Microneurographic evidence in healthy middle-aged humans for a sympathoexcitatory reflex activated by atrial pressure

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Millar PJ, Murai H, Morris BL, Floras JS. Microneurographic evidence in healthy middle-aged humans for a sympathoexcitatory reflex activated by atrial pressure. Am J Physiol Heart Circ Physiol 305: H931–H938, 2013. First published July 12, 2013; doi:10.1152/ajpheart.00375.2013.—Atrial mechanoreceptors, stimulated by increased pressure or volume, elicit in healthy humans a net sympathoexcitatory response. The co-existence of an atrial reflex eliciting muscle sympathoexcitation has been postulated but undetected by conventional multi-unit muscle sympathetic nerve activity (MSNA). We hypothesized that in response to a selective increase in atrial pressure, single-unit MSNA would reveal a subpopulation of efferent sympathetic nerves with firing patterns opposite to the integrated multi-unit MSNA envelope. Multi- and single-unit MSNA recordings were acquired in eight healthy middle-aged subjects (age, 57 ± 8 years; body mass index, 25 ± 2 kg/m²) to analyse decreases or increases in atrial pressure by nonhypotensive lower body negative pressure (LBNP; −10 mmHg) or nonhypertensive lower body positive pressure (LBPP; +10 mmHg), respectively. Single-unit MSNA firing responses were classified as anticipated if spike frequency and incidence increased with LBNP or decreased with LBPP and paradoxical if they decreased with LBNP or increased with LBPP. LBNP decreased (3.2 ± 2.8 to 1.4 ± 3.1 mmHg, P < 0.01) and LBPP increased (3.3 ± 2.7 to 4.9 ± 2.8 mmHg, P < 0.01) estimated central venous pressure without affecting stroke volume, systemic pressure, or resistance. Multi-unit MSNA increased with LBNP (31 ± 17 to 38 ± 19 bursts/min, P < 0.01) and diminished with LBPP (33 ± 15 to 28 ± 15 bursts/min, P < 0.01). Of 21 single-units identified, 76% exhibited firing responses to both LBNP and LBPP concordant with multi-unit MSNA, whereas 24% demonstrated discordant or paradoxical responses. The detection of two subpopulations of single-units within the multi-unit MSNA recording, exhibiting opposite firing characteristics, establishes the first evidence in humans for the existence of an excitatory cardiac-muscle sympathoexcitatory reflex activated by increasing atrial pressure.

IN HUMANS, THE LOW PRESSURE cardiopulmonary baroreflex, when stimulated by increases in pressure or volume, is considered inhibitory to muscle sympathetic nerve activity (MSNA) (19, 31). This conclusion is supported by experiments involving multi-unit microneurography in which mechanoreceptor afferents, unloading by nonhypotensive lower body negative pressure (LBNP; −5 to −10 mmHg) increased postganglionic MSNA (11, 13), whereas mechanoreceptor stimulation by nonhypertensive lower body positive pressure (LBPP; +10 mmHg) suppressed MSNA (16).

An assumption underlying the interpretation of such responses in humans is that all single postganglionic sympathetic units comprising the multi-unit fascicle studied fire concordantly in response to these two stimuli. However, this assumption may be incorrect. In animal preparations, subpopulations of efferent sympathetic nerves have been shown to respond discretely to specific afferent input. DiBona and colleagues (7, 8), for example, have identified subgroups of renal sympathetic nerve fibers that respond selectively to baroreceptor, chemoreceptor, peripheral thermoreceptor, and somatic receptor stimulation, resulting in functionally different responses. Other groups have demonstrated specificity of single-unit sympathetic firing in cardiac, renal, and skeletal muscle sympathetic nerves (22, 23, 36). From a detailed analysis of multi-unit recordings of 60 healthy subjects, Kienbaum and colleagues (24) concluded that different mechanisms regulate the occurrence and strength of sympathetic bursts (and hence multi-unit burst amplitude, a function of single-unit firing number) and proposed the presence of two central neural sites for modulation of MSNA by arterial baroreceptors. A potential interaction with, and contribution of, cardiopulmonary mechanoreceptor reflexes to their findings was not specifically discussed.

Cardiac mechanoreceptor afferents represent a functionally heterogeneous population. In experimental preparations with normal ventricular function, stimulation of unmyelinated vagal afferents, located primarily in the left ventricle, elicits peripheral sympathoinhibition, whereas myelinated vagal afferents located primarily at right and left veno-atrial junctions elicit a weaker but opposite sympathoexcitatory cardiac and peripheral response (14, 34). Left atrial and ventricular sympathetic afferents also can elicit reflex sympathoexcitatory responses to mechanical stimuli (29, 30, 38).

Similar heterogeneity is likely to exist also in humans. In subjects with normal cardiac function, reduction of atrial pressure by nonhypotensive LBNP elicits discordant peripheral and cardiac sympathetic responses: total body norepinephrine (NE) spillover and MSNA increase reflexively, as anticipated, but cardiac NE spillover and power spectral estimates of sympathetic heart rate modulation do not (2, 13). In heart failure patients, nonhypotensive LBNP caused a paradoxical reduction in cardiac NE spillover, a finding consistent with the concept that the elevated atrial pressure engaged a normally quiescent cardio-cardiac sympathoexcitatory reflex. The anticipated increase in total body NE spillover was blunted, relative to control subjects, as if nonhypotensive LBNP had unloaded two populations of mechanoreceptor reflexes exerting directionally opposite peripheral efferent sympathetic responses (2). We postulated that if nonhypertensive LBPP activates (and its converse, nonhypotensive LBNP, unloads) functionally distinct cardiac and pulmonary mechanoreceptor afferents, eliciting...
ing qualitatively or quantitatively discordant efferent sympathetic responses, single-unit recordings (28, 32, 33) should detect fiber subpopulations with distinctly different efferent firing patterns.

The purpose of the present experiment was to determine whether isolation of single-units within the multi-fiber MSNA preparation would permit the identification and characterization of an excitatory muscle sympathetic reflex stimulated by increasing atrial pressure in humans. We hypothesized that in response to a selective increase in atrial pressure by nonhypotensive (+10 mmHg) LBPP, single-unit microneurography recordings would reveal a subpopulation of efferent sympathetic neurons with a paradoxical (i.e., opposite to the multi-unit MSNA envelope) increase in firing. As a corollary, we hypothesized that in response to a selective reduction in atrial pressure by nonhypotensive (–10 mmHg) LBNP, the discharge frequency of this subpopulation of paradoxical single-units would decrease.

METHODS

Participants. We studied eight nonobese [body mass index, 25 ± 2 kg/m² (means ± SD)] healthy middle-aged volunteers (1 woman) in sinus rhythm (age, 57 ± 8 years) and without frequent (>5% of total) premature ventricular complexes. All were screened to ensure they were not prescribed medications with known autonomic or cardiovascular actions. The Research Ethics Boards of the University Healthy Network and the Mount Sinai Hospitals approved this protocol. All subjects provided informed written consent and in advance of their study were introduced to the laboratory environment.

Experimental protocol. After a 12- to 24-h abstention from alcohol and caffeine, a single morning experimental session was conducted in a quiet, light, and temperature-controlled room. After voiding, participants were positioned, supine, within a custom-built lower body tank, sealed at the level of the iliac crest and fitted with a pressure gauge to monitor the gradual adjustment (positive or negative) of internal pressure with the use of a modified vacuum cleaner motor. A removable side panel permitted simultaneous microneurographic recording of sympathetic traffic from the right peroneal (fibular) nerve (11, 13).

Electrocardiography (Lead II) was used to acquire beat-to-beat heart rate. Blood pressure was recorded every minute on the left arm by using an upper-arm cuff (Dinamap Pro 100; Critikon, Tampa, FL), and continuously on the right hand using a digital cuff system (Portapres; Finapres Medical Systems, The Netherlands). To ensure that all signals were obtained during spontaneous breathing, a pneumobelt was connected to a pressure transducer. A polyethylene catheter was inserted in a right antecubital vein suitable for monitoring peripheral venous pressure (forearm vein catheter) as a surrogate for estimated central venous pressure (eCVP) (1). Echocardiography (1.7–3.4 MHz probe, Vivid 7; GE Healthcare, Pittsburgh, PA) was used to calculate stroke volume from aortic mean time-velocity integrals and aortic root diameter (11, 20), enabling determination of cardiac output and calculation of total peripheral resistance.

Postganglionic MSNA was recorded from the right peroneal nerve, as described (11, 13, 33). Briefly, the common peroneal nerve was located posterior to the head of the fibular bone by palpation and surface electrical stimulation. 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 multi-unit bursts of sympathetic activity were observed. Further adjustment of the microelectrode was required until a large unitary spike discharge could be easily separated from the background noise in the raw nerve recording (Spike2, ver.5; Cambridge Electronics Design, Cambridge, UK) to permit single-unit MSNA analysis. Single- and multi-unit MSNA were recorded simultaneously from the same microelectrode.

After a 15-min rest period, heart rate, blood pressure, MSNA, and eCVP were recorded over a 7-min baseline period, after which echocardiography was performed. Upon completion of the baseline recording, LBPP was initiated to unload atrial mechanoreceptors and gradually augmented over at least 30 s until –10 mmHg was achieved. LBNP was subsequently maintained at –10 mmHg for a period of 7 min. After eCVP and blood pressure re-equilibrated, a second 7-min baseline period was recorded after which LBPP was initiated to stimulate atrial mechanoreceptors and gradually increased over at least 30 s until +10 mmHg was achieved. LBPP was maintained at +10 mmHg for a period of 7 min. Echocardiography was performed during the last 3 min of both LBNP (–10 mmHg) and LBPP (+10 mmHg). All procedures were well tolerated by all subjects.

Data analysis. Continuously acquired data were digitized and stored with LabView (National Instruments, Austin, TX) and Spike2 (ver.5; Cambridge Electronics Design). Signal output to LabView (all signals except single-unit MSNA) was acquired by a Gould Viper recorder (Gould Instrument Systems, Valley View, OH). The electrocardiogram was sampled at 1,000 Hz, and all other signals were sampled at a frequency of 200 Hz. Signal output to Spike2 (all signals) was acquired by a Micro1401 acquisition unit (ADC12; Cambridge Electronics Design) and sampled at 12,000 Hz for single-unit spike analysis; all other signals were sampled at 1,000 Hz. After conversion from analog to digital format, data were stored both on a personal computer desktop and laptop for subsequent offline analyses.

From the integrated mean voltage neurogram multi-unit MSNA burst frequency (bursts/min) and burst incidence (bursts/100 heartbeats) were determined using customized software. Morphologies of single-unit MSNA spikes in the raw neurogram were inspected carefully by an experienced investigator who isolated all action potentials discharging above a selected threshold amplitude (Spike2, ver.5; Cambridge Electronics Design). Three published criteria were applied to confirm that single-unit spikes of MSNA originated from a single fiber: 1) spike synchronization with multi-unit MSNA bursts, 2) triphasic spike morphology with the main phase being negative, and 3) superimposition of candidate action potentials with minimal variation (28, 32, 33). If present, additional single-units were isolated from the raw neurogram by adjusting the threshold and confirming, in the same way, the uniqueness of their morphologies. Figure 1 represents a typical single-unit recording with an expanded raw neurogram detailing three different single-units, classified according to spike morphology (amplitude and shape).

Single-unit MSNA was quantified in terms of spike firing frequency (spikes/min) and incidence (spikes/100 heartbeats) and the probability of multiple single-unit spike firing (percentage of heartbeats that contain ≥2 spikes among heartbeats with any spike) (33). We classified single-unit MSNA firing responses as anticipated if spike frequency and incidence decreased with LBPP or increased with LBNP and paradoxical if they increased with LBPP or decreased with LBNP.

Statistical analysis. Data presented as means ± SD. Paired t-tests were performed to compare group means for the dependent variables in the two conditions (LBPP and LBNP). All data were analyzed using Sigma Plot for Windows (version 10.0; Jandel Scientific, San Rafael, CA), and an α level of ≤0.05 was considered statistically significant.

RESULTS

All participants completed the full protocol. Mean baseline values for blood pressure, eCVP, heart rate, and cardiac output were within their normal ranges. Twenty-one single-units were identified with, at most, three single-units detected in a single subject (Tables 1 and 2). Data from a single representative individual are illustrated in Figs. 2 and 3.
LBNP. Unloading, or deactivation, of atrial mechanoreceptors by −10 mmHg LBNP reduced eCVP (3.2 ± 2.8 to 1.4 ± 3.1 mmHg, P < 0.01) without affecting heart rate, arterial blood pressure, stroke volume, cardiac output, or total peripheral resistance (all P > 0.05) (Tables 1 and 3).

LBNP increased both multi-unit MSNA burst frequency and incidence and single-unit MSNA spike frequency and incidence (all P < 0.01), whereas the probability of multiple spike firing was unchanged (P > 0.05).

Single-units with anticipated firing patterns increased spike frequency and incidence (both P < 0.01), whereas the spike frequency and incidence of paradoxically firing single-units diminished (both P < 0.05) when atrial pressure fell. The probability of multiple spike firing was unchanged in either group (P > 0.05). Reductions in eCVP with LBNP were similar in the subjects with anticipated (n = 3) and paradoxical (n = 5) single-unit responses (−2.5 ± 1.8 vs. −1.4 ± 0.5 mmHg, P = 0.57).

LBPP. Stimulation of atrial mechanoreceptors by +10 mmHg LBPP increased eCVP (3.3 ± 2.7 to 4.9 ± 2.8 mmHg, P < 0.01) without affecting heart rate, arterial blood pressure, stroke volume, cardiac output, or total peripheral resistance (all P > 0.05) (Tables 2 and 3).

LBPP decreased multi-unit MSNA burst frequency and incidence (both P < 0.01) but did not change single-unit MSNA spike frequency and incidence (both P > 0.05). The probability of multiple spike firing diminished (P < 0.05).

In response to nonhypertensive LBPP, 16 (76%) of these single-units exhibited anticipated firing properties, i.e., decreased spike frequency and incidence (both P < 0.01), whereas 5 (24%) had paradoxical single-unit firing patterns, i.e., a parallel increase in atrial pressure, spike frequency, and spike incidence (both P < 0.05). The probability of multiple spike firing was reduced in the anticipated firing fibers (P < 0.01) but unchanged in those exhibiting paradoxical firing (P > 0.05). LBPP elicited similar changes in eCVP in subjects with anticipated (n = 3) and paradoxical (n = 5) single-unit discharge (1.3 ± 0.6 vs. 1.8 ± 1.1 mmHg, P = 0.50).

All 16 units exhibiting anticipated and all five units with paradoxical single-unit firing patterns during LBNP maintained this behavior during LBPP. Paradoxical single-unit firing patterns were present in five of the eight subjects. In one subject, one of two (50%) identified fibers behaved in this manner, and in four subjects one out of three (33%) single-units discharged paradoxically.

Fig. 1. Expanded raw neurogram demonstrating the identification of 3 single-units (marked as stars; 1 discharging twice) based on spike morphology (amplitude and shape). MSNA, muscle sympathetic nerve activity; AU, arbitrary units.
Both anticipated and paradoxical single-units exhibited discharge hysteresis in response to changes in atrial pressure. Whereas the majority of single-units displayed a greater change in spike frequency from baseline when atrial pressure was lowered than raised, for paradoxically discharging single-units the opposite was observed (Tables 1 and 2). The anticipated single-unit multiple spike firing probability also differed in response to atrial loading and unloading. In addition to such hysteresis, there also may be different operating set-points or thresholds for the pressure-unit firing stimulus-response curves of these two sets of atrial-MSNA reflexes.

The experimental design presupposes that LBNP and LBPP produced sufficient changes in left atrial pressure to unload and stimulate low-pressure mechanoreceptors. Previous studies have demonstrated strong correlations between peripheral venous pressure and central venous pressure (1), and in our previous investigation of healthy middle-aged control subjects with normal ventricular systolic function, involving direct measurement using pulmonary artery catheters, nonhypotensive LBPP elicited simultaneously tightly correlated changes in right atrial and pulmonary capillary wedge pressure (2). In healthy subjects rapid intravenous infusion of saline (100–200 ml/min) exerts similar parallel effects (17). In the present experiments both interventions (LBNP and LBPP) changed estimated central venous pressure significantly.

It is recognized that in some young subjects application of LBNP in the range of 10–15 mmHg can unload the arterial baroreflex and increase sympathetic activity reflexively (13, 15), but in the present cohort of healthy middle-aged adults, lower body pressure changes of ± 10 mmHg had no effect on any measure of blood pressure, heart rate, stroke volume, or mean arterial pressure.

### Table 1. Effects of LBNP on hemodynamics and MSNA

<table>
<thead>
<tr>
<th>Hemodynamic variable</th>
<th>Baseline</th>
<th>LBNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>60 ± 12</td>
<td>61 ± 12</td>
</tr>
<tr>
<td>Pressure, mmHg</td>
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<td></td>
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<tr>
<td>Central venous</td>
<td>3.2 ± 2.8</td>
<td>1.4 ± 3.1**</td>
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<tr>
<td>Blood</td>
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<tr>
<td>Systolic</td>
<td>125 ± 7</td>
<td>126 ± 5</td>
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<td>Diastolic</td>
<td>71 ± 10</td>
<td>71 ± 10</td>
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<tr>
<td>Mean arterial</td>
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<td>89 ± 6</td>
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<tr>
<td>Multi-unit MSNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burst frequency, bursts/min</td>
<td>31 ± 17</td>
<td>38 ± 19**</td>
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<tr>
<td>Burst incidence, bursts/100 heart beats</td>
<td>54 ± 29</td>
<td>64 ± 30**</td>
</tr>
<tr>
<td>Single-unit MSNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of fibers, n</td>
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<td>21</td>
</tr>
<tr>
<td>Spike frequency, spikes/min</td>
<td>31 ± 18</td>
<td>43 ± 24**</td>
</tr>
<tr>
<td>Spike incidence, spikes/100 heart beats</td>
<td>52 ± 35</td>
<td>70 ± 41**</td>
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<tr>
<td>Probability of multiple spikes, %</td>
<td>23 ± 8</td>
<td>23 ± 9</td>
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<tr>
<td>Units with anticipated responses</td>
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</tr>
<tr>
<td>Number of fibers, n</td>
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<td>16</td>
</tr>
<tr>
<td>Spike frequency, spikes/min</td>
<td>33 ± 19</td>
<td>51 ± 21**</td>
</tr>
<tr>
<td>Spike incidence, spikes/100 heart beats</td>
<td>57 ± 38</td>
<td>83 ± 38**</td>
</tr>
<tr>
<td>Probability of multiple spikes, %</td>
<td>24 ± 9</td>
<td>24 ± 10</td>
</tr>
<tr>
<td>Units with paradoxical responses</td>
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</tr>
<tr>
<td>Number of fibers, n</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Spike frequency, spikes/min</td>
<td>22 ± 12</td>
<td>19 ± 12*</td>
</tr>
<tr>
<td>Spike incidence, spikes/100 heart beats</td>
<td>36 ± 18</td>
<td>30 ± 20*</td>
</tr>
<tr>
<td>Probability of multiple spikes, %</td>
<td>20 ± 4</td>
<td>19 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 compared with baseline; **P < 0.01 compared with baseline. MSNA, muscle sympathetic nerve activity; LBNP, lower body negative pressure.

### Table 2. Effects of LBPP on hemodynamics and MSNA

<table>
<thead>
<tr>
<th>Hemodynamic variable</th>
<th>Baseline</th>
<th>LBPP</th>
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<tr>
<td>Heart rate, beats/min</td>
<td>58 ± 9</td>
<td>60 ± 9</td>
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<tr>
<td>Pressure, mmHg</td>
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<td></td>
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<tr>
<td>Central venous</td>
<td>3.3 ± 2.7</td>
<td>4.9 ± 2.8**</td>
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<tr>
<td>Blood</td>
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<tr>
<td>Systolic</td>
<td>127 ± 7</td>
<td>128 ± 5</td>
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<tr>
<td>Diastolic</td>
<td>74 ± 7</td>
<td>74 ± 9</td>
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<tr>
<td>Mean arterial</td>
<td>92 ± 6</td>
<td>92 ± 7</td>
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<tr>
<td>Multi-unit MSNA</td>
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<td></td>
</tr>
<tr>
<td>Burst frequency, bursts/min</td>
<td>33 ± 15</td>
<td>28 ± 15**</td>
</tr>
<tr>
<td>Burst incidence, bursts/100 heart beats</td>
<td>58 ± 26</td>
<td>48 ± 26**</td>
</tr>
<tr>
<td>Single-unit MSNA</td>
<td></td>
<td></td>
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<tr>
<td>Number of fibers, n</td>
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<td>21</td>
</tr>
<tr>
<td>Spike frequency, spikes/min</td>
<td>31 ± 18</td>
<td>28 ± 17</td>
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<tr>
<td>Spike incidence, spikes/100 heart beats</td>
<td>54 ± 33</td>
<td>47 ± 28</td>
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<tr>
<td>Probability of multiple spikes, %</td>
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<td>17 ± 8*</td>
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<td>Units with anticipated responses</td>
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<td></td>
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<tr>
<td>Number of fibers, n</td>
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<td>16</td>
</tr>
<tr>
<td>Spike frequency, spikes/min</td>
<td>34 ± 19</td>
<td>27 ± 18**</td>
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<tr>
<td>Spike incidence, spikes/100 heart beats</td>
<td>59 ± 36</td>
<td>45 ± 29**</td>
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<tr>
<td>Probability of multiple spikes, %</td>
<td>26 ± 8</td>
<td>16 ± 9**</td>
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<tr>
<td>Units with paradoxical responses</td>
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<td>Number of fibers, n</td>
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<td>5</td>
</tr>
<tr>
<td>Spike frequency, spikes/min</td>
<td>22 ± 12</td>
<td>32 ± 13*</td>
</tr>
<tr>
<td>Spike incidence, spikes/100 heart beats</td>
<td>37 ± 19</td>
<td>53 ± 23*</td>
</tr>
<tr>
<td>Probability of multiple spikes, %</td>
<td>19 ± 5</td>
<td>20 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 compared with baseline; **P < 0.01 compared with baseline. MSNA, muscle sympathetic nerve activity; LBPP, lower body positive pressure.

### DISCUSSION

This is the first study to report the effects of selective decreases and increases in atrial pressure with nonhypotensive LBNP and nonhypertensive LBPP on single-unit MSNA in humans. The multi-unit mean voltage neurograms of these healthy middle-aged subjects responded classically, in that LBNP evoked sympathoexcitation and LBPP elicited sympathoinhibition. However, single-unit firing patterns did not demonstrate consistently concordant or homogenous parallel firing characteristics. Rather, single-unit recordings identified two functionally distinct subpopulations. In response to changes in atrial pressure induced by LBNP and LBPP, 76% of units exhibited anticipated firing characteristics, whereas the firing properties of 24% of the single-units identified were paradoxical. The latter responded to reductions in filling pressure with sympathoinhibition and to increases in filling pressure with sympathoexcitation. We interpret these findings as indicating that the stimulus of decreased or increased atrial pressure engages simultaneously two (or more) populations of mechanoreceptor afferents exerting different functions, resulting in the simultaneous elicitation of excitatory and inhibitory efferent muscle sympathetic nerve firing responses. In these healthy subjects the net integrated multi-unit MSNA response was driven by the behavior of the greater proportion of anticipated single-units, since paradoxically firing units were not only fewer in number but also had lower baseline spike frequency and incidence. The detection of two subpopulations of single-units within the multi-unit MSNA recording, exhibiting opposite firing characteristics, provides the first evidence in humans for the existence of an excitatory cardiac-muscle sympathetic reflex activated by increasing atrial pressure.
total peripheral resistance. It is important to appreciate, however, that arterial mechanoreceptors elicit homogenous efferent sympathoinhibitory responses; thus, even if LBPP or LBNP had engaged or unloaded arterial baroreceptors, any potential co-afferent stimulus could not explain the paradoxical single-unit responses detected.

In healthy subjects, single-unit MSNA responses to isometric handgrip exercise and the Valsalva maneuver parallel consistently simultaneously elicited increases in multi-unit MSNA (33). This absence of single-units exhibiting paradoxical firing characteristics would be anticipated, given the potent reflex sympathoexcitatory responses to the exercise pressor reflex and arterial baroreceptor unloading. In contrast, the present stimuli of slow graduated application and removal of lower body pressures was designed to selectively target low pressure cardiopulmonary mechanoreceptor afferents.

The concept of functionally specific efferent responses enveloped into a multi-fiber recording preparation has been validated by the demonstration by DiBona et al. (8) of a subpopulation of postganglionic renal sympathetic fibers that do not respond to arterial baroreflex or central chemoreflex stimulation but are activated by peripheral thermal stimulation. Other investigators have identified within postganglionic sympathetic vasoconstrictor neurons distinct subpopulations whose spike frequency respond differently to exogenous infusion of peripherally and centrally acting pressor agents (36). Kidd and colleagues (23) stimulated left atrial receptors using balloon distention and noted that the activity of five of eight renal sympathetic fibers decreased; that of three fibers was unchanged. However, balloon distension also altered heart rate and systemic arterial pressure. Common carotid occlusion evoked firing in a unit unresponsive to atrial dilatation. The concept of dual- or multi-site regulation of sympathetic nerve firing, which has been proposed to account for the differential modulation of multi-unit burst occurrence and strength observed in healthy humans (24), may apply also to the control of functionally specific postganglionic sympathetic pathways by independent afferent stimuli.

Using efferent renal sympathetic preparations, DiBona (6) has shown that specific nerve firing frequencies and discrete afferent inputs exert functionally distinct and important electrolyte, humoral, and vascular regulatory responses. In human studies, Lambert and colleagues (25) observed that a high incidence of multiple within-burst spike firing was accompanied by greater cardiac NE spillover. In much the same way, the capacity to adjust differently anticipated and paradoxical
single-unit firing patterns would provide greater flexibility when changing local metabolic requirements, such as with exercise, require rapid adjustment of neurotransmitter release. In the present experiments only 24% of the single-units studied behaved paradoxically, but all subjects exhibited normal central venous pressure and, by inference, normal atrial pressures. A local single-unit modulating capacity may assume greater functional importance in pathological states characterized by increased atrial volume or pressure or by abrupt distention of veno-atrial junctions, e.g., by the augmented reflection waves of mitral and tricuspid regurgitation or supraventricular tachycardia. Indeed, in so far as myelinated ventricular afferents demonstrate in response to volume loading greater increases in firing frequency than do unmyelinated ventricular afferents (18), if the relative proportion of paradoxically to appropriately firing single-units increases in response to chronic elevations in atrial pressure, this would represent a hitherto underappreciated stimulus to generalized sympathetic activation, with important functional and clinical consequences for a condition of volume overload, such as congestive heart failure, that is characterized by elevated atrial pressures and initially adaptive but subsequently pathological increases in cardiac, renal, and total body NE spillover and in multi- and single-unit MSNA discharge (2, 12, 21, 27).

In patients with untreated heart failure, saline loading elicits paradoxical increases in forearm vascular resistance (39), a finding consistent with the concept of a paradoxical muscle sympatoexcitatory response to stimulation of atrial mechanoreceptors. Strong positive correlations have been reported between filling pressures and resting plasma NE and MSNA (9, 26). Dibner-Dunlap et al. (5) found similar gains in heart failure patients and healthy controls with respect to the arterial baroreflex control of multi-unit MSNA. By contrast, responses to lowered atrial pressure without effect on arterial pressure were attenuated. These investigators concluded that the principal neural regulatory defect responsible for the sympathoex-

Table 3. Effects of LBNP and LBPP on hemodynamic variables estimated by Doppler-echocardiography

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>LBNP</th>
<th>LBPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke volume, ml</td>
<td>92 ± 10</td>
<td>92 ± 10</td>
<td>93 ± 10</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>5.6 ± 1.5</td>
<td>5.6 ± 1.4</td>
<td>5.6 ± 1.2</td>
</tr>
<tr>
<td>Total peripheral resistance, mmHg·l·min⁻¹</td>
<td>17 ± 5</td>
<td>17 ± 4</td>
<td>17 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SD.
citation of heart failure was impairment of cardiopulmonary
(not arterial) baroreflex-mediated inhibition of sympathetic
discharge (4, 5). The present findings offer an alternate inter-
pretation, namely that the blunted multi-unit MSNA response
observed reflects in its summation the firing properties of a
greater proportion (as compared with healthy controls) of
paradoxical versus anticipated single-unit discharge. Indeed, it
was our group’s previous observation of a paradoxical reduc-
tion in cardiac NE spillover in response to nonhypotensive
LBNP (2), and recognition by others of the potential clinical
importance of a sympathoexcitatory positive-feedback cardio-
pulmonary baroreflex loop (29), that stimulated the present
experiments.

Methodological considerations. The intent of this study was
to examine independently, relative to the preceding baseline,
the effect of LBNP and LBPP on single-unit discharge. For this
purpose we selected a sequential study design, which included
ample time for recovery between stimuli, as is evident from the
similarity of baseline data in Tables 1 and 2. Importantly, in
each instance, it was the identical unit, not newly recruited
units or different units, that behaved paradoxically in response
to these opposite stimuli. In our view these two findings
preclude attribution of the principal observations to any poten-
tial order-bias introduced as a result of this nonrandomized
experimental design. Future investigations of this concept
could randomize the sequence of interventions to assure the
absence of any potential carry-over effect.

The measurement and analysis of single-unit MSNA is
technically demanding, with high-quality stable recordings
required of all participants. We adopted established stringent
criteria, including synchronization with multi-unit MSNA, tri-
phasic spike morphology, and superimposition with minimal
variation (28, 32, 33) to minimize the possibility that the
identified spikes originated from more than one sympathetic
fiber.

With pulmonary veins, atria, and ventricles all populated by
mechanically and chemically sensitive vagal unmyelinated
afferent receptors, mechanically sensitive vagal myelinated
afferent receptors, and primarily chemically sensitive sympa-
thetic afferents, it may be questioned whether the present
experimental design permits determination of the specific an-
atomical site of the stimulus to the paradoxical single-unit
response identified. It is important to re-emphasize that our
objective was not to report the anticipated multi-unit responses
to LBNP or LBPP; those stimuli have been known for several
decades to elicit, respectively, net excitatory and inhibitory
multi-unit MSNA responses as a consequence of unloading and
loading atrial, ventricular, and pulmonary venous vagal unmy-
elinated afferents. Rather, we recorded from single-units to
determine whether we could identify within the multi-unit
preparation paradoxically firing single-units behaving as would
be anticipated from the unloading or stimulation of vagal
myelinated afferents eliciting, respectively, opposite efferent
sympathoinhibitory and sympathoexcitatory responses.

The latter, mechanically sensitive, afferents are located prin-
cipally within the atria, with their greatest density at veno-atrial
junctions. The left ventricle, by comparison, is much less
innervated (14, 37), although myelinated afferents exhibiting
higher resting firing rates and greater responsiveness to in-
creases in ventricular volume have been identified in cats (18).
Thus, with the preponderance of the myelinated mechanore-
ceptors of specific interest with respect to the present hypoth-
thesis (in so far as they have been characterized previously as
eliciting a reflex sympathoexcitatory response opposite to that
of stimulation of unmyelinated afferents by increases in vol-
ume) situated in atrial tissue, we considered it reasonable
anatomically to assume that it was these that represented the
principal and most probable stimulus to the documented parado-
Xical response.

There is also functional evidence that these stimuli of −10
mmHg and +10 mmHg were likely insufficient to stimulate,
significantly, ventricular myelinated mechanoreceptor affer-
ents. By combining hemodynamic measurements with cine
computed tomography, Oren et al. (35) demonstrated that when
LBNP −10 mmHg was applied to healthy subjects, right atrial
pressure decreased significantly (by 2.1–2.3 mmHg) and left
atrial volume fell on average by 27%, but right and left
ventricular end-diastolic volume and right or left ventricular
stroke volume did not change.

In the present series, the 1.6 to 1.8 mmHg changes in
estimated atrial pressure (2, 17) were more subtle than those
induced by Oren et al. (35) and would represent an ~15%
change in left ventricular end-diastolic pressure. A respective
decrease or an increase in stroke volume would be anticipated
had LBNP or LBPP exerted any significant effect on left
ventricular end-diastolic volume (and consequently ventricular
mechanoreceptor stretch), but no such changes occurred (Table
3). Importantly, we studied an older healthy cohort in whom
ventricular mechanoreceptors might be less responsive to sud-
den change, whether due to hypertrophy, or fibrosis, or other
subtle age-related deterioration. Based upon these anatomical
and functional considerations, we consider it reasonable to
conclude that the observed paradoxical single-unit responses
are affected primarily by deactivation and by stretch of my-
elinated atrial afferents.

Conclusions

In healthy middle-aged humans, single-unit MSNA record-
ings identified two populations of postganglionic sympathetic
fibers exhibiting opposite responses to changes in atrial pres-
sure induced by both nonhypotensive LBPP and nonhypoten-
sive LBNP. This demonstration of a subpopulation of paradox-
ically firing efferent single-units establishes the first evidence
in humans for an atrial-skeletal muscle sympathoexcitatory
reflex. Although the prevalence of such paradoxical single-
units in these healthy subjects was low (24% of all fibers
identified, and present in only 5 of 8 subjects studied),
documentation that postganglionic muscle sympathetic neu-
rons are not a functionally uniform population has relevance
for patients with heart failure and high atrial pressure, in
whom increased MSNA is a sign of increased risk for prematu-
re death (3).

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

AUTHOR CONTRIBUTIONS

REFERENCES