript{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 (NE) spillover\textsuperscript{5} or muscle sympathetic nerve activity (MSNA).\textsuperscript{4} Non-hypotensive lower body negative pressure (LBNP), a stimulus which reduces selectively cardiac filling pressure and in healthy subjects increases MSNA reflexively,\textsuperscript{9} causes a paradoxical reduction in cardiac NE spillover in HFrEF.\textsuperscript{10} In experimental HFrEF renal sympathetic activity also increases in response to atrial distension.\textsuperscript{11}

Importantly, the interpretation of attenuated cardiopulmonary reflex responsiveness in human HFrEF is predicated on the assumption that within the conventionally measured multi-unit MSNA envelope all postganglionic sympathetic single-units discharge concordantly. However, in recent experiments, single-unit MSNA recordings identified, in 5 of 8 healthy middle-aged men, two sub-populations of efferent postganglionic 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 non-hypertensive 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 non-hypotensive LBNP lowered filling pressure. The implication of this finding is that the conventional use of multi-unit 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 non-hypertensive 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 multi-unit MSNA responses to non-hypotensive LBNP and non-hypertensive LBPP. Our primary hypothesis was that HFrEF patients would demonstrate a greater proportion of single-units that respond to LBPP (i.e. increased filling pressure) with increased firing rates, resulting in a lesser than ‘anticipated’ attenuation of multi-unit 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 (LV ejection fraction 25±6% [mean±SD]) (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/or frequent premature ventricular contractions (>5% of total beats). Control subjects (data from 8 published as proof-of-concept12) 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 electrocardiogram (ECG). Blood pressure was recorded continuously from a right hand digit (Portapres, Finapres Medical Systems B.V., The Netherlands) and at timed intervals using an upper-left arm cuff (Dinamap Pro 100, Critikon, Tampa, FL, USA). Respiratory movement was tracked by a pneumobelt connected to a pressure transducer. Central venous pressure was estimated (eCVP) in 9 controls and 6 HFrEF patients from a polyethylene catheter inserted in a suitable right antecubital vein.13 Echocardiography (Vivid 7, GE Healthcare, Pittsburgh, PA, USA) was used to calculate LV ejection fraction (Teichholz method) and stroke volume14 permitting determination of cardiac output and total peripheral resistance.

Postganglionic single- and multi-unit MSNA was recorded simultaneously from the right fibular nerve using previously reported methods.12,15 A high-impedance (10mΩ) tungsten microelectrode (UNP35G0S; Frederick Haer, Brunswick, ME, USA) was inserted percutaneously into a motor fascicle and then adjusted until spontaneous pulse-synchronous
multi-fiber bursts 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 12-24 hour abstention 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, LBNP was applied incrementally over at least 30 seconds, maintained at -10 mmHg for 7 minutes to reduce selectively eCVP (Figure 1, Upper panel), then gradually reversed. After eCVP and blood pressure re-equilibrated, values were recorded over a second 7 minute baseline. Next, LBPP was applied incrementally over at least 30 seconds, maintained at +10 mmHg for 7 minutes to increase selectively eCVP (Figure 1, Lower panel), then gradually withdrawn.

Echocardiographic assessment of transmitral flow and stroke volume was performed before and during the last 3 minutes of both -10 mmHg LBNP and +10 mmHg LBPP. In 5 HFrEF and 2 control subjects this sequence of LBNP and LBPP application was reversed.

**Data Acquisition and Analysis**

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

Multi-unit MSNA was calculated as burst frequency (bursts/min) and burst incidence (bursts/100 heartbeats). Up to 3 single-units were detected in each subject. Briefly, 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 multi-unit burst; triphasic spike morphology with the main phase being negative; superimposition of candidate action potentials with minimal variation.12,15 Single-unit MSNA was calculated as spike frequency (spikes/minute) 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 using unpaired t-tests. The primary hypotheses was tested by comparing the proportion of expected versus observed single-unit MSNA responses in HFrEF and control subjects using a 2 x 2 contingency table and Fisher’s exact test. Prior identification, in healthy individuals, of single units within the multi-fiber preparation exhibiting qualitatively different discharge properties in response to the same stimulus12 indicates independence of single-unit behavior within the same subject. Nonetheless, to account for the possibility of
clustering of ‘anticipated’ or ‘paradoxical’ firing responses within an individual, a generalized estimating equation (GEE) 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 GEE model used an exchangeable correlation structure.

Secondary hypotheses concerning within-group multi-unit and single-unit responses to each independent intervention (LBNP, LBPP) were evaluated by Wilcoxon signed rank tests. Echocardiographic data were analyzed within each group (HFrEF, control) for condition (baseline, LBNP, LBPP) using a Freidman repeated measures ANOVA 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 using a two-way repeated measures ANOVA to determine main group (HFrEF, control) and condition (baseline, stimulus) effects and their interaction (group x condition). Bonferroni post-hoc tests were applied to establish pairwise differences for any significant interaction effects. All data were analyzed using SigmaPlot™ for Windows (version 10.0; Systat Software Inc, Richmond, CA). An alpha level of ≤ 0.05 was considered statistically significant.

**Results**

Compared to healthy controls of similar age, under baseline conditions HFrEF patients had higher eCVP \( (P<0.05) \) and multi-unit 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 mmHg LBNP lowered eCVP and +10 mmHg LBPP increased eCVP without affecting blood pressure, heart rate, stroke volume, cardiac output, or total peripheral resistance (Tables 2-4). The relative change in eCVP between controls and HFrEF was similar
during LBNP (P=0.77) and LBPP (P=0.17). Interrogation of trans-mitral 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, non-hypotensive LBNP, as expected, increased multi-unit 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 (P<0.05) and incidence (P=0.063).

Non-hypotensive LBPP, as expected, decreased multi-unit 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 LBNP was applied. With respect to the sixth unit, we could not confirm with certainty corresponding firing data for LBNP as 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 non-hypotensive LBNP, as expected, increased multi-unit 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 non-hypotensive LBNP was similar between the HFrEF cohort and controls (\(P>0.05\)) (Figure 2).

In contrast to the reduction in multi-unit MSNA documented in controls, in HFrEF subjects, non-hypotensive LBPP did not change multi-unit 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 multi-unit 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) (\(P=0.0001\), using Fisher’s exact test) (Figure 2). The generalized estimating equation analysis yielded concordant findings. With LBNP, there was a non-statistically significant relative increase of paradoxical firing in HF of 1.7 (95% CI 0.50-5.7;
p=0.40). With LBPP, the relative increase was larger and statistically significant at a value of 3.67 (95% CI: 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 (i.e. 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 similar age, a significantly greater proportion of single-units within the multi-unit MSNA preparation discharged ‘paradoxically’ in response to acute stimulation of cardiopulmonary mechanoreceptors by LBPP. Second, as a consequence, the net multi-unit response to non-hypertensive 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, multi-unit 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 non-hypertensive LBPP. When non-hypotensive 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 two 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 two interventions (Figure 3). In HFrEF subjects, 12 or 48% of the single-units identified exhibited ‘paradoxical’ firing only in response to LBPP; their discharge in response to non-hypotensive 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 multi-unit MSNA cardiopulmonary reflex response to LBPP and the previously documented loss of reflexive multi-unit sympathoexcitation during LBNP. As well, such summation illuminates why elevations in cardiac norepinephrine spillover in mild to moderate HFrEF are not accompanied by parallel increases in multi-unit MSNA.

With the weight of evidence arguing for preserved arterial baroreflex regulation of MSNA in human HFrEF, attention has focused on 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 mmHg) 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). Prior to the introduction of contemporary HFrEF drug therapy, Dunlap et al reported marked attenuation of the multi-unit MSNA response to non-hypotensive LBNP in HFrEF patients relative to healthy control subjects. This finding, which assumes that within the multi-unit envelope all postganglionic sympathetic neurons respond uniformly to an acute change in filling pressure, was interpreted as indicating impaired cardiopulmonary reflex sympathoinhibition.

However, in experimental preparations, efferent sympathetic nerves, such as those innervating the kidney, have been shown to incorporate sub-populations that respond discretely to different afferent stimuli; postganglionic sympathetic efferent fibers supplying the heart,
kidneys, skin, and muscle can be categorized into two distinct types based on opposite discharge patterns to reflex input. In 5 of 8 healthy middle-aged subjects, we identified two single-unit MSNA populations that responded oppositely to both non-hypotensive LBNP and non-hypertensive LBPP, raising the concern that a dissimilar proportion, in HFrEF and healthy subjects, of such single-unit populations with distinct firing properties would confound any interpretation of between-condition differences in multi-unit MSNA. The present findings provide the first definitive evidence that the attenuated gain of cardiopulmonary reflex regulation of multi-unit MSNA documented in HFrEF results in part from 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 anatomical distribution and neural response initiated. The classic or ‘anticipated’ cardiopulmonary reflex response (i.e., 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 veno-atrial 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 intra-vascular or atrial volumes increase, as in HFrEF. 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 HFrEF subjects displayed U-shaped firing characteristics (see the ‘anticipated’ unit 2 and 3 with LBNP in Figure 1 [Upper panel])
and ‘paradoxical’ unit 2 and 3 with LBPP in Figure 1 [Lower panel] 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 extra-thoracic mechanoreceptors\(^ {31-33}\) by the rostral volume shift induced by LBPP.

Our novel finding of increased single-unit spike frequency and incidence in response to non-hypertensive LBPP 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 expansion;\(^ {34}\) paradoxical positive correlations between filling pressures and efferent sympathetic activity, plasma NE or multi-unit MSNA;\(^ {3-5}\) and acute forearm vasodilation\(^ {35}\) and a reduction in cardiac NE spillover\(^ {10}\) with non-hypotensive 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 one means of restoring autonomic balance and improve 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. Activation of this reflex during dynamic exercise, when central venous pressure is increased, may contribute to reflex neurogenic vasoconstriction; multi-unit MSNA recorded during one-legged cycling relates inversely with maximal exercise capacity. 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 beta-adrenoceptor antagonism. This class has no chronic effect on multi-unit MSNA in HFrEF, but the majority of subjects were receiving also angiotensin-converting enzyme inhibition which has been shown in HFrEF to lower multi-unit burst incidence and to improve the sensitivity of the cardiopulmonary reflex multi-unit MSNA responses to LBNP. Consequently, the present findings may underestimate the magnitude of differences characteristic of the untreated HFrEF state. Due to patient and recruitment considerations, our independent variable was eCVP, not pulmonary capillary wedge pressure. However, in a previous non-hypotensive 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. Owing to our relatively small sample size, single- and multi-unit MSNA responses were analyzed conservatively, using non-parametric statistics. We did not determine the specific anatomical location of mechanoreceptors eliciting these ‘paradoxical’
responses but presume these to be myelinated vagal afferents situated at the veno-atrial junctions\(^22\) stretched by changes in local pressure or volume. Our experimental intervention has been applied extensively as a stimulus selective to the cardiopulmonary baroreflex (notably moreso in younger subjects than 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 circulation\(^31,32\) or (as suggested by data from a few healthy subjects exposed to substantially higher positive pressure) the abdomen cannot be excluded.\(^33\) 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 two 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 heart failure population.

Conclusions

In HFrEF, compared to healthy controls, acute increases in central venous pressure induced by
non-hypertensive LBPP cause a greater proportion of two 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.\textsuperscript{1,7,8} As increased MSNA is associated with premature mortality,\textsuperscript{39} 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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**Conflict of Interest Disclosures:** None.

**References:**


860.


### Table 1. Baseline characteristics

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<th>Control n=14</th>
<th>HF n=11</th>
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<td>Age, years</td>
<td>56 ± 7</td>
<td>53 ± 11</td>
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<tr>
<td>Male/Female</td>
<td>11/3</td>
<td>10/1</td>
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<tr>
<td>Left ventricular ejection fraction, %</td>
<td>66 ± 8</td>
<td>25 ± 6**</td>
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<tr>
<td>Heart rate, bpm</td>
<td>59 ± 10</td>
<td>71 ± 13</td>
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<tr>
<td>Estimated central venous pressure, mmHg</td>
<td>3.1 ± 2.7</td>
<td>5.8 ± 2.6*</td>
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<tr>
<td>Systolic blood pressure, mmHg</td>
<td>26 ± 11</td>
<td>108 ± 11**</td>
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<td>Diastolic blood pressure, mmHg</td>
<td>72 ± 9</td>
<td>69 ± 7</td>
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<td>Mean arterial pressure, mmHg</td>
<td>90 ± 9</td>
<td>82 ± 8*</td>
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<td>MSNA, bursts/min</td>
<td>32 ± 16</td>
<td>55 ± 20**</td>
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<td>MSNA, bursts/100 heart cycles</td>
<td>54 ± 24</td>
<td>73 ± 18*</td>
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**Therapy**

- β blockade
- ACE inhibitor
- Angiotensin-receptor blocker
- Calcium-channel blocker
- Vasodilator
- Statin
- Loop diuretic
- Mineralocorticoid receptor antagonist
- Digoxin

Upper panel values presented as mean ± SD. *, P≤0.05; **, P≤0.01 vs. healthy controls.

### Table 2. Effects of lower body negative and positive pressure on hemodynamic variables estimated by Doppler-echocardiography

<table>
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<th></th>
<th>Baseline</th>
<th>LBNP</th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
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<tr>
<td>Stroke volume (ml)</td>
<td>93 ± 16</td>
<td>93 ± 16</td>
<td>95 ± 18</td>
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<td>Cardiac output (L/min)</td>
<td>5.6 ± 1.5</td>
<td>5.7 ± 1.5</td>
<td>5.7 ± 1.5</td>
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<tr>
<td>Total peripheral resistance (dyn·s/cm²)</td>
<td>1392 ± 334</td>
<td>1379 ± 298</td>
<td>1398 ± 289</td>
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<tr>
<td><strong>HF</strong></td>
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<tr>
<td>Stroke volume (ml)</td>
<td>74 ± 17**</td>
<td>73 ± 15**</td>
<td>75 ± 16**</td>
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<tr>
<td>Cardiac output (L/min)</td>
<td>5.3 ± 1.6</td>
<td>5.3 ± 1.2</td>
<td>5.3 ± 1.5</td>
</tr>
<tr>
<td>Total peripheral resistance (dyn·s/cm²)</td>
<td>1352 ± 436</td>
<td>1306 ± 355</td>
<td>1342 ± 419</td>
</tr>
</tbody>
</table>

Values presented as mean ± SD. ***, P≤0.01 vs. controls. 
LBNP, lower body negative pressure; LBPP, lower body positive pressure.
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 (mmHg)</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 (mmHg)</td>
<td>125 ± 11</td>
<td>126 ± 11</td>
<td>126 ± 11</td>
<td>127 ± 9</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73 ± 10</td>
<td>72 ± 10</td>
<td>73 ± 9</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>90 ± 10</td>
<td>90 ± 10</td>
<td>91 ± 9</td>
<td>92 ± 8</td>
</tr>
</tbody>
</table>

**Multi-unit MSNA**

| Burst frequency (bursts/min)   | 32 ± 16 | 38 ± 15*** | 32 ± 16 | 30 ± 16* |
| Burst incidence (bursts/100hb) | 54 ± 23 | 63 ± 22*** | 54 ± 25 | 49 ± 23  |

**Single-unit MSNA**

<table>
<thead>
<tr>
<th>Number of fibers (n)</th>
<th>32</th>
<th>32</th>
<th>30</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike frequency (spikes/min)</td>
<td>27 ± 17</td>
<td>43 ± 23***</td>
<td>29 ± 17</td>
<td>25 ± 16*</td>
</tr>
<tr>
<td>Spike incidence (spikes/100hb)</td>
<td>46 ± 33</td>
<td>70 ± 40***</td>
<td>48 ± 32</td>
<td>41 ± 27*</td>
</tr>
</tbody>
</table>

**Units with anticipated responses**

<table>
<thead>
<tr>
<th>Number of fibers (n)</th>
<th>27</th>
<th>27</th>
<th>24</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects (n)</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Spike frequency (spikes/min)</td>
<td>28 ± 18</td>
<td>47 ± 22***</td>
<td>31 ± 18</td>
<td>24 ± 17***</td>
</tr>
<tr>
<td>Spike incidence (spikes/100hb)</td>
<td>48 ± 35</td>
<td>78 ± 39***</td>
<td>52 ± 34</td>
<td>40 ± 27***</td>
</tr>
</tbody>
</table>

**Units with paradoxical responses**

<table>
<thead>
<tr>
<th>Number of fibers (n)</th>
<th>5</th>
<th>5</th>
<th>6</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects (n)</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Spike frequency (spikes/min)</td>
<td>22 ± 12</td>
<td>19 ± 12</td>
<td>20 ± 12</td>
<td>28 ± 15*</td>
</tr>
<tr>
<td>Spike incidence (spikes/100hb)</td>
<td>36 ± 18</td>
<td>30 ± 20</td>
<td>32 ± 19</td>
<td>46 ± 26*</td>
</tr>
</tbody>
</table>

Values presented as mean ± SD. *, P<0.05; **, P<0.01; ***, P<0.001 compared to baseline. Hb, heartbeats; MSNA, muscle sympathetic nerve activity.
Table 4. Effects of lower body negative pressure and positive pressure on hemodynamics and muscle sympathetic nerve activity in heart failure patients.

<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 (mmHg)</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 (mmHg)</td>
<td>110 ± 11</td>
<td>109 ± 12</td>
<td>109 ± 11</td>
<td>110 ± 12</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>67 ± 6</td>
<td>67 ± 6</td>
<td>67 ± 5</td>
<td>67 ± 6</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>82 ± 8</td>
<td>81 ± 8</td>
<td>81 ± 7</td>
<td>81 ± 7</td>
</tr>
</tbody>
</table>

**Multi-unit MSNA**

| Burst frequency (bursts/min)             | 55 ± 20   | 59 ± 17**| 54 ± 20†  | 57 ± 22†  |
| Burst incidence (bursts/100hb)          | 73 ± 18   | 79 ± 17* | 72 ± 18   | 76 ± 20†  |

**Single-unit MSNA**

| Number of fibers (n)                     | 25        | 25       | 25        |          |
| Spike frequency (spikes/min)             | 35 ± 15   | 39 ± 23  | 34 ± 13   | 44 ± 24† |
| Spike incidence (spikes/100hb)           | 52 ± 28   | 58 ± 35  | 52 ± 25   | 62 ± 34† |

**Units with anticipated responses**

| Number of fibers (n)                     | 18        | 18       | 7         | 7         |
| Number of subjects (n)                   | 9         | 9        | 3         | 3         |
| Spike frequency (spikes/min)             | 35 ± 16   | 48 ± 21***| 32 ± 19   | 21 ± 23*  |
| Spike incidence (spikes/100hb)           | 54 ± 30   | 70 ± 32***| 54 ± 36   | 36 ± 41*  |

**Units with paradoxical responses**

| Number of fibers (n)                     | /         | /        | 18        | 18        |
| Number of subjects (n)                   | 3         | 3        | 8         | 8         |
| Spike frequency (spikes/min)             | 35 ± 14   | 17 ± 9*  | 35 ± 10   | 53 ± 18***|
| Spike incidence (spikes/100hb)           | 49 ± 21   | 24 ± 13* | 51 ± 19   | 74 ± 24***|

Values presented as mean ± SD. *, P≤0.05; **, P≤0.01; ***, P<0.001 compared to baseline. †, P<0.05 compared to controls at same time point. Hb, heartbeats; MSNA, muscle sympathetic nerve activity.
Figure Legends

Figure 1. (Upper panel). Representative tracing from one heart failure patient acquired before and during non-hypertensive lower body negative pressure (LBNP; -10 mmHg). A: typical recording of single- and multi-unit MSNA, arterial and estimated central venous pressure (eCVP), and electrocardiography. Unit 1 is ‘paradoxical’ while units 2 and 3 exhibit ‘anticipated’ responses to LBNP. B: identified single-units superimposed. (Lower panel). Representative tracing from one heart failure patient (same as upper panel) acquired before and during non-hypertensive lower body positive pressure (LBPP; +10 mmHg). A: typical recording of single- and multi-unit MSNA, arterial and estimated central venous pressure (eCVP), and electrocardiography. All units exhibit ‘paradoxical’ responses to LBPP. B: identified single-units superimposed.

Figure 2. Number of identified ‘anticipated’ and ‘paradoxical’ single-units in healthy controls and heart failure patients in response to non-hypotensive lower body negative pressure (LBNP; -10 mmHg) and non-hypertensive lower body positive pressure (LBPP; +10 mmHg) with P values for proportion of anticipated:paradoxical responses observed in heart failure patients compared to healthy controls.

Figure 3. Mean single-unit discharge characteristics of 8 fibers demonstrating a U-shaped discharge pattern acquired from 4 heart failure patients with simultaneous estimated central venous pressure measurements. Solid line represents the LBNP condition and dashed line represents the LBPP intervention.
**Figure 4.** Conceptual schematic illustrating emergence of paradoxical sympathetic reflex activation in human heart failure with reduced ejection fraction (HFrEF). In healthy control subjects ('Normal') with normal atrial pressure (P\text{atrial}), an increase in P\text{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 multi-unit (MSNA) consequence is sympatho-inhibition. In HFrEF with elevated P\text{atrial}, a further increase in P\text{atrial} (↑↑) stimulates a population of normally quiescent sympatho-excitatory (+) mechanoreceptors (thicker line), whereas the gain of the sympatho-inhibitory 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 multi-unit (MSNA) response differed significantly from control subjects, as a consequence of loss of sympatho-inhibition or in some HFrEF subjects’ net sympatho-excitation.
Figure 1
Figure 1, cont’d
Figure 2

- **Response to LBNP**
  - Healthy Control (n=27)
  - Heart Failure
  - Paradoxical (n=5)
  - Anticipated (n=18)
  - P=0.33

- **Response to LBPP**
  - Healthy Control
  - Heart Failure
  - Paradoxical (n=6)
  - Anticipated (n=24)
  - P=0.0001
Figure 4

Normal
- $P_{\text{atrial}}$ increase
- Afferent:
- $20\%$ increase
- Efferent:
- $80\%$ decrease
- MSNA decrease

HFrEF
- $P_{\text{atrial}}$ increase
- Afferent:
- $72\%$ increase
- Efferent:
- $28\%$ decrease
- MSNA
- $\leftrightarrow$ or $\uparrow$
Paradoxical Muscle Sympathetic Reflex Activation in Human Heart Failure
Philip J. Millar, Hisayoshi Murai and John S. Floras

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