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, were hypothesized to mediate cardio-cardiac sympathoexcitation in healthy humans. Microneurographic recording demonstrated consistent reflex sympathoexcitation in response to increases in atrial pressure. The reflex consisted of a decrease in cardiopulmonary sympathetic nerve activity and an increase in muscle sympathetic nerve activity. Although the reflex was consistently activated by nonhypertensive lower body positive pressure (LBPP; +10 mmHg), it was not activated by nonhypertensive lower body negative pressure (LBNP; −10 mmHg). The reflex was concordant with multi-unit muscle sympathetic nerve activity (MSNA), whereas 24% demonstrated discordance with respect to single-unit responses. Additionally, the reflex was present in patients with normal cardiac function but not in patients with nonhypertensive left atrial pressures who demonstrated increased atrial filling and decreased cardiac sympathectomy. An assumption underlying the interpretation of such responses is that all single postganglionic sympathetic units comprising the multi-unit fascicle studied fired concordantly in response to these two stimuli. However, this assumption may be incorrect. In animal preparations, subpopulations of efferent sympathetic fibers have been shown to respond discretely to specific afferent input.

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 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).

Asthma exacerbation is a common clinical scenario that is associated with increased airway responsiveness, but the underlying mechanisms are not well understood. In recent years, there has been growing interest in the role of the vagus nerve in asthma. The vagus nerve is a somatic and parasympathetic nerve that innervates a wide range of organs and structures, including the respiratory tract. It has been hypothesized that the vagus nerve may play a role in regulating airway responsiveness and that its activation may be involved in the pathogenesis of asthma exacerbations.

In this study, we aimed to investigate the potential role of the vagus nerve in asthma exacerbations. We hypothesized that the vagus nerve may be involved in the pathogenesis of asthma exacerbations and that its activation may be associated with increased airway responsiveness.

Methods

The study was approved by the institutional review board, and all participants provided written informed consent. The study consisted of two phases: a baseline phase and an exacerbation phase. In the baseline phase, participants underwent a standard protocol that included airway resistance measurements, bronchodilator response tests, and measurements of respiratory and cardiovascular parameters. In the exacerbation phase, participants were exposed to a standardized stimulus that was known to induce asthma exacerbations, and airway resistance measurements were repeated. Bronchodilator response tests and measurements of respiratory and cardiovascular parameters were also performed. The stimulus used in the exacerbation phase was allergen exposure, which is known to induce asthma exacerbations in a substantial proportion of patients. The allergen exposure protocol was designed to induce a moderate to severe exacerbation in the majority of patients.

Results

The results of the study showed that exposure to allergen induced a significant increase in airway resistance in the majority of patients, compared to baseline. Furthermore, the results showed that exposure to allergen was associated with a significant increase in respiratory and cardiovascular parameters, consistent with the clinical presentation of asthma exacerbations. In addition, the results showed that exposure to allergen was associated with a significant increase in vagal activity, as measured by respiratory sinus arrhythmia (RSA) and heart rate variability (HRV) parameters. These findings are consistent with the hypothesis that the vagus nerve may play a role in the pathogenesis of asthma exacerbations.

Conclusion

In conclusion, our study provides evidence that the vagus nerve may play a role in the pathogenesis of asthma exacerbations. The results suggest that the vagus nerve is activated during asthma exacerbations, and that its activation may be associated with increased airway responsiveness. These findings have important implications for the understanding of asthma exacerbations and for the development of new therapeutic strategies aimed at targeting the vagus nerve in this context.
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 noneobese [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 percutaneously into a motor fascicle and then adjusted until a polyethylene catheter was inserted in a right antecubital vein suitable for monitoring peripheral venous pressure (forearm 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 (UNP35GS; 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, LBNP 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 recorded 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 recorded by a Micro1401 acquisition unit (ADCl2; 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.
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).

All 16 units exhibiting anticipated and all five units with paradoxical single-unit firing patterns during LBPP 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.
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 LBNP 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 blood flow.
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 sympatoexcitatory 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

Fig. 2. Representative tracing in 1 subject acquired before and during nonhypotensive 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. Examination of the single-units demonstrates paradoxical firing in unit 1 and anticipated firing of units 2 and 3. B: identified single-units superimposed.
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 sympathoexcitatory 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>Variable</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>
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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 interpretation, 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 reduction 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 potential 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, triphasic 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 sympathetic afferents, it may be questioned whether the present experimental design permits determination of the specific anatomical 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 unmyelinated 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 principally 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 increases in ventricular volume have been identified in cats (18). Thus, with the preponderance of the myelinated mecha-noreceptors of specific interest with respect to the present hypothesis (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 volume) 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 paradoxical response.

There is also functional evidence that these stimuli of −10 mmHg and +10 mmHg were likely insufficient to stimulate, significantly, ventricular myelinated mechanoreceptor afferents. 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 sudden 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 myelinated atrial afferents.

Conclusions

In healthy middle-aged humans, single-unit MSNA recordings identified two populations of postganglionic sympathetic fibers exhibiting opposite responses to changes in atrial pressure induced by both nonhypotensive LBPP and nonhypotensive LBNP. This demonstration of a subpopulation of paradoxically 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 neurons 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 premature death (3).

GRANTS

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AUTHOR CONTRIBUTIONS


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