Differential sympathetic nerve and heart rate spectral effects of nonhypotensive lower body negative pressure

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Floras, John S., Gary C. Butler, Shin-Ichi Ando, Steven C. Brooks, Michael J. Pollard, and Peter Picton. Differential sympathetic nerve and heart rate spectral effects of nonhypotensive lower body negative pressure. Am J Physiol Regulatory Integrative Comp Physiol 281: R468–R475, 2001.—Lower body negative pressure (LBNP; −5 and −15 mmHg) was applied to 14 men (mean age 44 yr) to test the hypothesis that reductions in preload without effect on stroke volume or blood pressure increase selectively muscle sympathetic nerve activity (MSNA), but not the ratio of low- to high-frequency harmonic component of spectral power (P1/P3), a coarse-graining power spectral estimate of sympathetic heart rate (HR) modulation. LBNP at −5 mmHg lowered central venous pressure and had no effect on stroke volume (Doppler) or systolic blood pressure but reduced vagal HR modulation. This latter finding, a manifestation of arterial baroreceptor unloading, refutes the concept that low HR modulation. This discordance is consistent with selectivity of efferent sympathetic responses to nonhypotensive LBNP and with unloading of tonically active sympathoexcitatory atrial reflexes in some subjects. Hypotensive LBNP (−15 mmHg) increased MSNA and P1/P3, but there was no correlation between these changes within subjects. Therefore, HR variability has limited utility as an estimate of the magnitude of orthostatic changes in sympathetic discharge to muscle.

IN THE PRESENT EXPERIMENT, two generally accepted concepts concerning reflex regulation of the heart and circulation in normal humans were reevaluated using spectral analysis of heart rate (HR) variability (HRV) and microneurographic recordings of efferent sympathetic discharge to skeletal muscle [muscle sympathetic nerve activity (MSNA)]. The first concept is that incremental levels of lower body negative pressure (LBNP), which simulates upright posture by pooling blood in the venous compartment of the lower extremities, can be applied to discriminate between reflexes arising from afferent nerve endings situated in the atria, pulmonary veins, and left ventricle, which sense changes in filling pressures and chamber volumes, and reflexes arising from afferent nerve endings located in the aortic arch and carotid sinus, which sense changes in blood pressure and stroke volume (SV) (1, 20, 29, 34, 49). The second concept is that of selectivity or nonuniformity of responses to stimulation or inhibition of the afferent limbs of these two reflex pathways. In young sodium-replete subjects, the application of nonhypotensive LBNP lowers cardiac filling pressure and glomerular filtration rate and activates sympathetic vasoconstrictor outflow to skeletal muscle without affecting plasma renin activity or HR (13, 30, 49). Further reductions in preload cause a fall in systemic arterial pressure and splanchic blood flow and a reflex increase in plasma renin activity and HR (1, 20, 49). Whereas efferent sympathetic nerve traffic to skeletal muscle and other vascular beds can be modulated by both sets of reflexes, the HR response appears to be governed selectively by the arterial baroreflex (13, 20, 34, 44, 49).

However, definitive proof of these concepts is lacking. Previous experiments involving selective reductions in central venous pressure (CVP) by nonhypotensive LBNP as a means of evaluating, in isolation, sympathoneural responses to unloading of low-pressure mechanoreceptors have not demonstrated consistent increases in MSNA (38, 39, 43, 47). Blood pressure was often measured intermittently in these investigations, and additional influences on high-pressure baroreceptor discharge, such as SV or cardiac output (CO) (18), were not assessed. Some studies in which LBNP at up to −15 mmHg was applied as a nonhypotensive stimulus comprised small numbers of healthy volunteers and therefore may have been underpowered to detect significant lowering of arterial blood pressure by this intervention. The concept of selective regulation of efferent responses originated from experiments comparing reflex changes in regional blood flow with HR. However, HR represents the integrated response to perturbation of several reflexes arising from the heart, great veins, and arteries, with discrete and often

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opposing actions on cardiac sympathetic and parasympathetic outflow. Determining whether sinoatrial discharge in conscious humans is indeed indifferent to the stimulus of nonhypotensive LBNP requires a method of discriminating between its adrenergic and vagal modulation. Power spectral analysis of HRV has achieved widespread acceptance as a noninvasive technique that can be applied for this purpose (8, 14, 27, 31, 35–37, 41, 44).

The effects of nonhypotensive LBNP on MSNA, HR, or HRV have been examined individually (1, 3, 4, 7, 8, 13, 20, 34, 38, 39, 40, 43, 44, 47, 49), but no previous experiment has recorded these variables simultaneously in conjunction with SV and CO. Most previous studies of graded LBNP report responses in young men. Because advancing age influences reflexes arising from cardiopulmonary afferents and their interaction with the arterial baroreflex control of MSNA (19), it may not be possible to generalize their conclusions to older subjects. Indeed, results of experiments in youths may have limited applicability to the interpretation of disturbances in neurogenic regulation of the circulation in cardiovascular diseases, which in general afflict an older population.

We therefore applied low levels of LBNP (−5 and −15 mmHg) to middle-aged subjects to test the hypothesis that small reductions in cardiac filling pressure lacking effect on SV, CO, or systemic arterial pressure would increase MSNA reflexively without evoking an increase in power spectral representations of the modulation of cardiac adrenergic drive or a decrease in the modulation of efferent vagal discharge. If present, such dissociation would provide novel frequency domain evidence in support of these two important concepts. Conversely, detection of a decrease in the vagal modulation of sinoatrial discharge in the absence of any change in HR would effectively refute the concept that incremental levels of LBNP can be applied to discriminate between reflexes arising from low- and high-pressure mechanoreceptor afferents, whereas demonstration of a concordant increase in MSNA and the spectral representation of cardiac adrenergic drive would refute the concept that nonhypotensive LBNP increases selectively efferent sympathetic nerve traffic to skeletal muscle, without affecting discharge directed at the sinoatrial node.

METHODS

Subjects

Fourteen healthy middle-aged men [44.5 ± 1.7 (SE) yr] were studied. Their mean weight was 81.4 ± 3.5 kg, and their height was 180.4 ± 1.4 cm. None were taking medication. All subjects provided informed written consent as approved by our Institutional Human Subjects Review Committee, in accordance with the Declaration of Helsinki.

Procedures

After voiding, subjects lay supine in a chamber custom-built for recording MSNA from the right peroneal nerve during LBNP (13). An airtight seal was obtained by a Neo-prene kayak skirt fitted at the iliac crests. The right leg was held in position by form-fitting supports, and caudal displacement was prevented by bilateral foot plates. LBNP was applied briefly to familiarize subjects with this sensation and to lessen the possibility of inadvertent muscular contraction during the actual protocol.

A volume-clamp cuff (Finapres 2300, Ohmeda) was then placed on the left middle finger for continuous noninvasive beat-by-beat blood pressure recording. After local anesthesia, a CVP catheter was introduced into an antecubital vein of the right arm and advanced to an intrathoracic position. A respiratory belt was secured around the abdomen. CVP and breathing excursions were measured continuously by pressure transducers (Statham P23ID, Gould, Cleveland, OH) and recorded simultaneously with lead II of the electrocardiogram, HR, and the MSNA neurogram by an ink recorder onto paper. Postganglionic MSNA was recorded from the right peroneal nerve (13, 33). SV was calculated over −10 consecutive cardiac cycles from two-dimensional echocardiograms, and continuous-wave Doppler recordings using previously reported methods (32). CO was determined from HR and SV.

Protocol

A 10-min baseline recording followed 20 min of supine bed rest. Then LBNP was applied at −5 and −15 mmHg. Each level was sustained for 10 min. BP, HR, CVP, and MSNA were recorded continuously. SV was derived over the last 2 min of baseline and each level of LBNP.

Data Analysis

MSNA. Pulse synchronous bursts of MSNA were identified by inspection of the mean voltage neurogram (13) by two trained but blinded assessors. Any inconsistency with respect to burst identification was resolved by the senior investigator. MSNA was expressed as burst frequency (bursts/min), incidence (bursts/100 cardiac cycles), and integrated nerve activity (the product of burst frequency and mean burst amplitude, in mm). In this multifiber recording preparation, the latter serves as a quantitative representation of postganglionic discharge.

Spectral analysis of HRV. The analog output of the electrocardiogram amplifier was discriminated to yield a train of rectangular impulses corresponding to the QRS complexes. The impulse train was processed on a real-time basis with a microcomputer via a 12-bit analog-to-digital converter (DAS-16, Metrabyte) at a sampling frequency of 1,000 Hz and stored sequentially for coarse-graining spectral analysis. The specific details of this technique have been reported elsewhere (8, 48). Seven minutes of data (2nd-8th min) were analyzed to determine HRV during each level of LBNP. Extra or missing beats were replaced by substitute R-R intervals calculated by linear interpolation from adjacent cycles. Spectra were calculated as ensemble averages of 256-beat sequences taken from a time series containing −400–500 beats.

Total spectral power (P T) was divided into its fractal (P F), low-frequency harmonic (0.0–0.15 Hz, P L), and high-frequency harmonic (0.15–0.50 Hz, P H) components, with total harmonic power (P T) comprising the sum of P L and P H. The parasympathetic nervous system is responsible for generating high-frequency power (27, 37, 41, 45). Therefore, the ratio P L/P T was used to estimate vagal contributions to P T. By convention (45), P L/P H was used to estimate sympathetic neural contributions to HR modulation. These two harmonic contributions to HRV are superimposed on a broad-band...
Table 1. Hemodynamic and MSNA responses to −5 and −15 mmHg LBNP

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>−5 mmHg</th>
<th>−15 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>122 ± 4</td>
<td>119 ± 3</td>
<td>118 ± 3*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>78 ± 2</td>
<td>76 ± 2</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>44 ± 2</td>
<td>43 ± 2</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>CVP, mmHg</td>
<td>4.9 ± 1.0</td>
<td>3.3 ± 0.9§</td>
<td>1.7 ± 2.0§</td>
</tr>
<tr>
<td>SV, ml</td>
<td>69 ± 4</td>
<td>66 ± 3</td>
<td>57 ± 3§</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>62 ± 2</td>
<td>64 ± 2</td>
<td>67 ± 2‡</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>4.3 ± 0.2</td>
<td>4.2 ± 0.2</td>
<td>3.8 ± 0.2§</td>
</tr>
<tr>
<td>MSNA, bursts/min</td>
<td>34 ± 2</td>
<td>37 ± 3†</td>
<td>43 ± 2§</td>
</tr>
<tr>
<td>MSNA, bursts/100 heartbeats</td>
<td>53 ± 3</td>
<td>58 ± 4*</td>
<td>64 ± 3§</td>
</tr>
<tr>
<td>MSNA burst amplitude, mm</td>
<td>7.2 ± 0.3</td>
<td>8.4 ± 0.6*</td>
<td>9.6 ± 0.9*</td>
</tr>
<tr>
<td>MSNA, units</td>
<td>251 ± 25</td>
<td>321 ± 43*</td>
<td>423 ± 52‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 14, except for central venous pressure (CVP, n = 12). MSNA, muscle sympathetic nerve activity; LBNP, lower body negative pressure; SV, stroke volume; HR, heart rate; CO, cardiac output. Normally distributed data were submitted to 1-way repeated-measures ANOVA; nonnormally distributed data were submitted to nonparametric Friedman repeated measures on ranks. For all pairwise comparisons, normally distributed data were submitted to Student-Newman-Keuls test. *P < 0.05; †P = 0.05; §P < 0.01; ¶P < 0.001 compared with baseline values.

RESULTS

Hemodynamic Responses to LBNP

A continuous measure of CVP was obtained in 12 subjects. LBNP at −5 mmHg caused a significant reduction in CVP, from 4.9 ± 1.0 to 3.3 ± 0.9 mmHg (P < 0.00001), but had no effect on systolic or diastolic blood pressure, pulse pressure, HR, SV, or CO (Table 1). LBNP at −15 mmHg lowered CVP further (to 1.7 ± 2.0 mmHg, P = 0.004 compared with −5 mmHg LBNP) and, in addition, reduced systolic blood pressure (from 122 ± 4 to 118 ± 3 mmHg, P < 0.04), SV (from 69 ± 4 to 57 ± 3 ml, P < 0.0001), and CO (from 4.3 ± 0.2 to 3.8 ± 0.2 l/min, P < 0.001). HR rose, from 62 ± 2 to 67 ± 2 beats/min (P = 0.004; Table 1). Diastolic pressure and pulse pressure were not affected by this stimulus.

MSNA During LBNP

LBNP at −5 mmHg increased mean values for MSNA burst frequency (from 34 ± 2 to 37 ± 3 bursts/min, P = 0.051), burst incidence (from 53 ± 3 to 58 ± 4 bursts/100 heartbeats, P < 0.05), mean burst amplitude (from 7.2 ± 0.3 to 8.4 ± 0.6 mm, P < 0.05), and the product of burst frequency and amplitude (integrated MSNA; from 251 ± 25 to 321 ± 43 units, P < 0.05; Table 1).

With −15 mmHg LBNP there were significant additional increases in MSNA burst frequency (to 41 ± 2 bursts/min, P < 0.01 compared with baseline and −5 mmHg LBNP) and burst incidence (to 64 ± 3 bursts/100 heartbeats, P = 0.00005 compared with baseline and P = 0.009 compared with −5 mm Hg LBNP). Burst amplitude (from 7.2 ± 0.3 to 9.6 ± 0.9 mm, P < 0.05) and integrated MSNA (from 251 ± 25 to 423 ± 52 units, P < 0.01) increased significantly above baseline, but not −5 mm Hg LBNP values (Table 1).

HR and HRV During LBNP

LBNP at −5 mmHg had no effect on pulse interval, the reciprocal of HR, on P L, or on mean values for the power spectral estimate of sympathetic nervous system modulation of HR, P L/P H (from 5.4 ± 2.1 to 15.5 ± 7.0, q = 2.646; Table 2). In contrast, the power spectral estimate of parasympathetic nervous system modulation of HR, P H/P F, fell from 0.09 ± 0.01 to 0.06 ± 0.02 (P = 0.018).

Individual responses appear in Fig. 1. In the majority of subjects, P L/P H rose and P H/P F fell. However, in four subjects, there was a clear decrease in P L/P H. In two of these four subjects, this was accompanied by a distinct increase in P H/P F. In a third subject, −5

Table 2. R-R interval and HR variability responses to −5 and −15 mmHg LBNP

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>−5 mmHg</th>
<th>−15 mmHg</th>
</tr>
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<tbody>
<tr>
<td>R-R interval, ms</td>
<td>941 ± 39</td>
<td>935 ± 34</td>
<td>890 ± 34‡</td>
</tr>
<tr>
<td>P L, ms²</td>
<td>1,159 ± 251</td>
<td>1,398 ± 369</td>
<td>1,465 ± 398</td>
</tr>
<tr>
<td>P H, ms²</td>
<td>709 ± 313</td>
<td>990 ± 296</td>
<td>1,063 ± 286</td>
</tr>
<tr>
<td>P F/P H</td>
<td>0.68 ± 0.04</td>
<td>0.70 ± 0.04</td>
<td>0.72 ± 0.02</td>
</tr>
<tr>
<td>P L/P F, ms²</td>
<td>449 ± 149</td>
<td>408 ± 97</td>
<td>409 ± 94‡</td>
</tr>
<tr>
<td>P H, ms²</td>
<td>151 ± 64</td>
<td>76 ± 34</td>
<td>62 ± 17</td>
</tr>
<tr>
<td>P H/P F</td>
<td>0.09 ± 0.01</td>
<td>0.06 ± 0.02*</td>
<td>0.05 ± 0.01†</td>
</tr>
<tr>
<td>P L, ms²</td>
<td>299 ± 88</td>
<td>332 ± 89</td>
<td>343 ± 103</td>
</tr>
<tr>
<td>P F/P H</td>
<td>5.4 ± 2.1</td>
<td>15.5 ± 7.0</td>
<td>20.5 ± 6.5*</td>
</tr>
<tr>
<td>β</td>
<td>1.2 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
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</tbody>
</table>

Values are means ± SE; n = 14. P L, total spectral power; P F, fractal component of spectral power; P H, low- and high-frequency harmonic components of spectral power; β, slope of linear regression. Normally distributed data were submitted to 1-way repeated-measures ANOVA; nonnormally distributed data were submitted to nonparametric Friedman repeated measures on ranks. For all pairwise comparisons, normally distributed data were submitted to Student-Newman-Keuls test. *P < 0.05; †P = 0.01; ¶P < 0.001 compared with baseline values.
mmHg LBNP evoked a small increase in P_H/P_T, with virtually no change in P_L/P_H. There was no significant correlation between changes in P_L and changes in MSNA with −5 mmHg LBNP, whether expressed as burst frequency (r = −0.45) or units (r = −0.18).

LBNP at −15 mmHg LBNP had no effect on P_T, P_F, P_H, and P_L but decreased pulse interval (from 941 ± 39 to 890 ± 32 ms, P < 0.001) and P_H/P_T, the power spectral estimate of parasympathetic nervous system modulation of HR (to 0.05 ± 0.01, P < 0.01), and increased P_T/P_H, the power spectral estimate of sympathetic nervous system modulation of HR (from 5.4 ± 2.1 to 20.5 ± 6.5, P = 0.03; Table 2). However, there was no significant correlation within subjects between changes in P_T/P_H and MSNA from baseline values (r = 0.20).

**DISCUSSION**

The primary objective of this experiment was to test the hypothesis that small reductions in cardiac filling pressure without effect on SV, CO, or systemic blood pressure activate selectively sympathetic outflow to skeletal muscle without triggering an increase in cardiac adrenergic drive. For this purpose, the microneurographic technique provided a direct and quantitative measure of sympathetic outflow to skeletal muscle, whereas spectral analysis was applied to obtain an indirect estimate of the relative contribution of the sympathetic nervous system to the modulation of sinoatrial discharge frequency before and during graded increases in LBNP. No previous experiment has recorded MSNA, HR, and HRV simultaneously and in conjunction with SV and CO. The present study is also novel, in that these comparisons were performed in a middle-aged, but otherwise healthy, population.

There were four principal findings. First, reductions in CVP with −5 mmHg LBNP had no effect on blood pressure, SV, CO (determinants of arterial baroreceptor discharge), or HR but elicited a significant reflex rise in sympathetic discharge to skeletal muscle. This was not accompanied by any increase in mean values for P_L/P_H or P_L, the two frequency domain representations of cardiac sympathetic modulation of HRV. Second, −5 mmHg LBNP decreased P_H/P_T, the power spectral estimate of the vagal contribution to HRV. Third, cardiac sympathetic modulation of HR increased significantly with the application of −15 mmHg LBNP. This stimulus caused further reductions in preload, lowered SV, CO, and systolic blood pressure, and increased MSNA burst frequency, burst incidence, and HR. Fourth, there was concordance between the effect of −15 mmHg LBNP on group mean values for these microneurographic and frequency domain estimates of sympathetic outflow to skeletal muscle and the sinoatrial node but no significant correlation between these two variables within subjects.

Results of previous experiments involving reductions in CVP of similar magnitude have been inconsistent. Initially, −5 mmHg LBNP was reported to reduce CVP in young adults by 2–2.5 mmHg and increase MSNA by 60–90% (43, 47). Because HR was unaffected, these observations were considered evidence in support of the concept of selective regulation of MSNA by low-pressure mechanoreceptor reflexes. However, blood pressure was measured intermittently and noninvasively in these studies, and important influences on high-pressure baroreceptor discharge, such as SV and carotid or aortic arterial dimensions (24, 46), were not assessed (43, 47). Thus the possibility that −5 mmHg LBNP increased MSNA by reducing discharge from arterial and low-pressure mechanoreceptors could not be excluded. Importantly, in a subsequent study from the same institution, −5 mmHg LBNP lowered the CVP of young adults by 1.8 mmHg but evoked only a nonsignificant 17% increase in integrated MSNA (38). Other investigators have also failed to detect a significant effect of −5 mmHg LBNP on MSNA (39).
LBNP up to and including −15 mmHg has been considered a nonhypotensive intervention. In a study of eight healthy subjects, Baily et al. (3) reported no effect on blood pressure but parallel increases in MSNA, forearm norepinephrine spillover, and HR in response to −15 mmHg LBNP. At first pass, these observations appear to refute the concept of selectivity of reflex responses to unloading of low-pressure receptors in humans. However, this conclusion assumes that LBNP had no significant effect on blood pressure or on other determinants of arterial baroreceptor discharge. In the present series, comprising 14 subjects, LBNP at −15 mmHg caused a small (−4 mmHg, or 3%), but nonetheless significant, reduction in systolic blood pressure as well as significant reductions in SV (17%) and CO (12%). Thus a more plausible explanation for such findings (3) and for inconsistencies in this literature is that many studies, comprising fewer subjects than in the present experiment, may have been underpowered to detect a true hypotensive effect of −15 mmHg LBNP. Our recent radiotracer kinetic studies in middle-aged subjects with normal left ventricular function, demonstrating increased total body norepinephrine spillover with nonhypotensive LBNP, in the setting of reduced SV and CO (2), add to the concern that graded application of LBNP cannot dissociate reliably reflex responses arising from unloading of low-pressure mechanoreceptors from those arising from high-pressure mechanoreceptors (10).

LBNP at −10 mmHg has been shown to reduce left atrial volume without affecting left ventricular end-diastolic volume or SV. This finding has reinforced the concept that this particular intensity of LBNP can be applied to elucidate the role of nonventricular cardio-pulmonary baroreceptor afferents in circulatory regulation (34). However, in the present series, even LBNP at −5 mmHg elicited a significant decrease in mean values for P_{H}/P_{T}. The obvious interpretation of this finding is that arterial baroreceptors were indeed unloaded, resulting in a reflex reduction in the parasympathetic modulation of sinoatrial discharge. If this conclusion is correct, it then follows that LBNP at or greater than −5 mmHg cannot be applied to middle-aged men to discriminate between these two sets of baroreceptor reflexes. Three potential objections to this conclusion should be considered. The first is that a similar decrease with −5 mmHg was not noted in a previous study by other investigators involving eight healthy young subjects (7). However, there was a strong trend in this direction, suggesting that their study was not powered to detect a true effect on a ratio with such high between-subject variability as P_{H}/P_{T}. The second objection is the lack of any detectable hemodynamic stimulus to arterial baroreceptor unloading (such as reductions in blood pressure, SV, or CO). However, arterial baroreceptors can respond to 1- to 2-mmHg changes in blood pressure (12), participate in the maintenance of systemic blood pressure during LBNP at less than −5 mmHg (9), and may also function as rheoreceptors (18). In humans, nonhypotensive hypovolemia has been shown to reduce carotid artery and ascending aortic caliber (24, 46). This, in turn, could alter baroreceptor discharge properties. The third objection is the absence of any parallel reflex increases in spectral representations of cardiac sympathetic modulation of HR, or in HR itself, in response to this stimulus. However, in some subjects, such activation may have been offset, or obscured, by the simultaneous unloading of atrial, ventricular, or aortic receptors that activate cardiac-specific sympathoexcitatory reflexes (26).

In some subjects, LBNP at −10 mmHg can induce a slight fall, rather than an increase, in HR (4, 29), and bradycardia with syncope can occur during LBNP in patients with ventricular deafferentation after transplantation (16). Conversely, volume loading to raise left ventricular end-diastolic (and presumably atrial) pressure, but with no effect on end-systolic pressure, can cause a modest, but significant, tachycardia (6). In experimental preparations, stimulation of right and left atrial mechanoreceptors by volume loading increases cardiac sympathetic and reduces cardiac vagal nerve discharge (5, 6, 17, 21, 23, 25). Efferent cardiac sympathetic nerves, which fire in response to stimulation of these atrial receptors, differ from those responsive to alterations in arterial baroreceptor discharge (25). If a tonically active Bainbridge reflex was functionally important in humans, nonhypotensive LBNP should exert the opposite effect, i.e., decrease cardiac sympathetic and increase efferent vagal firing. As revealed by Fig. 1, in several of these subjects, −5 mmHg LBNP did elicit a marked decrease in P_{H}/P_{T} and a clear increase in P_{H}/P_{T}, as might be anticipated if the predominant effect of this stimulus in these individuals was to unload atrial receptors mediating cardiac-specific excitatory reflexes, possibly via sympathetic afferent fibers (26).

Whether spectral analysis of HRV is capable of quantifying, specifically, the intensity of cardiac sympathetic nerve activity is a subject of vigorous debate (11, 22, 27, 28). In healthy subjects, the low-frequency component of the HR power spectrum and P_{H}/P_{T} rise, appropriately, in response to an orthostatic stimulus (8, 31, 35, 37), and infusion of sodium nitroprusside to lower blood pressure results in concordant increases in the low-frequency component of the HR and MSNA power spectra (36). Because power spectra estimate the relative contribution of oscillations in parasympathetic and sympathetic discharge at these specific frequencies to the modulation of HR, rather than the intensity of these autonomic inputs to the sinoatrial node, any relationship between these frequency domain indexes and a direct measure of sympathetic nerve firing, such as MSNA, might well be tenuous. Under resting conditions, there is no between-subject relationship between MSNA and HRV estimates of cardiac sympathetic tone (22, 33, 42). Saul et al. (42) noted a weak but significant correlation between P_{H}/P_{T} and MSNA when nitroprusside was infused to unload arterial baroreceptors, but only when burst frequency exceeded 40/min.
The present experiment addresses the issue of within-subject comparisons in response to an orthostatic stimulus. Of the proposed spectral representations of sympathetic modulation of HR, low-frequency power was not affected significantly by either level of LBNP. There was qualitative concordance between mean P1/P1 and MSNA responses but no significant within-subject correlation between the effect of LBNP at −15 mmHg on these two indexes. By contrast, in a recent experiment the stimulus of 75° tilt enhanced the coupling between low-frequency oscillations in HR and low-frequency oscillations in MSNA in healthy volunteers (15).

Conclusions

The present observations have implications for concepts concerning neural regulation of the circulation and also for the application of spectral analysis of HRV as a noninvasive estimate of changes in the intensity of central sympathetic outflow. LBNP at −5 mmHg had no effect on HR but caused a significant reduction in P1/P1, the power spectral representation of parasympathetic modulation of HR, a response that cannot be attributed to unloading of inhibitory reflexes arising from low-pressure mechanoreceptors. This observation therefore refutes the concept that low levels of nonhypotensive LBNP can be used to interrogate, selectively, cardiopulmonary reflexes without perturbing arterial baroreceptor reflexes. LBNP at −5 mmHg also elicited a selective increase in sympathetic discharge to skeletal muscle without altering spectral representations of cardiac adrenergic drive.

The mechanism or mechanisms responsible for this selectivity, i.e., the discordance between HR power spectral and microneurographic representations of central sympathetic outflow, cannot be established with certainty. Recent observations, from our laboratory, utilizing the isotope-dilution technique indicate that nonhypotensive LBNP can increase total body norepinephrine appearance rate in plasma without affecting left ventricular norepinephrine spillover (2). However, neither sympathetic outflow to the sinoatrial node nor its modulation can be quantified by this radiotracer method. Power spectral analysis may lack the sensitivity to detect changes in sympathetic, as opposed to parasympathetic, modulation of HR in response to modest baroreceptor unloading. Finally, this intervention may have engaged several reflexes with independent and opposite effects on cardiac sympathetic and parasympathetic tone. In some subjects, there was evidence for withdrawal of cardiac sympathetic modulation and enhanced vagal modulation of sinoatrial discharge, perhaps due to diminished stimulation of atrial sympathetic afferents or inhibition of the Bainbridge reflex. This may have obscured activation of sympathetic outflow to the sinoatrial node, in the remainder, in response to this stimulus, when mean P1/P1 responses were considered.

In contrast, LBNP at −15 mmHg caused significant reductions in blood pressure, SV, and CO and, therefore, arterial baroreceptor discharge and elicited concordant increases in MSNA, the spectral representation of cardiac adrenergic modulation, and HR. However, the absence of any significant within-subject relationship between changes in MSNA and P1/P1 in response to hypotensive LBNP indicates that neither variable can be considered representative of the intensity, in a particular individual, of the sympathetic response to orthostatic stimuli directed at other vascular beds.

Perspectives

LBNP, MSNA, and spectral analysis of HRV have yielded novel and important insights into mechanisms of cardiovascular regulation by the autonomic nervous system in intact conscious humans. The strengths and limitations of these methods continue to be explored and debated. The present observations signal caution in interpreting the results of experiments involving nonhypotensive LBNP. Responses elicited by this stimulus should not be attributed exclusively to unloading of low-pressure receptors with vagal afferents. Our observations, which indicate that arterial baroreceptor reflexes, and possibly sympathetic afferents, are also modified by this intervention, should stimulate careful reevaluation of previous conclusions based on studies that may have inadequate power to detect such changes. Frequency domain analysis should be appreciated primarily for the insight it provides into the oscillations of regulatory systems and for its prognostic value in patients with cardiovascular disease. Enthusiasm for its uncritical application as a method for quantifying the intensity of neural discharge to the heart and regional vascular beds should be tempered. These recommendations should not be considered exclusive to the investigation of healthy subjects but given perhaps even greater emphasis when considering the design or interpretation of studies of cardiovascular regulation in pathological states, such as hypertension or heart failure, that are characterized by altered neural regulation of the circulation.

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